IMPROVING SANITIZATION AND FERTILISER VALUE OF DAIRY MANURE AND WASTE PAPER MIXTURES ENRICHED WITH ROCK PHOSPHATE THROUGH COMBINED THERMOPHILIC COMPOSTING AND VERMICOMPOSTING

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DECLARATION

I declare that this thesis describes my original work, except where specific acknowledgement is made of the work of others.

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Dated this 19 August 2010.

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ABSTRACT

Thermophilic composting (TC) and vermicomposting (V) are the two most common methods used for biological stabilization of solid organic wastes. Both have their advantages and disadvantages but the proposed method of combining composting and vermicomposting (CV) borrows pertinent attributes from each of the two methods and combines them to enhance overall process and product qualities. Dairy manure and waste paper are two wastes produced in large quantities at the University of Fort Hare. The study was carried out to address the following specific objectives, to determine (i) the effectiveness of combined thermophilic composting and vermicomposting on the biodegradation and sanitization of mixtures of dairy manure and paper waste, (ii) an optimum precomposting period for dairy manure paper waste mixtures that results in vermicomposts of good nutritional quality and whose use will not jeopardize human health, (iii) the effectiveness of phosphate rock (PR) in increasing available P and degradation and nutrient content of dairy manure-paper vermicomposts, (iv) the physicochemical properties of vermicompost substituted pine bark compost and performance of resultant growing medium on plant growth and nutrient uptake.

Results of this study revealed that wastes with a C: N ratio of 30 were more suitable for both V and CV as their composts were more stabilized and with higher nutrient contents than composts made from wastes with a C: N ratio of 45. Both V and CV were effective methods for the biodegradation of dairy manure and paper waste mixtures with C: N ratio of 30 but the latter was more effective in the biodegradation of waste mixtures with a C: N ratio of 45. The combinination of composting and vermicomposting eliminated the indicator pathogen *E. coli* 0157 from the final composts whereas V only managed to reduce the pathogen population.

A follow up study was done to determine the effects of precomposting on pathogen numbers so as to come up with a suitable precomposting period to use when combine composting dairy manure-waste paper mixtures. Results of this study showed that over 95% of fecal coliforms, *E. coli* and of *E. coli* 0157 were eliminated from the wastes within one week of precomposting and total elimination of these and protozoan (oo)cysts achieved after 3 weeks of precomposting. The vermicomposts pathogen content was related to the waste's precomposting period. Final vermicomposts pathogen content was reduced and varied according to precomposting period. Vermicomposts from wastes precomposted for over two weeks were less stabilized, less humified and had less nutrient contents compared to vermicomposts from wastes that were precomposted for one week or less. The findings suggest that a precomposting period of one week is ideal for the effective vermicomposting of dairy manure-waste paper mixtures.

Results of the P enrichment study indicated an increase in the inorganic phosphate and a reduction in the organic phosphate fractions of dairy manure-waste paper vermicompost that were enriched with PR. This implied an increase in mineralization of organic matter and or solubilization of PR with vermicomposting time. Applying PR to dairy manure-waste paper mixtures also enhanced degradation and had increased N and P contents of dairy manure-waste paper vermicomposts. Earthworms accumulated heavy metals in their bodies and reduced heavy metal contents of vermicomposts.

A study to determine the physicochemical properties of vermicompost substituted pine bark compost and performance of resultant growing medium on plant growth and nutrient uptake was done. Results obtained revealed that increasing proportions of dairy manure vermicomposts in pine bark compost improved tomato plant height, stem girth, shoot and root dry weights.

Tomatoes grew best in the 40 to 60% CV substituted pine bark and application of Horticote (7:2:1 (22)) fertilizer significantly increased plant growth in all media. Progressive substitution pine bark with dairy manure vermicomposts resulted in a decrease in the percentage total porosity, percentage air space whilst bulk density, water holding capacity, particle density, pH, electrical conductivity and N and P levels increased.

Precomposting wastes not only reduced and or eliminated pathogens but also improved the stabilisation and nutrient content of dairy manure waste paper mixtures. The application of PR to dairy manure waste paper mixtures improved the chemical and physical properties of vermicomposts. Earthworms bio-accumulated the heavy metals Cd, Cr, Cu, Pb and Zn whilst the contents of these in the vermicomposts declined. It is, therefore, recommended that dairy manure waste paper mixtures be precomposted for one week for sanitization followed by PR application and vermicomposting for stabilization and improved nutrients contents of resultant vermicomposts. Substitution of pine bark compost with 40 to 60 % PR-enriched vermicompost produced a growing medium with superior physical and chemical properties which supported good seedling growth. However, for optimum seedling growth, supplementation with mineral fertilizer was found to be necessary.

PREFACE

The work summarized in the Abstract of this thesis is explained in detail in the six chapters that follow. Chapter 1 is a general introduction and literature review which establishes the justification for the study. The literature review establishes the need for the study, the causes of the problem, possible solutions to the problem and the potential of the studied solution in resolving the identified problem. Chapter 2 reports on the evaluation of different composting methods to degrade dairy manure-paper mixtures of different C: N ratios and their effectiveness in eliminating/reducing pathogens in vermicomposts. This chapter has been accepted for publication in the African Journal of Biotechnology. Chapter 3 reports on findings of a study that sought to determine the optimum precomposting period for dairy manure paper waste mixtures that results in vermicomposts of good nutritional quality and whose use will not jeopardize human health. This chapter has been accepted for publication by the Waste Management Research (WMR) journal. Chapter 4 reports on the effectiveness of using phosphate rock (PR) to enrich dairy manure with phosphorus as well as effects of PR on degradation and nutrient content of vermicomposts. The chapter also explains the effects of earthworms on heavy metal contents of vermicomposts. Chapter 5 looks at the possible use of vermicompost to substitute for pine bark composts in order to improve the pine bark compost media’s physical and chemical properties. Chapter 6 gives the general discussion, conclusions and recommendations for future studies.

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LIST OF ABBREVIATIONS AND ACRONYMS

ANOVA	analysis of variance
CV	composting and vermicomposting
C_{EX}	extractable carbon
C_{FA}	fluvic acid carbon
C_{HA}	humic acid carbon
EC	electrical conductivity
FA	fluvic acid
FC	fecal coliforms
HA	humic acid
HI	humification index
HR	humification ratio
LSD	least significant difference
MC	moisture content
MBC	microbial biomass carbon
MBP	microbial biomass phosphorus
MPN	most probable number
ns	not significant
WSC	water soluble carbon
RCBD	randomized complete block design
PR	phosphate rock

CHAPTER ONE

1.0 GENERAL INTRODUCTION

Rapidly increasing human population, intensive agriculture and industrialization have resulted in the need for efficient disposal and management of organic solid wastes. Institutions such as universities generate a lot of solid wastes in form of paper from the various Faculties. Currently at Fort Hare, waste paper is incinerated in an open dump but this practice is of concern as it causes air pollution through the production of carbon dioxide and nitrous oxides, both potent greenhouse gases as well as dioxins. As well, high density livestock operations such as the Fort Hare Dairy project generate large quantities of nutrient rich manures. James *et al.* (2004) estimated that dairy cows in free stall barns produce approximately 1986 kg of manure/Animal Unit (AU)/yr on a dry weight basis (1 Animal Unit=370 kg). However, overproduction of this waste substance has led to inappropriate disposal practices such as indiscriminate and inappropriately timed application to agricultural fields. Such practices cause serious environmental problems including an excessive input of potentially harmful trace elements, inorganic salts and pathogens, increased loss of nutrients through leaching, erosion and run off caused by lack of consideration of nutrient requirements of the crop (Hutchinson *et al*., 2005). Dairy manure, therefore, needs to be stabilized before it is applied to the land as a fertilizer or used as a growing medium in the nursery industry.

Stabilization involves the decomposition of a waste substance to the extent where the hazards are reduced and is normally reflected by decrease in microbial activity and concentrations of labile compounds (Benito *et al*., 2003). Because of the slurry nature of dairy manures it is not an uncommon practice to bulk the manure. Bulking has been done with a variety of bulking agents such as wood chips, wood shaving, cereal straw (Larney & Hao, 2007). Waste paper has been used successfully as a bulking agent in the stabilization of biosolids (Ndegwa *et al*., 2000) but there are no local reports on the use of waste paper as an amendment in the stabilization of manure.

Composting and vermicomposting are the two best known processes for the biological stabilization of solid organic wastes. Benefits of thermophilic composting include reduction of manure mass, volume, and haulage requirements (Larney *et al*., 2000); and elimination of weed seed viability (Larney & Blackshaw, 2003), coliform bacteria (Larney *et al*., 2003), human parasitic protozoa (Van Herk *et al*., 2004), and malodours on spreading (Rynk *et al*., 1992). However, thermophilic composting is associated with a long duration of the process, high frequency of turning of the material, need to reduce size of the material to provide the required surface area, loss of nutrients during the prolonged composting process, and the heterogeneous nature of the product (Ndegwa and Thompson, 2001). In addition, the very high temperatures (>60ºC) associated with thermophilic composting are also known to inhibit decomposition (Bardos & Lopez-Real, 1991). This could be due to the decline in microbial numbers as well as diversity at high temperatures (>60 ºC) during thermophilic composting (Hansen *et al*., 2001).

Vermicomposting involves the use of earthworms to mix, fragment and aerate organic waste material, making it more conducive to microbial activity and generally avoiding the exothermic stage (Hand *et al*., 1988). The result is a highly humified product (Vincelas-Akpa & Louquet, 1997), which contains most nutrients in plant-available forms such as nitrates, phosphates, and exchangeable calcium and soluble potassium (Orozco *et al*., 1996). The low operating temperatures (<35º C) in vermicomposting, however, are not high enough for acceptable pathogen and weed seed kill (Ndegwa & Thompson, 2001).

Logsdon, (1994), reported that combining traditional composting and vermicomposting shortened the time for curing and stabilization of the compost. Frederickson *et al*. (1997) and Nedgwa & Thompson, (2001) corroborated the findings of Logson when they reported reduction in composting time when the two systems are combined. Combining the two systems also resulted in vermicomposts that were nutritionally superior to either of the individual processes (Ndegwa & Thompson, 2001; Alidadi *et al*., 2005; Lazcano *et al*., 2008). However, long pre-composting periods could reduce the quality of the wastes in relation to easily degradable substances such as sugars, carbohydarates and proteins (Hsu & Lo, 1999) that are necessary for growth and reproduction of earthworms and thus degradation of wastes. It is important then that a suitable precomposting period be determined which is long enough to eliminate pathogens but short enough not to negatively affect the nutritional value of precomposted wastes for earthworms.

The role of organic carbon (C) and inorganic nitrogen (N) for cell synthesis, growth and metabolism in all living organisms is critical (Ndegwa and Thompson, 2001). For proper nutrition, carbon and nitrogen for optimal earthworm digestion is necessary too. For rapid microbial decomposition Diaz *et al*., (1993) recommended C: N ratio of between 25 and 30. Different earthworm species are impacted differently by C: N ratio and feed mixture type. Aira *et al*., (2006) reported higher *Eisenia fetida* earthworm populations in high C: N ratio wastes with more juveniles and hatchlings than in the low C: N wastes where earthworms were fewer and bigger with more mature adults than juveniles. Ndegwa & Thompson (2000) recommended that for vermicomposting mature earthworms be used as these will break down wastes faster than the juveniles. Therefore, studies are necessary to establish optimal C: N ratio for a specific earthworm species and a specific feed mixture.

Composts are poor plant nutrient sources when compared to inorganic fertilizers and are particularly low in phosphorus (P) (Mupondi *et al*., 2006) which compromises their adoption as growing medium for the nursery industry or as sources of nutrients for plants. Biswas & Narayanasamy (2006) reported that phosphate rock (PR) enriched composts had significantly higher content of total P (2.20%) compared to straw composts (0.3%) where no PR was added. The PR-compost also had higher citric acid soluble P (0.72% P) compared to straw compost (0.1% P). Most of work done on PR enrichment of composts has been done using rice straw and thermophilic composting was used to degrade the rice straw. South Africa has large deposits of igneous phosphate rock (PR) in Paraborwa (van der Linde, 2004) which could be used to enrich vermicomposts and increase their P content and availability. There is, however, little information on PR incorporation as a means of increasing the P content of vermicomposts. All PRs contain hazardous elements including heavy metals cadmium (Cd), chromium (Cr), mercury (Hg) and lead (Pb) and radioactive elements, uranium (U) that are considered to be toxic to human and animal health (Mortvedt & Sikora, 1992). Incorporation of PR into dairy manure-waste paper mixtures may therefore increase the heavy metal content of the vermicompost produced. Earthworms on the other hand have been reported to bioaccumulate heavy metals cadmium (Cd), chromium (Cr), copper (Cu), nickel (Ni), lead (Pb) and zinc (Zn) in their bodies (Shahmansouri *et al*., 2005) and their presence in the PR enriched dairy manure-waste paper mixtures may reduce the heavy metal composition of the resultant vermicomposts.

Pine bark is the growing medium of choice in South Africa due to its availability, affordability, and desired physical properties (high air porosity). However, it has low nutrient retention properties and little ability to provide nutrients to the substrate solution. Several studies have investigated benefits of vermicompost amended peat moss based substrates and have reported increased plant growth of greenhouse crops, both with and without fertilizer added, as well as increased water holding capacity compared to non-amended controls (Atiyeh *et al*., 2000; Atiyeh *et al*., 2001). However, studies investigating vermicompost amended pine bark in South Africa are not documented. Research is therefore required to determine the effects of partially substituting local pine bark with vermicomposts on the chemical, physical and microbiological properties of the plant growing medium and how these changes are related to seedling growth responses.

Aim of the study

The aim of the study is to improve sanitization and fertilizer value of dairy manure and waste paper mixtures enriched with rock phosphate through combined thermophilic composting and vermicomposting.

Objectives of the study

The objectives of this study were:

(i) To determine the effectiveness of combined thermophilic composting and vermicomposting on the biodegradation and sanitization of mixtures of dairy manure and paper waste.

(ii) To determine an optimum precomposting period for dairy manure-waste paper waste mixtures that results in vermicomposts of good nutritional quality and whose use will not jeopardize human health.

(iii) To determine the effectiveness of PR in increasing available P and degradation and nutrient content of dairy manure-paper vermicomposts.

(iv) To determine the effects of PR incorporation on the contents of the heavy metals Cd, Cr, Cu, Pb and Zn in earthworms and the resultant vermicomposts.

(v) To determine the physicochemical properties of pine bark compost substituted with various amounts of PR enriched vermicompost and performance of the resultant growth media on seedling growth and nutrient uptake.

Hypotheses of the study

(i) Combining composting and vermicomposting of dairy manure-waste paper mixtures will produce vermicomposts which are more humified and stabilised, and have higher nutrient content and lower pathogen *E. coli* 0157 compared with vermicomposting alone.

(ii) Reducing the period of precomposting dairy manure and waste paper mixtures to one week will eliminate faecal coliforms, the pathogen *E. coli* 0157, and protozoan oocysts.

(iii) Reducing the period of precomposting dairy manure and waste paper mixtures to one week will increase humification, stabilisation and nutrient content of dairy manure-waste paper based vermicomposts.

(iv) Amending dairy manure-waste paper mixtures with phosphate rock will improve stabilization of the vermicompost, its total P content and bioavailability, as well as the overall nutrients content of the vermicompost.

(v) Vermicomposting of PR amended wastes will result in increased concentrations of the heavy metals Cd, Cr, Cu, Pb and Zn in earthworms and a reduction of the same in the resultant vermicomposts.

(vi) Partial substitution of pine bark compost growing medium with vermicomposts will improve its physical and chemical properties, and enhance plant growth.

1.2 LITERATURE REVIEW

1.2.1 Introduction

The increase in industrialization has resulted in increases in both industrial and agricultural wastes production due to improved standards of living and intensification of agricultural production. These waste now present disposal and management challenges. Composting and vermicomposting are sustainable ways of managing the ever increasing amounts of wastes as populations continue to grow. The literature reviewed here is focused on waste production, waste management through composting, vermicomposting and their combination. Vermicomposts can either be used as a source of nutrients for crops or it can be used as a growing medium and in this review focus is on the use of vermicomposts to amend peat based media.

1.2.2 Solid waste generation and management

Over the last few years, the problem of efficient disposal and management of organic solid wastes has become more rigorous due to rapidly increasing population, intensive agriculture and industrialization (Garg *et al.*, 2006). In intensive livestock farming enterprises, such as the University Of Fort Hare Dairy farm, there is a huge amount of animal excreta being generated. According to James *et al*. (2004) dairy cows in free stall barns (1 Animal Unit=370 kg) produce approximately 1986 kg of manure/AU/yr on a dry weight basis with a moisture content of 80-88%. The current project at the University of Fort Hare Dairy farm has over 700 dairy cattle and that generates thousands of tones of nutrient rich dairy manure annually. The huge manure quantities generated present management and disposal problems.

Besides the manure, institutions such as universities also generate large quantities of other wastes such as printing and writing paper from different Faculties, food wastes from canteens and residential hostels as well as green wastes (mainly grass and twigs). At the University of Fort Hare, waste paper forms the bulk of the wastes produced. According to Richard Scott (26-05-2010, University of Fort Hare Xerox Manager, personal commnication) the amount of printing paper waste used at the university of Fort Hare has increased from 2.4 tons per month in 2007 to 3.2 tons per month in 2010. Nationally with rapid urbanization, increasing literacy, changing life style, consumeristic attitude and industrial growth, paper consumption in South Africa increased by 125 000 tons to 2.1 million tons from 2004 to 2005 (PAMSA, 2006/7). In the year 2006, 1 056 000 tons of printing and writing paper were produced in South Africa of which 56% (587 000 tons) was consumed domestically and the rest exported (PAMSA, 2007). Due to poor collection and segregation practices in South Africa, the wastes paper recovery rate is low and has been reported to be as only 20%. (PASMA, 2007).

Land application of manure is one practice the farmers have adopted as a way of recycling nutrients and disposal of the manure. However, research has shown that application of manure to fields can lead to high nitrate, phosphate and salt levels in the soil and run off (Tillman & Surapaneni, 2002; McLeod & Hegg, 1984). The narrow nitrogen to phosphorus ratio in cattle manure often results in excess loading of phosphorus when application rates are based on crop nitrogen demands (Dao, 1999). Besides the elevated nutrients levels problem manure also contains human pathogens. *Escherichia coli* 0157H:7 is one of the many strains of the bacterium *Escherichia coli (E. coli*) and this has been reported to occur in cattle manure (O'Connor, 2002) and has been reported to cause serious diarrhoea especially in immonodeficient people such as those with HIV and acquired immune deficiency syndrome (AIDS). Land application of raw manure can also potentially spread pathogens to the wider environment (Bach *et al.*, 2002). In Canada, *E. coli* 0157H:7 contaminated water caused seven deaths and made more than 2000 people ill in Walkerton, Ontario in May 2000 (O' Connor, 2000). The outbreak was linked to contamination of the town's water supply from land application of livestock manure from a nearby farm (O'Connor, 2002). Even land application of stock piled manure poses a threat since Kudva *et al*. (1998) reported that *E. coli* 0157H:7 survived for more than a year in a non aerated ovine manure pile that was exposed to environmental conditions. Reports of *E. coli 0157H:7* infections after consumption of spinach, lettuce and other crop produce after the use of animal manures or manure based soil amendments as fertilizers have been made (Ackers *et al*., 1998; CDCP, 2006; Rangel *et al*., 2005). Besides *E. coli* bacteria, animal manures are also sources of *Cryptosporidium* spp and *Giardia* spp protozoan parasites (Garber *et al*., 1994). *Cryptosporidium* spp is an important human pathogen as evidenced by several outbreaks of cryptosporidiosis in the past decade; the most severe of these outbreaks occurred in Milwaukee, Wis. USA, where more than 40 000 people were infected (MacKenzie *et al*., 1994). Cryptosporidiosis is a particularly serious health threat to immunodeficient individuals because there is no effective cure for the disease. Likewise giardiasis is also an infection of the small intestines caused by the parasites *Giardia* spp but unlike the former disease the latter has a cure (MacKenzie *et al*., 1994).

Other methods of disposal methods include use of dump sites and incineration. Some of the measures adopted to solve disposal problems have created more serious problems. At the University of Fort Hare, paper and other green wastes from the university grounds are usually incinerated in an open dump just outside the university and this could be a major source of air pollution with the production of carbon dioxide as well as oxides of nitrogen which are major greenhouse gases. The use of dump sites is being discontinued by many municipal authorities as land has become scarce and expensive. Dumpsites are also environmental disaster areas due to a variety of reasons. Firstly there is the danger of contamination of underground water through leaching of poisonous chemicals in these dump sites. Secondly fire out breaks can occur in these places due to production of methane in the dumps. Composting of wastes can therefore be used to address the management and disposal problems being currently faced.

However, there are a number of challenges that may arise with use of composts as sources of nutrients due to their often low nutrients content coupled with low availability of present nutrients (Brady & Weil, 2008). Low nutrient availability is partly attributed to the fact that composted materials when applied to soil decompose and mineralize more slowly than uncomposted organic materials (Brady & Weil, 2008). Composts also have high P to N ratios in comparison to plant needs and as such attempts to use composts as principal sources of plant nutrients can result in application of polluting levels of P (*ibid*.). During composting more labile organic substances are decomposed and composts usually provide less benefit to soil aggregation than would fresh residues (Brady & Weil, 2008).

1.2.3 Thermophilic composting

Composting is an accelerated biooxidation of organic matter passing through a thermophilic stage (45 to 65 ºC) where microorganisms (mainly bacteria and fungi and actinomycetes) liberate heat, carbon dioxide and water (Dominguez & Edwards, 1997). Finished compost is a more stable product made up of microbial residues and the more resistant organic compounds from the raw materials (Dominguez & Edwards, 1997).

Elements Necessary for Composting

Five factors that are critical for composting process are: temperature, air, moisture, carbon-nitrogen ratio, and pH levels. Temperature is a major factor in determining the type of microorganisms, the rate of metabolic activities, and the rate of biodegradation of the organic waste (Hobson *et al*., 1993). Air supplies aerobic microorganisms with oxygen and air movement is necessary to remove water from wet substrates through drying, and to remove heat and carbon dioxide generated by organic decomposition which will help to control process temperatures (Haug, 1993).

Moisture is essential to maintain microbial activity. Lack of moisture can impose severe rate limitations on the composting process. Moisture levels must be high enough to assure adequate rates of biological stabilization, yet not so high that free airspace is eliminated which can reduce the rate of oxygen transfer and in turn the rate of biological activity (Haug, 1993). The majority of composting processes should operate with moisture content in the 40 - 60% range (Haug, 1993).

The C: N ratio has a fundamental significance because nitrogen is necessary to support cellular synthesis and carbon makes up the largest fraction of organic molecules in the cell (Haug, 1993). Carbon is oxidized (respired) to produce energy and metabolized to synthesize cellular constituents. Nitrogen is an important constituent of protoplasm, proteins, and amino acids. An organism can neither grow nor multiply in the absence of nitrogen in the form that is accessible to it. Although microbes continue to be active without having a nitrogen source, the activity rapidly dwindles as cells age and die (Diaz et *al.*, 1994). During active aerobic metabolism, microbes use about 15 to 30 parts of carbon for each part of nitrogen. Hence, rapid composting is favored by maintaining a C: N ratio of approximately 30 or less. A higher C: N ratio can slow the compost process, however a C: N ratio that is too low can lead to a loss of nitrogen as ammonium N (Inbar *et al*., 1996).

Very low or very high pH levels can limit the rate of microbial activity. The optimum range for most bacteria is between 6.0 and 7.5, whereas the optimum range for fungi is 5.5 to 8.0 (Diaz *et al.,* 1994). Fortunately, composting has a rather unique ability to buffer both high and low pH back to a neutral range as composting proceeds. This ability to buffer extremes of pH is caused by the fact that both carbon dioxide (a weak acid) and ammonia (a weak base) are released as a result of organic decomposition (Haug, 1993). Carbon dioxide is an end product of all organic decomposition, while ammonia is an end product of protein decomposition. These components will tend to neutralize extremes of low or high pH, therefore, pH adjustment of the starting substrates is usually not required.

1.2.4 Compost stability, maturity and quality

According to Bernal *et al.* (1998a) maturity and stability of compost imply the absence of phytotoxic compounds and plant or animal pathogens. More specifically, they cite stability as generally related to microbial activity, whereas maturity is more associated with the absence of phytotoxins. Microbial activity is widely accepted as the most reliable indicator of compost stability and several studies have attempted to correlate various physical and chemical parameters to respiration (Bernal *et al.,* 1998a; Belete *et al*., 2001; Eggen & Vethe, 2001; Brewer & Sullivan, 2002). Microbial respiration, as measured by O_2 uptake and CO_2 production, generally decreases with the loss of readily biodegradable carbon and the subsequent stabilization of the remaining fractions (Brewer & Sullivan, 2002). Brewer & Sullivan (2002) reported high respiration rates during the first 27 days of aerated yard waste compost, and stable respiration during the curing period of 70 to 133 days. Microbial biomass is another measure of stability (Bernal *et al*., 1998b). Belete *et al*. (2001) estimated microbial populations not only by respiration, but also by measuring colony forming units (CFUs) and cell counts using epiflourescence microscopy. In a forced-aeration windrow of household and yard waste compost bulked with wood chips, they found a significant correlation between compost age and decrease in microbial CFUs, respiration, and bacterial cell counts. Water-soluble organic carbon generally decreases with time and is often used as another indicator of compost stability (Bernal *et al*., 1998b; Belete *et al*., 2001; Eggen & Vethe, 2001). Bernal *et al*. (1998b) reported reductions in water soluble organic C in various mixtures of sewage, municipal solid waste (MSW), pig slurry, crop residues, and olive (*Olea europaea*) mill wastewater, and attributed the decreased C to the rapid degradation of sugars, amino acids, and peptides.

As compost stabilizes, the ratio of humic to fulvic acids (humification) increases, due to loss of readily degraded fulvic acids (Bernal *et al*., 1998b). C: N is measured either in a solid compost or a compost water extract. The N concentration in mature compost is generally very low and the C: N in compost is generally higher in the solid compost than in the water extract (Bernal *et* al., 1998b; Eggen & Vethe, 2001). Other research has correlated C: N with other stability indices (Bernal *et al*., 1998a; Bernal *et al*., 1998b; Eiland *et al*., 2001). While the C: N ratio will vary depending on the compost feedstock, C: N ratios less than 12 are often considered stable (Bernal *et* al., 1998b).

Nitrification is another measure of compost maturity. Since temperatures greater than 40 ºC inhibits nitrifiers, nitrification generally occurs after the thermophilic phase. Mineralization of organic N is limited during the final phases of composting when little ammonium is available to bacteria (Sánchez-Monedero *et al*., 2001). As phytotoxic NH_4^+ concentrations decrease and NO^-_3

concentration increases, the compost is considered mature (Bernal *et al.*, 1998b; Eiland *et al*., 2001).

Compost quality refers to absence of foreign materials such as glass, plastics, nails, absence of pathogens and low heavy metal content as well as high nutrient content. Heavy metals should be within set limits and different countries have different limits for the different heavy metals. With pathogens there are also set limits depending on the country. In South Africa there are no set limits for compost quality parameters and as such the USEPA guidelines are usually used. In the case of pathogens, composts are considered safe for human handling and application to food and none food crops when the fecal coliform numbers in the composts < 1000 MPN fecal coliforms per gram (USEPA, 1994).

1.2.5 Effects of thermophilic composting on pathogens content and nutrient composition of composts.

Thermophilic composting has been shown to reduce/eliminate pathogenic organisms in wastes such as biosolids (Dumontet *et al*., 1999). Other workers have reported elimination of coliform bacteria in composted dairy manure feedlot manure (Larney *et al*., 2003). Composting also eliminates parasitic protozoa *Giardia* and *Cryptosporidium* (Larney *et al*., 2003). The principal mode of disinfection is based on time-temperature relationships that destroy pathogens although antagonistic microorganisms and ammonia may also play a role (Epstein, 1997). For pathogen elimination during windrow composting of biosolids, temperature should be maintained at > 55 $^{\circ}$C for 15 days or longer (USEPA, 1992).

However, even though the elimination of pathogens by composting is well documented (Krogmann *et al*., 2002, Tiqua *et al*., 2002) composting regimes (time, temperature) required to achieve elimination of pathogens vary widely. Turner (2002) demonstrated inactivation of *E. coli* in pig manure feces and cereal straw after only 2 h at 55 ºC. In contrast, Schleiff & Dorn (1997) reported that *E.coli* could be cultured from dry poultry manure after 88 days of composting. Research is required to establish composting regime (time, temperature) required to achieve elimination of pathogens in dairy manure-waste paper mixtures.

The thermophilic stage of composting is when these disinfecting temperatures are attained and as well it is the period of intense organic matter degradation. Composts which do not attain thermophilic temperatures are usually less stabilized and contain less nutrients than composts that attain thermophilic temperatures (Mupondi *et al*., 2006).

1.2.6 Vermicomposting

Vermicomposting as a practice started in the middle of 20th century and the first serious experiments were established in the Netherlands in 1970, and subsequently in England, and Canada. Later vermiculture practices were followed in USA, Italy, Phillipines, Thailand, China, Korea, Japan, Brazil, France, Australia, and Israel (Edwards 1988).Vermicomposting involves the use of earthworms to mix, fragment and aerate source material, making it more conducive to microbial activity and generally avoiding the thermophilic temperatures (Hand *et al*., 1988). It is usually carried out by epigeic earthworms (surface feeders) and the species commonly utilized for this purpose are *Eisenia fetida* and its related species *Eisenia Andrei*. These two species are prolific, have a wide temperature tolerance, and can grow and reproduce well in many kinds of organic wastes with a wide range of moisture contents (Edwards, 1988).

Vermicomposting results in a highly humified product (Vincelas-Akpa & Louquet, 1997) which contains most nutrients in plant-available forms such as nitrates, phosphates, and exchangeable calcium and soluble potassium (Orozco *et al*., 1996). The low operating temperatures (<35º C) in vermicomposting, however, are not high enough for acceptable pathogen and weed seed kill (Ndegwa and Thompson, 2001).

Basic Requirements for vermicomposting

In order for vermicomposting to successfully take place there are a number of basic requirements that have to be met. Some of these are discussed below.

(i) Moisture

Water constitutes 75 - 90% of the body weight of earthworms, so prevention of water loss is important for earthworm survival (Edwards and Lofty, 1977). Earthworms also need a moist skin in order to breathe and therefore a moist environment is required for their growth and survival. Edwards (1988) found the optimum moisture conditions for *E.fetida* to be 80 - 90% with a limit of 60 - 90%. However, in an earlier study, Reinnecke & Venter (1987) observed that earthworms have different moisture requirements at different stages of growth. The moisture preference of clitellate cocoon-producing (adult) *E. fetida* in separated cow manure ranged from 50% to 80% for adults, but juvenile earthworms had a narrower moisture range of 65% to 70%. Furthermore clitellum development occurred in earthworms at moisture contents from 60% to 70% but is delayed at lower moisture content ranges from 55% to 60% (*ibid*.). Clitellum is part of the reproductive system of earthworms that secretes a viscid sac in which eggs are deposited Edwards, (1998).

(ii) Temperature

Temperature just like moisture content of organic wastes is also a major factor in determining the growth of earthworms. The activity, metabolism, growth, respiration, and reproduction of earthworms are all greatly influenced by temperature (Edwards & Bloom, 1996). The temperature limit for survival of earthworms varies between species (Lee, 1985). *Eisenia fetida* thrives well in at temperatures of between 25-30ºC but can tolerate temperatures ranging from 0 to 35 °C (Edwards, 1988). Reinecke *et al*. (1992) found *E. fetida* to be more suited for vermiculture in Southern Africa compared to two other species they tested because it had a wider temperature tolerance. During the reproductive stages of earthworms require lower temperatures of less than 25 ºC which are less than those required for rapid growth Aston (1988).

(iii) Worm stocking density

Neuhauser *et al*. (1980) studied the effects of population density on growth and reproduction of of *E. fetida* and showed that the earthworm growth decreased with increased population density. Using regression analysis they estimated the ideal stocking density for *E. fetida* to be approximately 0.8 kg-worms m^{-2} on horse manure and 2.9 kg-worms m^{-2} on activated sludge. Later, Domiguez & Edwards (1997) working with *Eisenia andrei* concluded that, whereas individual worms grew more and faster at the lowest population density, the total biomass production was maximum at the highest population density. They observed that at higher densities, the worms sexually matured faster than at lower stocking densities. In a related study, Ndegwa *et al*. (2000) reported that a density of 1.60 kg-worm m^{-2} resulted in the highest bioconversion of biosolids into earthworm biomass and also produced the best vermicompost though at a lower feeding rate. From the above it is clear that a high stocking rate is to be preferred if rapid turnover of materials is desired.

(iv) pH

The distribution, numbers, and species of earthworms that live in any particular environment are limited by the pH of the environment. Edwards (1988) found the optimal pH for *E. fetida* to be a pH of > *5* and < 9. However, Edward (1995) found that when given a choice in a pH gradient, *E. fetida* tended to move towards the more acid materials with a pH preference of 5.0.

(v) C: N ratio

Organic carbon and inorganic nitrogen are important for cell synthesis, growth and metabolism in all living organisms including earthworms (Ndegwa & Thompson, 2000). Aira *et al*. (2006) reported that low C: N ratio materials resulted in an earthworm population that consisted mainly of mature adults with a higher mean individual weight than in high C: N ratio. However, in high C: N ratio materials the population consisted of mainly juveniles and hatchlings. A suitable C: N ratio is, therefore, required for the different materials used in vermicomposting that will allow for high earthworm growth and reproduction rates in order to accelerate waste breakdown and stabilization.

(vi) Feedstock

The organic material fed to earthworms in vermicomposting is usually referred to as feedstock. Feedstocks are important in that their quality not only determines the speed of breakdown by earthworms and microorganisms but also the nutrient content of the resultant vermicomposts. Most feedstocks used in vermicomposting are animal manures as they happen to be the most palatable to earthworms. Some of the commonly used feedstocks in vermicomposting and their benefits and limitations are discussed below.

(a) Pig manure.

In terms of nutritional value for earthworm, pig manure has been reported to have the highest nutrient levels in comparison to other manures (Edwards *et al*., 1994; Phillips, 1988; Edwards, 1998). Pig manure is usually in a slurry form necessitating the need for separating solids from the liquid fraction before the manure can be used for vermicomposting. Fresh pig manure from the pens may contain high amounts of salts as well as ammonia levels and may not be suitable for immediate vermicomposting (Edwards, 1998). Therefore, separated solids and fresh pig manure may need to be stabilized first to avoid overheating during vermicomposting (Philips, 1988).

(b) Cattle manure.

Cattle manure can be dairy manure or feedlot manure from intensive cattle production units or kraal manure from small scale farmers. The quality of manure has been reported to vary according to a number of factors. According to Donahue *et al*., (1981), the composition of domestic animal manures varies with type and age of animal, feed consumed, bedding used, the waste management system and climate. Accordingly dairy manures and feedlot manures are more suitable for vermicomposting as these manures have high nutritional values due to the feed given to these cattle. In contrast manures from communal areas are of low quality as these animals depend on natural grasslands/forests with no supplements. Animal manures are of high nutritional value and of special value for both composting and vermicomposting as they contain large and diverse populations of microorganisms (Raviv, 1998).

Dairy manure is usually in form of slurry after washing of this manure from milking sheds or parlours. Solids have to be separated from the slurries before they can be used to grow earthworms satisfactorily, but the liquids can be re-fed back to the solids at a later stage (Edwards, 1998). Hartenstein (1981) has pointed out that for cattle manure to be productive for earthworm biomass it has to be relatively free of urine as urine contains high salt levels which tend to dehydrate earthworms. Manures contaminated with urine may perhaps be suitable sources to *Eisenia fetida after* the ionic components become assimilated into microorganisms (Hartenstein 1981).

Extensive work has been done about the potential of cattle solids (either alone or mixed with other substrates) as a suitable substrate for vermicomposting using different species of earthworms in different temperatures, different moisture contents and different population densities (Chaudhri & Bhattacharjee, (2002), Dominguez *et al*. (2001), Reinecke & Viljoen (1990), Reinecke *et al*. (1992, Edwards *et al*. (1998)) and they all reported that cattle manure was suitable for vermicomposting alone or with additives. Recently Gupta & Garg (2009) successfully vermicomposted mixtures of cattle manure with non recyclable paper. They reported that vermicomposting of dairy manure with recyclable paper increased the nutrient content of the resultant vermicomposts thus providing a method of recycling nutrients from waste paper.

(c) Poultry manure

Poultry manure contains high levels of ammonia and soluble salts and is thus not a suitable feedstock for vermicomposting because nitrate and salt levels affect the growth of earthworms and can result in mortality of earthworms (Edwards, 1998). Nitrate content greater than 1 g kg^{-1} can result in earthworm mortality which may reach 100% at nitrate levels between 3 and 4 g kg^{-1} (Edwards, 1998). Salt levels greater than 5 g kg^{-1} have been reported to be toxic to earthworms

(Edwards, 1998). During vermicomposting of poultry manure on its own Leon et al. (1992) reported a 100% mortality but 84 – 100% survival with other undiluted and manure substrates. They attributed the 100% mortality to presence of high levels of ammonium and salts in the fresh manure. In order to vermicompost poultry manure on its own it is necessary to reduce the nitrate and salt levels by stockpiling the manure (Philips, 1998) or by leaching the poultry manure or pre-composting it for at least three months before addition of earthworms (Edwards, 1998).

(d) *Paper*. There is not much information on the use of paper on its own as a feedstock in vermicomposting. It has been established that the nutrient content of composts is dependent on the initial materials and the degree of decomposition (Gaur & Singh, 1995) and paper being nutritionally poor has traditionally been used to blend other composting materials. Paper has been shown to be low in nitrogen and high in carbon resulting in a material with a high C: N ratio (Gupta & Garg, 2009). Due to its poor nutritional status paper has traditionally been used as a bulking agent during composting of dairy manure slurry and biosolids (Ndegwa *et al*., 2000). A bulking agent is used to increase the C: N ratio, porosity, aeration as well as absorb excess water of the composting mass.

1.2.7 Compost enrichment

The nutrient content of vermicomposts differs greatly depending on the chemical nature of the feedstock. In general most composts/vermicomposts are poor in phosphorus in comparison with nitrogen. This could be related to the higher nitrogen content than phosphorus content in plant and or animal wastes though sewage sludge has been reported to have higher P contents probably due to anthropogenic inputs thorough detergents and industrial wastes processing (McLaughlin, 1984). There are possibilities of increasing the nitrogen and phosphorus content of composts by inoculation with nitrogen fixing microorganisms and the phosphorus content by addition of phosphate rock and then possible inoculation with phosphate solubilizing microorganisms (Kumar & Singh, 2001).

(i) Phosphate Rock

Phosphate Rock (PR) is a naturally occurring mineral source of phosphate mined and processed into soluble phosphate fertilizers. It is much less expensive than soluble phosphorus fertilizers, and is an amendment that is allowed by organic certification bodies (Zapata & Roy, 2004) Phosphate rocks generally are apatitic, containing varying percentages of P_2O_5 in a calcium matrix (*ibid*). In South Africa, PR in mined mainly in the Paraborwa area in the Mpumalanga Province (van Linden, 2004). The PR produced there is a low grade granitic type with low P and heavy metal contents (van Linden, 2004).

All PRs contain hazardous elements including heavy metals, e.g. cadmium (Cd), chromium (Cr), mercury (Hg) and lead (Pb), and radioactive elements, e.g. uranium (U), that are considered to be toxic to human and animal health (Mortvedt & Sikora, 1992; Kpomblekou & Tabatabai, 1994). The amounts of these toxic elements are dependent on whether the PR is sedimentary or granitic. More of these toxic elements have been reported in the more reactive sedimentary type of PR than the less reactive granitic PR (Van Kauwenbergh, 1997). The reactivity of the PR influences the availability of Cd to the plant because Cd is bound with P in the apatite structure (Sery & Greaves, 1996). McLaughlin *et al.* (1997) found that Cd concentrations in clover grown on the soil treated with a lower reactive Hamrawein PR (Egypt) containing 5.3 mg of Cd kg^{-1} per kilogram were lower than that treated with highly reactive North Carolina PR containing 40.3 mg of Cd kg^{-1} at the same P rates.

(ii) Principles of phosphor-composting

Phosphocomposting is a technique of applying PR to organic materials and then composted. During decomposition of organic wastes a lot of microbial activity occurs and these microorganisms in turn produce a large amounts of organic acids and humic substances (Gray *et al*., 1971). These acids and humic substances can solubilize PR thereby release phosphorus through acidification of PR by organic acids and through their chelating ability on calcium, iron and aluminum (Pohlman & McColl, 1986). The ratio of PR to organic wastes which has been reported to be effective is four parts of organic waste material to one part of PR (dry-weight basis) (Singh & Amberger, 1991).

(iii) Practical considerations of phospho-composting

The type of organic materials being composted and the rate of decomposition largely determine the effectiveness of composts in solubilizing PR. According to Mahimairaja *et al.* (1995) plant materials such as chopped leaves, crop residues (e.g. cereal straw) and lawn clippings composted with animal wastes are favoured because they produce more organic acids and humic substances. Poultry manure, on the other hand, is to be avoided as it will reduce dissolution of PR due to the high amounts of calcium carbonate it contains (*ibid*.)

Most phospho-composting work involving PR has been mostly done using thermophilic composting (Biswas & Narayanasamy, 2006) with rather limited work involving the use of earthworms to improve the solubility of PR. Vermicomposts have been shown to have high and diverse microbial populations (Edwards *et al*., 2004) and have also been reported to be more humified and contain more humic substances than the thermophilically processed aerobic composts (Senesi *et al*., 1992; Masciandaro *et al*., 1997). It is possible that these microorganisms and humic substances can enhance the dissolution of PR during vermicomposting and studies need be done to ascertain this.

1.2.8 Effects of heavy metals on earthworms

Heavy metals present in sewage sludge, municipal solid wastes and other organic wastes are toxic and can accumulate in earthworms. Malecki *et al*. (1982) investigated heavy metal toxicity in *E. fetida* and found minimum concentrations for different elements (in ppm dry weight) that retarded earthworm growth to be: 50 for Cd, 100 for Cu, 12 000 for Pb, 200 for Ni, and 2000 for Zn. Minimum concentrations for suppression of reproduction (ppm) were 25 for Cd, 100 for Cu, 4000 for Pb, 200 for Ni, and 500 for Zn (*Ibid*.).

Reviews by Beyer (1981) and Ireland (1983), have reported that earthworms can accumulate heavy metals from both contaminated and non-contaminated environments. Storage ratios or concentration factors (interchangeable terms that refer to the ratio of metal in worm tissue to that in the substrate) tend to be highest in infertile soil and lowest in media high in organic matter such as sewage sludge. Shahmansouri *et al.*, (2005) have demonstrated that the ability of earthworms to bioaccumulate heavy metals can be exploited to reduce heavy metals in vermicomposts.

1.2.9 Combining thermophilic composting and vermicomposting

Vermicomposting can accelerate organic matter stabilisation when compared with thermophilic composting. Hartenstein &Hartenstein (1981) reduced mean sludge volatile solids content by 9% in 4 weeks with *E. fetida* which was approximately one third more than the control without earthworms. Neuhauser *et al.* (1988) used *E. fetida* to vermicompost sludge for 4 weeks and achieved a reduction in the mean volatile solids content of approximately 28% which was at least twice that achieved for the control without worms. It is possible that an important factor in the enhancement of stabilisation with vermicomposting was the lower temperature vermicomposting regime compared to composting. For example fungi, associated with the decomposition of cellulose and lignin, are known to favour the mesophilic, rather than the initial thermophilic, phase of the composting process (de Bertoldi *et al.*, 1982).

The major drawback in the vermicomposting process is that, in contrast to traditional thermophilic composting the temperature must be below 35°C for survival of earthworms which is not high enough for acceptable pathogen kill and weed seed destruction (Ndegwa and &Thompson, 2001). An integrated system approach that borrows pertinent attributes from both the traditional thermophilic composting process and the vermicomposting process would be preferable to provide a product free of pathogens, and with desirable characteristics at a faster rate than either of the individual processes (Ndegwa & Thompson, 2001)..

Researchers have investigated the feasibility of combining thermophilic composting (precomposting) and vermicomposting to hasten the stabilisation of organic matter (Graziano & Casalicchio, 1987; Frederickson *et al.*, 1997, Ndegwa & Thompson, 2001) and as well to reduce pathogens (Nair *et al*., 2006). Most commercial vermicomposters use pre-composted material as a feedstock to guarantee a weed-free product. Graziano & Casalicchio (1987) recommended the integration of thermophilic composting and mesophilic vermicomposting as a means of sludge processing, using the coarser compost for “less specialized” crops and the finer, concentrated vermicompost for specialty horticulture.

An important factor in relation to improving the quality and suitability of wastes for vermicomposting maybe the degree of stabilisation or precomposting of the wastes. Pre-composting could reduce pathogenic organisms, and potentially toxic components, such as ammonia or salts in animal manures or the tannins and acids in green wastes and improve the survival of the worms (Gunadi *et al*., 2001). Long pre-composting periods could, however, reduce the quality of the wastes in relation to nutrient availability for growth and reproduction of earthworms. Frederickson *et al.* (1997) noted that precomposting period affected degradation rates during vermicomposting phase and recommended that precomposting be kept to a minimum consistent with wastes sanitization to avoid a reduction in stabilisation rates during the ensuing vermicomposting phase.

Though precomposting of wastes before they are fed to earthworms appears to be an important practice, there in no consensus on a suitable precomposting period of wastes. Ndegwa & Thompson (2001) precomposted biosolids and waste paper mixtures for four weeks whereas other workers like Nair *et al*. (2006) used a precomposting period of 9 days for kitchen wastes and recently Lazcano *et al.* (2008) precomposted dairy manure for 15 days. Further research is needed then to find an optimum precomposting period which is long enough to destroy pathogen but at the same time short enough not to alter the chemical composition of dairy manure-waste paper drastically.

The combination of thermophilic composting and vermicomposting results in composts of better quality than either process. Research has shown that combining thermophilic composting and vermicomposting results in wastes that are more degraded and stabilized with more available nutrients than composts from either vermicomposting or thermophilic composting alone

(Ndegwa & Thompson, 2001; Alidadi *et al*., 2005, Lazcano *et al*., 2008) and with less pathogens than vermicomposting alone (Nair et al, 2006).

1.3.0 Uses of vermicomposts

Vermicomposts can be used in several ways: 1) as a container growing medium, 2) as a component of a growing medium 3) as mulch or top dress, or 4) as a field soil amendment. The use of vermicompost in a container-growing medium is one that requires the best quality vermicompost.

Growing medium Physical Properties

Physical properties are the most important parameters related to plant performance in potting media (Chen *et al*., 1988). Physical properties are those properties that can be seen and felt, including colour, structure, texture and behaviour towards air and water (Van Schoor *et al*., 1990). Typically a growing medium consists of solid matter interspaced with pores. The pores may be filled with air or water, and the relative proportions of pore space filled with water and air affect plant growth (Van Schoor *et al*., 1990).

The bulk density (BD) of a growing medium is the ratio of the mass of dry solids in the medium to the bulk volume of the medium (Reed, 1996). For porous media, the bulk volume is the sum of the volume occupied by solids and that occupied by pore space. The mass of dry solids can be determined by drying the material in a forced-draught oven at a temperature of 70 ^{o}C until constant mass.

The volume of a growing medium is usually equated to the content of the container holding the medium (Reed, 1996). Media with low bulk densities are favoured, because they require little effort during the mixing process. Low bulk density also limits the mass of the containers when filled with medium, making their transport easy (Bunt, 1988). According to Nelson (1991), the wet bulk density of a growing medium should not be less than 640 kg m^{-3}, and not more than 1112 kg m^{-3}.

Total porosity (TPS) of a growing medium is the volume proportion of a medium that consists of pores (Reed, 1996). Pore space provides a medium with the capacity to contain water and air. Particle size distribution and the porosity of individual particles determine the total pore space and the volume of water and air a medium can hold. On average, a good peat medium can have a TPS of up to 96%. An average bark medium has a TPS of 85 % to 90 % (Smith, 1985).

The water holding capacity (WHC) of a medium is the volume of water that is retained by a medium after it has been saturated, and free water has been allowed to drain out of the medium. The amount of water a medium can hold depends upon the particle size distribution of the medium and the container height and should ideally be greater than 20% by volume (Ingram, *et al*., 1993).

Air filled porosity (AFP) of a medium is the volume proportion of a medium that contains air after it has been saturated with water and allowed to drain. AFP depends on container size. It increases as the container increases in height (Nelson, 1991). For growing medium an AFP of between 10 and 20% is considered suitable (Bunt, 1988).

There is lack of information on the effects of PR addition on physical properties of vermicompost. Addition of PR to vermicomposts can alter the physical properties of the resultant vermicompost especially so where the PR is applied in a powder form. The PR will increase the mass and particles in the vermicomposts thus can increase the bulk density and reduce porosity of vermicompost. It is necessary then to study the effects of PR addition with regards to physical properties of the resultant vermicomposts.

Chemical properties

According to Reed (1996), the pH of a growing medium is a measure of the concentration of hydrogen ions (H^+) found in the media solution. The pH influences the availability of nutrients in the medium solution. For this reason, plants usually grow best in a medium with a pH of 5.5 to 6.5 (Ingram *et al*., 1993).

Electrical conductivity (EC) is used as an indication of the salt concentration in a growing medium. Considering that a medium is expected to supply plants with nutrients, and nutrients appear in solution as mineral salts, the electrical conductivity should not be too low. It should also not be too high, because plants have difficulty in extracting water from solutions with a high salt concentration. According Hanlon *et al.* (2002), the optimum EC of growing medium extracts for use in vegetable seedling production ranges between 200 and 350 mSm^{-1}, depending on the plant to be grown.

Vermicomposts as components of potting medium

Vermicomposts have successfully been used as potting media substitutes in a bid to find an outlet for the product as well as reduce dependence on the ever declining peat. Several studies have evaluated the effect of vermicompost-amended potting media on plant growth greenhouse production. Generally, potting medium with 10 to 20% vermicompost by volume provides adequate fertilization for transplant growth (Atiyeh *et* al., 2000).

In one study, germination rates of greenhouse tomatoes increased up to 15% when vermicomposted pig manure was mixed with peat potting medium at 20, 30, and 40% by volume. The highest marketable yield of fruit was reported in the 20% mixture. Treatments consisting of 100% vermicompost led to smaller growth and fewer leaves than other treatments, due to high-moisture and possible phytotoxicity (Atiyeh *et al*., 2000). Because vermicomposts alone are not suitable to be used as potting medium due to their poor porosity and aeration, they have been shown instead to be capable of improving peat based potting medium's physical and chemical properties namely water holding capacity and nutrient content (Atiyeh *et al*., 2001). Most of the reported work on vermicompost incorporation has been done using peat based growing media and very few reports on substitution of pine bark compost growing media. Pine bark compost is the choice growing medium in South Africa due to its availability and good physical properties. Studies therefore needed to establish a suitable substitution rate for dairy manure-waste paper vermicompost into pine bark compost growing medium.

The foregoing literature review has revealed combined thermophilic composting and vermicomposting is a novel way of that can be used to sanitize and degrade organic wastes. There is however lack of information on the feasibility of using the combined system to sanitize and degrade dairy manure waste-paper mixtures. Precomposting wastes for sanitization should be kept to a minimum to avoid depleting wastes of nutrients for earthworms. However, there is no consensus on a suitable precomposting period for various organic wastes and a suitable precomposting period for dairy manure-waste paper mixtures has to be found. Dairy manure composts are poor sources of nutrients in particular P and research elsewhere has shown that phosphate rock can be used to enrich composts. However no work has been done with PR incorporation into dairy manure waste paper mixtures with a view to improve P availability and degradation of the wastes during vermicomposting. Pine bark compost is by far the most widely used growing medium in South Africa because of its availability and good physical properties. However it is acidic and does have sufficient nutrient to meet plant needs. There is no reported work in South Africa on the use of nutrient rich vermicomposts to amend pine bark compost medium in order to improve its chemical and physical properties. This study was therefore carried out to address the above mentioned concerns

CHAPTER TWO

EFFECTIVENESS OF COMBINED THERMOPHILIC COMPOSTING AND VERMICOMPOSTING ON BIODEGRADATION AND SANITIZATION OF MIXTURES OF DAIRY MANURE AND WASTE PAPER

2.1 ABSTRACT

Thermophilic composting is commonly used for bioconversion of organic wastes or for production of organic fertilizers but vermicomposting is also increasingly becoming popular. These two techniques have their inherent advantages and disadvantages. In this study vermicomposting and a combination of thermophilic composting and vermicomposting were compared as ways of sanitizing and biodegrading dairy manure and waste paper mixtures with C: N ratios of 30 and 45. Wastes with a C: N ratio of 30 proved more suitable for both vermicomposting and combined thermophilic composting and vermicomposting as their composts were more stabilized and had higher nutrients contents than composts made from wastes with a C: N ratio of 45. Both vermicomposting and combined composting and vermicomposting were effective methods for the biodegradation of dairy manure and paper waste mixtures with C: N ratio of 30 but the latter was more effective in the biodegradation of waste mixtures with a C: N ratio of 45. Combining therrmophillic composting and vermicomposting eliminated the indicator pathogen *E. coli* 0157 from the final composts whereas vermicomposting only managed to reduce the pathogen population.

Keywords: Biodegradation; C: N ratio; Dairy manure; *Eisenia fetida*; *E. coli* 0157; Humification index; Thermophilic composting; Vermicomposting; Waste paper

2.2 INTRODUCTION

The increasing rate at which organic wastes are being generated has created major waste disposal challenges in both developed and developing countries (Triphati & Bhardwaj, 2003). Among the solids wastes of concern is waste paper whose production and consumption has increased in South Africa by 125 000 tons to 2.1 million tons from 2004 to 2005 (PAMSA, 2006/7). Although no research has been done on the amounts of paper generated at the University of Fort Hare, the amounts have since increased with the increase in the enrollment from 8876 students in 2007 to 10116 students in 2009 according to Tracey Gardner (2010, 05-05-2010, Fort Hare Planning Unit, personal communication). According to Richard Scott (26-05-2010, University of Fort Hare Xerox Manager, personal communication) the amount of printing paper used at the university of Fort Hare increased from 2.4 tons per month in 2007 to 3.2 tons per month in 2010. The large volumes of paper produced at Fort Hare are incinerated in an open dump, contributing to greenhouse gases, like CO_2, in the atmosphere (Ndegwa & Thomson, 2001).

Besides paper the University of Fort Hare Dairy Farm has well over 700 head of cattle that generate equally large amounts of wastes in the form of animal excreta. James *et al.* (2004) estimated that dairy cows in free stall barns produce approximately 2000 kg of manure/Animal Unit (AU)/yr on a dry weight basis (1 Animal Unit=370 kg) and it is apparent then that the amount of manure produced at the university dairy farm is huge.

A common disposal avenue for animal manures is their use in agriculture as soil amendments. Cattle manure is, however, reported to contain pathogenic faecal bacteria and recent studies 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 (O'Connor, 2002). In fact, in the United States of America, outbreaks of *E. coli* 0157:H7 infections associated with consumption of spinach, lettuce and other produce crops have implicated animal manures as sources of pathogens in fruits and vegetables (Ackers *et al*., 1998; CDCP, 2006; Rangel *et al*., 2005). Therefore, the management of animal manures for recycling in agriculture must, of necessity, incorporate sanitization to minimize potential disease transmission.

Thermophilic composting and vermicomposting are two of the best-known processes for the biological stabilization of solid organic wastes. Thermophilic composting allows sanitization of the waste by the elimination of pathogenic microorganisms (Lung *et al*., 2001). However, it requires a long duration, frequent turning of the material, and results in loss of nutrients during the prolonged composting process, while the heterogeneous nature of the final product makes it less desirable (Ndegwa & Thompson, 2001). The high temperatures (>60°C) associated with the process are also known to inhibit decomposition (Bardos & Lopez-Real, 1991). Vermicomposting, which involves the bio-oxidation and stabilization of organic material by the joint action of earthworms and microorganisms, results in a more homogenous product. However, pathogen removal is not ensured since the temperature is always in the mesophilic range, although some studies have provided evidence of suppression of pathogens (Monroy *et al*., 2008).

The combination of thermophilic composting and vermicomposting could provide the combined benefits of the two systems (Ndegwa & Thompson, 2001; Alidadi *et al.*, 2005; Nair *et al*., 2006; Tognetti *et al*., 2007). While thermophilic composting enables sanitization of wastes and elimination of toxic compounds, the subsequent vermicomposting reduces particle size and increases nutrient availability (Ndegwa & Thompson, 2001). Combined thermophilic and vermicomposting has been studied with a variety of materials including biosolids (Ndegwa &

Thompson, 2001; Alidadi *et al.*, 2005), kitchen wastes and green waste mixtures (Nair *et al*, 2006) as well as cattle manure (Lazicano *et al*., 2008). They all reported enhanced stabilization and higher nutrient contents in vermicomposts from the combined system than from either individual processes. There is need therefore to determine the feasibility of combined composting of dairy manure-waste paper mixtures.

The role of organic carbon (C) and inorganic nitrogen (N) in cell synthesis, growth and metabolism is critical for all living organisms including earthworms (Ndegwa & Thompson, 2000). Different earthworm species are impacted differently by C: N ratio and feed mixture type (Aira *et al*.,2006). Besides the impact on earthworms, precomposting wastes low in C: N ratio could not only result in high nitrogen losses but also the production of wastes which could be unsuitable to earthworms due to high levels of ammonium nitrogen and salinity (Kirchman &Widen, 1994). Wastes with high C: N ratio would benefit from such precomposting through a reduction in the C: N ratio and hence an improvement in nutritional value of wastes. Therefore, studies are necessary to establish optimal C: N ratios for dairy manure-waste paper mixtures vermicomposted using *Eisenia fetida (E. fetida).*

There is little or no local information available on the effectiveness of combined thermophilic composting and vermicomposting on biological stabilization and sanitization of mixtures of dairy manure and wastes paper. The aim of this study was to combine thermophilic composting and vermicomposting in the bioconversion of dairy manure and waste paper mixture. Specific objectives of this study were to determine (i) the comparative effectiveness of combined thermophilic composting and vermicomposting on the biodegradation of mixtures of dairy manure and paper waste with different C: N ratios, and (ii) the effectiveness of combined thermophilic composting and vermicomposting on the sanitization of biodegraded dairy manure-waste paper mixtures.

2.3 MATERIALS AND METHODS

2.3.1 Experimental site, wastes and earthworms utilized

The study was carried out at the University of Fort Hare located 32°46´ S and 26°50´ E in the Eastern Cape Province of South Africa in an open but shaded yard. Dairy manure used in the study was obtained from the Keiskammerhoek Dairy Project located about 60 km North East of the University of Fort Hare, while shredded waste paper was obtained from the Duplicating Center of the University of Fort Hare. Representative samples of dairy manure and shredded paper were air dried, and ground to pass through a 2 mm sieve and then analyzed for pH, EC, total N, C and P, available N and P, volatile solids (organic matter), and ash. The earthworm species *Eisenia fetida* (commonly known as red wigglers) (Edwards and Bohlen, 1996) obtained from East London, Eastern Cape Province, South Africa was used in the study. The earthworms had been fed on grass cuttings and vegetable wastes for three months before use.

2.3.2 Composting treatments

Composting was done using mixtures of dairy manure and waste paper mixed to obtain C: N ratios of 30 and 45. The composting methods were (i) control (C) (ii) vermicomposting (V) and (iii) combined thermophillic composting and vermicomposting (CV). ,

Control (C)

In the control, mixtures of dairy manure and shredded waste paper having C: N ratios of 30 and 45 were put into vermicomposting boxes (similar size as those for vermicomposting) and watered to 80% moisture level recommended for vermicomposting but no earthworms were introduced. The waste mixtures were allowed to incubate for eight weeks without further treatment except moisture adjustment.

Vermicomposting (V)

Vermicomposting was performed in boxes measuring 0.50 x 0.40 x 0.30 m^3 (length x width x depth) which provided a 0.2 m^2 of exposed surface area. Mature earthworms were introduced at a stocking rate of 1.6 kg-worms/m^{-2} into the worm boxes and fed at the recommended rate of 0.75 kg-feed/kg-worm/day (Ndegwa *et al*., 2000). Enough feedstock (14 kg dry weight basis) consisting of mixtures of dairy manure and shredded waste paper having C: N ratios of 30 or 45 was provided to meet the needs of the earthworms for the entire eight week period. Moisture levels were maintained at about 80% MC level. At the end of the eighth week the worms were separated from the vermicompost.

Combined thermophilic composting and vermicomposting (CV)

Dairy manure was mixed with shredded waste paper to give feedstock materials with ratios of 30 and 45. Thermophilic composting of the wastes was then done in boxes measuring 1m x 1m x 1m (length x width x height) for four weeks. The wastes were weighed and mixed manually on a polythene sheet using shovels. Mixing was done repeatedly from one end to the other adding water to 60% moisture content, before the materials were loaded into the composting boxes. Compost and ambient temperatures were taken daily for four weeks while MC was determined weekly and used as a basis for its adjustment to 60%. Turning was done biweekly. At the end of four weeks the waste mixtures were vermicomposted. Enough of the composted feedstock was provided to meet the needs of the earthworms for the entire four weeks (28 days) of this phase of the experiment. The vermicomposting process was terminated at the end of the fourth week at which time worms were separated from the vermicompost.

Samples were taken for analysis at the beginning of the experiment (feedstock), four weeks (intermediate composting phase) and at eight weeks (final composts) for all the treatments.

2.3.3 Analyses

The samples were analyzed for moisture content (MC), volatile solids (VS), ash content, total carbon (C), total nitrogen (N), inorganic N (nitrate- N and ammonium – N), total and available phosphorus P, humic substances and *E. coli* 0157 population.

2.3.3.1 Methods of analyses

Moisture content (MC) was determined gravimetrically by oven drying samples at 70ºC to constant mass and expressed on a wet-weight basis. For other determinations, representative samples were dried in an oven at 60ºC until constant weight and then ground (< 2mm) to provide a homogenous sample. Volatile solids were obtained by ashing at 550ºC for 4h (Atiyeh *et al*., 2000). Electrical conductivity (EC) and pH were determined potentiometrically in a 1:10 (compost: distilled water) suspension as described by Ndegwa & Thompson (2001). This suspension was shaken on a mechanical shaker at 230 rpm for 30 minutes and allowed to stand for an hour prior to pH or EC measurement

Total nitrogen (N) and carbon (C) were determined using a Truspec CN Carbon / Nitrogen Determinator (LECO Corporation, 2003). Total phosphorus (P) was extracted by wet digestion using the concentrated sulphuric acid, selenium, lithium sulphate and hydrogen peroxide mixture as described by Anderson & Ingram (1996). The concentration of P in the digest was then determined by the molybdenum blue colorimetric method.

Mineral nitrogen (NH_4^+-N and NO_3^--N) was extracted from fresh compost samples with 0.5 *M* K_2SO_4 (Okalebo *et al.*, 2002). The nitrate and ammonium concentrations were determined using a spectrophotometer after development of a yellow colour using 5% salicylic acid in concentrated sulphuric acid for nitrate-N and a blue colour using salicylate-nitroprusside colorimetric method (Okalebo *et al.*, 2002). Available P of organic wastes was estimated using the Bray 1 extractant for nitrate (Okalebo *et al*., 2002). The amount of phosphorus extracted was determined by the molybdenum blue colorimetric method.

Humic substances were extracted by treating samples with 0.1 *M* NaOH (1:20 w/v ratio) and constantly shaking for 4 h (Garcia *et al.*, 1993). After centrifugation for 15 min, at 8000 rpm, the supernatants were divided into two fractions, one of which was stored for later analysis of total extractable fraction (C_{EX}), and the other adjusted to pH 2 with concentrated H_2SO_4 and allowed to coagulate for 24 h at 4°C. The precipitates that formed constituted the humic acid fraction (HA) while fraction that remained in solution constituted the fulvic acids (FA).

The two fractions were separated by centrifugation as described earlier and stored for carbon (C) analyses by the Walkely Black method as described by Anderson and Ingram (1996). The carbon concentration of the humic acid fractions (C_{HA}) was calculated by subtracting the fulvic acid fraction C (C_{FA}) from the total extractable C fraction (C_{EX}). The humification ratio (HR) and humification indexes (HI) were calculated using Equations 1 and 2 respectively:

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

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

Presence of *E. coli* in the initial and final composts was determined as outlined by Berry and Wells (2008). A sample (5g) of either waste mixture or compost was added to 45 ml of peptone buffer and mixed using a blender. Serial dilutions of 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5} and 10^{-6} were prepared from the 1: 10 dilution and the 10^{-5} and 10^{-6} dilutions were used for *E. coli* enumeration. A 50 µl portion was plated onto CHROMagar O157 (DRG International) containing 5 mg/L novobiocin and 2.5 mg/L potassium tellurite (ntCHROMagar O157). The CHROMagar O157 plates were incubated at 42ºC for 24 hours and enumerated. *E. coli* O157 colonies are flat, mauve-colored colonies without distinct centers. Plates with less than 20 or more than 300 colonies were discarded. The presumptive *E. coli* O157 colonies were identified via an agglutination test using *E. coli* O157:H7 latex. Presence of agglutination confirmed the presence of *E. coli* O157 bacteria. Colonies subjected to agglutination test were then indentified as *E. coli* with API 20E kits.

2.3.4 Statistical Analysis

Analysis of variance (ANOVA) was carried out on the various data sets using GENSTAT Release 4.24 (Lawes Agricultural Trust, 2008) while means were separated using least significant difference method (LSD) at $P \leq 0.05$ level of significance.

2.4 RESULTS

2.4.1 Chemical characteristics of the wastes

Dairy manure had higher nitrogen content and lower carbon content than waste paper resulting in a lower C: N ratio (Table 2.1). The pH of both dairy manure and waste paper was alkaline but EC was higher in dairy manure than in paper. The ash content of paper was also much lower than that of dairy manure.

Table 2.1: Selected chemical properties of wastes used in the study

Chemical Property	Raw material	
	Dairy Manure	Waste paper
pH	7.8 ± 0.1	8.2 ± 0.3
EC (mSm^{-1})	440 ± 2.0	0.18 ± 0.1
Total N (gkg^{-1})	24 ± 2.5	3 ± 0.6
Total C (gkg^{-1})	321 ± 3.0	370 ± 5.3
C:N	13.2 ± 0.6	123 ± 3.7
Total P (gkg^{-1})	2.9 ± 0.4	0.5 ± 0.1
C:P	110 ± 5.0	740 ± 15.5
Ash (gkg^{-1})	379 ± 8.5	178 ± 4.5

Parameters reported as mean ± standard deviation

The P content of manure, at 2.9 g kg^{-1}, was much higher than that of waste paper, which only contained 0.39 g kg^{-1} resulting in a much lower C: P ratio for manure.

2.4.2 Temperature profiles during the thermophilic composting phase

Composts with C: N ratio of 30 maintained thermophilic temperatures from the second day up to the end of the composting period, reaching a maximum temperature of 72ºC on the 5th day (Figure 2.1). By contrast, composts with a C: N ratio of 45 attained a maximum temperature of only 54 ºC after 4 days of composting which dropped to 40 ºC by day 7. After the first turning, temperature increased to thermophilic ranges briefly but decreased thereafter until termination of this phase of the experiment after 28 days.

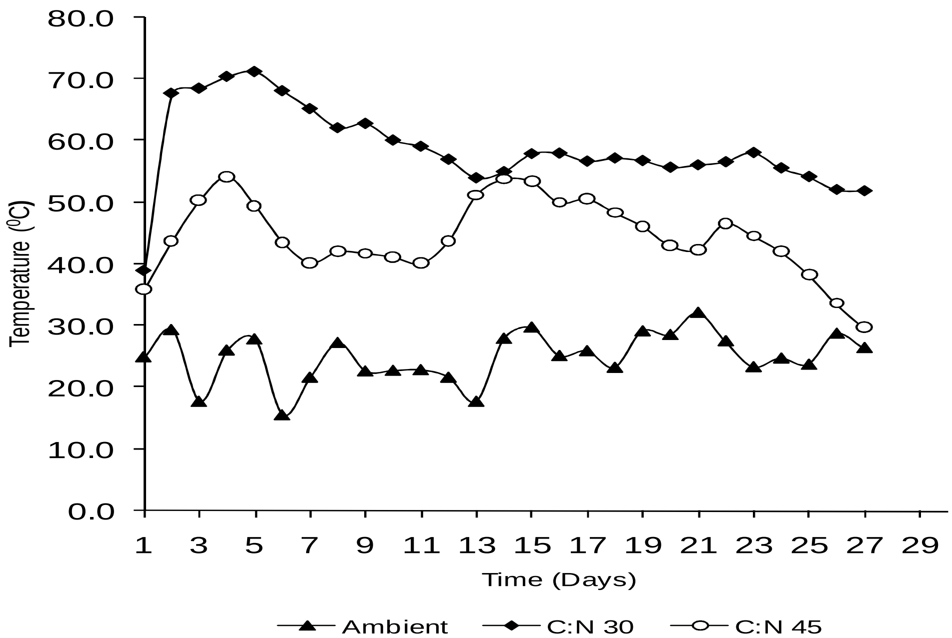


Figure 2.1: Temperature profiles of dairy manure – paper waste mixtures during the thermophillic composting phase prior to vermicomposting.

2.4.3 Degradation and humification of composted wastes

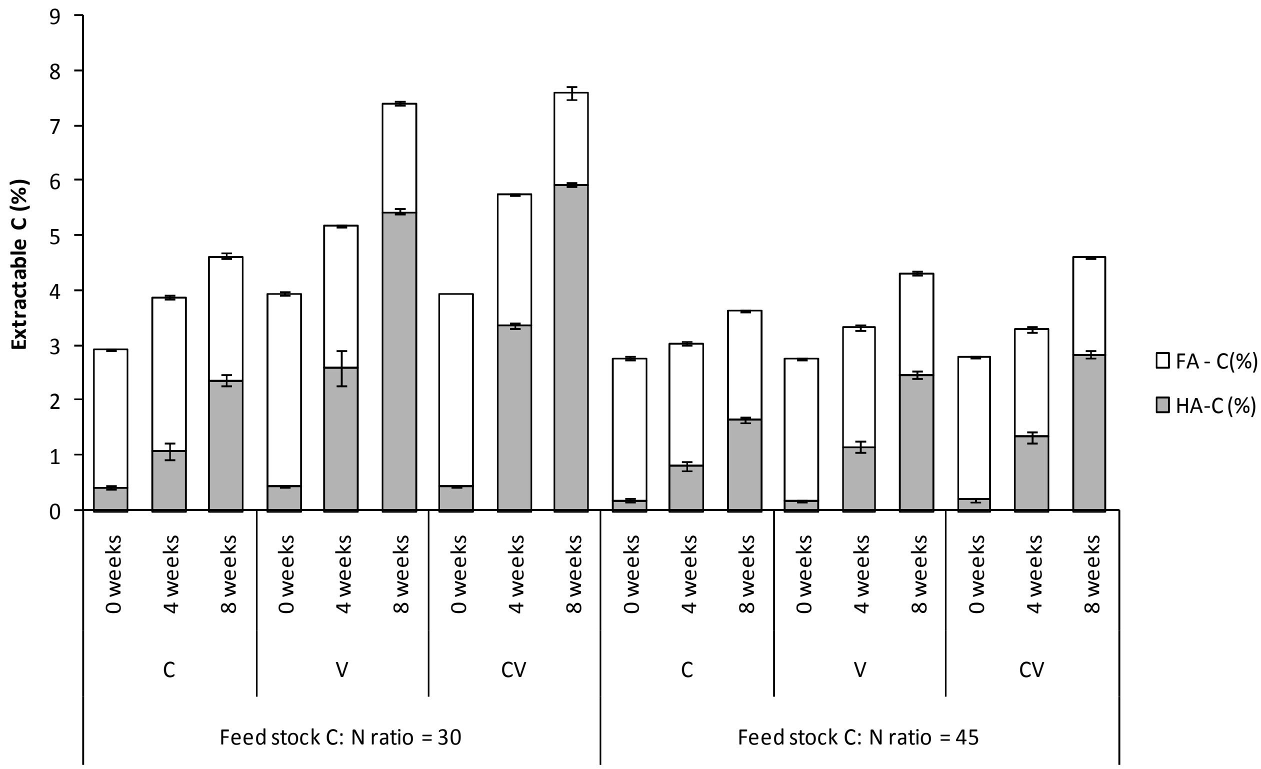
Feed-stocks with a C: N ratio of 30 had higher ash and lower VS contents than feed-stocks with a C: N ratio of 45 (Table 2.2). Volatile solids decreased by between 4% and 27% as a result of composting and the decreases were influenced by both composting method and the C: N ratio of waste mixtures. The greatest reductions in VS were observed in waste mixtures with a C: N ratio of 30 where VS in final composts was reduced by 27% and 23% by vermicomposting and combined composting and vermicomposting, respectively.

Table 2.2: Effect of composting method and C: N ratio of dairy manure and paper waste mixtures on ash, volatile solids (VS) contents and mean percentage change in these parameters in the final composts in response to treatments.

Feedstcok C: N ratio	Composting Method	Composting Stage	Ash	VS	% Change (Feedstock vs Final Product)	
					Ash	VS
30	C	0 Weeks	24	76		
		4 Weeks	26	74		
		8 Weeks	27	73	21[a]	-7[b]
	V	0 weeks	25	75		
		4 weeks	32	68		
		8 weeks	46	54	79[d]	-27[d]
	CV	0 weeks	25	75		
		4 weeks	34	66		
		8 weeks	42	58	72[d]	-23[d]
45	C	0 Weeks	19	81		
		4 Weeks	20	80		
		8 Weeks	21	79	21[a]	-4[a]
	V	0 weeks	18	82		
		4 weeks	22	78		
		8 weeks	26	74	41[b]	-9[b]
	CV	0 weeks	18	82		
		4 weeks	26	74		
		8 weeks	28	72	53[c]	-12[c]

C= Control (Dairy manure-paper waste mixtures allowed to decompose on their own); V= Vermicomposting; CV = Combined composting and Vermicomposting.] Volatile solids decreased by between 4% and 27% as a result of composting and the decreases

By contrast, the ash contents of the composted mixtures increased by between 21% and 79%. The increase in ash was similarly affected by both composting method and C: N ratio. The increase in ash followed the order V30 > CV30 > CV45 > C30 = C45, which was similar to reductions in VS (Table 2.2).



[C= Control (Dairy manure-paper waste mixtures allowed to decompose on their own);

V= Vermicomposting CV = Combined thermophilic composting and Vermicomposting.]

Figure 2.2: Effect of feed stock C: N ratio of dairy manure-paper waste mixtures and composting method on extractable fulvic acid (FA) and humic acid (HA) carbon contents.

Dairy manure and waste paper mixtures with C: N ratio of 30 had higher C_{FA} and C_{HA} contents than mixtures with ratio of 45 at the beginning of the experiment and this relative difference was maintained even in the final composts (Figure 2.2). The C_{HA} of waste mixtures of both C: N ratios increased with composting time while the C_{FA} content decreased (Figure 2.2).

Table 2.3: Effect of composting method and C: N ratio of dairy manure and paper waste mixtures on selected compost maturity parameters

Composting Method	Composting Stage	Selected maturity parameters		
		C_{HA}/C_{FA}	HI (%)	HR (%)
		Mixtures with C: Nratio = 30		
C	0 weeks	0.2	1.4	13
	4 weeks	0.4	9.4	13
	8 weeks	1.0	10	19
V	0 weeks	0.1	1.5	14
	4 weeks	1.0	11.2	22
	8 weeks	2.8	39	53
CV	0 weeks	0.1	1.4	13
	4 weeks	1.4	9.7	24
	8 weeks	3.6	40	52
		Mixtures with C: N ratio = 45		
C	0 weeks	0.1	0.6	6
	4 weeks	0.4	5.3	7
	8 weeks	0.8	4.4	9
V	0 weeks	0.1	0.4	6
	4 weeks	0.5	5.4	8
	8 weeks	1.3	6.6	12
CV	0 weeks	0.1	0.5	6
	4 weeks	0.7	5.1	9
	8 weeks	1.6	8.1	13
LSD		1.0	13.4	16. 8

C= Control (Dairy manure-paper waste mixtures allowed to decompose on their own); V= Vermicomposting; CV = Combined composting and Vermicomposting. C_{HA}= Extractable humic acid carbon; C_{FA} = Extractable fulvic acid carbon HI = Humification index; HR= Humification ratio.

The increase in C_{HA} and decrease in C_{FA} translated to increases in the C_{HA}/ C_{FA} ratio with composting time (Table 2.3). The C_{HA}/ C_{FA} ratio was highest in the final vermicomposts of C: N ratio of 30 from the V and CV composting systems whilst the least C_{HA}/ C_{FA} were recorded for all vermicomposts from wastes of C: N ratio of 45. The HI and HR values in final composts of vermicomposting and combined systems of wastes of C: N ratios of 30 were comparable and far greater than those of all vermicompost from wastes of C: N ratio of 45 from similar composting systems. For both C: N ratios there was no difference in HR and HI indices between the different CV and V composting methods though HI and HR were significantly higher in vermicomposts from wastes of C: N ratio of 30 than C: N ratio of 45 (Table 2.3).

2.4.4 Effect of composting on C, total and extractable N and P contents

Total C decreased with composting time while total N increased for all three composting methods (Table 2.4). These changes were greater for feedstock materials with a C: N ratio of 30 than those of C: N ratio of 45 and translated to final composts with corresponding narrower C: N ratio at the end of week eight. For dairy manure- waste paper mixtures of C: N ratio 30, the C: N ratio decreased to 29, 22, and 23 after four weeks and further decreased to 25, 14, and 15 in final (week 8) composts in the control, vermicompost, and combined thermophilic and vermicomposting treatments, respectively (Table 2.4). The C: N ratios of dairy manure- waste paper mixtures with a C: N ratio of 45 also decreased with time reaching values of 38, 36, and 33 in final (week 8) composts of the control, vermicompost, and combine compost vermicompost treatments, respectively (Table 2.4).

Table 2.4: Effect of composting method and C: N ratio of dairy manure and paper waste mixtures on total and extractable N and P contents

Composting method	Composting stage	Total and extractable nutrients							
		Total N (%)	Total C (%)	C: N	NH_4^+ mgkg^{-1}	NO_3^- mgkg^{-1}	NH_4^+: NO_3^-	Bray 1 P mgkg^{-1}	Total P (%)
			Mixtures C: N= 30						
C	0 weeks	1.16	36	31	6.5	2.6	2.5	59	0.18
	4 weeks	1.20	35	29	50.6	8.6	5.8	72	0.22
	8 weeks	1.34	34	25	5.1	28.4	0.2	83	0.31
V	0 weeks	1.15	36	30	6.3	2.5	2.5	59	0.17
	4 weeks	1.46	33	22	89.7	19.8	4.5	92	0.24
	8 weeks	2.06	28	14	10.5	139	0.1	141	0.71
CV	0 weeks	1.17	36	30	6.5	2.6	2.5	58	0.18
	4 weeks	1.43	33	23	197	48	4.1	97	0.38
	8 weeks	1.93	28	15	9.9	134	0.1	130	0.65
			Mixtures C: N= 45						
C	0 weeks	0.80	37	46	2.0	1.6	1.3	29	0.07
	4 weeks	0.83	36	43	18	2.8	6.5	32	0.15
	8 weeks	0.92	35	38	2.5	6.7	0.3	37	0.18
V	0 weeks	0.80	36	46	2.0	1.7	1.2	31	0.08
	4 weeks	0.86	35	41	18.4	5.3	3.5	40	0.18
	8 weeks	0.93	34	36	2.7	17.3	0.1	56	0.22
CV	0 weeks	0.81	36	45	2.1	1.6	1.3	32	0.07
	4 weeks	0.92	34	37	38.8	11.5	3.4	51	0.22
	8 weeks	0.98	33	33	3.1	29.7	0.1	61	0.29
LSD		0.10	2.5	1.3	2.2	4.8	1.4	5.0	0.03

C= Control (Dairy manure-paper waste mixtures allowed to decompose on their own); V= Vermicomposting; CV = Combined composting and Vermicomposting

Total N increased with composting time for each of the different composting methods (Table 2.4). The largest increases occurred in vermicomposted wastes followed by those that were pre-composted before vermicomposting. Ammonium N increased and reached maximum levels in week 4 and decreased thereafter for all composting methods and waste mixtures of both C: N ratios. By contrast, nitrate N levels increased steadily with time reaching maximum levels in the final composts (Table 2.4). The largest increases in nitrate N were, however, observed in feedstock materials of C: N ratio 30 which were vermicomposted or composted followed by vermicomposting. Total and extractable P also increased with composting time and followed patterns similar to those observed for total N and nitrate-N, respectively (Table 2.4).

For mixtures with a C: N ratio of 30 both vermicompost and combined compost and vermicomposting resulted in total N, ammonium N, nitrate N, total P and extractable P levels in the final composts that far exceeded levels observed in the control composts (Table 2.4). Vermicomposting resulted in higher nutrient levels than the combined system (CV). By contrast, for mixture with C: N ratio of 45, an opposite trend was observed whereby the combined system resulted in generally higher total N, ammonium N, nitrate N, total P and extractable P levels than vermicomposting.

2.4.5 Effect of composting on *E. coli* 0157

At the beginning of the experiment (week 0) there was no difference in the pathogen numbers in the mixtures of both C: N ratios (Table 2.5). After 4 weeks of thermophilic composting only the composting method had an effect on *E. coli* 0157 while C: N ratio had no effect on *E. coli* 0157 numbers in composts. CV completely eliminated the pathogen *E. coli* 0157 while V resulted in insignificant reductions in pathogen numbers (Table 2.5). By contrast, *E. coli* 0157 numbers increased in the control, though not significantly so. A similar trend was observed in the pathogen numbers dynamics in week 8, with the CV method having no detectable levels of *E. coli* 0157 while the V system significantly reduced pathogen numbers and in the control the numbers actually increased. (Table 2.5).

Table 2.5: Changes in numbers of E. coli 0157H: 7 with composting time

Composting period (weeks)	Composting method		
	CV	V	C
0	2330 a	2340 a	2330 a
4	0 b	2120 a	2890 a
8	0 c	1120 b	4820 a

C= Control (Dairy manure-paper waste mixtures allowed to decompose on their own);

V= Vermicomposting; CV = Combined composting and Vermicomposting.

Means in the same row followed by the same letter are not significantly different according to LSD at $P \leq 0.05$.

2.5 DISCUSSION

2.5.1 Effectiveness of composting methods on the chemical properties of composts from dairy manure-waste paper mixtures

The decrease in VS and corresponding increases in the ash content in the final composts relative to the feedstock materials indicated degradation of organic matter (OM) in the dairy manure-paper waste mixtures during composting as reported by other workers (Bernal *et al*., 1998a; Levanon & Pluda, 2002). The decrease with composting time of the C content of the waste mixtures composted by the different methods confirmed the loss in OM reflected by reduction in VS contents. The greater effectiveness of vermicomposting in the degradation of OM in waste mixtures with C: N ratio of 30 than combined system and vermicomposting and *vice versa* for waste mixtures with C: N ratio of 45 indicated that the effectiveness of the two composting methods in degrading organic matter was dependent on the C: N ratio of the mixtures being composted. Increases in total N with composting time was probably due to a concentration effect caused by weight reduction of the composting mixtures as a result of loss in C. It also suggests that limited nitrogen loss occurred during composting by the different methods. The narrower C: N ratio of vermicomposts of wastes of C: N 30 than C: N 45 suggested that both vermicomposting and vermicomposting were equally effective in degrading wastes of an initial C: N ratio of 30.

The combined system was more effective than vermicomposting alone in degrading wastes with a C: N ratio of 45 as reflected by the higher ash, less VS and higher, C_{HA}/C_{FA} ratio, HI, and HR; as well as greater quantities of total and extractable N and P in final composts. It would seem that composting of these wastes prior to vermicomposting resulted in wastes that were more suitable for breakdown by earthworms. This was probably due to an increase in water soluble carbon in the wastes due to the liberation of simple, soluble organic compounds at rates exceeding their degradation during the initial stages of composting (Castaldi *et al*., 2005). This was in agreement with the results of Ndegwa & Thompson (2001) and Alidadi *et al*. (2005), which showed that the combined system produced composts with more nutrients than vermicomposting alone. However, the results for wastes with a C: N ratio of 30 contradicted those of the above authors in that the combined system produced composts with similar nutrient contents when compared with vermicomposting alone.

According to Iglesias-Jiminez & Perez-Garcia (1992) a C: N ratio lower than 12 indicated a good degree of maturity for municipal waste compost, so none of our final composts could be considered sufficiently matured as all of them had C: N ratios greater than 12. However, according to Allison (1973) composts with final C: N ratios of 14 and 15 could be added to soil without altering the microbiological equilibrium of the soil such that vermicomposts from dairy manure-waste paper mixtures can safely be applied to the soil.

Increases in the amount of extractable C (C_{EX}) with time indicated conversion of organic matter into humus. The decline in the fulvic acid fraction with time and increase of the humic acid fraction indicated transformation of the easily degradable molecules that make the fulvic acid fraction to the more recalcitrant molecules of higher molecular weight which make up the humic acid fraction. This translated to increases in the C_{HA}/C_{FA} ratio with time with values in the final (week 8) compost samples which ranged from 0.8 to 3.6 (Table 2.3). Only vermicomposted mixtures and composts from the combined system with an initial C: N ratio of 30 resulted in C_{HA}/C_{FA} ratio values that exceeded the critical level of 1.9, which Iglesias-Jiminez & Perez-Garcia (1992) proposed as a maturity index for city-refuse and sewage sludge compost. Increases in HR and HI values throughout the process indicated humification of organic matter in the composted mixtures. Comparable HI and HR values in final vermicomposting and composts from the combined system for C: N ratio of 30 indicated that the two methods were equally effective for wastes of this C: N ratio. However, observed greater HI and HR values in combined system compared to vermicomposting for mixtures with a C: N ratio of 45 indicated that the latter is more effective in the humification of such mixtures.

The ammonium levels increased during thermophilic composting as mineralization of organic wastes occurred. As temperatures fell, during vermicomposting there was a gradual decline in ammonium concentration with a corresponding increase in nitrate levels as nitrification occurred. The decline of $NH_4^+/N0_3^-$ ratio to values of about 0.1 in all final (week 8) composts which was less than the critical level of 0.16 suggested by Bernal *et al*. (1998b) for mature composts indicated that all final composts except the controls had desirable ratio of NH_4^+ to $N0_3^-$ ratios.

Observed increases in extractable nutrients as a result of composting and vermicomposting, is consistent with results of previous studies (Tognetti *et al*., 2007; Zhang *et al*., 2000). The greater effectiveness of vermicomposting than CV in releasing larger quantities of nutrients from wastes with a C: N ratio of 30 than those of C: N ratio of 45 indicated that the former was more suitable for vermicomposting. It is likely that a lower ratio encouraged higher earthworm and microbial activity leading to greater degradation, higher nutrient content and stabilization of wastes with C: N ratio of 30 than 45.

2.5.2 Effectiveness of composting methods on sanitization of composts

The increase in *E. coli* 0157 in controls of both C: N ratios with composting time could be attributed to creation of a good environment for multiplication of this pathogen through rehydration and subsequent availability of easily degradable substrates by dissolution following rehydration. These results indicate that dairy manure –waste paper mixtures allowed to simply compost in place could pose a health hazard to users and that alternative ways of handling the wastes that will result in a safer product are necessary.

Vermicomposting alone failed to eliminate *E. coli* 0157 but it significantly reduced its numbers by the 8th (final) week of vermicomposting. Similar observations were made by Brown and Mitchell (1981) who reported that *Eisenia fetida* feeding on a growing medium inoculated with S*almonella eneritidus,* reduced the populations of this enteric pathogen after 28 days by 42 times compared to controls. The reduction in pathogen numbers by earthworms is ascribed to the digestion of some of the microbial constituents as they pass through the earthworm gut (Edwards *et al*., 1984). Complete elimination of pathogens through vermicomposting is reportedly achievable only with high earthworm populations (earthworm biomass: biosolids = 3:2 weekly) (Eastman *et al*., 2001).

The combined compost-vermicompost system, on the other hand, eliminated *E. coli* 0157 within the first 4 weeks of pre-composting. The elimination of *E. coli* 0157 in this system could be attributed to thermophilic temperatures attained by these composts during the thermophilic composting phase. According to USEPA (1992), a temperature of 55^{o}C must be maintained for 15 consecutive days for efficient composting and pathogen reduction. In this study, mixtures with a C: N ratio of 30 maintained such high temperatures for four weeks. Mixtures with a C: N ratio of 45 maintained temperatures of 55^{o}C or higher for only four consecutive days, but *E. coli* 0157 was still eliminated in the final composts. A similar observation was made by Larney *et al*. (2003) who reported that 99.9% of *E. coli* 0157 was eliminated within 7 days when average windrow temperatures were only 33.5 – 41.5^{o}C (within the mesophilic temperature range) which is lower than the thermal kill limit of 55^{o}C (USEPA, 1992). No measurements were taken earlier than the 4 weeks of composting in this study but it is likely that the elimination of *E. coli* from the composts could have occurred much earlier. Further studies will explore shorter composting periods before vermicomposting. Nevertheless, the results of this study indicated that precomposting of dairy manure-waste paper mixtures before vermicomposting was a more practical option for elimination of pathogens.

2.6 CONCLUSIONS

Both vermicomposting and the combined system were effective methods for the biodegradation of mixtures of dairy manure and waste paper with a C: N ratio of 30 but the combined system was more effective in the biodegradation of waste mixtures with a C: N ratio of 45. Composting before vermicomposting eliminated the pathogen *E. coli* 0157:H7 from dairy manure and waste paper mixtures whereas vermicomposting alone could only reduce pathogen levels. Therefore, pre-composting the waste mixtures prior to vermicomposting is recommended where elimination of pathogens from composts is a critical consideration. Studies to explore the shortest possible pre-composting period for elimination of pathogens in dairy manure-waste paper mixtures are necessary.

CHAPTER THREE

EFFECTS OF PRECOMPOSTING PERIOD ON SANITIZATION AND PROPERTIES OF VERMICOMPOSTS PREPARED FROM DAIRY MANURE-WASTE PAPER MIXTURES

3.1 ABSTRACT

Combining thermophilic composting with vermicomposting is promoted as a means of sanitizing animal manures before vermicomposting to ensure pathogenic bacteria are not spread to a wider environment during land application. The duration of precomposting is, however, critical to ensure success of the subsequent vermicomposting process. This study evaluated the effects of precomposting dairy manure and waste paper mixtures for 0, 1, 2, 3 and 4 weeks on coliform bacteria and protozoa oocyst numbers, earthworm growth, biodegradation of the waste mixtures, and nutrient content of vermicomposts. Over 95% of fecal coliforms, *E. coli* and of *E. coli* 0157 were eliminated from the wastes within one week of precomposting but total elimination of these and protozoan (oo)cysts was only achieved after 3 weeks of precomposting. Precomposting reduced microbial biomass carbon and water soluble carbon of precomposted wastes and had a negative effect on earthworm growth and degradation of organic matter. Vermicompost from wastes precomposted for over two weeks were less stabilized, less humified and had less nutrient contents compared to vermicomposts from wastes that were precomposted for one week or less. The findings indicated that a precomposting period of one week is ideal for producing safe vermicomposts from dairy manure-waste paper mixtures.

Keywords: dairy manure, *Eisenia fetida, Escerichia coli*, precomposting, vermicomposting

3.2. INTRODUCTION

Raw animal manures and manure-based soil amendments have been implicated in outbreaks of *E. coli* 0157:H7 infections of spinach, lettuce and other produce crops (Ackers *et al*., 1998; CDCP, 2006; Rangel *et al*., 2005). Cattle manure has been reported to contain the pathogenic fecal bacteria, *Escherichia coli* 0157 (O'Connor, 2002), and parasitic protozoa such as *Giardia* and *Cryptosporidium* (Smith and Smith, 1990). *E. coli* 0157 and parasitic protozoa can be transmitted to food crops when manure containing these organisms is used as a fertiliser or soil amendment or because of other inadvertent contact (Cieslak *et al*., 1993; Natvig *et al*., 2002). Manure management strategies that can eliminate these pathogenic organisms are needed.

Thermophilic composting of organic wastes (precomposting) before vermicomposting could reduce pathogenic organisms, and potentially toxic components, such as ammonia or salts in animal manures or the tannins and acids in green wastes and improve the survival and growth of the worms (Gunadi *et al*., 2001). Early work on combining thermophilic composting and vermicomposting used rather long periods of precomposting of four weeks (Ndegwa & Thompson, 2001). The previous work reported in Chapter 2 revealed that precomposting dairy manure-waste paper mixtures for four weeks was sufficient to eliminate *E. coli* 0157H: 7 from dairy manure-waste paper mixtures. Such long periods of pre-composting however, have been reported to reduce the quality of the wastes in relation to nutrient availability for growth and reproduction of earthworms (Frederickson *et al*., 1997). Water-soluble carbon (WSC) is the organic fraction that is most readily utilized by both earthworms and microorganism and gets depleted with time (Bernal *et al*., 1998a). The length of precomposting of wastes may reduce this important fraction and impact negatively on earthworm growth and thus effectiveness of vermicomposting and impact negatively on degradation and stabilization of these wastes by earthworms.

Earthworms have been reported to obtain their nutrition from microorganisms associated with ingested organic matter as they have minimal capacity to digest organic wastes (Doube & Brown, 1998). Precomposting wastes for long periods of time could result in a decline in microbial population and diversity and this could mean less nutrition for earthworms and thence an ineffective vermicomposting phase. There is need therefore to balance between sanitization (pathogens) and need to have a good nutrition for earthworms.

In order to integrate thermophilic composting and vermicomposting in the processing of dairy manure and paper wastes it is necessary to establish an optimum precomposting period both for elimination of *E. coli* 0157 and parasitic protozoa as well as to minimize loss in nutritional quality of the wastes for the earthworms. The objective of this study was to determine an optimum precomposting period for dairy manure paper waste mixtures that results in vermicomposts of good nutritional quality and whose use will not jeopardize human health.

3.3 MATERIALS AND METHODS

This composting study was carried out in an open but shaded yard at the University of Fort Hare ($32^0 46^{\prime}$ S and $26^0 50^{\prime}$ E) in the Eastern Cape Province of South Africa. The waste paper used was obtained from Grocott Printers in Graham's Town. The earthworms (*E. fetida*) and dairy manure used in this study were the same as that described previously in Chapter 2. The chemical properties of these wastes are given earlier in Chapter 2.

3.3.1 Precomposting

Dairy manure and waste paper were mixed as detailed in Chapter Two to give mixtures with C: N ratio of 30. Precomposting of the mixtures was done in three replicates, compost boxes measuring 1m x 1m x 1m (length x width x height). Weighed mixtures of manure and paper were mixed using shovels on a polythene sheet and water was added while mixing, to 60% moisture content before loading into composting boxes. Compost and ambient temperatures were taken daily for the entire precompostingperiod. Turning was done weekly after moisture content determination. Samples for microbiological analyses were stored at 4º C, and analyzed within 24 hours for faecal coliforms, *E. coli* and *E. coli* 0157, presence or absence of *Cryptosporidium* oocysts and *Giardia* cysts and microbial biomass carbon (MBC). Samples were also taken weekly for the determination of volatile solids (VS), ash content, total carbon (C), water soluble carbon (WSC), nutrients: nitrogen (N) both total and inorganic N (nitrate- N and ammonium – N), total and available phosphorus P as well as humic compounds. Small volumes of precomposted mixtures were taken weekly from each of the three boxes pooled and mixed together to form a composite sample which was then vermicomposted in three replicates.

3.3.2 Vermicomposting

The waste mixtures that were precomposted for 0, 1, 2, 3 and weeks were vermicomposted in worm boxes measuring 0.50 x 0.40 x 0.30 m^3 (Length x Width x Depth) with an exposed surface area of 0.2 m^2. The treatments were arranged in a randomized complete block design (RCBD) replicated three times. Mature earthworms were introduced into boxes at a recommended stocking rate of 1.6 kg-worms/m^2 and were fed at a rate of 0.75 kg-feed/kg-worm/day (Ndegwa *et al*., 2000). 14 kg (dry weight basis of the dairy manure waste paper mixtures enough feed for the entire vermicomposting period was supplied at the beginning of the vermicomposting phase.

The precomposted wastes were all vermicomposted for the same period of eight weeks. Moisture levels were maintained at 80% by spraying the surface with water after each weekly analysis for moisture. At the termination of the vermicomposting phase earthworms were separated from the vermicompost by spreading out the vermicomposts on a polythene sheet and then picking out the earthworms. Samples were then taken and analyzed for parameters mentioned earlier. Mean individual biomass of earthworm was determined before and after the vermicomposting phase.

3.3.3 Microbiological analyses

Samples were subjected to a pretreatment procedure before microbiological analysis (Anon, 2003). Aseptically weighed 10 g samples were suspended and homogenized in 90 ml of maximum recovery diluent in sterile stomacher bags. The suspensions were vortexed to ensure thorough mixing and serial dilutions up to 10^{-6} dilution were prepared from the 1: 10 dilution. The 10^{-2} to 10^{-6} dilutions were then used for the enumeration of each desired micro-organism as described below.

a) Faecal coliforms and E. coli.

Faecal coliforms and *Escherichia coli* (*E. coli*) were enumerated by the Most Probable Number (MPN) technique (Anon, 2003). The 10^{-2} to 10^{-6} dilutions were inoculated into tubes containing tryptose broth (Merck) and incubated at 36 ± 1ºC for 24 h (presumptive test) and positive tubes (with acid production characterized by yellow colouration) were inoculated in brilliant green lactose bile broth (Merck) and incubated at 44 ± 1 ºC for 24 h as a confirmatory test. From tubes showing a positive reaction 1ml was used to inoculate tryptone water (TW; Merck) and incubated at 44 ± 1 ºC for 24 h. Indole-positive *E. coli* strains were counted after addition of the Kovac's reagent. Indole production was demonstrated by the rapid appearance of a deep red colour in the upper non-aqueous layer. Bacterial levels were estimated from MPN tables and results were reported as MPN grams per dry weight (g_{dw}^{-1}).

b) E. coli O157:H7

Enrichment, dilution and plating

Compost samples (10 g) were added to 90 ml of modified *E. coli* (mEC) broth containing 20 μgml^{-1} of novobiocin (n) (Merck) (Heuvelink *et al*. 1998). The samples were homogenized by blending for 1 min (Cagney *et al*. 2004) and incubated in an incubator with rotary shaker (Gallenkamp, Loughborough, UK) at 37 ºC for 8 h at 143 rpm. Serial dilutions up to 10^{-6} dilution were prepared in distilled water and 10^{-3} to 10^{-6} dilutions were used for *E. coli* counts. These dilutions (1 ml) were plated in duplicate onto Sorbitol-MacConkey agar supplemented with 0.05 mg l^{-1} of cefixime and 2.5 mg l^{-1} of potassium tellurite (CT-SMAC) (Merck) (Muller *et al*., 2002) and incubated at 37 ºC for 24 h. Non-sorbitol fermenting colonies were enumerated and then up to five colourless colonies per plate per sample were randomly selected and further plated by streaking onto Eosin Methylene Blue agar (EMBA) (Merck).

Identification of presumptive E. coli O157

The presumptive *E. coli* O157 colonies from the EMBA were identified via an agglutination test using *E. coli* O157:H7 latex. Presence or absence of agglutination confirmed the presence or absence of *E. coli* O157 bacteria. Colonies subjected to the agglutination test were identified as *E. coli* 0157:H7 with API 20E kits.

c) Cryptosporidium and *Giardia* oocysts.

Samples (10g) were suspended in 90 ml of an elution buffer (1% Tween 80, 1% sodium dodecyl sulphate, 0.001% antifoam A; Merck), shaken for 1h and then filtered through a 400 µm pore size filter. Eluates were concentrated by centrifugation at 500 rpm and purified through floatation in a 40% saline solution. Aliquots of the sediments were examined by a direct immunofluorescence antibody test used according to the manufacturer's instructions (Davies Diagnostics (Pty) Ltd, Johannesburg, South Africa). Briefly, 20 µl aliquots of the sediments were air dried on glass slides at room temperature and fixed in methanol. When the samples were completely dry, they were incubated with monoclonal antibody in a humid chamber at 37 °C for 30 min. *Cryptosporidium* oocysts and *Giardia* cysts were identified on the basis of their size, shape and the pattern and intensity of the immunofluorescence assay staining (i.e., bright green fluorescence of the oocyst and cyst walls). Results were reported as Presence or Absence of the helminth ova in a given sample.

d) Microbial biomass carbon

Microbial biomass carbon (MBC) was determined in vermicomposts using the chloroform fumigation and extraction method (Vance *et al*., 1987). Briefly, 2 g (oven-dry basis) moist compost was fumigated for 24 h at 25 °C with ethanol free chloroform. Following fumigant removal, the sample was extracted with 60 ml of 0.5 *M* potassium sulphate solution. The organic C in the extracts was measured by dichromate oxidation (Kalembasa & Jenkinson, 1973) and the microbial biomass carbon (MBC) was calculated using the equation:

$$BC = {}^{E_c}\!/_{K_{EC}}$$

Where E_C is organic carbon extracted from fumigated soil minus organic carbon extracted from unfumigated soil and K_{EC} = 0.38 (Vance *et al*., 1987).

3.3.4 Earthworm growth

Earthworm biomass was determined by removing 50 worms from each container, washing them in distilled water and drying them on paper towels. They were then weighed in a weighing boat with water using a balance. This was done to prevent worms from desiccating and affect the weight of the earthworms.

3.3.5 Chemical analysis

Moisture content (MC) was determined gravimetrically as described in Chapter 2. For other determinations, representative samples were dried in an oven at 60ºC until constant weight and ground (< 1mm) to provide a homogenous sample for chemical analyses.

Water-soluble carbon (WSC) was measured after suspending 5 g of vermicompost sample in 50 ml of distilled water in a 250 ml Erlenmayer flask followed by shaking for 24 h on rotary shaker at 160 revolutions per minute (rpm). The suspensions were then centrifuged at 8000 rpm and then and filtered through Whatman No. 1 filter paper. Carbon in the extract was measured by oxidation with potassium dichromate as described by Anderson & Ingram (1996).

Humic substances were extracted and analysed as described in detail in Chapter 2. Humification ratio (HR) and and humification index (HI) were also calculated. Volatile solids, total nitrogen (N) and carbon (C), total phosphorus (P), mineral nitrogen (NH_4^+-N and NO_3^--N) and available P were all determined as described in detail in Chapter 2.

3.3.6 Data Analysis

Pathogen numbers, microbial biomass, water soluble organic carbon, ash, volatile solids and nutrients in dairy manure-waste paper mixtures precomposted for different times were subjected to analysis of variance (ANOVA) using GENSTAT Release 4.24 (Lawes agricultural Trust, 2008) and mean separation was done using least significant differences (LSD) at $p \leq 0.05$. Relationships between earthworm biomass with precomposted wastes properties were determined using regression analysis. The contributions of mixtures' properties on the earthworm biomass were examined using the maximum r^2 improvement stepwise model building procedure (SPSS, 2001).

3.4 RESULTS

3.4.1Temperature profiles during the composting phase

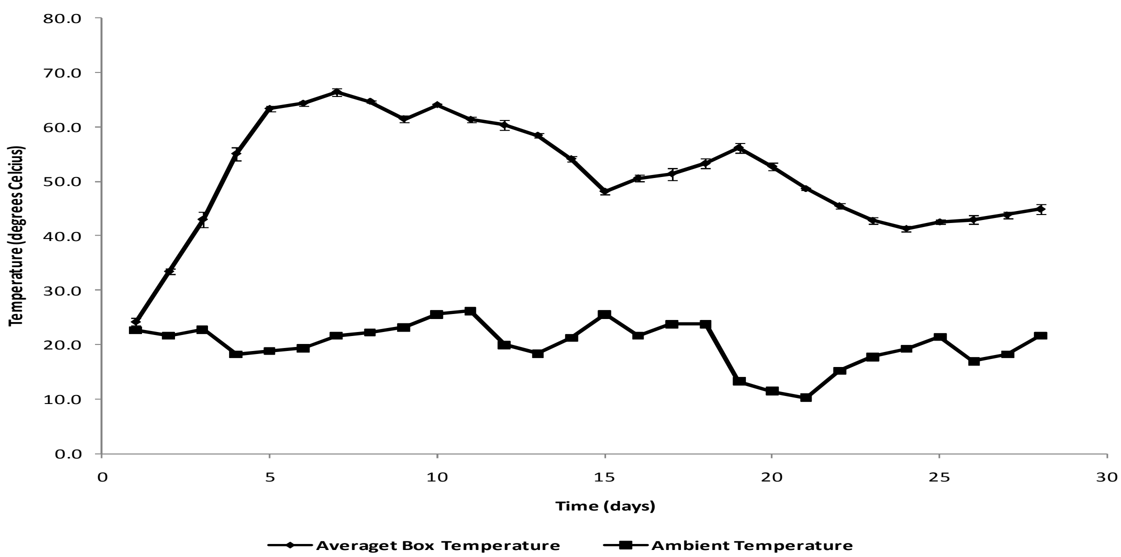


Figure 3.1: Temperature profile of dairy manure-waste paper mixtures during the 4 week precomposting stage (Each point is an average of the 3 boxes). Error bars represent standard deviations.

Thermophilic temperatures were reached within the first three days of composting, attaining a highest mean temperature of 66 ºC after 7 days (Figure 3.1). Two more peaks at 64 and 56 ºC were reached after the first and second turnings, respectively. At the end of the fourth week of the composting phase the mean temperature of the three boxes was still thermophilic at 44.9 ºC.

3.4.2 Effects of precomposting on coliform bacteria and protozoan (oo)cysyst.

Table 3.1: Effects of precomposting period on numbers of FC, *E. coli*, *E. coli* 157 and presence of absence (P – A) of *Cryptosporidium* cysts and *Giardia* oocysts in precomposted wastes (M) and in final vermicomposts (V). Effects precomposting period on MBC shown in M only

Precomposting period (weeks)	Fecal coliforms (MPN gdw^{-1})	*E.coli* (MPN gdw^{-1})	*E. coli* 0157 (MPN gdw^{-1})	*Cryptosporidium* (P or A 10g dw^{-1})	*Giardia* (P or A 10g dw^{-1})	MBC (mg/g)
			M			
0	9125 a	7608a	2516a	P	P	10.9a
1	525 c	400 c	76 c	P	P	8.6b
2	167 c	67 c	31 c	P	P	7.7c
3	0 c	0 c	0 c	A	A	6.3d
4	0 c	0 c	0 c	A	A	6.1d
			V			
0	4375 b	3625b	820b	P	P	nd
1	417 c	292 c	32 c	P	P	
2	108 c	33 c	12 c	P	P	
3	0 c	0 c	0 c	A	A	
4	0 c	0 c	0 c	A	A	

* Effects of precomposting period on MBC are shown for M only. For each parameter, means followed by different letters are significantly different according to LSD at $p < 0.05$

Numbers of faecal coliform, *E. coli*, *E. coli* 0157 and protozoa oo(cysts) were influenced by precomposting period in both the precomposted wastes and the final vermicompost. Dairy manure-waste paper mixtures that were not precomposted had the highest fecal coliforms followed by their corresponding vermicomposts (Table 3.1). Numbers of fecal coliforms in all precomposted wastes and their corresponding vermicomposts were not different though precomposting materials for three weeks and over resulted in total elimination of fecal coliforms in both the precomposted wastes and their corresponding vermicomposts. Results also indicated that up to 94% of fecal coliforms were eliminated after one week of precomposting whereas fecal coliforms were not detectable in mixtures that were precomposted mixtures for at least three weeks. Results for *E. coli* and *E. coli* 0157 followed the same trend (Table 3.1). The (oo)cysts were absent from wastes that were precomposted for at least three weeks both before and after vermicomposting (Table 3.1). MBC was observed to decline with increase in precomposting period with the highest quantities in wastes that were not precomposted and least in wastes precomposted for 4 weeks though this was not different from mixtures precomposted for 3 weeks (Table 3.1).

3.4.3 Effects of precomposting on WSC and earthworm growth.

Water soluble carbon (WSC) declined with increase in precomposting period with the highest quantities in wastes that were precomposted for one week and least in wastes precomposted for at least 3 and 4 weeks (Table 3.2). At the beginning of the experiment the earthworms had the same mean weight of 390 mg but after vermicomposting, the highest mean individual earthworm biomass of 470 mg was recorded for earthworms fed on wastes that were precomposted for one week while the least mean biomass of 380 mg was recorded for earthworms fed on wastes precomposted for at least 3 weeks (Table 3.2).

Table 3.2: Effects of precomposting period on water soluble carbon (WSC) content of mixtures and mean individual earthworm biomass after vermicomposting

Precomposting Period (weeks)	WSC (mg g^{-1})	Mean Individual earthworm biomass (mg)
0	30.7 b	450 b
1	34.4 a	470 a
2	28.4 bc	440 c
3	25.7 c	390 d
4	22.8 c	380 d

Numbers followed different letters in each column are significantly different according to LSD ($p \leq 0.05$)

Earthworms fed on wastes precomposted for up to two weeks increased in individual biomass whereas mixtures precomposted for at least three weeks did not result in an increase in individual biomass. Percentage increases in individual biomass ranged from 21% to -3% (a decline) with the mixtures precomposted for four weeks recording a decline in individual earthworm biomass.

Using stepwise regression procedure ($p \leq 0.05$), a combination of WSC and MBC explained 95.8% of the variation in mean individual earthworm biomass of which 92.5% of variation was explained by WSC alone. The functions best fitting data were:

Mean Individual Earthworm Biomass = 2.78 *WSC $r^2 = 0.925$

Mean Individual Earthworm Biomass = 2.78 *WSC – 1.824 *MBC $r^2 = 0.958$

From these functions mean earthworm biomass increased with increase in water soluble carbon but decreased with increase in microbial biomass

3.4.5 Chemical properties of vermicomposts

Precomposting of mixtures for longer periods resulted in higher ash (4w > 3 w > 2 w > 1 w > 0 w) and lower VS contents (4w < 3w < 2w < 1w < 0 w). (Table 3.3).

Table 3.3: Effect of precomposting of dairy manure and paper waste mixtures on selected compost maturity parameters

Precomposting period (weeks)	Selected maturity parameters							
	Ash (%)	VS(%)	C_{HA}:C_{FA}	HI (%)	HR (%)	Total C (%)	Total N (%)	C: N
M								
0	24g	76a	0.1f	1.4i	11f	39a	1.31i	30a
1	27f	73b	0.3ef	2.7hi	12f	38b	1.42h	28b
2	29e	71c	0.4e	4.0g	13ef	38b	1.47gh	26c
3	32d	68d	0.4e	5.8f	15e	37c	1.52fg	24d
4	34c	66e	0.6e	8.2e	16e	36d	1.55f	23e
V								
0	36b	64ef	4.1a	21b	28b	28f	2.14b	13i
1	39a	61g	3.4b	27a	33a	26g	2.31a	11j
2	39a	61g	3.1c	27a	33a	26g	2.31a	11j
3	36b	64ef	2.8d	17d	23bc	34e	1.88d	18g
4	35bc	65e	2.7d	15d	21d	34e	1.69e	20f

Numbers followed by different letters for the same parameter are significantly different according to LSD ($p \leq 0.05$)

M = Precomposted wastes: V= Vermicompost. C_{HA}= Extractable humic acid carbon; C_{FA} = Extractable fulvic acid carbon; HI = Humification index; HR= Humification ratio.

However, only vermicomposts that were precomposted for 1 week had higher ash content and lower VS than the rest of the vermicompost (Table 3.3). Mixtures that were not precomposted had a lower C_{HA}: C_{FA} than those that were precomposted for at least one week. There was a general increase in the C_{HA}: C_{FA} ratio with precomposting and wastes precomposted for one week and above had a greater ratio than wastes which were not precomposted. Longer precomposting periods resulted in vermicomposts with lower C_{HA}: C_{FA} ratio. Humification index and HR of precomposted wastes increased with precomposting time and were highest in mixtures precomposted for 4 weeks and least in wastes that were not precomposted (Table 3.3). For the vermicomposts HI and HR decreases were highest for the one week precomposting period (higher than the no precomposting) and decreased with precomposting period thereafter.

Total C of precomposted wastes decreased with precomposing time while total N increased with precomposting time (Table 3.3). Wastes precomposted for 4 weeks had the least carbon content and the highest total N content whilst wastes which were not precomposted had the highest C and least total N. The C: N ratio of the wastes decreased with increase in precomposting period and followed the order: 0w > 1w > 2 > 3> 4w. The highest C was recorded in vermicomposts that were precomposted for 3 – 4 weeks and the least C was recorded in vermicomposts precomposted for one week. On the other hand, total N was highest in vermicomposts that were precomposted for one week and least in those precomposted for 4 weeks (Table 3.4). As a result the C: N ratios of vermicomposts were in the order 4w > 3w > 2w > 0w > 1w.

Ammonium, nitrate, available P and total P content of both precomposted wastes and vermicomposts were affected by precomposting period (Table 3.4). In the precomposted wastes nitrate, ammonium, available P and total P increased with precomposting period. The highest nitrate, ammonium, available P and total P concentrations were recorded in wastes that were precomposted for 4 weeks whilst the least amounts of these nutrients were recorded in wastes that were not precomposted.

Table 3.4: Effects of precomposting period on total N, ammonium, nitrate, available and total P feedstocks and final vermicomposts

Precomposting Period (Weeks)	Ammonium	Nitrate	NH_4^+: NO_3^-	Bray 1	Total P
			mg kg^{-1}		g kg^{-1}
M					
0	2.9 gh	5.3 i	0.6e	51.7 i	2.23 g
1	20.8 f	14.0 gh	1.5d	57.1 h	2.66 f
2	72.2 c	21.5 g	3.4c	60.0 h	3.06 e
3	113.7 b	28.9 g	3.9b	71.9 g	3.26 e
4	165.5 a	39.2 f	4.2a	81.3 f	3.43 d
V					
0	5.9 g	168.9 b	0.02h	148.7b	5.96 b
1	2.5 gh	201.4 a	0.02h	160.5a	6.36 a
2	3.2 g	132.7 c	0.05h	138.8c	5.80 c
3	32.6 e	115.2 d	0.2g	120.1d	5.80 c
4	44.1 d	110.4 e	0.4f	107.7e	5.76 c

Means followed different letters for the same parameter are significantly different according to LSD ($p \leq 0.05$) PW = precomposted wastes, V = vermicompost

Vermicomposts that were precomposted for 4 weeks had the highest ammonium levels followed by those that were precomposted for 3 weeks which were higher than those of other precomposting periods. The highest nitrate, available P and total P were recorded in vermicomposts that were precomposted for one week after which they declined with precomposting period. The changes in ammonium and nitrate content with precomposting resulted in an increase in NH_4^+: NO_3^- ratio of the precomposted waste as the ammonium content of precomposted wastes increased more than the nitrate content during the precomposting stage (Table 3.4).

The increase in nitrate and subsequent reduction in ammonium levels with vermicomposting resulted in a reduction in the NH_4^+: NO_3^- ratio of vermicomposts. The ratio was least in vermicomposts derived from wastes precomposted for 1 week and highest in vermicomposts derived from wastes precomposted for 4 weeks (Table 3.4).

3.5 DISCUSSION

3.5.1 Effects of precomposting and subsequent vermicomposting on sanitization of dairy manure-waste paper mixtures and resultant vermicomposts

The observed reduction in microbial numbers in dairy manure-waste paper mixtures was mostly due the high thermophilic temperatures attained during the composting phase of the experiment. The temperatures recorded during precomposting in this study were well above the stipulated thermal kill limit of 55 ºC in composting guidelines (USEPA, 1992). These findings were in agreement with those of Larney *et al*. (2003) , who reported a 99.9% reduction (from $\log_{10}$ 7.86. to $\log_{10}$ 3.38 cells g^{-1} (dry wt) in *E. coli* 0157 numbers in the first 7 days of composting dairy manure when the average windrow temperatures ranged from 33.5 to 41.5 ºC.

According to the U.S. Environmental Protection Agency (USEPA) (1994), Class A compost contains safe and acceptable levels of pathogens, and is considered safe for application to food and non-food plants if it contains < 1000 MPN of fecal coliforms per gram. The results of this study showed that one week of precomposting was enough to bring down the fecal coliforms

(417 MPN per gram) in dairy manure wastes paper mixtures to levels lower than recommended minimum acceptable safe levels.

Although vermicomposts alone significantly reduced fecal coliform number it was not able to reduce to safe levels according USEPA (1994). The further reduction of faecal coliforms, *E. coli* and *E. coli* 0157 numbers in the final vermicomposts could be attributed to earthworm action as the temperatures involved during this phase were in the mesophilic range throughout. The mechanism of pathogen elimination by earthworms is not fully understood but Edwards *et al*. (1984) suggested that as wastes pass through the earthworm gut pathogens can be digested resulting in a reduction of their numbers. Similar observations were made by Brown & Mitchell (1981), who reported that *Eisenia fetida,* feeding on a growing medium inoculated with S*almonella eneritidus*, reduced the populations of this enteric pathogen by 42 times, compared to controls, after 28 days with the greatest reduction occurring in the first 4 days. While earthworms can reduce pathogen numbers in wastes, complete elimination of pathogens is reported to be feasible only when high earthworm populations are used in the vermicomposting process (earthworm biomass: biosolids = 2:3) (Eastman *et al*., 2001). Such high earthworm population levels may, however, not be practically sustainable.

The reduction in bacterial coliform numbers during the precomposting period was related to the decline in microbial biomass carbon of the precomposted wastes. As wastes were exposed to thermophilic temperatures the numbers of bacterial coliforms eliminated were higher and the microbial biomass carbon of such wastes was low. Similar observations of a reduction in microbial populations was made by Hansen *et al*. (2001), who reported a decline in microbial population during the thermophilic stages of composting of municipal solid wastes.

The reduction and eventual elimination of protozoan (oo)cysts could be attributed to the high temperatures attained during the precomposting period. In a study of composting of fecal samples containing *Giardia* cysts and *Cryptosporidium* oocysts, Van Herk *et al*. (2004) found that the percentage of viable Cryptosporidium oocysts declined gradually over a 31 d period. In our experiment the period required to eliminate the (oo)cysts (< 21 days) was shorter probably because of the higher temperatures of greater than 60 ºC attained compared with the 55 ºC reported in their study.

3.5.2 Effects of precomposting on earthworm biomass, MBC and WSC of vermicomposts

Earthworm food quality has been reported to influence not only the size of the earthworm populations but also their growth and reproduction (Aira *et al*., 2006). Nutrient depletion is reported to be one cause of reduced earthworm food quality (Fredrickson *et al*., 1997). Growth of earthworms was affected by WSC content of precomposted wastes. This biologically active parameter consisting of sugars, hemicellulose, phenolic substances, amino acids, peptides and other easily biodegradable compounds (Hsu & Lo, 1999), declined with precomposting period. Thus wastes that were precomposted for one week may have promoted high growth and reproduction rates which accelerated waste breakdown and stabilisation. On the other hand wastes precomposted for longer periods of time (> two weeks) were less nutritious and hence did not promote earthworm growth and thus were less degraded and stabilized. A positive relationship was observed between WSC and earthworm biomass as was shown by the multiple stepwise regression equation best fitting the data viz: Individual earthworm Biomass = 2.78 *WSC – 1.824* MBC. These results are similar to those reported by Neuhauser *et al* (1988), who observed that the mean individual weight of *Eisenia fetida* decreased from 500 mg to 100 mg in sewage sludge as a result of precomposting for 4 weeks.

Besides WSC, it was also expected that MBC of precomposted wastes would have an impact on the earthworm growth as Edwards *et al*., (1984), reported that earthworms not only feed on organic wastes but also on the microorganisms present. Our results, however, showed a negative relationship between MBC and earthworms biomass as was shown by the multiple stepwise regression equation best fitting the data viz: Individual earthworm Biomass = 2.78*WSC – 1.824 MBC.

3.5.3 Effects of precomposting on chemical composition of vermicomposts

The decrease in VS and corresponding increases in the ash content in the final composts relative to the feedstock materials indicated degradation of organic matter (OM) in the dairy manure-paper waste mixtures during composting as reported by other workers (Bernal *et al*., 1998a; Grigatti *et al*., 2004). Precomposting wastes for more than one week significantly reduced the effectiveness of the vermicomposting phase in the degradation of OM in these waste mixtures as was reflected by higher VS and lower ash contents of vermicomposts precomposted for over a week. Taking the C: N ratio as an indicator of decomposition, the results indicated that precomposting wastes for one week was a more effective way of combining thermophilic composting and vermicomposting as these vermicomposts had the lowest C: N ratio and hence more decomposed. According to Bernal *et al.* (1998b) a ratio less than 12 indicated a good degree of maturity so vermicomposts from wastes precomposted for one week could be considered sufficiently matured as they had C: N ratios of less than 12.

Increases in the amount of extractable C (C_{EX}) with time indicated conversion of organic matter into humus. The decline in the fulvic acid fraction with time and increase of the humic acid fraction indicated transformation of the easily degradable molecules to the more recalcitrant molecules of higher molecular weight. This translated to increases in the C_{HA}: C_{FA} ratio with time with values in the final vermicompost samples (week 8) which ranged from 2.7 to 4.1 (Table 3.4). Precomposting wastes for periods of over two weeks resulted in a significant decrease in humification rate of vermicomposts though all vermicomposts had C_{HA}: C_{FA} ratio values that exceeded the critical level of 1.9 which Iglesias-Jiminez & Perez-Garcia (1992) proposed as a maturity index for city-refuse and sewage sludge compost. Increases in HR and HI values throughout the process indicated humification of organic matter in the composted mixtures. Comparable HI and HR values in final vermicomposts of wastes precomposted for two weeks or less indicated that the vermicomposting phase was equally effective for wastes precomposted for not more than two weeks.

Increases in nutrient content (total N, P extractable nitrogen and P) with composting time were probably due to a concentration effect caused by weight reduction of the composting mixtures as a result of OM degradation and loss of C as carbon dioxide. During the process of vermicomposting, the earthworms usually enhance nitrogen mineralization in the substrate, so that the mineral nitrogen is retained in the nitrate form (Atiyeh *et al*., 2000). Gupta & Garg (2009) observed that nitrogen increased significantly during vermicomposting of cattle manure and consumer paper waste due to mineralization of C-rich materials, and possible action of free living N-fixing bacteria. Earthworms have also been reported to add nitrogen to vermicompost in the form of mucus, nitrogenous excretory substances, growth stimulating hormones and enzymes during the fragmentation and digestion of organic matter (Tripathi & Bhardwaj, 2004). As with stabilization parameters, precomposting of wastes affected the efficiency of the vermicomposting phase and the highest N and P concentrations were in the wastes which were precomposted for one week. The low degradation rate of wastes precomposted for more than two weeks could be attributed mainly to the depletion in WSC which resulted in low earthworms activity and low rates of degradation and mineralization of wastes.

The nutrient content of composts is a function of the initial feedstock and as well as the degree of degradation undergone by the wastes (Gaur & Singh, 1995). Because of the nature of the feed stock materials used in our experiment (dairy manure and waste paper), even for the most degraded wastes (wastes precomposted for 1 week) the amounts of nutrients in general and P in particular recorded much lower than nutrient contents for vermicompost reported in similar studies. Gunadi *et al.* (2001) reported very high nitrate contents, ranging from 1511 to 3889 mg kg^{-1} in vermicompost from cattle wastes precomposted for different times whereas we recorded nitrate values averaged 178 mg kg^{-1} in this study. This could have been due to the fact that the above workers used cattle manure alone in their study which is nutritionally richer than mixtures of dairy manures and waste paper used in this study. Edwards & Burrows (1988), reported total P contents of 0.4, 1.7, 2.9 and 2.7% in vermicomposts derived from separated cattle, pig, duck and chicken solids, respectively.

In this study the total P contents were as low as 0.6%. The most likely reason for this is that the above mentioned materials except for cattle solids are rich in P whereas the dairy manure-waste paper mixtures in this study are poor in P with the bulk of the P being found in cattle manure. Enrichment of dairy manure-waste paper mixtures is necessary to make it more acceptable as a growing medium or as an organic fertilizer. Enrichment of composts for P can be done through inoculation using P solubilizing microorganisms or through incorporation of phosphate rock (PR) into the dairy manure-waste paper mixture. Further studies will investigate the effectiveness of PR incorporation on improving P nutrition of dairy manure-waste paper vermicomposts.

3.6 CONCLUSIONS

Precomposting of wastes for one week reduced faecal coliforms, *E. coli* and *E. coli* 0157 on to below the safe levels of < 1000 MPN fecal coliforms per gram. It required 3 weeks of precomposting to totally eliminate faecal coliforms, *E. coli, E. coli* 0157 and protozoan oocysts. Precomposting of wastes for more than one week reduced WSC of precomposted wastes resulting in reduced earthworm growth in wastes. Vermicompost precomposted for more than two weeks were less stabilized and had less nutrient contents than vermicompost from wastes that were precomposted for one week. Results indicated that precomposting of dairy manure-waste paper mixtures for one week was ideal for efficient vermicomposting of dairy manure-waste paper mixtures. Generally, vermicomposts prepared from dairy manure-waste paper mixtures had low nutrient contents, and in particular total and available P. Further studies will explore the feasibility of enriching precomposted wastes with phosphate rock as a way of increasing the P content of dairy manure wastes paper vermicomposts.

CHAPTER 4

PHOSPHORUS ENRICHMENT OF DAIRY MANURE-WASTE PAPER VERMICOMPOST USING PHOSPHATE ROCK

4.1 ABSTRACT

Dairy manure-waste paper vermicomposts were found to be low in nutrients and in particular phosphorus (P) in the earlier study. The present study evaluated the effectiveness of a low grade South African phosphate rock (PR) in enriching dairy manure-waste paper vermicomposts with P and its possible effects on stabilization, nutrient content and physical properties of the vermicomposts. The ability of earthworms to bioaccumulate heavy metals was also assessed. Results indicated an increase in inorganic phosphate and a reduction in the organic phosphate fractions of dairy manure-waste paper vermicompost that were enriched with PR. Incorporation of PR also enhanced the degradation of the dairy manure-waste paper mixtures and increased the nutrient contents of the resulting vermicomposts. Earthworms accumulated heavy metals in their bodies and reduced their contents in vermicomposts. Air filled porosity and total porosity were decreased while bulk density, particle density and container capacity of vermicompost were increased by the incorporation of PR. Thus the low grade South African PR could be effectively used to improve the growing medium value of dairy manure-paper waste vermicomposts.

Keywords: phosphate rock, dairy manure-waste paper, earthworms, heavy metals, physical properties

4.2 INTRODUCTION

Nutrient contents of composts differ greatly depending on the raw materials used and the extent of composting (Gaur & Singh, 1995). Results reported in Chapter 3 revealed that vermicomposts prepared from dairy manure and waste paper mixtures were low in P due to the low contents of P of both dairy manure and waste paper materials. The average total P content of these vermicomposts was 0.6% which was much lower than that of vermicomposts derived from phosphate rich organic wastes such as separated pig solids, duck and chicken manure which were reported by Edwards & Burrows (1988) to have total P contents of 1.7, 2.9 and 2.7%, respectively.

To increase acceptability of dairy manure-waste paper vermicomposts as a source of nutrients or growing medium it is necessary to increase their P content and availability. There are a number of ways of enriching vermicomposts with nutrients and these include the inoculation of composts with N fixing bacteria and P solubilizing microorganisms (Kaushik *et al*., 2008; Kumar & Singh, 2001) and inoculating composts with phosphate rock (PR) (Biswas & Narayanasamy, 2006). Biswas & Narayanasamy (2006) reported that PR enriched composts had significantly higher content of total P (2.20%) compared to straw composts (0.3%) where no PR was added. The PR-compost also had higher citric acid soluble P (0.72% P) compared to straw compost (0.1% P). Preparation of PR-enriched composts may be the most feasible method of enriching vermicompost as other methods of using microorganisms require farmers to buy necessary inoculants that may not be readily available in South Africa. The PR enrichment studies mentioned above have been done with rice straw using the thermophilic composting method. There is little information on the effectiveness of PR incorporation to improve the P availability of dairy manure-waste paper mixtures using the vermicomposting process.

The P released from PR or organic wastes can undergo a number of transformations in the vermicomposts. Most of the released P is taken up by microflora and some is refixed due to abundance of Ca in the system (Singh *et al*., 1982). Earthworms also assimilate P for their body synthesis while some of the P can be loosely held by organic colloids (saloid-bound P) (Gosh *et al*., 1999). It is therefore important to study the changes in the forms of P during phospho-vermicomposting in order to understand how P availability in the resulting vermicomposts is likely to be affected.

All PRs contain hazardous elements including the heavy metals cadmium (Cd), chromium (Cr), mercury (Hg) and lead (Pb) and radioactive elements, uranium (U) that are considered to be toxic to human and animal health (Mortvedt & Sikora, 1992). Incorporation of of dairy manure-waste paper mixtures with PR may therefore increase the heavy metal contents of the resulting vermicompost. This is especially true for Cd which is usually bound with P in the apatite structure (Sery & Greaves, 1996) and is thus concomitantly released with P during solubilization. Earthworms have, however, been reported to accumulate heavy metals in their bodies (Ireland, 1983) and so it is possible then that their involvement in the biodegradation of wastes could reduce the amounts of heavy metals present in the final vermicomposts.

Application of PR (in powder form) can alter physical properties of the resulting vermicompost. According to Chen *et al*. (1988), physical properties are the most important parameters related to plant performance in potting medium. If these vermicomposts are to be used as growing medium it would therefore be of interest to find out how PR incorporation affects the physical properties of resultant dairy manure-waste paper vermicomposts.

The aim of this study was to improve toal and available P of dairy manure waste paper vermicomposts through PR application to dairy manure waste paper mixtures. The specific objectives were to determine the: a) effectiveness of a low grade South African PR in increasing available P and other biochemical properties of dairy manure-waste paper vermicomposts b) effects of PR and earthworms on heavy metal composition of vermicomposts, and c) physical properties of the final vermicomposts.

4.3 MATERIALS AND METHODS

The dairy manure used in this study was the same as that previously described in Chapter 2 whilst the wastes paper was from Grocott Publishing in Grahamstown which was previously described in Chapter 3. The phosphate rock used in this study was obtained from Paraborwa in Mpumalanga Province, South Africa. According to van der Linde (2004) it is granitic in nature and has low P contents as well as being insoluble. The PR sample used is known by its industry name as PALFOS 88P and has the following chemical properties: P_2O_5 – 40.3%.; CaO – 54.6%; MgO – 0.26%, cadmium – 1.2 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} (Forskor, Paraborwa, South Africa).

4.3.1 Precomposting

The study was carried out at Fort Hare University. The precomposting procedure was carried out as describe earlier in Chapter 3. Moisture content (MC); volatile solids (VS), ash content, total carbon (C), nitrogen (N) both total and inorganic N (nitrate- N and ammonium – N), total P, and easily available P were determined at the time of loading the mixtures into compost boxes.

4.3.2 Phosphorous enrichment of vermicomposts with PR

The P enrichment treatments consisted of 4 rates of 0, 2, 4 and 8 kg (elemental P basis) as ground PR each mixed with 100 kg (dry matter basis) of precomposted dairy manure wastes paper mixtures.The PR enriched mixtures were then vermicomposted in worm boxes as described earlier in Chapter 2. An absolute control, where dairy manure-waste paper mixtures left to decompose on their own without addition of earthworms or PR was included. Moisture content; VS, ash content, total C, total N and inorganic N (nitrate- N and ammonium – N), total P, microbial biomass carbon and microbial biomass P were determined at the time of loading. Samples were taken on day 0, 14, 28 and 56 of vermicomposting and analyzed for the above mentioned parameters. At the beginning and end of the vermicomposting period Cd, Cu, Cr, Pb and Zn contents of both substrate and earthworms were determined. Sequential P fractionation was carried out on precomposted wastes (day 0) and vermicompost (day 56 samples). Physical properties were determined on the final vermicomposts only.

4.3.3 Chemical analysis

For all determinations, except for moisture content, representative samples were dried in an oven at 60ºC until constant weight and then ground (< 1 mm) to provide a homogenous sample. Samples for microbiological analysis were stored at temperatures below 4ºC and analyzed within 24 hours of sampling.

4.3.3.1 P Fractionation in vermicomposts

Vermicompost samples collected at the start (0 day) and end (56 days) of vermicomposting were subjected to sequential P fractionation using a modified version of the Hedley *et al.* (1982) procedure as described by Reddy *et al.* (2005). Moist compost samples of 0.5 g (on oven-dry basis) were placed in 50 ml centrifuge tubes with two resin strips (anion and cation exchange resin) in 30 ml deionised water and shaken at 175 rev min^{-1} for 16 h at room temperature . The P adsorbed by resin strips was recovered in 20 ml of 0.5 M HCl after shaking for 60 min and inorganic P was determined by the molybdenum blue colorimetric method (Murphy and Riley, 1962). The suspension was centrifuged for 10 min at 8000 rpm and the supernatant discarded. The compost residues left in the centrifuge tubes were then sequentially extracted with 30 ml each of 0.5 M $NaHCO_3$ (pH 8.5), 0.1 M NaOH and 1.0 M HCl. After shaking with each extractant for 16 h, the suspensions was centrifuged and filtered. A portion of $NaHCO_3$ and NaOH extracts was acidified to precipitate extracted organic matter and supernatant analysed for inorganic P (Pi). Another portion of $NaHCO_3$ and NaOH extracts were digested with acidified potassium persulphate oxidation and analysed for total P (Pt). The organic P (Po) in $NaHCO_3$ and NaOH extracts was obtained as the difference between Pt and Pi of respective extracts. The P concentration in all extracts and digests was determined by the molybdenum blue colorimetric method of Murphy & Riley (1962).

4.3.3.2 Microbial biomass indices

Microbial biomass carbon (MBC) was estimated by fumigation– extraction (Vance *et al.,* 1987) as described in Chapter 3. Compost microbial biomass P was measured by fumigation–extraction (Brookes *et al*., 1982) as described by Joergensen *et al.* (1995). Three portions equivalent to 1 g oven-dry compost were each extracted with 100 ml of 0.5 M $NaHCO_3$ (pH 8.5). The first portion was used for the fumigated treatment the second portion for the non-fumigated treatment, and the third portion for estimating P fixation by the addition of 25 µg P g^{-1} compost as KH_2PO_4 to the extractant. P was analysed by the ammonium molybdate–ascorbic acid method (Murphy and Riley, 1962). Microbial biomass P (B*p*) was calculated as described by Brookes *et al*. (1982).

Bp (mg kg^{-1} compost) = $(p_f - P_{nf})/(K_p*100/R)$

Where: P*f* = P extracted from $CHCl_3$ fumigated samples

P*nf* = P extracted from non-fumigated samples

K*p* = 0.4, the fraction of microbial biomass P extracted after fumigation (Brookes *et al.* (1982).

R = % recovery of added P = 100(P*s*-P*n*)/50 (Brookes *et al.* 1982)

where:P*s* = P extracted by exchange resins from non-fumigated soil spiked with P and

P*n* = P extracted from non-fumigated soil.

4.3.3.3 Chemical analyses

Moisture content, VS, total N, C and P were determined as described in Chapter 2. Mineral nitrogen (NH_4^+ and NO_3^-) and humic substances were extracted and determined as described in Chapter 2. For the heavy metal contents of the earthworms prior to the vermicomposting and at termination, substrate samples and 20 earthworms per group (treatment) were removed from substrate and used to determine the levels of cadmium (Cd), chromium (Cr), copper (Cu), lead (Pb) and zinc (Zn) . Afterwards the earthworms were placed on wet filter paper for a period of 24 h to allow the depuration of their gut contents. This was to prevent misleading results concerning the actual heavy metals in the body tissues as a result of heavy metals present in the gut contents. After the 24h period the earthworms were washed in distilled water, dried on paper towels and killed by freezing. Earthworms and vermicompost samples were dried and ground (< 1 mm) then digested using the aqua regia method as described by Faithful (2002). The concentration of heavy metals in worms and substrate was determined using an atomic absorption spectrophotometer (AAS). For vermicomposts, dry samples were ground (< 1 mm) and then digested using the aqua regia method (Faithful, 2002). Dry PR samples were also digested as above. The concentrations of heavy metals in the digested vermicompost and PR samples were determined using an AAS.

The physical properties of the various vermicomposts were determined following the procedures described by Bragg & Chambers (1988) and Gabriels *et al.* (1993) and equations shown in Table 4.1.

Table 4.1. Equations used to determine the physical properties of soilless container media (adapted from Inbar *et al*., 1993)

Bulk density (BD) (g cm^{-3}) = dry weight/volume
Particle density (PD) (g cm^{-1}) = 1/ [% organic matter/ (100 x 1.55) +% ash/ (100 x 2.65)][a]
Total porosity (% volume) = (1 - BD/PD) x 100
Water holding capacity (% volume) = [(wet weight- dry weight)/volume] x 100
Air space (% volume) = total porosity - water holding capacity

[a] 1.55 and 2.65 are the average particle densities of soil organic and mineral matter, respectively.

Samples from each of the potting mixtures were wetted thoroughly in bulk batches. Samples of the media were placed into containers of known volumes and weights, with a fine mesh cloth attached to the base. After initial drainage, the mixture level in the container was adjusted such that it was level with the top of the container, saturated with water for 48 h, then allowed to re-drain. The containers were weighed twice, before and after drying in an oven for 4 days at 60°C. The ash contents and organic matter contents of the potting mixtures were determined in samples that had been incinerated in muffle furnace at 550°C for 5 h. From these measurements, the bulk density, particle density, porosity, and air and water capacities were calculated using the equations of Inbar *et al*. (1993) (Table 4.1).

4.3.4 Data analysis

The data on P fractionation, MBP, MBC, ash, VS, nutrients, HI, HR, heavy metals in earthworms and wastes as well as physical properties of vermicomposts were subjected to analysis of variance (ANOVA) and mean separation was done using least significant differences (LSD) at $p \leq 0.05$ using GENSTAT Release 4.24 (Lawes Agricultural Trust, 2008).

4.4 RESULTS

4.4.1 Effects of PR application on P fractions

There was increase in the amounts of the inorganic P (Pi) fractions and a decline in the organic P fraction (Po) of the vermicomposts with time (Figure 4.1). The highest P fraction in the uncomposted wastes was the bicarbonate Po whilst the least P fraction was the HCl Pi. Concentrations of the different P fractions were not affected by PR application rates initially but the different P fraction responded after 56 days.

In the vermicomposts, the absolute control had the least Pi concentrations for Resin, Bicarbonate, NaOH and HCl extractions whilst the highest concentrations were observed for vermicomposts treated with PR at a rate of 8% P (8 kg P per 100 kg dry weight of waste mixture) (Figure 4.1). Organic P was highest in the absolute control and least in the 8% P treatment for both bicarbonate and NaOH extractants. Introduction of earthworms to wastes reduced the P_o

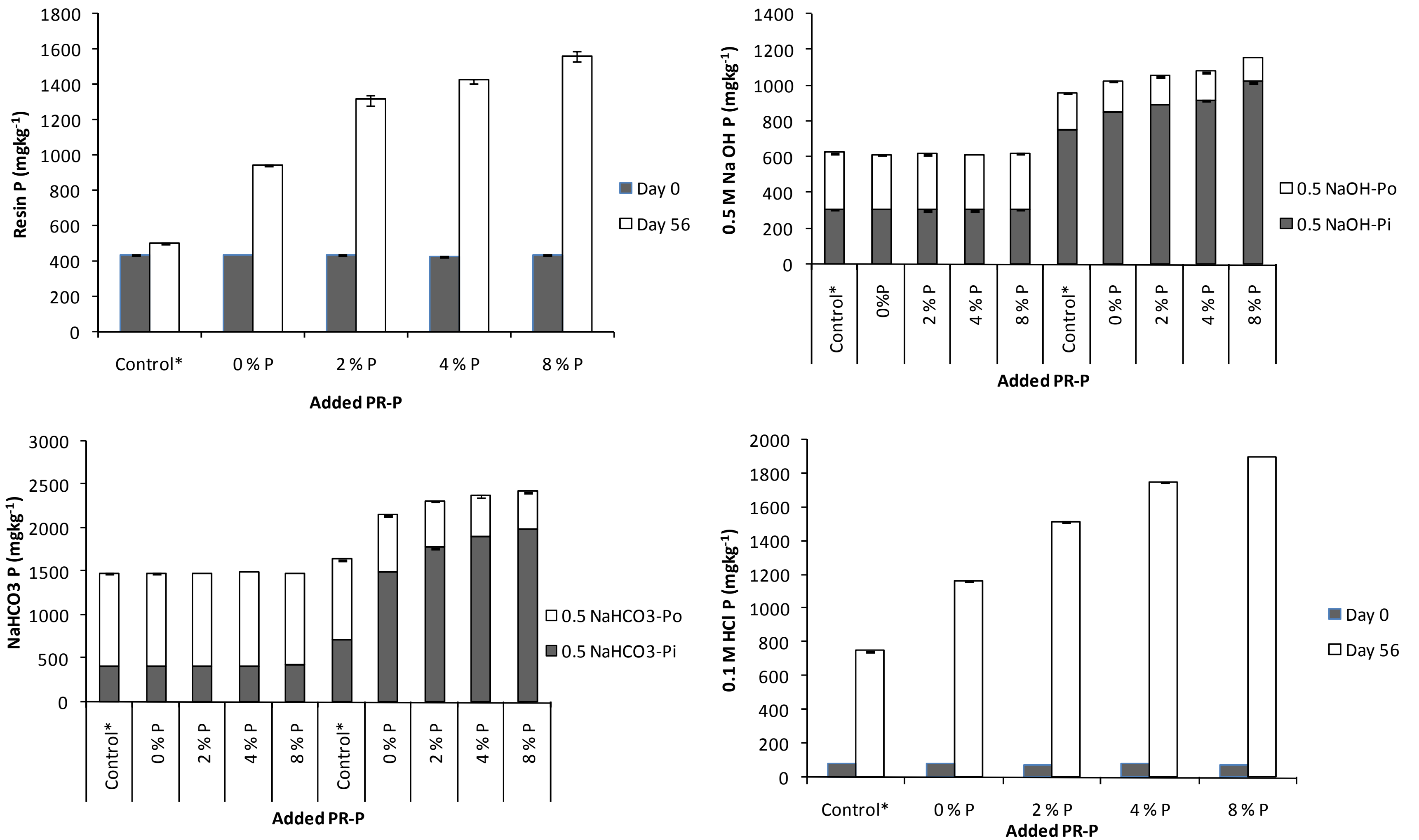


Figure 4.1. Effects of PR application and time on different Pi and Po fractions of vermicomposts. (Error bars represent standard deviation)

fractions and increased the Pi fractions of the various extractants. PR addition resulted in significant increases in the Pi fraction and reductions in the Po fractions of the various extractants (Figure 4.1).

4.4.2 Effects of PR application on MBC and MBP contents of vermicomposts

There was an increase in both MBC and MBP during the first 14 days of vermicomposting which was followed by a sharp decline in the 28th day and a steady decline to the end of the 56th day of vermicomposting (Figures 4.2; 4.3). The increase in MBC was greater than the increase in MBP and both were influenced by PR application. At the beginning of vermicomposting there was no difference in the MBC and MBP contents of the precomposted wastes but from day 14 onwards application of PR significantly affected the MBC and MBP content of vermicomposts. At the end of the vermicomposting period the MBC and MBP of vermicomposts was higher than their initial content whilst for the control the opposite was true. The decrease in MBP was steeper between days 14 and 28 than between 28 and 56 days. However, the MBC decreased linearly between 14 and 56 days.

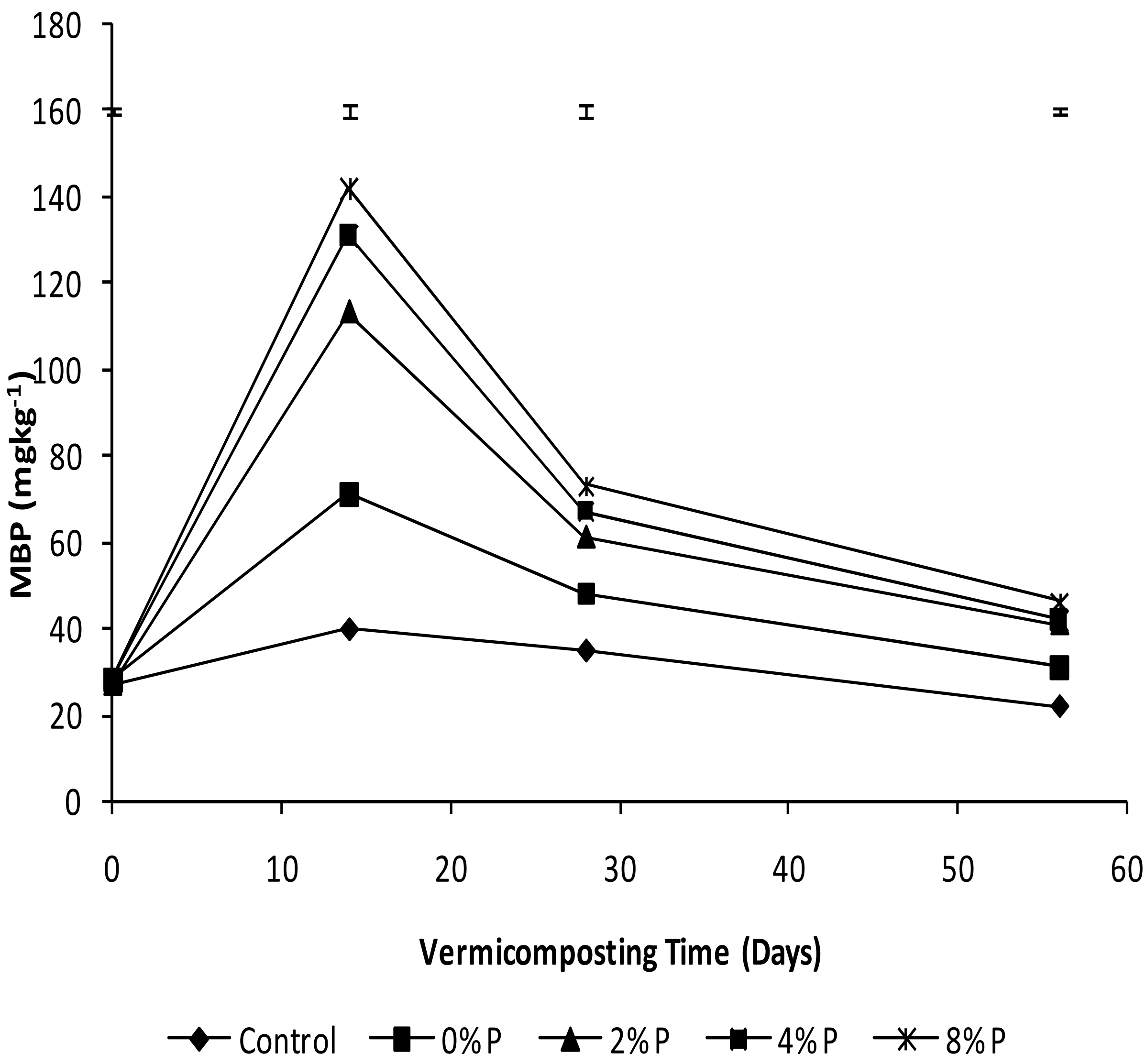


Figure 4.2: Evolution of microbial biomass phosphorus (MBP) with vermicomposting time. Errors bars represent LSD ($p < 0.05$).

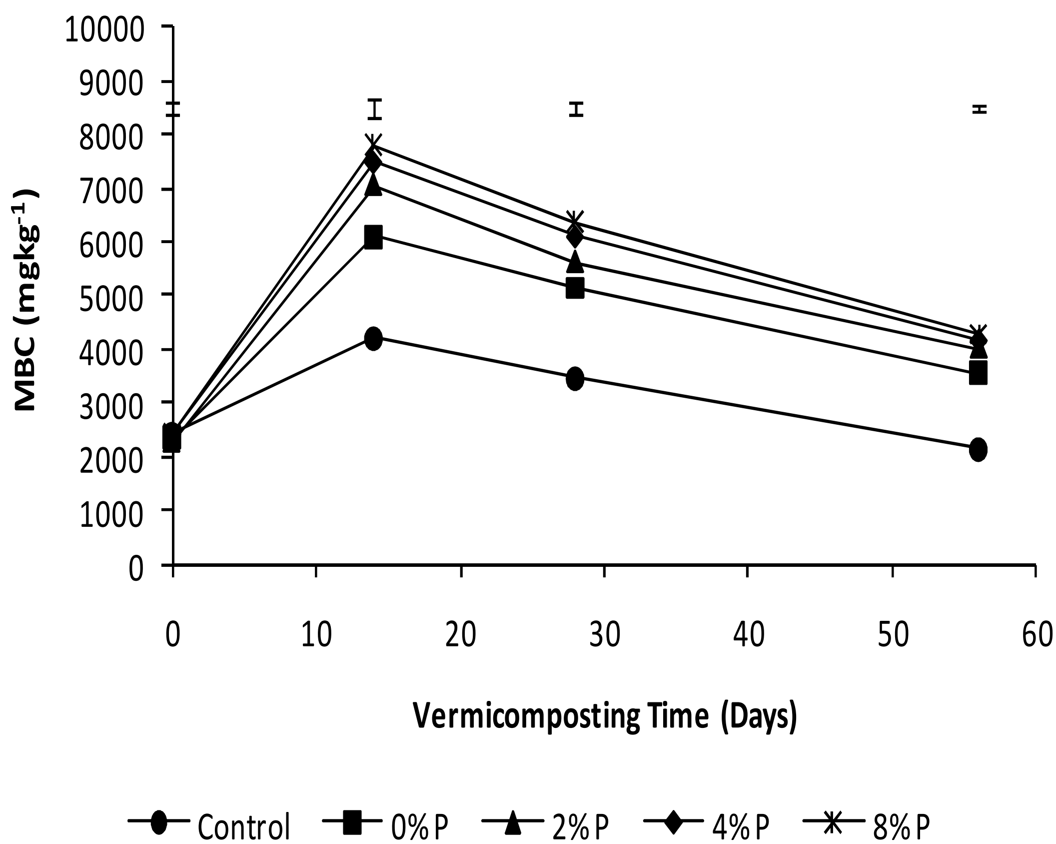


Figure 4.3: Evolution of microbial biomass carbon (MBC) with vermicomposting. Error bars represent LSD ($p < 0.05$).

4.4.3 Effects of PR application on selected chemical and maturity parameters of final vermicomposts

The various chemical and maturity parameters for the feedstock and vermicomposts are shown in Table 4.2. All measured and derived chemical and maturity parameters were affected by both PR application and time and there was an interaction between PR application and time. All the measured nutrients in vermicomposts were significantly affected by PR application.

Table 4.2: Selected chemical and maturity parameters of dairy manure-waste paper mixtures and vermicomposts.

Treatment	Compost Age	Total N	Total P	Total C	C: N ratio	HR	HI	NH_4^+	NO_3^-	NH_4^+/NO_3^-	Resin P	pH	EC µS/cm
			gkg^{-1}				(%)	mgkg^{-1}			mg/kg		
Day 0													
Control*	M	1.30a	4.4a	38e	29e	1.27a	6.2a	48.9f	15.2a	3.21c	435a	8.05b	868b
O% P	M	1.29a	4.5a	37de	29e	1.28a	6.2a	47.8f	15.2a	3.14c	431a	8.05b	893ab
2 % P	M	1.32a	14.2c	38e	29e	1.33a	6.4a	47.8f	15.2a	3.15c	434a	8.35d	989bc
4 % P	M	1.31a	19.8d	38e	29e	1.37a	6.8a	48.7f	15.4a	3.17c	428a	8.49e	921ab
8 % P	M	1.28a	30.9g	37de	28e	1.53b	7.6b	48.5f	15.3a	3.17c	434a	8.56f	929 bc
Day 56													
Control*	V	1.45b	4.9a	36d	26d	4.0c	7.6b	34e	30b	1.13b	583b	8.07b	1362d
O% P	V	2.13c	8.0b	29c	14c	16.2d	18.9c	16d	147c	0.11a	945c	7.84a	858a
2 % P	V	2.24d	24.5e	26b	12ab	20.5e	23.3d	13c	231d	0.06a	1313d	8.18bc	889b
4 % P	V	2.25d	29.8f	25ab	11a	25.3f	28.4e	11b	289e	0.04a	1421e	8.23c	778a
8 % P	V	2.28d	36.7h	24a	10a	32.9g	36.4f	9a	332f	0.03a	1562f	8.11b	717a

M = mixtures of dairy manure-waste paper, V = vermicompost. Means in the same column followed by the same letter are not different according to LSD ($p < 0.05$). *Control = No earthworm and no PR applied

The highest total N level was recorded in the vermicomposts where 8% P was added though this was not different from those where 2% and 4% was added and the least total N was in the absolute control (Table 4.2). Initially all the wastes had the same amount of C but after the vermicomposting the highest C content was in the absolute control whilst the least C was in the treatment with the highest P application rate. Composts derived from absolute control had the least total N. The combination of an increase in N and a corresponding decline in C resulted in lower C: N ratio in vermicomposts (Table 4.2). Though the C: N ratio of mixtures was the same and not affected by P application rate, at the end P application rate strongly influenced the C: N ratio of vermicomposts. The least C: N ratio of 10 was recorded for wastes which had the highest P application rate whilst the highest C: N ratio of 26 was in wastes that had no earthworms or P added.

Initial total P levels were affected by P application with the highest P recorded for the mixtures with the highest P application and least in the absolute zero treatment. The same trend was observed for the total P content of vermicomposts. Resin P content of wastes was initially the same but there was an increase in vermicomposts with the highest amount recorded where the highest P applied and the least in the absolute controls. Highest levels of ammonium were recorded in the precomposted wastes and the levels were not affected by PR addition (Table 4.2). After vermicomposting the ammonium levels dropped and the least ammonium concentration was recorded in the treatment with the highest P application rate (8% P) whilst the highest ammonium level was in vermicomposts derived in the absolute control.

Like other nutrients, nitrate was low in the precomposted wastes and was not affected by PR addition (Table 4.2). At the end of the vermicomposting phase, the nitrate levels had risen in all treatments and PR application had an influence on the nitrate content of vermicomposts. The highest nitrate levels were recorded in the treatment with the highest P application and the least nitrate levels in the absolute control. Like the C: N ratio of wastes, there was a decline in the NH_4^+:NO_3^- ratio of the vermicomposts as the nitrate levels increased at the expense of the ammonium levels (Table 4.2). Initially the NH_4^+: NO_3^- ratio was not affected by application of PR and was high due to the high initial levels of ammonium in the wastes. The least NH_4^+: NO_3^- ratio was recorded for vermicomposts derived from wastes with the highest P application and the highest NH_4^+: NO_3^- ratio was for the absolute control. Similar trends were observed for the HR and HI indices.

The initial pH and EC of feedstock were affected by PR application and these parameters got higher with P application rate (Table 4.2). Both parameters were reduced with increasing vermicomposting time and in the final vermicomposts the highest EC was recorded in the absolute control whilst the least was recorded in wastes that had the highest P application (8% P). The lowest pH was recorded in the 0% PR application rate (wastes with earthworms but no P applied) and highest in wastes with 4% P application rate. The changes in pH were small though.

. **4.4.4 Effects of PR application on earthworm and vermicompost heavy metal content**

Table 4.3: Heavy metal composition of mixtures (M) and final vermicomposts (V)

Treatments			Cd	Cr	Cu	Pb	Zn
Day	Added PR – P (%)	Earthworms			$mgkg^{-1}$		
					M		
0	0	N	1.11b	4.54b	5.82b	2.37b	8.41b
	0	P	1.09b	4.56b	5.79b	2.43b	8.39b
	2	P	2.35e	20.56e	10.85g	8.33f	20.28g
	4	P	3.13g	37.56h	12.90h	13.52h	28.53h
	8	P	4.05h	59.99i	15.66i	17.82i	32.74i
					V		
56	0	N	1.51c	5.49c	6.12c	2.52c	9.06c
	0	P	0.71a	2.74a	3.76a	1.46a	4.67a
	2	P	1.53c	12.40d	7.05d	5.00d	11.16d
	4	P	2.03d	22.53f	8.38e	8.11e	15.69e
	8	P	2.63f	34.78g	10.18f	10.69g	18.01f

N = none P = present M = dairy manure-waste paper mixtures V = vermicomposts

Means followed by the same letter under each parameter are not significantly different according to LSD ($p < 0.05$).

The highest concentrations of heavy metals were in feedstock and vermicomposts that received the highest PR application rate and least in the treatments without any PR application (Table 4.3). In the absolute control the concentration of all heavy metals significantly increased with composting time. For wastes with earthworms it was observed that there was a decline in the concentration of all the heavy metals in the vermicomposts (Table 4.3). The greatest reduction in heavy metals occurred with Zn whilst Cu and Cd had the least reductions.

Table 4.4: Heavy metal composition of earthworms before (I) and after vermicomposting (F)

Treatments Day	Added PR-P mg kg^{-1} (%)	Cd	Cr	Cu	Pb	Zn
				mg kg^{-1}		
			I			
0	0	0.27a	0.53a	0.85a	0.39a	1.44a
	2	0.30a	0.57a	0.86a	0.40a	1.42a
	4	0.30a	0.58a	0.83a	0.42a	1.40a
	8	0.28a	0.57a	0.87a	0.37a	1.39a
			F			
56	0	0.30a	1.65b	1.32b	0.56b	2.18b
	2	0.53b	2.68c	2.47c	1.75c	5.21c
	4	0.64c	3.70d	2.85d	2.73d	6.90d
	8	0.83d	4.62e	3.32e	3.53e	7.80e

Means followed by the same letter under each parameter are not significantly different according to LSD ($p< 0.05$). I = initial earthworm heavy metal. F = final earthworm heavy metal content

At the beginning of the vermicomposting period it was observed that earthworm heavy metal content was not affected by PR application (Table 4. 4). Zn was the found in highest amounts whilst Cd was in the least amounts in the earthworms. After vermicomposting there was a significant increase in the amounts of all heavy metals in the earthworms and this increase was influenced by PR application rate (Table 4.4). For all the heavy metals, the highest levels were in earthworms fed on wastes with an 8% P application rate whilst the least was in earthworms fed on wastes without any PR.

4.4.5 Physical properties of vermicomposts.

The bulk densities (BD) of the vermicomposts were observed to increase with PR application (Table 4.5)

Table 4.5: Physical properties of vermicomposts

Treatment (PR added)	Bulk Density (g cm^3)	Particle Density (g cm^{-3})	Water holding capacity (%)	Total Porosity (%)	Air filled porosity (%)
0% P	0.305a	1.90a	75c	83c	15c
2% P	0.431b	1.96b	73c	80b	13b
4% P	0.440c	2.04c	74b	78a	10a
8% P	0.465d	2.08c	69a	77a	9a

Means followed by the same letter under each parameter are not significantly different according to LSD ($p < 0.05$)

The least bulk density of 0.305 g cm^{-3} was recorded for vermicomposts without any PR application and the highest bulk density of 0.465 g cm^{-3} recorded for vermicomposts with the highest PR application.

The same trend was observed for particle densities (PD) of the vermicomposts. Water holding capacity (WHC), total porosity (TP) and air filled porosity (AFP) all decreased with increased PR application. The least PD, BD and highest WHC, TP and AFP were recorded for the vermicomposts that had no P applied to them whilst the opposite was true for wastes that had the highest PR applied to them.

4.5 DISCUSSION

4.5.1 Effects of PR application on P fractions

The presence of earthworms enhanced P availability as was evidenced by the increase in all the Pi levels and decrease in Po levels in vermicomposts with earthworms only without P applications compared with wastes without earthworms. Such effects of earthworms in mineralizing wide ranges of organic materials with the help of various bacteria and enzymes in their intestines have been described in detail by Edwards & Lofty (1972) and others. Similar improvements in P contents due to earthworm presence have been reported by Gosh *et al.* (1999). PR application to dairy manure-waste paper mixtures enhanced P availability through increases in the labile (Resin Pi and $NaHCO_3$Pi) and less labile pools of P (NaOH Pi and Po). The increase in both labile and less labile P was probably due to the combined effects of degradation of organic matter and the dissolution of applied PR. The more PR applied the more the increase in labile P and less labile P fractions in vermicomposts. The increase in the Pi and corresponding decrease in Po implied solubilisation of PR as well as of organic P in the mixtures.

A possible explanation for the enhanced increase in available P could be the presence of high microbial populations as evidenced by presence of high microbial biomass C and P during the early stage of vermicomposting. The increase in microbial biomass C and P could have been a result of an increase phosphate solubilizing microorganisms. Though these microorganisms were not enumerated in this study, these microorganisms have been reported to be able to solubilize PR through acidification of the media (Raju & Reddy, 1999). Similar results of increases in available P with vermicomposting were reported by Bhattacharya & Chattopadhyay (2002) who reported an increase in phosphate solubilising microorganisms during vermicomposting of cattle manure amended with flyash. Presence of humic substance could also have aided in the dissolution of PR. In this study high levels of humic substances were recorded in vermicomposts where PR was applied. According to Singh & Amberger, (1990), fulvic acid can adsorb significant amounts of Ca^{2+} and releasing H^{+} ions, thereby enhancing PR dissolution while humic acid may form complexes with P and Ca, and create a sink for further dissolution of PR.

4.5.2 Effects of PR application to selected chemical and maturity parameters of vermicomposts

Increases in total N and P with vermicomposting time were probably due 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. During the process of vermicomposting, the earthworms usually enhance nitrogen mineralization in the substrate, so that the mineral nitrogen is retained in the nitrate form (Atiyeh *et al*., 2000). Earthworms have also been reported to add nitrogen to vermicompost in the form of mucus, nitrogenous excretory substances, growth stimulating hormones and enzymes during the fragmentation and digestion of organic matter (Tripathi & Bhardwaj, 2004).

The observed increase in the total N content of vermicomposts together with the loss in carbon as carbon dioxide, resulted in a decline in the C: N ratio of composting materials. Vermicomposts with the highest added P had the least C: N ratio of 10 whilst the absolute control had the highest C: N ratio of 26. Application of earthworms to the wastes caused a significant reduction in the C: N ratio of wastes to 13. According to Bernal *et al.* (1998a) a C: N ratio of less than 12 and or an NH_4^+: NO_3^- ratio of ≤ 0.16 indicated a good degree of maturity such that all vermicomposts with added P can be considered sufficiently matured.

The pH of the control was not different from that of the original mixtures whereas that of the different treatments were reduced after vermicomposting. Other authors have found similar results in vermicomposting experiments and have suggested that the mineralization of N and P compounds, and the production of humic and fluvic cids as possibly the causes of the decrease in pH during vermicomposting (Ndegwa & Thompson, 2001; Kaushik & Garg, 2004). The electrical conductivity (EC) of the different vermicompost after the different treatments did not exceed the threshold value of 3 dS m^{-1} indicating that the material can be safely applied to soil (Soumare *et al*., 2002). Low EC values recorded for the vermicompost could be attributed to vermicomposting process whereby the minor production of soluble metabolite such as ammonium as well as the precipitation of dissolved salts may lead to lower EC values (Mitchell, 1997).

Increases in the amount of extractable C (C_{EX}) with time indicated conversion of organic matter into humus. The decline in the fulvic acid fraction with time and increase of the humic acid fraction indicated transformation of the easily degradable molecules that make the fulvic acid fraction to the more recalcitrant molecules of higher molecular weight which make up the humic acid fraction. The increases in C_{EX} and C_H with vermicomposting resulted in increases in HR and

HI values which indicated humification of organic matter in the vermicomposts. Application of PR increased microbial biomass of vermicomposts and this could have resulted in increased rates of degradation and increased humification as more organic matter was degraded in these vermicomposts.

Results from this study revealed that there was a significant reduction in C: N ratio, increase in nutrient contents (Total N and P as well as available N and P), higher HI and HR in vermicomposts with PR application over the vermicompost with earthworms but no PR application. This could have been due to a reduction in the C: P ratio of dairy manure-waste paper mixtures and resulted in a proliferation in microorganisms whose activities resulted in enhanced decomposition of the organic wastes. This suggested that P could be critical in vermicomposting and that more work is needed to establish critical P levels necessary for the efficient vermicomposting of dairy manure-waste paper mixtures.

4.5.3 Effects of PR application on heavy metal contents of vermicomposts and earthworms

The concentrations of the heavy metals Cd, Cr, Cu, Pb and Zn in mixtures were observed to increase with PR application. This could be attributed to addition of PR to dairy manure-waste paper mixtures. In the vermicomposts, the concentrations of Cd, Cr, Cu, Pb and Zn declined whilst in earthworms and the absolute control there was an increase in their concentrations. The observed increase in heavy metal contents of earthworms and reduced levels in vermicomposts can be attributed to the ability of the earthworms (*E. fetida*) to accumulate heavy metals (Ireland, 1983). Similar observations of an increase in earthworm heavy metal content and a corresponding decrease of heavy metals in sewage sludge based vermicomposts were reported by Shahmansouri *et al*. (2005). However, in their study they recorded much higher values for both vermicompost and earthworm heavy metal contents due to the fact that they used sewage sludge which naturally has higher heavy metal contents than the dairy manure wastes paper mixtures used in this study.

The levels of heavy metals Cd, Cu, Pb and Zn in PR amended vermicomposts reported in this study are far below the toxicity their levels reported by Malecki *et al*., (1982) for *E. fetida* which on ppm per dry weight basis are 50, 100, 200, and 12000 for Cd, Cu, Pb and Zn, respectively. This implies that the growth of earthworms may not have been affected by the presence of these heavy metals in the composting mixtures. The high concentration of heavy metals in vermicomposts amended with the highest the rate of PR application implies that in order to reduce heavy metal loading to the environment from applied vermicomposts, it would be necessary to amend waste mixtures with lower rates of PR application. An optimum low rate needs to be established experimentally. Compared to sewage sludge derived composts reported by Mupondi *et al*., (2006), the heavy metals Cd, Cu and Zn in the PR amended vermicomposts are much lower and will not pose an immediate danger to the environment through their use as nutrient sources for gardens. The pHs of the vermicomposts were also high and when applied to acidic soils may help to increase soil pH thereby suppressing solubility of Cd.

4.5.4 Effects of PR application on vermicompost physical properties

The increase in bulk density of vermicomposts with increased PR application could be due to the fact that PR application increased the mass of the mixture and the resultant vermicomposts compared to where no PR was added. The same explanation holds for particle density which was highest in vermicomposts with the highest PR application and least in vermicomposts without any PR application. As for the WHC, TP and AFP, application of PR caused a decrease in each of these properties. Addition of PR particles resulted in lower WHC as the PR particles do not hold much water. After the addition of PR the WHC was lowered from 75% where no PR was added to 69% after addition of 8% PR but this was still within acceptable limits according to de Boodt & Verdonck (1972) who proposed WHC range for growing medium of between 55% and 75%. For good plant growth a minimum of 85% total porosity is considered suitable for growing medium (de Boodt & Verdonck, 1972). Total porosity of the vermicompost without PR application (83%) was observed to be below the recommended limit of 85% (de Boodt & Verdonck, 1972) and addition of PR further reduced the amount of air spaces in the vermicomposts and thus a decline in both total and air filled porosity. Air filled porosity of vermicomposts without PR application (15%) was within the recommended range of between 10 and 20% considered suitable for growing medium (Bunt, 1988). At 8% PR application the air filled porosity of the vermicomposts fell below the 10% limit which made these unsuitable for use growing medium because of the limited aeration.

Pine bark is the substrate of choice in the South Africa due to its availability, affordability, and desired physical properties (high air porosity). However, pine bark is acidic and has low nutrient retention properties (Mupondi *et al*., 2006) with little ability to provide nutrients to the substrate solution. Substitution of pine bark with the alkaline and nutrient rich dairy manure vermicomposts could help improve the pH and provide nutrients to the substrate solution. The vermicompost could also help improve water holding capacity of pine bark compost medium. A subsequent study to be reported separately evaluated the technical feasibility of dairy manure-waste paper vermicomposts as components of pine bark compost based growing medium.

4.6 CONCLUSIONS

The addition of PR to dairy manure-waste paper mixtures improved chemical properties of vermicomposts. It increased the total P and available P as well as total N and available N of vermicomposts. PR application also enhanced the degradation process as was evidenced by increases in humification indices. Earthworms reduced Cd, Cr, Cu, Pb and Zn concentrations in vermicomposts and increased the levels of these heavy metals in their bodies. Phosphate rock application can, therefore, be used to improve chemical and physical properties of vermicomposts but must be incorporated at low rates in order to reduce heavy metal loadings into the environment.

CHAPTER 5

DAIRY MANURE-WASTE PAPER VERMICOMPOSTS AS COMPONENTS OF VEGETABLE GROWING MEDIUM: EFFECTS ON PHYSICOCHEMICAL PROPERTIES, TOMATO SEEDLINGS GROWTH AND NUTRIENT UPTAKE.

5.1 ABSTRACT

A glasshouse experiment was carried out to determine the effects of amending a commercial pine bark compost with dairy manure-paper vermicompost (0%, 20%, 40%, 60%, and 100% by volume) on the physicochemical properties of the medium and plant growth. The study was a 5 x 4 factorial experiment in a randomized complete block design (RBD) with three replicates. Four fertilizer levels, 0, 2, 4, and 6 granules per cavity were used. Tomato (*Lycopersicon esculentum* Mill.) seeds were sown and seedlings allowed to grow for 4 weeks. The percentage total porosity and percentage air space, decreased significantly, after substitution of pine bark composts with equivalent amounts of dairy manure-paper vermicomposts whereas bulk density, particle density, container capacity, pH, electrical conductivity and N and P levels increased. The growth of tomato seedlings in the potting mixtures containing 100% dairy manure-paper vermicompost was reduced and was greatest after substitution of pine bark compost with 60% dairy manure-waste paper vermicompost, with more growth occurring in combinations of dairy manure-paper vermicompost where fertilizer rate of 6 granules per cavity was applied than in those with no fertilizer applied. The findings of this study suggest that the best medium planting tomatoes is the 60% vermicompost substitution into pine bark.

Keywords: fertilizer; **g**rowing medium; physicochemical properties; pine bark compost; plant growth; vermicompost;

5.2 INTRODUCTION

Pine bark compost is the growing medium of choice in South Africa due to its availability, affordability, and desired physical properties (high air porosity) and good structure but it has low water holding capacity and available water (Smith, 1985). Pine bark is acidic with a pH of between pH 4 to 4.3 (Germishuisen, 1988) and does not have sufficient available nutrients to supply plant needs (Roberts, 1987). Liming of pine bark-based composts, and fortification with nutrients, are required in order to improve its effectiveness as a growing medium for seedlings. Mupondi *et al*., (2006) produced composts with a high pH of 6.7 when they co-composted acidic pine bark with kraal manure. The P enriched dairy manure-waste paper vermicomposts produced in the Chapter 4 had an alkaline pH of 7.9. However, vermicomposts from the 8% PR application had poor air filled porosity and would therefore require an improvement in aeration.

According to Ingram & Henley (1991), roots growing in poorly aerated media are weaker, less succulent and more susceptible to micronutrient deficiencies and root rot pathogens such as *Pythium* and *Phytophtora* than those growing in well-aerated media. The P enriched dairy manure-paper vermicomposts could therefore improve pine bark compost growth medium's bulk density, particle density and water holding capacity as well as raise the pH of pine bark compost. The mixing of dairy manure vermicomposts with pine bark will mean a compromise in their nutritional value and necessitates the use of fertilizer to raise bigger and healthier seedlings.

Several studies assessed the effect of vermicompost amendments in potting substrates on seedling emergence and growth of a wide range of marketable fruits cultivated in greenhouses

Arancon *et al*., 2004 (a); Atiyeh *et al*., 2000), as well as on growth, yields Atiyeh *et al*., 2000; Arancon *et al*., 2004 (b). The greatest plant growth responses occurred where vermicomposts constituted a relatively small proportion (10–20%) of the total volume of the substrate mixture, with higher proportions of vermicomposts in the mixture not always improving plant growth (Subler *et al.,* 1998).

The aim of this study was to formulate P-enriched growing media through substitution of pine bark compost with PR enriched vermicomposts. The specific objectives were: (1) to determine the effect of amending pine bark composts with dairy manure vermicomposts on physical and chemical properties of medium and on tomato seedling growth. (2) to evaluate the possibility of optimizing the growth of seedlings grown in the compost using Horticote (7: 2: 1 (22)), a slow release NPK fertilizer.

5.3 MATERIALS AND METHODS

The plant growth media consisted of a commercial pine bark compost (Rances Timbers, Sutterheim, South Africa) as a control and amendments of the pine bark compost with 20%, 40%, 60% and 100% (by volume) of P- enriched dairy manure-waste paper vermicompost. The P-enriched dairy manure- waste paper vermicompost was prepared at the University of Fort Hare as described in Chapter 4.

5.3.1 Analyses of physical and chemical properties of growing media

The initial physical properties of the various potting mixtures were determined following the procedures described by Bragg and Chambers (1988) and Gabriels *et al.* (1993) as detailed in Chapter 4.

EC and pH were determined potentiometrically in a 1: 10 suspension of sample in distilled water water. These suspensions were placed on a mechanical shaker at 230 rpm for 30 minutes prior to the measurements. Mineral nitrogen (NH_4^+ and NO_3^-) and Bray 1 P were extracted and determined as described in Chapter 2.

5.3.2 Seedling growth experiment

A 5 x 4 factorial experiment in a randomized complete block design (RBD) with three replicates was carried out in a glasshouse which has a controlled environment. Five levels of growing media, consisting of amendments of pine bark growing medium with vermicomposts as follows 0% (control), 20, 40, 60 and 100% (by volume), were used. Four fertilizer levels of 0, 2, 4 or 6 granules of Horticote (7:1:2 (22)) were applied to each growing medium before sowing. The test crop used was tomato (*Lycopersicon esculentum* Mill.). Polystyrene trays with 40 cavities per tray were filled with the appropriate medium as per treatment and two tomato seeds were sown into each cavity. At the end of one week emergence counts were done and used for calculating the emergence percentages for the different media. After two weeks the plants were thinned to one plant per cavity. Plants were monitored regularly for deficiency symptoms as well as for possible disease and pest attack. The experiment was terminated after 4 weeks and plant height (distance from potting medium level to the top node) and stem girth were determined. Plants were removed from potting mixtures, separated into shoot and root portions and their fresh and dry weights determined. Dry weights were determined by oven drying samples at 60 ºC for 3 days (Atiyeh *et al*., 2001). The dried shoots were ground (< 1 mm) and analysed for tissue N, P and K by wet digestion using a mixture of concentrated sulphuric acid, selenium, salicylic acid and hydrogen peroxide as described by Okalebo *et al*. (2004).

5.3.3 Data Analysis

Plant height, stem girth, shoot and root dry weights as well as tissue N, P and K were statistically analyzed using the GENSTAT RELEASE 4.24 (Lawes Agricultural Trust, 2008) statistical package and means separation done using the least significant difference (LSD) method at 0.05 level of significance**.**

5.4 RESULTS

5.4.1 Physical properties of growing media

Dairy manure-waste paper vermicompost had a bulk density more than 1.6 times that of pine bark composts and its particle density was also significantly higher than that of pine bark compost. Substitution of pine bark compost with dairy manure-waste paper vermicompost resulted in an increase in both bulk and particle densities of the resultant medium (Table 5.1).

The percentage water holding capacity of dairy manure-waste paper vermicompost (74%) was significantly greater than that of pine bark compost (48%) (Table 5.1). The air filled porosity (10%) of dairy manure-waste paper vermicompost was significantly less than that of pine bark (40%). The percentages of total porosity of the potting mixtures, after the incorporation of 20% up to 60% vermicompost into pine bark compost medium, were reduced by 1.1% to 3.4% whilst air filled porosity was lowered by 12.5% to 57.5%. Percentages of water holding container capacities were increased by 8.3% to 39.5% (Table 5.1).

Table 5.1: Physical Properties of growing media constituted by substituting pine bark compost with different proportions of dairy manure-waste paper vermicompost.

Proportions of pine bark compost and Vermicompost		Bulk density[A] (g cm^{-3})	Particle density (g cm^{-3})	Total porosity (%)	Water holding capacity (%)	Air space (%)
Pine bark (%)	Vermicompost (%)					
100	0	0.19 e	1.64 c	88 a	48 e	40 a
80	20	0.23 d	1.76 b	87 b	52 d	35 b
60	40	0.26 c	1.83 b	86 c	62 c	24 c
40	60	0.29 b	1.94 a	85d	67 b	17 d
0	100	0.33 a	1.95 a	83 e	74 a	10 e

[A]Means within the same column followed by the same letter are not significantly different at $P \leq 0.05$

5.4.2 Effect of vermicompost on chemical properties of growth medium

The dairy manure-waste paper vermicompost had higher levels of the different chemical parameters analyzed than pine bark compost (Table 5.2). Substituting pine bark compost with increasing proportions of dairy manure-waste paper mixture vermicompost resulted in significant increases in nitrate and ammonium-nitrogen and Bray 1 P concentrations in the resulting media (Table 5.2). The pH and EC followed a similar trend. The pH of the constituted media became progressively less acidic with each increment of the vermicompost whose pH was alkaline (pH 7.59). Corresponding increases in the electrical conductivity were also observed.

Table 5.2: Chemical properties of a standard growing medium (Pine bark compost) substituted with different concentrations of dairy manure-waste paper vermicompost.

Proportions of pine bark compost and vermicompost		Nitrate (mg/kg)[A]	Ammonium (mg/kg)	Bray 1 P (mg/kg)	pH	EC (dS/m)
Pine bark (%)	Vermicompost (%)					
100	0	46 e	11 d	39 e	4.32 e	6.9 e
80	20	77 d	16 c	93 d	4.71 d	27.8 d
60	40	105 c	26 b	123 c	5.19 c	40.9 c
40	60	163 b	28 b	139 b	6.17 b	80.9 b
0	100	198 a	32 a	152 a	7.59 a	129.5 a

[A]Means within the same column followed by the same letter are not significantly different at $P \leq 0.05$

5.4.3 Effects of vermicompost on seedling emergence

Neither media nor fertilizer levels had an effect on tomato seedling emergence (Table 5.3). The mean tomato emergence percentages, in the five mediawere (100% pine bark compost) - 94.5%;

20% vermicompost - 95.3%; 40% vermicompost - 95.3%; 60% vermicompost - 94.5%nad 100% vermicompost – 94%.

Table 5.3: Effects of medium and fertilizer levels on tomato seedling emergence

Fertilizer level (granules/cavity)	Growth medium				
	Control	20% V	40% V	60 %V	100% V
	Seedling emergence (%				
0	94	96	97	96	95
2	96	93	96	95	94
4	93	95	95	94	93
6	95	96	93	93	96

C – Control 100% Pine bark compost; V – vermicompost

5.4.4 Effects of vermicompost and fertilizer application on tomato seedling growth and tissue nutrient content

Stem girth, plant height, shoot and root dry weights of tomato seedlings receiving no fertilizer and grown in 100% dairy manure-waste paper vermicompost differed significantly from those of seedlings grown pine bark compost alone (Figure 5.1). Tomato seedling growing in unfertilized growing media showed visible N and P deficiency symptoms within a week after germination. Visual observations showed that addition of 2 granules of fertilizer helped alleviate N deficiency symptoms in all growing media but P deficiency symptoms persisted in the PB compost medium. Successive additions of dairy manure-waste paper vermicompost improved growth of tomato seedlings. There was an interaction between medium type and fertilizer level

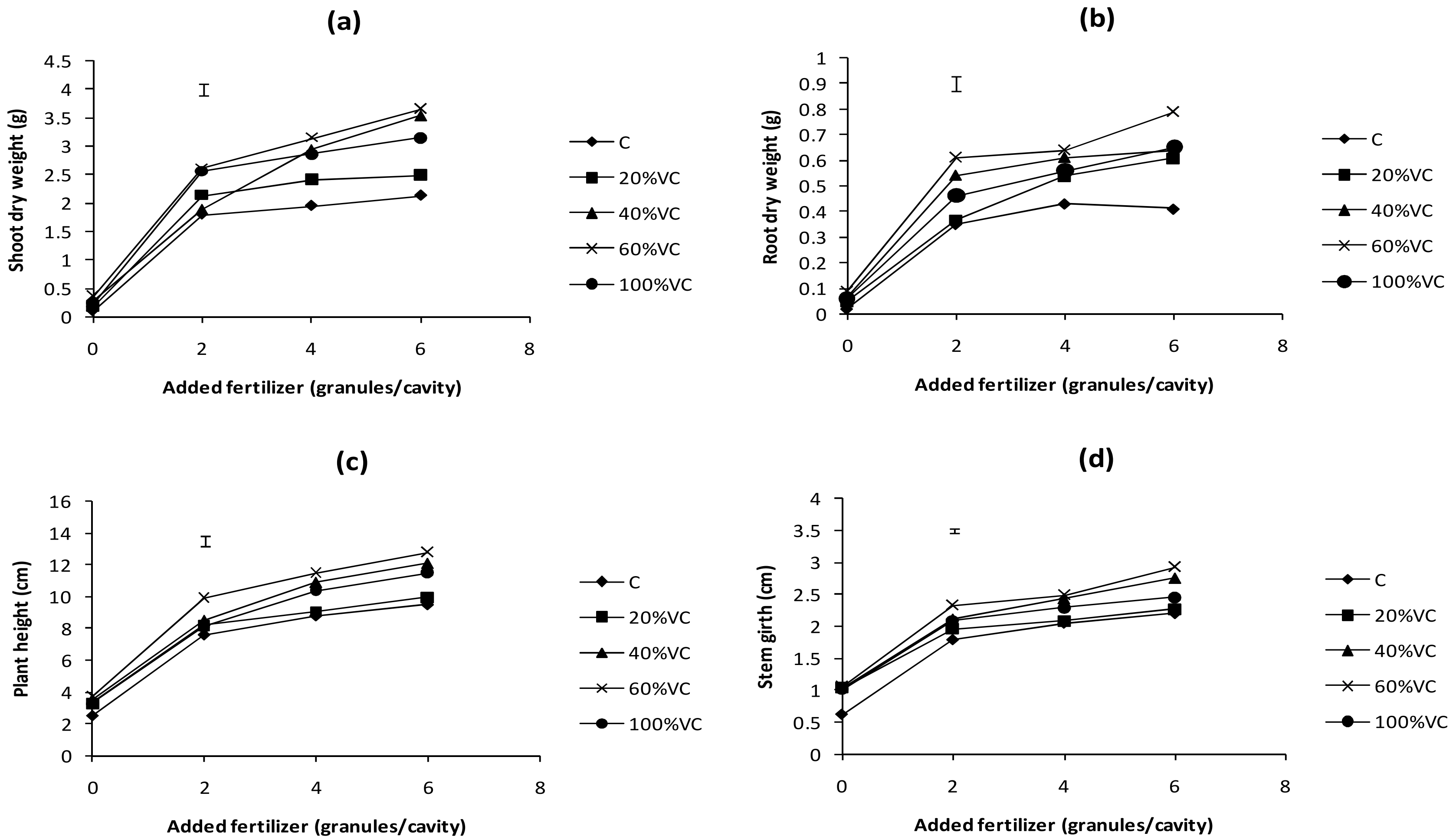


Figure 5.1. Effects of medium and fertilizer on (a) shoot dry weight, (b) root dry weight, (c) plant height and (d) stem girth.

Errors bars represent LSD ($p \leq 0.05$) .The average fertilizer granule mass was 116 mg and contained 8.95 mg N, 1.25 mg P, and 2.55 mg K.

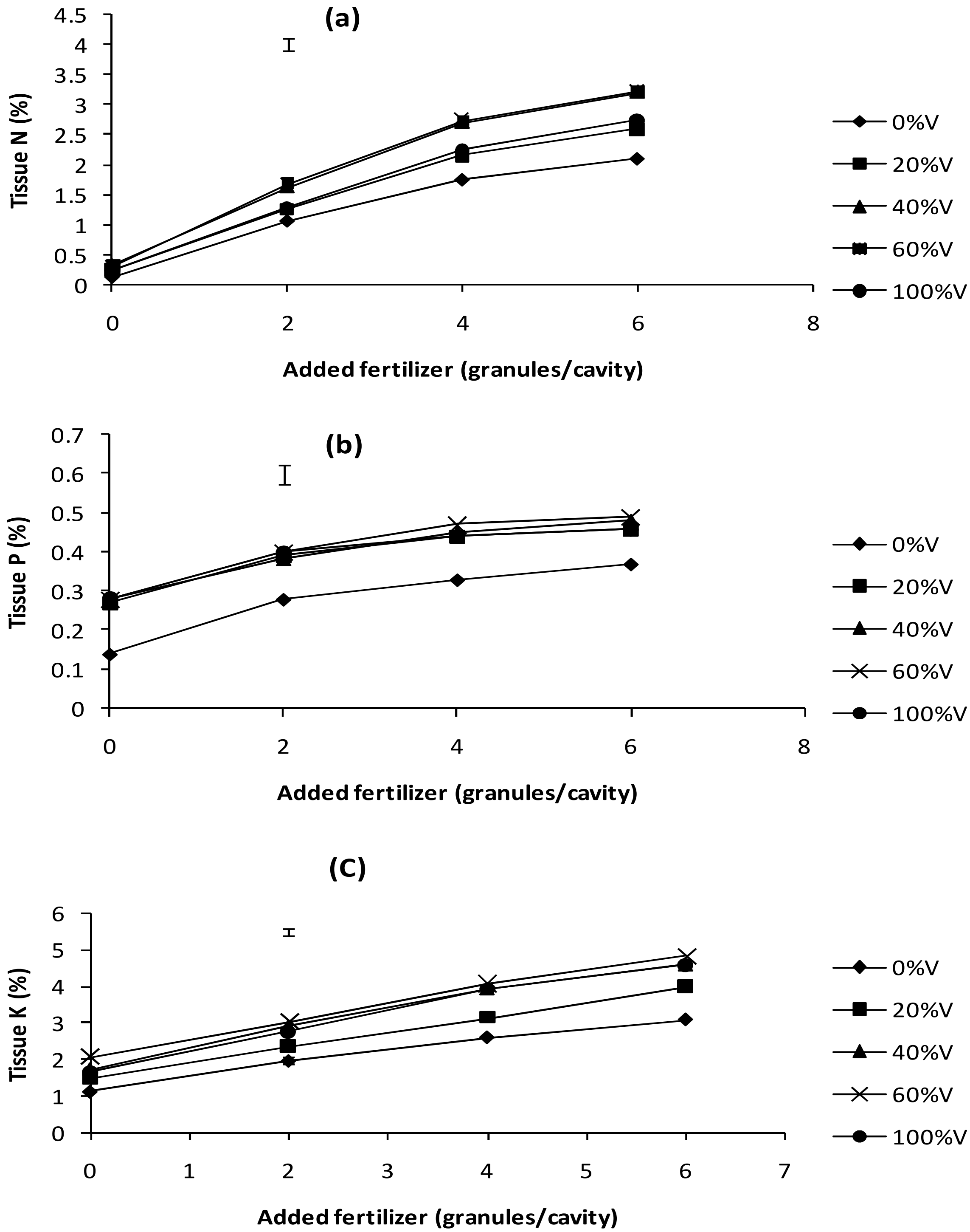


Figure 5.2. Effects of medium and fertilizer level on (a) N (b) P and (c) K tissue content in tomato tissue. Errors bars represent LSD ($p \leq 0.05$)

on the stem girth, plant height, shoot and root dry weights of tomato seedlings. The stem girth, plant height, shoots and root dry weights of tomatoes were dependent on the amount of fertilizer added and the type of medium. Increase in tomato stem girth, plant height, shoot and root dry weights with fertilizer addition was in the order amended pine bark compost < 20% V < 100% V ≤ 40% V < 60% V at the highest fertilizer application rate of 6 granules per cavity.

The tomato tissue nutrient content (N, P and K) was affected by the medium and fertilizer application and there was an interaction between growth medium and fertilizer application level (Figure 5.2). Combinations of dairy manure-waste paper vermicompost with Horticote fertilizer increased nitrogen, phosphorus and potassium uptake by the plants, which was demonstrated by the greater concentrations of tissue N, P and K in tomato plants treated with fertilizer compared with the plants with no fertilizer, and consequently greater seedling growth (Figure 5.2). The tissue N, P and K concentrations of tomato seedlings increased with increasing proportions of dairy manure-waste paper vermicompost in the potting mixtures (up to 60%) and fertilizer application rate.

5.5 DISCUSSION

According to Jansen (21-05-2010), commercial producers of vegetable transplants consider an emergence percentage of 90% or more as satisfactory and values lower than 90% as low Therefore all growing media tested provided optimal conditions since the percentage emergence for all media was greater that the critical level of 90%.

Visual observations of N and P deficiency symptoms were made in all unfertilized media. This suggested that the media used in this study were not able to supply tomato seedlings with sufficient amounts of nutrients. The decreases in plant growth, when the concentration of dairy manure-waste paper vermicompost in the potting medium approached 100%, could possibly be attributed to poor aeration. According to Bunt, (1988) a good growing medium should have an AFP of between 10 and 20%. The air filled porosity of the 100% vermicompost growing medium (10%) was at the lower end and that may have negatively affected root growth and proliferation and result in reduced plant growth. By contrast when AFP was optimal as was the case with the 20% to the 60% vermicompost substitutions seedling growth increased progressively. Initially the pH of both pine bark compost (4.32) and dairy manure-waste paper vermicompost (7.59) were both outside the optimum range of between 5.0 and 6.5 (Goh & Haynes, 1977). Upon incorporation of dairy manure-waste paper vermicomposts into pine bark compost the pH of the growing media mixtures increased progressively with increasing substitution of vermicompost. This improvement in pH from could have improved plant growth by its direct effect on nutrient availability. At high pHs the availability of all micronutrients except for molybdenum is very low as well as that of P whilst at low pHs the availability of P, N, Mg is low whilst levels of micronutrients can reach toxic levels (Havlin *et al*., 2005). Similar improvements in plant growth as a result of an improvement in substrate pH were reported by Atiyeh *at al*., (2001) Without fertilizer application, there was an improvement in tomato dry matter with increasing proportions of dairy manure-waste paper vermicomposts up to 60%. This was probably due to the high nitrogen content and P content of the substituted substrates, compared to the pine bark compost control. The dairy manure-waste paper vermicompost contained more nitrates and more available P, than pine bark compost. In agreement with results of Atiyeh *et al*. (2001), the substitution of pine bark compost with increasing proportions of dairy manure-waste paper vermicompost into increased, P and content of the leaves. This seems directly attributable to the higher concentration of these elements in the dairy manure-waste paper vermicompost than in the pine bark compost medium.

Fertilizer application improved the growth and nutrient uptake of tomato seedlings across all growing media. This suggested that the growing media used in this study were not able to supply tomato seedlings with sufficient amounts of readily available nutrients and that the addition of Horticote fertilizer increased tomato seedling growth further. Similar results of increased seedling growth with fertilization of growing medium were reported by Atiyeh *et al*. (2001).

The growth of tomato seedlings treated with fertilizer was greatest in potting mixtures substituted with 60% dairy manure-waste paper vermicompost, probably as a result of combined improved physical conditions and nutritional factors of the growing medium. The highest application rate of 6 granules of fertilizer per cavity resulted in the greatest growth and nutrient uptake across all media.

Results of this study revealed that the best media for raising tomato seedling were the 40 to 60% vermicompost substituted pine bark media. This is similar to the studies reported by Atiyeh *at al.* (2000), where the greatest plant growth responses and largest yields have usually occurred when vermicomposts constituted only a relatively small proportion (20 to 40%) of the total volume of a greenhouse container medium mixture, with greater proportions of vermicomposts substituted into the plant growing medium not always improving plant growth further.

5.6 CONCLUSIONS

The Substitution of pine bark compost with 40 to 60 % PR-enriched vermicompost produced a growing medium with superior physical and chemical properties which supported good seedling growth. However, for optimum seedling growth, supplementation with mineral fertilizer was found to be necessary. Horticote (7: 2: 1 (22)), a slow release NPK fertilizer, proved to be quite effective in this regard. Therefore, PR-enriched vermicompost has the potential to enhance the growing medium value of pine bark composts.

CHAPTER SIX

GENERAL DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS FOR FOLLOW UP STUDIES

6.1 GENERAL DISCUSSION

The massive production of organic wastes calls for management approaches that are ecologically sound. Composting is the best known process for biological stabilization of solid organic wastes. Traditional thermophilic composting requires long duration and high frequency of turning. The material may also need to be reduced in size, resulting in loss of nutrients and a heterogeneous product (Ndegwa & Thompson, 2001). The thermophilic temperatures reached provide adequate pathogen kill and weed seed kill. Vermicomposting is driven by earthworms and results in a more homogenous product. Earthworms aerate, condition and fragment the substrate, thereby altering the microbial activity (Lazcano *et al*., 2008). However, pathogen removal is not ensured since the temperature is always in the mesophilic range, although some studies have provided evidence of suppression of pathogens (Monroy *et al*., 2008). Compost products are generally low in some nutrients like P which affects their value as nutrient sources for plants. The objective of this study was to evaluate the possibility of combining thermophilic and vermicomposting of dairy manure-waste paper mixtures including enrichment with phosphate rock on physical, chemical and microbiological properties of the product and their effects on seedling growth.

The aim of combining thermophilic composting and vermicomposting is to eliminate pathogens during the thermophilic stages of the precomposting phase. The study revealed that the 4 week precomposting period result in undetectable levels of *E. coli* 0157 in waste mixtures of both C: N ratios of 30 and 45 compared to vermicomposting which significantly reduced pathogen numbers whilst pathogens actually increased in the control. Elimination of pathogens could have been effected through one or a combination of modes of disinfection. High temperature attained during the thermophilic stage of composting could have been responsible for pathogen elimination. A temperature of 55^{o}C must be maintained for 15 consecutive days for efficient composting and pathogen reduction (USEPA, 1992). As well during thermophilic decomposition ammonia is produced and this may also have been responsible for pathogen elimination as ammonia has been reported to kill microorganisms (Epstein, 1997). On the other hand pathogen reduction in vermicomposts could be attributed to the ingestion and the digestion of pathogen by earthworms as well as production of enzymes such as peroxidase capable of killing bacteria (Edwards *et al*., 1984). Similar observations in pathogen reduction through vermicomposting were made by Brown & Mitchell (1981), who reported that *Eisenia fetida,* feeding on a growing medium inoculated with S*almonella eneritidus*, reduced the populations of this enteric pathogen by 42 times, compared to controls, after 28 days.

A study by Larney *et al*. (2003) reported a 99.9% reduction in *E. coli* 0157 pathogen within 7 days of composting when average windrow temperatures were only 33.5 – 41.5^{o}C, lower than the thermal kill limit of 55^{o}C (USEPA, 1992). It is possible then that precomposting wastes for 4 weeks may be too long as elimination of pathogens may have occurred much earlier than the 4 week precomposting used. A follow up study reported in Chapter 3, showed that more than 95% of fecal coliforms, *E. coli* and of *E. coli* 0157 were eliminated from the wastes within one week of precomposting leaving less than 1000 fecal coliforms per gram of compost thus making them safe (USEPA, 1994). The reduction of pathogens in dairy manure-paper mixtures was in agreement with Larney *et al*. (2003) , who reported 99.9% reduction (from $\log_{10}$ 7.86. to $\log_{10}$ 3.38 cells g^{-1} (dry wt) in *E. coli* 0157 numbers in the first 7 days of composting dairy manure.

These findings suggested that a precomposting period of one week is ideal for the effective vermicomposting of dairy manure-waste paper mixtures.

While sanitization is vital in composts the chemical and physical properties are equally essential. The importance of humic substances in soil ecology, fertility and structure and their beneficial effects on plant growth make humification a key factor in quality of composts (Inbar *et al*., 1990). Results from Chapter 2 revealed that only composts from vermicomposted and from the combined system, with an initial C: N ratio of 30, had C_{HA}: C_{FA} ratio values that exceeded the critical level of 1.9, which was proposed as a maturity index for city-refuse and sewage sludge compost by Iglesias-Jiminez & Perez-Garcia (1992). Application of PR to the wastes after precomposting for one week resulted in increases in the C_{EX}, C_{HA} with a corresponding decrease in C_{FA} and increases in HR and HI indices. This could have been due to the possible improvement in P nutrition of the wastes mixtures which could have resulted in more microbial activity and degradation in the PR amended mixtures as high microbial biomass carbon and phosphorus were recorded in the vermicomposts during vermicomposting of dairy manure-waste paper mixtures amended with PR.

Nutrient content of composts is one of the important aspects of composts which influences their adoption and use as nutrient sources for plants. The study in Chapter 2 revealed total N, P and available N and P of composts to be greater in dairy manure-waste paper mixtures of C: N ratio of 30 than those of C: N ratio of 45. Total N and P increased with composting due to loss of carbon as carbon dioxide during biooxidation of organic wastes thus concentrating these nutrients (Lazicano *et al*., 2008). Increases in total N may also have been due to the addition of nitrogenous excretory substances and enzymes from earthworms (Bhadauria & Ramakrishnan, 1996). Nitrate–N concentration in vermicomposts was higher than ammonium-N due to the nitrification process which converts available ammonium to nitrate.

The nutrient content of dairy manure-waste paper vermicomposts produced was generally low and particularly P. The study reported in Chapter 4 showed that application of PR to precomposted wastes resulted in higher total and available P and greatly improved the total and available N contents of the resultant vermicompost compared to vermicomposting alone. Fractionation of P revealed that there was an increase in the inorganic P (Pi) fractions extractable with Resin, $NaHCO_3$ and NaOH while the organic P (Po) fractions extractable with bicarbonate and sodium hydroxide declined. Increases in the Pi fractions of vermicompost meant that there was an increase in available P as a result the combined effects of organic matter degradation and dissolution of PR. The increased nutrient contents in PR amended vermicomposts could have been a direct result of an improvement in P nutrition which resulted in higher microbial activity with the resultant PR dissolution and breakdown of organic matter. This suggested that P could be critical in vermicomposting and that more work is needed to establish critical P levels necessary for the efficient vermicomposting of dairy manure-waste paper mixtures. These results were supported by increased humification as a result of amendment with PR. Similar results were reported by Biswas and Narayanasamy (2006) who reported that PR enriched composts had significantly higher content of total P (2.20%) compared to straw composts (0.3%) where no PR was added. The PR-compost also had higher citric acid soluble P (0.72% P) compared to straw compost (0.1% P).

While precomposting of dairy manure-waste paper mixtures eliminated pathogens it inadvertently affected the nutritional value of waste paper mixtures for earthworms and this affected earthworm growth during vermicomposting. Earthworms in wastes precomposted for one week or not at all gained weight whilst those in wastes precomposted for two or more weeks lost weight. The earthworm biomass was related to the water soluble carbon content of the wastes which declined with precomposting period of wastes. Water soluble carbon is a biologically active parameter consisting of sugars, hemicellulose, phenolic substances, amino acids, peptides and other easily biodegradable compounds (Hsu & Lo, 1999) and is utilized by earthworms and microorganisms to break down organic wastes while they get acclimatized to the recalcitrant materials. A positive relationship was observed between WSC and earthworm biomass as was shown by the multiple stepwise regression equation best fitting the data viz: Individual earthworm Biomass = 2.78*WSC – 1.824*MBC, $r^2 = 0.958$. These results are similar to those reported by Neuhauser *et al*. (1988), who observed that the mean individual weight of *Eisenia fetida* decreased from 500 mg to 100 mg in sewage sludge as a result of precomposting for 4 weeks.

Besides depletion of waster soluble carbon there are some chemical species present during vermicomposting which have been reported to be harmful to earthworms. Nitrate is one such chemical species harmful to earthworms when present at certain concentrations. According to Edwards (1988), nitrate content greater than 1 g kg^{-1} can result in rapid increases in mortality, with 100% morality occurring between 3 and 4 g kg^{-1}. Precomposting of wastes usually results in an increase in ammonium levels which will subsequently be converted to nitrate as temperature drops resulting in elevated nitrate levels. Results from Chapter 3 showed that for all the five precomposting period of 0, 1, 2, 3 and 4 weeks the amounts of nitrate-N recorded were lower than the threshold level of 1g kg^{-1}. Therefore, nitrate levels played no significant role in the observed decline of earthworm biomass with precomposting period. From a nutrition stand point one week of precomposting is suitable for vermicomposting of dairy manure-waste paper mixtures as this does not cause depletion of nutrients for earthworms and has already been shown to be long enough for pathogen reduction to safe levels.

While it is important to get improved sanitization, humification and nutrient composition as a results of the combined system with PR amendment, physical properties of the vermicomposts are equally important. Application of PR to dairy manure-waste paper mixtures not only changed the chemical properties of vermicomposts but also affected the physical properties of these composts. The addition of PR increased bulk density and particle density but reduced total porosity, air filled porosity as well as water holding capacity of vermicompost compared to where no RP was added. This is because PR does not degrade much throughout the vermicomposting period and that mass added results in the observed increases bulk density and particle density and decreases total porosity, air filled porosity as well as water holding capacity of vermicompost. Most of the physical properties of the resultant vermicomposts were within the desired ranges for growing medium except for air filled porosity of vermicomposts with 8% PR added which fell below the recommended range of between 10 and 20% is considered suitable for growing media (Bunt, 1988). Aeration is an important parameter in seedling production as roots growing in poorly aerated medium are weaker and less succulent and more susceptible to micronutrient deficiencies and root pathogens such as *Pythium* and *Pythophtora* than roots growing in well aerated medium (Ingram & Henley, 1991).

Pine bark compost is a popular growing media in South Africa as it is ubiquitous and has excellent physical properties though its water holding capacity is generally lower than the recommended 55 – 75% (de Boodt & Verdonck, 1972). Pine bark is also acidic and has low nutrient contents (Mupondi *et al*., 2006). Because of their high nutrient contents and high pH the dairy manure-waste paper mixtures produced in Chapter 4 can be used to amend pine bark compost growing medium. Results reported in Chapter 5 showed that amending pine bark with various portions (0, 20, 40, 60 and 100%) of dairy manure-waste paper vermicomposts improved its water holding capacity, nutrient content and pH. Without fertiliser application, progressive substitution of pine bark with dairy manure-waste paper vermicomposts resulted in an increase in tomato plant growth and tissue nutrient contents up to the 60% substitution compared to pine bark alone. This could have been due to improvement in nutrient content and pH with increased proportions of vermicompost added to pine bark medium. Similar results on improvement of plant growth and leaf nutrient content on amending pig manure vermicompost into Metro Mix 33 (a peat based growing medium) were reported by Atiyeh *et al*., (2001). Results from Chapter 5 also showed that fertilizer addition to all media used improved tomato seedling growth and nutrient uptake with the best response being in the 40 to 60% vermicompost substitution of pine bark composts probably because of the ideal condition created after the substitution. There was an improvement in available N and P contents as well as in improvement in pH of the amended pine bark compost growing media. The response to fertiliser application suggested that all the media used in this study had low nutrient contents and could not supply sufficient available nutrients to plants. In other similar studies the greatest plant growth responses and largest yields have usually occurred when vermicomposts constituted between 25 to 50% of the total volume of a greenhouse container medium mixture, with greater proportions of vermicomposts substituted into the plant growth medium not always improving plant growth further (Atiyeh *et al*., 2000).

6.2 GENERAL CONCLUSIONS.

1. Mixtures of dairy manure and waste paper with C: N ratio of 30 was more suitable for composting as these produced more mature and humified compost with higher ash, more and total and extractable N and P contents than wastes with a C: N ratio of 45.

2. A precomposting period of one week was found to be suitable for a combined system for stabilizing dairy manure-waste paper mixtures. Precomposting of wastes for one week reduced fecal coliforms, below the USEPA minimum content of < 1000 coliforms per gram of compost and was thus ideal for combine composting of dairy manure-waste paper mixtures. Precomposting dairy manure-waste paper mixtures for one week resulted in the most humified vermicompost with the highest C_H: C_{FA}, HI and HR indices.

4. Phosphate rock incorporation into dairy manure-waste paper mixtures resulted in increased total P, N and available N and P. Higher humification indices; C_H: C_{FA}, HI and HR were recorded for the PR amended vermicomposts than vermicomposting alone. Thus, PR incorporation enhanced the degradation of the waste mixtures in addition to the nutrient content of the resultant vermicomposts.

5. Emergence of tomato seedling was not affected by medium type or fertiliser application rate but the growth and nutrient uptake of tomato seedlings improved with progressive substitution of pine bark with dairy manure vermicomposts to a maximum at 60% vermicompost substitution. Fertiliser application improved tomato seedling growth as well as tissue nutrient content across all growing media

6. Amending pine bark with dairy manure vermicomposts increased the available N and P, pH and water holding capacity of growing medium mixtures whilst the total porosity and air filled porosity were reduced.

6.3 GENERAL RECOMMENDATION FOR FOLLOW-UP STUDIES

This study is the first of its kind in South Africa and as such many questions remain unanswered. The following are a few such areas which may need attention in the near future:

1) The mechanisms of PR solubilization need to be investigated. The organic acid content of the vermicomposting wastes need to be determined as well as the phosphate solubilization microorganism numbers need to be monitored so that the mechanism of PR solubilization can be established.
2) Phosphate rock application intensified degradation of dairy manure-waste paper mixtures resulting in vermicomposts that were highly humified and had high total P and N as well as available P and N. It could be that P is critical for vermicomposting process and thus it would be interesting to see how other sources of P such as soluble P sources would affect nutrient content and humification. The question of how much P is required to enhance vermicomposting also needs to be addressed.
3) The use of PR as an ameliorant is a novel idea in vermicomposting but the environmental risks associated with use of the end product as well effects of PR application on earthworm populations are not fully understood and need to be explored needs to be explored.
4) There is need to study the water use efficiency as well as the ability of vermicomposts to replace conventional fertilizer input. Vermicomposts have a high water holding capacity

and as well are rich in easily available nutrients and it is possible their use reduce water and conventional fertilizer usage.

5) There are different types of paper produced by different institutions. It may be necessary to see of this approach could be effective for other types of waste paper. Paper used in this study was newsprint but there are types of paper whose composition is different from newsprint and could give different results.

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