CHEMICAL CHARACTERISTICS, FERTILIZER VALUE AND *ESCHERICHIA COLI AND SALMONELLA* COMPOSITION OF VERMICOMPOSTED SEWAGE SLUDGE

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DECLARATION

I, Busisiwe Mashologu, declare that:

The research reported in this dissertation, except where otherwise indicated, is my original work.

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Busisiwe Mashologu

We, as the candidate's supervisors have/have not approved this dissertation for submission

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Dr R. Zengeni (Co-supervisor)

ABSTRACT

Treatment and disposal of high volumes of domestic sewage sludge is among the most crucial environmental and health challenges facing urban communities due to the increase in population and unplanned urbanization. Sewage sludge is produced from the treatment of waste water through the removal of solids, soluble and fine suspended organic pollutants both by biological oxidation and adsorption processes, and excess water is either removed using drying beds or dewatering with centrifuges. The conversion of this solid organic waste into useful organic fertilizers, through vermicomposting has gained major interest to close the gap between disposal challenges and fertiliser value of sewage sludge

A vermicomposting study was carried out to investigate the effect of vermicomposting of two types of domestic sewage sludge on stabilization, composition of pathogenic organisms and fertilizer value of the vermicomposts with or without biochar. This composting study was conducted as a 2 × 2 factorial experiment laid out in randomized complete block design (RCBD) and replicated 3 times. The treatments used were (i) drying bed sludge without biochar (DB), (ii) drying bed sludge with biochar (DBB), (iii) dewatered sludge without biochar (DW), (ii) dewatered sludge with biochar (DWB). Vermicomposting was done using mature and juvenile *Eisenia fetida* earthworms for a period of 9 weeks. The results from this study revealed that dewatering method of sewage sludge had a major influence on the nutrient and pathogenic content of the resultant vermicomposts. Dewatered sludge vermicompost (DW) without biochar had more nutrients (N, P, K, Mg, Ca and reduced C content) than dewatered sludge vermicompost with biochar (DWB) and drying bed sludge vermicompost (DB) with or without biochar (DBB). The DB vermicompost with biochar was the second most nutrient-rich vermicompost compared to the other vermicomposts used. Pathogen reduction especially of *Salmonella spp* was mostly achieved in the DB vermicompost than DW vermicompost and the presence of biochar significantly decreased *E.coli* in the DB vermicompost while there was no significant difference in *E.coli* population of DW vermicompost with or without biochar.

A follow up study was conducted to determine the N, P release and fertilizer value of the sewage sludge vermicompost with or without biochar. An incubation experiment was conducted in a constant temperature room (25˚C) using a Clovelly soil form (200g/container) that was amended with drying bed sewage sludge vermicomposts with and without biochar (DB and DBB) respectively at application rates equivalent to 0 (control), 10, 50 and 100 t/ha. The design of the experiment was arranged in a completely randomized design and treatments were replicated 3 times. Holes were poked on the side of the containers to minimize anaerobic conditions during the 8 weeks incubation period. Water was added up to 100% field capacity of the soil and moisture correction was after every 4 days, based on weight loss. The same experiment was repeated with vermicomposts from dewatered sludge DW and DWB. The setup, management, sampling and analyses were the same as for the drying bed sludge vermicomposts (DB). The results showed that vermicomposts from dewatered sludge (DW) had greater nutrient release than those from drying bed (DB) sludge. Nitrogen release in the form of NH_4-N and available P was higher in soils amended with vermicomposts from D than DB. Increasing the application rate of vermicompost increased its nutrient release in soil. Soils amended with vermicompost containing biochar had higher Ca.

The effects of sewage sludge vermicompost on crop (spinach) drymatter and nutrient uptake was also tested in a glasshouse experiment where spinach seedlings were grown in pots containing 1.5kg of soil (Clovely form). The soil was amended with DB, DBB, DW and DWB vermicomposts at recommended application rates of 10t/ha, 50t/ha and 100t/ha. The experiment included a negative control and a positive control (2:3:2 (30) +5% Zn and was conducted for a period of 6 weeks. It was found that applying vermicompost at application rates higher than 10t/ha significantly increased spinach yields and uptake of major nutrients. The concentration of N in the soil from which spinach was planted decreased with increasing application rate. The growth response was higher for DB vermicompost. Maximum crop response and yields were obtained from soils amended with the DB, DBB and DW vermicomposts. The findings of this study suggested that vermicomposting of sewage sludge is a sound waste management strategy that is well suited for improving soil fertility and plant nutrition and also reduces the risk to public health by decreasing human pathogenic microorganisms.

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LIST OF ABBREVIATIONS AND ACRONYMS

ANOVA	analysis of variance
DB	drying bed sludge vermicompost
DBB	drying bed sludge vermicompost containing biochar
DW	dewatered sludge vermicompost
DWB	dewatered sludge vermicompost with biochar.
EC	electrical conductivity
LSD	least significant difference
MPN	most probable number
ns	not significant
RCBD	randomized complete block design

CHAPTER 1: INTRODUCTION

Treatment and disposal of sewage sludge is one of the most crucial environmental issues facing today's community due to the increase in population and unplanned urbanization with high demand for better water quality (Gupta and Garg, 2008). Sewage sludge is generated in huge quantities and its disposal on land creates major problems in both soil and human health (Nayak *et al*. 2013). Domestic sewage sludge is generated by wastewater treatment plants from waste produced from different sources including homes, medical facilities, street runoff and businesses (Hait and Tare, 2011). The sewage treatment processes involve; (1) removal of solids by screening or sedimentation and (ii) removal of soluble and fine suspended organic pollutants by biological oxidation and adsorption processes (Woods. 2010). The by- product of these processes is sludge, which is further treated to reduce biological oxygen demand, pathogens, volume and water content. Water removal is essential to reduce costs of transport and handling costs of disposal (Woods. 2010). As a result the sludge is either dewatered through centrifugation or through evaporation on drying beds.

During dewatering, the sludge is centrifuged to reduce water and volume to 50% which produces dewatered sludge. Water removal from this process could reduce the nutrient content during water removal. Another method of water removal involves drying of the sludge on drying beds, where the sludge is pumped out into sand layered beds for the water to evaporate resulting in dryer material which can be disposed of.

Common disposal methods include incineration, dumping on land fill sites, land application and composting. The drawback from incineration is the pollution of air from the gases produced during the burning of sludge whereas leachates from land fill sites may lead to the contamination of ground water bodies. Sewage sludge contains high concentrations of nutrients and therefore has high potential to be used as a fertilizer and soil conditioner (Gupta and Garg, 2008).

The application of sewage sludge was found to significantly increase mean foliar P (from 3.0 to 4.0mg/kg) and trunk diameter of pine trees (Egiart *et al*. 2005) while Mohammed and Athamneh, (2009) reported an increase in fresh and dry weight of lettuce. Sinha *et al*. (2009) found that sewage sludge had a pH of 9.5 before vermicomposting, 0.5g nitrate-N /kg and 6.2g phosphate /kg. Hait *et al.* (2012) found primary sewage sludge (PSS) and waste activated sludge (WAS), to contain 22.79% and 28.29% total carbon, 1.7% and 2.29% total nitrogen, 0.72% and 0.98% P and 5.50% and 6.66% K with a pH of 7.5 and 7.6, respectively. In addition to macro nutrients, sewage sludge also contains organic compounds, micro nutrients, pollutants and pathogenic microorganisms (Sinha *e t al*. 2009).

The composition of the sludge could depend on whether the water is removed by dewatering and drying bed methods. While sewage sludge may contain heavy metals, the concentrations are generally lower in sewage sludge produced from smaller less industrialized cities than large cities. Whereas the application of sewage sluge as a fertilizer is attractive, untreated sewage sludge results in rapid decomposition, which causes anaerobic conditions in the soil, which is responsible for the odor. The presence of pathogens in domestic sewage sludge also presents major concerns for its application on agricultural land since these can enter human bodies through ingestion of crops grown with such a fertilizer, with serious health risks. There is need for alternative methods of treating sewage sludge for safer disposal and composting could be a viable strategy.

Thermophilic composting has shown positive results in terms of removing volatile material which cause odours (stabilizing) and removal of pathogens (sterilization) from sewage sludge (Nakasaki *et al*., 1985; Fang and Wong, 1999; Manios and Stentiford, 2006). Thermophilic composting reduces the population of pathogenic organisms but this method could reduce the nutrient value of the resultant compost through gaseous nitrogen losses. High temperatures used during thermophilic composting lead to nitrogen losses due to volatilisation and essential microorganisms are also killed during this process (Hait and Tare, 2011). Moreover, the process is labour intensive as it requires frequent turning of the material and as such, vermicomposting could be a better option.

The use of earthworms for treating organic wastes has been widely practiced and recommended (Chaoui, 2010). Vermicomposting is a low cost system for the treatment or processing of organic waste through the use of earthworms (Gupta and Garg, 2008; Chaoui, 2010). As such the earthworms do the turning and the lower temperatures, compared to thermophilic composting, suggest that nutrients, and possibly pathogens, are retained. The process involves both physico-chemical and biochemical conversion of organic substrate in the gut of the earthworms which is then excreted as casts. The substrate is aerated, mixed and ground and this constitutes the mechanical and physical processing while the microbial biomass in the gut of the earthworm is responsible for the degradation of the substrate (Gupta and Garg, 2008). In vermicomposting the inoculated earthworms maintain aerobic conditions in the organic waste and convert organic material into worm biomass, respiration products and excrete casts as stabilized vermicompost. These earthworms stabilize and homogenize the sewage sludge and increase rate of destruction of sludge volatile solids due to the increased aeration and turnover of waste (Gupta and Garg, 2008). Apart from homogenizing and stabilizing the sludge, earthworms are also capable of bioaccumulating heavy metals in their body tissue reducing heavy metal concentrations in the sludge.

Vermicomposting is a mesophilic process therefore complete sanitization of the waste material cannot be expected because there is no thermal stabilization to remove pathogens (Aria *et al*., 2011). Moreover, the lack of oxygen during vermicomposting under high relative humidity and high temperatures has negative implications on earthworm activity, thus complete removal of pathogens may not be achieved (Hait and Tare. 2012). Low levels of pathogens in vermicast have been reported on by Mupondi *et al.* (2010). Chaoui, (2010) stated that earthworms consume fungi, and the vermicast allows for the survival of disease-suppressing organisms which feed or destroy the pathogens. The content of volatiles, heavy metals and pathogens in the vermicast could depend on the method of dewatering used. Centrifuged sludge is likely to contain less of the volatiles, heavy metals and pathogens since some of these could be leached during draining of water out of the sludge as compared to sludge that is poured on drying beds to remove water. These dewatering methods could, therefore, have an effect on the quality of the resultant vermicompost.

The Howick Wastewater Works (HWW), near Pietermaritzburg is currently facing problems of sludge treatment and disposal of waste activated sludge. Sludge is either dewatered or dried on the drying beds onsite and is then disposed of on the land fill sites. A dewatering unit has been installed to treat the sludge to the required solid concentration of 18-20% in the aeration tanks but the resultant sludge is often not acceptable at land fill sites as it is not dry enough and thus too thick to be pumped to the drying beds. Therefore, vermicomposting could be a convenient and cost effective method for the treatment of the sludge to a more acceptable and useful product for land application. There is limited literature on comparison of quality of vermicomposts from drying bed sludge and dewatered sludge.

Earthworms function better under moist conditions and as such water management during vermicomposting is essential. The required moisture levels for vermicomposting are 60-80%. This helps in the interchange of gases and prevents the worm from dehydration. If the moisture levels in the tissue drops, the worm losses its weight, hence the maturity of the worm can also be determined in terms of its moisture (Rodriguez-Canché *et al*. 2010). Because of this, some leachate is released during periodic watering which could result in the loss of nutrients. Biochar, a carbonaceous material with high surface area, high water retention capacity and cation exchange capacity (CEC), could be a useful for retaining nutrients during the vermicomposting of sewage sludge.

Vermicomposted sewage sludge has more available nutrients per kg weight as compared to the initial substrate, thus it has high nutrient value and its use as fertilizer is a common practice. Its application as a soil amendment is a common practice due the nutritional benefits. Nitrogen and phosphorus mineralization as a result of earthworm activity qualifies the end product to be used for plant growth since these nutrients become bio-available for plant uptake (Sinha *et al*., 2009). The vermicompost is homogenized and contains beneficial soil microorganisms such as nitrogen fixing bacteria and mycorrhizal fungi which all aid in crop growth.

1.2 Aim of the study

The main objective of this study was to investigate the effect of vermicomposting of two types of domestic sewage sludge on stabilization, composition of pathogenic organisms and fertilizer value of the vermicomposts with or without biochar.

1.3 Objectives

(1) To determine the effect of water removal method from sewage sludge and addition of biochar on nutrient composition and population of selected pathogenic organisms in domestic sewage sludge vermicomposts.

(2) To determine the N, P release of the sewage sludge vermicompost with or without biochar

(3) To determine effects of sewage sludge vermicompost on crop (spinach) drymatter and nutrient uptake.

1.4 Hypotheses

(1) Water removal method and biochar addition do not have an effect on the chemical composition and population of selected pathogenic organisms in vermicomposted domestic sewage sludge.

(2) There is no difference in the N, P release and fertilizer value of sewage sludge vermicomposting with and without biochar.

(3) There are no effects of vermicompost application on spinach yield and nutrient uptake.

CHAPTER 2: VERMICOMPOSTING OF DOMESTIC SEWAGE SLUDGE, FERTIIZER VALUE AND COMPOSITION OF PATHOGENIC ORGANISMS (*E.COLI AND SALMONELLA*): A REVIEW

2.1 Introduction

Domestic waste water sludge is a "resource" that is produced in abundance in all societies. Its use in agriculture is receiving great interest as a source of fertilizer and supplement for water needs. Being a semi-arid and water scarce country, South Africa receives an average annual rainfall of 500 mm per annum, and irrigated agriculture uses about 62% of the water from the scarce resources while commercial farmers use 95% of agriculture's share in irrigation (Blignaut *et al*. 2009). This means that waste water needs to be recycled and domestic waste water sludge has major benefits for agricultural practices. The literature reviewed below reports on the positives and drawbacks of vermicomposting organic waste material including sewage sludge. It is of outmost interest to investigate the issues raised in this review so as to better understand the technology of vermicomposting sewage sludge and make conclusions along with recommendations for future use and adoption of vermicomposting in our society**.**

This review covers the following topics:

- Sewage sludge production
- Vermicomposting as an alternative method of sewage sludge treatment and utilization
- Elements necessary for vermicomposting
- Effect of vermicomposting on pathogenic content and nutrient composition of vermicompost
- Vermicomposting with the inclusion of biochar: Advantages and disadvantages

- Nutrient release and fertilizer value of vermicomposted sewage sludge and its effect on crop growth and residual soil.

2.2 Sewage sludge production.

Sewage sludge is an inevitable, odorous and hazardous by-product that is produced from the treatment of water and waste water by treatment plants. This by-product is produced in large quantities both globally and locally, such that it creates problems in terms of its disposal. Mexico was reported to have generated an estimated 493,000-1,500, 000MgL/year of sewage sludge in its treatment plants over the last decade alone (Ludibeth *et al*. 2012). All this production was due to the biological processes that are used to digest organic matter from households (Ludibeth *et al*. 2012). While countries such as Malaysia produce an estimated 5 million cubic meters of domestic sludge per year an amount which is estimated to increased to 7 million cubic meters per year by 2022 (Kala *et al*. 2009). A study by Wei *et al*. (2003) revealed that treatment and disposal of sewage sludge from wastewater treatment plants (WWTPs) accounts for about half or even 60% of the total cost of wastewater treatment in Europe. The author further states that in 1991/1992, the EU operated 40,300 WWTPs which produced 6.5million tonnes of dry solids per year. Due to Urban Wastewater Treatment Directives (UWWTD) which require more extensive wastewater treatment and an end to sewage disposal to the sea, sewage sludge production in EU was estimated to increase by at least 50% by the end of the year 2005 producing 10.1million tonnes of dry solids per year (Wei *et al*. 2003).

Waste is generated in huge quantities all over the world and the growing concerns over environmental issues raise a lot of despair for communities at large. The South African Department of environmental affairs (DEA) categorizes waste production in two categories namely general (municipal waste) and hazardous (health care risk and certain industrial waste) (Mundeza. 2014). According to a report by Mundeza. (2014), South Africa generated an estimated 59 million tonnes of general waste in the year 2011 of which 5.9 million tonnes were recycled and the remaining 53.9 million tonnes were land filled. These waste are produced in both urban and rural areas due to intensive livestock farming enterprises, small-holder enterprises, businesses and other waste generating activities (Mupondi *et al*. 2011). Over the last few years, the problem of efficient disposal and management of organic solid wastes has become more rigorous due to the rapidly increasing population, intensive agriculture and industrialization (Garg *et al*. 2006). The unhealthy and unstable environment occurring as a result of this waste is causing concerns of environmental contamination. Minimization of excess sludge production for biological waste water treatment include:

(i) Lysis-cryptic growth, the processes where by an organic autochthonous substrate is produced from cell lysis and used in microbial metabolism, and liberation of carbon as product of respiration later results in reduced overall biomass production. Lysis-cryptic growth involves two stages namely (lysis and biodegradation) (Wei *et al*. 2003). An increase in lysis efficiency reduces sludge production. Ozonation is another process of lysis-cryptic growth which consists of a sludge ozonation and biodegradation stages whereby a fraction of the recycled sludge passes through the ozonation unit and then the treated sludge is decomposed in the subsequent biological treatment (Wei *et al*. 2003). According to Yasui and Shibata (1994), throughout the operational periods of full-scale plants with sludge ozonation for treating municipal wastewater and industrial wastewater respectively, no excess sludge was withdrawn and no significant accumulation of inorganic solids occurred in the aeration tank at optimal ozone doses. This means that the process was more efficient in reducing excess sludge production than other processes. Other lysis techniques include the use of temperature to control the amount of sludge produced and chlorination. In work done by Tian et *al*. (1994), sludge production at 8ºC in activated sludge process had an increased volume by an average of 12-20% in comparison with that at 20ºC. This confirmed that low temperatures can lead to increased volume in sludge production due to high accumulation of cell protoplasm within flocculants in the form of chemical oxygen demand (COD). The drawbacks with thermal treatment is that it causes odor and the cost of maintenance is too high. The volume of excess sludge production was reportedly reduced by 65% as a result of chlorination at doses of 0.066g Cl_2/ ML was reported in a study by Chen *et al*. (2001). However, the major disadvantages reported for this technique were the formation of trihalomethanes (THMs) which pose increased risk of cancer and DNA alteration in humans, bad sludge stability and also increase soluble chemical oxygen demand (SCOD) in the effluent.

(ii) Uncoupling metabolism: this involves bacterial anabolism (breakdown of complex substances into simpler substance by living cells through biochemical activities) coupled with catabolism of substrate through rate limiting respiration. The dissipation of energy for anabolism without reducing the removal rates of organic pollutants in biological wastewater treatment provides a direct metabolism for the reduction in sludge production (Wei *et al*. 2003).

(iii) Maintenance metabolism:

Waste disposal strategies that are currently being used in most municipalities include incineration, thermophilic composting, landfilling and the use of some of the waste in agriculture. The drawbacks with these methods make them inefficient for their primary role of waste minimization. For instance, the use of incineration for the minimization of waste contributes to the climate change problem due to the gases produced during the burning process. Thermophilic compost requires intensive labour and very high temperatures which in turn kill some of the useful microorganisms responsible for the decomposition of the substrate (Hait and Tare. 2011). Moreover, nutrients such as nitrates are not easily retainable during thermophilic composting due to process of denitrification. The use of landfill sites for the disposal of waste is also unreliable and not environmentally friendly due to the leaching of nutrients such as nitrates to ground water bodies (Gupta and Garg. 2008). In addition, some of the waste is at times rejected at landfill site because it does not meet the necessary requirements for disposal, and in such cases more efficient methods need to be adopted (Gupta and Garg. 2008). Waste needs to be treated before it can be used in agriculture land to minimize the risk of transferring diseases and contaminants which can later be transferred to humans (Ludibeth *et al*. 2012).

2.3 Vermicomposting for minimization and utilizing organic wastes

Vermicomposting is a low cost system for the treatment or biological processing of organic wastes into useful soil amendments (Domínguez *et al*. 1997) through the use of earthworms (Chaoui, 2010; Gupta and Garg. 2008). It 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. During vermicomposting, earthworms do the turning; and the lower temperatures compared to thermophilic composting suggest that nutrients are retained and pathogen may sometimes be reduced or otherwise. The process involves both physico-chemical and biochemical conversion of organic substrate in the gut of the earthworms which is then excreted as casts. The substrate is aerated, mixed and ground and this constitutes the mechanical and physical processing while the microbial biomass in the gut of the earthworm (Figure 2.1) is responsible for the degradation of the substrate (Gupta and Garg, 2008). During vermicomposting, the inoculated earthworms maintain aerobic conditions in the organic waste and convert organic material into worm biomass, respiration products and excrete casts as stabilized vermicompost. These earthworms stabilize and homogenize the sewage sludge and increase the rate of destruction of sludge volatile solids due to the increased aeration and turnover of waste (Gupta and Garg, 2008). The presence of contaminants such as organic compounds, heavy metals and human pathogens in sewage sludge could cause changes in microbial activity and alter the composting potential of earthworms. Low levels of pathogens in vermicast have been reported on by Mupondi *et al*. (2010). Vermicomposting is a mesophilic process and therefore, complete sanitization of the waste material cannot be expected because there is no thermal stabilization to remove pathogens (Aria *et al*. 2011). Moreover, the lack of oxygen during vermicomposting under high relative humidity and high temperatures has negative implications on earthworm activity, thus complete removal of pathogens may not be achieved (Hait and Tare, 2011). The content of volatiles, heavy metals and pathogens in the vermicast could depend on the method of dewatering used, since centrifuged sludge is likely to contain less of the volatiles, heavy metals and pathogens because some of these could be leached during draining of water out of the sludge as compared to sludge that is poured on drying beds to remove water. These dewatering methods could have an effect on the quality of the resultant vermicompost. In addition, earthworms function better under moist conditions and as such water management during vermicomposting is essential. Rodriguez-Canché *et al*. (2010) states that required moisture levels are 60-80%, which help in the interchange of gases and prevents the worm from dehydration, as a result some leachate is released during periodic watering which could result in the leaching of nutrients. Vermicomposting is also used in sewage sludge and can result in more available nutrients per kg weight as compared to the initial substrate, thus it has high nutrient value and its use as fertilizer is a common practice. Nitrogen and phosphorus mineralization as a result of earthworm activity qualifies the end product of vermicomposting to be used for plant growth since these nutrients become bio-available for plants (Sinha *et al*. 2009).

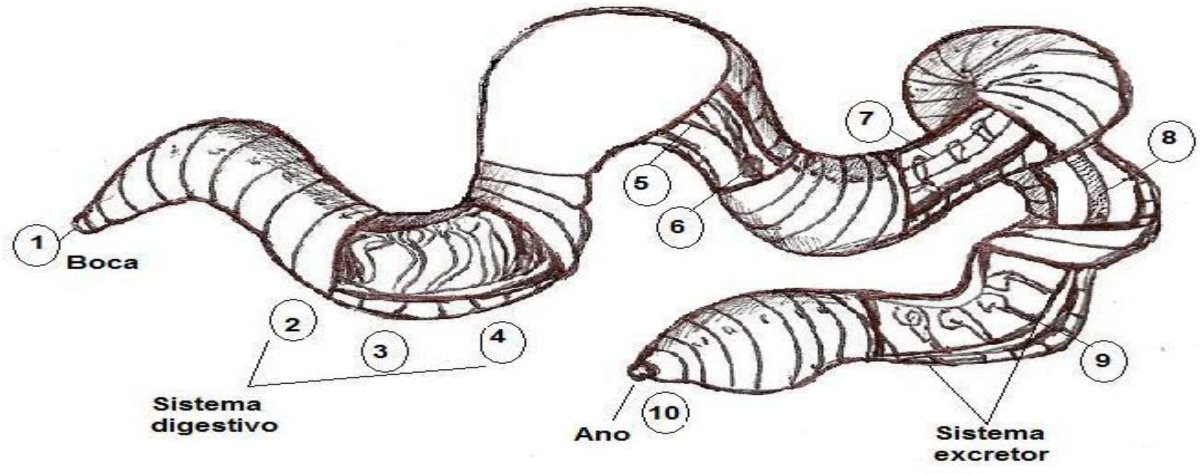

Figure 2.1. General schema of organic matter (OM) processing by the earthworm: (1) sucks OM; (2) triturates and grinds particles; (3) mix substrates; (4) modifies and levels acidity; (5) inoculates with microorganisms; (6) promotes and multiplies microorganisms; (7) creates regulating compounds; (8) homogenizes and homogeniza palletizes; (9) covers proteins in muco; and (10) excretes organic manure. (Source: Juarez. 2011)

2.4 Elements necessary for vermicomposting

Vermicomposting is the biological conversion of organic matter into humus like material with the agency of earthworms. In order for vermicomposting to successfully take place there are a number of basic requirements that have to be met which include the following:

(i) Earthworms suitable for vermicomposting

Several reports are available on the use of earthworms in composting and degradation of organic wastes including sewage sludge. Earthworm species that are mostly employed are epigeic species (earthworms found on the surface and which feed on dead leaves and organic matter) due to their voracious feeding habits on organic matter and these include, *Eisenia Fetida, Lumbricus rubellus, Amyanthes diffrigens, Eudrillus engineac*, *Perionyx excavates* (Theunissen *et al*. 2010). The adaptability of earthworm species varies with different soil types and different agro-climatic conditions (Padmavathiamma *et al*. 2008), hence different species are found to be suitable for vermicomposting under different regions. When planning to practice vermicomposting, the worms have to be introduced to the waste materials that are about to decompose to allow them to acclimatize to the conditions. This allows the researcher or farmer to be able to determine how much time the worms are likely to take to do the actual decomposting, produce eggs and multiply.

(ii) Stocking density of worms

Stocking density of earthworms is an important factor to consider in order to avoid over feeding and allow for optimum conditions for reproduction. Using the correct stocking density prevents the earthworms from being overcrowded hence Chaoui (2010) recommended stocking density of 150 earthworms/L of waste. Ndegwa *et al.* (2000) investigated the effects of stocking density and feeding rate on vermicomposting of biosolids and concluded that stocking density of 1.6kg worms/m^2 and feeding rate of 1.25kg feed/kg worm/day were the optimum combinations. Furthermore, the use of the same stocking density with a 0.75kg feed/kg worm/day resulted in a completely digested vermicompost. A stocking density of 0.8kg-worms/m^2 for horse manure and 2.9kg/m^2 for activated sludge was obtained by Neuhauser *et al*. (1980) for *E.foetida* while

Domingez and Edwards, (1997), found that at lower population densities, individual *Esenia Andrei* worms grew more and faster whereas at higher population densities there was faster sexual maturation and biomass production.

(iii) Moisture

Water constitutes 75 - 90% of the body weight of earthworms and so, the 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. Shama *et al*. (2005) found the optimum moisture conditions for *E.fetida* to be 80 - 90% with a limit of 60 - 90%. According to Chaoui. (2010), a moisture level of 75% (field capacity) for waste material should not be exceeded especially when dealing with food waste such as fruits and vegetables as these contain about 90% of moisture. However, in an earlier study, Reinnecke and 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%. In essence the clitellum development occurred in earthworms at moisture contents from 60% to 70% but is delayed at lower moisture content ranges from 55% to 60% .Clitellum is part of the reproductive system of earthworms that secretes a viscid sac in which eggs are deposited (Shama *et al*. 2005).

(iv)Temperature

Earthworms can survive in most climatic conditions due to their wide adaptability range. Survival and reproduction of earthworms including activity is largely influenced by temperature, as earthworms tend to be less active under cold conditions. Temperatures of 0-35ºC are considered favorable for survival of earthworms but this varies with different species and 25ºC is considered as the most optimum temperature for most species (Chaoui. 2010). Earthworm species such as *E.foetida* perform best with temperatures between 25-30ºC (Mupondi *et al*., 2010). The process of vermicomposting is not a thermophilic one, so temperatures exceeding 35ºC may result in complete fatality of the worms.

(v) Drainage and Aeration

Passage of gases is influenced by the drainage and aeration of the system, as any lack of these two factors may result in the system being waterlogged and suffocated. During decomposition of the organic material, gases such as CH_4, CO_2, NO_2 are released and escape the system as volatile gases, with no ventilation these gases circulate and kill the earthworm. Drainage allows for leaching of excess moisture (i.e. worm wee) from the system making the material more porous and conditions more favorable for earthworms to thrive. For appropriate drainage and aeration, container walls bottom and side walls are perforated, most commonly the bottom part for ease of drainage. The bedding material also becomes an important component necessary for drainage. Commonly used bedding types are grass cuttings (because earthworms do not feed on the grass), a layer of vermicast, which provides room for keeping the worm stocking density lower than the maximum 300 worms/L and also improves aeration as the earthworms will burrow through the vermicast creating holes (Chaoui . 2010).

(vi) Salinity and pH

Earthworms are sensitive to saline conditions, and ammonium levels greater than 5mg/g repel earthworms. In fresh vermicast, ammonium mineralized in the earthworm gut is nitrified over 2 weeks. Optimum pH conditions for earthworm survival are in the range of 6.5-8 (Chaoui 2010).

However, Edwards (1988) reported that pH of >5 and < 9 was optimum for *E. foetida* but from further studies Edwards (1995) later concluded that *E. foetida* can thrive and survive in more acidic material of pH 5.

(vii) Feeding preferences

The ideal feed for earthworms is kitchen waste, animal manure, crop residues, waste rich in nitrogen , industrial and domestic waste and pre-composted (for up to two weeks for ease of digestion) waste and paper waste (Chaoui.2010). Many studies have been carried out on different types of feedstock used for vermicomposting. Animal manures (sheep, goat and cow dung) have topped the list of most researched and studied materials along with chicken manure (Arancon *et al*., 2005); cattle manure with food waste and paper waste (Azarmi *et al*., 2008); sheep manure (Padmavathiamma *et al*., 2008); fresh banana leaves and cow dung (Ansari. 2010); cattle dung (Gutierrez-Miceli *et al*., 2007); sheep manure (Atiyeh *et al*., 2001) and pig manure.

(viii) C/N ratio

Organic carbon and inorganic nitrogen are important for cell synthesis, growth and other metabolic activities in all living organisms including earthworms (Ndegwa and Thompson, 2000). The ideal earthworm feed must have a C/N ratio of 25:1 and this value is lowered as decomposition of organic matter proceeds (Chaoui.2010). Lowering of the C/N ratio is also an important indicator of the maturity of the end product (Padmavathiamma *et al*. (2008). According to Padmavathiamma *et al*. (2008), A C/N ratio of ≤15 is most desirable and based on studies by Nayak *et al*. (2013), material with a C/N ratio of 30 (composed of 0.87 kg sewage sludge, 0.45 kg cattle manure and 0.18 kg sawdust) produced the most stable vermicompost after 45 days as compared to materials which had lower C/N ratio at the beginning. The use of bulking material such as shredded tree leaves, wood chips or wheat straw with more organic carbon help to reduce nitrogen losses during decomposition (Dominguez *et al*. 1997).

2.5. Effect of vermicomposting on pathogenic content and nutrient composition of vermicompost.

In South Africa sewage sludge is classified into three (3) classes based on microbial stability and pollutant classification. The pathogen classification is divided into A (unrestricted use quality), B (general use quality) and C (limited use quality). Table 2.1 below illustrates this classification.

Table 2.1: Compliance and classification criteria: Microbiological class (DWAF. 2006)

Microbiological class	**Unrestricted use quality**		**General use quality**		**Limited use quality**
	A		**B**		**C**
	Target value	Maximum permissible value	Target value	Maximum permissible value	
Faecal coliforms (CFU/g $_{\text{dry}}$	< 1000 (5 log reduction)	10 000 (4 log reduction)	<1x10^{6} (2 log reduction)	1x10^{7} (log reduction)	> 1x10^{7} (log reduction)
Helminth ova (viable ova/g $_{\text{dry}}$)	< 0.25 (or one ova/4g)	1	<1	4	>4
			Compliance requirements		
Requirements for classification purposes (minimum 3 samples)	All the samples submitted for classification purposes must comply with these requirements	Not acceptable	Two of the samples submitted for classification must comply with these requirements	The samples that failed must not exceed the minimum permissible value	not acceptable

Dominguez *et al*. (1997) vermicomposted pig manure and observed 50-60 % higher nutrients in the earthworm treatments than in the control. Further on the basis of the preliminary studies it was also observed that human pathogens do not survive during vermicomposting. After 60 days of vermicomposting; faecal coliform bacteria in bio-solids dropped from 39000 MPN/g to 0 MPN/g, Salmonella sp. also dropped from < 3 MPN/g to < l MPN/g. Based on Table 2.1, the vermicompost produced by Dominguez *et al*. (1997) was well in compliance with the restrictions and compliance standards. A study by Mupondi *et al*. (2010) revealed that pre-composting reduced faecal coliforms in dairy manure wastes paper mixes to levels lower than those recommended as safe levels in a period of one week. Vermicomposting is a mesophilic process (temperature are below 40ºC) and therefore temperatures involved are not sufficient to completely eliminate all pathogens or faecal coliforms. This was also proven in the previously mentioned study by Mupondi *et al*. (2010), as shown in Table 2.2 where vermicompost significantly reduced faecal coliforms in pre-composted waste to the safe levels that are commended by USEPA (1994). To support these findings, the author argues that the additional reduction in *E. coli*, faecal coliforms and *E. coli* 0157 (a pathogenic strain causing severe hemorrhagic diarrhea and kidney failure, mostly found in animal manures) could be a result of earthworm action.

Table 2.2: Effects of pre-composting period on umbers of Faecal coliforms, C, *E. coli*, *E. coli 157* and presence or absence (P – A) of Cryptosporidium cysts and Giardia oocysts in precomposted wastes (M) and in final vermicomposts (V).

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

Effects of precomposting period on MBC are shown for M only. Most probable number (MPN). For each parameter, means followed by different letters are significantly different according to LSD at $p<0.05$. (Mupondi *et al*. 2010).

The low levels of pathogens in vermicasts have also been reported on by Chaoui. (2010), who stipulates that earthworms consume fungi, and the vermicast allows for disease-suppressing organisms to survive which feed or destroy the pathogens.

Results from Rodriguez-Chanche. (2010) show that fecal coliforms, salmonella spp and helminth ova all decrease in sewage sludge as a result of vermicomposting (Table 2.3). The results also indicate that increasing earthworm population densities completely eliminated helminth ova while reducing fecal coliforms and salmonella spp to safe levels (Table 2.3).

Table 2.3. Faecal coliform, Salmonella spp. and helminth ova in Class A biosolids, raw sludge (RS), pre-composted sludge (PCS), PCS without earthworms (control) and vermicomposted sludge treated with the three earthworm population densities. (Rodriguez- Chanche. 2010).

Substratum	**Variable**		
	Faecal coliform (MPN/g dry wt.)	***Salmonella* spp (MPN/g dry wt.)**	**Helminth ova (viable ova/g dry wt.)**
Class A biosolids	<1000	<3.0	<1.0
RS	1600	>2400	10
PCS	1600	>2400	12.5
Control	7.75	<3.0	0
1.0 kg m^{-2}	3.25	<3.0	0
2.0 kg m^{-2}	3.25	<3.0	0
2.5 kg m^{-2}	60	<3.0	0

MPN (Most probable number)

Rodriguez-Chanche. (2010) states that, microbes (composters) that are released by earthworms compete for nutrient resources with pathogenic organisms hence pathogens decrease in vermicasts. Pierre *et al*. (1982) mentions that earthworms preferentially consume protozoa, bacteria and fungi as food thus reducing their proliferation and in the process release coelomic fluids that have antibacterial properties and destroy most pathogens in the waste bio-mass. Some bacteria and fungi (*Penicillium spp. and Aspergillus spp*.) have also been found in the intestine of earthworms, (Singelton *et al*. 2003). These bacteria produce antibiotics which kill pathogenic organisms in the sewage sludge making it sterile. The removal of pathogens, faecal coliforms (*E. coli*), Salmonella spp., enteric viruses and helminth ova from sewage and sludge appears to be much more rapid when they are processed by E. foetida, of all these*, E. coli and Salmonella* are greatly reduced (Bajsa *et al.* 2003). Bajsa *et al*. (2004, 2005) studied pathogen removal in vermi- composting of sewage sludge spiked with *E. coli, S. typhimu- rium and E. faecalis*. In the study, composting was done with different bulking materials such as lawn clippings, sawdust, sand and sludge alone for about 9 months. A safe product was achieved in 4–5 months of vermicomposting and the product quality remained the same in the remaining months of the test. Lotzof (2000) also showed that pathogens like enteric viruses, parasitic eggs and *E. coli* were reduced to safe levels in sludge vermicasts. Cardoso and Remirez (2002) reported a 90% removal of faecal coliforms and 100% removal of helminths from sewage sludge after vermicomposting. Brah- mbhatt (2006) also confirmed complete removal of coliforms by earthworms. Nair *et al.* (2006) studied a combination of thermophilic treatment followed by vermicomposting and observed that the combination often leads to a faster reduction of pathogens than the same 21 days period of thermophilic composting. The study also indicated that vermicomposting leads to greater reduction of pathogens even after 3 months of storage, while samples subjected to thermophilic composting alone retained higher levels of pathogens even after 3 months. These finding are similar to those reported by Mupondi *et al.* (2010), on comparisons of thermophilic composting with vermicomposting.

2.6. Nutrient release from vermicomposted organic waste and its effect on crop growth

The method of using vermicomposting to supply nutrients in crop or vegetable production is receiving great interest as most farmers are moving away from the use of synthetic fertilizers. This is due to the minimal cost that are incurred when using vermicompost and the benefits that it comes with. Vermicomposts contain nutrients in the forms that are readily taken up by the plants such that when applied as a fertilizer, nutrient release is much faster (Atiyeh *et al*. 2000). These nutrients include nitrates, exchangeable phosphorus, and soluble potassium, calcium and magnesium. Moreover; vermicompost also has benefits such as supplying the soil with much useful microorganisms for decomposition (Atiyeh *et al*. 2000). According to Shoba *et al.* (2012), the germination rate of tomato seeds and soil quality were highly improved with the addition of 15% vermicompost. Baskar *et.al.* (2011) used different vermicompost concentrations in his study, where vermicompost was added to red soil and maize plants were grown for 90 days. Based on the collected data, the experiment clearly showed that the application of vermicompost greatly enhanced the kernel yield. On the other hand Laubscher *et.al*. (2010) reported the uptake of vermicompost to have a positive effect on plant productivity, be it plant nutrition, photosynthesis, and leaf chlorophyll content. It also improves the nutrient content of the different plant components such as roots, shoots and the fruits.

Based on a study by Padmarathiamma *et al*. (2008), the addition of vermicompost to an acidic-agricultural soil greatly influenced nutrient absorption, while stimulating root growth and increased the yield of bananas, cassava and cowpea. It was also found that the biometric character of the soil was improved leading to improved quality of the planted crops. According to a study by Atiyeh *et al*. (2000), the shoot dry weight of raspberry plants planted in a mineral soil amended with vermicomposted pig waste was higher than those that were grown in an unfertilized soil but same as those planted in a soil fertilized with inorganic fertilizers. According to Arancon and Edwards (2005), vermicompost have many beneficial biological properties, namely high populations of actinomycetes, fungi and cellulose-degrading bacteria. Among its superior chemical qualities, vermicomposts made from animal waste sources contained more mineral elements such as nitrates, exchangeable phosphorus, potassium, calcium, and magnesium. Many of these elements were changed to forms that could readily be taken up by plants. Bera *et.al.* (2013) reported that the physical properties of the soil improved with increasing proportion of vermicompost addition. Bulk density of soil declined steadily with increasing addition of vermicompost, while water holding capacity of the mixture steadily increased as well from 39.6 to 72.4%. It was then concluded that addition of vermicompost increased the porosity of the soil and hence decreased the bulk density. Total fungal and bacterial count per gram of sample also showed an increase.

Aside from influencing crop growth and yield, vermicompost application to soils for fertilization purposes has also been reported to affect the chlorophyll content of plant leaves. Increased photosynthesic pigment and leaf gas exchange in red chilli (*Caspsicum annum L.*) due to the application of vermicompost was observed by Berova and Karanatsidis (2009). Moreover, Golchini *et al*. (2006) reported findings that leaf area index (LAI) and chlorophyll content of the leaves of pastichio *(Pistacia vera L.)* seedlings were better in vermicompost treatments when compared to the treatments without vermicomposting.

2.7. Conclusion

Domestic sewage sludge is a byproduct with a high nutritive value therefore reclycing and re-using it becomes imperative for the sake of the environment. Vermicomposting is the most sensible, environmentally friendly and cost effective method for recycling and re-using this type of waste. Since vermicomposting enhances nutrient release and availability along with microbial properties in organic waste, the use of vermicompost produced from domestic sewage sludge could support crop growth and influence yields. Based on the literature reviewed in this chapter, it important to investigate how vermicomposting will influence chemical properties of the soil and those of planted crops along with crop yields.

CHAPTER 3: VERMICOMPOSTING OF DIFFERENT TYPES OF SEWAGE SLUDGE AND THE EFFECT OF BIOCHAR ADDITION ON COMPOST QUALITY AND POPULATION OF SELECTED PATHOGENS

3.1 Introduction

The large quantities of domestic sewage sludge require proper management to minimise environmental pollution, and vermicomposting could be useful to convert the sludge in to a useful product; compost. Vermicomposting is a low cost system for the biological treatment or processing of organic wastes into useful soil amendments (Domínguez *et al*. 2000) through the use of earthworms (Chaoui, 2010; Gupta & Garg. 2008). The vermicomposting process involves both physico-chemical and biochemical conversion of organic substrate by the microbial biomass in the gut of the earthworms which is then excreted as casts **(**(Gupta & Garg. 2008), Suthar *et al*. 2008**)**. The lower temperatures compared to thermophilic composting suggest that nutrients are retained, pathogen could be reduced while the inoculated earthworms maintain aerobic conditions in the organic waste and convert organic material into worm biomass and respiration products (Mupondi *et al*. 2010). The presence of contaminants such as organic compounds, heavy metals and human pathogens in sewage sludge could cause changes in microbial activity and alter the composting potential of earthworms. The turning over of waste results in a stabilized and homogenized product with less volatile solids (Gupta & Garg. 2008).

Vermicomposting is a mesophilic process therefore complete sanitization of the waste material cannot be expected because there is no thermal stabilization to remove pathogens (Aria *et al*. 2011). Chaoui. (2010) reported that earthworms consume fungi and the vermicast allows for the disease-suppressing organisms to survive, and feed on or destroy the pathogens. The content of volatiles, heavy metals and pathogens in the sewage sludge vermicast could depend on the method of dewatering used.

Centrifuged sludge is likely to contain less of pathogens, nutrients and heavy metals than drying bed sludge, as they could be leached during draining the water out of the sludge as compared to sludge that is poured on drying beds to remove water. These dewatering methods could have an effect on the quality of the resultant vermicompost. In addition, earthworms function better under moist conditions and as such water management during vermicomposting is essential. The required moisture levels of 60-80%, helps in the interchange of gases and prevents the worm from dehydration, and if the moisture levels in the tissue drops, the worm losses its weight (Rodriguez-Canché *et al*. 2010). As a result some leachate could be released during periodic watering, which could result in the leaching of nutrients. Retention of nutrients could however, be enhanced by addition of biochar.

Biochar is known to have high surface area, water retention capacity and CEC (Laydygina and Rineau. 2013) and as such could be useful in retaining nutrients during the vermicomposting of sewage sludge. According to Ok *et al*. (2016), application of bocarto the soil can significantly increase CEC, water holding capacity and the bioavailability of nutrients like P, N, Ca, Zn due to its porous nature. The N use efficiency of plants is also enhanced by biochar application to croplands and thereby decreasing the N demand and the emission of greenhouse gases (Zhang *et al*. 2012). Laird *et al*. (2010) reported an increase in the adsorption capacity of plants for soil nutrients and reduced leaching losses of nutrients. The incorporation of biochar into agriculutural soils can increase leaching of P and K in short term (Ok *et al*. 2016). Leaching losses of 30-70% of dissolved P occurred as PO_4^{-3}-P as a result of applying biochar produced from chicken manure at 800ºC. However, the impact of biochar application to dissolved P depends on the type of feed stock used and soil properties. Biochar derived from wood significantly decreased leaching of P and increased leaching of K at 0,6m depth of a low-fertility acidic soil while leaching of the nutrient s was significantly decreased at depth of 1.2m on the same soil (Major *et al*. 2012). The addition of biochar increased the CEC of a Mollisol by 20% (Laird *et al*. 2012), while similar findings were reported by Chinatala *et al*. (2014b) who found that soil CEC significantly increased as a result of adding corn stover-derived biochar in relation to the switchgrass biomass derived biochar. The authors also reported an increase in CEC with increasing application rate of biochar. Vermicomposted sewage sludge has more available nutrients per kg weight as compared to the initial substrate, thus it has high nutrient value and its use as fertilizer is a common practice. Nitrogen and phosphorus mineralization as a result of earthworm activity qualifies the end product to be used for plant growth since these nutrients become bio-available for plants (Sinha *et al*. 2009). The objective of this study was to determine the effect of water removal method from sewage sludge and addition of biochar on nutrient composition and population of pathogenic organisms in vermicomposts.

3.2 Materials and Methods

3.2.1 Sludge and worms used in the study

Domestic sewage sludge used in this study was collected from Howick Waste Water Works (HWWW), 15 km north-west of Pietermaritzburg in South Africa. The HWWW receives waste water from six pump stations, which transfer waste water from around Mpopomeni and Howick, has a design capacity of 6.8 ML/day and currently treats 6.4 ML of sludge per day. Two different types of sludges (drying bed sludge and dewatered sludge) were used in this composting experiment. Drying bed sludge was collected from the beds where the sludge was pumped in a liquid form and allowed to dry into a cake form before being scrapped off and disposed of to land-fill sites. The dewatered sludge was produced by dewatering centrifuges to remove excess water from the sludge. The two types of sludge were sampled and analyzed for initial chemical composition and pathogenic organisms. The chemical parameters of the domestic sewage sludge are presented in Table 3.1.

The epigeic *Eisenia fetida* earthworm species, purchased from a commercial producer (Wizzard Worm cc.), was used in this study. The worms used in the study were both mature and juvenile (including eggs) and were grown and condition on horse manure. The vermicomposting studies were carried out at Howick WWW.

3.2.2 Effects of biochar inclusion on compost quality from two sludge types

This composting study was conducted as a 2×2 factorial experiment laid out in randomized complete block design (RCBD) and replicated 3 times. Two sludge types (drying bed and dewatered sludge) and two biochar levels (with and without biochar) were used. The treatment combinations with drying bed sludges, were (i) drying bed sludge without biochar (Db), (ii) drying bed sludge with biochar (Db+char), (iii) dewatered sludge without biochar (Dw), (ii) dewatered sludge with biochar (Dw+char). Biochar was added at an application rate equivalent to 50t/ha (based on exposed surface area). The experiment was conducted using bins with an exposed surface area of 0.102 m^2. Mature and juvenile *Eisenia fetida* earthworms were introduced at the recommended stocking density of 1.6 kg-worms/m^2 which was calculated as 1.632 kg-worms/ m^2

and added to the bins and sludge was added based on the feed requirements 0.75 kg-feed/kg-worms/day (Ndegwa *et al*. 2000) for the entire vermicomposting period of 9 weeks. The moisture level was maintained at 80% throughout the vermicomposting period using tap water. During the experiment the cover of one of the bins with Dw +char was blown away by wind and some of the earthworms escaped, which should account for any differences with the replicates.

Approximately 500g of vermicompost was sampled from each bin after every three weeks (i.e., 0, 3, 6, 9 weeks). A subsample was dried at 70^{o}C to constant weight before analysis of pH, EC, total C and N, extractable P, exchangeable bases (Na, Mg, K and Ca), NH_4-N and NO_3-N. The other portion, for analysis of *E.coli* and *Salmonella* species, was stored at 4˚C.

3.2.3 Laboratory analysis

Vermicompost pH was determined at 1: 5 (vermicompost: distilled water) ratio and the suspension was stirred for 2 minutes and left to stand for 1 hour before measurement of pH using the pH 210 standard pH meter. Electrical conductivity (EC) was also determined on 1:5 (vermicompost: distilled water) using a CDM 210 conductivity meter. Exchangeable bases (Na, Mg, K, and Ca) were extracted with 1M NH_4Cl (pH 7) as outlined by Rayment and Lyson (2011). Vermicompost samples (5.0 g) were placed in centrifuge tubes and 50 ml of 1M NH_4Cl solution added, and shaken on a reciprocating horizontal shaker at 180 oscillations per minute for 30 minutes. The suspension was filtered through Whatman no 5 filter paper. The extracts were analyzed using the atomic absorption spectrophotometer (Varian AA 280 FS).

For the determination of total C and N, samples were ground and passed through a 0,5mm sieve and analyzed with the Leco- TruMac CNS Autoanalyser (Leco-cooperation, 2012). This procedure entails the dry combustion of air dried 0.2 g compost samples in crucibles and subjected to 1450^{o}C furnace temperature for about 6 minutes per sample. Ammonium-N and Nitrate-N were extracted from the vermicompost samples using 2M KCl (Kalra and Maynard, 1991). The vermicompost (5g) was placed into a 100 ml centrifuge tube and 50 ml of 2M KCl was added. The suspension was shaken for 30 minutes and filtered through Whatman no 5 filter paper and analyzed on the Gallery Discrete Autoanalyser (Scientific Thermo Fisher, 2014).

Available phosphorus was extracted with ammonium bicarbonate (AMBIC 2) solution (The Non-Affiliated Soil Analysis Work Committee, 1990). The solution contained 0.25 M ammonium bicarbonate, 0.01M EDTA disodium salt and 0.01 M ammonium fluoride. The suspension (2.5g compost:25ml of solution) was shaken on a reciprocating horizontal shaker at 400 revolutions per minute (rpm) for 10 minutes, and then filtered through a Whatman no 5 filter paper. The filtrate was analysed for P using the molybdenum blue method (Murphy and Riley, 1962). Pathogenic organisms were tested after 0, 3 and 9 weeks during the vermicomposting period using the MPN technique at the Allerton Laboratories near Pietermaritzburg.

3.2.4 Statistical Analysis

The results of the experiment were subjected to analysis of variance (ANOVA) using the Genstat 14th edition (Lawes Agricultural 425 Trust, 2012) and the comparison of means was done using the fishers test. Mean separation was performed using the LSD at $p<0.05$ level of significance.

3.3 Results

3.3.1 Sludge characteristics

Dewatered sludge had a significantly higher pH than drying bed sludge ($p<0.001$). Drying bed sludge had higher electric conductivity (EC) and exchangeable Ca, total C % and Mg, than dewatered sludge (Table 3.1). Total N, extractable P and K were higher in dewatered sludge than drying bed sludge (Table 3.1). There was no significant difference in mineral N between the two sludges ($p= 0.384$).

Table 3.1: Characteristics of domestic sewage sludge used in vermicomposting

Parameters	**Drying bed sludge**	**Dewatered sludge**
pH	6.3	6.7
EC (mS/cm)	8.29	4.9
Ca ($cmol_c$/kg)	49.33	36.4
*E.coli (*x 10^4 *cfu/g)*	5.93	3.57
Mg ($cmol_c$/kg)	42.1	34.0
K ($cmol_c$/kg)	51.3	72.9
Total C (%)	17.05	13.51
Total N (%)	2.89	3.22
Mineral -N (mg/kg)	141. 5	142.1
Extractable P (mg/kg)	1508	1597

3.3.2. Effects of biochar inclusion on selected physico-chemical properties of the composts

Interaction effects of the treatments and vermicomposting time were not significant for most of the parameters except total- and Ammonium-N, extractable P and K. Most of the parameters in both sludge types were significantly affected by the inclusion of biochar (Table 3.2). There were no significant differences among the vermicomposts in terms of pH and EC (Table 3.2). Biochar had no significant effect on Ca content of Dw vermicomposts but the Db +char had higher Ca than the Db vermicompost. Addition of biochar did not result insignificant differences in Mg regardless of sludge type. However, the Dw vermicompost contained higher Mg than Db vermicompost (Table 3.2). The Db and Db+char vermicomposts had less total C than the Dw and Dw+char vermicomposts. The presence of biochar significantly increased total C and decreased *E. coli* in the Db vermicompost while there was no significant effect in the Dw vermicompost. Nitrate-N was not significantly affected by sludge type with or without biochar.

Table 3.2: Characteristics of vermicomposted sewage sludge with or without biochar after 9 weeks of vermicomposting.

Chemical parameters	Treatments				
	Db	Dw	Db+char	Dw+char	LSD
pH (H_2O)	6.57a	6.57a	6.57a	6.46a	0.102
EC (mS/cm)	4.86a	4.69a	4.86a	4.43a	0.428
Ca ($cmol_c$/kg)	42.2a	78.75b	59.59ab	74.98b	22.517
Mg ($cmol_c$/kg)	35.96a	40.76b	38.75ab	36.65ab	3.043
Total C (%)	2.94a	3.53ab	3.07a	3.83b	0.513
NO_3 (mg/kg)	6.75a	4.57a	5.56a	5.89a	2.191
E.coli (x 10^4 cfu/g)	5.89b	3.67ab	1.71a	2.85ab	2.645

Db = drying bed sludge vermicompost, Dw= dewatered sludge vermicompost, DB+char= drying bed sludge vermicompost with biochar and DW+char= dewatered sludge vermicompost with biochar. Values with same letter subscript are not significantly different at ($p< 0.05$).

3.3.3 Total and ammonium nitrogen

All vermicomposts had similar total N at all sampling times, except after three weeks of vermicomposting (Figure 3.1). Sludge vermicomposted without biochar had higher N content after three weeks of vermicomposting than those with biochar (Figure 3.1). At week 6 all the vermicomposts with or without biochar had the similar N contents.

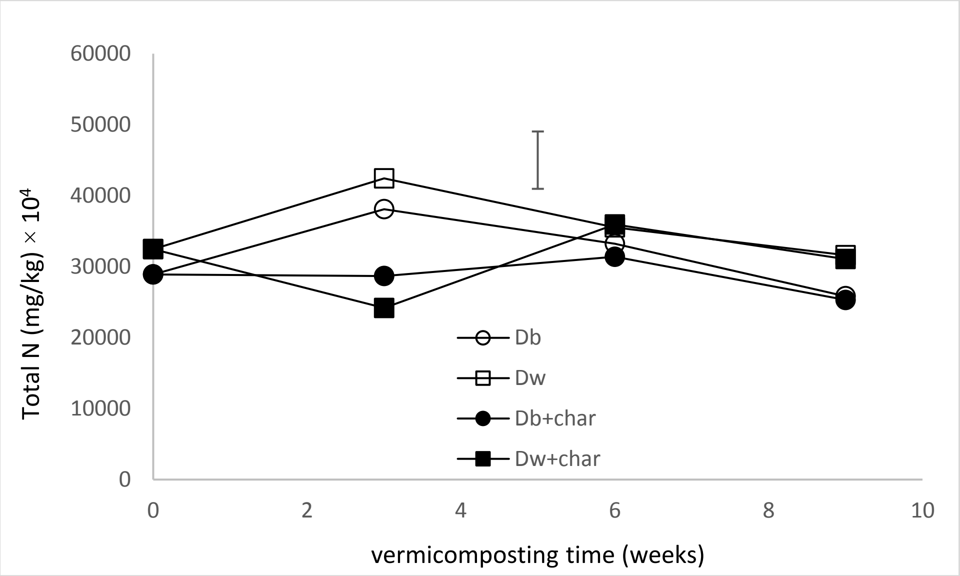

Figure 3.1. Changes in total nitrogen during vermicomposting of the sludges with or without biochar. Db = drying bed sludge vermicompost, Dw= dewatered sludge vermicompost, DB+char= drying bed sludge vermicompost with biochar and DW+char= dewatered sludge vermicompost with biochar. The error bar indicates least significant difference at $p<0.05$.

Ammonium-N was significantly increased with vermicomposting time up to week 6, and levelled off for all vermicomposts, except the Db vermicompost, which did not increase between week 3 and week 6, and then increased upto week 9 (Figure 3.2). After 9 weeks (end of the experiment) all vermicomposts had similar ammonium-N levels. The Db+char vermicompost had higher ammonium-N than Dw+char after three weeks of vermicomposting, while Db had lower levels than the rest after six weeks.

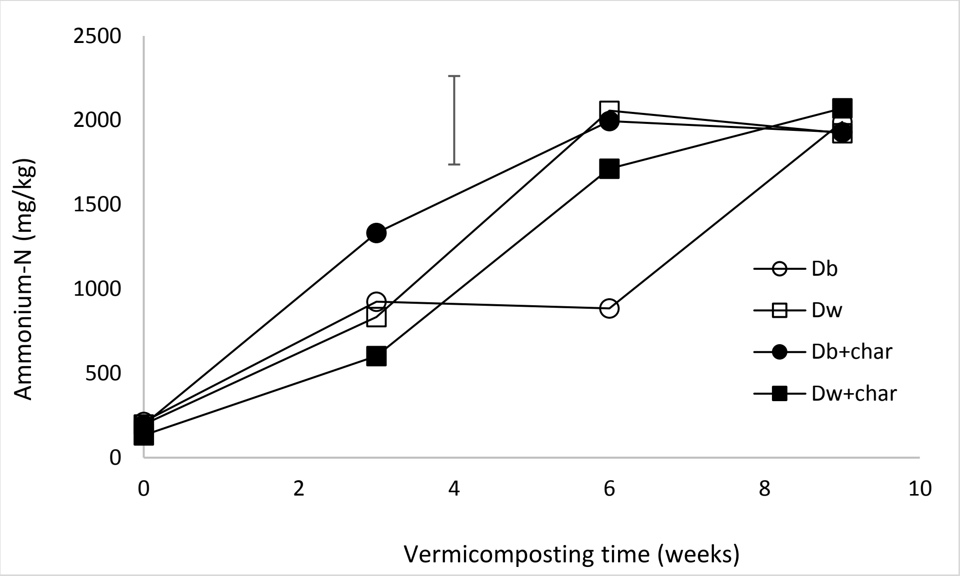

Figure 3.2. Changes in ammonium-N during vermicomposting of sewage sludge with or without biochar. Db = drying bed sludge vermicompost, Dw= dewatered sludge vermicompost, DB+char= drying bed sludge vermicompost with biochar and DW+char= dewatered sludge vermicompost with biochar. Bar represents least significant difference at $p < 0.05$.

3.3. 4 Available phosphorus and exchangeable K

There was a significant interactive effect on extractable P content between vermicompost type and incubation period (Figure 3.3; p=0.005). Extractable P increased within the first three weeks of vermicomposting after-which it declined. The P content in Dw vermicomposts was affected by the inclusion of biochar especially after 3 and 6 weeks of vermicomposting, where the Dw had higher extractable P than the Dw+char. However, all vermicomposts had the same extractable P at week 9 (Figure 3.3).

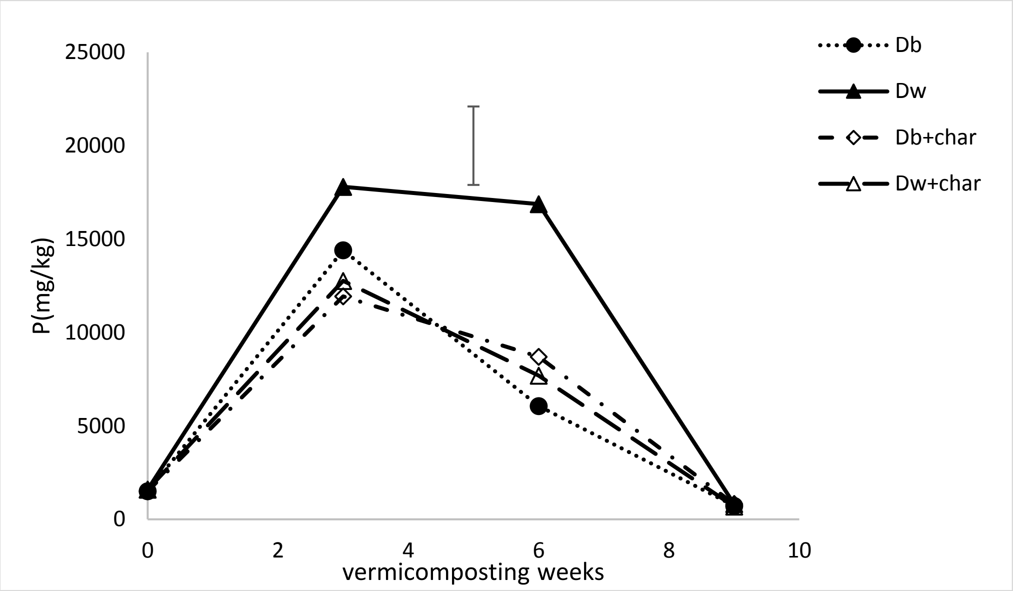

Figure 3.3. Changes in available P during vermicomposting of the sludges with or without biochar. Db = drying bed sludge vermicompost, Dw= dewatered sludge vermicompost, DB+char= drying bed sludge vermicompost with biochar and DW+char= dewatered sludge vermicompost with biochar. The error bar indicates least significant difference at $p<0.05$.

Extractable K was significantly affected by vermicomposting time and type of sludge. Initial K was higher in dewatered than drying bed sludge vermicomposts irrespective of the presence or absence of biochar (Figure 3.4). The K levels declined with time and at 3 and weeks there were no significant differences in K among all vermicompost irrespective of sludge type with or without biochar. The extractable K in all vermicomposts increased between 6 and 9 weeks. Only the Dw vermicompost (without biochar) had significantly higher K content than the other 3 treatments at the end of vermicomposting (Figure 3.4).

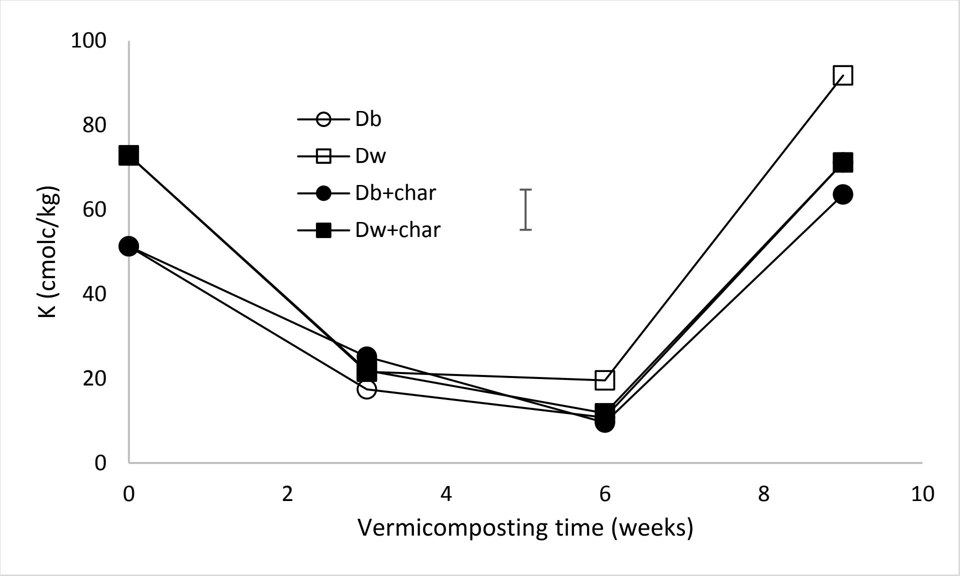

Figure 3.4. Changes in exchangeable potassium during vermicomposting of the sludges with or without biochar. Db = drying bed sludge vermicompost, Dw= dewatered sludge vermicompost, DB+char= drying bed sludge vermicompost with biochar and DW+char= dewatered sludge vermicompost with biochar. The error bar indicates least significant difference at p<0.05.

3.3.5 **Salmonella**

At the beginning of the experiment, group B Salmonella was only detected in Dw and Dw+char vermicomposts (Table 3.3). After 3 and 9 weeks of vermicomposting no Salmonella was detected in all the treatments.

Table 3.3: Existence of Salmonella during the 9 weeks of vermicomposting

Treatments	**week0**	**week3**	**week9**
Db	ND	ND	ND
Dw	Group B	ND	ND
Db+char	ND	ND	ND
Dw+char	Group B	ND	ND

ND=not detected. Db = drying bed sludge vermicompost, Dw= dewatered sludge vermicompost, DB+char= drying bed sludge vermicompost with biochar and DW+char= dewatered sludge vermicompost with biochar.

3.4. Discussion

3.4.1. Sludge characteristics

The higher EC of the drying bed sludge (Db) than the dewatered sludge could be explained by the higher Ca and Mg in solution. The higher Ca, Mg and total C could be explained by the method of water removal. When water evaporates from drying beds all the dissolved elements and soluble organic matter are left in the sludge (McLaughlin. 1983), while in dewatered sludge these components are removed with the supernatant solution, leaving the sludge with lower concentrations. In addition to the method of water removal, the lower concentration of total C in dewatered sludge could be a dilution effect caused by addition of flocculating agents before centrifugation. According to Zagyvai *et al*. (1987), a typical sludge treatment operation, 1.5L of

40% aqeous formaldehyde solution is added along with 100g of polyacrylamide- base flocculating agent per 1m^3 of sludge (aerobic or anaerobic).

The higher total N, extractable P and K and pH in the dewatered sludge could be explained by the composition of the flocculating agent. According to Wong *et al*. (2016), a flocculating agent comprises of microbial aggregates and their extracellular polymetric substances (EPS), which constitutes protein, polysaccharides, lipids and humic like substances which entrap water and cause high viscosity. Digested sewage sludge generally contains 97-99% water content and remaining as organic and inorganic matter in the form of suspended and dissolved solids (Wong *et al*., 2016). Before the addition of fermaldehyde and flocculating agents the pH of the sludge has to be raised to pH of 10-12 using calcium milk or lime (Zagyvai *et al*. (1987), this contributes to the difference in pH, P and other nutrients that are higher in dewatered sludge compared to the drying bed sludge. Drying bed sludge had higher *E.coli* population (5.93 x10^4) compared to the dewatered sludge (3.57 x10^4) this difference maybe a result of dewatering of the sludge as dewatered sludge is done mechanically for the DWS and not for the DBS.

3.4.2. Vermicomposting and biochar inclusion on compost quality

Vermicomposting of domestic sewage sludge results in changes in the chemical characteristics of the initial sludge due to biochemical processes taking place. Nutrients are released and some are lost due to leaching, volatilization and or are taken up as earthworm biomass. The initial (week 0) pH of the two sludges was in the range of 6.3 (dbs) - 6.7 (dws) which did not alter much as the pH remained in this range at the end of the vermicomposting. Any shift in pH is related to the mineralization of N and P into nitrites/nitrates and orthophosphate and production of organic acids from organic materials (Suthar (2009); Lopez-Valdez *et al*. (2010) and Ndegwa and Das. (2000)). Rodriguez *et al*. (2012), reported that initial pH of the sludge was in the alkaline range and shifted towards the more neutral range under experimental conditions. Epigeic earthworms are more tolarent to the acidic range and have high preference for the acidic material (Rodigez-canche *et al*. 2012) and can adjust the pH of the medium by secreting Ca (Sinha *et al*. 2009). Garg *et al*. (2006) explained the decrease in pH to be integral to the retention of N as this nutrient is lost as gaseous ammonia at high pH. Hait and Tare (2012) reported a decrease in pH under all experimental conditions with maximum decrease recorded under temperature of 20ºC and 90% relative humidity. Furthermore, the pH was in the range of 7.9-8.5 for primary sewage sludge and waste activated sludge had a pH range of 7.8-8.3 while the pH of the control was always higher under all experimental conditions. It also important to mention that pH has a decisive influence on the availability of nutrient for plant uptake because at low pH some micro nutrients become unavailable.

The results of EC could be explained by the EC values of the original sewage sludges, which were higher for the Db sludge than Dw sludge. EC during vermicomposting is dependent on the solutes being released in solution during ingestion and excretion by earthworms (Garg *et al*., 2006). In this case, the lower EC in the Dw+char than the Dw vermicompost could be explained by the sorption of the solutes on the surfaces of biochar, leaving lower concentrations in solution The threshold value for EC is 400mS/m which is the moderate but significantly higher for earthworm mortality. There is reduced earthworm population growth associated with higher pH and higher amounts of dissolved salts (Ludibeth *et al*. 2012). Hait and Tare. (2011) reported a significant increase in EC of sludge mixed with different ratios of composted material at different environmental conditions (temperature and humidity). Based on their study, the final EC ranged from 6.65- 8.52dSm^{-1}and this result was attributed to the loss of organic matter and release of mineral salts such as ammonium, phosphates and potassium.

Although there was little difference among the vermicomposts throughout the period, the higher total N in the Dw sludge vermicompost than those with biochar, after three weeks, could be explained by more rapid loss of C. Ludibeth *et al*. (2012) studied the vermicomposting of sewage sludge mixed with cow dung at different ratios and found that sewage sludge alone contained largest amount of total N (3.1%) followed by treatment C (90% sludge and 10% cow dung) which resulted in a vermicompost with high N content. Whereas, Gupta and Garg (2008) reported an increase in total N of vermicompost when sewage sludge was mixed to equal proportions (50% Sludge and 50% Cow dung) with cow dung . The higher ammonium-N in the Db+char than the Dw+char suggested that mineralization of N in drying bed than dewatered sludge was higher in the first three-six weeks. The similarity of ammonium-N for all treatments at the end of vermicomposting suggested that most of the N had been converted to ammonium-N than nitrate-N. This view was supported by the low nitrate-N and pH results, which were not different among the treatments. According to Hait and Tare (2012), higher nitate-N concentration than exchangeable ammonium-N in vermicompost implies that ammonification and nitrification were not inhibited. From the study of Hait and Tare (2012), the increase in nitrate- N was higher than the increase in ammonium- N which they attributed to the presence of heterotrophic bacteria, fungi and actinomycetes which are able to oxidize amino-N to nitrate- N. Vermicompost containing 70, 80 and 90% sewage sludge had more available N (689.66, 1081.69 and 2189.80) compared to sewage sludge vermicompost (162.7) alone (Ludibeth *et al*. 2012). The lack of nitrification could have been a result of moist conditions that were maintained, which could have limited oxygen. Nitrification would have resulted in higher nitrate-N and lower pH (Benitez *et al*. 1999). However, Benitez *et al*. (1999), reported a decrease in ammonium –N during the first 2 weeks of while nitrate-N increased significantly after 4 weeks of vermicomposting equal portions (50%) of anaerobically digested industrial and aerobically digested municipal sewage sludges

The increase in extractable P for all treatments in the first three weeks could be explained by the degradation of the organic materials during composting but the decline thereafter could be a result of uptake by earthworms into their own bodies as the biomass increased. Some of the P could have been leached as a result of moisture adjustment to provide a comfortable environment for the worms. Unfortunately, the leachates collected could not be analyzed as it got contaminated by extraneous material due to windy conditions. Ludibeth *et al*. (2012) found no significant increase in available P after vermicomposting of sewage sludge nor significant difference between treatments which were mixtures of cow dung and sewage sludge. Similar results were reported by Benitez *et al.* (1999) where soluble PO_4^{-3} was undetectable after 6 weeks of vermicomposting sewage sludges. These results were related to the increase in earthworm total biomass. Gupta and Garg. (2008) however, reported a two-fold increase in total P of the final vermicompost compare to the initial feed mixtures. The original DW sludge had higher P than Db sludge, which explains the difference between during the vermicomposting of these sludges. The higher extractable P in DW vermicompost than DW+char could be explained by sorption of P on biochar. Column leachate of a Norfolt loamy soil amended with pecan shell biochar made at 70º C contained less amount of P (by about 35%) compared to the control soil with no biochar (Schneider and Haderlien. 2016). This sorption may reduce the availability of P for plant uptake where biochar is added to soils.

Ca was higher in DW vermicompost with and without biochar compared to the DB vermicomposts. This difference could be attributed to addition of calcium milk/lime to increase ph of sludge before the addition of a flocculating in the dewatered sludge. So during vermicomposting worms could have enhanced the release of organically bound Ca. While the lower content in DB vermicompost could be attributed to the secretion of Ca by earthworms (Sinha *et al*., 2009). The increase of Mg in DW vermicompost also suggests it was as a result of the addition of the of calcium milk/lime even though it did not affect the vermicompost with biochar. DW vermicompost also increased in K content and was low in all the other treatment. Low K in vermicompost may be attributed to the loss during draining of excess water and leachate has been found to be high in K content (Hait and Tare. 2011). The higher initial extractable K in the DW and DW+charvermicomposts could be explained by the higher K in the original sludge than Db sludge. The lower extractable K in the DWB than DW vermicompost at the end of vermicomposting could be explained by sorption of K by biochar, removing it from solution. In a column leachate study by Schneider *et al*. (2016), loamy soil amended with pecan shell biochar contained higher amounts K, Na but less Ca. This sorption may reduce the availability of K for plant uptake where biochar is added to soils. Biochar made from baggase has the ability to absorb significant amounts of NO_3 and possibly decrease the amount of available nutrients in soil (Mukherjee and Zimmerman. 2013). Hait and Tare. (2011) also reported that vermicomposting of waste activated sludge and primary sewage sludge resulted in significantly increased content of total macro nutrients (Na, Ca, Mg and K) as well as their water soluble contents when compared to the initial material. These findings were also collaborated by Gupta and Garg. (2008), where they found total macro nutrients to increase during vermicomposting compare to compost material.

The reason for undetectable levels of Salmonella in vermicomposts from Db sludge at the beginning of vermicomposting while there were some in Dw composts, is not clear. The elimination of *Salmonella* in three weeks could be explained by the effects of consumption of the sludge by the worms and the interaction with worm gut microbes. This suggestion was also proposed by Edwards *et al*. (1984), stating that pathogen contained in waste are reduced during the ingestion of wastes in the gut of the earthworms. In addition, Brown and Mitchell (1981), reported that *Eisena fetida* reduced *Salmonella* by 42 times compared to the control after 28 days with the greatest reduction occurring during the first 4 days of feeding these earthworms with growing medium inoculated with *Salmonella enteritidas*. In a study by Rodriguez-canche *et al*. (2010) fecal coliforms, helminth ova and Salmonella in vermicomposted sewage sludge were reduced to low levels and the vermicompost was classified as Class A biosolid according to the Mexican official standard NOM-004 for class A biosolids. Vermicomposting can reduce human pathogens through the action of intestinal enzymes and with the secretion of certain fluids which contain antibacterial features earthworms have the ability to eliminate pathogens (Rodriguez-canche *et al*., 2010). This finding was in agreement with Mupondi *et al*. (2010). Vermicomposting alone did not significantly reduce *E. coli* this was evident as he population did not decrease as a result of vermicompost. However, *E.coli* results were higher in the Db than Db+char vermicomposts, which suggested that the presence of biochar limited *E. coli* growth, possibly as a result of limited nutrients, like P and K. Biochar produced from wood chips at 350ºC removed more *E.coli* from storm water biochar effluent in relation to the commercial biochar (Mohanty *et al*., 2014).

3.5. Conclusion

The dewatering of sewage sludge has a major influence in the nutrient content of the sludge and resultant vermicomposts. Drying bed sludge had higher Ca, Mg and therefor higher EC while the dewatered sludge contained higher amounts of N, P, K and higher Ph. Chemical composition of dewatered sludge makes it the most nutritious sludge. Vermicomposting of sludge did not have an effect on the PH of both sludges but decreased electrical conductivity for dewatered sludge vermicomposts containing biochar. Total nitrogen, exchangeable calcium, exchangeable magnesium and extractable potassium were high in dewatered sludge vermicompost while drying bed sludge contained higher NH_4-N in the mineral form. The inclusion of biochar in vermicompost decreased extractable P, N, K, Nitrate-Nitrogen, ammonium nitrogen, electrical conductivity and ph especially in dewatered sludge vermicomposts. Vermicomposting completely eliminated salmonella by the end of the study period while presence of biochar decreased *E.coli* population in both sludge types. Further studies need to test the following (i) nutrient release of vermicomposted sewage sludge with or without biochar at different application rates (ii) the fertilizer value of the vermicompost when used to grow vegetable crops (Spinach).

CHAPTER 4: INCUBATION OF VERMICOMPOST PRODUCED FROM DOMESTIC SEWAGE SLUDGE FOR THE DETERMINATION OF NUTREINT RELEASE

4.1 Introduction

Soil fertility and productivity are the major components in sustaining agricultural production. The loss of organic matter and nutrients due to run-off and soil erosion have a huge impact on soil fertility and therefore stimulate the need to improve the quality and overall productivity of the soil. Organic wastes such as crop residues, animal manures and sewage sludge are some of the materials commonly used for improving soil fertility and productivity due to their high organic matter and nutrient contents (Prabha *et al*., 2007). Some of these organic wastes (e.g. crop residues) take longer to degrade or decompose in soil; hence are not reliable for immediate amelioration of soils. Others (e.g. sewage sludge) can have negative impacts due to the presence of pathogens, heavy metals and production of toxins (Masciandaro *et al*., 2000). While domestic sewage sludge contains low levels of heavy metals, its composition of pathogenic organisms could be high (Hait and Tare. 2012). Vermicomposting of these materials reduces some of these problems (toxins and pathogens) and speeds up the process of decomposition. Vermicomposting induces biochemical activities and the earthworm casts are used as soil amendments due to their high nutritive value (Chaoui *et al*. 2003).

When vermicomposted, the nutrient availability of sewage sludge is increased resulting in a high value as a fertilizer. Its application as a soil amendment is a common practice due the nutritional benefits. Nitrogen and phosphorus mineralization as a result of earthworm activity qualifies the end product to be used for plant growth since these nutrients become bio-available for plants (Sinha *et al*. 2009). The vermicompost is homogenized and contains beneficial soil microorganisms such as nitrogen fixing bacteria and mycorrhiza fungi which all aid in crop growth when applied to soils (Padmavathiamma *et al*. 2008). Results obtained from the previous vermicomposting experiment (Chapter 3) conducted with sewage sludge show a low nutrient content. As Ammonium-N was high while Nitrate-N was low in the resultant vermicompost produced from sewage sludge, possibly due to slow nitrification process. Therefor, N and P release patterns of the vermicompost in soil need to be understood. This study was carried out with the objective of determining the nitrogen and phosphorus release of vermicomposts applied to soil at increasing rates.

4.2. Materials and Methods

4.2.1 Soil sampling and Preparation

The soil used in the experiment was a Clovelly soil form (Soil Classification Working Group, 1991) collected from the Ferncliff Nature reserve in Pietermaritzburg: Sites (29.55574E, 30.32917S) and 29.58207E, 30.346154S). The samples were collected from both A (0-30cm) and B horizons (30-50cm) depth. The soil was manually ground, mixed and air dried for 7 days before sieving to (< 2mm). The soil had a pH (H_2O) of 5.2, EC of 15.85 mS/cm, 5.1 mg available P/kg and 167.15mg/kg of mineral N.

Vermicomposts used in this study were produced as detailed in Chapter 3 from drying bed and dewatered sludges with or without biochar (Drying sludge vermicompost, dewatered sludge vermicompost, drying bed sludge with biochar vermicompost and dewatered sludge with biochar vermicompost) and their characteristics (Table 4.1) are discussed in Chapter 3.

Table 4.1 Characteristics of the vermicomposts used in the study

Treatments	Chemical parameters					
	pH (H_2O)	**EC (mS/cm)**	**N %**	**P (mg/kg)**	**K (cmol+/kg)**	**NH_4-N (mg/kg)**
Db	6.57	4.85	3.15	5676	71.17	1002.8
Dw	6.56	4.69	2.77	9271	91.8	1252.8
Db+char	6.57	4.86	2.86	5739	63.6	1362.8
Dw+char	6.46	4.43	3.01	5689	80.58	1130

Db = drying bed sludge vermicompost, Dw= dewatered sludge vermicompost, DB+char= drying bed sludge vermicompost with biochar and DW+char= dewatered sludge vermicompost with biochar.

4.2.2 Nitrogen and phosphorus release from the vermicomposts

An incubation experiment was conducted to evaluate the fertilizer value and nutrient release pattern of drying bed sewage sludge vermicomposts. The design of the experiment was arranged in a completely randomized design and treatments were replicated 3 times. The vermicompost treatments were drying bed sludge (Db), drying bed sludge with biochar (Db+char) at increasing rates. The soil (200g) was weighed into 500ml clear plastic containers and amended with vermicomposts, with (Db+char) and without (Db) biochar at application rates equivalent to 0 (control), 10, 50 and 100 t/ha. Holes were poked on the side of the containers to minimize anaerobic conditions during the incubation period. Water was added up to 100% field capacity of the soil and moisture correction was after every 4 days, based on weight loss. The soil was incubated for a period of 8 weeks (56 days) in a constant temperature room (25˚C). Samples were collected after 0 (day 1 of the study), 2, 4, and 8 weeks of incubation using the destructive sampling method. The samples were analyzed for pH, EC, NO_3-N and NH_4-N, available P.

The same experiment was repeated with vermicomposts from dewatered sludge. The setup, management, sampling and analyses were the same as for the drying bed sludge vermicomposts. The vermicompost treatments were dewatered sludge (Dw), dewatered sludge with biochar (Dw+char).

4.2.3. Chemical analysis

The pH and EC of the soil were determined on 1: 2.5 soil: distilled water suspension, which was stirred for 2 minutes and left to stand for 1 hour before being measured using the pH 210 standard pH meter with a standard glass electrode and a CDM 210 conductivity meter, respectively. Determination of mineral nitrogen (ammonium-N and nitrate-N) from the soil samples was done by using 2M KCl (Kalra and Maynard, 1991). Air dry soil (5 g) was placed into 100 ml centrifuge tubes and 50 ml of 2M KCl was added. The suspension was shaken for 30 minutes and filtered through Whatman no 5 filter paper and analyzed on the Gallery Discrete Autoanalyser (Scientific Thermo Fisher, 2014). Phosphorus was extracted from 2.5g of soil with 25ml of 0.25 M ammonium bicarbonate, EDTA disodium salt and 0.01M ammonium fluoride (Ambic 2) solution (The Non-Affiliated Soil Analysis Work Committee, 1990). The suspension was shaken on a reciprocating horizontal shaker at 400 revolutions per minute (rpm) for 10 minutes, and then filtered through a Whatman no 5 filter paper. The filtrate was analysed for P using the molybdenum blue method (Murphy and Riley, 1962).

4.2.4. Statistical analysis

Results are reported as the means of determinations made on three replicates using the analysis of variance (ANOVA) using the Genstat 14th edition (Lawes Agricultural 425 Trust, 2012). The means were compared by using least significant difference values calculated at $p < 0.05$ (benferroni test).

4.3. Results

4.3.1. Incubation experiment with drying bed sludge vermicomposts

4.3.1.1 pH and electrical conductivity

The pH of soils treated with db vermicompost, increased with increased application rate for all incubation periods except for 50t/ha at week8 (Table 4.2). The presence of biochar was not significant at application rates higher than 10t/ha as soils containing treatments with biochar had lower pH than those without biochar. At week 4 all the vermicompost increased in pH except for control. pH decreased for all vermicomposts at week 8 except for 10t/ha (+B) and the control (Table 4.2).

Table 4.2. pH of an incubated soil amended with drying bed sludge vermicompost with (+B = char) and without biochar at different application rates.

				pH				
weeks	10t/ha	10 t/ha(+B)	50t/ha	50t/ha(+B)	100t/ha	100t/ha(+B)	control	LSD
0	5.14ab	5.02a	5.33b	5.06a	5.68c	5.21ab	5.15ab	0.118
4	4.80a	5.03ab	5.28bc	5.14bc	5.44c	5.19bc	5.31bc	0.187
8	4.90ab	5.05bc	4.71a	4.85ab	4.81ab	4.79ab	5.2c	0.156

EC increased as the incubation period progressed for all application rates except for Db+char at 10t/ha. Biochar had no significant difference on EC in all the application rates with the exception of 10t/ha at week 8, 50t/ha at week 0 and 100t/ha at week 2 (Table 4.3).

Table 4.3. Electric conductivity of soils treated with vermicompost derived from drying bed sludge at different application rates (0, 10, 50 and 100t/ha).

	Electric conductivity (mS/cm)						
Weeks	Control	10t/ha	10t/ha (+B)	50t/ha	50t/ha (+B)	100t/ha	100t/ha (+B)
0	15.9a	51.9ab	61.0ab	80.4b	98.40bc	160.2c	162.7c
4	39.9a	102.4a	78.7a	227.6b	213.0b	456.3c	396.3c
8	51.2a	131.4a	247.6ab	349.8ab	280.3ab	769.3c	477.3bc

Different letters after means indicate significant difference at $p<0.05$ (Bonferroni LSD test).

Ammonium (NH_4-N)

In soils amended with Db vermicomposts, there was no significant difference in NH_4-N release between the different application rates at week 0, but it was generally higher for most treatments than that obtained at week 4 except for DB100 (Figure 4.1). However, a significant difference was observed at week 4 as soils containing Db without biochar had higher NH_4-N concentration than those with biochar at the same rates. A similar trend to week 4 was also observes at week 8, though NH_4 concentrations were now appreciably lower and treatments with biochar were all similar in concentration.

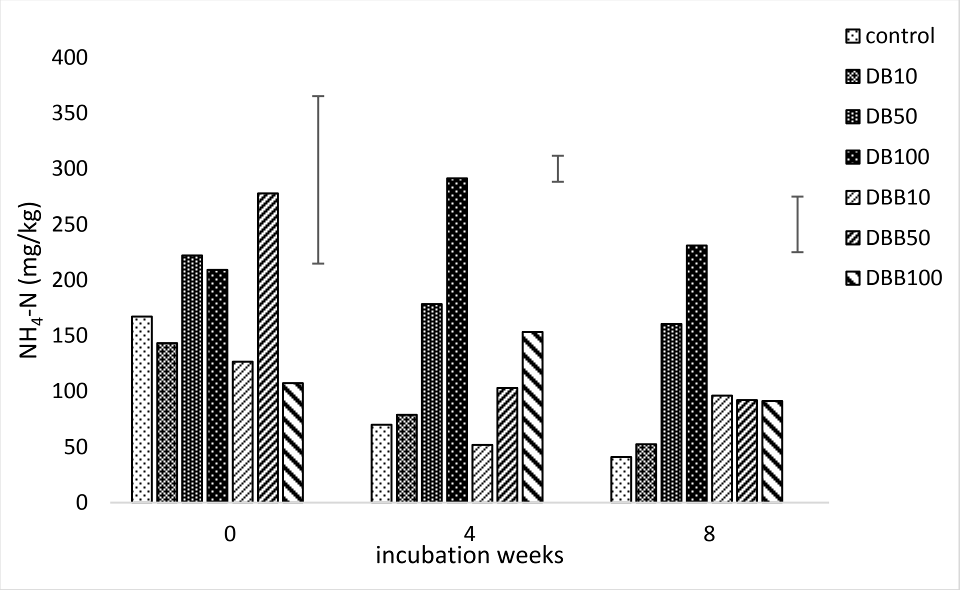

Figure 4.1. Extractable NH_4-N levels from incubated soils amended with drying bed sludge vermicompost at application rate of 10, 50 and 100t/ha for a period of 8 weeks.

Nitrate (NO_3-N)

Although the concentration of nitrate increased with incubation period, all the concentrations were lower than 5 mg/kg throughout the incubation study, compared to ammonium results which got as high as 300 mg/kg during part of the incubation study. At week 0 the control and soils amended with 100t/ha DB+char had higher nitrate than other rates while at week 4 there was no significant difference in the control and soils amended with 10t/ha treatments as well as with the 50 and 100t/ha of db vermicomposts. The 50t/ha and 100t/ha DB+char treated soils had the highest concentration and were significantly different. the concentration of NO_3-N increased significantly in the control and was higher than all other treatments except for the DB 100t/ha at week 8 (Figure 4.2). There was no significant difference between 10t/ha amended soils for both db and dbb as well the 50 and 100t/ha DB+char amended soils at week 8. The DB 100t/ha amended soils had significantly highest NO3-N at the end of the incubation that all application rates for all treatments (Figure 4.2).

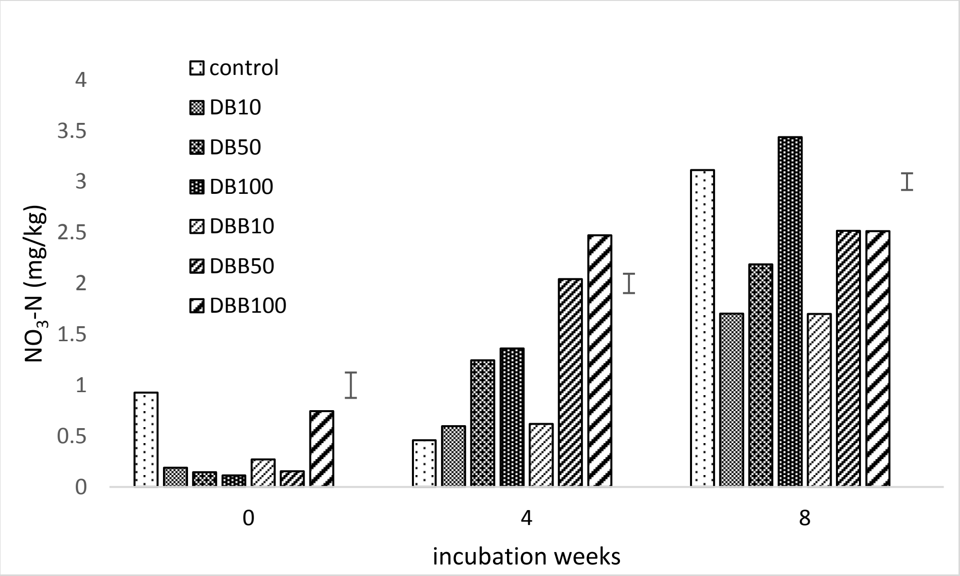

Figure 4.2. NO_3 release from incubated soils amended with drying bed sludge vermicompost with application rate (0, 10, 50 and 100t/ha) for a period of 8 weeks.

Available Phosphorus (P)

There was a constant increase of available P in the Db vermicompost amended soils through out the incubation period except for the DB+char10t/ha amended soils (Figure 4.3). The release of P increased with increasing application rate for both vermicomposts. Like ammonium and EC, the increase in available P was lower, where biochar was included.

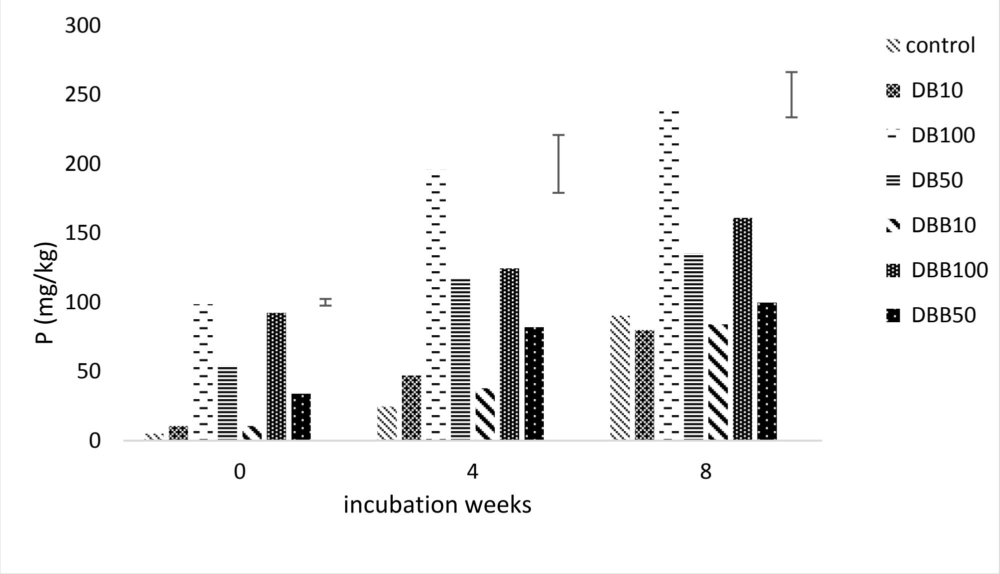

Figure 4.3. Available phosphorus release from incubated soils amended with drying bed sludge vermicompost at application rate of 10, 50 and 100t/ha for a period of 8 weeks.

Incubation experiment with dewatered sludge vermicomposts

pH and electrical conductivity

The pH of the soils amended with DW vermicompost was significantly ($p<0.001$) affected by the interaction between treatment (with and without biochar) and application rates (Table 4.4). Soil pH generally increased with increasing application rate of vermicompost in both treatments with or without biochar. Biochar inclusion however did not significantly change the pH of the soils. Incubation period also did not have a clear effect on pH of the different vermicomposts. At week 0 the pH of the control was similar to that of the 10t/ha amended soils and no significant difference between control pH was recorded with progression of time except for week 8.

Table 4.4. pH of an incubated soil amended with dewatered sludge vermicompost with (+B = biochar) and without biochar at different application rates.

	pH						
wees	10t/ha	10 t/ha(+B)	50t/ha	50t/ha(+B)	100t/ha	100t/ha(+B)	control
0	5.10a	5.09a	5.32ab	5.27ab	5.53b	5.34ab	5.15a
4	5.24a	5.26a	5.38a	5.34a	5.71b	5.61b	5.31a
8	5.06ab	5.17ab	4.93a	5.13ab	5.20ab	5.26b	5.24b

Values followed by same letters are not significantly different at $p<0.05$ (Bonferroni LSD test).

Application of Dw vermicompost significantly increased the EC of the soil ($p<0.001$). Similar to the Db vermicomposts, the increase in EC increased with incubation time with soils amended with biochar incorporated biochar having lower EC than without biochar (Table 4.5).

Table 4.5. Electric conductivity of soils treated with vermicompost derived from dewatered sludge at different application rates (0, 10, 50 and 100t/ha).

	EC (mS/cm)						
(weeks)	Control	10t/ha	10t/ha (+B)	50t/ha	50t/ha (+B)	100t/ha	100t/ha(+B)
0	15.89a	31.3ab	31.6ab	83.1abc	95.57abc	115.13bc	163.9c
4	39.9a	83.4a	61.2a	283.7b	203.8b	443c	437c
8	51.2a	106.4a	82.6a	351.2b	259.2bc	577.3cd	454.3d

Different letters after means indicate significant difference at $p<0.05$ (Bonferroni LSD test).

Ammonium (NH_4-N)

For soils amended with Dw vermicomposts, the 50 and 100t/ha treatments without biochar had significantly lower NH_4 than vermicomposts with biochar at the beginning of incubation (Figure 4.4). Again at this stage, the control also had higher ammonium-N than most of the vermicompost treatments except for the 50 and 100t/ha vermicomposts with biochar. At week 4, soils amended with 0, 10 and 50t/ha had significantly lower NH_4 than those amended with 100t/ha vermicompost and the effect of biochar was insignificant biochar. In the last week of the incubation, ammonium-N release significantly increased with progressive increase in vermicompost application rate, and those treatments with biochar had lower ammonium-N than those without. Nevertheless, the release of NH_4-N in week 8 had decreased when compared to that in week 4 (Figure 4.4).

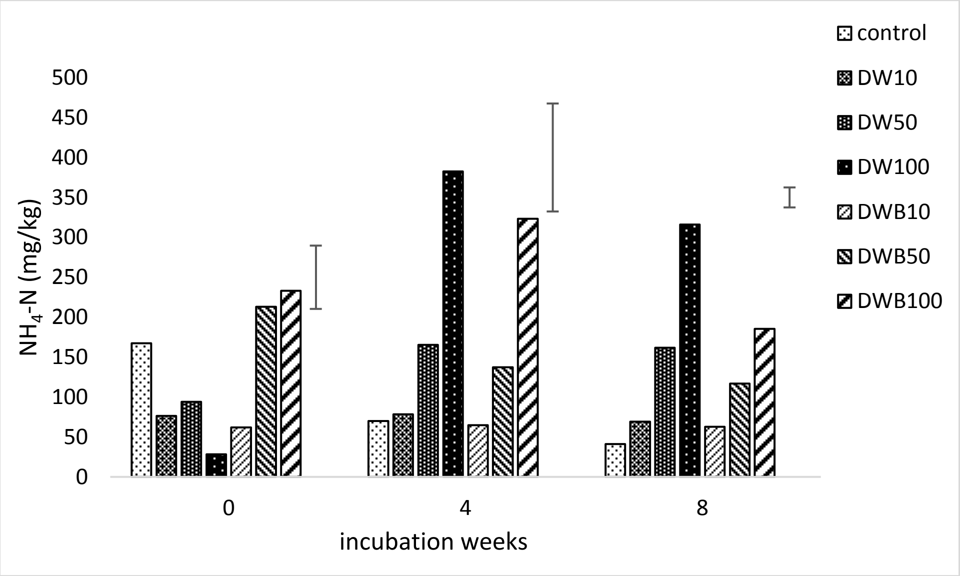

Figure 4.4. Ammonium-N levels in soils amended with dewatered sludge vermicompost at application rate of 10, 50 and 100t/ha for a period of 8 weeks.

Nitrate (NO_3-N)

The release of nitrate in the soils amended with DWS vermicompost was not significantly affected by application rate excluding the control at the same incubation period but progressively increased with time. Thus week 4 had higher nitrate release than week 0 for all compost rates serve for the control. In week 8, the control had significantly higher nitrate than the other application rates which did not differ much (Figure 4.5). Biochar inclusion did not have a significant input on nitrate release patterns of DWS at all application rates.

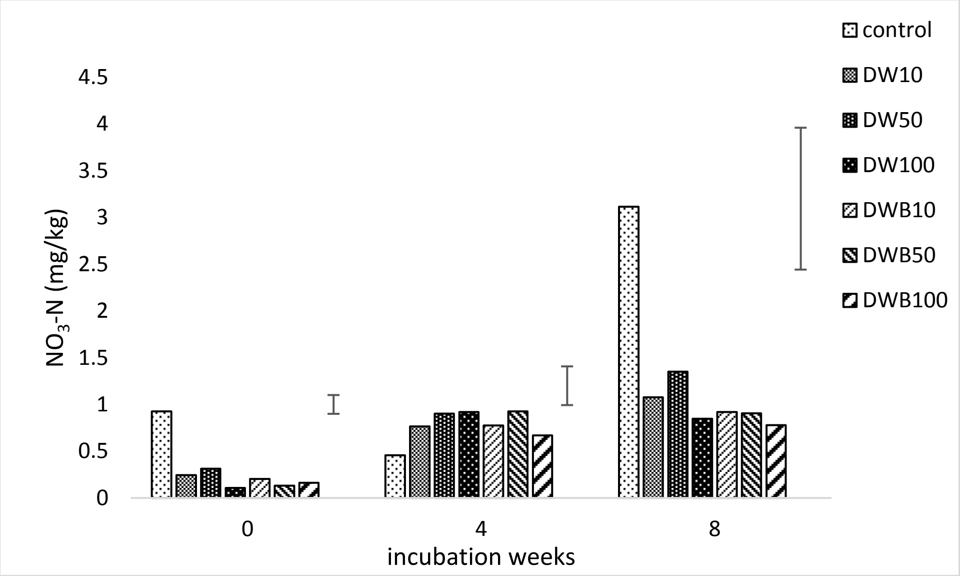

Figure 4.5. Nitrate-N release in soils amended with dewatered sludge vermicompost with application rate (0, 10, 50 and 100t/ha) for a period of 8 weeks.

Available Phosphorus (P)

There was a constant increase of available P in the DW vermicompost amended soils throughout the incubation period with the exclusion of week 4 (Figure 4.6). The release of P increased with increasing application rate for both vermicomposts. Like ammonium and EC, the increase in available P was lower, where biochar was included.

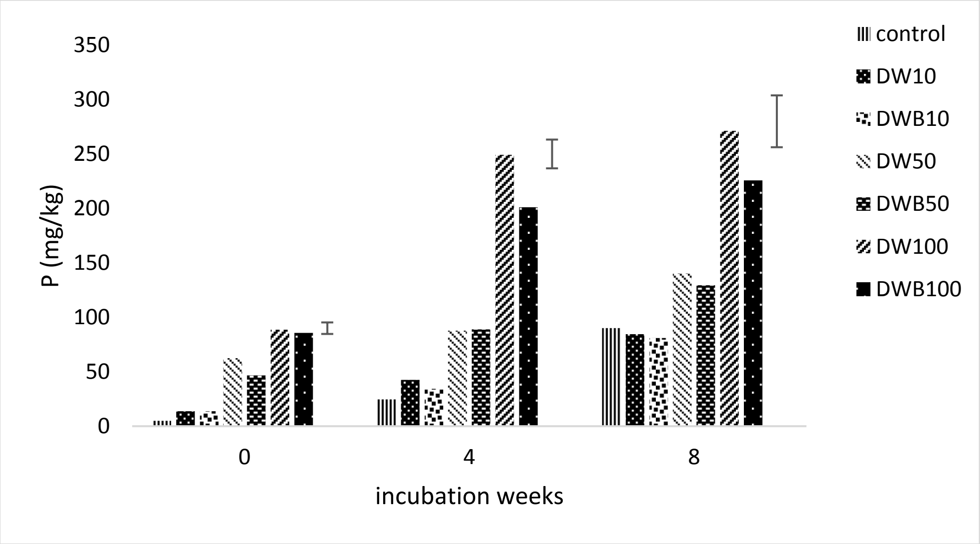

Figure 4.6. Available phosphorus release from incubated soils amended with and dewatered sludge vermicompost at application rate of 10, 50 and 100t/ha for a period of 8 weeks.

4.4. Discussion

The quality and value of agricultural organic soil amendments is determined by their contribution to soil fertility as well as the chemical properties of the soil. The nutrient release and chemical properties of the soil following vermicompost amendment have been evaluated in this study and it was found that increasing the application rate of vermicompost also increased the nutrient release, pH and EC in soil. The trends of all the results were similar for Db and Dw vermicomposts, and as such the results are discussed without distinguishing the original sewage sludge from which the vermicomposts were made.

The increase in pH with incubation time and rate of vermicompost could be explained by the results of ammonium-N release, and the low nitrification as shown by the low NO_3-N. NH_4-N concentration had significantly increased as result of vermicomposting (Chapter 3 figure 3.7) which would explain the increase when the vermicompost is applied to soils. Although the concentration of NO_3-N increased with incubation period, the measured concentrations were lower than 5 mg/kg throughout the incubation study, compared to ammonium results which got as high as 300 mg/kg during part of the incubation study. According to Jat and Ahlawat. (2006), application of vermicompost increased the soil nutrient status (N and P) and influenced the uptake of nutrients by the cropping system. Moreover, vermicompost have been reported to enhance the presence of nutrient solubilising microorganisms such as phosphatase and nitrogen fixing bacteria (Ansari and Sukhraj. 2010). On the other-hand, nitrification and release of high concentrations of nitrate-N is associated with the release of H+ ions resulting in decline in pH (Fertilizer Society of South Africa. 2007). Arancon *et al*. (2006) also reported similar results where an increase of NH_4-N, NO_3-N and othorphosphates and higher dehydrogenase activity was recorded in vermicompost treated soils than the controls. The low levels of nitrate N-suggested that nitrification did not occur. The soils could have been poorly aerated (lack of oxygen) during the incubation study resulting in minimal nitrification. This view is further supported by the high ammonium-N which increased with incubation time, without declining. Contrasting results were reported by Gutierrez-Miceli *et al*. (2007) who reported a lower NH_4-N and NO_2 concentrations compared to NO_3-N as a result of vermicompost application as a soil supplement. Gutierrez-Miceli *et al* (2007) future stipulated that these results were an indication that ammonification and nitrification were not inhibited and that a stable compost was achieved.

The results of EC could be explained by the ammonium-N and available P results which increased with vermicompost rate and incubation time. The higher ammonium-N and available P over time could be explained by mineralisation of these elements during incubation. Organic NH_2 compounds are converted to NH_4^+ by ammonifying bacteria and the NH_4^+ is later converted to NO_3^- by nitrifying bacteria. The high temperature and moist conditions during incubation were conducive for mineralisation of N. The higher EC with increase in vermicompost rate of pure vermicompost could be explained by greater release of ammonium-N and available P from the decomposition of higher rate of vermicomposts containing these elements. EC is greatly influenced by the presence of salts in solution hence it was increased with the release of available P and ammonium-N.

The lower EC, ammonium-N and available P in the presence of biochar than without could be explained by the sorption of ammonium-N and P by biochar removing them from solutions, lowering EC. According to Mukherjee and Zimmerman.(2013), biochar reduces the amount of available nutrients in soil through absorption hence making them unavailable in solution. However, other authors have reported that presence of biochar reduces the leaching of nutrients such as NH_4, NO_3 (Mohanty *et al*., 2014), PO_4^{-3}, NH_4, NO_3 and K (Yuan *et al*. 2016; Ca, P, Mn, Zn (Schneider *et la*. 2016). The presence of biochar could therefore limit availability of ammonium-N and P. Based on a study by Rajkovich *et al*. (2011), the use of different biomass feed stocks (chicken manure, 8 different woods, crop residues, manure and sludges) and temperature between 330 and 500ºC yielded a great variation in growth of corn with greatest response from chicken manure. It was concluded that application rates above 2% did not improve corn growth and biochar produced from dairy manure, paper sludge and food waste decreased growth when applied at higher rates. Crop N uptake was 15% higher when biochar was applied at 0.2% than fully fertilized controls but the uptake of N decreased at higher application rates. An increase in canopy dry weight and leaf area in tomato and pepper plants as result of amending soils with biochar produced from different levels (0,1,3,5 weight%) of citrus wood was reported by Graber *et al*. (2010). The authors further reported a significant increase in pepper bud, flower and fruit yields in this greenhouse pot experiment. As stated by Yang *et al*., (2015) vermicompost is an excellent soil amendment due to its high porosity, aeration, drainage, water holding capacity and its ability to enhance microbial activity**.**

4.5. Conclusion

Incubation of vermicomposts in soils results in increase in Ammonium-N and available P in the soil. Increasing the application rate of vermicompost increased EC, the release ammonium- N and available P and also the pH of the soil. The effect of biochar inclusion on vermicompost reduced ammonium-N and available P. It is worthy to study the ability of these vermicomposts as soil amendments/fertilizer sources in support of crop/ vegetable growth.

CHAPTER 5: EFFECTS OF SEWAGE SLUDGE VERMICOMPOSTS ON DRY MATTER YIELDS AND NUTRIENT COMPOSITION OF SPINACH (*SPNACIA OLERACEA*) TISSUE AND RESIDUAL SOIL.

5.1 Introduction

The use of vermicompost from organic wastes as organic fertilizer/ soil conditioning purposes is practiced globally as farmers and producers reduce the use of chemical fertilizers (Theunissen *et al*. 2010). This shift is due to the minimal costs that are incurred with the use of vermicompost and the nutritional benefits that vermicompost provide. Vermicomposts contain nutrients in forms that are readily taken up by the plants such that when applied as a fertilizer, nutrient release is much faster (Atiyeh *et al*. 2000). Nutrients contained in vermicomposts include nitrates, available phosphorus and soluble potassium. Sheep manure vermicompost used as potting media by Gutierrez-Micel *et al.* (2007) contained 243 mg NO_3-N /kg and 9.14mg/kg of NH_4-N, while vermicompost derived from sewage sludge contained 2189.80 mg available N /kg in the form of NH_4-N, 2310.63mg P /kg and 1801 mg K /kg (Ludibeth *et al*. 2012). Vermicompost also has additional benefits of supplying the soil with organic matter and the much useful microorganisms for decomposition (Atiyeh *et al*. 2000). These benefits contribute to crop productivity of the vermicompost amended soils.

According to Shoba *et al*. (2012), the germination rate of tomato seeds and soil quality were highly improved with the addition of vermicompost produced from chicken manure to soil 15% by weight. The addition of vermicompost from a mixture of banana leaves and cow-dung (1:1) to an acidic-agricultural soil was reported to greatly influence nutrient absorption, stimulated root growth and increased yields of bananas, cassava and cow-pea (Padmarathiamma *et al*., 2008). It was also found that the agronomic properties of the soil were improved leading to improved quality of the planted crops. Although vermicompost has been confirmed and recommended for use in the production of vegetables by many researchers (Gutierrez-Micel *et al*. 2007; Singh *et al*. 2008; Arancon *et al*. 2004), its value as a fertilizer depends on nutrient composition and release, which are both affected by the feedstock material, and maturity of the vermicompost. Pig manure vermicompost was found to contain 27.38% organic carbon, 2.36% N, 4.50% P, 0.40% K by Atiyeh *et al*. (2001). Vermicompost derived from a combination of horse manure, chicken manure and warmcast contained 317.3g/kg organic matter (31.7%), 7.0g/kg total N (0.7%), 4.4g/kg total P (0.44%) and 5.6g/kg total K (0.56%) (Yang *et al*., 2015). The production of vermicompost from sewage sludge could also make a contribution to soil fertility.

Large quantities of sewage sludge are being produced in South Africa due to urbanization and improved living standards of South African citizens. Currently municipalities are faced with problems of waste disposal and alternatives such as vermicomposting need to be explored. It has been confirmed that vermicomposting has the capacity to supply both macro and micronutrients to the soils for optimum plant growth. Using vermicompost as a waste minimization and nutrient recycling strategy seems to be a sound and socially acceptable method of dealing with the excess waste produced by our society. Based on findings from vermicomposting studies and their nutrient release patterns in earlier chapters (Chapter 3 and Chapter 4), it becomes necessary to understand the effects of vermicomposts on plant growth, nutrient uptake and the residual nutrients in the soil. Therefore, the objective of this study was to evaluate the effects of application sewage sludge vermicomposts, with or without biochar, at different application rates on the dry matter yields and nutrient uptake of spinach, and nutrient composition of the residual soil. It was hypothesized that sewage sludge vermicomposts will increase dry matter yield and nutrient uptake of spinach and improve residual soil nutrient composition.

5.2 Materials and Methods

The study was conducted at the University of KwaZulu-Natal (UKZN), Pietermaritzburg Campus ($29^{o}36'$S, $30^{o}23'$E) under glasshouse conditions. The soil used in the incubation study (Chapter 4) was also used in this pot experiment. Details of the soil characteristics are given in Chapter 4 under section 4.2.1. The vermicomposts used in this study were produced as detailed in Chapter 3 from drying bed (drying bed sludge vermicompost) and dewatered sludges (dewatered sludge vermicompost) with or without biochar, and their characteristics (Table 4.1).

5.2.1. Spinach response to the application of vermicomposts

The pot experiment was laid out in a completely randomised design (CRD) with the three replications in the glasshouse. The minimum and maximum temperatures in the glasshouse were 16^{o}C and, 26^{o}C respectively, throughout the experimental period. Air-dried (2 kg) and sieved soil (<2 mm) was placed in pots, then limed at recommended liming rate equivalent to 13.5t/ha. Vermicompost was then applied as a source of N and P to achieve the recommended rate but K was supplemented with K_2SO_4 as it was not sufficient in the vermicompost. These were based on the recommended application rates for spinach from DAFF (Department of Agriculture Forestry and Fisheries). The application rates were calculated on per hectare (ha) basis as 0t/ha (control), 10t/ha 50t/ha and 100t/ha. The 10t/ha was the lowest application rate and this gave a minimum of 129mg N/1.5kg, 30mg P/ 1.5kg and 0.083mg K/1.5kg. The vermicompost was low in K so it was supplemented with 90 mg K/1.5kg as K_2SO_4. A positive control was included using a chemical fertilizer (2:3:2 (30) +5% Zn) N: P: K for comparison purposes of the vermicompost to a chemical fertilizer. Spinach seedlings (*Beta valgaris var cicla*) were bought from sunshine seedlings at 2 weeks old and were transplanted on the amended soils. Moisture correction was done after every other day by weighing the pots on a weighing balance. The experiment was conducted for 6 weeks after which the spinach was harvested by cutting it from just above ground, the roots were not recovered from the soil. The spinach was rinsed with distilled water and weighed to determine fresh weight, before being oven dried for 48 h at 60 ºC, for the determination of dry matter. The dried spinach was milled to pass through a 5µm sieve. The soil was air-dried and analyzed for total N and available P.

5.2.2. Spinach tissue analysis

A part of each sample was analysed for total C and N by dry combustion using the Leco9-TruMac CNS instrument using 0.5g of the sample. The other part was used for the extraction of K, Ca, Mg in soil and available P (in both soil and plant tissue) after acid digestion as outlined by Hsue (2004), then analysed using the inductively coupled plasma-atomic emission spectroscopy (ICP-AES) Varian 720 ES. The extracted available P was determined using the molybdenum blue method (Murphy and Riley, 1962) with the Ultra Violet Spectrophotometer.

5.2.3. Statistical analysis

Statistical analysis of results was performed using Genstat statistical software (Version 12.1; 2009). Results are reported as the means of determinations made on three replicates using the analysis of variance (ANOVA) test. The means were compared by using least significant difference values calculated at $p < 0.05$.

5.3. Results

5.3.1. Effects of vermicompost type and application rate on dry matter yield of spinach.

There was a significant interaction between vermicompost type and application rate (p<0.001) on the spinach yields (Table 5.1). The yields increased with increasing application rate of vermicompost treatments with 100t/ha vermicompost (for both vermicomposts) showing highest yields than lower rates (Table 5.1). There was no significant difference on the spinach grown with 10 and 50t/ha of vermicompost except on the DBB where higher yields were obtained compared to other treatments. Biochar containing vermicompost amended soils were not significantly different from sols treated without biochar.

Table 5.1: Dry matter yields of spinach grown in soil amended with different application rates of vermicomposted sewage sludge.

Treatments	**Dry matter yields (g/pot)**		
	10t/ha	50t/ha	100t/ha
DB	13.1b	13.5b	16.58a
DW	13.1b	13.8b	16.13a
Db+char	16.1a	13.3b	15.55a
Dw+char	13.0b	11. 9b	16.37a
Control+	12.0b	12.0b	12.0b
Control-	11.4b	11.4b	11.4b

Means with same letter subscript within a column are not significantly different. DB = vermicompost from drying bed sewage sludge; DW = vermicompost from dewatered sewage sludge; DB+char= vermicompost from drying bed sewage sludge amended with biochar from pine bark; DW+char= vermicompost from dewatered sewage sludge amended with biochar from pine bark.

5.3.2. Effects of vermicompost type on plant characteristics.

Plant characteristics were not significantly affected by the interaction between treatments and application but these two factors were significant separately. Phosphorus in plant tissue was significantly ($p<0.001$) affected by treatments as plants receiving DW+char had highest P content followed by the positive control and negative control while other treatments were significantly lower (Table 5.2). Maximum uptake of P was also recorded in plants that received DW+char, positive control and negative control of which they were all significantly ($p<0.001$) different from other treatments. Plant height was recorded as highest but not significantly different in the plants that received DW, DB and DW+char and lowest in positive and negative control. Maximum number of laves per plant was recorded in plants that received DB+char followed by DB and DW treatments while controls and DW+char treated plants had less number of leaves.

Table 5.2. Plant characteristics of spinach grown in soil amended with different vermicomposts.

	Plant characteristics			
Vermicompost treatments	Plant tissue P	P uptake	Plant height	Leaves/plant
DB	61a	0.87a	24.17c	22.33bc
DW	115ab	1.61ab	24.56c	21.00bc
DB+char	152abc	2.33ab	21.11bc	22.56c
DW+char	436d	6.06c	23.11c	17.00abc
Positive C	403cd	4.87bc	16.83ab	12.67ab
Negative C	373bcd	4.12abc	15.50a	11.00a
LSD	*167.8*	*2.253*	*3.180*	*6.309*

DB = vermicompost from drying bed sewage sludge; DW = vermicompost from dewatered sewage sludge; DBB= vermicompost from drying bed sewage sludge amended with biochar from pine bark; DWB= vermicompost from dewatered sewage sludge amended with biochar from pine bark.Means with same letter subscript within a column are not significantly different.

5.3.3. Effects of vermicompost application rate on plant characteristics

P in plant tissue was not significantly different amongst application rates, this was also the same case with P uptake. Plant height and number of leaves had a similar trend as there was no significant difference between 10t/ha and 50t/ha while the 100t/ha application rate was significantly different and higher than the lower rates (Table 5.3).

Table 5.3. Plant characteristics of spinach as affected by application rate.

	Application rates of vermicompost			
	10t/ha	50t/ha	100t/ha	*LSD*
P in plant tissue	236a	249a	286a	*118.7*
P uptake	2.97a	2.96a	4.00a	*1.593*
Plant height	18.92b	19.69b	24.03a	*2.249*
Leaves/plant	16.67b	16.06a	20.56a	*4.461*

Means with same letter subscript within a row are not significantly different. Db = vermicompost from drying bed sewage sludge; Dw = vermicompost from dewatered sewage sludge; Db+char = vermicompost from drying bed sewage sludge amended with biochar from pine bark; Dw+char = vermicompost from dewatered sewage sludge amended with biochar from pine bark.

5.3.4. Effects of vermicompost type and application rate interaction on tissue N and N uptake

There was a significant interactive effect between application rate and vermicompost type on the N content of spinach ($p<0.001$), as shown but the high concentration of tissue N at the 100t/ha vermicompost treatments (Table 5.4). Greater response to vermicompost fertilization in terms of tissue N and uptake was recorded for the 100t/ha rate. At the 100t/ha, the Db and Dw had the same tissue N uptake while the Db+char and the Dw+char had similar levels which were lower than those without biochar, but higher than the 10 and 50 t/ha rates and the controls (Table 5.4).

Table 5.4. Total tissue N and N uptake of spinach grown with different types of vermicomposts at different application rates.

	Tissue N concentration (%)			N uptake (g/pot)		
	Application rates (t/ha)					
Treatments	10	50	100	10	50	100
DB	2.177	2.204	4.217	0.286	0.298	0.694
DW	1.892	1.996	4.508	0.248	0.275	0.728
DB+char	1.807	2.047	3.623	0.292	0.273	0.563
DW+char	1.851	2.033	3.14	0.239	0.243	0.515
Negative	1.882	1.882	1.882	0.225	0.225	0.225
Positive	2.216	2.216	2.216	0.255	0.255	0.255
LSD	*0.638*			*0.102*		

LSD (least significance difference) was used to compare the treatments. Db = vermicompost from drying bed sewage sludge; Dw = vermicompost from dewatered sewage sludge; Db+char = vermicompost from drying bed sewage sludge amended with biochar from pine bark; Dw+char = vermicompost from dewatered sewage sludge amended with biochar from pine bark.

5.3.5. Effects of vermicompost type and application rate on residual soil N after Spinach harvesting.

The interaction effects between treatments and application rates did not affect the residual soil N results (p=0.605). There was no significant difference between application rates on soil residual N (Table 5.5).

Table 5.5. Soil residual N after harvesting of spinach fertilized with vermicompost at different application rates.

Application rates	Residual N%
10t/ha	0.2755a
50t/ha	02736a
100t/ha	0.2809a
LSD	*0.01617*

LSD represents all application rates and means with the same letter subscript are not significantly different. Db = vermicompost from drying bed sewage sludge; Dw = vermicompost from dewatered sewage sludge; Db+char = vermicompost from drying bed sewage sludge amended with biochar from pine bark; Dw+char = vermicompost from dewatered sewage sludge amended with biochar from pine bark.

Treatments had a significant effect on the residual N content of the soil (p<0.001), however, treatments were not significantly different except for the negative control which had lower N content than all the other treatments (Table 5.6).

Table 5.6. Soil residual N after harvesting of spinach fertilized with different vermicompost.

Treatments	Residual N%
DB	0.2922b
DW	0.2703b
DB+char	0.2838b
DW+char	0.2842b
Positive	0.3056b
Negative	0.2238a
LSD	*0.02287*

DB = vermicompost from drying bed sewage sludge; DW = vermicompost from dewatered sewage sludge; DBB= vermicompost from drying bed sewage sludge amended with biochar from pine bark; DWB= vermicompost from dewatered sewage sludge amended with biochar from pine bark

5.3.8. Effects of vermicompost type and application rate on residual soil P after Spinach harvesting.

The interaction between application rate and treatment significantly affected P concentration ($p=0.013$). The vermicompost application rate significantly affected residual soil P concentration ($p<0.001$), as there was an increased concentration of P in the residual soil as the application rate was increased (Figure 5.1).

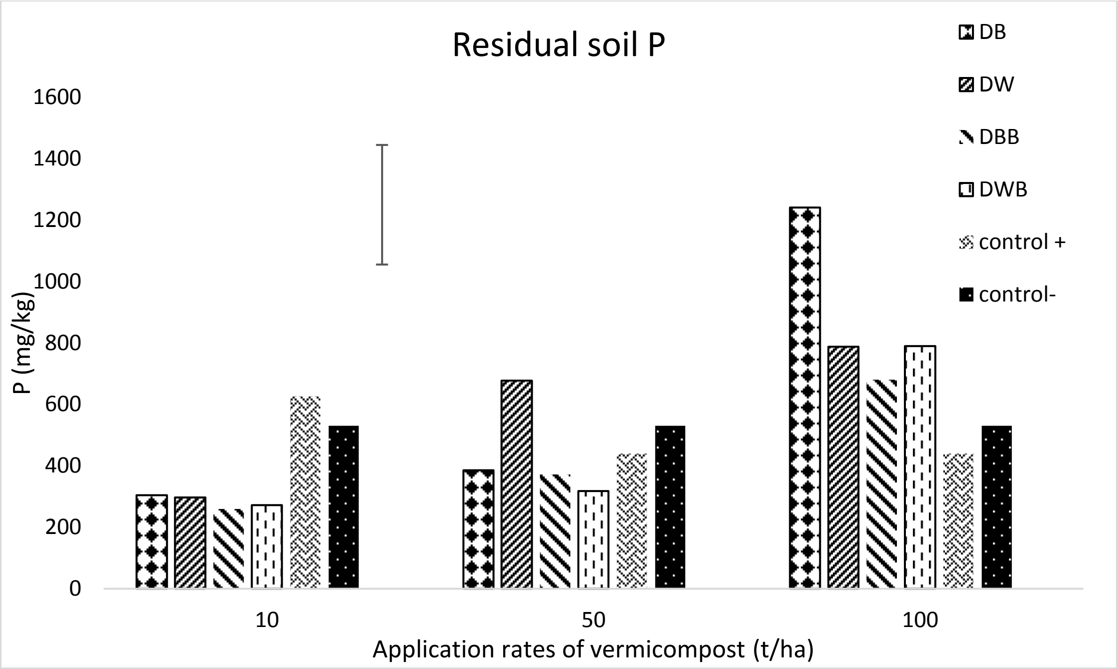

Figure 5.1. Concentration of available P on the residual soil after spinach harvesting. Db = vermicompost from drying bed sewage sludge; Dw = vermicompost from dewatered sewage sludge; Db+char = vermicompost from drying bed sewage sludge amended with biochar from pine bark; Dw+char = vermicompost from dewatered sewage sludge amended with biochar from pine bark.

5.4. Discussion

The increase in plant height, number of leaves and dry matter yield with higher vermicompost rates could be explained by the higher N uptake, and not P uptake, which was higher in the tissue of plants with lower dry matter. Nitrogen is the building block of chlorophyll in plants and is responsible for the green colour on plant leaves hence it is a major nutrient required for crop production. Aside from influencing crop growth and yield, vermicompost application to soils has also been reported to affect the chlorophyll content of the plant leaves. For example, increased photosynthetic pigment and leaf gas exchange in red chilli (*Caspsicum annum L.*) due to the application of vermicompost was observed by Berova and Karanatsidis (2009). Leaf area index (LAI) and chlorophyll content of the leaves of pastichio *(Pistacia vera L.)* seedlings were reported to be better in vermicompost treatments when compared to the treatments without vermicomposting (Golchini *et al*., 2006). All these findings are in support of the fact that, increased N tissue and yield, is a result of increased N uptake by spinach.

Higher vermicompost rates supplied more available N, and further mineralization in the soil supplied even more when compared to lower rate than the controls. These findings are similar to those reported by Arancon *et al*. (2004) who reported yield increase of 45% from peppers grown on potting media substituted with 40% and 60% vermicompost compared to 100% Metro-Mix 360 alone. Similar findings were reported by Atiyeh *et al*. (2001) stating that increasing vermicompost application beyond 40% of the potting medium did not significantly improve yields. Atiyeh *et al*. (2000) concluded that substituting the Metro-Mix 360 potting medium with 10 or 50% pig manure vermicompost increased the weights of tomato seedlings significantly as compared to the ones grown in 100% potting medium alone. Effect of vermicompost application rate to crop response was also investigated by Singh *et al*. (2008) where they used strawberry (*Fragaria x ananassa Duch*) and found that application rates of 2.5 to 7.5t/ha significantly increased dry matter yields by 20.7%, plant spread by 10.7% and leaf area by 23.1% while doses beyond 7.5t/ha did not significantly affect the growth parameters. The lower drymatter in the negative control than the positive control and vermicompost treatments could also be explained by the lower N, which was only supplied by the soil without external input. The similarity in plant heights and yields between the 10 and 50t/ha, suggest that the N supplied by the 50 t/ha was not high enough to result in a significant change. The higher plant height and drymatter in the vermicompost treatments than the positive control could be explained by availability of other plant essential nutrients, including K, other bases and micronutients, from the vermicomposts. Ansari and Sukhraj (2010) reported that plant height was maximum for plants grown with vermiwash and vermicompost relative to chemical fertilizers and this observation was explained in terms of the impact of microbes in bio-fertilizers. The lower plant heights in vermicompost treatments with biochar, suggested that biochar limited availability of nutrients. These results are supported by the extractable K in the vermicomposts which were lower where biochar was incorporated, biochar sorbs nutrients and reduces their availability. According to Ok *et al.* 2016, biochar can increase the adsorption capacity of plants for soil nutrients and thereby reduce the leaching losses of nutrients, however, contrasting findings by Lehmann *et al*. 2003, suggest that biochar addition might limit soil N availability in N deficient soils due to increased C/N ratio of biochar and therefore lead to reduced crop yields. The ability of vermicompost to improve plant quality and growth can be associated with the availability of plant growth regulators and humic acids in vermicompost which are cause by microbial actions during decomposition (Singh *et al*. 2008). Spinach plants receiving vermicomposts derived from drying bed sludge showed greater response compared to those derived from dewatered sludges. Arancon *et al*. (2004), reported that mineral N in the growth mixtures increase with increasing substitution rate of vermicomposted food waste into potting medium in all sampling dates. According to Atiyeh *et al*. (2001) increasing the rate of pig manure vermicompost greatly influenced the N tissue of tomato plants.

N and P results from the residual soil indicate that N was the nutrient mostly taken up by the plants as P still remained in higher quantities. Residual nitrogen in the soil was the same indicating that all that was available was taken up by the growing plants. There was more P remaining in the soil as a result of increased application rate, this could mean that the spinach took up P according to its requirements not based on increasing application rate of vermicompost. Increasing the application rate of vermicompost increased the P content in the soil but did not increase uptake of P.

5.5. Conclusion

The effects of applying vermicompost at increasing application rates had a major effect on plant height, spinach yield and uptake of N as these plant characteristics increased with increasing application. The concentration of N in the residual soil decreased with increasing application rate while the plant uptake increased. The addition of biochar to sewage sludge vermicomposts resulted in lower plant heights compared to pure vermicomposts. Residual soil P increased with increasing application rate but the uptake was the similar for all the rates.

CHAPTER 6: GENERAL DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS FOR FUTURE STUDIES.

6.1. General discussion

The rapid urbanization has resulted in massive production of both domestic and industrial sewage sludge. Environmental and health hazards presented by this massive production are a major concern in our society. However, the conversion of this product to a useful organic fertilizer seems to be a sound solution to the despair caused by its disposal. The use of sewage sludge in agriculture is becoming a growing practice but concerns arise from the contamination of their products by pathogens and heavy metals and the subsequent transfer of these to humans. Through the use of earthworms to convert sewage sludge into an organic fertilizer, both farmers and society can benefit from the pathogen free and highly nutritious vermicomposted product. The use of vermicompost in agriculture has been widely used for both crop production and as means of waste management. Vermicomposting results in the production of finer material in relation to the parent material that is high in plant essential nutrients and has reduced pathogen content. The main objective of this study was to investigate the effect of vermicomposting two types of domestic sewage sludge on stabilization, composition of pathogenic organisms (*E.coli* and *Salmonella*), nutrient release and fertilizer value of the vermicomposts with or without biochar.

The quality of the sewages (nutrient composition), vermicompost quality (with or without biochar) and their nutrient release could explain the results of spinach dry matter yield, nutrient uptake and the residual elemental composition in the soil.

Dewatered sludge had higher total N than drying bed sludge which resulted in higher total N in the dewatered sludge vermicompost than those with biochar and in relation to the drying bed sludge and this could be explained by more rapid loss of C. Gupta and Garg (2008) reported an increase in total N of vermicompost when sewage sludge was mixed to equal proportions (50% Sludge and 50% Cow dung) with cow dung. Ludibeth *et al*. (2012) studied the vermicomposting of sewage sludge mixed with cow dung at different ratios and found that sewage sludge alone contained largest amount of total N (3.1%) followed by treatment C (90% sludge and 10% cow dung) which resulted in a vermicompost with high N content. The amount of total N contained in raw sludge has implication for the resultant vermicompost (chapter3) and therefore its release on soil. In chapter 3, this difference in sludge type and nutrient trends was associated with the method of drying the sludge as dewatered sludge was centrifuged and therefore leached out more nutrients than the drying bed sludge where drying was through evaporation. The release of nitrogen was recorded in the mineral form (NH_4-N and NO_3-N) and the results (chapter 4) revealed that, NH_4-N concentration of the soil had significantly increased as result of vermicompost application. On the other hand, the concentration of NO_3-N also increased with incubation period, but all the concentrations were lower than 5 mg/kg, while NH_4 results were as high as 300 mg/kg during part of the incubation study. The low levels of nitrate N-suggested that nitrification did not occur maybe due to overwatering leading to poor aeration (lack of oxygen) during the incubation study resulting in minimal nitrification. This view is further supported by the high NH_4-N which increased with incubation time without declining. Nitrification and the release of high concentrations of NO_3-N is associated with release of H+ ions resulting in decline in pH (Fertilizer Society of South Africa. 2007). Arancon (2006) also reported an increase of NH_4-N, NO_3-N and othorphosphates and higher dehydrogenase activity was recorded in vermicompost treated soils than the controls.

Contrasting results were reported by Gutierrez-Miceli *et al*. (2007) who reported a lower NH_4-N and NO_2 concentrations compared to NO_3-N as a result of vermicompost application as a soil supplement. Gutierrez-Miceli *et al.* (2007) future stipulated that these results were an indication that ammonification and nitrification were not inhibited and that a stable compost was achieved

Biochar presence did not have a significant input on NO_3-N release patterns of DWS at all application rates and the result were the same for DB vermicompost amended soils. NH_4-N release was higher in DB vermicompost amended soils than those containing biochar. Whereas soils amended with DW vermicompost amended with or without biochar had similar concentration of NH_4-N. Biochar has an absorbing effect on soil nutrients and as a result makes them unavailable. According to Mukherjee and Zimmerman. (2013), biochar reduces the amount of available nutrients in soil through absorption hence making them unavailable in solution. However, other authors have reported that presence of biochar reduces the leaching of nutrients such as NH_4, NO_3 (Mohanty *et al*. 2014), PO_4^{-3}, NH_4, NO_3 and K (Yuan *et al*. 2016; Ca, P, Mn, Zn (Schneider *et la*. 2016). Nutrient release of vermicompost to soils determines the ability of the vermicompost to support crop growth by supplying nutrients. Nutrient in plant tissue, uptake and yields are the determining factors of this ability. According to Jat and Ahlawat. (2006), application of vermicompost increased the soil nutrient status (N and P) and influenced the uptake of nutrients by the cropping system. Chapter 5 of this study reveals that application of vermicompost at different application rates supported spinach growth. Greater response to vermicompost fertilization in terms of tissue N and uptake was recorded for the 100t/ha rate. Moreover, Db and Dw had the same tissue N uptake while the Dbb and the Dwb had similar levels which were lower than those without biochar. The positive control showed similar results to those obtained for 100t/ha in tissue N but not for N uptake. The yields increased with increasing application rate of vermicompost treatments with no significant difference between vermicompost type and the presence and the absence of biochar. Increased photosynthetic pigment and leaf gas exchange in red chilli (*Caspsicum annum L*.) due to the application of vermicompost was observed by Berova and Karanatsidis (2009). Leaf area index (LAI) and chlorophyll content of the leaves of pastichio *(Pistacia vera L.)* seedlings were reported to be better in vermicompost treatments when compared to the treatments without vermicomposting (Golchini *et al*. 2006). Higher vermicompost rates supplied more available N, and further mineralization in the soil supplied even more when compared to lower rate than the controls. These findings are similar to those reported by Arancon *et al*. (2004) who reported yield increases of 45% from peppers grown on potting media substituted with 40% and 60% vermicompost compared to 100% Metro-Mix 360 alone. Similar findings were reported by Atiyeh *et al*. (2001) stating that increasing vermicompost application beyond 40% of the potting medium did not significantly improve yields. Atiyeh *et al*. (2000) concluded that substituting the Metro-Mix 360 potting medium with 10 or 50% pig manure vermicompost increased the weights of tomato seedlings significantly as compared to the ones grown in 100% potting medium alone. In addition to N, sewage sludges and vermicomposts contained other nutrients including significant levels of P**.**

The original DW sludge had higher P than Db sludge, which explains the difference between during the vermicomposting of these sludges. The increase in extractable P for all treatments in the first three weeks could be explained by the degradation of the organic materials during composting but the decline thereafter could be a result of uptake by earthworms into their own bodies as the biomass increased. Some of the P could have been leached as a result of moisture adjustment to provide a comfortable environment for the worms. Unfortunately, the leachates collected could not be analysed as it got contaminated by extraneous material due to windy conditions. Similar results were reported by Benitez *et al.* (1999) where soluble PO_4^{-3} was undetectable after 6 weeks of vermicomposting sewage sludges. Gupta and Garg. (2008) however, reported a two-fold increase in total P of the final vermicompost compare to the initial feed mixtures. The higher extractable P in DW vermicompost than DWB could be explained by sorption of P on biochar. Column leachate of a Norfolt loamy soil amended with pecan shell biochar made at 70º C contained less amount of P (by about 35%) compared to the control soil with no biochar (Schneider *et al*., 2016). This sorption may reduce the availability of P for plant uptake where biochar is added to soils. Lower available P in the presence of biochar than without was recorded during the incubation study (Chapter4) and these results could be explained by the sorption of P by biochar removing them from solutions. Furthermore, the P release of vermicompost translate to plant tissue P and uptake of this element by the plant. Results from chapter 5 of this study showed that increasing the application rate of vermicompost did not significantly increased the tissue and uptake P of spinach but these characteristics were significantly affected by the type of vermicompost applied. Spinach plants fertilized with DWB vermicompost and positive control had higher tissue and uptake P compared to other treatments. N and P results from the residual soil indicate that N was the nutrient mostly taken up by the plants as P still remained in higher quantities and therefore could have limited the uptake of P. There was more P remaining in the soil as a result of increased application rate, this could mean that the spinach took up P according to its requirements not based on increasing application rate of vermicompost. In addition to N and P, the composition in sludges and vermicomposts of bases (K, Ca and Mg) could also have contributed to growth and yield of spinach. K was higher in dewatered sludge and dewatered sludge vermicompost (DWS) compared to Ca and Mg which were lower. The lower extractable K in the DWB than DW vermicompost at the end of vermicomposting could be explained by sorption of K by biochar, removing it from solution. In a column leachate study by Schneider *et al*. (2016), loamy soil amended with pecan shell biochar contained higher amounts K, Na but less Ca. The difference in exchangeable bases of vermicompost could have had a significant contribution to the yields (although not measure in the spinach), compared to negative and positive control. Whereas the sewage sludges and their vermicomposts contained significant nutrients, the composition of pathogenic organisms could have implications of the value of these materials as nutrient sources. Drying bed sludge had higher *E.coli* population (5.93 x10^4) compared to the dewatered sludge (3.57 x10^4) this difference maybe a result of dewatering of the sludge as dewatered sludge is done mechanically for the DWS and not for the DBS. Vermicomposting alone did not significantly reduce *E. coli* this was evident as he population did not decrease as a result of vermicompost. However, *E.coli* results were higher in the DB than DBB vermicomposts, which suggested that the presence of biochar limited *E. coli* growth, possibly as a result of limited nutrients, like P and K. Biochar produced from wood chips at 350ºC removed more *E.coli* from storm water biochar effluent in relation to the commercial biochar (Mohanty *et al*. 2014). Based on the results presented on *E.coli*, vermicomposting with biochar addition reduces the population of this pathogen and there for reducing the risk to human health. Moreover, *Salmonella* was completely eliminated during the vermicompost of the sludge, this implies that the transfer of this pathogen to humans is not possible through the consumption of spinach grown with these vermicomposts. Comparing these vermicomposts to the controls on the basis of pathogen population is not realistic as the controls would not be expected to contain any pathogens, as opposed to a comparison on the basis of fertilizer costs, nutrient value and obtainable yields.

6.2. General conclusions

Water removal methods sewage sludge have a major influence in the chemical composition of the sludge and resultant vermicomposts. Drying bed sludge had higher Ca, Mg and EC while the dewatered sludge contained higher amounts of N, P, K and Ph which makes dewatered sludge the most nutritious sludge. Vermicomposting of sludge did not have an effect on the pH of both sludges. Total nitrogen, exchangeable calcium, exchangeable magnesium and extractable potassium were high in dewatered sludge vermicompost while drying bed sludge contained higher NH_4-N. The inclusion of biochar in vermicompost decreased extractable P, N, K, Nitrate-Nitrogen, ammonium nitrogen, electrical conductivity and pH especially in dewatered sludge vermicomposts. Vermicomposting completely eliminated salmonella by the end of the study period while presence of biochar decreased *E.coli* population in both sludge types. Incubation of vermicomposts in soils resulted in increased Ammonium-N and available P in the soil. Increasing the application rate of vermicompost increased EC, the release ammonium- N and available P and also the Ph of the soil. The effect of biochar inclusion on vermicompost reduced ammonium-N and available P, and subsequently.

When applied at increasing application rates as a fertilizer source, vermicompost increased spinach plant height, spinach yield and uptake of N compared to the controls. The concentration of N in the residual soil decreased with increasing application rate while the plant uptake increased. The addition of biochar to sewage sludge vermicomposts resulted in lower plant heights compared to pure vermicomposts. Residual soil P increased with increasing application rate but the uptake was the similar for all the rates. It can be recommended that vermicomposted sewage sludge be applied at rates higher than 50t/ha to obtain maximum crop response and yields.

6.3 Recommendations for future studies

Dewatered sludge (mechanically dewatered) sewage sludge is recommended based on the nutrient composition and composition of pathogenic organisms in the resultant sludge and resultant vermicompost. Even though the inclusion of biochar in sewage sludge vermicomposting did not enhance the quality (nutrient content) of vermicomposts, it is recommended in reducing pathogens in both sludge types. Another recommendation would be to use dewatered sludge vermicompost for crops as this type showed to be a better fertilizer and its porous material makes it easier to work with compared to the cake form of the drying bed sludge. The sludge should be vermicomposted for extended periods than 12 weeks not only to achieve maximum stability and availability of nutrients but also to reduce pathogen levels even further. It is also recommended that vermicomposted sewage sludge especially Db derived vermicompost be applied at rates higher than 50t/ha to obtain maximum nutrient release, crop response and yields.

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