

**OPTIMIZATION OF BIO-CONVERSION AND NUTRIENT RELEASE FROM  
COAL FLY ASH AMENDED COW DUNG – WASTE PAPER COMPOSTS USING  
EARTHWORMS (*EISENIA FETIDA*) AND EFFECTIVE MICRO-ORGANISMS**

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**A thesis submitted in fulfilment of the requirements for the degree of Doctor of  
Philosophy in Agriculture (Soil Science)**

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**April 2015**

## DECLARATION

I, **Hupenyu Allan Mupambwa**, declare that the thesis hereby submitted for the degree of Doctor of Philosophy (PhD) at the University of Fort Hare is entirely my work with the exception of such quotations or references which have been attributed to their sources or authors. This thesis has not been previously submitted to this or any other University for a degree.

**Signature:**

A handwritten signature in black ink, consisting of a large, stylized 'H' followed by a series of loops and a final upward stroke.

**Date:** 07 April 2015

**Place:** University of Fort Hare (Alice Campus)

## GENERAL ABSTRACT

Improving the fertilizer value of fly ash can create an abundant nutrient source in agriculture, whilst reducing the landfill deposition of this coal waste. This study was undertaken to address this challenge and was guided by the following specific objectives, (i) to characterize fly ash samples from selected South African coal powered electricity stations (ii) to optimize the fly ash incorporation into cow dung – waste paper mixtures for enhanced biodegradation and nutrient mineralization (iii) to determine the ideal *Eisenia fetida* (*E. fetida*) stocking density for the vermicomposting of fly ash - cow dung-waste paper mixtures (FCP) (iv) to determine the effectiveness of Effective Micro-organisms (EM) during vermicomposting in enhancing the biodegradation of FCP (v) to determine the effectiveness of FCP vermicompost substituted into pine bark compost on media physico-chemical properties and marigold growth.

The eight South African fly ashes evaluated had a total phosphorus content ranging from 553.3 to 1514 mg/kg and the Olsen extractable P from 130 to 345.5 mg/kg. Across all three soils, fly ash incorporation increased extractable P content from a P deficient level to about 20 mg P/kg. Except for Cu, all metal species investigated (Cr, Pb, Ni and Fe) showed significantly ( $P \leq 0.05$ ) low extractability under fly ash treated soils compared to the soil alone control. To enhance the nutrient mineralization in fly ash, it was mixed with optimized cow dung – waste paper (CP) mixtures at ratios of (F: CP) 1:1, 1:2, 1:3, 2:1, 3:1 and CP alone and composted for 14 weeks with or without *E. fetida* earthworms . Based on C: N ratio, the extent of vermi-degradation of the waste mixtures followed the decreasing order (F: CP) of 1:3 > 1:2 > 1:1 > CP alone > 2:1 > 3:1. Olsen P was significantly higher ( $P < 0.05$ ) where earthworms were added. The mean percentage increase in extractable P was in the order CP alone > 1:2 > 1:3 > 1:1 > 2:1 > 3:1, with earthworm addition almost doubling P release across

the 1:1; 1:2 and CP alone treatments. Fly ash incorporation at the 1:2 ratio proved to be the most appropriate, as it enhanced biodegradation and nutrient release to a greater extent than all other treatments with fly ash incorporation.

To optimize stocking density of fly ash vermicompost, the optimum fly ash incorporation level of 1: 2 was treated with four stocking densities of 0; 12.5; 25 and 37.5 g –worm/kg; including and control with cow dung- waste paper only (CP alone). Though the treatments 12.5; 25 and 37.5 g – worm/kg all resulted in mature vermicompost, a stocking density of 25 g-worm/kg and above resulted in faster maturity; higher humification parameters and a low C: N ratio range (11.1 – 10.4). The activity of  $\beta$ -glucosidase and fluorescein di-acetate hydrolysis enzymes showed faster stabilization at stocking densities of 25 g-worm/kg and above, indicating vermicompost stability and maturity. These enzymes also showed significant ( $P < 0.05$ ) correlation with changes in C: N ratio in the vermicompost. Similarly, a stocking density of 25 g-worm/kg resulted in the highest potential to increase nitrate/nitrite and Olsen extractable P. The treatments 0; 12.5; 25 and 37.5 g-worm resulted in a net Olsen P increase of 16.3%; 38.9%; 61.0% and 53.0%, respectively, after 10 weeks. Though vermicompost maturity can be attained at a stocking density of 12.5 g-worm/kg, for faster more humified and nutrient rich fly ash vermicompost, a stocking density of 25 g-worm/kg seemed most appropriate.

Inoculation of fly ash vermicompost with EM alone did not result in significantly ( $P < 0.05$ ) different changes in C: N ratio and dissolved organic matter (DOC) compared to the control. Also, though inoculation with EM together with *E. fetida* resulted in greater changes in C: N ratio and DOC compared to the *E. fetida* alone treatment, this difference was not statistically significant. Inoculation with EM coupled with *E. fetida*, however, resulted in significantly ( $P$

< 0.05) higher rates of Olsen phosphorus release compared to the *E. fetida* alone treatment. On average, the EM + *E. fetida* treatment resulted in a rate of weekly Olsen P release of 54.32 mg/kg, with the *E. fetida* alone, EM alone and control releasing 48.39; 28.71 and 16.56 mg-P/kg/week, respectively. It was observed that inoculation of fly ash based composts with EM alone is not beneficial, whilst combining EM with *E. fetida* may result in faster compost maturity and greater Olsen P release.

By substituting pine bark growing media with fly ash vermicompost up to 50% significant improvements in water holding capacity, total porosity and air filled porosity was observed, with germination over 90%. The 25% FA treatment also resulted in significantly higher number of flowers and buds compared to the 50% and 75%, despite the higher concentration of essential nutrients in the 50% FA treatment. For effective marigold seedling germination and growth, the 50% FA: 50% PB growing medium is recommended whilst for maturity and flower production, the 25% FA: 75% PB combination is to be preferred as it performed better than all treatments regardless of the nutrient composition of the media.

The results of this study have shown that fly ash can be an effective phosphorus source when vermicomposted at an incorporation ration of 1:2 (fly ash: cow dung – waste paper) and a stocking density of 25 g-worm/kg. The fly ash vermicompost can be an important part of planting media which can be used for land reclamation and horticultural crop production. However, optimization of EM incorporation may be required for significant effects of on biodegradation and mineralization of fly ash vermicomposts following EM inoculation.

**Key words:** Fly ash incorporation; stocking density; phosphorus; enzyme activity; marigold growth.

## PREFACE

This thesis is made up of eight separate but linked chapters. Chapter 1 gives a general introduction to the reader on fly ash, cow dung and waste paper as waste materials with potential as a nutrient source. Chapter 2 gives a thorough review of current literature on fly ash, cow dung and waste paper, the potential of composting and vermicomposting in improving their fertilizer value. Chapter 3 gives an insight into the chemical characteristics of various fly ashes from South African power stations and their potential and limitations for direct land application. Chapter 4 follows by looking at the optimization of fly ash incorporation into cow dung waste paper mixtures which had been adjusted to a C: N ratio of 30, for effective biodegradation and nutrient release. Chapter 5 then evaluates the effects of various earthworm (*Eisenia fetida*) stocking densities on degradation and nutrient release of optimized fly ash – cow dung – waste paper mixtures. Chapter 6 evaluates the potential of using earthworms and a special cocktail of microbes called Effective micro-organisms (EM) in enhancing degradation and nutrient release of fly ash – cow dung – waste paper composts. Chapter 7 wraps the experimental chapters by evaluating the potential of using the fly ash – cow dung – waste paper vermicomposts as a component in pine bark growing media for growth of ornamental marigold (*Tagetes spp*) flowers. Finally, Chapter 8 gives a synthesis in the form of general discussion of the entire research chapters, also giving conclusions and recommendations for further research on fly ash composting. The chapters in the thesis have been treated as separate though linked studies and thus some repetition between chapters could not entirely be avoided. It is my hope that the results of this thesis will form a basis for effective, optimized vermicomposting of fly ash to produce high nutrient content composts and facilitate use of coal fly ash as an important source of nutrients in agriculture.

## **DEDICATION**

*To Jean Chirimuuta, Jenipher Anne Tafadzwa Mupambwa, Tennia my wife and Ropafadzo*

*Zowie; the four generations that nurtured, inspired and loved me*

## ACKNOWLEDGEMENTS

I started this Ph.D. study within the Department of Agronomy at the University of Fort Hare (UFH) in 2012 under a supervisor linked bursary. My study aimed at optimizing the biodegradation and nutrient release in coal fly ash (a product of coal combustion at electricity power stations) which had been incorporated into cow dung – waste paper mixtures using an epigeic earthworm specie (*Eisenia fetida*) and effective micro-organisms and further evaluating the suitability of the fly ash vermicompost as a planting media. The study was carried out in the field at the research farm and in the laboratory all at the University of Fort Hare, Alice campus. However, with only my two hands and intellectual limitations, I would have not been able to complete and enjoy my studies and this called for participation of several individuals in different respects within my study.

To undertake this Ph.D., I was nominated by Professor Pearson N.S. Mnkeni for a supervisor linked bursary under the Govan Mbeki Research and Development Centre (GMRDC) at the University of Fort Hare. Prof Mnkeni became my chief mentor throughout my studies and without his diligent mentorship, positive views, critical ideas and fatherly attitude, I would have not realized this dream and I am forever grateful to him. Despite his busy schedule, Prof Mnkeni always made time for me instantly without ever making an appointment. His patience and leadership is incomparable and he will remain my true inspiration and I feel privileged to have worked under his supervision. I got financial assistance mainly from GMRDC which was responsible for payment of my tuition fees and leaving allowance. Throughout my research work, I got funds to undertake the various experiments from the National Research Foundation (NRF) through its incentive research funding to rated researchers. I thank the GMRDC and NRF for generously funding my studies, which again contributed to me achieving my degree.

To undertake the various experiments, I got assistance from ESKOM<sup>®</sup> power stations, Ash Resources<sup>®</sup> and Ulula Ash<sup>®</sup> with the supply of the various fly ash samples and am truly grateful. During the various experiments that contributed my study, I was privileged to get more than two hands to assist me from both undergraduate and postgraduate students. Siyabonga Msani literally volunteered to assist me in my laboratory work and his hand made a great difference in my progress. I also worked with Dr B. Ravindran and Noxolo Lukashe in the EM work and vermicompost evaluation in the glasshouse, respectively, as partners in my studies which significantly improved the depth of my work and reduced some of the work load giving me more time to write my thesis. During my study, I got encouragement from Dr Ernest Dube to write a thorough literature review, and together we wrote a review paper (published) which highlighted possibilities of fly composting in improving its fertilizer value. I am forever grateful to the contribution of these colleagues in my studies. As we did the microbiology work in the Department of Biochemistry and Microbiology at the University of Fort Hare, we received a warm welcome from Dr Ezekiel Green who gave us unlimited access to the well-equipped microbiology laboratory. This made it possible for me to evaluate the various microbiological characteristics of my EM composts.

In my final year in May 2014, I was privileged to be assigned a hardworking NRF intern, Nolukholo Mketso, to assist me in my huge laboratory work. Her presence made an instant difference in my final year, which happened to be the busiest involving thesis and manuscript writing together with laboratory work. I thank the NRF internship programme and the intern herself for the assistance rendered. I also worked with a hard working young man, Mphakhamisi Trato, who not only provided me with labour assistance, but also helped me with his vast experience with working with composts. His dedication to duties assigned made

it possible for me to spend my time doing other things as he took away some of the manual load off me.

After the various tiresome, hard days in office, laboratory or farm, I had friends that helped me regain my energy through relaxing, giving me positive ideas and encouragement until I finished these studies. These friends include Dr A.D. Nciizah, Mr Carlos Nantapo, Mr Patrick Nyambo, Mr Tendayi Kadango and Mr Cosmas Parwada. Your presence and positive spirit helped me keep upright and indeed made me a better social being, regardless of the work pressure I endured. I also thank my colleagues in the Department of Agronomy who made it possible for me to co-exist with them regardless of my weaknesses, working with similar research machines and resources. The list is endless but include Mr Bothwell Musunda, Mr Frank Unuofin, Mr Godwin Nebo, Ms Linda Muzangwa, Ms Cleopatra Pfunde, Mr Isaac Gura, all postgraduate students, together with the various undergraduate students. I also acknowledge the various contributions to my studies both direct and indirect of the staff in my department (Dr J van Tol, Prof I. I. C. Wakindiki, Ms M Maphaha, Dr B.K. Eiasu, Mr T. Mthoko, Mr T. Ngqangweni, Mrs P. Macingwane).

Though I was far away from home, I kept getting encouragement from my mother Jenipher (a great woman), siblings and my in-laws, to soldier on as I wilted under the pressure of work. Thank you for understanding my absence from all those family issues that required attention, allowing me to focus more and entirely, during my study period.

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## CHAPTER ONE

### 1.0 GENERAL INTRODUCTION

---

#### 1.1 INTRODUCTION

The rapid urbanization and population growth in the 21<sup>st</sup> century has resulted in increased demand for most resources, including food, on the planet. This has led to intensification of agriculture and rapid industrialization in most countries, consequently increasing production of agricultural and thermal energy generation wastes. In most developing countries, including South Africa, more than 90% of the electricity generation is met through coal fired power stations. This intensification of agriculture and industrialization increases the carbon footprint and waste generation and yet there are no clear policies, infrastructure and financial resources for effective and sustainable management of these wastes (Gupta and Garg 2009).

In South Africa, where there are huge coal deposits, thermal electricity generation is a cheaper option versus nuclear or hydro electricity generation, making the country largely dependent on coal combustion for power generation (Petrik et al. 2003). Combustion of coal at thermal stations generates huge quantities of fly ash due to the relatively low calorific value of the coal utilized at most of these power stations (Kruger and Krueger 2005). Electricity generation through coal combustion in South Africa produces more than 28 million tons of fly ash annually, for which only 5% is utilized with the remainder being disposed in fly ash dams or heaps at sites near power stations (Kruger and Krueger 2005).

This fly ash poses a potential environmental hazard due to the presence of a mixture of potentially important nutrients together with toxic heavy metals (Basu et al. 2009). Due to the process involved with coal formation and leaching of rocks into coal beds, fly ash contains a broad range of elements suggesting that it can be a source of plant nutrients in agriculture (Bhattacharya and Chattodhyay 2002; Basu et al. 2009; Belyaeva and Haynes 2009). However, if applied directly into soil, the presence of potentially toxic heavy metals in fly ash, has potential to be leached in to soil and ground water, posing an environmental challenge. Also, most of the potentially essential nutrients present in fly ash are associated with the mineral phase and thus only small quantities can be immediately released for plant uptake (Schumann and Sumner 2000). Phosphorus is one of the primary fertilizer nutrients in fly ash with a relatively high total concentration with much of this P being however fixed into insoluble forms which are unavailable for plant uptake (Ram and Masto 2010). Improving the bioavailability of essential plant nutrients such as P in fly ash could increase its acceptability as a source of plant nutrients (Ram and Masto 2010).

Recently, there is also a growing realization of the role of vermicomposting in organic waste bio-degradation and stabilization (Ndegwa and Thompson 2000; Lazcano et al. 2008). Various species of earthworms e.g. *Eisenia fetida*, *E. andrei* (Ndegwa and Thompson 2000; Garg et al. 2006; Mupondi et al. 2010); *Limpito mauritii* (Ananthankrishnasamy et al. 2009) and *Eudrilus eugeniae* (Ravikumar et al. 2008) can consume diverse waste materials rapidly (Gupta and Garg 2009). However, the *Eisenia* species have been identified as the most effective organic waste decomposers during vermicomposting (Dominguez et al. 2011). These species are ubiquitous, resilient, can feed on a wide range of organic materials and have good tolerance to wider temperature and moisture regimes (Atiyeh et al. 2000a). Fly ash incorporation during vermicomposting can be a very effective way of utilizing this potentially

toxic waste product in agriculture (Bhattacharya and Chattopadhyay 2002). Gupta et al (2005) and Bhattacharya and Chattopadhyay (2002; 2006) studied the vermicomposting of fly ash mixed, however, with non-optimized organic material for vermicomposting. It would be interesting to investigate the potential nutrient benefits and biodegradation of fly ash incorporated into organic material whose carbon: nitrogen ratio has been optimized for *E. fetida* activity (Mupambwa et al. 2015).

Dairy cattle manure and waste paper are examples of waste products that are produced in large quantities in South Africa (Mupondi 2010). Waste paper can be used as carbon source and bulking agent during composting of organic waste materials like cattle manure mixed with industrial wastes (Gupta and Garg 2009; Mupondi 2010). During the vermicomposting of industrial waste like fly ash mixed with organic materials like cow dung requires an appropriate earthworm stocking density for effective biodegradation and nutrient release. Different stocking densities have been reported in the literature; e.g. Lazcano et al. (2008) working with cattle manure used 250 g of earthworms per kg of manure; Ananthakrishnasamy et al. (2009) working with cattle manure and fly ash used 15 g of earthworms per kg of substrate; Garg et al. (2006) using a mixture of organic materials used 14 worms per kg of material used; Ndegwa et al. (1999) recommended a stocking density of 1.60 kg worms/m<sup>2</sup> using bio-solids. Moreover, much of the work on stocking density in vermicomposting has focused mainly on the growth and development of the earthworms and not on compost maturity (Dominguez and Edwards 1997; Garg et al. 2008). Thus, there appears to be no agreed stocking density specific for various organic waste materials, during the vermicomposting process, for the attainment of a high quality vermicompost. It is therefore, of interest to establish the optimum earthworm stocking density for the bioconversion of cow dung-waste paper mixtures mixed with coal fly ash.

During composting, microorganisms have been shown to play a crucial role (Dominguez 2011). Some investigators have therefore suggested that microorganisms can be added to composting mixtures as a starter package to enhance the composting process. Many blends of beneficial organisms (BM) are used in different parts of the world. The microorganisms in these blends are added to compost to hasten the composting process and control undesirable odours and flies during composting. One blend which is being promoted in South Africa is EM (effective microorganisms) (Ncube et al. 2011). According to the Japanese inventors of the technology, EM is a multi-functional media that consists of natural and beneficial microorganisms, which form clusters to make a food chain, living in a symbiotic relationship. Effective microorganisms include predominant populations of lactic acid bacteria, yeasts, actinomycetes and photosynthetic bacteria (Yamada and Xu 2001). Anecdotal evidence suggests that the use of EM as an activator can bring down the thermophilic composting period from twelve weeks to four weeks. There is, however, no information available on the possible influence of EM on the vermicomposting of coal fly ash- organic waste mixtures. It would therefore be of interest, to determine if inclusion of EM would speed up the biodegradation of cow-dung, waste paper and fly ash mixtures.

Furthermore, the product from the composting of cattle manure incorporated with fly ash may have great potential as a planting medium for establishing of ornamental crops (Atiyeh et al. 2000a). Composting of potentially toxic organic waste materials like fly ash mixed with other organic wastes has great potential in creating “manufactured soils” which can be effectively utilized in the horticulture industry (Belyaeva and Haynes 2009). It is against this background that research was established to address the following objectives:

## 1.2 GENERAL OBJECTIVE

To optimize the bio-conversion and nutrient release from coal fly ash amended cow dung – waste paper composts using earthworms (*Eisenia fetida*) and Effective Micro-organisms.

### 1.2.1 SPECIFIC OBJECTIVES

- To characterize fly ash samples from selected South African coal powered electricity stations for elemental composition and nutrient release.
- To optimize the fly ash incorporation into cow dung – waste paper mixtures for enhanced biodegradation and nutrient mineralization.
- To determine the ideal *Eisenia fetida* stocking density for the vermicomposting of fly ash - cow dung-waste paper mixtures.
- To determine the effectiveness of Effective Micro-organisms (EM) during vermicomposting in enhancing the biodegradation of fly ash incorporated into cow dung-waste paper mixtures.
- To determine the effectiveness of fly ash – cow dung – waste paper mixture vermicompost substituted into pine bark compost on media physico-chemical properties and ornamental crop growth.

### Specific hypothesis

- Fly ash samples from selected power stations in South Africa differ in their elemental composition and nutrient release properties.
- There is a difference in rate of biodegradation and nutrient mineralization during vermicomposting of cow dung – waste paper mixtures incorporated with various levels of fly ash.

- Stocking density affects the rate of bio-degradation and nutrient mineralization in fly ash – cow dung – waste paper mixtures during vermicomposting.
- Effective Micro-organisms inoculation and earthworm presence affect the chemical and biological processes during the vermicomposting fly ash – cow dung – waste paper mixtures
- Fly ash – cow dung – waste paper vermicompost improve pine bark compost physico-chemical properties and is an effective source of plant nutrients for ornamental plants.

## CHAPTER TWO

### 2.0 LITERATURE REVIEW

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This Chapter is based on the manuscript entitled:

Mupambwa HA, Dube E, Mnkeni PNS. Forth coming 2015. Fly ash composting to improve fertilizer value – A review. *South African Journal of Science (Accepted)*.

## **2.1 Abstract**

South Africa is increasingly reliant upon coal fired power stations for electricity generation. Fly ash, a by-product of coal combustion, contains a high total content of essential plant nutrients such as phosphorus (P), as well as heavy metals. If the plant nutrient bio-availability in fly ash could be improved, and the toxic element content reduced, fly ash could contribute significantly as a fertilizer source in South African agriculture. The review aims to summarize up-to-date information on the soil fertility and detoxification benefits of fly ash composting, and to identify information gaps in this regard. Scientific studies investigating the potential of fly ash-based composts to supply plant nutrients and to contaminate the environment are presented and discussed. The roles of earthworms and micro-organisms in improving the decomposition process, hence fertilizer value of fly ash composts, are explored. Although much progress has been made, further research efforts are required to optimise microbial and earthworm activity in the decomposition process, which could further enhance nutrient supply benefits and reduce toxic elements at higher fly ash incorporation rates.

## 2.2 Introduction

There has been rapid increase in population growth, urbanization, expansion in agricultural production and industrialization in South Africa over the past two decades. This has greatly increased electricity demand, and South Africa generates more than 90% of this electricity through coal combustion (Seshadri et al. 2010a). South Africa has vast coal deposits, mainly in the Central Basin, covering the Witbank, Highveld and Ermelo (Eberhard 2011). Coal seams that are relatively thick and close to the surface (15 - 50 m) allow low cost coal mining, making thermal electricity power stations a much cheaper option than hydro and nuclear power stations in South Africa (Eberhard 2011). Coal fired power stations will remain the principal power generation source for South Africa in the foreseeable future as evidenced by construction of two new coal fired stations (the 4764 MW Medupi and 4800 MW Kusile plants) (Eberhard 2011; Baker 2011). During coal combustion, fly ash, which is the fine airborne solid residue captured from exhausts through electrostatic precipitators, is obtained, constituting more than 70% of the solid waste (Haynes 2009). Fly ash is composed of oxidised, non-combustible materials which are very fine in size (0.01 to 1000  $\mu\text{m}$ ) and is generally greyish in colour (Seshadri et al. 2010a). Most fly ash particles are spherical in shape and the average diameter is 10  $\mu\text{m}$  (Ukwattage et al. 2013). The chemical characteristics of fly ash vary widely and are influenced by coal combustion processes, age of the ash and most importantly, coal characteristics (Basu et al. 2009; Ram and Masto 2010).

Coal is classified into three broad groups based on organic maturity – namely; lignite, bituminous and anthracite coal (Seshadri et al. 2010a). Bituminous and sub-bituminous coals constitute more than 90% of South African coal and they are characterised by higher contents of CaO (5 - 40%), MgO (1 - 10%) and SO<sub>3</sub> (0 - 10%) when compared to the higher grade anthracite coals (Seshadri et al. 2010b; Gitari 2006; Blissett and Rowson 2012). These

different groups of coal also yield different classes of fly ash. Class F fly ash has a low total Ca content which ranges from 1 - 12%, and is derived mainly from bituminous and anthracite coals, while Class C fly ash has a Ca content as high as 30 - 40% and is derived from lignite and sub-bituminous coals (Seshadri et al. 2010a; Gitari et al. 2010). Class F fly ash is mostly used in the construction industry for cement making, brick making and as road bed material because of its high pozzolanic (cementing) effect (Seshadri et al. 2010a). Class C fly ash has a high Ca content, which makes it a potential neutralizing agent in acid mine drainage and acidic soils (Gitari 2006).

The physico-chemical properties of fly ash are determined primarily by the type of coal burned to produce the fly ash, hence there is significant variation in fly ash quality between and even within regions of production. The general chemical composition of fly ash is metal oxides that occur in the order  $\text{SiO}_2 > \text{Al}_2\text{O}_3 > \text{CaO} > \text{MgO} > \text{K}_2\text{O} > \text{NaO} > \text{TiO}_2$  as highlighted in Table 2.1 (Blissett and Rowson 2012). Using X-ray diffraction (XRD), Gitari et al. (2009) showed that the major crystalline mineral phase in typical fly ashes are quartz ( $\text{SiO}_2$ ) and mullite ( $3\text{Al}_2\text{O}_3 \cdot 2\text{SiO}_2$ ), with lesser amounts of magnetite, maghemite together with lime and calcite, which give fly ash its alkaline pH. The lime occurs as particles on the surface of the fly ash spheres and is thought to originate from decarbonation of dolomite or limestone impurities during coal formation (Gitari 2006). Fly ash also contains toxic heavy metals which originate from rock weathering into coals basins (Table 2). From an agronomic point of view, fly ash is a potential fertilizer for crop production as it contains essential elements such as phosphorus (P), potassium (K), sulphur (S), sodium (Na) and magnesium (Mg) that are potentially beneficial to crop growth. The various concentrations of plant available and easily available (water soluble) nutrients, heavy metals and metalloids in fly ash are presented in Table 2.2 (Gitari et al. 2009; Izquierdo and Querol 2012).

**Table 2. 1:** Typical chemical concentrations of major elements as oxides in different fly ashes as analysed using X-ray fluorescence (XRF).

Component	Range (mass %)			
	Europe <sup>a</sup>	China <sup>a</sup>	India <sup>a</sup>	South Africa <sup>b</sup>
SiO <sub>2</sub>	28.5 – 59.7	35.6 – 57.2	50.2 – 59.7	50.1 – 67.0
Al <sub>2</sub> O <sub>3</sub>	12.5 - 33.6	18.8 – 55.0	14.0 – 32.4	23.4 – 27.0
Fe <sub>2</sub> O <sub>3</sub>	2.6 – 21.2	2.3 – 19.3	2.7 – 14.4	2.7 – 4.7
CaO	0.5 – 28.9	1.1 – 7.0	0.6 – 2.6	6.4 – 8.7
MgO	0.6 – 3.8	0.7 – 4.8	0.1 – 2.1	1.9 – 2.7
Na <sub>2</sub> O	0.1 – 1.9	0.6 – 1.3	0.5 – 1.2	0 – 1.3
K <sub>2</sub> O	0.4 – 4.0	0.8 – 0.9	0.8 – 4.7	0.5 – 0.9
P <sub>2</sub> O <sub>5</sub>	0.1 – 1.7	1.1 – 1.5	0.1 – 0.6	0.3 – 0.89
TiO <sub>2</sub>	0.5 – 2.6	0.2 – 0.7	1.0 – 2.7	1.3 – 1.6
MnO	0.03 – 0.2	nd	0.5 – 1.4	0.04 -0.5

Source: <sup>a</sup> Blissett and Rowson (2012); <sup>b</sup> Gitari et al. (2009), Gitari (2006) and Gitari et al. (2005)

**Table 2. 2:** Typical elemental concentrations of total, plant available (mg/kg) and easily available fraction (%) of selected fly ash samples.

Element	Total concentration mg/kg	Plant available fraction mg/kg	Easily soluble fraction <sup>d</sup> %	Element	Total concentration mg/kg	Plant available fraction mg/kg	Easily soluble fraction <sup>d</sup> %
P	553.3 – 1197.3 <sup>a</sup>	130.0 – 256.2 <sup>a</sup>	nd	Cu	32 – 54 <sup>a</sup>	0.2 – 0.9 <sup>a</sup>	0.17 – 0.92
K	0.15 – 3.5 <sup>b</sup>	nd	0.23 – 0.25	B	17 – 38 <sup>c</sup>	0.5 – 0.8 <sup>c</sup>	nd
Ca	0.11 – 22.2 <sup>b</sup>	nd	15.84 – 24.23	As	1.0 – 4.0 <sup>c</sup>	BDL <sup>c</sup>	BDL
Mg	0.04 – 7.6 <sup>b</sup>	nd	0.0047 – 0.0062	Cd	5 – 10 <sup>c</sup>	0.03 – 0.07 <sup>c</sup>	BDL
Na	0.01 – 2.03 <sup>b</sup>	nd	0.76 – 0.82	Cr	143.7 – 488.3 <sup>a</sup>	0.36 – 1.0 <sup>a</sup>	0.22 – 0.54
Al	0.1 – 17.3 <sup>b</sup>	nd	0.0005 – 0.0019	Ni	33.3 – 69.8 <sup>a</sup>	0.2 – 0.3 <sup>a</sup>	BDL
Fe	3000 – 6111 <sup>a</sup>	4.83 – 136.0 <sup>a</sup>	0.00049 – 0.001	Pb	26.5 – 121.3 <sup>a</sup>	0.17 – 0.42 <sup>a</sup>	BDL
Mn	500 – 750 <sup>c</sup>	0.9 – 1.5 <sup>c</sup>	BDL	Co	10 – 50 <sup>c</sup>	0.05 – 0.15 <sup>c</sup>	BDL
Zn	9.7 – 23.7 <sup>a</sup>	0.6 – 0.7 <sup>a</sup>	0 – 0.12	Se	0.6 – 2.6 <sup>c</sup>	0.1 – 0.4 <sup>c</sup>	2.17 – 4.83

Source: <sup>a</sup>Mupambwa and Mnkeni (2015a); <sup>b</sup>Basu et al. (2009); <sup>c</sup>Ram and Masto (2010); <sup>d</sup>Gitari et al. (2009); nd = no data; BDL = below detectable limits.

### **2.3 Agricultural application of fly ash**

Fly ash is an abundant waste material which has a high total concentration of essential plant nutrients, but low bioavailability of the nutrients greatly limits its direct use in agriculture (Bhattacharya and Chattopadhyay 2006; Yadav and Garg 2011). The low nutrient bioavailability is apparent with essential nutrients such as P as highlighted in Table 2. This is partly due to the low microbial activity in fly ash, which limits its mineralization. Even when applied to the soil, fly ash has been reported to severely inhibit microbial respiration, enzyme activity and nitrogen (N) cycling processes (Jala and Goyal 2006; Pandey and Singh 2010). The inhibitory effects of fly ash when applied to soil have been mainly observed in alkaline fly ashes such as the ones existing in South Africa and this is mainly attributed to the high salinity, pH, B toxicity and lack of substrate carbon (C) and N (Haynes 2009). Schumann and Sumner (2000) also highlighted that the major pitfalls in direct use of fly ash include low supply of major plant nutrients, nutrient deficiencies caused by unfavourable pH, slow nutrient release and fixation of other nutrients already present in soil solution, such as P.

Globally, the utilization of the various classes of fly ash falls within the 0 - 30% range, with most developing countries including South Africa utilising less than 5% of the fly ash that they produce, and mostly in the construction industry (Haynes 2009; Gitari et al. 2010). In the USA, China, and India, fly ash generation ranges from 30 - 130 million t/year, and for South Africa it is more than 28 million t/year (Haynes 2009; Gitari et al. 2010). Enormous quantities of fly ash remain unused in South Africa, and they are deposited into fly ash heaps or dams close to the power stations. They are not only an eyesore, but also a public health and environmental hazard through fly ash erosion and leachate generation, which may result in sub soil siltation and heavy metal pollution (Malik and Thapliyal 2009). Information on

leachate chemistry and contaminants attenuation in acid mine drainage by fly ash and its derivatives in South Africa is available from work done by Gitari (2006).

Much research has been carried out to demonstrate that direct application of fly ash to the soil increases the heavy metal concentration in crops and sometimes, in the soil. For example, Pandey et al. (2009) mixed fly ash with garden soil at various ratios i.e. 0%, 25%, 50% and 100% (w/w) and used it as a planting media for *Cajanus cajan*. They observed that heavy metal (Fe, Zn, Cu, Cr, Cd) accumulation in the crop was highly responsive to increases in the fly ash application rate. Similarly, Bilski et al. (2012) observed higher concentrations of all heavy metals in fly ash treatments compared to the soil alone when they evaluated the germination and subsequent heavy metal accumulation during early growth of selected cereal crops. This higher bioaccumulation of heavy metals in fly ash amended treatments has been reported by several other researchers (Pandey et al. 2010; Gautam et al. 2012). Apart from the low nutrient bioavailability, it appears that another major concern from direct application of fly ash to the soil in crop production is the potential accumulation of toxic heavy metals in crops. Direct application of fly ash to the soil has some positive effects, but these tend to be outweighed by the negative effects as summarised in Table 2.3.

It is generally agreed that addition of large quantities of fly ash to soils should be done with special consideration of pH and intensive monitoring of heavy metals (Ukwattage 2013). There is much concern about the possible loading effects of these heavy metals through continual soil application of fly ash and the possible leaching of the metals into ground water (Yunusa et al. 2009). This limits direct utilisation and approval of fly ash as a source of plant nutrients for most edible crops. In order to address this challenge, much research has since been dedicated towards bio-remediation strategies for fly ash, such as composting.

**Table 2. 3:** Some liming and crop nutrient supply effects from direct soil application of different fly ashes under various agricultural systems.

Experimental objective, fly ash and soil characteristics	Experimental conditions and treatments	Observations	References
To evaluate the effects of soil (Ultic and Typic Hapoxeralf) application of aged alkaline fly ash from two power stations on pH, salinity, available B and P, growth and uptake of B and P by rye grass. Soils had a pH of 4.7 and 5.8. Fly ash was (6 month old) and had a pH of 8.9.	Pot experiments were carried out with fly ash being incorporated at 0, 5, 20 and 50 g/kg – soil. Rye grass was grown in the pots for 300 days and well watered as well as fertilised. Plant samples were harvested five times for nutrient determination.	Direct fly ash addition to the soil increased pH to an average of 7.03 compared to 5.25 for the control. Originally, all the fly ashes had very low plant available P and B, hence application did not result in any significant increase in soil P and B. However, the application of fly ash did significantly increase plant P and B. This highlights the need to consider the effects of fly ash on toxic nutrient concentrations in plants, even when it may not have apparent effects on soil concentration. Boron is toxic to plants at very low concentrations.	Matsi and Keramidas. (1999)
To determine the impact of fly ash from Western Australia on soil physical and chemical properties, heavy metals and subsequent growth of turf grass. Fly ash had a pH of 5.5 to 7.9 and the sandy soil had a pH of 4.7 (CaCl <sub>2</sub> ).	Fly ash was applied at 0, 73, 150 and 300 t/ha. It was incorporated into the soil and 7 days later, turf grass ( <i>Cynodon dactylon</i> ) was planted.	Direct fly ash incorporation into the soil at all levels resulted in a significant increase of soil extractable P (18.5, 42.6, 46.1 and 51.2 mg/kg, respectively) but not leaf tissue P. However, a significant increase in heavy metals was also realized for Cd, Mn, Se and Zn.	Pathan et al. (2001)

Experimental objective, fly ash and soil characteristics	Experimental conditions and treatments	Observations	References
<p>To clarify the differences among plant species in their response to fly ash amendment. Two types of fly ash that were used were derived from sub-bituminous and alkaline coal, and had a pH of 10.8 and 9.0, respectively. The potting mixture (50:50 sand/peat mixture) had a pH of 6.5.</p>	<p>The test crops were canola, radish, field peas, lucerne, barley and rye. Radish and rye grass were planted in potting mixture and the other crops were planted in soil. Ashes were applied to the pots at 0, 2.5, 5.0, 10 and 25 t/ha and fertilizer (8:3:8) applied at 20 days after planting.</p>	<p>Both types of fly ash significantly increased growth rates and concentrations of chlorophyll <i>a</i> and <i>b</i> at application levels of 5 t/ha, but reduced carotenoid concentrations. Addition of ash at all rates increased CO<sub>2</sub> assimilation of barley and radish. Application of ashes up to 5 t/ha also increased transpiration in barley only. In this study, all crops showed a general difference in response to fly ash application rates, highlighting the need for crop specific recommendations for field application of fly ash.</p>	Yunusa et al. (2009)
<p>To investigate the impact of fly ash amendment of soil on microbial responses, extent of heavy metal accumulation in the soil and rice crop growth. Unweathered fly ash with a pH of 7.7 and soil (Inceptsol) with a pH of 5.8 (in water) were used.</p>	<p>Pot experiments were carried out using 10 kg soil mass after fly ash amendment at 0, 5, 10, 20, 40 and 100 % on a volume basis. Each pot was planted with 25 day old rice seedlings and fertilizer (20-40-20) was applied to each pot. Destructive sampling was done at panicle initiation and at harvest.</p>	<p>Significant increases in crop growth parameters (chlorophyll content, plant height, leaf area index, number of panicles) were observed at fly ash application rates of 5 - 20%. Beyond 20% direct fly ash incorporation, significant differences were observed on heavy metals (Fe, Mn, Zn, Cu, Pb, Cr and Cd). Application of fly ash above 40% significantly decreased the microbial population dynamics and enzyme activity. These results highlighted the potential heavy metal toxicity effects of fly ash which are likely to be greater under repeated applications.</p>	Nayak et al. (2014)

## **2.4 Problem statement**

If the plant nutrient bioavailability in fly ash could be improved, and the toxic element content reduced, or bio-absorbed, fly ash could contribute significantly as a nutrient source in South African agriculture. As a potential solution to this problem, there is interest in research for refining the fly ash composting strategies in a cost effective and environmentally sustainable way. The present review summarises up-to-date information on the effects of fly ash composting. It identifies information gaps in regard to fly ash composting science, with the aim of guiding future research programs for use of fly ash as a nutrient source in agriculture. This review is guided by the following questions:

1. Can the bioavailability of plant nutrients from fly ash be improved significantly through refining the composting strategy?
2. Can the plant available fraction of heavy metals from fly ash be managed through refining the composting strategy?

## **2.5 Improving decomposition and plant available nutrients of fly ash-based composts**

The soil nutrition improvement capacity of fly ash composts is highly variable, largely depending on the chemical characteristics of the fly ash, incorporation ratio of the fly ash and composting technique. The variations in fly ash elemental content (total and plant available nutrients) have been presented in Table 2.2. The most abundant primary fertilizer nutrient in fly ash is P, and it is also a major limiting nutrient to crop production (Bhattacharya and Chattopadhyay 2006). Therefore, although fly ash composts supply other important plant nutrients, this review focuses mainly on P supply.

### ***2.5.1 Traditional composting of fly ash***

Traditional composting, known scientifically as thermophilic composting, is probably the oldest and most widely applied method of enhancing the fertilizer value of waste materials. It can be described as “the accelerated degradation of organic matter by microorganisms under controlled conditions, during which the organic material undergoes a characteristic thermophilic phase (45°C – 65°C), which allows sanitization of the waste by the elimination of pathogenic microorganisms” (Dominguez and Edwards 2011). During the thermophilic stage, high microbial activity increases respiration and C loss, resulting in a lower C: N ratio of the compost. A lower C: N ratio is one of the important determinants of a mature compost (Raji and Antil 2011). The end product of thermophilic composting is a stabilized and well humified compost which should have a higher fertilizer value than the constituent materials, and no pathogens. A major disadvantage of thermophilic composting is the loss of N through volatilization of ammonia during the thermophilic stage (Lazcano et al. 2009).

Fly ash contains 0 – 0.2% N and 0 – 0.34% C, making it an inorganic by - product (Basu et al. 2009; Jala and Goyal 2006). It cannot support microbial activity and it is not possible to decompose fly ash biologically, unless a rich and balanced C and N source is added to the compost (Anbalagan and Manivannan 2012). Hence, numerous studies have been carried out to evaluate various organic substrates as additives in biological decomposition of fly ash. Fang et al. (1999) tested the decomposition characteristics of alkaline fly ash and sewage sludge mixtures (C: N ratio of 25) and reported that fly ash incorporation rate for sewage sludge composts should not exceed 35% because the decomposition index, used to evaluate compost maturity, would be significantly decreased. This was attributed to the inhibitory effect of alkaline fly ash on thermophilic micro-organisms during decomposition. The high pH also causes loss of essential N through ammonification and volatilization, thus greatly

reducing microbial activity. A progressive decrease in thermophilic bacterial population and diversity was also observed when municipal green waste was amended with fly ash at 0, 25, 50, 75 and 100% (w/w) (Belyaeva and Haynes 2009). The amended treatments did not reach the thermophilic phase during composting, with no or little self-heating observed beyond 75% fly ash incorporation rates. A major challenge in thermophilic composting is therefore the substantial reduction of microbial activity and decomposition rate.

Microbial activity during biological decomposition process produces organic acids that can solubilise minerals associated with phosphates in fly ash, resulting in increased availability of P and other essential plant nutrients (Imran et al. 2011). Evidence of the occurrence of phosphate solubilizing microbes (PSM) has existed since the early 20<sup>th</sup> century and these have been used as bio-fertilizers since the 1950's (Khan et al. 2009). Within PSM, the phosphate solubilizing bacteria (PSB) are more effective compared to the phosphate solubilizing fungi (PSF), and they generally constitute from 1 - 50% of the soil microbial population (Khan et al. 2009). These PSM release low molecular weight organic acids that bind to cations associated with the phosphate, thus converting it into plant available forms (Chen et al. 2006). Much of the research to show the benefits of PSM has focused on solubilisation of P in rock phosphate, Sibi (2011); Aria et al. (2010), and limited information is available on the role of PSM strains in enhancing solubilisation of fly ash P.

It would be interesting to test the effects of special microbial cocktails such as “effective micro-organisms (EM)” on the decomposition rate in fly ash-based composts. According to Japanese inventors of the technology, EM is a mixed culture of natural and beneficial micro-organisms, which form clusters to make a food chain, living in a symbiotic relationship (Yamada and Xu 2013; Mupondi et al. 2006a). Effective microorganisms include

predominant populations of lactic acid bacteria, yeasts, actinomycetes and photosynthetic bacteria (Yamada and Xu 2013). Anecdotal evidence suggests that the use of EM as an activator can bring down the traditional composting period from twelve weeks to four weeks (Freitag 2000). At present, in reference to traditional composting of coal fly ash - organic waste mixtures, there is a paucity of information on the effects of EM on composting processes. It is necessary to determine if the groups of micro-organisms within the EM cocktail are sufficiently resilient to remain active during composting of fly ash mixtures, and if so, identify the optimal EM inoculation level.

### ***2.5.2 Vermicomposting of fly ash***

Earthworms have an important role to play in enhancing bio-degradation and stabilization of organic wastes. Vermicomposting has been defined as a process in which earthworms interact with micro-organisms and soil invertebrates within the decomposer community, strongly affecting decomposition processes, accelerating the stabilization of organic matter, and greatly modifying its physical and biochemical properties (Dominguez 2011). Earthworms are the crucial drivers of the process, as they mechanically fragment the waste with their gizzards and increase substrate surface area, thus altering micro-flora activity (Lazcano et al. 2008). Earthworms significantly increase conversion of micronutrients into plant available forms in fly ash and cow dung compost mixtures (Bhattacharya and Chattopadhyay 2006). However, as indicated previously, fly ash is devoid of C and N, which are essential components for any biological process, hence for vermicomposting, fly ash should also be amended with a C and N source. There is theoretical evidence suggesting that microbial activity in fly ash-based composts can be enhanced by adding earthworms to the composts (Bhattacharya and Chattopadhyay 2006). Earthworms modulate the microbial community and tend to selectively feed more on fungi than on bacteria (Dominguez 2011). The earthworms

carry microbes in their digestive system, possibly shielding them from the direct adverse environment brought about by fly ash addition. Thus, better results at higher incorporation rates of fly ash up to 50% to organic waste have been reported when earthworms were added to the compost (Bhattacharya and Chattopadhyay 2006). During vermicomposting, earthworms secrete mucus which moistens the waste and also provide a more habitable environment for waste biodegradation by the gut micro-organisms. Research is, however, required to determine the interactions of various earthworm species with combinations of EM.

There are more than 3000 known species of earthworms, and these can be divided into three categories based on their feeding behaviour, burrow habit, habitat, body size, fecundity, casting activity and mobility (Lazcano et al. 2009). Surface feeding earthworms, known as “epigeic earthworms” have an important role to play in organic waste bio-degradation and stabilization (Nayak et al. 2014; Fang et al. 1999). This group of earthworms is widely used for vermicomposting and include *Eisenia fetida*, *Eisenia andrei* and *Eudrilus eugeniae* (Yunusa et al. 2009; Fang et al. 1999; Belyeva and Haynes 2009). *Eisenia* species could be the most effective organic waste decomposers during vermicomposting (Pathan et al. 2001). This specie is ubiquitous, resilient, can feed on a wide range of organic materials and has good tolerance to a wide temperature and moisture range (Pathan et al. 2001; Yamada and Xu 2013).

Cow dung appears to be one of the most commonly preferred substrate for enriching fly ash with C during vermicomposting. Several studies have evaluated the transformation of nutrients during fly ash vermicomposting using various species of earthworms, mixed at various ratios with cow dung and other waste materials. Using an unspecified earthworm

specie, Bhattacharjee et al. (1999) evaluated cow dung, soil and fly ash mixtures. The cow dung was first mixed with soil at a ratio of 2: 5 and then fly ash was incorporated at six levels of 5, 10, 15, 25, 40 and 50% w/w, to achieve a final weight of 700 g. These mixtures were moistened to 40 – 45 % and then inoculated with 25 earthworms. The earthworms not only survived in cow dung – soil mixture amended with fly ash up to 25%, but they also bio-accumulated Pb in their bodies. However, the moisture content used in this study (40 – 45%) may not have been the optimum for maximum activity of the earthworms, which prefer moisture contents of 50 - 90% (Dominguez and Edwards 2011). The pH of the cow dung – soil – fly ash mixtures was not optimised in this study, and this could have affected the vermicomposting process. With an optimal moisture content and pH level, it is possible that earthworms can tolerate a higher fly ash amendment ratio than the 25% reported in this study.

In another cow dung-fly ash vermicomposting study, Bhattacharya and Chattopadhyay (2002) evaluated the potential of *E. fetida* in improving compost plant available P levels at 25%, 50% and 75% fly ash: cow dung mixing ratios. Earthworms proved superior in increasing the phosphate utilizing bacteria responsible for conversion of P to plant available forms compared to the no earthworm control. The fly ash incorporation ratios of 50%, 25% and 75% fly ash contributed 42.8; 10.8 and 12.7 mg/kg of P, respectively after 50 days. However, it also appears that in this study, the C: N ratio and earthworm stocking density were not optimized for effective vermicomposting. It is possible that even better results could have been obtained with an optimal substrate C: N ratio and earthworm stocking density. Other fly ash vermicomposting studies, e.g. Ananthakrishnasamy et al. (2009) did not report plant available nutrients in the composts, but rather measured the total nutrients which are most likely to increase due to the concentration effect from weight loss during composting, rather than exclusively earthworm activity. Substrate C: N ratio and earthworm stocking

density strongly influence the vermicomposting process (Ndegwa and Thompson 2000). There is also a lack of information on the types of microbes that flourish under different fly ash incorporation ratios from these studies. Such information is required as it could form a basis for development of specialized microbial cocktails for effective bio-conversion of fly ash. Recent studies at the University of Fort Hare in South Africa, using *E. fetida* and fly ash, cow dung and waste paper mixtures indicated that a 2: 1 (cow dung – waste paper: fly ash), which gives a C: N ratio of approximately 30 may be the most appropriate as reflected by rapid decomposition and increase in extractable P (Mupambwa and Mnkeni 2015b).

Fly ash-cow dung compost mixtures may, sometimes, have less extractable P compared to the cow dung alone (Bhattacharya and Chattopadhyay 2006). This problem is attributed to microbial community modification as evidenced by very low levels of PSB in fly ash composts (Bhattacharya and Chattopadhyay 2006). In India, Bhattacharya and Chattopadhyay (2002) composted cow dung and fly ash at various ratios (1:1; 1:3 and 3:1) for 50 days at room temperature and observed an average occurrence of PSB of  $0.067 \times 10^8/\text{g}$  for the fly ash treatments compared to  $4.63 \times 10^8/\text{g}$  for the cow dung alone. This microbial modification can be corrected by introducing earthworms as shown in the follow-up study, where the average occurrence of PSB significantly increased to  $30.3 \times 10^8/\text{g}$  compared to  $33 \times 10^8/\text{g}$  for cow dung alone. This also corresponded with the fly ash amended treatments which yielded 54.7% (79.9 mg/kg) more extractable P under vermicomposting than the same treatments without earthworms, which had 51.6 mg/kg (Bhattacharya and Chattopadhyay 2006)

## **2.6 Reducing the content of toxic heavy metals in fly ash composts**

Toxic heavy metals in fly ash limit its use as a direct source of nutrients in agriculture. A high soil concentration of toxic heavy metals hinders soil microbial activity, Pandey and Singh (2010), thus affecting vital soil processes such as nutrient mineralization and effectively sterilizing the soil. The plant availability of heavy metals following fly ash addition to soil tends to be variable and is controlled by the presence of Mn, Al and Fe oxides, carbonates, pH and other anions (Seshadri et al. 2010a; Adriano et al. 2002). For example, above pH 6, an increase in surface charge on oxides of Fe, Al and Mn which is pH dependent, coupled with binding by organic matter, greatly lowers metal availability in soil (Adriano et al. 2002). Whilst a once off application of fly ash compost to the soil at moderate levels does not seem to pose much challenge with heavy metals, the potential heavy metal load increase over time due to continuous application of fly ash is a cause for concern. Hence, any activity that will further reduce the level of heavy metals in fly ash composts is important as it will result in less risk associated with continuous fly ash compost application to soil. There is, however, a lack of information on the heavy metal dynamics in soil under continuous application of fly ash composts in the current literature, and it may be necessary to establish or model the cumulative heavy metal load associated with such.

Earthworms have the capacity to bio-accumulate heavy metals, suggesting that earthworm harvests from the composts can be used to reduce the heavy metal load (Mupondi 2010; Gupta et al. 2005; Neuhauser et al. 1995; Li et al. 2010). The effects of vermicomposting as a possible way of reducing the heavy metal concentrations of fly ash and cow dung mixtures have been investigated (Gupta et al. 2005). Gupta et al. (2005) in an experiment, started with 2 kg of feed consisting of proportions (20%, 40%, 60% and 80% w/w) of fly ash in cow dung with 125 mature earthworms (*E. fetida*). After 30 days, the earthworms and casting were

separated and the reactor contents discarded, and a new set of 2 kg material added to which the earthworms from previous 30 day period, were added. A total of 6 runs of 30 days each were done following which various parameters were determined. Reductions of 85%, 77.2%, 68.8% and 33.5% for Cr and 78.8%, 69.4%, 83.7% and 25.3% for Pb when fly ash was incorporated at rates of 20%, 40%, 60% and 80%, respectively, were reported from these studies. Gupta et al. (2005) reported that earthworms bio-accumulated on average 58.1 mg/kg Cr and 42.8 mg/kg Pb after 180 days of vermicomposting fly ash and cow dung mixtures which had on average 52.5 mg/kg Cr and 43.5 mg/kg Pb (Gupta et al. 2005). A decrease in levels of easily extractable Cr, Cd and Pb in all treatments due to vermicomposting after 50 days compared to the respective treatments without earthworms was also reported from a vermicomposting study by Bhattacharya and Chattopadhyay (2006).

A refinement of the composting strategy will indeed improve the nutrient bioavailability and reduce the heavy metals in the large quantities of fly ash produced by coal fired power stations. Although there is currently limited scientific information in literature on the economics of composting, and more especially fly ash composting, the proposed technologies for fly ash composting make use of cheap and abundantly available waste materials such as cow dung, food waste, saw dust and waste paper. The earthworms and microbial populations do not require specialized, artificial conditions. As such, the cost associated with the composting of huge fly ash quantities should be minimal and the composting can be done at subsistence or commercial scale, enabling the production of cheaper fertilizer.

## **2.7 Conclusions**

A comprehensive, up-to-date review of research on improving the fertilizer value of fly ash-based composts has hitherto been unavailable. In this article, scientific studies on fly ash composting have been discussed to explore information gaps towards refining fly ash composting science. Sewage sludge, cow dung, paper and food waste are the organic substrates that are most commonly tested as sources of C and N in fly ash composting. In this case, decomposition rate, hence nutrient release, is strongly influenced by fly ash: organic waste mixing ratio, as well as the C: N ratio of the organic waste. The composts show a great potential to supply the major elements, especially P, for crop production. A major drawback to biological decomposition of fly ash appears to be the reduction of microbial activity, population and diversity. Earthworms and special microbial cocktails such as EM, PSM and other bio-inoculants are a potential solution to this problem. Research is required to identify the microbes that tolerate high concentrations of fly ash amendment during composting. Fly ash composting appears viable mostly at low incorporation rates ranging from 5% to 25% and at these low application rates, the heavy metals emanating from fly ash composting may not be a serious challenge as they fall within permissible limits outlined for other wastes such as sewage sludge in South Africa. However, repeated applications of fly-ash composts to the soil over time may increase the heavy metal load to toxic levels. In this regard, research efforts aimed at further reducing the heavy metal load in fly ash composts are required.

## CHAPTER THREE

### 3.0 ELEMENTAL COMPOSITION AND RELEASE CHARACTERISTICS OF SOME SOUTH AFRICAN FLY ASHES AND THEIR POTENTIAL FOR LAND APPLICATION

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This Chapter is based on a manuscript entitled:

Mupambwa H.A. and Mnkeni P.N.S. 2015a. Elemental composition and release characteristics of some South African fly ashes and their potential for land application. *Archives of Agronomy and Soil Science*. doi: 10.1080/03650340.2015.1017567.

**(See Appendix 1)**

### 3.1 ABSTRACT

Eight fly ash samples collected from South African power stations were evaluated for various chemical properties, liming potential and metal species release under incubation. All fly ashes had alkaline pH ranging from 10.97 to 12.75, with much wider variations observed on electrical conductivity (range 0.46 to 8.27 dS/m). Their total P content ranged from 553.3 to 1514 mg P/kg and Olsen extractable P from 130 to 345.5 mg P/kg. Application of two of the fly ashes to three different soils showed high ability to neutralize acidity in the three different soils used, resulting in an average 41 % change in pH after 8 weeks of incubation. Across all three soils, the fly ash incorporation increased extractable P content from a P deficient level to levels above 25 mg P/kg in two of the three soils. Except for Cu, all metal species (Cr, Pb, Ni and Fe) showed significantly ( $P \leq 0.05$ ) low extractability under fly ash treated soils compared to the soil alone control. These results suggest that the South African fly ashes studied are effective liming materials and can provide essential elements like P with minimum risk to soil contamination from metal species release.

**Keywords:** Fly ash, phosphorus release, incubation study, soil contamination, heavy metal species

### 3.2 INTRODUCTION

Throughout the world availability of huge coal deposits favour thermal electricity generation as a much cheaper energy option over nuclear or hydro-electricity generation. However, the use of coal combustion for electricity generation results in production of large quantities of fly ash coupled with large quantities of greenhouse gases. Fly ash is the fine airborne solid residue captured from exhausts through electrostatic precipitators during coal combustion and it constitutes approximately 75% of the residues of coal combustion (Haynes 2009). Among the highly industrialised countries like the United States, China and India; South Africa ranks highest with more than 95% dependency on coal for energy generation (Seshadri et al. 2010a). Coupled with the relatively low calorific value of South African coals, this greatly increases the quantities of fly ash residue during coal combustion at power stations (Kruger & Krueger 2005). Globally, fly ash is mainly utilized in the construction industry for road bed material, brick making and cement making, with this utilisation falling within the 30% range whilst most developing countries utilize around 5% only of the millions of tonnes of fly ash generated each year (Haynes 2009; Gitari et al. 2010). The production of coal combustion products is likely to increase as the generation of energy through coal continues to increase, creating more disposal challenges (Seshadri et al. 2010a). With close to 95% of fly ash being disposed in ash dams or heaps, this has driven researchers to evaluate the potential for utilisation and the consequences associated with usage of fly ash (Gitari et al. 2010; Izquierdo & Querol 2012; Bhattacharya et al. 2012). Several researchers, including in South Africa, have evaluated the potential of fly ash as a soil ameliorant mixed with organic materials like sewage sludge (Reynolds et al. 1999; Reynolds et al. 2002; Truter et al. 2001; Tsadilas et al. 2002; Belyaeva & Haynes 2009). Truter et al. (2013) also evaluated the potential of using class F (Ca range 1 to 12%) fly ash to amend exposed acidic surface coal mine cover soil in order to regain its agricultural potential. However, research has so far been limited to

evaluation of the potential effects and uses of South African fly ashes in degraded agricultural soils and land reclamation.

The chemical properties of fly ash are largely dependent on the parent coal, combustion conditions and emission control practices (Haynes 2009). Fly ash contains almost all of the natural elements, with the major elements existing mainly as oxides of Fe, Al and Si. Depending on the origin of coal, fly ash may also contain toxic metal species such as Zn, Pb, Cu, Cd, Hg, Ni, Cr, Mo which originate from the rock weathering into coal basins (Izquierdo & Querol 2012; Gitari et al. 2005). The high temperature during combustion of coal renders these toxic metal species more susceptible to leaching when the fly ash comes in contact with water and organic acids (Izquierdo & Querol 2012). The wide range and mixture of beneficial and toxic elements found in fly ash complicates the utilisation of fly ash as a source of plant nutrients, as evidenced by the conflicting reports in literature (Yadav & Garg 2011; Schumann and Sumner 2000). When applied to soils, fly ash has been shown to act both as a source and sink of various metal species and metalloids, with various researchers reporting various effects (Kumpiene et al. 2008; Seshadri et al. 2010b).

The presence of metal species and metalloids in fly ash makes it a potential environmental hazard (Gitari et al. 2005). Nevertheless, in addition to the metal species and metalloids, fly ash also contains anhydrite ( $\text{CaSO}_4$ ), lime ( $\text{CaO}$ ) and periclase ( $\text{MgO}$ ) and other elements like P, K, S and Mg which are potentially beneficial to plant nutrition (Haynes 2009). The alkalinity and nutrition of fly ash has resulted in it being evaluated as a neutralizing agent in the mining industry (Gitari et al. 2005; Kumpiene et al. 2008), and as a source of plant nutrients, respectively (Reynolds et al. 1999; Rethman & Truter 2001; Bhattacharya & Chattopadhyay 2002; Ndoro 2008; Basu et al. 2009). In a study in India, Kalra et al. (1998)

evaluated the potential of fly ash as a soil conditioner and fertilizer for maize, mustard, wheat, and rice after application at levels ranging from 0 to 50 t/ha. Though increased crop yields were observed in the range of 10 to 20 t/ha of fly ash application, crops grown in the fly ash treated soils had a higher metal content in the range of 0.5 to 4.3% compared to where no fly ash was applied. In another study, Yunusa et al. (2008) also evaluated the effects of fly ash application on crop growth and metal accumulation. Fly ash application at 25 t/ha was observed to increase CO<sub>2</sub> assimilation, plant weight and seed yield in canola by up to 21% mainly due to improvement in phosphorus uptake under fly ash amendment. Variable uptake was, however, observed on metal species and metalloids (B, Cu, Mo and Zn) in different plant parts under fly ash amended soils. The effects of fly ash application to soil on plant metal species and metalloid uptake has been mainly observed at higher application rates and these effects vary across different crops as reported by Schumann and Sumner (2000) and in a review by Pandey and Singh (2010).

Though much work on fly ash application on soils has been done internationally, the majority of studies in South Africa have evaluated the potential of fly ash as a soil ameliorant when mixed with other organic sources of plant nutrients. Reynolds et al. (1999); Rethman and Truter (2001) evaluated the potential of manufactured soil made from fly ash and sewage sludge mixture as a source of plant nutrients. Due to the classification of fly ash in South Africa as an industrial waste and environmental hazard according to the Department of Water and Sanitation (DWAF 2006), limited research has been done on the nutrient dynamics of fly ash applied directly to the soil. Hitherto, the potential of fly ash application to soils either as a liming material or source of nutrients and their potential metal and metalloid release to soils has been barely researched. Metal species and metalloid contamination of soil is, however, a

serious environmental challenge and it is essential to evaluate the potential metal species and/or metalloid load associated with the direct soil application of fly ash (Kalra et al. 1998).

The disposal and management of waste materials in South Africa is the responsibility of the Department of Water and Sanitation and is governed by the National Environmental Management: Waste Act (Act 59 of 2008) among others as outlined by Ndoro (2008). Under South African regulations, fly ash is considered a high volume – low hazard waste which may pose environmental pollution problems. However, these regulations do not state the maximum permissible soil metal species and metalloid content limits following fly ash application as has been done for sewage sludge (WRC 1997).

There is great variation in environmental and geological conditions under which coal is formed and it is important to characterise fly ash for beneficial nutrient and toxic metal species and metalloid content (Pandey & Singh 2010). Knowledge on the chemical behaviour and nutrient release dynamics of fly ashes is essential for predicting the behaviour and potential beneficial utilization of fly ashes in agricultural ecosystems. Therefore, the objectives of this study were to: (i) evaluate the elemental composition and phosphorus release of some South African fly ash samples when used as a liming material in different soils, and (ii) to determine the potential heavy metal release in South African fly ash amended soils and hence their potential for utilization as sources of plant nutrients for landscaping, and re-vegetation of degraded lands.

### **3.3 MATERIALS AND METHODS**

#### **3.3.1 Fly ash samples**

Eight fly ash samples were used for the study, with six of the samples collected directly from the hoppers at the power stations and the other two samples being commercially processed fly ash sourced from two local companies in South Africa. The six ESKOM<sup>®</sup> power stations from which fly ash was collected are Hendrina (26° 01' S; 29 ° 36' E), Camden (26° 37' S; 30 ° 05' E), Grootvlei (26° 46' S; 28° 30' E), Duvha (25° 57' S; 29 ° 20' E), Matla (26° 16' S; 29° 08' E) all located in Mpumalanga Province and Lethabo (26° 44' S; 27° 58' E) in Free State Province. The commercial fly ash samples were sourced from Ash Resources<sup>®</sup> and Ulula Ash<sup>®</sup>. The fly ash samples required no milling as they were already in powder form and were kept in airtight plastic containers at room temperature before use.

#### **3.3.2 Chemical characterization**

##### **3.3.3 pH and Electrical conductivity (EC)**

The pH was determined both in KCl and water at the ratio of 1: 2.5 (w/v) using a glass electrode pH meter (Crison Instruments, Spain) (AgriLASA 2004). Electrical conductivity was measured in distilled water at the ratio of 1:2.5 (w/v) (Okalebo et al. 2002).

##### **3.3.4 Elemental concentrations**

###### *Total elements*

The microwave digestion method was used for total element dissolution within the fly ash samples (Jackson & Miller 1998). The combination of *aqua regia* [3:1 v/v hydrochloric acid (37%): nitric acid (65%)] plus hydrofluoric acid (HF) was used for digestion.

A 250 mg fly ash sample was placed in a 55 mL Teflon tube and mixed with 2 mL of hydrofluoric acid (48 %) and 5 mL of *aqua regia*. The digestion was done in a MARS 5 microwave digester (CEM Corporation, Matthews, North Carolina) as described by Jackson & Miller (1998). Total elemental concentrations of Pb, Zn, Fe, Ni, Cr and Cu were determined in the digests using an atomic absorption spectrometer (iCE 3300 Atomic Absorption Spectrometer, Thermo Scientific, USA). Total phosphorus in the filtrates was determined using a continuous flow analyser (San 2++ Skalar Continuous Flow Analyser, Skalar Analytical B.V. The Netherlands).

#### *Plant available elements*

The plant available metal elements Pb, Zn, Fe, Ni, Cr and Cu were extracted using Diethylenetriaminepentaacetic-acid-triethanolamine (DTPA-TEA) as described by Reed & Martens (1996) and ammonium nitrate ( $\text{NH}_4\text{NO}_3$ ) as described by DIN (1997); which both extract the mobile pool of soil nutrients through chelation and cation ion exchange reactions, respectively. The ammonium nitrate method is particularly recommended in South Africa by the Department of Water Affairs and Sanitation guidelines, for characterisation of sewage sludges which contain significant amounts of extractable metal species and metalloid elements (DIN 1997). Plant available phosphorus was extracted using the sodium bicarbonate (Olsen) method (Schoenau & O'Halloran 2006).

#### *$\text{NH}_4\text{NO}_3$ method (DIN 1997)*

A 20 g of fly ash sample was extracted with 50 mL of 1 M  $\text{NH}_4\text{NO}_3$  solution on a horizontal shaker for 2 hrs. After shaking, the solution was then filtered through Whatman No. 42 paper and the metals analysed using an Inductively Coupled Plasma – Optical Emission Spectrometer (ICP-OES, Varian Inc., The Netherlands).

*DTPA-TEA method* (Reed & Martens 1996)

The DTPA-TEA extraction solution which contained 0.005 M DTPA, 0.01 M CaCl<sub>2</sub> and 0.1 M TEA adjusted to pH 7.3 ± 0.05 with 1 M HCl was used for the extraction. After shaking on a horizontal shaker for 2 hrs, the suspension was filtered through Whatman No. 42 filter paper and the metals on the filtrate analysed using the ICP-OES (Varian Inc., The Netherlands).

*Sodium Bicarbonate (Olsen) – Extractable Phosphorus* (Schoenau & O'Halloran 2006)

In this extraction, 0.5 M Sodium bicarbonate (NaHCO<sub>3</sub>) which had been adjusted to pH of 8.5 using 1 M Sodium hydroxide (NaOH) was used. A 2.5 g sample of fly ash was extracted with 50 mL of the 0.5 M NaHCO<sub>3</sub> solution on a horizontal shaker for 30 minutes. After shaking, the suspensions were filtered through Whatman No. 42 filter paper and the filtrates were then adjusted to pH 5 with 2.5 M sulphuric acid (H<sub>2</sub>SO<sub>4</sub>) before analysis. The orthophosphate in the extracts was automatically determined using a continuous flow analyser (San 2++ Skalar CFA, Skalar Analytical B.V., The Netherlands) employing the ammonium molybdate – antimony potassium tartrate – ascorbic acid method.

### **3.3.5 Incubation study**

#### *Soils*

Three acidic soils with a high acid saturation, one collected from Mbinja - (31° 21'S, 28° 29'E) and two from Hogsback (32° 33'S, 26° 54' E), all located in the Eastern Cape Province, were used for the incubation study. Approximately twenty individual samples were randomly collected from 0 - 20 cm depth at each site with a spade and mixed thoroughly to make a composite sample. The collected soil samples were air dried, milled and then sieved through a 2 mm sieve before being used in the incubation study. Selected characteristics of the soils used in the study are given in Table 3.1.

The various characteristics of the soils used in the incubation study were determined by an independent accredited laboratory, the Soil Fertility and Analytical Services under the Department of Agriculture, Fisheries and Forestry in South Africa, employing the standard analytical methods outlined by the Agri Laboratory Association of Southern Africa – Soil Handbook (AgriLASA 2004).

**Table 3. 1:** Selected characteristics of the three soil samples used in the incubation study.

Soil	SA System <sup>a</sup>	WRB System <sup>b</sup>	pH (H <sub>2</sub> O)	Field capacity (m <sup>3</sup> /m <sup>3</sup> )	Acid saturation	Organic C %	Total N	Clay	P	Fe	Ni	Pb	Cu	Cr
											(mg/kg)			
<b>Hogsback</b> – H	Hutton	Ferralsols	4.89	0.38	55.0	2.5	0.17	46	4.80	152.63	1.20	1.82	2.72	0.14
<b>Mbinja</b>	Hutton	Acrisols	5.50	0.32	21.0	0.7	0.09	25.0	2.73	13.80	1.14	0.07	1.62	0.04
<b>Hogsback</b> - Cv	Clovely	Lixisols	4.55	0.35	68.0	0.7	0.13	30.0	3.49	112.87	0.82	3.13	0.45	0.04

<sup>a</sup> = Soil Classification Working Group (1991)

<sup>b</sup> = IUSS Working Group WRB (2014)

### ***Lime requirement and calcium carbonate equivalence***

Two non-commercial fly ash samples from Matla and Duvha with high total and extractable phosphorus and low metal species contents were selected for the incubation study. In this incubation study, the alkaline fly ashes were used as a liming materials, which thus formed the basis of calculating the fly ash application rates. Therefore, initially the calcium carbonate equivalence of the fly ashes together with the lime requirement (LR) of the soils was determined. The calcium carbonate equivalence (CCE) was determined using the titration method outlined by Horneck et al. (1989). The lime requirements for the three soils was determined using the Shoemaker-McLean-Pratt (SMP) buffer method. Based on the soils' lime requirements and the fly ash CCE, the lime application rate (LAR) for the various soils was formulated to quench 100% of the lime requirement of the soils to reach neutral pH of 7. The lime application rate (LAR) for the various treatments was calculated as shown in Equation 3.1.

$$LAR (t ha^{-1}) = LR \times \frac{100\% \text{ CCE for commercial lime}}{\text{CCE for fly ash}} \quad (\text{Equation. 3.1})$$

### ***Experimental design, treatments and incubation procedure***

A 4 × 3 factorial experimental arrangement was used with the two factors being liming material (Matla, Duvha, Calcium carbonate and no lime (soil alone control)) and soil type (Hogsback - Clovely; Hogsback – Hutton and Mbinja). This design gave 12 treatment combinations which were replicated 3 times and laid out in a completely randomized design. A weight of 30 g of the soil was amended with the various liming materials based on the soils lime requirement and the CCE of the liming materials. The fly ash-soil mixtures were put into

plastic containers and moistened to field capacity using de-ionised water. The amount of water required to reach field capacity was determined earlier using the pressure plate apparatus as described by Dane and Hopmans (1996) and is shown as moisture content in Table 1. These moistened soils were then placed in an incubator in the dark at a constant temperature of 25 °C, with water trays to maintain humidity. Aerobic conditions were maintained during the incubation by regularly opening the containers without mixing. The moisture content of the samples was adjusted according to weight once they had lost more than 10% of their initial moisture. The mixtures were incubated for periods of 0, 1, 2, 4, 6 and 8 weeks with separate samples for each sampling period to allow for destructive sampling. The combinations of three soil types × three liming materials (including no-liming controls) × three replicates × five sampling times gave 216 observations. At each sampling, the soil-fly ash mixtures were oven dried at 65 °C, sieved through a 1 mm sieve and then analysed for pH, EC, extractable P and DTPA - TEA extractable Pb, Fe, Ni, Cr and Cu as described above.

### **3.3.6 Statistical analysis**

Analysis of variance was performed using the JMP<sup>®</sup> Release 11.0.0 statistical package (SAS Institute, Inc., Cary, North Carolina, USA, 2010). Mean separations were performed using Fisher's protected least significant difference (LSD) test at  $P \leq 0.05$ . Based on X-ray Fluorescence (XRF) chemical composition results obtained from Eskom<sup>®</sup> unpublished reports, correlation analysis was performed to identify the relationship between pH and free lime (CaO) and periclase (MgO). The relationship between the extractable and total phosphorus was also explored using correlation analysis with the JMP statistical package.

### **3.4. RESULTS**

#### **3.4.1 pH and Electrical Conductivity (EC) of fly ash samples**

All eight fly ash samples evaluated were alkaline with pH values of more than 10 in both water and 1 M KCl (Table 3.2), with significant differences between the fly ash samples ( $P < 0.05$ ). Fly ash from Hendrina had the highest pH of 12.75 and 12.62 in KCl and water, respectively. The fly ash from Grootvlei had the lowest pH in both solutions. However, excluding Grootvlei fly ash, pH in water and KCl showed a strong linear relationship ( $R^2 = 0.95$  and  $0.91$ , respectively) with MgO and a much weaker relationship with CaO. A trend almost similar to that of pH was observed for EC (Table 3.2). Hendrina and Ulula fly ash samples had the highest EC with Grootvlei fly ash showing again the lowest EC. The EC for all fly ashes studied showed a wider range from 0.46 to 8.27 dS/m with significant differences ( $P < 0.05$ ) between fly ash samples.

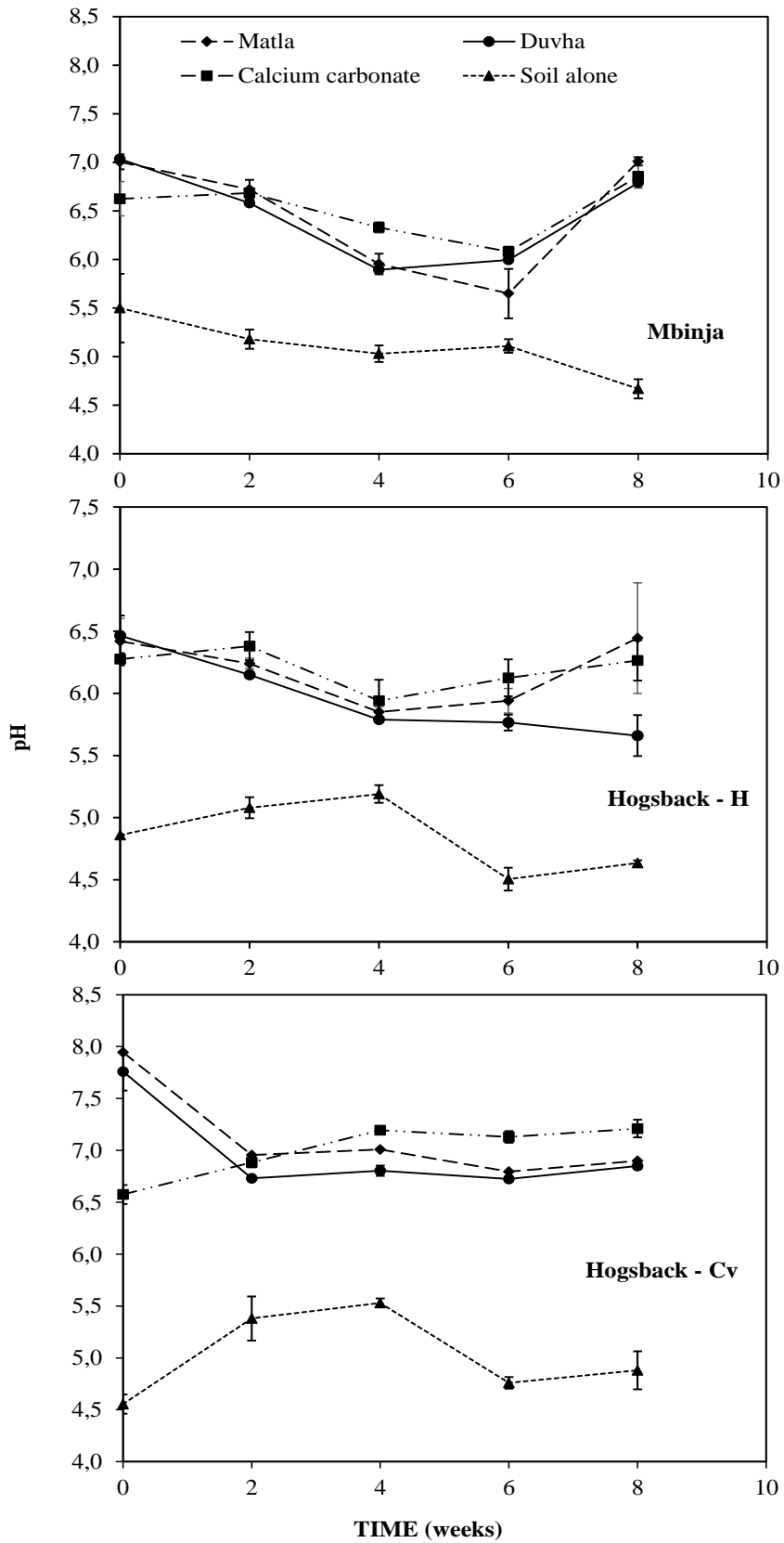
**Table 3. 2:** pH, Electrical Conductivity (EC) (dS/m) and chemical composition (XRF) of fly ash samples from selected electricity power stations in South Africa.

Fly ash source	pH in KCl	pH in H <sub>2</sub> O	EC (dS/m)	SiO <sub>2</sub> <sup>†</sup>	AlO <sub>3</sub>	Fe <sub>2</sub> O <sub>3</sub>	CaO	MgO	S
HENDRINA	12.75a	12.62a	7.91a	54.9	25.9	4.1	4.7	1.8	3.8
CAMDEN	12.72b	12.60a	7.08b	46.9	27.8	6.6	6.6	1.7	4.3
GROOTVLEI	10.97g	11.01e	0.46f	nd	nd	nd	nd	nd	nd
DUVHA	12.44d	12.38b	3.93c	53.5	28.1	5.4	4.3	1.4	2.7
MATLA	12.69c	12.58a	6.84b	54.0	31.7	2.7	5.1	1.7	2.4
LETHABO	12.14f	11.93d	1.25e	53.3	30.7	3.3	4.1	1.1	3.0
ASH RESOURCES	12.34e	12.00c	2.03d	54.28	34.14	3.5	4.1	1.1	nd
ULULA	12.72b	12.60a	8.27a	54.6	28.7	3.1	6.9	1.6	nd
ANOVA <i>P</i> value	< 0.001*** (0.09)	< 0.001 *** (0.19)	< 0.001 *** (4.67)	-	-	-	-	-	-

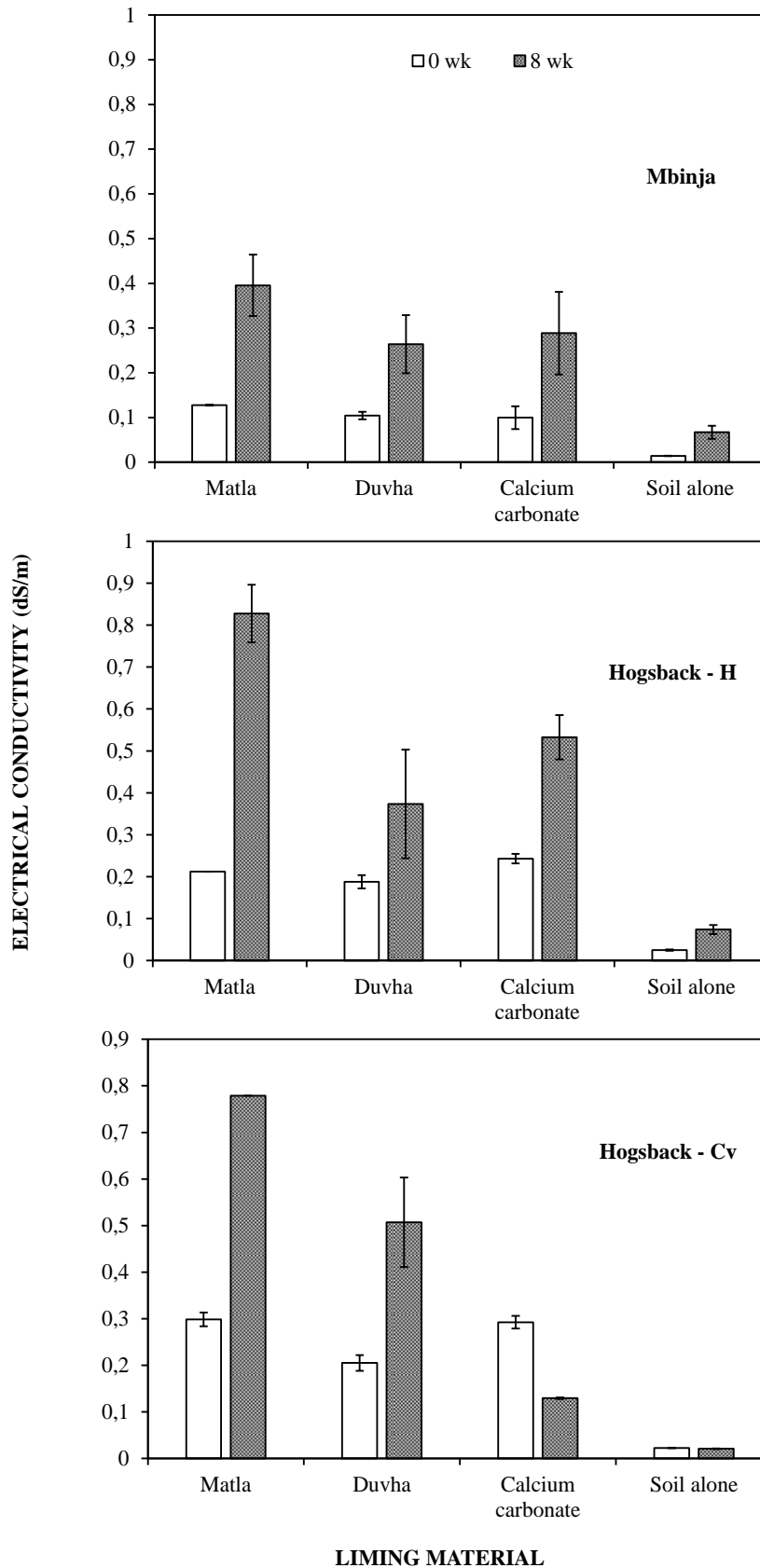
<sup>†</sup> - Chemical composition in percentage (XRF) data obtained from Eskom<sup>®</sup> reports (unpublished); nd - no data; \*\*\* significant at  $P \leq 0.001$ , different letters within a column indicate a significant difference among fly ash samples, values in parentheses are the coefficient of variation (CV %).

### **3.4.2 Changes in pH and EC during incubation of 3 acidic soils following application of fly ash as a liming material**

The reaction of the three acidic soils, with different lime requirements, to the various sources of lime was quite variable as indicated by the highly significant difference ( $P < 0.0001$ ) in pH (Table 3.3). Generally, pH in all the three soils significantly ( $P < 0.0001$ ) changed from almost neutral to slightly acidic from zero to 4 weeks and then either increased or remained almost constant for the remaining incubation period (Figure 3.1). All the three liming materials (Matla and Duvha fly ash and calcium carbonate) showed a similar ability to neutralize acidity in the different soils resulting in an average 41% change in pH from 4.7 to 6.7 after 8 weeks of incubation. The changes in pH with time and variations between the liming materials and the control of soil alone resulted in highly significant interactions ( $P < 0.001$ ; Table 3.3), revealing that pH change depended on soils, fly ashes and time. Application of the different fly ashes to quench 100% of the lime requirements of the soils resulted in significant changes in electrical conductivity following 8 weeks of incubation (Figure 3.2). After 8 weeks of incubation, the EC of the fly ash amended soils ranged from 0.26 to 0.83 dS/m, with much lower values for calcium carbonate and the control.



**Figure 3. 1:** Changes in pH during 8 weeks incubation of three soils mixed with fly ash (Matla and Duvha) and calcium carbonate. Error bars indicate standard deviation.



**Figure 3. 2:** Changes in electrical conductivity of three soils after incubation for 8 weeks with fly ash (Matla and Duvha) and calcium carbonate. Error bars indicate standard deviation.

**Table 3. 3:** Analysis of variance (completely randomized design) for changes in various parameters analysed following destructive sampling over an 8 week incubation period.

Source of variation		pH in water	Olsen P (mg/kg)	Diethylenetriaminepentaacetic acid- triethanolamine (DTPA- TEA) extractable metal species (mg /kg)				
				Chromium	Lead	Nickel	Iron	Copper
Liming material	$F_{(2,60)}$	677.3	1071.0	6.9	54.0	34.4	151.1	11.2
(L)	$P$	< 0.0001	< 0.0001	0.0005	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Soil type (S)	$F_{(3,60)}$	207.2	368.9	32.3	609.7	75.0	562.0	336.1
	$P$	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Time (T)	$F_{(4,60)}$	36.6	7.1	171.5	12.8	124.0	14.5	30.8
	$P$	< 0.0001	< 0.0001	0.0295	< 0.0001	< 0.0001	< 0.0001	< 0.0001
L × S	$F_{(6,60)}$	27.3	110.6	2.5	26.0	8.6	41.7	2.8
	$P$	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	0.0169
S × T	$F_{(8,60)}$	10.2	4.0	32.3	8.1	6.2	4.8	3.1
	$P$	< 0.0001	0.0007	< 0.0001	< 0.0001	< 0.0001	< 0.0001	0.0059
L × T	$F_{(12,60)}$	21.8	13.2	3.4	2.9	3.5	9.2	0.9
	$P$	< 0.0001	< 0.0001	0.0007	0.0031	0.0006	< 0.0001	ns
L × S × T	$F_{(24,60)}$	9.9	2.2	3.5	0.9	2.4	3.6	1.1
	$P$	< 0.0001	0.0064	< 0.0001	ns	0.0035	< 0.0001	ns

### 3.4.3 Total and extractable P in fly ash samples

The total P and extractable P contents of the fly ashes ranged from 553 to 1514 mg P/kg and 171 to 345 mg P/kg, respectively (Table 3.4). The proportion of extractable P ranged from 11 to 34% with four fly ashes having more than 20% of their P extractable by the Olsen method (Table 3.4). The Grootvlei and Duvha fly ashes had among the highest total P contents but they had the lowest percentage of extractable total P (Table 3.4). A significant but weak linear relationship ( $R^2 = 0.21$ ;  $P = 0.0251$ ) was observed between the sodium bicarbonate (Olsen) extractable P fraction and total P, but a stronger significant linear relationship ( $R^2 = 0.72$ ;  $P < 0.001$ ) was observed when the Grootvlei and Duvha fly ashes were excluded from the data set.

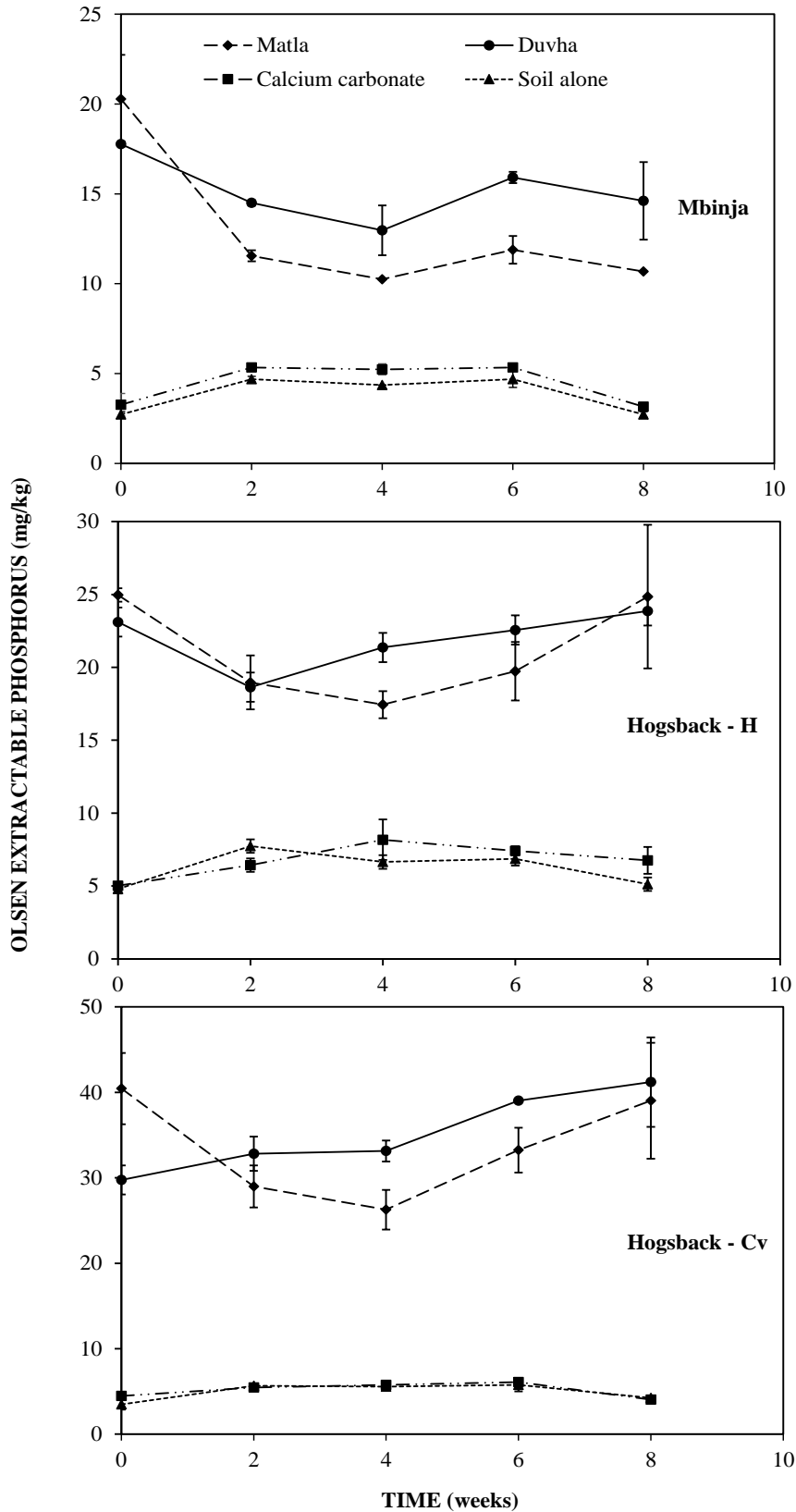
**Table 3. 4:** Total and extractable Phosphorus in selected fly ash samples from South African power stations.

FLY ASH SOURCE	Phosphorus		Extractable Phosphorus
	Total (mg/kg)	Olsen-P (mg/kg)	(% of total P)
HENDRINA	910.8e	171.4f	18.8
CAMDEN	553.3f	188.6e	34.1
GROOTVLEI	1197.3c	130.0g	10.9
DUVHA	1376.6b	205.3d	14.9
MATLA	1028.0d	256.2b	24.9
LETHABO	1012.7d	200.3d	19.8
ASH RESOURCES	947.3e	232.2c	24.5
ULULA	1514.0a	345.5a	22.8
ANOVA- <i>P</i> value	< 0.001*** (3.14)	< 0.001*** (2.48)	-

\*\*\* Significant at  $P \leq 0.001$ , ns = not significant at  $P \leq 0.05$ , different letters within a column indicate a significant difference among fly ash samples. Values in parentheses are the coefficients of variation (CV, %).

#### **3.4.4 Olsen P release from fly ash amended soils under incubation**

Across all three soils, the fly ash incorporated treatments (Matla and Duvha) released significantly ( $P < 0.0001$ ; Table 3.3) the highest amount of Olsen extractable P under incubation (Figure 3.3) consistent with their high total and extractable P content (Table 3.4). There was a general decrease in P release following fly ash application from 0 weeks to around 4 weeks of incubation, followed by a general increase until 8 weeks for all the three soils. Application of fly ash as a liming material to soil whose original P content was around 5 mg/kg (Hogsback – H and Hogsback – Cv) raised the Olsen extractable P content from a P deficient level to levels above more than 25 mg/kg. It was also noteworthy in this study that liming a soil with a P deficient material like calcium carbonate did not result in significant increase in P release, showing that the fly ashes contributed much of the P when applied directly to the soil as observed in Figure 3.3.



**Figure 3. 3:** Changes in Olsen extractable Phosphorus during 8 weeks incubation of three soils mixed with fly ash (Matla and Duvha) and calcium carbonate. Error bars indicate standard deviation.

### **3.4.5 Total and DTPA and Ammonium nitrate extractable metal species (Fe, Cr, Cu, Zn, Ni, Pb)**

Among all elements measured, total Fe was highest in all eight fly ash samples, with most samples having concentrations of more than 2000 mg Fe/kg. Fly ash from Grootvlei had the highest total Fe concentration of 6191 mg Fe/kg. The lowest total Fe concentration was observed in the Matla fly ash (Table 3.5). No consistent trend was observed between total and DTPA or ammonium nitrate extractable Fe. For the extractable fractions, only the DTPA extractable fraction showed significant ( $P \leq 0.001$ ) differences between fly ash samples from different power stations. The Camden fly ash had the significantly ( $P \leq 0.001$ ) highest DTPA extractable Fe. The fly ash from Duvha and Lethabo had the lowest DTPA extractable Fe. The Hendrina fly ash had the significantly ( $P \leq 0.001$ ) lowest total Cu concentration among all the eight fly ash samples. The highest total concentration of Cu was observed in the commercial sample from Ulula (Table 3.5). The Lethabo fly ash had the lowest concentration of extractable Cu though it was among the fly ash samples with the highest total Cu content. Zinc was found in detectable amounts only in fly ash from Duvha and Camden (Table 3.5).

Highly significant ( $P \leq 0.001$ ) differences were observed between fly ash samples from the eight power stations on total and extractable Ni. The fly ash from Ulula and Duvha had the highest content of total Ni. All fly ash samples except for Hendrina had a total Ni content greater than 55 mg/kg (Table 3.6). The trend for DTPA and ammonium nitrate extractable Ni was almost similar to that of total Ni except for Grootvlei and Ash Resources fly ash. Camden fly ash had the highest Ni content extracted by the two extracting solutions. The two commercial fly ash samples from Ulula and Ash Resources had the highest total Pb content (Table 3.6). Again, this trend for total Pb was not reflected in the extractable Pb fractions as Ulula fly ash had the lowest DTPA extractable Pb and was among the lowest for the

ammonium nitrate extractable Pb. The fly ash from Grootvlei which was among the lowest in total Pb content was among the highest in extractable Pb.

The concentration of total Cr in fly ash from Grootvlei was significantly ( $P \leq 0.001$ ) different and highest from all the other seven fly ash samples (Table 3.6). The total Cr concentration of the Grootvlei fly ash was almost twice the content of all the other fly ash samples. The fly ash from Hendrina, Camden and Matla had the lowest total Cr content. However, though the Grootvlei fly ash had the highest total Cr content, it had the significantly lowest DTPA and ammonium nitrate extractable Cr content. The commercial fly ash from Ash Resources had the highest extractable Cr for both DTPA and ammonium nitrate. For all the metal elements determined in the study, no consistent trend was observed between total and extractable contents of the elements.

**Table 3. 5:** Total and extractable Iron, Copper and Zinc in selected fly ash samples from South African power stations.

FLY ASH SOURCE	Iron			Copper			Zinc		
	Total	DTPA	NH <sub>4</sub> NO <sub>3</sub>	Total	DTPA	NH <sub>4</sub> NO <sub>3</sub>	Total	DTPA	NH <sub>4</sub> NO <sub>3</sub>
	(g/kg)	(mg/kg)	(mg/kg)	(mg/kg)	(mg/kg)	(mg/kg)	(mg/kg)	(mg/kg)	(mg/kg)
HENDRINA	30.79b	106.88b	1.37	31.98e	0.67b	0.79b	nd	nd	nd
CAMDEN	23.43d	136.25a	1.37	50.91bc	0.78b	0.27f	9.7b	0.60b	0.51a
GROOT VLEI	61.91a	43.41d	1.35	43.48d	0.91a	0.54c	nd	nd	nd
DUVHA	32.40b	8.24f	1.36	50.83bc	0.46c	0.50cd	23.7a	0.67a	0.44b
MATLA	18.26e	39.36d	1.38	45.75cd	0.71b	0.51c	nd	nd	nd
LETHABO	27.01d	4.83f	1.38	53.76ab	0.19e	0.36e	nd	nd	nd
ASH	23.26d	22.91e	1.37	53.56b	0.55c	1.10a	nd	nd	nd
RESOURCES									
ULULA	20.27e	50.61c	1.38	60.37a	0.31d	0.43de	nd	nd	nd
ANOVA- <i>P</i>	<0.001***	<0.001***	ns	<0.001	<0.001***	<0.001***	<0.001***	<0.001***	<0.001***
value	(4.73)	(5.9)	(2.09)	(7.81)	(11.21)	(7.45)	(29.69)	(20.45)	(10.78)

\*\*\* Significant at  $P \leq 0.001$ , different letters within a column indicate a significant difference among fly ash samples. Values in parentheses are the coefficients of variation (CV, %). nd – not detected.

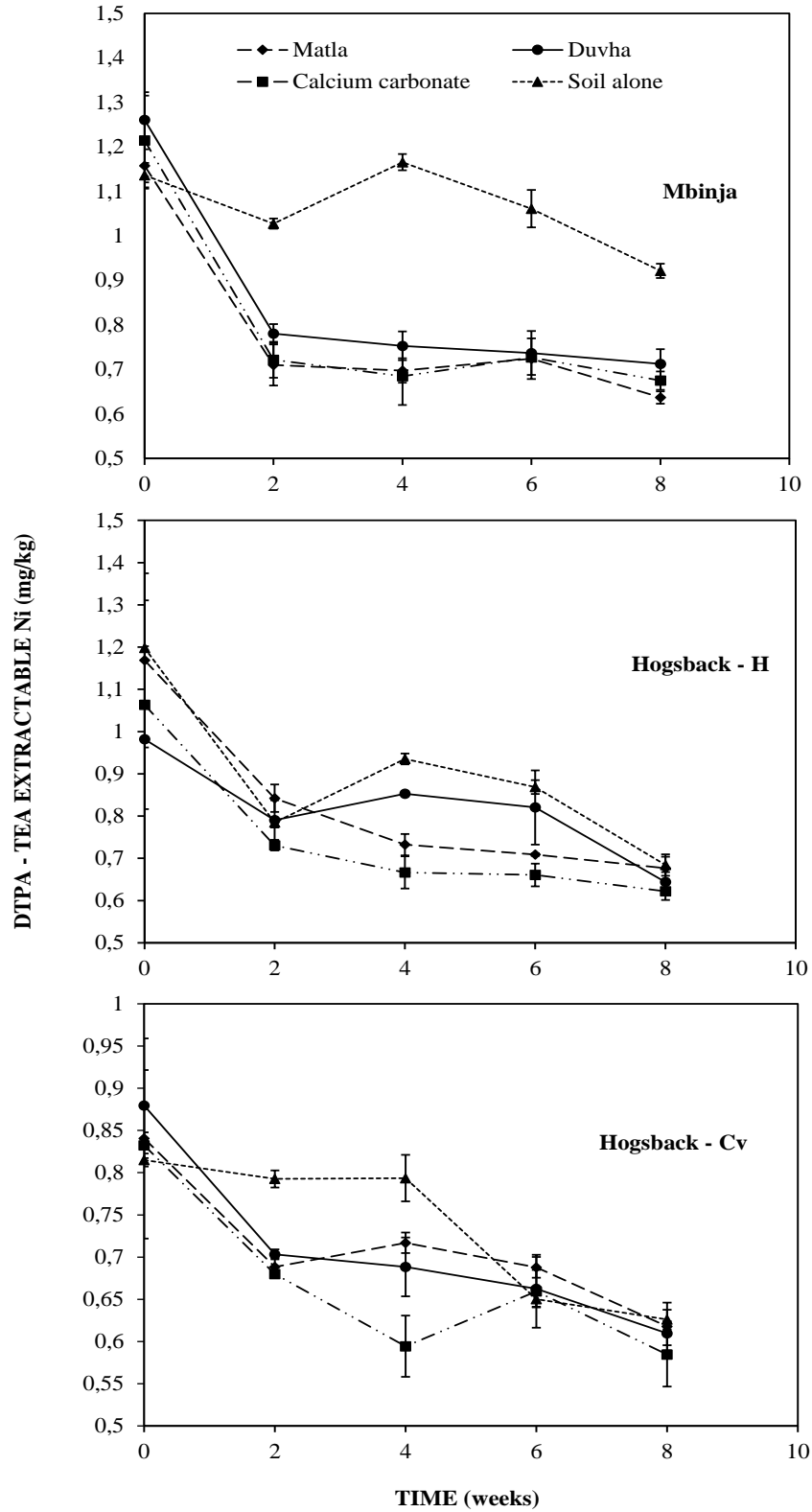
**Table 3. 6:** Total and extractable Nickel, Chromium and Lead in selected fly ash samples from South African power stations.

FLY ASH SOURCE	Nickel			Chromium			Lead			
	Total	DTPA	NH <sub>4</sub> NO <sub>3</sub>	Total	DTPA	NH <sub>4</sub> NO <sub>3</sub>	Total	DTPA	NH <sub>4</sub> NO <sub>3</sub>	
					mg/kg					
HENDRINA	33.25e	0.20c	0.28c	143.69e	0.91c	1.40d	26.48f	0.25cd	0.40ab	
CAMDEN	57.68cd	0.34a	0.42a	164.96de	0.97c	1.72c	74.36d	0.38b	0.28cd	
GROOT VLEI	57.11cd	0.25b	0.14d	488.29a	0.36e	0.64f	40.01e	0.41ab	0.42ab	
DUVHA	69.79a	0.26b	0.40a	178.84cd	0.47e	1.06e	102.61c	0.21de	0.19d	
MATLA	52.25d	0.19c	0.39ab	162.81de	0.68d	1.42d	98.51c	0.17e	0.33bc	
LETHABO	66.73ab	0.21bc	0.40a	232.31b	0.88c	1.40d	121.29b	0.25c	0.35abc	
ASH RESOURCES	62.83bc	0.32a	0.36abc	229.05b	1.53a	2.51a	141.52a	0.42a	0.44a	
ULULA	71.13a	0.20c	0.30bc	199.69c	1.33b	1.97b	138.15a	0.17e	0.28cd	
ANOVA- <i>P</i>	< 0.001	<0.001***	<0.001***	<0.001***	<0.001***	<0.001***	<0.001***	<0.001***	<0.002**	
value	*** (6.23)	(10.52)	(15.84)	(5.99)	(7.75)	(4.48)	(6.44)	(8.16)	(18.05)	

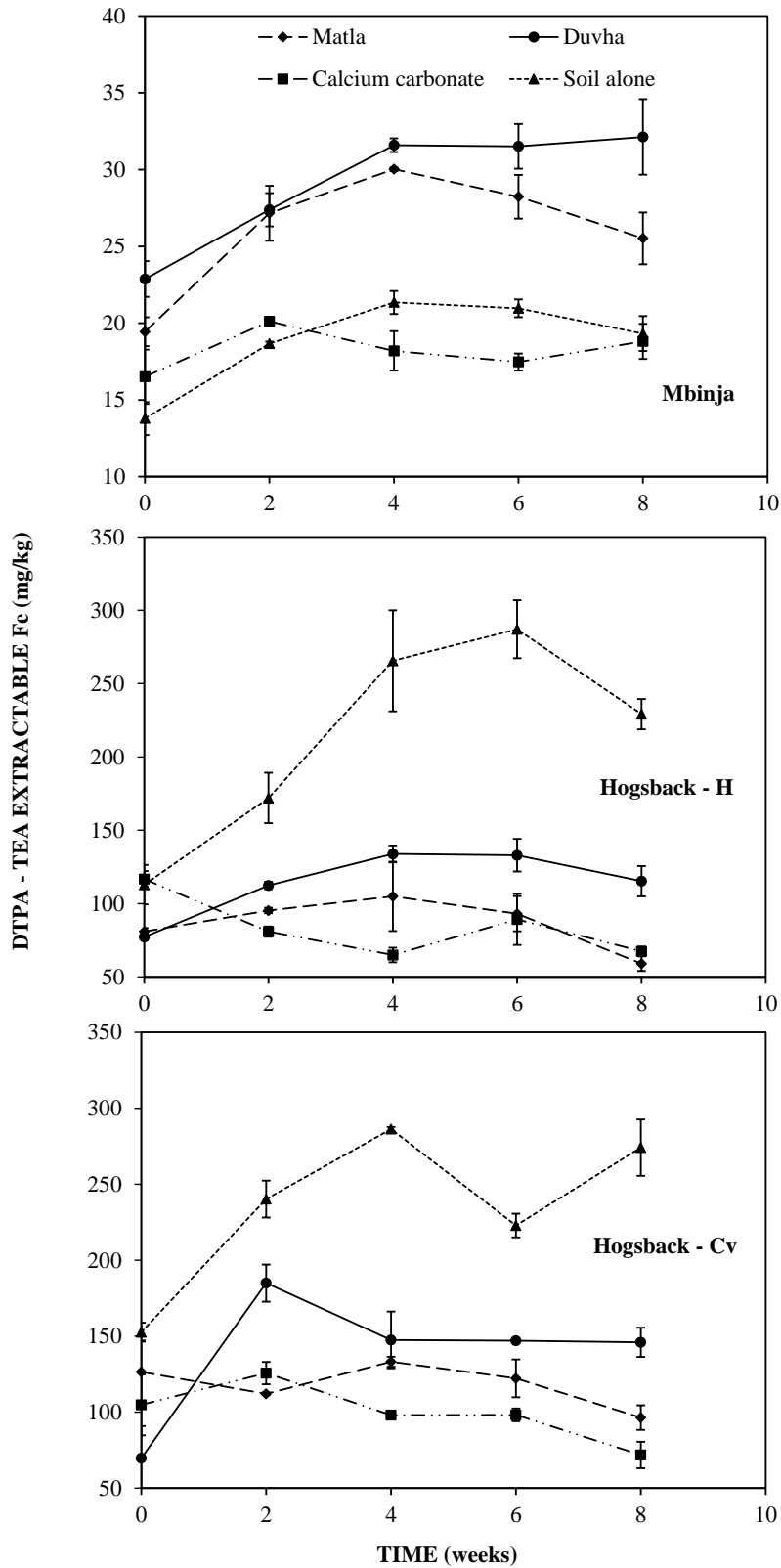
\*\*\* Significant at  $P \leq 0.001$ , different letters within a column indicate a significant difference among fly ash samples. Values in parentheses are the coefficients of variation (CV, %).

### **3.4.6 Metal species release in soil following application of fly ashes**

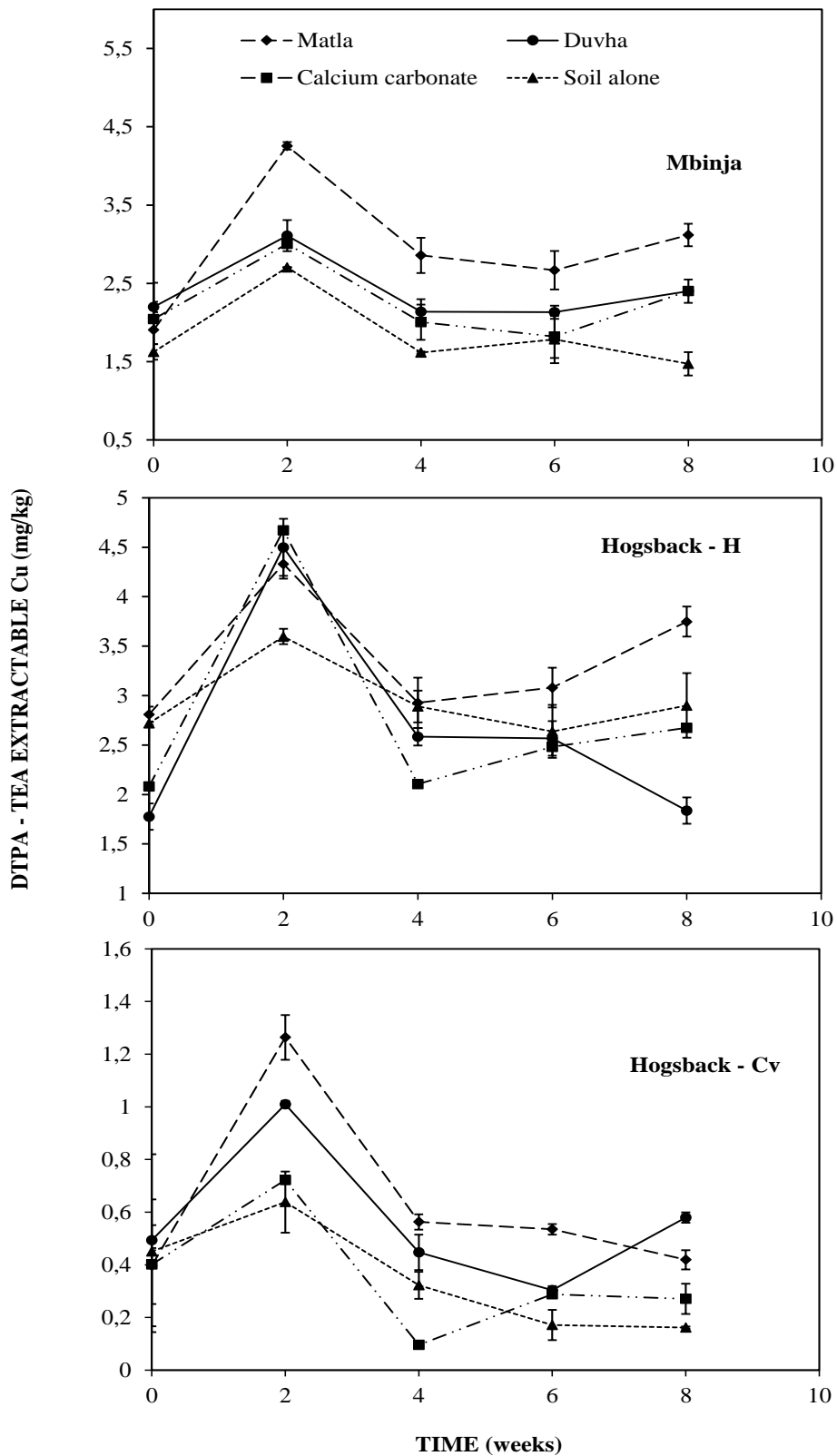
During incubation, there were significant differences ( $P < 0.05$ ) between the liming materials, soils and time as indicated also by the various significant interactions (Table 3.3). Results of the incubation study showed that fly ash use as a liming materials across all three soils, except for Cu, resulted in release of less Cr, Pb (data not shown), Ni and Fe compared to the soil alone control (Figures 3.4 to 3.6). Only Cr showed a significant increase throughout the incubation period, having more extractable Cr after 8 weeks compared to zero weeks, in contrast to other metals which were either constant or less over the 8 week incubation period. For most of the metals determined in this study, there was, however, no consistent trend on the metal species release pattern showing the influence of soil chemistry and mineralogy.



**Figure 3. 4:** Changes in DTPA – TEA extractable Nickel (Ni) during incubation of three soils incorporated with fly ash (Matla, Duvha) and calcium carbonate. Error bars indicate standard deviations.



**Figure 3. 5:** Changes in DTPA – TEA extractable Iron (Fe) during incubation of three soils incorporated with fly ash (Matla, Duvha) and calcium carbonate. Error bars indicate standard deviations.



**Figure 3. 6:** Changes in DTPA – TEA extractable Copper (Cu) during incubation of three soils incorporated with fly ash (Matla, Duvha) and calcium carbonate. Error bars indicate standard deviation.

## **3.5 DISCUSSION**

### **3.5.1 Fly ash pH and electrical conductivity and potential of fly ash as liming material**

The high pH values confirm the view that South African coals are largely bituminous and tend to generate fly ash with higher free lime (CaO) and periclase (MgO) contents (Gitari et al. 2010). The minimal variations in pH observed among the fly ash samples confirms the view that little variations in pH are observed in fly ashes derived from a similar group of coal. Larger variations in pH are normally expected in fly ashes derived from anthracite and bituminous coals due to the wide variation in Ca, Mg and S contents (Pandey & Singh 2010). The electrical conductivity of a fly ash is largely determined by the total soluble salt concentration (Friedman 2005). The concentration of the salts (cations and anions) is likely to vary due to variation in sample chemistry, hence the wide range in EC values between fly ash samples in our study. The range of the EC of the fly ashes studied here (0.46 – 8.27 dS/m) is within the range reported not to pose a challenge to crops when the fly ash is applied to the soil even at application rates of up to 50 t/ha because it is diluted by the soil (Schuman & Sumner 2000).

The increase in pH following application of fly ash shows the ability of fly ash to act as an effective liming material, which is in agreement with other studies (Wang et al. 2008; Moon et al. 2014). The significant interactions between fly ash, soils and time are in agreement with work by Matsi and Keramidas (1999) who made similar observations using two soils with different pH and lime requirements, in which the changes in pH varied depending on the fly ashes, soils and fly ash application rate. The changes in pH over longer time periods is of critical importance in soil solution chemistry as they affect precipitation, co-precipitation and

adsorption reactions which govern metals and metalloid availability (Gitari et al. 2008). In an experiment using surface coal mine cover soils with pH 4.3, Truter et al. (2013) applied fly ash once off as a liming material to achieve a pH of 6.5 and observed acid neutralizing and nutrient supply potential even after 6 years. Such observations indicate that limited changes in soil chemistry with some metal precipitation over longer time periods are expected once equilibrium is attained following fly ash application. Using fly ash from Duvha power station, Ndoro (2008) observed similar small changes in EC after incubating fly ash amended soil for 112 days. These results also confirm that amendment of an acidic soil with fly ash to quench 100% of the lime requirement will pose no serious salinity challenge as there is a dilution effect from the soil which keeps the EC values way below the maximum of what most crops can tolerate (Schumann & Sumner 2000).

### **3.5.2 Total and extractable P release from fly ash amended soils under incubation**

The total P elemental composition of fly ash is usually high but availability is generally low (Bhattacharya & Chattopadhyay 2002), and variable due to differences in their mineralogical composition. Phosphorus is one the most important nutrient elements especially in South African soils (Gichangi et al. 2009), so the relationship between total P with extractable P and the P release potential of the fly ashes was investigated.

The general decrease in Olsen P release observed from 0 to 4 weeks could be attributed to the decrease in pH observed between 0 and 4 weeks (Figure 3.1) which resulted in the slight P fixation (Seshadri et al. 2010b), hence the observed decrease in P release between this period (Figure 3.3). From these results, it seems that for effective P supply following fly ash application to a P deficient soil, it may be prudent to wait for a 4 week period before

establishing a crop. It was important to note that application of fly ash as a liming material to soil raised the Olsen extractable P content from a P deficient level to levels above more than 25 mg/kg which is above the critical P level for most crops (Johnson et al. 2013). In spite of the potential P nutrient benefits of fly ash, care should be taken to avoid soil crusting and hardening that may result from application of fly ashes with low Ca contents (class F, Ca: 1 to 12 %). The pozzolanic properties of fly ash can result in soil particle cementing which results in formation of hard pans on soil surface which reduce infiltration, seedling emergence and root growth (Truter et al. 2013). The silt size particles of fly ash also result in soil pore clogging thus inducing crust formation which reduces infiltration, when fly ash is applied at large quantities. However, limited information is available on maximum quantities of fly ash that can be applied to various soils. On the other hand, the application of fly ash is not expected to result in deterioration of physical properties of soils but is likely to improve them in most cases (Matsi & Keramidas 1999).

### **3.5.3 Metal species in fly ash and their release in soils**

The high Fe content in the fly ash samples (Table 3.5) is consistent with the findings of other researchers in that up to 95% of fly ash is dominated by ferro-alumino-silicates (Gitari et al. 2008; Malik & Thapliyal 2009). The extractable fraction of all metal species in the fly ashes was very low in comparison to the total element content. This may be attributed to the fact that most of the elements in fly ash are associated with the mineral phase which greatly limits their extractability (Bhattacharya et al. 2012). Land application of fly ash and other coal combustion products (CCPs) affects the bioavailability of nutrients and metal species and metalloids by both acting as their source and sink in soils (Seshadri et al. 2010a). The mobility of metal species like Zn, Pb and Cu in soil has been shown to be modified by the

presence of Mn, Al, oxides, carbonates, other anions and pH (Lee et al. 2006; Adriano et al. 2002). When fly ash is incorporated into soil, chemi-sorption, precipitation and complexing of the metal species in the fly ash has been observed to occur, thus greatly limiting the extractability of the metals (Brown et al. 2005; Kumpiene et al. 2008). Results consistent with ours were observed by Ndoro (2008) and Tsadilas et al. (2002) using alkaline fly ash on an acidic soil, where decreased extractability of the metal species was observed in the fly ash treated soils compared to the soil alone control.

#### **3.5.4 Suitability of fly ash as a soil liming material**

Though the soils used in this study differed in their cation, metal chemistry and acid saturation, the fly ash application was done based on the acid saturation and lime requirement as recommended by Matsi and Keramidas (1999). The acid saturation and pH are two parameters that critically affect the mobility of heavy metals in soils by influencing their chemical breakdown, solubility and adsorption (Brady and Weil 2008). Thus each soil received only enough of the fly ash required to neutralize the acidity. It was noted that there was no much difference in heavy metal release in the soils compared to the control of soil alone. These results demonstrate that even with varying soil chemistries, if fly ash is applied based on the soils lime requirements, no adverse heavy metal release is expected. As the soil pH becomes neutral, for the heavy metals analysed in this study, their solubility decreases as observed by Brown et al. (2005) and Izquierdo and Querol (2012).

### **3.6 CONCLUSIONS**

Though originating from a mostly similar coal type, the fly ash samples studied varied significantly in elemental concentrations. Thus characterization is essential for customized recommendations for their utilization as amendments in agriculture, landscaping, and degraded land reclamation. Application of the fly ashes evaluated here as acidic soil ameliorants based on the soils lime requirements does not pose any potential salinity challenge and can provide a cheap liming source and a source of essential phosphorus with minimum risk to soil contamination from heavy metal species. However, establishment of potential changes over time in pH under field conditions need to be considered as this may result in increased metal species and metalloid bio-availability. Future studies should further investigate the P fertilizer value of the fly ashes and ways of further enhancing their ability to release P, more especially for fly ashes with high total P but low extractable P such as the one from Duvha. Increasing the fraction of extractable P and thus potentially bioavailable P has been suggested as one of the ways of increasing fly ash utilizations and acceptability in agriculture, and vermicomposting is suggested to be one such technology.

## CHAPTER FOUR

### 4.0 OPTIMIZATION OF FLY ASH INCORPORATION INTO COW DUNG – WASTE PAPER MIXTURES FOR ENHANCED VERMI - DEGRADATION AND NUTRIENT RELEASE

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This Chapter is based on a manuscript entitled:

Mupambwa H.A. and Mnkeni P.N.S. 2015b. Optimization of fly ash incorporation into cow dung – waste paper mixtures for enhanced vermidegradation and nutrient release. *Journal of Environmental Quality*. doi:10.2134/jeq2014.10.0446.

**(See Appendix 2)**

#### 4.1 ABSTRACT

This study was conducted to establish an appropriate mixture ratio of fly ash (F) to optimized cow dung-waste paper mixtures (CP) in order to develop a high quality vermicompost using earthworms (*Eisenia fetida*). Fly ash was mixed with cow dung – waste paper mixtures at ratios of (F: CP) 1:1, 1:2, 1:3, 2:1, 3:1 and CP alone and composted for 14 weeks. Olsen P; inorganic N ( $\text{NO}_3$ ,  $\text{NO}_2$  and  $\text{NH}_4$ ); C: N ratio, ash content, microbial biomass C (MBC) and humification parameters were measured together with scanning electron micrograph (SEM) images, to determine compost maturity. Based on C: N ratio, the extent of vermi-degradation of the waste mixtures followed the decreasing order (F: CP) of  $1:3 > 1:2 > 1:1 > \text{CP alone} > 2:1 > 3:1$ . Similarly, Olsen P was significantly higher ( $P < 0.05$ ) where earthworms were added. The mean percentage increase in extractable P was in the order  $\text{CP alone} > 1:2 > 1:3 > 1:1 > 2:1 > 3:1$ , with earthworm addition almost doubling P release across the 1:1, 1:2 and CP alone treatments. Fly ash incorporation enhanced conversion of organic N to the plant available inorganic forms, with the 1:3 treatment resulting in the highest conversion. SEM images confirmed the extent of vermi – degradation reflected by the various humification parameters determined. Fly ash incorporation at the 1:2 ratio proved to be the most appropriate as it will allow processing of more fly ash whilst giving a vermicompost with desirable maturity and nutritional properties.

**Key words:** Biodegradation, compost maturity, nutrient release, *Eisenia fetida*

## 4.2 INTRODUCTION

Coal fired power stations generate more than 90% of the total electricity generated in South Africa. This trend is most likely to increase as evidenced by the construction of two new high capacity coal powered electricity generating stations (the 4700 MW Medupi and 4800 MW Kusile) (Eberhard, 2011). Coal combustion generates large quantities of greenhouse gases together with fly ash. Fly ash, which is the powdery particulate material collected from flue gasses by electrostatic or mechanical devices, forms more than 70% of the solid residue during coal combustion at power stations (Haynes, 2009). Of the more than 28 million tonnes of fly ash generated in South Africa, only around 5 % is utilized in the construction industry with the rest being disposed at sites near the power stations.

Fly ash has potential to supply essential plant nutrients in agriculture as evidenced by the wide range of essential nutrients in fly ash such as P, K, Mg, Ca and S among others. Though the levels of total nutrient content of nutrients like P in fly ash are high, the major limitation of direct fly ash utilization in agriculture is their very low plant availability (Bhattacharya and Chattopadhyay, 2006; Basu et al., 2009). This is aggravated by the low microbial activity in fly ash which further limits mineralization even when fly ash is applied directly to soil (Bhattacharya and Chattopadhyay, 2006). Against this background, several researchers have evaluated the potential of using earthworms during composting to improve the nutrient release in fly ash, thus improving its fertilizer value.

Bhattacharya and Chattopadhyay (2002) evaluated the potential of *Eisenia fetida* in improving the P levels when fly ash was incorporated in cow dung at 25%, 50% and 75% levels. The earthworms proved superior in increasing the extractable P levels and phosphate utilizing bacteria even though the C: N ratio and earthworm stocking density were not

optimized for effective vermicomposting. In other studies, the incorporation ratio of fly ash to cow dung has been evaluated and recommendations made following composting under varying C: N ratios of the total material mix, stocking density and moisture content (Gupta et al., 2005; Bhattacharya and Chattopadhyay, 2006; Ananthakrishnasamy et al., 2009). These parameters have been shown to greatly influence bioconversion effectiveness and nutrient release during vermicomposting of organic and inorganic waste mixtures with *E. fetida* (Dominguez and Edwards, 2011).

Mupondi (2010) and Ndegwa and Thompson (2000) reported a C: N of 30 as the most appropriate for vermicomposting using *E. fetida*. Earthworm population growth during vermicomposting has been observed to be limited by the C: N ratio, with the earthworms allocating more resources to growth than reproduction under low C: N ratios like that observed in most cow dungs (Aira et al., 2006a). During vermicomposting, both high reproduction and growth rates are required in order to accelerate waste biodegradation and stabilization (Aira et al., 2006a). The biodegradation of waste materials can thus be accelerated by optimizing the C: N ratio during vermicomposting and this can be achieved by using carbonaceous waste materials as bulking agents. One such bulking material is waste paper which can also be a good feed for earthworms in the vermicomposting process (Gupta and Garg, 2009). Using waste paper as a bulking agent is a recycling opportunity that can control pollution, as the waste paper would otherwise be burned thereby releasing greenhouse gases. Several researchers have incorporated fly ash into various organic wastes during composting such as cow dung (Bhattacharya and Chattopadhyay, 2006); crop residues, press mud (Anbalagan and Manivannan, 2012); municipal waste (Belyaeva and Haynes, 2009); food waste and sewage sludge (Wong et al., 2009; Fang et al., 1999), with and without earthworms, with variable results. Limited research has been conducted to determine the

optimum levels of fly ash incorporation with organic wastes already optimized by the C: N ratio for efficient vermicomposting. Against this background, the objective of the experiment was to optimize the fly ash incorporation into cow dung – waste paper mixtures for enhanced biodegradation and mineralization of the fly ash – cow dung – waste paper mixtures.

## **4.3 MATERIALS AND METHODS**

### **4.3.1 Materials for composting**

The study was conducted at the vermicomposting research unit at the University of Fort Hare Farm, located in Alice, Eastern Cape Province (32°46' S and 26°50'E). Composting was done in cylindrical vermi-reactors measuring 0.5 m × 0.3 m (diameter × height; volume 0.0589 m<sup>3</sup>) with perforations on the bottom side. Cow dung was collected from a mainly pasture based dairy farm in Keiskammahoek, located about 60 km away from Alice. The cow dung was crushed to remove large lumps, air dried, and stored under a shed in a dry area. Shredded waste paper was obtained from the University of Fort Hare. The fly ash sample used for the study was obtained from Matla power station (26° 16' S; 29° 08'E), which is located in Mpumalanga Province in South Africa. Mature clitellated earthworms (*Eisenia fetida*) commonly known as red wigglers (Ndegwa and Thompson 2000), which had been feeding on a mixture of cow dung and waste paper, were obtained from our local wormery at the University of Fort Hare. The cow dung, waste paper and fly ash were characterised for selected characteristics (Table 4.1). Cow dung was mixed with waste paper to give an appropriate C: N ratio of  $30 \pm 1$  following recommendations of Mupondi et al. (2010).

**Table 4. 1:** Selected chemical characteristics of cow dung, waster paper and fly ash used in the study.

Parameter	Cow dung	Waste paper	Fly ash
pH (H <sub>2</sub> O)	7.88 ± 0.02	8.1 ± 0.1	12.58 ± 0.01
EC (dS/m)	5.94 ± 0.25	0.12 ± 0.02	6.84 ± 0.38
Total C (%)	15.06 ± 1.45	37.96 ± 1.01	nd
Total N (%)	1.36 ± 0.09	0.038 ± 0.01	nd
C:N ratio	11.07 ± 0.37	998.94 ± 1.74	-
Total P (g/kg)	2.8 ± 0.5	nd	1.028 ± 0.27
Olsen extractable P (mg/kg)	747.4 ± 6.94	nd	256.2 ± 5.24
Extractable NO <sub>2</sub> /NO <sub>3</sub> (mg/kg)	244.95 ± 0.64	nd	nd
Extractable NH <sub>4</sub> (mg/kg)	160.35 ± 8.84	nd	nd

Parameters reported as Means ± Standard Deviation, n = 3.

nd = not detected

#### 4.3.2 Treatments and experimental design

The experiment was a factorial with two factors i.e. fly ash incorporation level at six levels and earthworm presence at two levels. Fly ash (F) was incorporated into cow dung–waste paper mixtures (CP) at six ratios on a dry weight to weight basis: F+CP (1:1), F+CP (1:2), F+CP (1:3), F+CP (2:1), F+CP (3:1) and CP alone. Prior to starting the experiment, the mixtures were allowed to pre-compost for 1 week to eliminate volatile toxic gases (Mupondi et al. 2010). Following this pre-composting period, the earthworm absence or presence factor

was implemented. Earthworms were introduced into each vermireactor at a stocking density of 13.6 g-worm/kg of fly ash – cow dung – waste paper mixture based on recommendations of Ndegwa and Thompson (2000), with a total of 8 kg (dry weight) material being used. Three replicates were used for each treatment and these were laid in a completely randomized design. Samples were collected every two weeks for the duration of the experiment and analysed for various chemical parameters as described below.

#### **4.3.3 Ash content and Total C & N**

Samples originally dried at 105 °C for 48 h were used for determining ash content through dry ashing at 550 °C for 4 h (Okalebo et al. 2002). Total C and N was determined using the dry combustion method and analysed using a LECO TruSpec C/N auto analyzer (LECO Corporation 2003).

#### **4.3.4 Microbial biomass Carbon (MBC)**

The chloroform fumigation – extraction (CFE) procedure was used for determination of MBC in compost samples based on the methods of Anderson and Ingram (1993). For each sample, two 15 g of fresh treatment material with known moisture content were weighed into a crucible and placed into a separate desiccator. In one of the desiccators a 100 mL beaker containing 25 mL of alcohol free chloroform with boiling chips was placed and a vacuum applied. The vacuum was applied to the fumigated treatments until the chloroform was rapidly boiling, and then sealed and placed for 24 h in a the dark cupboard at room temperature. The non- fumigated treatments were also similarly incubated in the dark but without a vacuum. After incubation, the fumigated treatments were evacuated using a vacuum pump for 5 times with each evacuation lasting at least 2 mins. After evacuation, all the desiccators were opened and the organic carbon in the samples was extracted using 50

mL of 0.5 M potassium sulphate on a shaker at 180 rpm for 1 h and filtered using Whatman No. 42 filter paper. Organic C in the extracts was then determined using the dichromate oxidation method and calculated using Equation 1 (Anderson and Ingram 1993; Joergensen and Brookes 2005).

$$C (\mu\text{g/g} - \text{soil}) = \frac{(\text{HB} - \text{S}) \times \text{N} \times \text{E} \times \text{VD} \times (\text{VK} + \text{SW}) \times 1000}{\text{CB} \times \text{VS} \times \text{DM}} \quad [\text{Equation 4.1}]$$

**Where:**

**HB** = consumption of titration solution by hot blank (mL); **S** = consumption of titration solution by sample (mL); **N** = normality of the  $\text{K}_2\text{Cr}_2\text{O}_7$ ; **E** = 3, conversion of Cr (+VI) to Cr (+III) assuming all organic C on average as C (0); **VD** = added volume of  $\text{K}_2\text{Cr}_2\text{O}_7$  (mL); **VS** = added volume of sample (mL); **VK** = volume of  $\text{K}_2\text{SO}_4$  extractant (mL); **CB** = consumption of titration solution by cold blank (mL); **SW** = amount of water in compost sample (mL); **DM** = total mass of dry soil (g)

The microbial biomass carbon was then calculated as:

$$\text{MBC} = \frac{(\text{organic C in fumigated compost} - \text{organic C in unfumigated compost})}{k_{\text{EC}}} \quad [\text{Equation 4.2}]$$

**Where:** the extractable part of microbial biomass carbon,  $k_{\text{EC}} = 0.38$  (Vance et al. 1987)

### 4.3.5 Humic and fulvic acids

The humic and fulvic acid fractions in the composts were extracted using a method described by Sanchez-Monedero et al. (1996). Extraction was done using 0.1 M NaOH at the ratio of 1:20 w/v and shaken on a horizontal reciprocating shaker for 4 hrs. After the shaking, the extracts were centrifuged at 8000 rpm equivalent to  $8.2 \times 10^3$  g Relative Centrifugal Force; and half of the extracts were stored for subsequent analysis of total extractable carbon fraction ( $C_{\text{EX}}$ ) and the remainder acidified to pH 2 using concentrated sulphuric acid. The pH adjusted extracts were allowed to coagulate over 24 h at 4°C following which the extracts were centrifuged at 8000 rpm (8200 g) to separate the humic acid (HA) fraction from the fulvic acid (FA) fraction. The fulvic acid ( $C_{\text{FA}}$ ) portion in solution after centrifugation and the ( $C_{\text{EX}}$ ) fraction were then analysed for extractable carbon using the dichromate oxidation method. The extractable carbon in the extracts was then calculated using Equation 4.3 by Anderson and Ingram, (1993).

$$\text{Organic carbon (\%)} = \frac{(A \times M \times 0.003)}{g} \times \frac{E}{S} \times 100 \quad \text{[Equation 4.3]}$$

**Where:**

**A** = (mL heated blank (HB) – mL sample) × [(mL unheated blank (UB) – mL HB)/mL UB] + (mL HB – mL sample).

**M** = molarity of ferrous ammonium sulphate; **g** = dry soil mass (g); **E** = extraction volume (mL); **S** = digest sample volume (mL).

The C concentration of the humic acid ( $C_{HA}$ ) fraction was then calculated as the difference between the total extractable carbon and the fulvic acid carbon. Humification ratio (HR), humification index (HI) and polymerisation index (PI) which are indices used for the evaluation of humification level in the compost were then calculated as shown below, where C is total carbon determined by dry combustion.

$$HR = \frac{C_{EX}}{C} \times 100 \quad \text{[Equation 4.4]}$$

$$HI = \frac{C_{HA}}{C} \times 100 \quad \text{[Equation 4.5]}$$

$$PI = \frac{C_{HA}}{C_{FA}} \times 100 \quad \text{[Equation 4.6]}$$

#### **4.3.6 Scanning electron microscopy**

The morphological characteristics of the composts at the beginning and the end of composting were observed using a Scanning Electron Microscope (SEM) [JEOL, JSM-6390LV, Japan]. The samples were mounted on a stub using carbon double sided tape and then coated using gold – palladium at the ratio of 80:20 in an Ion coater (EIKO – IB3 Ion Coater, Japan). The images were then taken using a secondary electron detector at 15 kV.

#### **4.3.7 Olsen extractable P** (Schoenau and O'Halloran 2006)

The Olsen P was extractable using the procedure explained in Chapter 3. Section 3.3.4.

#### **4.3.8 Exchangeable ammonium and nitrate and nitrite** (Maynard et al. 2006)

Inorganic nitrogen ( $\text{NH}_4$  (N),  $\text{NO}_3$  (N) and  $\text{NO}_2$  (N)) were extracted using 50 mL of 0.5 M potassium sulphate (1:10 w/v). A 5 g homogenous sample was weighed into a 150 mL plastic bottle and shaken on a reciprocating shaker for 30 mins at 160 rpm. The suspension was then filtered using Whatman™ No. 42 filter paper. The concentration of ammonium was determined based on the Berthelot reaction involving salicylate whilst the concentration of nitrate and nitrite was determined based on the cadmium reduction method; all done automatically on a continuous flow analyser (San 2++ Skalar CFA, Skalar Analytical B.V. The Netherlands)

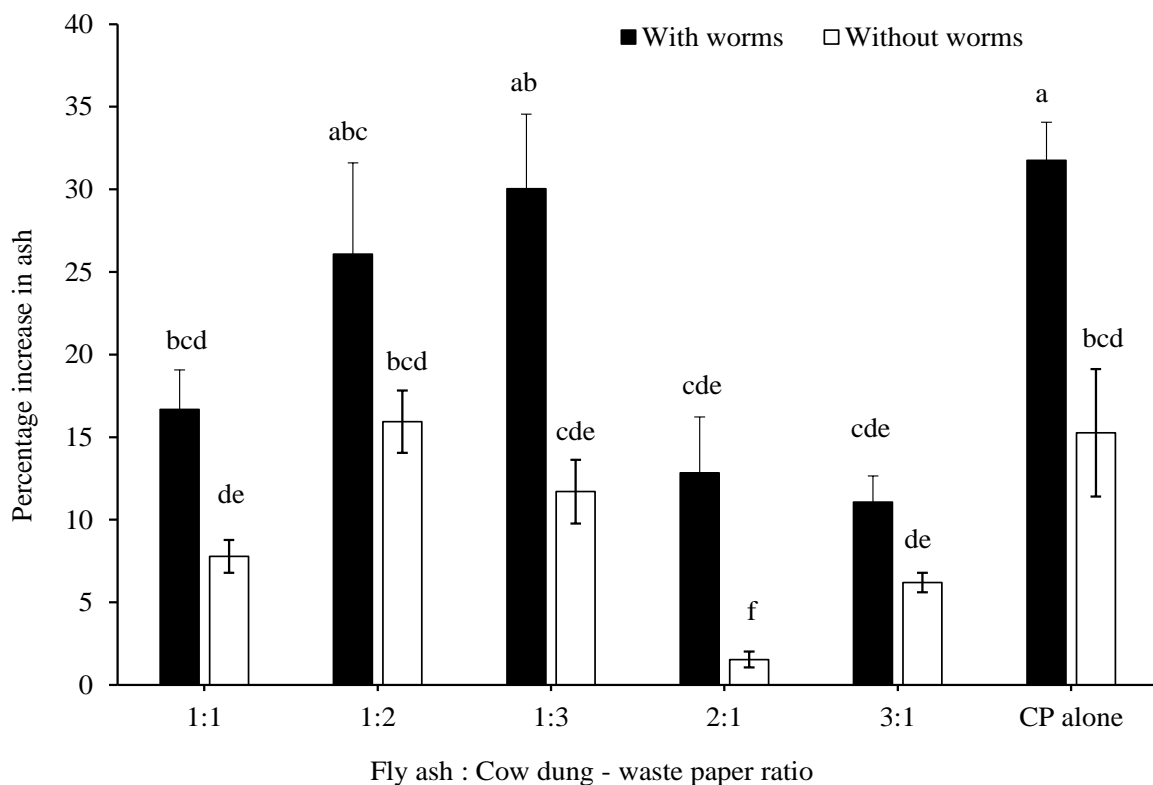
#### **4.3.9 Statistical analysis**

Sampling was not destructive and thus data were analysed using repeated measures analysis of variance (ANOVAR). Where sphericity assumptions could not be met, the Greenhouse-Geisser correction of  $P$  was used. All data were analysed using JMP version 11.0.0 statistical software (SAS Institute, Inc., Cary, North Carolina, USA, 2010).

## 4.4 RESULTS

### 4.4.1 Effects of fly ash rate and earthworm presence on ash content and C: N ratio

Following 14 weeks of composting, ash content was significantly ( $P < 0.001$ ) influenced by earthworm presence and the fly ash incorporation ratio (Table 4.2). Incorporation of fly ash into cow dung waste paper mixtures at ratios of 1:2 and 1:3 showed no difference with the CP alone control treatment, with or without earthworm incorporation on ash content (Figure 4.1). On average, across all treatments, addition of earthworms significantly ( $P < 0.05$ ) increased ash content by 21.4 % compared to 9.7 % without earthworms.



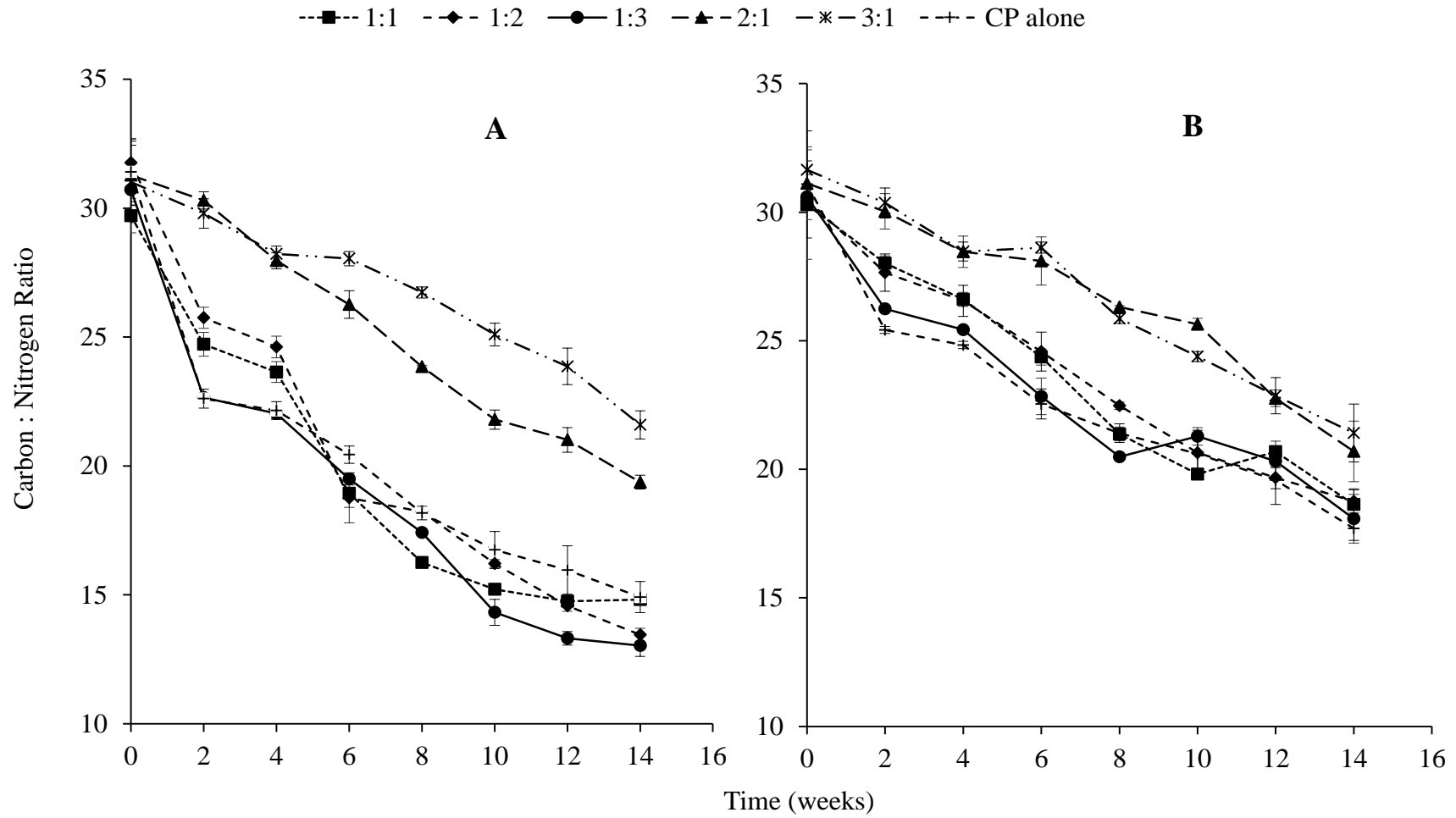
**Figure 4. 1:** Mean percentage increase in ash content following 14 weeks of composting (with or without earthworms) of fly ash incorporated into cow dung- waste paper mixtures. Error bars indicate standard deviation.

**Table 4. 2:** Repeated measures ANOVA for changes in several parameters following 14 weeks of fly ash-cow dung-waste paper composting.

Source of variation		Change in ash content (%)	C: N ratio <sup>a</sup>	Olsen extractable P (mg/kg) <sup>a</sup>	Nitrate N (mg/kg)	Ammonium N (mg/kg) <sup>a</sup>	Microbial Biomass Carbon (µg/g) <sup>a</sup>
<i>Between subjects</i>							
Fly ash incorporation level	$F_{5,288}$	383.3	1136.3	4059.5	3186.9	4213.53	146.8
	$P$	< 0.000	< 0.000	< 0.000	< 0.000	< 0.000	< 0.000
Earthworm presence (EP)	$F_{1,288}$	97.8	1851.5	657.9	2219.6	593.6	6.9
	$P$	< 0.000	< 0.000	< 0.000	< 0.000	< 0.000	0.0146
EP × Fly ash level	$F_{5,288}$	8.6	124.9	134.7	360.5	178.1	5.8
	$P$	< 0.000	< 0.000	< 0.000	< 0.000	< 0.000	0.0012
<i>Within subjects</i>							
Time	$F_{7,288}$	165.2	1747.8	200.6	437.02	37.7	184.4
	$P$	< 0.000	< 0.000	< 0.000	< 0.000	< 0.000	< 0.000
Time × EP	$F_{7,288}$	18.8	35.9	22.3	108.5	52.1	22.0
	$P$	< 0.000	< 0.000	< 0.000	< 0.000	< 0.000	< 0.000
Time × Fly ash level	$F_{35,288}$	3.8	18.8	51.5	43.4	43.3	12.3
	$P$	< 0.000	< 0.000	< 0.000	< 0.000	< 0.000	< 0.000
Time × Fly ash level × EP	$F_{35,288}$	1.5	6.1	7.2	19.6	20.3	9.6
	$P$	Ns	< 0.000	< 0.000	< 0.000	< 0.000	< 0.000

<sup>a</sup>Greenhouse-Geisser adjusted values of  $P$  for within subject factors.

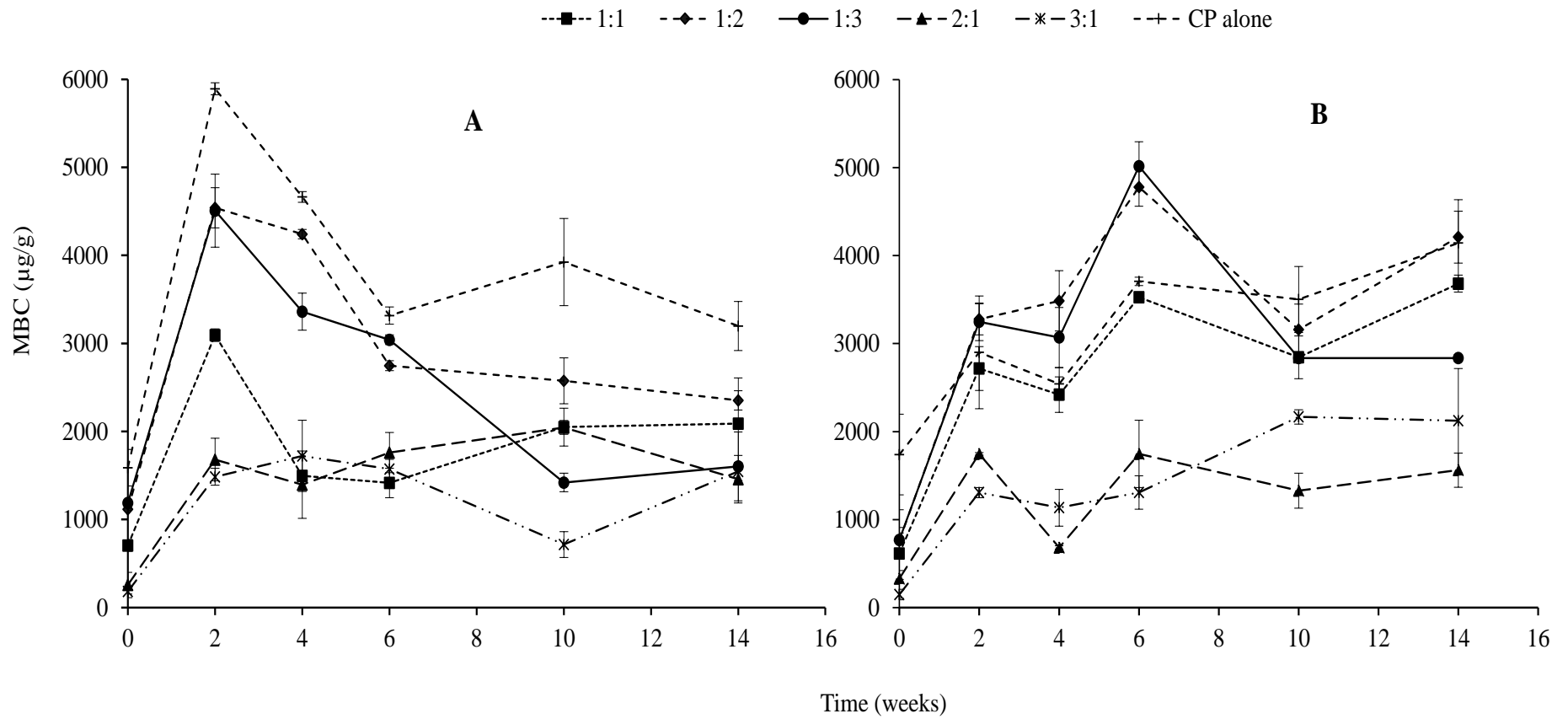
The C: N ratio of the compost decreased constantly with or without earthworm addition across all treatments. However, earthworm activity enhanced the loss of carbon, which resulted in a significantly faster decrease ( $P < 0.001$ ) in C: N ratio compared to the treatments without worms. With earthworm addition, the effects of fly ash incorporation level on changes in C: N ratio became more evident, as reflected by the wider significant differences between 2:1 and 3:1 treatments, compared to narrow differences where there were not earthworm addition (Figure 4.2). Both fly ash incorporation ratio and earthworm presence significantly affected the changes in C: N ratio, hence the significant ( $P < 0.001$ ) interaction between earthworm presence  $\times$  fly ash level as shown by ANOVAR (Table 4.2). With or without earthworms, the average decrease in C: N ratio throughout the 14 week vermicomposting period followed a highly significantly different ( $P < 0.0001$ ) order of 1: 3 > 1:2 > CP alone > 1:1 > 2:1 > 3: 1. Generally, addition of fly ash up to a ratio of 1:1 did not seem to significantly affect the changes in C: N ratio both with and without earthworms.



**Figure 4. 2:** Changes in C: N ratio over time during composting of fly ash incorporated into cow dung – waste paper mixtures A) with earthworm presence; B) without earthworms. Error bars represent standard deviation.

#### 4.4.2 Changes in microbial biomass carbon during fly ash composting

Microbial biomass in the composts was measured using microbial biomass carbon (MBC), and both earthworm presence and fly ash incorporation level significantly influenced changes in microbial biomass ( $P < 0.05$ ; Table 4.2). With or without earthworm presence, the treatments 1:1; 1:2; 1:3 and CP alone showed a significant increase ( $P < 0.05$ ) in microbial biomass compared to the treatment 2:1 and 3:1, from 0 to 2 weeks (Figure 4.3). However, with earthworm presence, microbial biomass in the 1:1; 1:2; 1:3 and CP alone treatments significantly ( $P < 0.05$ ) decreased beyond 2 weeks until 10 weeks, in which the values showed no significant difference from 10 to 14 weeks (Figure 4.3A). On the contrary, without earthworms, microbial biomass continued to increase beyond 2 weeks with the 1:2 and 1:3 treatments showing maximum microbial biomass at 6 weeks whilst the other treatments continued to increase up to 14 weeks (Figure 4.3B). There was a significant interaction between earthworm presence  $\times$  fly ash incorporation level on microbial biomass ( $P < 0.0012$ ; Table 4.2). The lower fly ash incorporation ratios of 1:1, 1:2 and 1:3 were associated with significantly ( $P < 0.05$ ) higher microbial biomass levels than the higher fly ash incorporation levels of 2:1 and 3:1 with or without earthworm addition (Figure 4.3). This indicated that microbial biomass was dependent on fly ash incorporation level.



**Figure 4. 3:** Changes in Microbial Biomass Carbon (MBC) over time during composting of fly ash incorporated into cow dung – waste paper mixtures A) with earthworm presence; B) without earthworms. Error bars represent standard deviation.

#### 4.4.3 Changes in humification parameters

The humification parameters are used to measure the degree of maturation and stabilization of composts. The presence of earthworms and fly ash incorporation level both significantly ( $P < 0.0001$ ) influenced the humification ratio (HR), humification index (HI) and the polymerisation index (PI) across all treatments. Generally, as the level of fly ash incorporation increased up to the 1: 1 level, the HR and HI significantly increased compared to the CP alone treatment (Figure 4.4 and 4.5). However, for PI incorporation of fly ash at the 1:3 ratio gave the highest value ( $P < 0.05$ ) compared to CP alone, 1:2 and 1:1 treatments (Figure 4.6). The influence of earthworm presence on the humification parameters was dependent on fly ash incorporation level as indicated by a significant interaction between earthworm presence  $\times$  fly ash incorporation level ( $P < 0.0001$ ). The increase in total extractable carbon resulted in a significant increase ( $P < 0.05$ ) in HR with earthworm presence, with the treatments 1:1; 1:2 and 1:3 resulting in a HR greater than 7 (Figure 4.4). A similar trend was observed on the humification index (HI), with earthworm presence resulting in a significantly ( $P < 0.05$ ) higher HI (Figure 4.5). However, incorporation of fly ash at 1:3 level resulted in the highest polymerisation index ( $P < 0.05$ ), compared to the CP alone and other treatments (Figure 4.6).

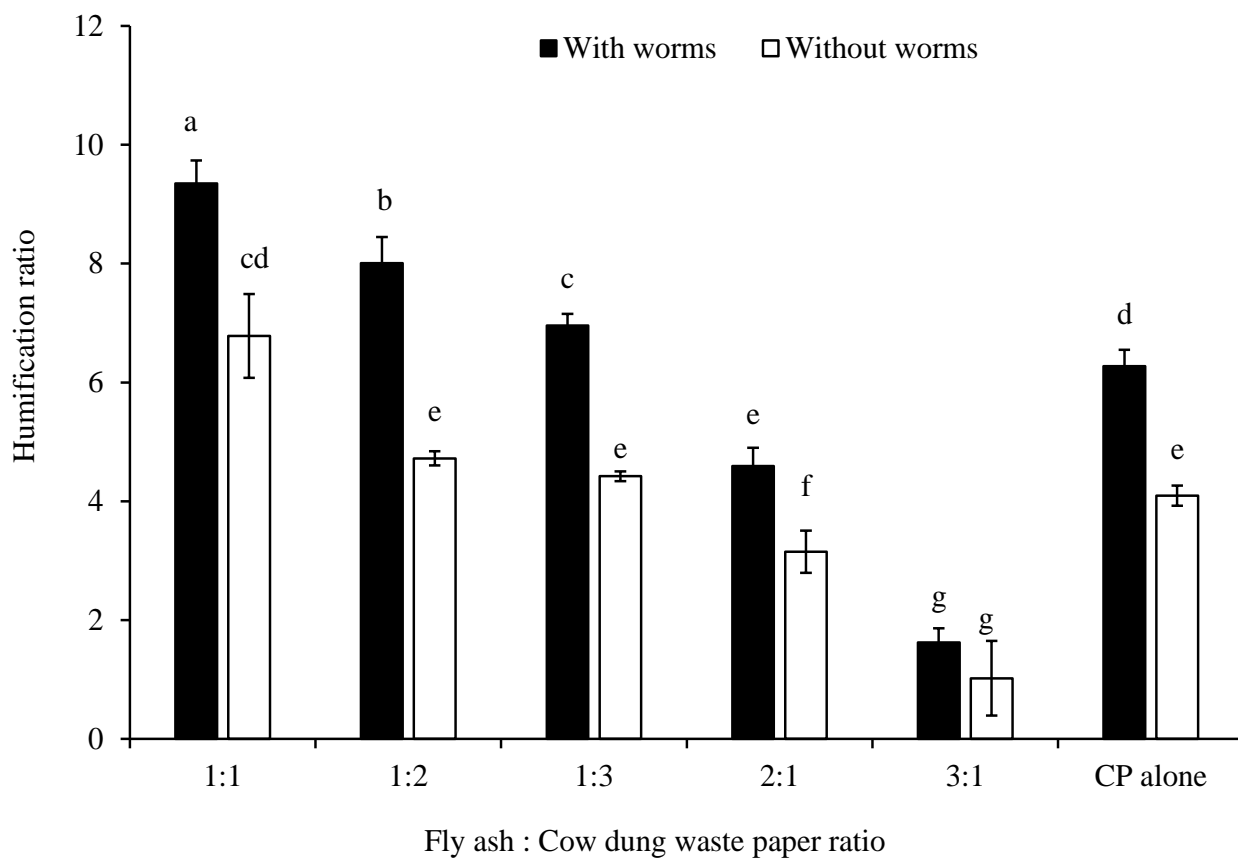
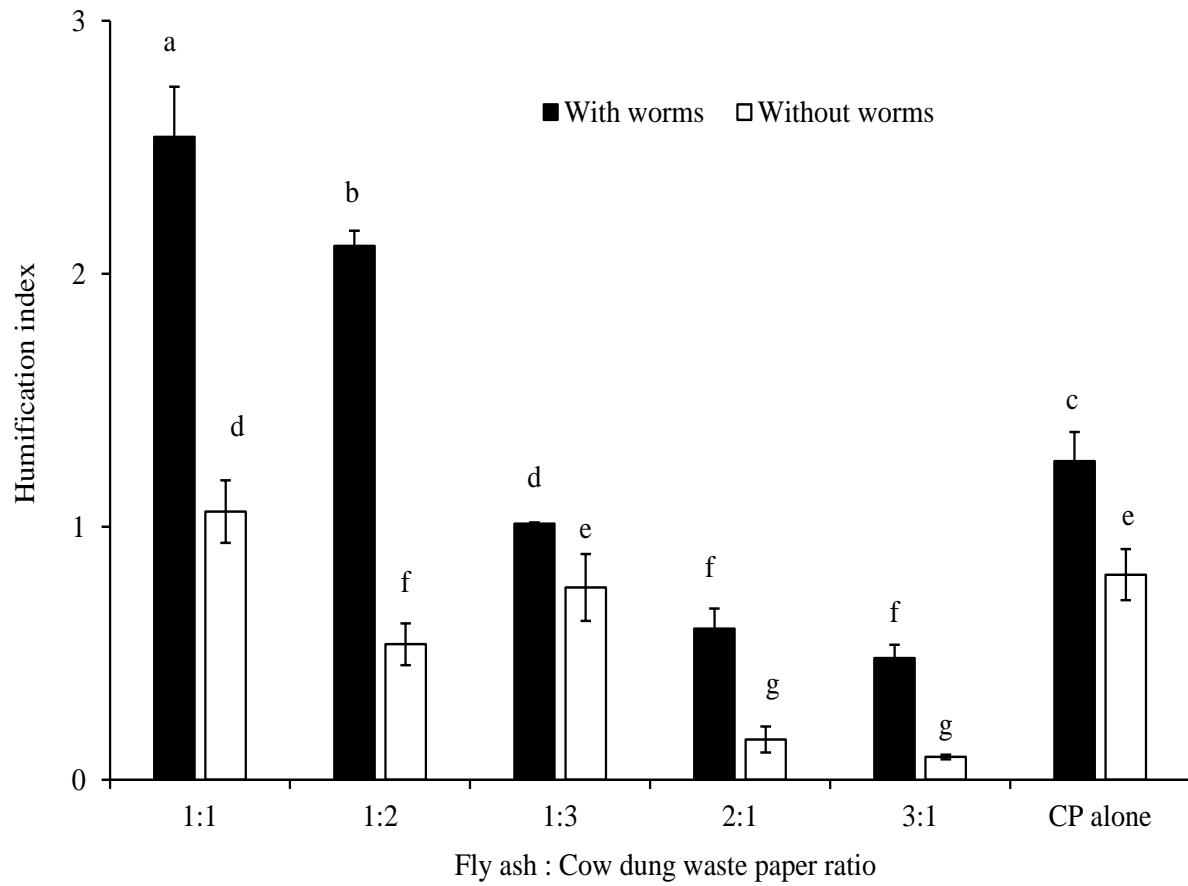
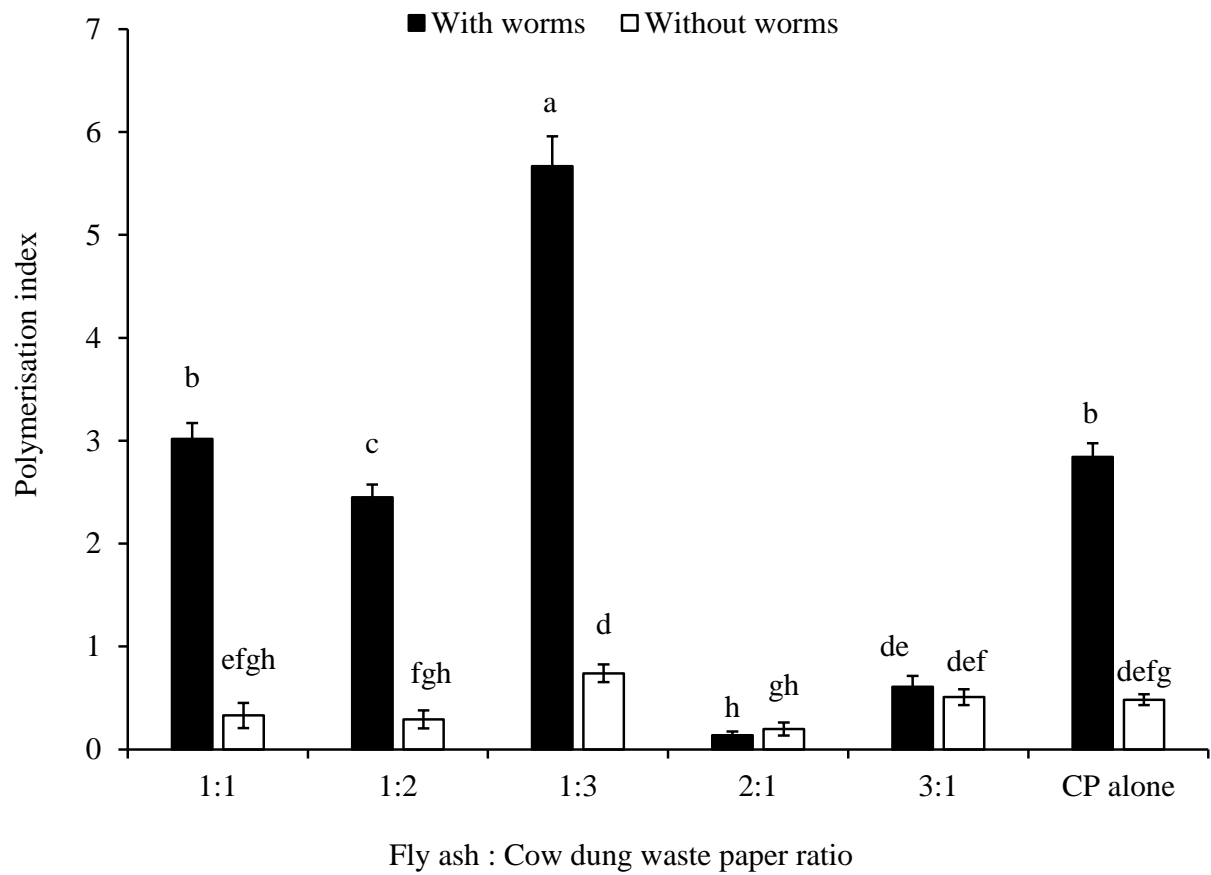


Figure 4. 4: Changes in humification ratio following 14 weeks of composting with or without earthworm presence. Error bars indicate standard deviation. Different lowercase letters indicating significant differences at  $P < 0.05$ .



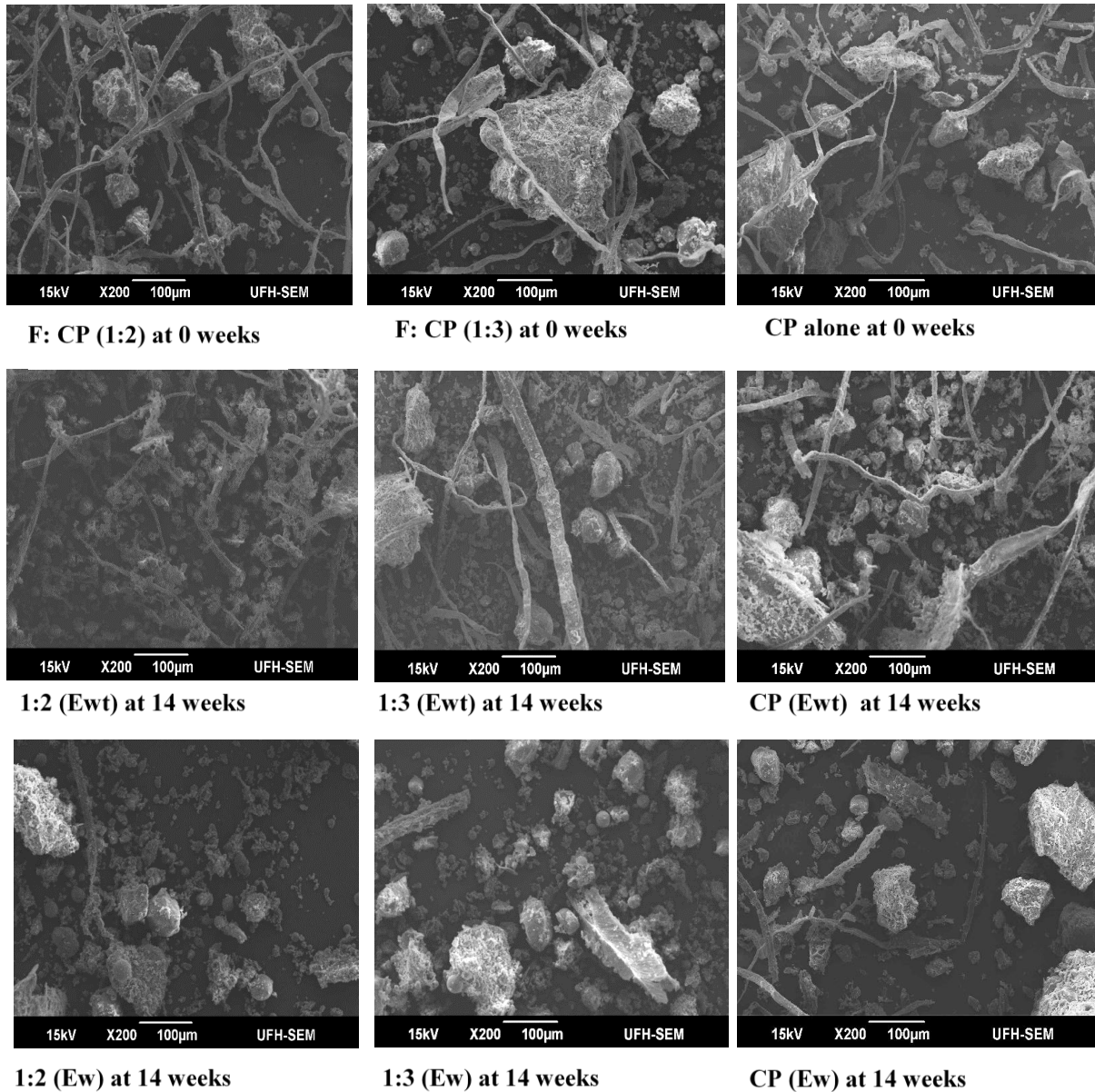
**Figure 4. 5:** Changes in humification index following 14 weeks of composting with or without earthworm presence. Error bars indicate standard deviation. Different lowercase letters indicating significant differences at  $P < 0.05$ .



**Figure 4. 6:** Changes in polymerisation index following 14 weeks of composting with or without earthworm presence. Error bars indicate standard deviation. Different lowercase letters indicating significant differences at  $P < 0.05$ .

#### **4.4.4 Changes in morphological properties of vermicomposts**

The scanning electron microscopy (SEM) images shown here for the 1:2; 1:3 and CP alone treatments (Figure 4.7) provided visual evidence to complement observations made on the maturity status and extent of humification of the composts based on the C: N ratio and humification parameters data. Initially, the morphological structure of the vermicomposts showed large fibrous materials originating from the waste paper which did not change much after 14 weeks in the composts with no earthworms. However, the SEM images showed that addition of fly ash at 1:2 and 1:3 ratio enhanced degradation as compared to the CP alone treatment where some fibres were still visible after 14 weeks, consistent with the observed humification parameters (Figures 4.4, 4.5, and 4.6).



**Figure 4. 7:** Scanning electron microscope images showing changes in morphological characteristics during composting; with earthworms (Ew) and without earthworms (Ewt) of fly ash incorporated into cow dung waste paper mixtures.

#### 4.4.5 Effects of fly ash level and earthworm presence on plant available phosphorus and nitrogen

The mean increase in Olsen extractable phosphorus following a 14 week period of composting is presented in Table 4.3. The highest increase in extractable phosphorus was observed in the CP alone treatment with and without earthworms. As fly ash incorporation level increased, the extractable phosphorus significantly ( $P < 0.05$ ) decreased. Earthworm addition significantly ( $P < 0.001$ ) increased the amount of extractable P released following the 14 weeks of composting. The treatments 1:2 and 1:3 proved to be the best in increasing extractable P during composting, with earthworms presence resulting in a significant 75.59 % increase in extractable P compared to where not earthworms were added, within the same 1:2 and 1:3 treatments. At the highest fly ash incorporation level of 3:1, a net decrease in extractable P was observed after 14 weeks of composting.

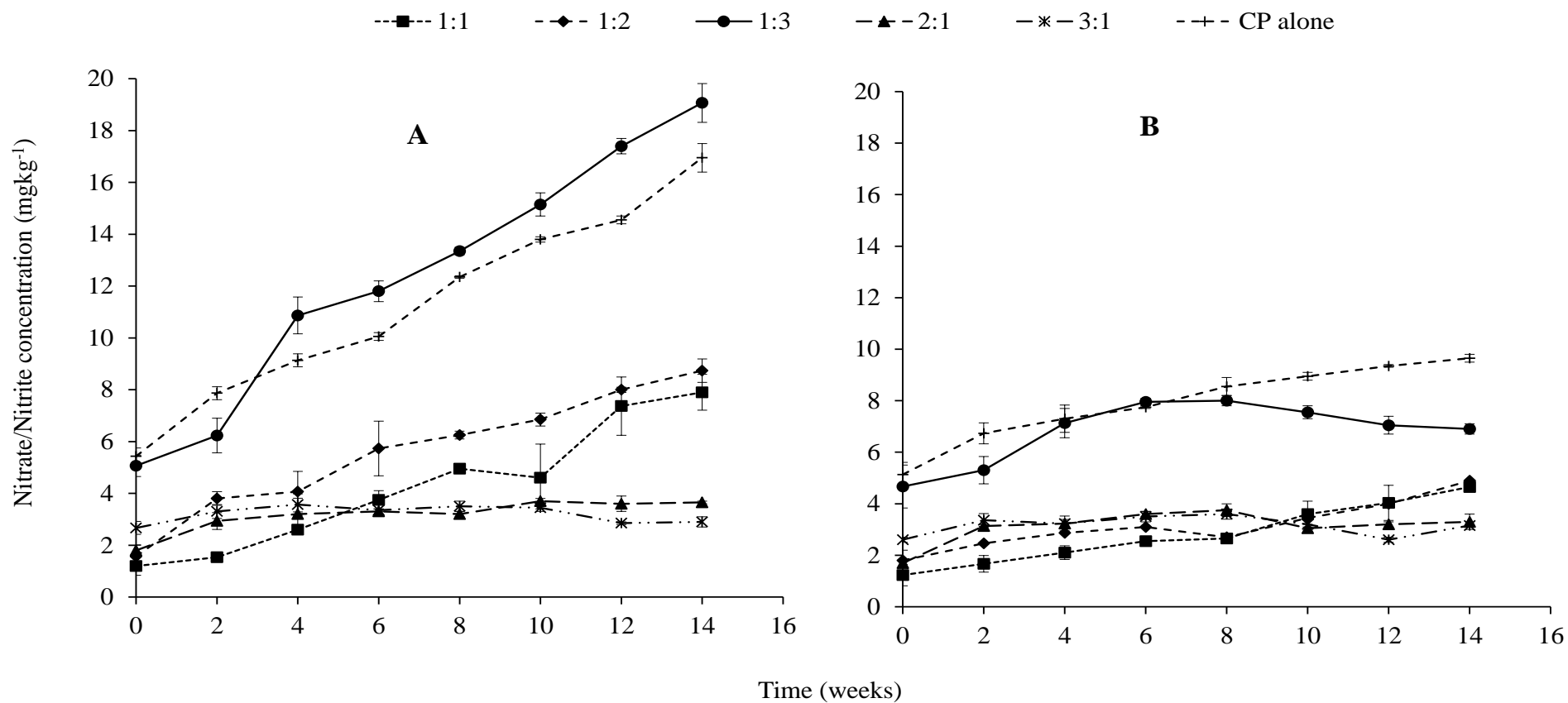
**Table 4. 3:** Mean percentage increase in Olsen extractable Phosphorus during composting after 14 weeks of incubation.

Composting	Treatments					
	F: CP (1:1)	F: CP (1:2)	F: CP (1:3)	F: CP (2:1)	F: CP (3:1)	CP alone
	%					
With earthworm presence	22.3c*	41.6b	40.4b	7.6d	-21.8f	87.5a
Without earthworms	8.0d	22.6c	24.1c	9.6d	-9.7e	39.3b

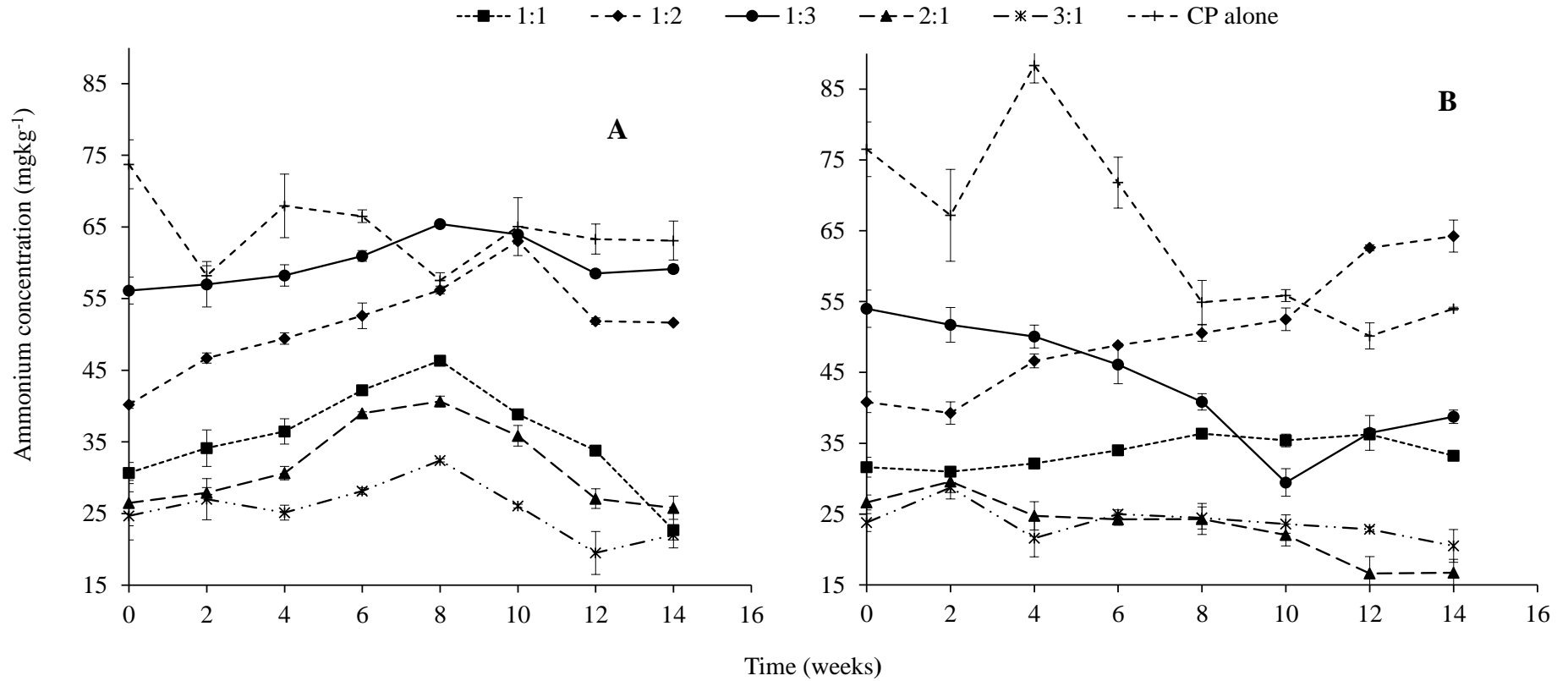
Values followed by the same lower case letter are not significantly different at  $P < 0.05$ . Fly ash (F), Cow dung – waste paper (CP)

\*mean percentage increase was calculated as the change in Olsen P for each treatment after 14 weeks of composting relative to the Olsen P at zero weeks.

The extractable N was measured as nitrate, nitrite and ammonium in the composts as shown in Figures 4.8 and 4.9. Generally, the nitrate/nitrite content was lower than the ammonium content across all treatments. Incorporation of fly ash at the 1:3 level resulted in the greatest release of nitrate followed by the CP alone treatment, across the entire composting period. Earthworm presence significantly ( $P < 0.001$ ) enhanced the release of nitrate/nitrite mostly in the 1:2, 1:3 and CP alone treatments. Fly ash incorporation level had a critical influence on the release of nitrate/nitrite as evidenced by the 10.34 mg/kg difference ( $P < 0.0001$ ) between the 1:3 and 1:2 treatments with earthworm presence after 14 weeks of composting. The changes in ammonium during the 14 weeks of composting did not show any consistent trend without earthworms. However with earthworm addition, from 10 weeks onwards, a general decline in ammonium was observed for all treatments except for the CP alone treatment, with the differences in changes in ammonium with time, earthworm presence and fly ash incorporation resulting in a significant interaction between time  $\times$  fly ash level  $\times$  earthworm presence ( $P < 0.0001$ ; Table 4.2). The importance of earthworm presence and the criticality of the fly ash incorporation level was also revealed in the ANOVAR which showed a significant ( $P < 0.001$ ) fly ash level  $\times$  earthworm presence interaction.



**Figure 4. 8:** Changes in extractable nitrate/nitrite over time during composting of fly ash incorporated into cow dung – waste paper mixtures A) with earthworm presence; B) without earthworms. Error bars represent standard deviation.



**Figure 4. 9:** Changes in extractable ammonium over time during composting of fly ash incorporated into cow dung – waste paper mixtures A) with earthworm presence; B) without earthworms. Error bars represent standard deviation.

## 4.5 DISCUSSION

The direct use of fly ash as a soil amendment has been limited by its poor mineralization potential and low nutrient concentration including the absence of N (Belyaeva and Haynes 2009). The present study investigated the optimization of fly ash incorporation into cow dung – waste paper mixtures for enhanced biodegradation and mineralization of the fly ash – cow dung – waste paper mixtures, during composting with and without earthworms. Biodegradation was evaluated by monitoring treatments effects on the humification of the composts. A successful composting process is determined by the maturity and stability of the compost such that the compost will not act detrimentally when used as a soil amendment (Henry and Harrison 1996). No one parameter can be effectively used to evaluate compost maturity (Raj and Antil 2011) therefore, in this study various compost humification indices were evaluated.

The observed percentage increase in ash content following 14 weeks of composting across all treatments indicated decomposition and mineralization of organic matter in the composts as highlighted by Mupondi et al. (2010). However, with the presence of earthworms, the higher ash content compared to treatments without earthworms indicated enhanced mineralization with earthworm presence, similar to observations made by Gupta and Garg (2009). Using the C: N ratio as an indicator of organic matter decomposition, the results indicated that earthworms enhanced the breakdown of the organic matter in the waste mixtures. Addition of earthworms has been observed to rapidly increase microbial biomass and diversity, with the microbes being the main drivers in organic matter losses mainly by using the carbon as an energy source (Gomez-Brandon et al. 2013; Gupta and Garg 2011).

In this study, the presence of earthworms enhanced microbial biomass within the early stages of composting which resulted in the rapid depletion of resources in the form of organic matter, for the microbes compared to the treatments without earthworms as also reported by Dominguez (2011). The rapid decrease in organic matter under earthworms, which is lost mainly as CO<sub>2</sub>, results in weight loss which has a concentration effect resulting in an increase in nitrogen concentration, thus reducing the C: N ratio (Bernal et al. 2009). This explains the rapid changes observed in the C: N ratio in the treatments with earthworms compared to where earthworms were not present. A C: N ratio of between 15 and 12 has been reported to represent mature stable compost, (Bustamante et al. 2008); and this was observed when fly ash was incorporated at ratios below 1:1 with earthworm presence, in our study. Incorporation of fly ash beyond the 1:1 level significantly reduced the organic matter turn over as reflected by the slow changes in ash content and C: N ratio relative to the 2:1 and 3:1 treatments. This is most likely due to the reduction in microbial biomass and possibly diversity under higher incorporation levels of fly ash (Mupambwa et al. 2015).

As highlighted above, the rapid depletion of resources available for the microbes causes a rapid decrease in microbial biomass observed in Figure 4.3. Pramanik and Chung (2011) also highlighted that the rapid decomposition of organic matter in the presence of earthworms could be responsible for the decrease in microbial biomass. The relatively higher organic matter in the 1: 2 and 1: 3 treatments with earthworm presence thus resulted in an initially higher microbial biomass during the first two weeks which decreased rapidly thereafter indicating faster degradation under lower fly ash incorporation similar to the CP alone treatment.

The increase in humification parameters with earthworm presence indicated the transformation of the easily degraded molecules to more recalcitrant molecules with higher molecular weight which is a good indicator of a mature compost (Mupondi et al. 2010). The inclusion of fly ash at levels beyond 1:1 with earthworms, resulted in a polymerisation index above 1, which indicates a mature compost (Bernal et al. 2009). It was noteworthy that inclusion of fly ash between 1:1 and 1:3 ratio, with earthworm presence, resulted in high HR, HI and PI relative to the CP alone treatment. These results are consistent with work done by Unuofin and Mnkeni (2014) using cow dung – waste paper and rock phosphate, in which composts with higher humification parameters were observed when earthworms were added to the composts. The maturation and extent of humification was also confirmed by the SEM images which showed that earthworms greatly increased degradation of the fibrous structure of the cow dung – paper based compost, resulting in a fine well degraded composts, similar to observations made by Aira et al. (2006b) and Ravindran and Sekeran (2010).

The sensitivity of the composting process to the levels of fly ash incorporation, possibly due to microbial activity modification as fly ash level increase, was quite apparent throughout this study. This could explain the decrease in P content and N transformations as the level of fly ash increased, with incorporation of fly ash into cow dung – waste paper mixtures seeming to inhibit the mineralization of phosphorus, in comparison to the CP alone treatment. This is contrary to reports by Bhattacharya and Chattopadhyay (2002; 2004) and Venkatesh and Eevera (2008), who reported a high contribution of extractable phosphorus from fly ash at an incorporation ratio of 1: 1. During vermicomposting of organic and inorganic waste mixtures, it is likely that the microbes attack the easily degradable organic material first followed by the inorganic material, as earthworms and microbes have been observed to derive their nutrition mostly from the organic materials (Sarojini et al. 2009). We suspect that in our

study, the high total phosphorus in the cow dung, could have provided much of the mineralizable phosphorus during organic matter decomposition, thus masking the contribution of fly ash to phosphorus in the vermicomposts. Ananthakrishnasamy et al. (2009) observed similar results using a different earthworm species (*Lampito mauritti*) in which the treatment with the highest cow dung and less fly ash resulted in the highest phosphorus content after vermicomposting. Though extractable nitrogen is not an important element to consider from fly ash, a similar influence to that observed on P, was observed on changes in inorganic nitrogen during fly ash composting. Nevertheless, incorporation of fly ash at 1:2 and 1:3 ratio resulted in vermicomposts that were rich in phosphorus and nitrogen making them potential nutrients sources in agriculture. It would be interesting to further evaluate the effects of optimizing the earthworm stocking density on phosphorus release in fly ash incorporated vermicomposts as higher stoking densities could produce more organic acids and enzymes and result in greater mineralization of fly ash phosphorus during composting with earthworms as the findings of Unuofin and Mnkeni (2014) and Ndegwa and Thompson (2000) seem to suggest.

#### **4.6 CONCLUSION**

Vermicomposting of fly ash incorporated into optimized cow dung resulted in effective degradation and nutrient release during composting. However, contrary to other reports in which non-optimized cow dung was used, incorporation of fly ash at a ratio of 1: 2 seemed most appropriate as this give a fly ash based vermicompost with desirable maturity and nutritional properties whilst allowing for more fly ash. Optimization of cow dung using waste paper and incorporation of fly ash during vermicomposting can be an effective waste management strategy for both fly ash and waste paper.

## CHAPTER FIVE

### **5.0 *EISENIA FETIDA* STOCKING DENSITY OPTIMIZATION FOR EFFICIENT BIODEGRADATION AND NUTRIENT RELEASE DURING VERMICOMPOSTING OF FLY ASH INCORPORATED INTO COW DUNG-WASTE PAPER MIXTURES**

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This Chapter is based on a manuscript entitled:

Mupambwa H.A. and Mnkeni P.N.S. Forthcoming. *Eisenia fetida* stocking density optimization for efficient biodegradation and nutrient release during vermicomposting of fly ash incorporated into cow dung-waste paper mixtures.

## 5.1 ABSTRACT

Though it's widely agreed that stocking density critically affects rate of vermicomposting, there appears to be no agreed stocking density specific for waste materials like fly ash, for the attainment of a highly degraded and nutrient rich vermicompost. This study sought to optimize the stocking density of fly ash – cow dung – waste paper mixtures for effective biodegradation and nutrient release. Four stocking densities of 0 g; 12.5 g; 25 g and 37.5 g worm/kg; including and control with cow dung- waste paper only (CP alone); were evaluated on their effects on biodegradation and nutrient release in fly ash vermicomposts over 10 weeks. Though the treatments 12.5 g; 25 g and 37.5 g all resulted in a mature vermicompost, a stocking density of 25 g-worm/kg and above resulted in faster maturity; higher humification parameters and a significantly low final C: N ratio range (11.1 – 10.4). The activity of  $\beta$ -glucosidase and fluorescein di-acetate hydrolysis enzymes showed faster stabilization at stocking densities of 25 g-worm/kg and above, representing compost stability and maturity. These enzymes also showed significant ( $P < 0.05$ ) correlation with changes in C: N ration in the compost. Similarly, a stocking density of 25 g-worm/kg resulted in the highest potential to increase in Olsen extractable P and nitrate/nitrite. The CP control; 0 g; 12.5 g; 25 g and 37.5 g treatments resulted in a net Olsen P increase of 32.4%; 16.3%; 38.9%; 61.0% and 53.0% respectively, after 10 weeks. Though compost maturity can be attained at stocking densities of 12.5 g-worm/kg, for faster more humified and nutrient rich fly ash vermicompost, a stocking density of 25 g-worm/kg seemed most appropriate for the fly ash vermicompost.

**Keywords:** Enzyme activity, phosphorus; fly ash incorporation, humification parameters.

## 5.2 INTRODUCTION

Fly ash is a product of coal combustion at thermal power stations, which constitutes more than 70 percent of the solid residue during coal combustion. Fly ash is generally categorised in most countries, including South Africa, as a waste material with potential to pollute the environment due to the presence of potentially toxic heavy metals in fly ash (Mupambwa et al. 2015). Apart from the environmental challenge, fly ash also contains essential nutrients like phosphorus, making it a potential source of nutrients in agriculture. With respect to the heavy metals, several researchers have demonstrated that the majority of these heavy metals are not readily available for plant uptake when fly ash is used in agriculture at lower levels of application (Ram and Masto 2014; Singh and Pandey 2013; Pandey et al. 2009). Recently fly ash research on plant nutrition has focussed on enhancing the bioavailability of essential plant nutrients in fly ash using epigeic earthworm species like *Eisenia fetida*, a process called vermicomposting (Mupambwa et al. 2015).

As fly ash is almost devoid of carbon, considerable research has been carried out on blending fly ash with various organic materials like animal manure, poultry manure, sewage sludge and press mud (Ram and Masto 2014). This has the advantage of enhancing nutrient availability, decreasing heavy metal bioavailability, pH reduction and stimulating microbial activity (Ram and Masto; Mupambwa et al. 2015). Cow dung appears to be one of the most commonly preferred substrates for enriching fly ash with C during vermicomposting, possibly due to its ubiquitous nature. Several researchers such as Bhattacharya and Chattopadhyay (2002; 2006); Venkatesh and Eevera (2008) and Ananthkrishnasamy et al. (2009), have evaluated the most appropriate fly ash incorporation for effective fly ash vermicomposting using cow dung but this was not done at the C: N ratio recommended by Ndegwa and Thompson (2000). Mupondi et al. (2010) used cow dung which had been

adjusted to an appropriate C: N ratio of 30 using waste paper and observed effective vermicomposting of rock phosphate with *Eisenia fetida* earthworms. Unuofin and Mkeni (2014) and Gupta and Garg (2009) have also demonstrated that waste paper that is usually incinerated in most municipalities in South Africa, can also be used as an important carbon source and bulking agent when mixed with cow dung, for effective vermicomposting.

Apart from the carbon and nitrogen balance, the stocking density of earthworms during vermicomposting has been identified as a critical factor as it directly influences microbial and enzyme activity, hereby affecting the quality and duration of the vermicomposting process. Intracellular and extracellular enzyme are known to be critical drivers involved in the degradation of different constituents of organic matter during composting, which include  $\beta$ -glucosidases related to C decomposition; phosphatases and arylsulfatases involved in P and S cycles and fluorescein di-acetate (FDA) representing total enzyme activity (Garcia-Gomez et al. 2003; Cayuela et al. 2008). The determination of various enzyme activities during vermicomposting can be a useful tool for evaluation of biological activity in vermicomposts under different conditions (Garcia-Gomez et al. 2003). However, the relationship between earthworm stocking densities and its influence on biological properties like enzymes and compost maturity has been seldom researched.

Different stocking densities have been reported in the literature; e.g. Lazcano et al. (2008) working with cattle manure used 250 g of earthworms per kg of manure; Ananthkrishnasamy et al. (2009) working with cattle manure and fly ash used 15 g of earthworms per kg of substrate; Bhattacharya et al. (2012) and Bhattacharya and Chattopadhyay (2006) mixed fly ash with cow dung and used 10 worms per kg substrate whilst Ndegwa et al. (1999) recommended a stocking density of 1.60 kg worms/m<sup>2</sup> using bio-

solids. Moreover, much of the work on stocking density in vermicomposting has focused mainly on the growth and development of the earthworms and not on compost maturity (Dominguez and Edwards 1997, Garg et al. 2008). Though it is widely agreed that stocking density critically affects rate of vermicomposting, there appears to be no agreed stocking density specific for various waste materials like fly ash, for the attainment of a highly degraded and nutrient rich vermicompost. It is therefore, of interest to establish the optimum earthworm stocking density for the bioconversion of coal fly ash which has been incorporated into cow dung adjusted to the optimum C: N ratio of 30 using waste paper. The objectives of this study were thus to (i) investigate the optimum stocking density of *E. fetida* for effective biodegradation of fly ash incorporated into cow dung – waste paper mixtures; (ii) to evaluate the effect of varying *E. fetida* stocking densities on the release of plant available phosphorus and inorganic nitrogen.

## **5.3 MATERIALS AND METHODS**

### **5.3.1 Materials for vermicomposting**

The study was carried out at the vermicomposting research unit at the University of Fort Hare, Alice campus, Eastern Cape Province (32° 46' S and 26° 50' E). Cylindrical vermireactors measuring 0.30 m (top diameter) × 0.23 m (height) and 0.21 base (diameter) with a total volume of 0.015 m<sup>3</sup>, having perforations underneath were used for the vermicomposting process. The cow dung was collected from a mainly pasture based dairy farm located in Keiskammahoek, located some 60 km away from the University of Fort Hare, Alice campus, crushed and air dried. The waste paper was sourced from within the Alice campus of the University of Fort Hare and shredded at the University of Fort Hare

Duplicating Centre. The fly ash used in this study was obtained from Matla power station (26° 16' S; 29° 08' E), located in Mpumalanga Province of South Africa. Mature *Eisenia fetida* earthworms were used in this study and these were sourced from our local wormery at the University of Fort Hare, mainly feeding on cow dung and waste paper, The cow dung, waste paper and fly ash used in this study was initially characterized for various chemical characteristics and these are shown in Table 4.1 (Chapter 4). The cow dung was originally mixed with waste paper to adjust the C: N ratio to an optimum of  $30 \pm 1$  for effective vermicomposting, as recommended by Unuofin and Mnkeni (2014). The fly ash (F) was then incorporated into the cow dung – waste paper mixtures (CP) at a ratio of 1: 2 F: CP (w/w basis) as recommended by Mupambwa and Mnkeni (2015b).

### **5.3.2 Treatments and experimental design**

Three stocking densities of *E. fetida* were evaluated which are 0; 12.5; 25.0 and 37.5 g-worms/kg dry mass of fly ash-cow dung- waste paper mixtures (FCP) based on a study by Unuofin and Mnkeni (2014). The experiment was laid down in a completely randomised design with three replications. The total weight of fly ash - cow dung – waste paper mixture used was 4 kg dry mass with no extra feed being added for the duration of the 10 week experiment. Prior to inoculating with earthworms, the mixtures were allowed to pre-compost for a period of 1 week at 60% moisture level to allow for elimination of toxic volatile gases (Mupondi et al. 2010). After pre-composting, the mixtures were adjusted to a moisture content of  $70 \pm 10\%$  after which the earthworms were inoculated at the different rates. Samples were collected at 2 week intervals during the entire vermicomposting process and analysed for the various chemical and biological parameters as described below.

### 5.3.3 Chemical Analysis

### 5.3.4 Total carbon and nitrogen

Total C: N was measured on dry, ground samples using a Truspec CN Carbon/ Nitrogen auto analyser (LECO Corporation, 2003).

### 5.3.5 Humification parameters

The humification parameters were determined using methods described in Chapter 4 section 4.3.5. Humification ratio (HR), humification index (HI), percentage of humic acids (Pha) and polymerisation index (PI) which are indices used for the evaluation of humification level in the compost were then calculated as shown in the equations below:

$$HR = \frac{C_{EX}}{C} \times 100 \quad \text{[Equation 5.1]}$$

$$HI = \frac{C_{HA}}{C} \times 100 \quad \text{[Equation 5.2]}$$

$$Pha = \frac{C_{HA}}{C_{tEX}} \times 100 \quad \text{[Equation 5.3]}$$

$$PI = \frac{C_{HA}}{C_{FA}} \times 100 \quad \text{[Equation 5.4]}$$

### 5.3.6 Enzyme activity

#### *β-Glucosidase* (Deng and Popova 2011)

The  $\beta$ -Glucosidase activity was measured based on the colorimetric determination of *p*-nitrophenyl released when a compost is incubated with *p*-Nitrophenyl -  $\beta$  - D- glucoside (PNG) at pH 6. A 1 g (fresh weight) compost sample was mixed with 0.2 mL of toluene, 4 mL of buffer solution and 1 mL of PNG and then incubated for 1 hour. A control with no PNG was also included for each respective sample. After incubating for one hour, 1 mL of 0.5 M CaCl<sub>2</sub> and 4 mL of 0.1 M of tris (hydroxymethyl) aminomethane (THAM) was added to all samples, with the PNG solution being added to the control samples at this time. The samples were then filtered using Whatman No.2 filter paper and the yellow colour intensity of the filtrate was measured using a Helios Thermo Spectrophotometer (Thermo Fisher Scientific, England) at 405 nm. The activity of  $\beta$ -Glucosidase was determined based on the  $\mu$ mol of *p*-nitrophenol released by reference to a calibration curve developed with standards containing *p*-nitrophenol. The  $\beta$ -Glucosidase activity ( $\beta$ ) was calculated as shown in equation 5.5 below:

$$\beta \text{ (}\mu\text{mol of } p\text{-nitrophenol/ g – dry weight compost/ hour)} = \frac{S-C}{DM} \quad [\text{Equation 5.5}].$$

Where S is mean concentration of *p*-nitrophenol in the sample

C is concentration of *p*-nitrophenol in the control

DM is the dry mass of the compost (determined based on moisture content)

***Alkaline Phosphatase*** (Acosta-Martinez and Tabatabai 2011)

Alkaline phosphatase activity was determined based on the colorimetric estimation of the *p*-nitrophenol released by phosphatase activity when a substrate is incubated with buffered sodium *p*-nitrophenyl phosphate solution at pH 11 and toluene. A 1 g (fresh weight) compost sample was placed into a 150 mL plastic bottle and 0.2 L of toluene, 4 mL of Modified Universal Buffer (MUB) at pH 11 and 1 mL of *p*-nitrophenol phosphate (PNP) solution was added. A control with no PNP was also included for each respective sample. All the samples were then incubated at 37 °C for 1 hour, following which 1 mL of 0.5 M of CaCl<sub>2</sub> and 4 mL of 0.5 M of NaOH was added, with the PNP being added to the controls at this moment. The suspensions were then filtered through Whatman No.2 filter paper and the yellow intensity of the filtrate measured using a Helios Thermo Spectrophotometer (Thermo Fisher Scientific, England) at 410 nm. The alkaline phosphatase activity was expressed as µg of *p*-nitrophenol released per g-dry weight compost per hour, determined based on a reference calibration curve. Alkaline phosphatase activity (A) was calculated as shown in equation 5.6 below:

$$A (\mu\text{g of } p\text{-nitrophenol/ g-dry weight compost/ hour}) = \frac{S-C}{DM} \quad \text{Equation 5.6.}$$

Where S is mean concentration of *p*-nitrophenol in the sample

C is concentration of *p*-nitrophenol in the control

DM is the dry mass of the compost (determined based on moisture content)

### ***Fluorescein di-acetate hydrolysis*** (Prosser et al. 2011)

Fluorescein di-acetate (FDA) activity was determined by measuring the fluorescein released following hydrolysis of fluorescein di-acetate. A 1 g (fresh weight) compost sample was placed into a 150 mL plastic bottle and 50 mL of THAM buffer (0.1 M, pH 7.6) was added to each flask and 0.5 mL of 47.6  $\mu$ M FDA. A control sample was included for each sample in which only acetone and not FDA was added. The samples were incubated at 37 °C for 3 hours following which 2 mL of acetone was added to all samples and the FDA was added to the controls at this time. The samples were then filtered using Whatman No. 2 filter paper and the intensity of the yellow green colour measured using Helios Thermo Spectrophotometer (Thermo Fisher Scientific, England) at 490 nm. The FDA hydrolytic activity was then expressed as  $\mu$ g of fluorescein released per g-dry weight of compost per hour, based on a reference calibration curve. The FDA activity was calculated as shown in equation 5.7 below:

$$FDA \text{ (}\mu\text{g of fluorescein/ g – dry weight compost/ hour)} = \frac{S-C}{DM} \quad \text{Equation 5.7}$$

Where: S is mean concentration of fluorescein in the sample

C is concentration of fluorescein in the control

DM is the dry mass of the compost (determined based on moisture content)

### **5.3.7 Exchangeable ammonium and nitrate and nitrite** (Maynard et al. 2006)

Exchangeable ammonium and nitrate/nitrite was extracted using 0.5 M potassium sulphate as described in Chapter 4 section 4.3.8.

### **5.3.8 Olsen extractable P (Schoenau and O'Halloran 2006)**

The Olsen P was extractable using the procedure explained in Chapter 3. Section 3.3.4.

### **5.3.8 Statistical Analysis**

Sampling was not destructive and thus data were analysed using repeated measures analysis of variance (ANOVAR). Where sphericity assumptions could not be met, the Greenhouse-Geisser correction of  $P$  was used. All data was analysed using JMP version 11.0.0 statistical software (SAS Institute, Inc., Cary, North Carolina, USA, 2010).

## 5.4 RESULTS

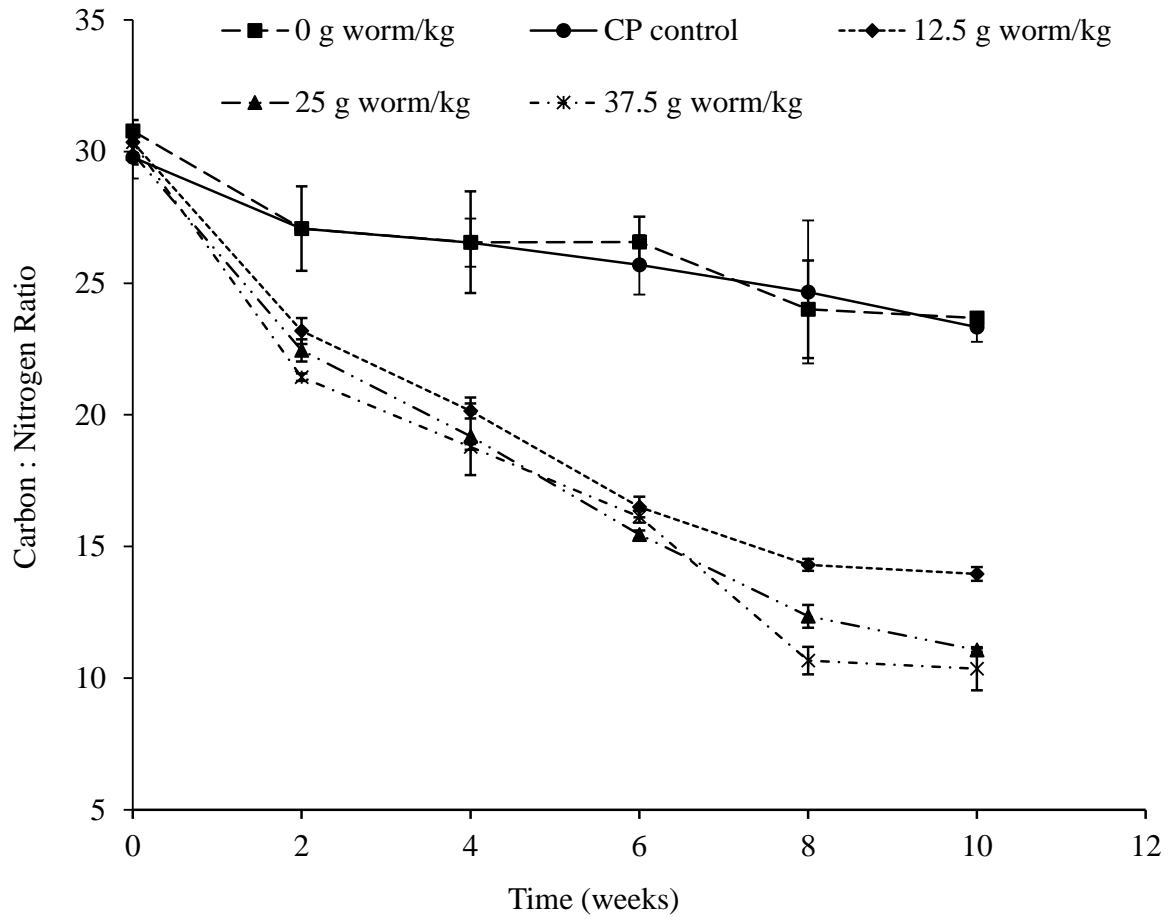
### 5.4.1 Effects of *E. fetida* stocking density on C: N ratio and humification parameters in fly ash fly ash vermicompost.

Across the 10 weeks of vermicomposting in all treatments, the carbon: nitrogen ratio generally decreased from the original C: N ratio of 30 to lower values (Figure 5.1). Earthworm stocking density, however, resulted in highly significant differences ( $P < 0.0001$ ) in the C: N ratio change, with time also significantly affecting the rate of C: N ratio decrease as indicated by the significant interaction between stocking density and time (Table 5.1). Throughout the vermicomposting process, the control of cow dung- waste paper with no earthworms and 0 g-worm fly ash- cow dung – waste paper treatments showed the least change in C: N ratio. It was noteworthy that across the three *E. fetida* stocking densities of 12.5; 25 and 37.5 g- worm/kg, significant differences between the three stocking densities on C: N ratio changes were observed only from 8 to 10 weeks (Figure 5.1). Following 10 weeks of composting, the final C: N ratio within the treatments followed the order CP control > 0 g-worm > 12.5 g- worm > 25 g-worm > 37.5 g-worm. The corresponding final C: N ratios were of 23.7, 23.3, 14.0, 11.1 and 10.4, respectively at the end of 10 weeks.

A significant difference between 0 and 10 weeks measurement was observed in all the humification parameters, with most of the treatments having higher humification parameters at 10 weeks. However, stocking density resulted in a much higher effect on the changes observed on the humification parameters (Figure 5.2 and 5.3).

**Table 5. 1:** Repeated measures ANOVA for changes in several maturity parameters following fly ash-cow dung-waste paper vermicomposting.

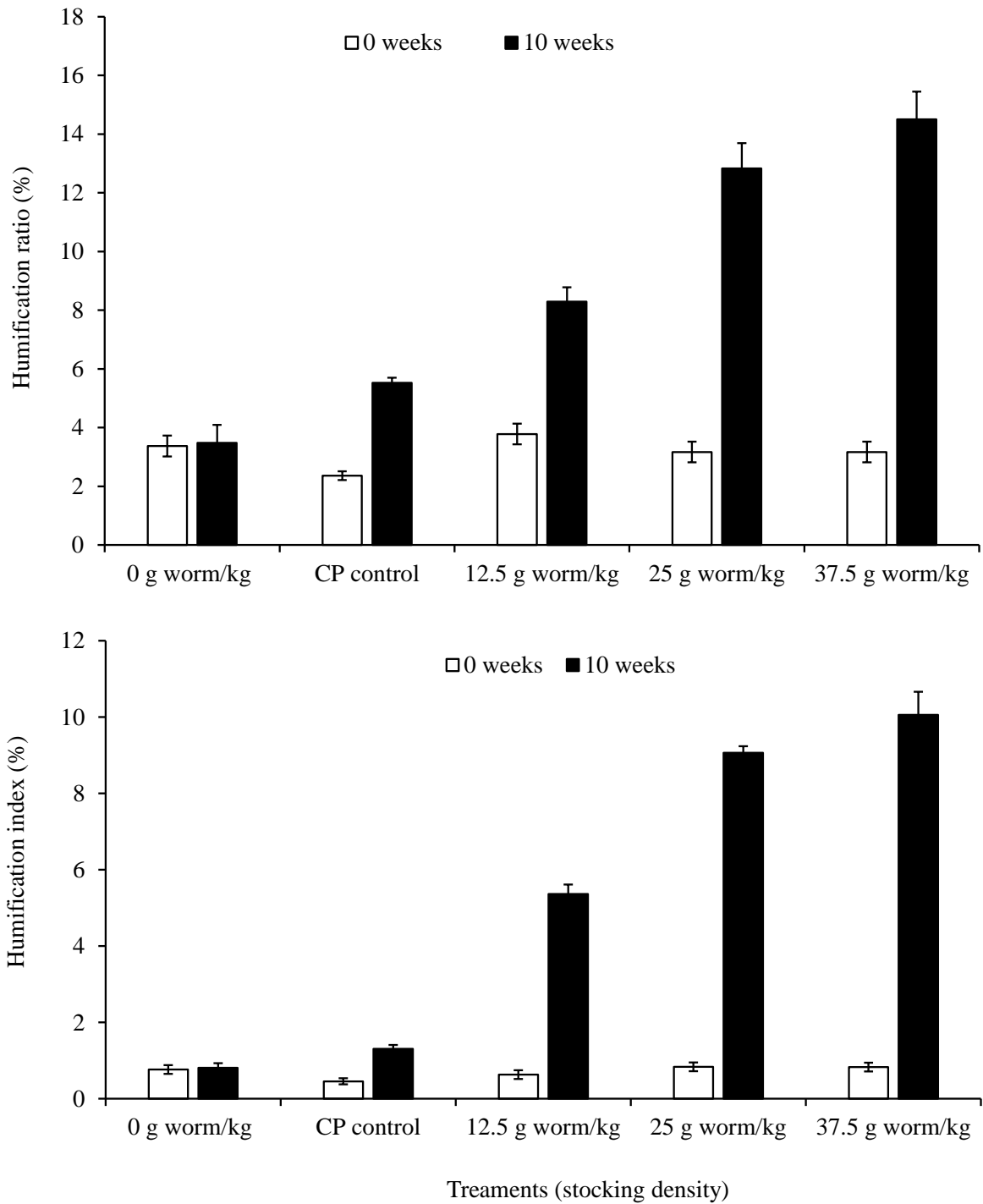
Effect	Stocking density ( <i>E. fetida</i> )		Time (weeks)		Stocking density × Time	
	F <sub>4,89</sub>	P	F <sub>5,89</sub>	P	F <sub>29,89</sub>	P
	C:N Ratio	293.77	< 0.0001	460.31	< 0.0001	24.07
Olsen P (mg/kg)	82.56	< 0.0001	156.98	< 0.0001	10.73	< 0.0001
NO <sub>3</sub> /NO <sub>2</sub> (mg/kg)	587.89	< 0.0001	878.89	< 0.0001	68.95	< 0.0001
NH <sub>4</sub> (mg/kg)	182.83	< 0.0001	202.06	< 0.0001	30.30	< 0.0001
Alkaline Phosphatase (µg-PNP/g-dry weight/h)	92.55	< 0.0001	51.79	< 0.0001	16.04	< 0.0001
β-Glucosidase (µmol-PNP/ g-dry weight/h)	6.73	0.0112	30.91	< 0.0001	4.78	0.0032
Fluorescein di-acetate (µg-FDA/g-dry weight/h)	36.79	< 0.0001	76.79	< 0.0001	6.69	0.0007



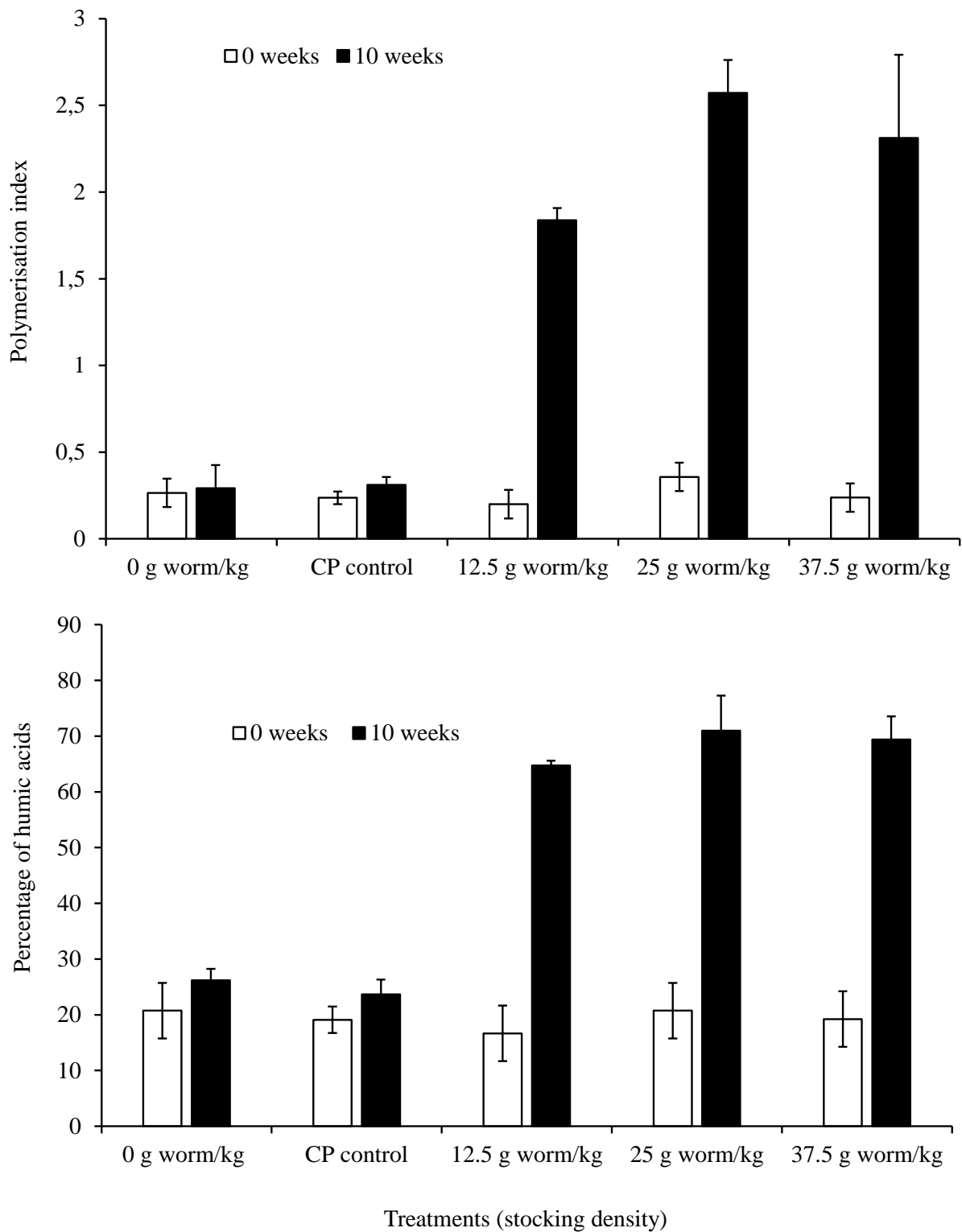
**Figure 5. 1:** Changes in Carbon: Nitrogen ratio during vermicomposting of fly ash – cow dung- waste paper mixtures at different *E. fetida* stocking densities. Error bars indicate standard deviation.

Significant differences between all treatments were observed on humification ratio and humification index after 10 weeks of vermicomposting, with an almost linear increase as stocking density increased. The humification ratio followed the order 37.5 g-worm (14.5%) > 25 g-worm (12.8%) > 12.5 g-worm (8.3%) > 0 g-worm (5.5%) > CP control (3.5%). Similarly, the humification index (HI) followed a similar order with the three stocking densities 12.5; 25 and 37.5 g-worm resulting in a HI greater than 3.5% whilst the 0 g-worm and CP control resulted in a HI of 0.81% and 1.3 %, respectively (Figure 5.2).

For the polymerisation index (PI) and percentage humic acids (Pha) it was noteworthy that no significant differences were observed between the three stocking densities 12.5; 25 and 37.5 g-worm at 10 weeks, with the 25 g-worm treatment having the highest values (Figure 5.3). However, compared to the CP control and 0 g – worm treatments, significant differences were observed between the treatments on PI and Pha, with the CP alone and 0 g-worm treatments resulting in PI of 0.31 and 0.29 whilst their Pha were 23.7 and 22.0, respectively.



**Figure 5. 2:** *E. fetida* stocking density effects on humification ratio and index following vermicomposting of fly ash – cow dung- waste paper mixtures. Error bars indicate standard deviation.

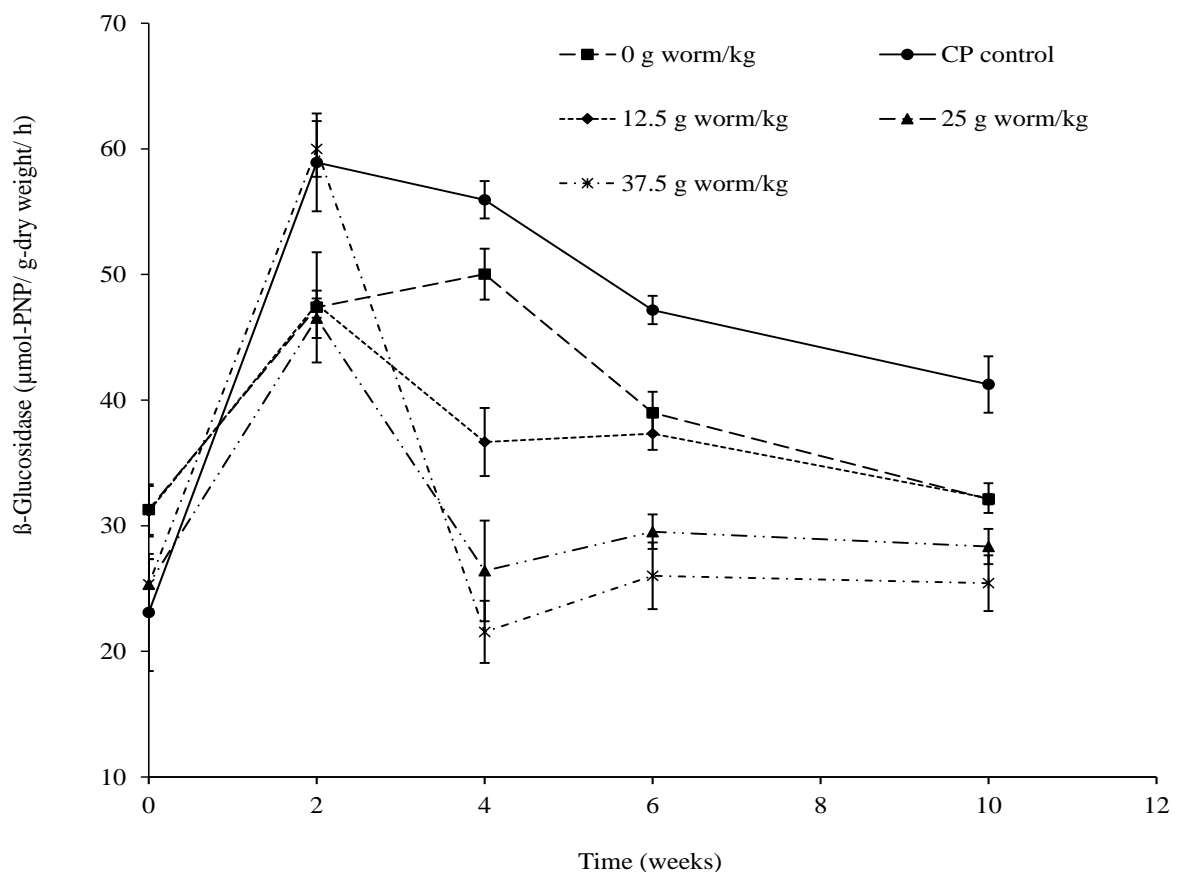


**Figure 5. 3:** *E. fetida* stocking density effects on polymerization rate and percentage of humic acids following vermicomposting of fly ash – cow dung- waste paper mixtures. Error bars indicate standard deviation.

#### 5.4.2 Effects of *E. fetida* stocking density on biological enzyme activity of fly ash vermicompost

##### *β* – Glucosidase

*β* – Glucosidase activity was significantly influenced by stocking density ( $P < 0.05$ ) with this relationship being dependent on time as indicated by the significant interaction between stocking density and time (Table 5.1). For all treatments, there was a significant increase in *β* – Glucosidase activity from 0 to 2 weeks with the CP control and 37.5 g-worm treatments showing the highest increase (Figure 5.4).

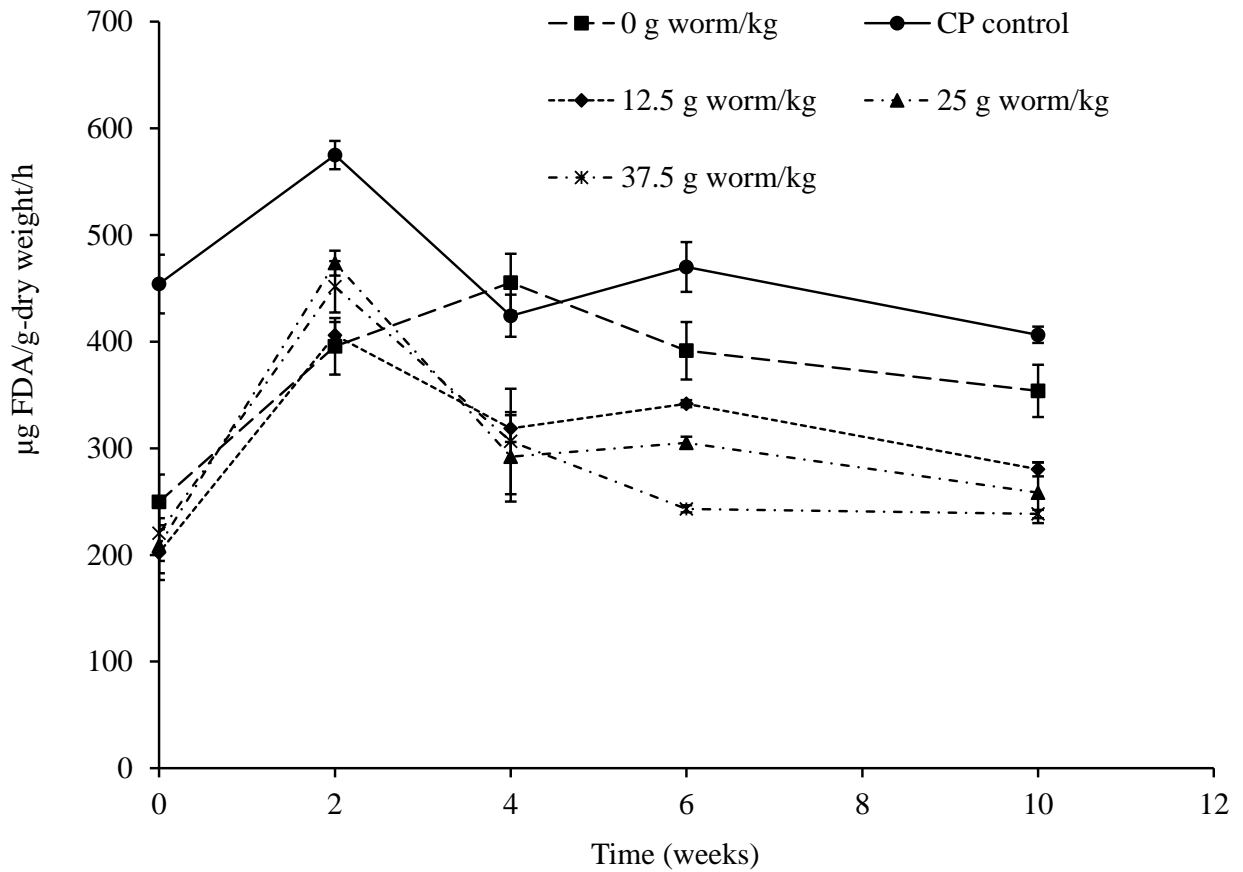


**Figure 5. 4:** Changes in *β*- Glucosidase enzyme activity during vermicomposting of fly ash – cow dung – waste paper mixtures under varying *E. fetida* stocking densities. Error bars indicate standard deviation.

However, from 2 weeks onwards, there was a decline in  $\beta$  – Glucosidase activity with the 25 g-worm and 37.5 g-worm treatments showing the greatest reduction in  $\beta$  – Glucosidase activity followed by 12.5 g-worm > 0 g-worm > CP control. The treatments 25 g-worm and 37.5 g-worm, showed a constantly lower activity of  $\beta$  – Glucosidase from 6 till 10 weeks, whilst the other treatments showed a slowly decreasing but higher  $\beta$  – Glucosidase activity. The activity of  $\beta$  – Glucosidase showed an almost similar trend to that of microbial biomass determined in Chapter 4, section 4.4.2 Figure 4.3a, showing an initial rapid increase up to 2 weeks, then followed by a decrease thereafter. The activity of  $\beta$  – Glucosidase enzyme showed a significantly correlation with the enzyme fluorescein di-acetate hydrolysis ( $r = 0.68$ ;  $P < 0.001$ ; Table 5.3).

#### ***Fluorescein di-acetate hydrolysis (FDA)***

There was a general increase in FDA activity across most treatments from 0 to 2 weeks almost similar to  $\beta$ - Glucosidase, except for the 0 g-worm treatment which showed maximum FDA activity at 4 weeks (Figure 5.5). Following the peaks at 2 and 4 weeks, the CP control and 0 g-worm treatments showed a significantly higher FDA activity across the entire 10 week vermicomposting period. However, for the treatments 12.5, 25 and 37.5 g-worm, there was a constantly lower FDA activity from 4 to 10 weeks, with FDA activity following the order 12.5 g-worm > 25 g-worm > 37.5 g-worm, across the entire vermicomposting period.

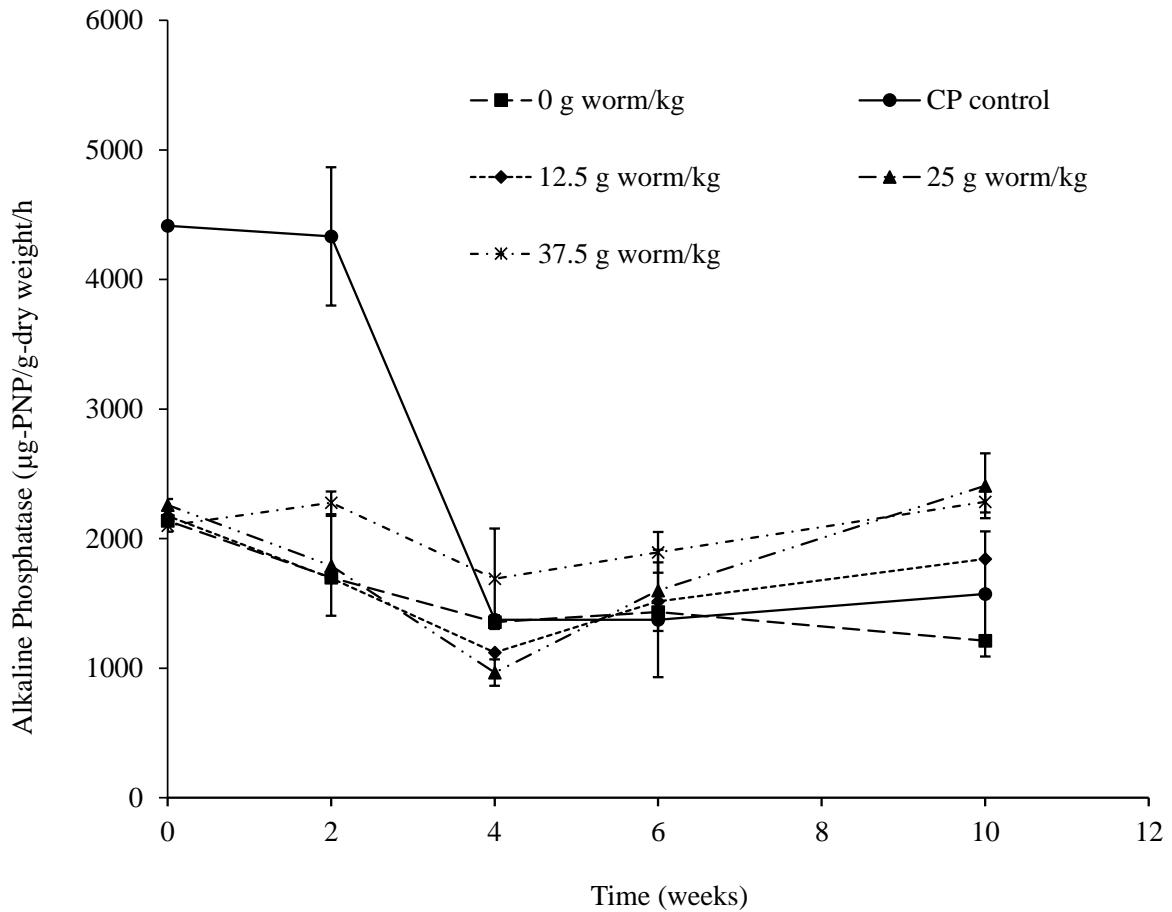


**Figure 5. 5:** Changes in Fluorescein di-acetate hydrolysis (FDA) enzyme activity during vermicomposting of fly ash – cow dung – waste paper mixtures under varying *E. fetida* stocking densities. Error bars indicate standard deviation.

### ***Alkaline Phosphatase***

Only the CP control showed the highest initial activity of alkaline Phosphatase from 0 to 2 weeks which then significantly decreased and stabilized at 4 weeks (Figure 5.6). However, across all stocking densities, including 0 g-worm, alkaline Phosphatase activity showed a gradual decrease up to 4 weeks and then a slight non-significant increase thereafter. After 10 weeks of composting, the treatments 25 and 37.5 g-worm showed a slightly higher significant

activity of alkaline phosphatase, though the activity was not much different to the activity recorded at the start of the vermicomposting process.

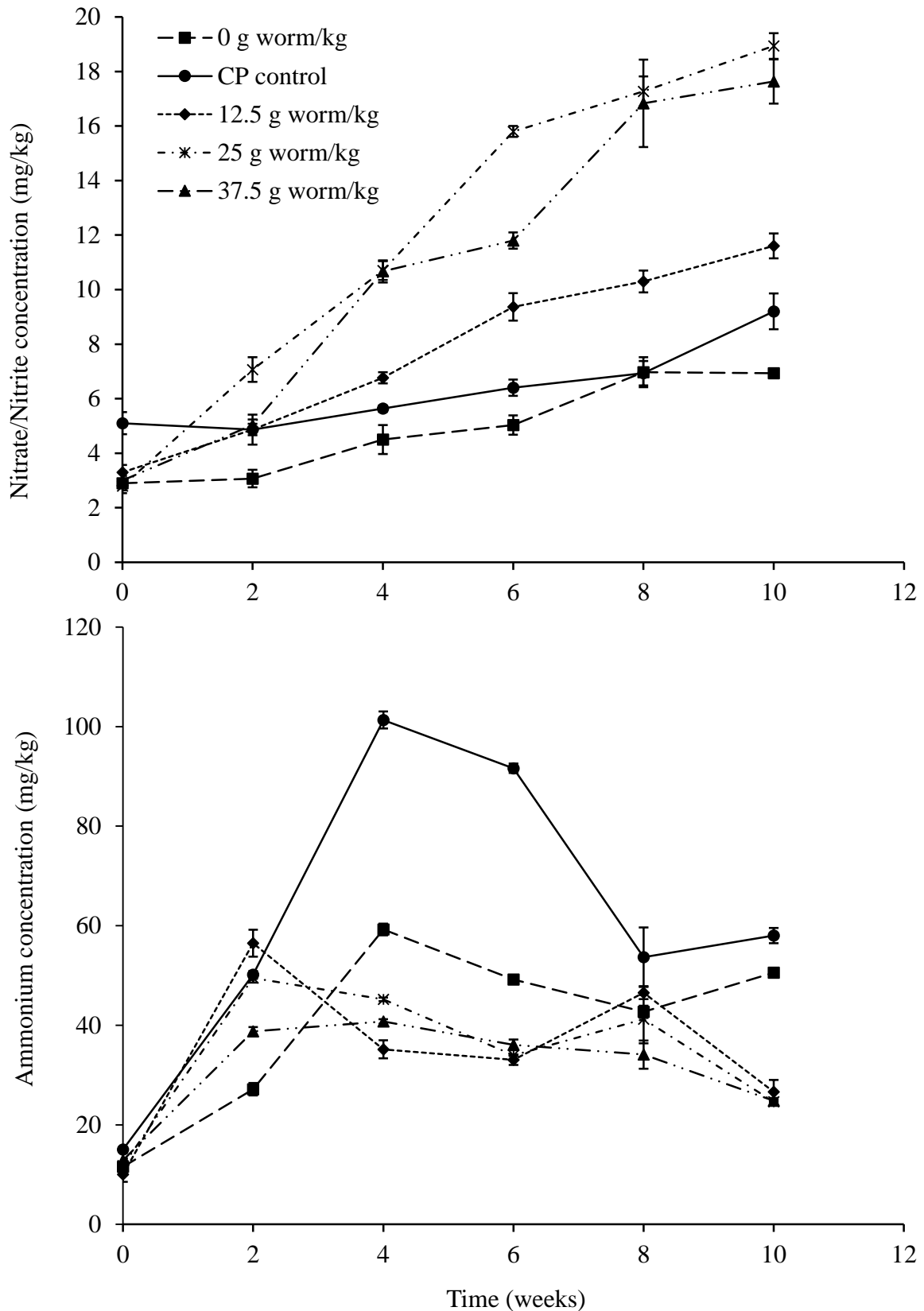


**Figure 5. 6:** Changes in alkaline Phosphatase enzyme activity during vermicomposting of fly ash – cow dung – waste paper mixtures under varying *E. fetida* stocking densities. Error bars indicate standard deviation.

### **5.4.3 Effects of *E. fetida* stocking density on Nitrogen and Phosphorus changes in fly ash vermicompost**

#### ***Inorganic nitrogen***

Generally, across all treatments, inorganic nitrogen measured as nitrate/nitrite showed an increase at different intensities from 0 to 10 weeks, whilst ammonium nitrogen showed an increase up to 4 weeks and a decrease thereafter (Figure 5.7). Stocking density significantly influenced ( $P < 0.001$ ) the rate of nitrate/nitrite release in the composts, with these effects being recognised more significantly from 4 weeks onwards. The 25 g-worm treatment resulted in the highest nitrate/nitrite concentration across all weeks, though it was not significantly different with the 37.5 g-worm treatment. Across the 10 weeks of vermicomposting, the treatments 37.5; 25; 12.5; 0 g- worm/kg and CP alone resulted in a respective 1.5 mg/kg; 1.6 mg/kg; 0.83 mg/kg; 0.4 mg/kg and 0.41 mg/kg rate of nitrate/nitrite release per week. It was also important to note that across all treatments, the nitrate/nitrite concentration showed a different but continued increasing trend even at 10 weeks after vermicomposting (Figure 5.7).

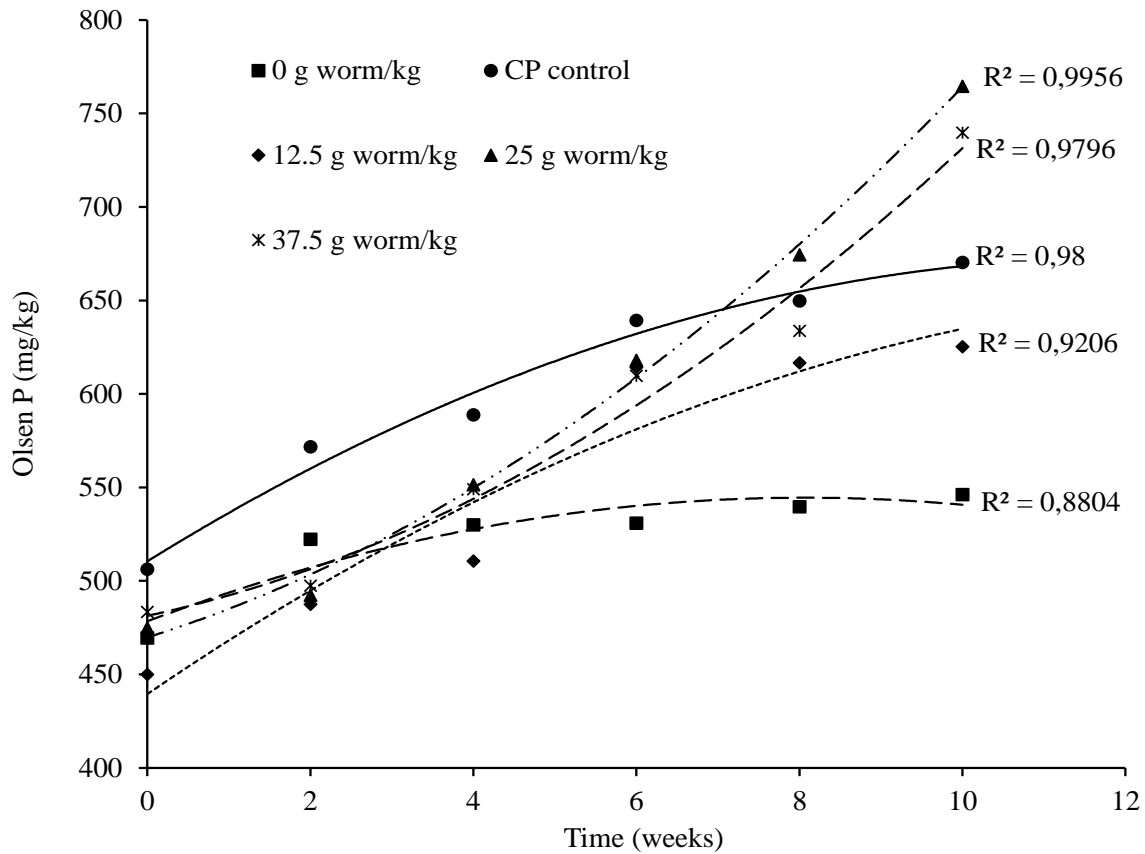


**Figure 5. 7:** Effects of *E. fetida* stocking density on changes in extractable inorganic nitrogen (NO<sub>3</sub>/NO<sub>2</sub> and NH<sub>4</sub>) over time. Error bars indicate standard deviation.

For the ammonium - nitrogen, the CP control showed highest concentration of ammonium across the 10 week period followed by the 0 g- worm treatment, resulting in a significant effect of stocking density on the changes in ammonium. After 10 weeks of vermicomposting, there were no significant differences in ammonium concentration between the treatments 12.5; 25 and 37.5 g-worms only. However, the CP control and 0 g-worm treatments showed a significantly higher concentration of ammonium nitrogen compared to the other treatments (Figure 5.7).

### ***Olsen extractable Phosphorus***

Phosphorus is one of the most important nutrients present in fly ash, hence the effects of stocking density on the release of phosphorus were measured in this study. Both stocking density and time had a highly significant effect on phosphorus release in the fly ash vermicompost (Table 5.1). Generally, Olsen P increased with time across all treatments with the 25 g-worm treatment having the highest value of 764.6 mg/kg of Olsen P after 10 weeks of composting compared to 546.2 mg/kg for the 0 g-worm treatment (Figure 5.8). Olsen P concentration after 10 weeks of vermicomposting followed the order 25 g-worm > 37.5 g-worm > CP control > 12.5 g- worm > 0 g-worm. On average, the net Olsen P increase after 10 weeks was 20.1%; 88.1% and 63.6% more for the 12.5 g-worm; 25 g-worm and 37.5 g-worm treatments, respectively compared to the CP control; whilst the 0 g-worm treatment was 49.6% less than the CP control (Table 5.2).



**Figure 5. 8:** Effects of *E. fetida* stocking density on changes in Olsen extractable Phosphorus over time.

Compared to the CP control, the rate of P release per week was 6.8%; 76.5% and 56.2% more in the 12.5 g-worm; 25 g-worm and 37.5 g- worm treatments respectively whilst the 0 g-worm treatment was 53.3% less than the CP control. Almost similar to inorganic nitrate/nitrite, the treatments 12.5 g-worm; 25 g-worm37.5 g-worm showed a continued increased Olsen P releasing trend even at 10 weeks ,whilst the CP control and 0 g-worm showed an almost decreasing trend (Figure 5.8).

**Table 5. 2:** Relationship between *E. fetida* stocking density and Olsen P during vermicomposting of fly ash-cow dung-waste paper vermicomposting.

<i>Eisenia fetida</i> stocking density (g worm/kg substrate)	Regression equation	R <sup>2</sup>	Rate of Olsen P release (mg P/kg/week)	Predicted Olsen P at 10 weeks (mg/kg)	Observed Olsen P at 10 weeks (mg/kg)	Net Olsen P increase after 10 weeks (%)
CP control	$-1.1223x^2 + 27.009x + 510.51$	0.980	16.41b	668.37	670.35b	32.41b
0	$-1.0149x^2 + 16.382x + 478.39$	0.880	7.67c	540.72	546.16c	16.33c
12.5	$-1.0164x^2 + 29.697x + 439.51$	0.921	17.52b	634.84	625.24b	38.94b
25	$1.5773x^2 + 13.665x + 469.82$	0.996	28.96a	764.20	764.57a	60.96a
37.5	$1.5637x^2 + 9.38x + 481.34$	0.980	25.63a	731.51	739.84a	53.02a

**Table 5. 3:** Pearson correlation matrix between chemical parameters and enzyme activity during fly ash – cow dung – waste paper vermicomposting.

	C: N ratio <sup>†</sup>	β- Glucosidase	FDA	Alkaline P	Olsen P	NO <sub>3</sub> /NO <sub>2</sub>
β- Glucosidase	0.258*					
FDA	0.239*	0.681***				
Alkaline P	0.216	0.043	0.237*			
Olsen P	-0.732***	-0.170	-0.059	-0.035		
NO <sub>3</sub> /NO <sub>2</sub>	-0.918***	-0.386***	-0.294*	-0.105	0.824***	
NH <sub>4</sub>	0.008	0.512***	0.596***	-0.328**	0.210	-0.043

<sup>†</sup>C: N ratio = carbon: nitrogen ratio; β – Glucosidase = β – Glucosidase enzyme; FDA = Fluorescein di-acetate hydrolysis enzyme; Alkaline P = Alkaline Phosphatase enzyme; Olsen P = Olsen extractable phosphorus; NO<sub>3</sub>/NO<sub>2</sub> = Inorganic nitrogen as nitrate/nitrite; NH<sub>4</sub> = Inorganic nitrogen as ammonium; \* Significant at  $P < 0.05$ ; \*\*\* Significant at  $P < 0.001$ .

## 5.5 DISCUSSION

### 5.5.1 Effects of *E. fetida* stocking density on maturation and biological activity in fly ash vermicompost

One of the major limitations to direct utilization of fly ash in agriculture is the limited availability to supply essential nutrients like phosphorus, which exist in high total concentrations but with limited bioavailability (Mupambwa et al. 2015). To improve the bioavailability of these nutrients in fly ash, vermicomposting with highly adaptable earthworm species like *Eisenia fetida* has been recommended, with the stocking density being one of the fundamental parameters that determines the rate of biodegradation and nutrient release during vermicomposting (Unuofin and Mnkeni 2014). This study looked at the influence of various *E. fetida* stocking densities on the rate of biodegradation, biological activity and nutrient release, contrary to other researchers who have focussed more on the influence of stocking density on the growth and development of earthworms (Garg et al. 2008).

The overall decline in C: N ratio over time observed in all treatments indicated the degradation of organic matter as a result of both natural microbes and earthworm activity. However, the presence of earthworms alone made a significant increase in the rate of organic matter degradation as observed in Figure 5.1. This highlights the ability of earthworms to enhance biodegradation by mechanically fragmenting the materials which greatly increases the surface area for microbe and enzyme activity and also promoting higher microbial activity especially in the early stages of decomposition (Aira et al. 2007; Dominguez 2011). A C: N ratio of less than 15 and preferably < 10, has been reported to represent a mature compost (Bernal et al. 2009). Though all treatments with earthworms resulted in C: N ratios

of less than 15, the stocking densities of 25 g-worm and 37.5 g- worm/kg gave a much lower C: N ratio of 11.1 and 10.4, respectively after 10 weeks, which shows higher maturity.

The influence of stocking density on faster degradation of organic matter leading to formation of more stable molecules was evident in the higher humification parameters observed with an increase in stocking density. A mature compost has been reported to have a humification ratio of greater than 7; humification index greater than 3.5; polymerisation index greater than 1 and percentage humic acids above 50% (Bernal et al. 2009). Though all stocking densities from 12.5 g-worm/kg to 37.5 g-worm/kg resulted in composts that satisfy the above criteria, it was important to note that doubling the stocking density from 12.5 to 25 g – worm/kg, significantly enhanced the humification of the composts, whilst there was no much difference by further increasing stocking density beyond 25 g-worm/kg. Similar observations were made by Unuofin and Mnkeni (2014), working with rock phosphate and cow dung waste paper mixtures, in which stocking densities above 7.5 g-worm/kg resulted in well humified composts, with subsequently increasing stocking density not resulting in a proportional increase in humification. Results of this study highlight that at stocking densities of 25 g-worm/kg, a highly mature fly ash vermicompost can be achieved at a faster rate.

The efficiency of the vermicomposting process under different stocking densities measured as the rate of biodegradation and maturity depends on largely on the various hydrolytic enzymes from micro-organisms (Kayikcioglu and Okur 2011). Enzymes are thus the main drivers of the various biochemical processes.  $\beta$  – Glucosidase enzyme plays an important role in catalysing the hydrolysis of glucosides, thus playing a major role in organic matter decomposition (Cayuela et al. 2008).

During the fly ash vermicomposting,  $\beta$  – Glucosidase activity was seen to decrease in all treatments, though at different rates, with the control having the lowest decrease and the highest stocking density having the highest decrease. The decrease and stabilization in  $\beta$  – Glucosidase activity has been attributed to a decrease in available organic substrates (Benitez et al. 1999). Our results support the idea that during vermicomposting of fly ash mixed with an organic base like cow dung and waste paper, a higher stocking density within the range 25 – 37.5 g – worm/kg biomass gives a faster decrease in organic matter compared to lower stocking densities like 12.5 g-worm/kg. This also means a faster maturing composting with higher humification parameters. Similar results were reported by Aira et al. (2007) during vermicomposting of pig slurry with or without *E. fetida*, in which initially  $\beta$  – Glucosidase activity was high in the treatments with earthworms and later decreased to levels lower compared where earthworms were excluded.

The activity of FDA enzyme during composting has been proposed to estimate non-specific enzyme activity and compost microbial biomass (Cayuela et al. 2008). This could explain the positive significant correlation observed between FDA enzyme activity and  $\beta$  – Glucosidase activity and C: N ratio changes. During the vermicomposting process, development of microbial biomass requires large quantities of organic substrate and the decrease in FDA activity in the earthworm treated composts in this study indicates compost maturity which was highly dependent on stocking density as shown in Figure 6. Similar results have been observed by Garcia – Gomez et al. (2003), in which compost maturity was correlated to stabilized microbial and FDA activity. This could mean that the various microbes involved in the vermicomposting process are responsible for releasing hydrolytic enzymes like  $\beta$  – Glucosidase, directly involved in organic matter decomposition. So as the organic substrate decreases, this consequently reduces the microbial biomass, as observed in Chapter 4 with microbial biomass data.

Alkaline Phosphatase activity showed no positive correlation with most measured parameters including changes in Olsen extractable phosphorus. These results are inconsistent with other researchers who have reported a higher alkaline phosphatase activity using pig slurry (Aira et al. 2007); sewage sludge (Benitez et al. 1999); olive mill waste (Cayuela et al. 2008). However, this is likely due to variations in pH and EC, which are high in fly ash vermicomposts made from alkaline fly ash (Mupambwa and Mnkeni 2015a). Similar reduced alkaline phosphatase activity has been reported by Kayikcioglu and Okur (2011), working with highly alkaline tobacco waste during composting. It would be interesting to further pursue other biological parameters like phosphate utilizing bacteria, under different stocking densities and their relationship with P turnover during fly ash vermicomposting.

#### **5.5.2 Effects of *E. fetida* stocking density on fly ash vermicompost nutrition**

Though fly ash amendment to organic materials during vermicomposting is not likely to contribute to any significant inorganic nitrogen, it was noted in this study that higher earthworm density resulted in higher release in nitrate/nitrite within the fly ash – cow dung – waste paper vermicompost. This shows the ability of earthworms to more efficiently mineralize the organic nitrogen within the cow dung at higher *E. fetida* stocking densities. Earthworms are indirectly responsible for organic matter degradation by maceration of organic matter thus reducing particle size and increasing surface area which results in enhanced microbiological activity (Dominguez 2011). Bhattacharya and Chattopadhyay (2004) also made similar observations of higher N-mineralization during fly ash vermicomposting where earthworms were present and this is attributed to higher populations of N – mineralizing bacteria under earthworms.

The influence of *E. fetida* stocking density in the phosphorus nutrition status was quite apparent in this study, with the stocking densities from 25 g- worm/kg and 37.5 g - worm/kg

resulting in significantly higher Olsen P after the 10 week of vermicomposting. The higher mineralization of Olsen P in the higher stocking densities may likely be due to the enhanced activity of microbes like phosphate solubilizing micro-organisms (Bhattacharya and Chattopadhyay 2002). Similar results have also been reported by Unuofin and Mkeni (2014), using rock phosphate, in which the higher stocking densities resulted in higher extractable phosphorus during vermicomposting. In this study, it was also important to observe that maturity as measured with C: N ratio, humification parameters and biological activity seemed to occur first, whilst the nutrient status of the compost tended to exponentially increase during the maturation stage. This is likely due to an increase in earthworm casts as degradation takes place which results in increase in nutrient concentration within the compost as earthworm cast have been observed to have high nutrient content compared to un-ingested material (Gomez-Brandon and Dominguez 2014). It would be interesting to evaluate the maximum potential nutrient benefits that can be observed beyond the 10 weeks used in this experiment, during vermicomposting of fly ash at stocking densities of 25 to 37.5 g – worm/kg.

## **5.6 CONCLUSIONS**

In this study, the inclusion of earthworms even at the lowest stocking densities of 12.5 g-worm/kg enhanced biodegradation of the fly ash based vermicompost measured as changes in C: N ration and humification parameters. However, vermicomposting fly ash – cow dung – waste paper mixtures at stocking densities of 25 g-worm/kg and above is likely to give a highly matured compost, within a shorter period with higher plant available phosphorus and

nitrogen, compared to 12.5 g- worm/kg. In addition to existing literature in which lower or generalized stocking densities have been used, our study clearly demonstrates the need for optimization of stocking density particularly in relation to nutrient release during vermicomposting.

## CHAPTER SIX

### **6.0 CHANGES IN CHEMICAL AND BIOLOGICAL PROPERTIES FOLLOWING INOCULATION OF FLY ASH – COW DUNG – WASTE PAPER MIXTURES WITH EFFECTIVE MICRO-ORGANISMS (EM) DURING VERMICOMPOSTING WITH *E. FETIDA***

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This Chapter is based on a manuscript entitled:

Mupambwa HA, Ravindran B and Mnkeni PNS. Forthcoming. Changes in chemical and biological properties following inoculation of fly ash – cow dung – waste paper mixtures with Effective micro-organisms (EM) during vermicomposting with *E. fetida*.

## 6.1 ABSTRACT

The link between earthworm activity and microbe activity has prompted several researchers to evaluate the potential of artificially inoculating vermicomposts with additional specific microbes, with intention of enhancing the vermicomposting process. This study evaluated the potential of inoculating fly ash based vermicomposts with a specialized microbial cocktail called Effective micro-organism (EM) together with *E. fetida* earthworms in enhancing the fly ash –cow dung waste paper vermicomposting. Inoculation with EM alone did not result in significantly ( $P < 0.05$ ) different changes in C: N ratio and dissolved organic matter (DOC) compared to the control with no EM or *E. fetida*. Also, though inoculation with EM together with *E. fetida* resulted in greater changes in C: N ratio and DOC compared to the *E. fetida* alone treatment, this difference was not statistically significant. Inoculation with EM coupled with *E. fetida*, however, resulted in significantly ( $P < 0.05$ ) higher rates of Olsen phosphorus release compared to the *E. fetida* alone treatment. On average, the EM + *E. fetida* treatment resulted in a rate of weekly Olsen P release of 54.32 mg/kg, with the *E. fetida* alone, EM alone and control releasing 48.39; 28.71 and 16.56 mg-P/kg/week, respectively. The Olsen P released was correlated to the total phosphate solubilizing bacteria (PSB) in the vermicompost. However, the PSB were not significantly influenced by the inoculation with EM, but rather by the presence of *E. fetida* in the vermicompost. It is concluded that inoculation of fly ash based composts with EM alone is not beneficial, whilst combining EM with *E. fetida* may result in faster compost maturity and greater Olsen P release. It would be interesting to evaluate higher rates of EM inoculation and fortifying EM cocktails with PSB on fly ash vermicompost degradation and phosphorus mineralization.

**Keywords:** Extractable phosphorus; phosphate solubilizing bacteria; enzyme activity; microbial activity.

## 6.2 INTRODUCTION

Vermicomposting has in recent years been adopted over traditional composting, as a faster and environmentally friendly method of creating organic fertilizer from organic waste (Lazcano et al. 2008). Traditional composting generally relies on naturally occurring microbes and the thermophilic temperatures to decompose and stabilize organic waste. This greatly slows the stabilization and maturation process during composting together with loss of essential nitrogen as ammonia during the thermophilic stage, thus reducing the quality of the resultant organic fertilizer (Lazcano et al. 2009). Vermicomposting is a mesophilic process, which makes use of natural micro-organisms and earthworms (Hong et al. 2011). Earthworms like *Eisenia fetida* have been shown to enhance the biodegradation of organic materials by mechanically fragmenting the waste material with their gizzards consequently increasing substrate surface area, thus altering micro-flora activity in the composts (Gomez-Brandon et al. 2013).

Within *E. fetida* intestines, endo-symbiotic micro-organisms that are capable of producing hydrolytic enzymes that degrade cellulose and phenolic compounds enhance the degradation of organic material ingested by the earthworms (Dominguez 2011). Due to the ability of earthworms to enhance microbial activity and mineralization in composts, they have even been used to process inorganic materials like rock phosphate and fly ash to enhance their low essential nutrient availability (Unuofin and Mkeni 2014; Bhattacharya and Chattopadhyay 2002). Bhattacharya and Chatopadhay (2002), for example, observed higher levels of Olsen extractable phosphorus in a fly ash amended vermicompost, which they attributed partly to the high levels of phosphate solubilizing micro-organisms in the presence of earthworms.

Due to the link between earthworm activity and microbe activity, several researchers have evaluated the potential of enhancing the vermicomposting - microbe community by

inoculating composts with additional microbes, with the intention of improving the quality of the compost (Mupondi et al. 2006a; Busato et al. 2012). Kumar and Singh (2001) inoculated a vermicompost made from chopped stalk and leaves of pearl millet and rock phosphate, with nitrogen fixing microbes (*Azotobacter chroococcum* and *Azospirillum lipoferum*) and phosphate solubilizing *Pseudomonas striata*. They reported an enhanced phosphate solubilization from the rock phosphate and enhanced nitrogen fixation in the vermicomposts. Kumar et al. (2010) inoculated a compost with a combination of *Pleurotus sajorcaju*, *Trichoderma viridae*, *Aspergillus niger* and *Pseudomonas striatum* 30 days prior to the vermicomposting process and observed an overall reduction in composting period by 20 days due to accelerated degradation together with a nutrient rich compost. Mupondi et al. (2006b) inoculated a pine bark goat manure or sewage sludge compost with and without earthworms, with a special cocktail of microbes called Effective micro-organisms (EM) but, contrary to expectations, inoculation with EM did not significantly influence the composting process.

According to the Japanese inventors of the technology, EM is a cocktail of natural and beneficial microbial populations of lactic acid bacteria, yeasts, actinomycetes and photosynthetic bacteria, living symbiotically (Yamada and Xu 2001). Anecdotal evidence suggests that EM is a cheap way for enhancing the compost biodegradation process, reducing odour and ammonia volatilization (Freitag 2000). At present, there is a paucity of information on the interactions between earthworm activity and EM during vermicomposting of phosphate rich inorganic materials like fly ash incorporated into organic materials like cow dung and waste paper. This study therefore sought to evaluate the effects of (i) EM inoculation during fly ash – cow dung – waste paper vermicomposting on compost chemical and biological activity, and (ii) EM inoculation during fly ash – cow dung – waste paper vermicomposting on extractable phosphorus and nitrogen in the resultant vermicompost.

## 6.3 MATERIALS AND METHODS

### 6.3.1 Materials for composting

This study was carried out at the University of Fort Hare Alice campus vermicomposting research unit, Eastern Cape Province (32° 46' S and 26° 50' E). The fly ash, cow dung and waste paper was sourced from similar locations as described in Chapter 5 section 5.3.1. Cylindrical vermireactors measuring 0.30 m (top diameter) × 0.23 m (height) and 0.21 base (diameter) with a total volume of 0.015 m<sup>3</sup>, having perforations underneath were used for the vermicomposting process. Mature, *Eisenia fetida* earthworms sourced from our local wormery at the University of Fort Hare, mainly feeding on cow dung and waste paper, were used. The selected chemical properties of the cow dung, fly ash and waste paper used in this study are shown in Table 6.1 below.

The cow dung was originally mixed with waste paper to adjust the C: N ratio to an optimum of  $30 \pm 1$  for effective vermicomposting (Unuofin and Mnkeni 2014). The fly ash (F) was then incorporated into the cow dung – waste paper mixtures (CP) at a ratio of 1: 2 F: CP (w/w basis) as recommended by Mupambwa and Mnkeni (2015b). The Effective micro-organisms (EM) that were used were ordered from a local South African supplier (Lindoros Whole Earth Consultants; [http://http://lindros.co.za/effective-micro-organismsem](http://lindros.co.za/effective-micro-organismsem)).

**Table 6. 1:** Selected chemical characteristics of cow dung, waster paper and fly ash used in the study.

Parameter	Cow dung	Waste paper	Fly ash
pH (H <sub>2</sub> O)	7.23 ± 0.28	8.1 ± 0.1	12.58 ± 0.01
EC (dS m <sup>-1</sup> )	6.07 ± 0.14	0.12 ± 0.02	6.84 ± 0.38
Total C (%)	26.8 ± 0.1	37.96 ± 1.01	nd
Total N (%)	2.08± 0.07	0.038 ± 0.01	nd
C:N ratio	12.88 ± 0.51	998.94 ± 17.44	-
Olsen extractable P (mg/kg)	1110.7 ± 35.86	nd	256.2 ± 5.24
Extractable NO <sub>2</sub> /NO <sub>3</sub> (mg/kg)	269.67 ± 11.84	nd	nd
Extractable NH <sub>4</sub> (mg/kg)	364.0 ± 3.6	nd	nd

Parameters reported as Means ± Standard Deviation, n = 3; nd = not detected.

### 6.3.2 Treatments, experimental design and sampling process

The experiment was laid down in in completely randomized design (CRD) with three replicates and two factors i.e. EM presence (with or without) and *E. fetida* presence (with or without). This gave four treatments namely: EM only; EM plus *E. fetida*; *E. fetida* only and control with no EM or *E. fetida*. These treatments were effected on the fly ash – cow dung – waste paper mixtures (FCP) described above, whilst a stocking density of 25 g – worm/ kg-dry material was used based on results of the stocking density study (Chapter 5). The total weight of the FCP mixtures used was 4 kg dry mass, with no extra feed being added for the duration of the 10 week experiment. Prior to effecting of the treatments, the FCP mixtures were allowed to pre-compost for a period of 1 week to allow volatile gases to escape

(Mupondi et al. 2010). During the vermicomposting process, samples were collected at the following intervals, 0; 1; 2; 3; 4; 6; 8 and 10 weeks; and further analysed for the various chemical and biological properties described below.

### **6.3.3 Chemical Properties**

#### **C and N determination**

Total C and N were measured using a Truspec CN Carbon/ Nitrogen Auto analyser (LECO Corporation, 2003).

#### **Dissolved organic carbon (DOC) (Jones and Willet 2006)**

Dissolved organic carbon was determined on a 5 g homogenous sample extracted with 50 mL of 0.5 M  $K_2SO_4$ . The samples were shaken on a reciprocating shaker for 30 mins at 160 rpm and the suspension was then filtered using Whatman number 42 filter paper. The concentration of DOC was determined colorimetrically using a Skalar continuous flow analyser (San 2++ Skalar CFA, Skalar Analytical B.V. The Netherlands).

#### **Olsen extractable P (Schoenau and O'Halloran 2006)**

The Olsen P was extractable using the procedure explained in Chapter 3. Section 3.3.4.

#### **Exchangeable ammonium and nitrate and nitrite (Maynard et al. 2006)**

Exchangeable ammonium and nitrate/nitrite was extracted using 0.5 M potassium sulphate as described in Chapter 4 section 4.3.8.

### 6.3.4 Enzyme activity

#### *β-Glucosidase* (Deng and Popova 2011)

β-Glucosidase activity was determined using the methods described in Chapter 5 section 5.3.6. Following the incubation, the activity of β-Glucosidase was determined based on the μmol of p-nitrophenol released by reference to a calibration curve developed with standards containing p-nitrophenol. The β-Glucosidase activity (β) was calculated as shown in the equation 6.1:

$$\beta \text{ (}\mu\text{mol of p - nitrophenol/ g - dry weight compost/ hour)} = \frac{S-C}{DM} \quad [\text{Equation 6.1}].$$

Where S is mean concentration of p-nitrophenol in the sample

C is concentration of p-nitrophenol in the control

DM is the dry mass of the compost (determined based on moisture content)

#### *Alkaline Phosphatase* (Acosta-Martinez and Tabatabai 2011)

The alkaline phosphatase activity was determined using the methods described in Chapter 5 section 5.3.6. Following the incubation, the alkaline phosphatase activity was expressed as μg of p-nitrophenol released per g-dry weight compost per hour, determined based on a reference calibration curve. Alkaline phosphatase activity (A) was calculated as shown in the equation 6.2 below:

$$A \text{ (}\mu\text{g of p - nitrophenol/ g - dry weight compost/ hour)} = \frac{S-C}{DM} \quad [\text{Equation 6.2}]$$

Where: S; C and DM are as defined above.

### **6.3.5 Microbiological analysis**

Samples collected during the vermicomposting process were analysed for microbiological counts of total bacteria, total fungi, total *E. coli* and total phosphate solubilising bacteria, using the serial dilution and standard spread plate counting method (Germida and Freitas 2006). A 1g (fresh weight) sample from each treatment was suspended in 10 mL of sterile distilled water and shaken for 1 min on a rotary shaker and serial dilutions were prepared up to  $10^{-5}$ . All samples were assayed by dilution with three replicates of each suspension.

#### ***Total bacteria***

Nutrient Agar was used for pour plating for total bacterial counts with a serial dilution of  $10^{-5}$ . The plates were incubated at 37° C for 24 h following which counts were made for determining the number of colony forming units (CFU).

#### ***Total E. coli***

The m-FC agar was used for total *E. coli* counts, with a serial dilution of  $10^{-3}$ . The plates were incubated at 44° C for 24 h following which counts were made to determine the number of CFU.

#### ***Total Phosphate solubilizing bacteria (PSB)***

The Pikovskaya's Agar was used for total PSB counts, with a serial dilution of  $10^{-4}$  being used. The plates were incubated for a period of 3 – 5 days at 30° C following which counts were made to determine the number of CFU.

### ***Total fungi***

The Rose Bengal Chloramphenicol agar was used for the determination of total fungi with a serial dilution of  $10^{-3}$  being used. The plates were incubated at 30° C for a period of 3 – 5 days, following which counts were made to determine the number of CFU.

### **6.3.6 Statistical Analysis**

Sampling was not destructive and thus data were analysed using repeated measures analysis of variance (ANOVAR). Where sphericity assumptions could not be met, the Greenhouse-Geisser correction of *P* was used. All data was analysed using JMP version 11.0.0 statistical software (SAS Institute, Inc., Cary, North Carolina, USA, 2010).

## 6.4 RESULTS

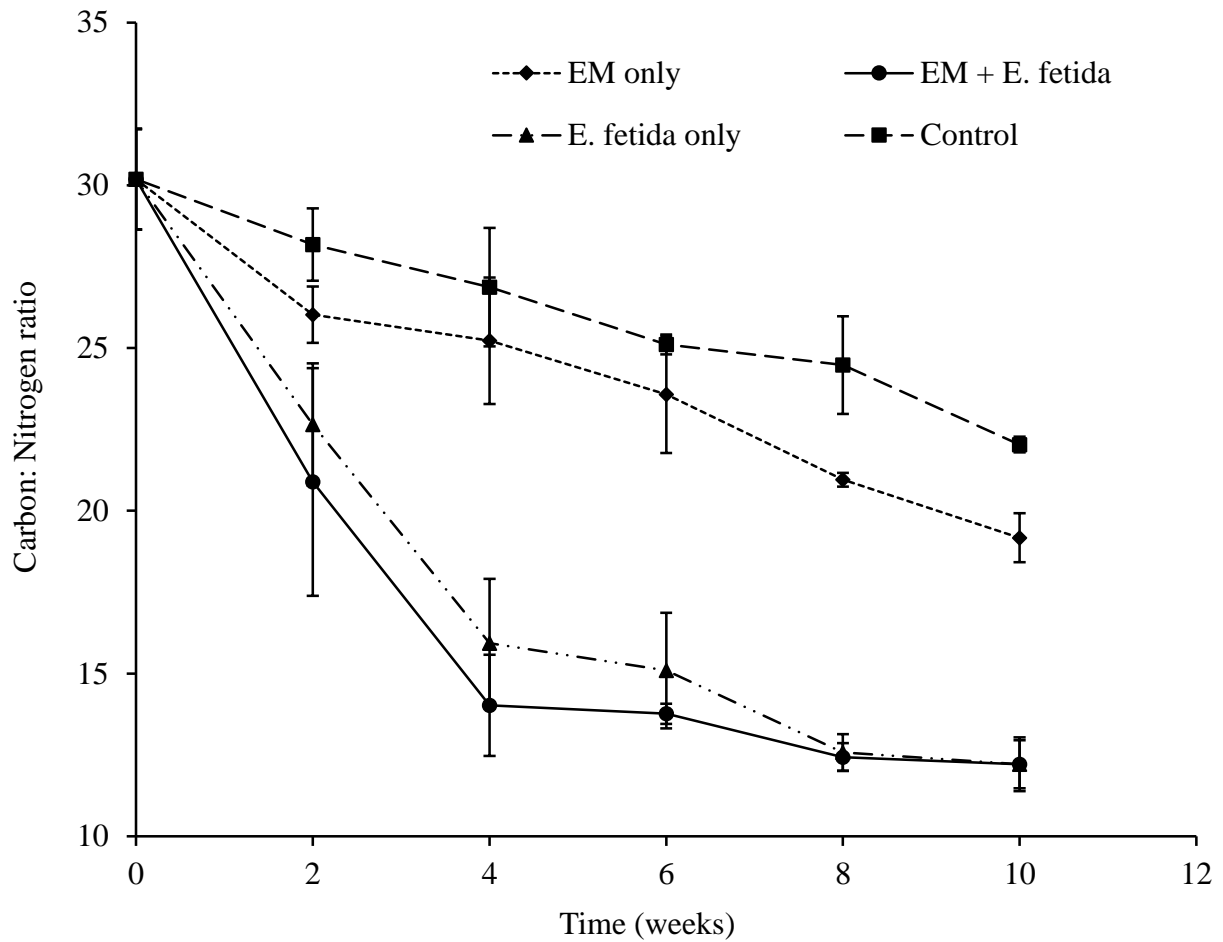
### 6.4.1 Effects of *E. fetida* and EM inoculation on C: N ratio and dissolved organic carbon (DOC)

#### *Carbon: Nitrogen ratio*

After 10 weeks of vermicomposting, the C: N ratio generally decreased for all treatments including the control (Figure 6.1). However, the presence of *E. fetida* and EM resulted in a significant difference ( $P < 0.0001$ ; Table 6.2) in overall decrease in C: N ratio, as shown by the very low average C: N value of 12.25 for the *E. fetida* only and *E. fetida* + EM treatments. The enhanced decrease in C: N ratio in the *E. fetida* + EM treatment compared to the EM alone treatment resulted in a significant interaction ( $P = 0.0076$ ; Table 6.2) between *E. fetida* presence and EM. Generally, though the *E. fetida* + EM treatment showed constantly lower C: N values, these values were not significantly different from the *E. fetida* only treatment. The EM only treatment showed lower C: N values across the 4 to 10 week period of vermicomposting compared to the control. The final C: N ratio for the four treatments were *E. fetida* only (12.2); EM + *E. fetida* (12.3); EM only (19.2) and control (22.0). The significant change in C: N ratio with time for all treatments with *E. fetida* also resulted in a significant interaction between *E. fetida*  $\times$  time ( $P < 0.0001$ ; Table 6.2). Changes in C: N ratio were significantly correlated with changes in  $\beta$  – glucosidase ( $r = 0.75$ ;  $P < 0.0001$ ).

**Table 6. 2:** Repeated measures ANOVA for changes in C: N; DOC; inorganic nitrogen and Olsen P following 10 weeks of fly ash vermicomposting.

Source of variation		Carbon : Nitrogen ratio	Dissolved organic carbon (mg/g)	NO <sub>3</sub> /NO <sub>2</sub> – inorganic nitrogen (mg/kg)	NH <sub>4</sub> – inorganic nitrogen (mg/kg)	Olsen Phosphorus (mg/kg)
<i>Between subjects</i>						
EM presence (EM)	$F_{1, 71}$	10.1	7.7	29.5	3.6	85.7
	$P$	0.0193	0.0326	0.0016	ns	< 0.0001
<i>E. fetida</i> presence (EP)	$F_{1, 71}$	285.1	306.8	1417.3	1708.0	297.5
	$P$	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
EM × EP	$F_{3, 71}$	1.5	21.8	0.359	2212.0	39.6
	$P$	0.0076	0.0034	ns	< 0.0001	< 0.0008
<i>Within subjects</i>						
Time	$F_{5, 71}$	125.4	43.9	1713.5	13767.4	154.9
	$P$	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Time × EM	$F_{11, 71}$	0.6	2.4	53.0	189.3	4.22
	$P$	ns	ns	< 0.0001	< 0.0001	0.0358
Time × EP	$F_{11, 71}$	21.1	34.9	661.3	1485.3	16.6
	$P$	< 0.0001	< 0.0001	< 0.0001	< 0.0001	0.0002
Time × EM × EP	$F_{23, 71}$	0.8	0.7	3.0	474.3	6.6
	$P$	ns	ns	ns	< 0.0001	0.0093

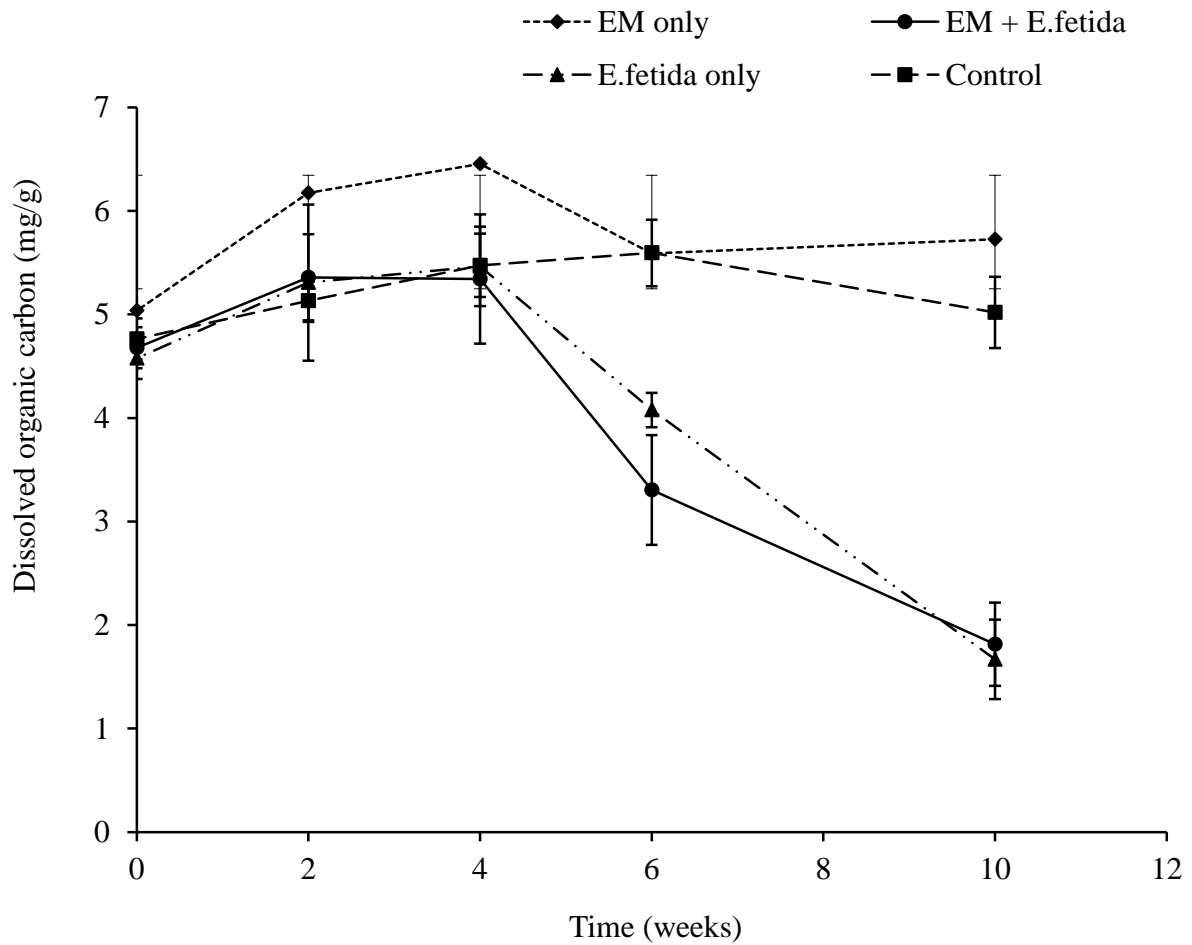


**Figure 6. 1:** Changes in carbon: nitrogen ratio following composting of fly ash – cow dung – waste paper mixtures with or without *E. fetida* and effective micro-organisms (EM). Error bars represent standard deviation.

### *Dissolved organic carbon*

Dissolved organic carbon (DOC) showed a general increase across all treatments from 0 to 4 weeks and subsequently decreased with significantly different intensities (Figure 6.2). Both *E. fetida* and EM presence significantly influenced the changes in DOC ( $P < 0.05$ ; Table 6.2), with also a significant interaction between *E. fetida* presence and EM ( $P < 0.0034$ ; Table 2). The DOC was highest in the EM only treatment followed by the control treatment, whilst the *E. fetida* and *E. fetida* + EM treatments had the lowest DOC concentration, particularly from 4 to 10 weeks (Figure 6.2). After 10 weeks of vermicomposting, the treatments EM only;

control; *E. fetida* + EM and *E. fetida* only, had final DOC concentrations of 5.73; 5.02; 1.82 and 1.67 mg/kg, respectively. Similar to C: N ratio, DOC was significantly correlated with the activity of  $\beta$  – Glucosidase ( $r = 0.51$ ;  $P < 0.001$ ).



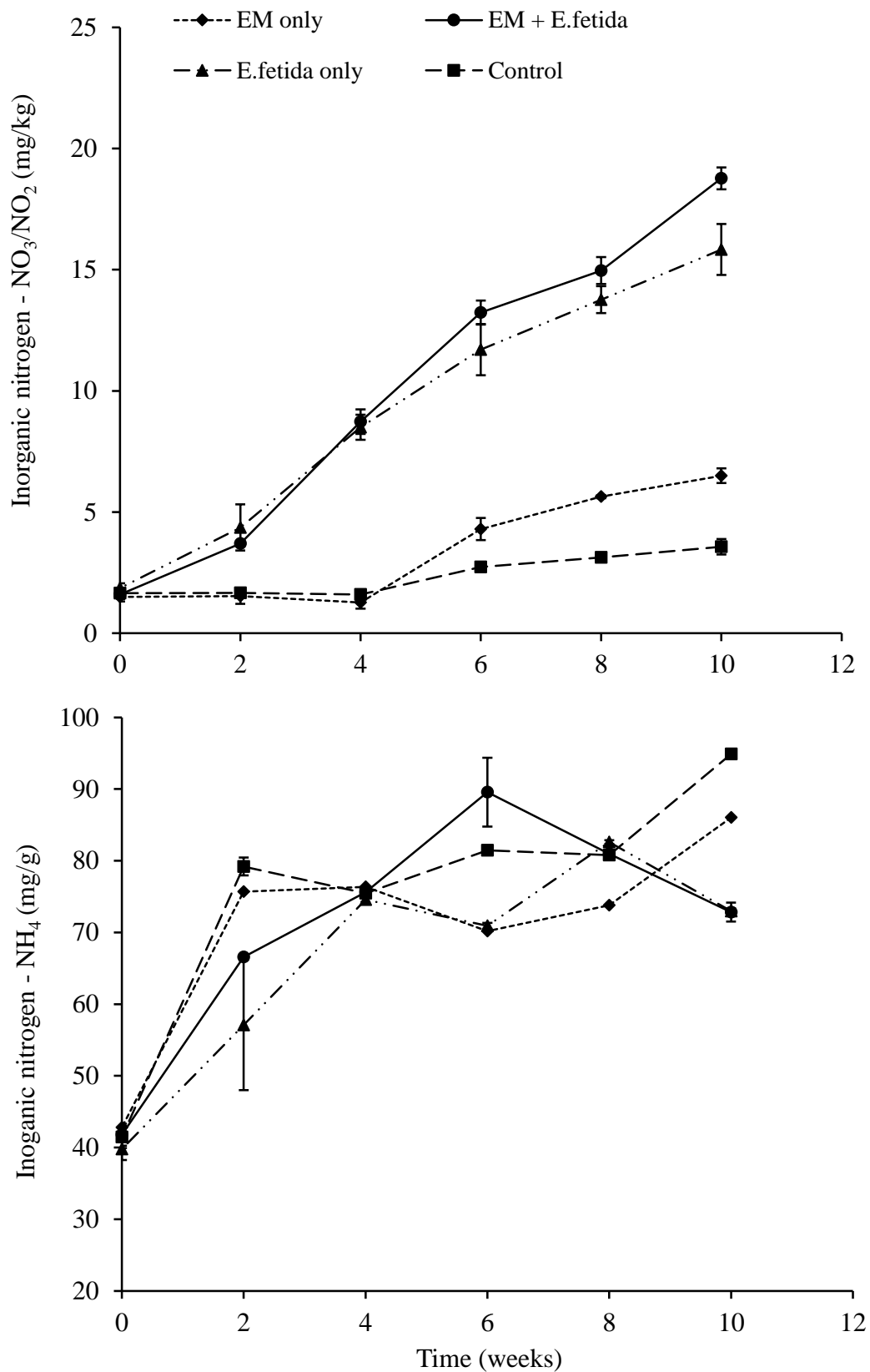
**Figure 6. 2:** Changes in dissolved organic carbon (DOC- mg/g) during composting of fly ash – cow dung – waste paper mixtures with or without *E. fetida* and effective micro-organisms (EM). Error bars represent standard deviation.

#### 6.4.2 Effects of *E. fetida* and EM inoculation on changes in extractable inorganic nitrogen and Olsen P

##### *Nitrate/nitrite and ammonium – Nitrogen*

The incorporation of EM and *E. fetida* significantly influenced the rate of change in nitrate/nitrite concentration in the composts ( $P < 0.01$ ; Table 6.2), with no interactions between *E. fetida* and EM presence. The increase in nitrate/nitrite concentration was more or less linear for all treatments until 10 weeks, showing a continued potential to increase even beyond 10 weeks for all treatments, as reflected by the significant influence of time (Table 6.2). Beyond 4 weeks, it was noted that the *E. fetida* + EM treatment showed a consistently higher though non-significant nitrate concentration compared to the *E. fetida* only treatment, whilst the EM only treatment showed a significantly higher nitrate concentration compared to the control (Figure 6.3). After 10 weeks of vermicomposting, the nitrate/nitrite concentration followed the order *E. fetida* + EM > *E. fetida* only > EM only > control with significant differences ( $P < 0.05$ ) between the treatments. The treatments *E. fetida* + EM; *E. fetida* only and EM only, resulted in 5.3; 4.4 and 1.8 times more nitrate/nitrite concentration compared to the control.

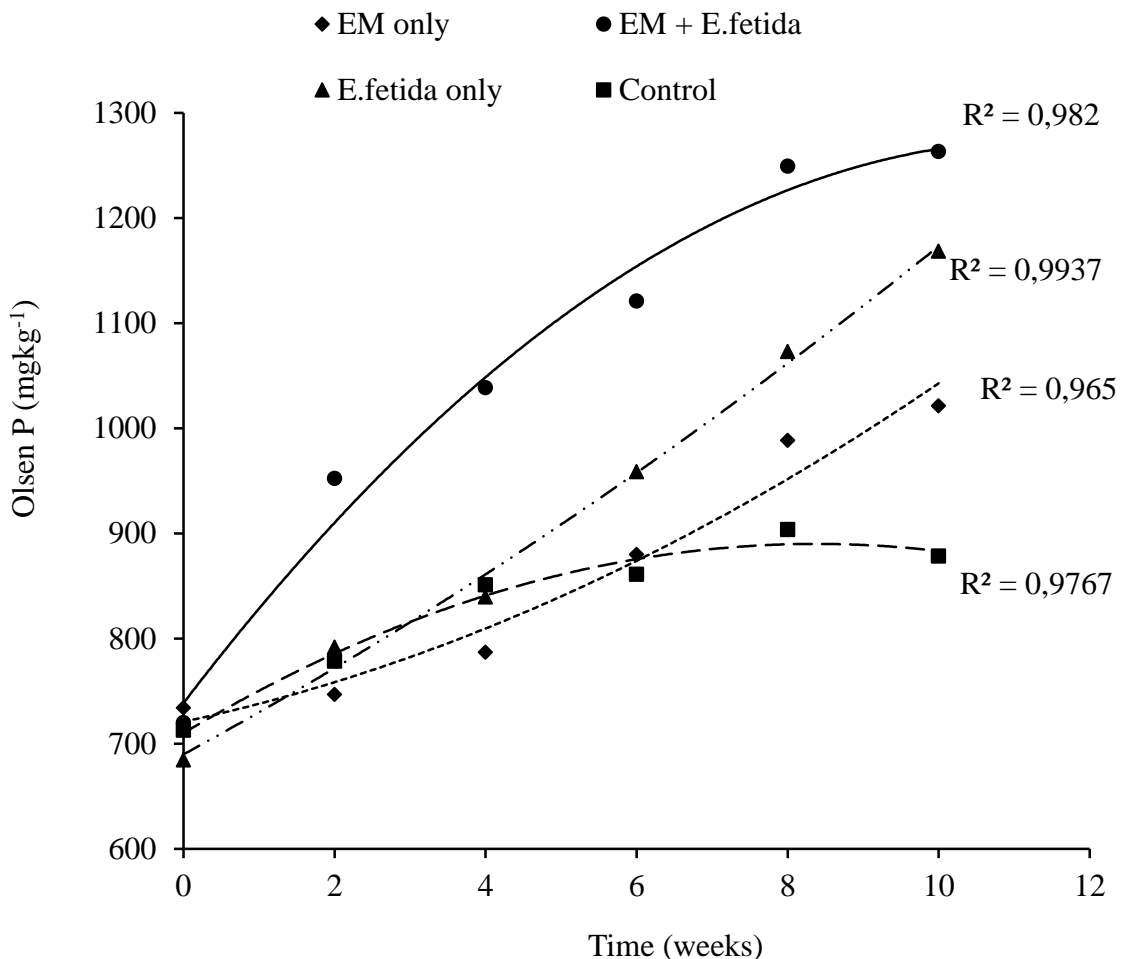
*E. fetida* presence significantly ( $P < 0.0001$ ; Table 6.2) influenced ammonium concentration within the composts, whilst EM presence did not. Generally, the ammonium concentration increased in all treatments with no consistent trend being observed between treatments. However, the increase up to 6 weeks and subsequent decrease beyond this for the *E. fetida* + EM treatment, resulted in a significant interaction between *E. fetida* presence  $\times$  EM (Table 6.2). On average, after 10 weeks of vermicomposting, the treatments *E. fetida* + EM and *E. fetida* only showed a significantly lower ( $P < 0.05$ ) ammonium concentration compared to the EM only and control treatment (Figure 6.3).



**Figure 6. 3:** Changes in inorganic nitrogen measured as nitrate/nitrite and ammonium during composting of fly ash – cow dung – waste paper mixtures with or without *E. fetida* and effective micro-organisms (EM). Error bars represent standard deviation.

### *Olsen extractable phosphorus*

One of the most important nutrients present in fly ash is phosphorus, and in this study, EM inoculation and *E. fetida* presence significantly influenced changes in Olsen phosphorus ( $P < 0.001$ ; Table 6.2). It was noteworthy that, throughout the entire 10 weeks of vermicomposting, the *E. fetida* + EM treatment showed significantly higher ( $P < 0.05$ ) ability to increase Olsen P in the fly ash vermicompost (Figure 6.4). The ability to increase Olsen P by the different treatments followed the order *E. fetida* + EM > *E. fetida* only > EM only > control.



**Figure 6. 4:** Effects of *E. fetida* and effective micro-organism (EM) presence on changes in Olsen extractable phosphorus during composting of fly ash – cow dung – waste paper mixtures.

The significantly higher increase in Olsen P in the *E. fetida* + EM treatment compared to the *E. fetida* only and EM only treatments is reflected by a significant interaction ( $P < 0.0008$ ; Table 6.2) between EM inoculation and *E. fetida* presence, whilst the consistent increase in Olsen P over time was indicated by the significant ( $P < 0.0001$ ) influence of time on Olsen P release. The treatments *E. fetida* + EM; *E. fetida* only and EM only treatments resulted in 43.8%; 33% and 16.3% increases in Olsen extractable P relative to the control after 10 weeks of vermicomposting. Except for the control, all treatments showed an increasing trend in Olsen extractable P even at 10 weeks after vermicomposting. The rates of Olsen P release were 54.3; 48.4 and 28.7 mg-P/kg per week for the treatments *E. fetida* + EM; *E. fetida* only and EM only, respectively (Table 6.3). The Olsen extractable P was significantly correlated ( $r > 0.55$ ;  $P > 0.001$ ) with total phosphate releasing bacteria, total bacteria and total fungi counts but no correlation was observed between Olsen P and the alkaline phosphatase enzyme activity.

**Table 6. 3:** Relationship between incorporation of Effective micro-organisms (EM) and *E. fetida* presence on changes in Olsen P during vermicomposting of fly ash – cow dung – waste paper mixtures.

Treatment	Regression equation	R <sup>2</sup>	Rate of Olsen P release (mg P/kg/week)	Predicted Olsen P at 10 weeks (mg/kg)	Observed Olsen P at 10 weeks (mg/kg)	Net Olsen P increase after 10 weeks (%)
Control (no <i>E. fetida</i> or EM)	$-2.5651x^2 + 42.981x + 709.9$	0.977	16.56d	883.20	878.23d	23.18d
EM only	$1.6694x^2 + 15.505x + 720.75$	0.965	28.71c	1042.74	1021.11c	43.35c
<i>E. fetida</i> only	$0.9187x^2 + 39.136x + 689.8$	0.994	48.39b	1173.03	1168.19b	64.03b
EM + <i>E. fetida</i>	$-4.1268x^2 + 93.977x + 738.67$	0.981	54.32a	1265.76	1263.02a	77.20a

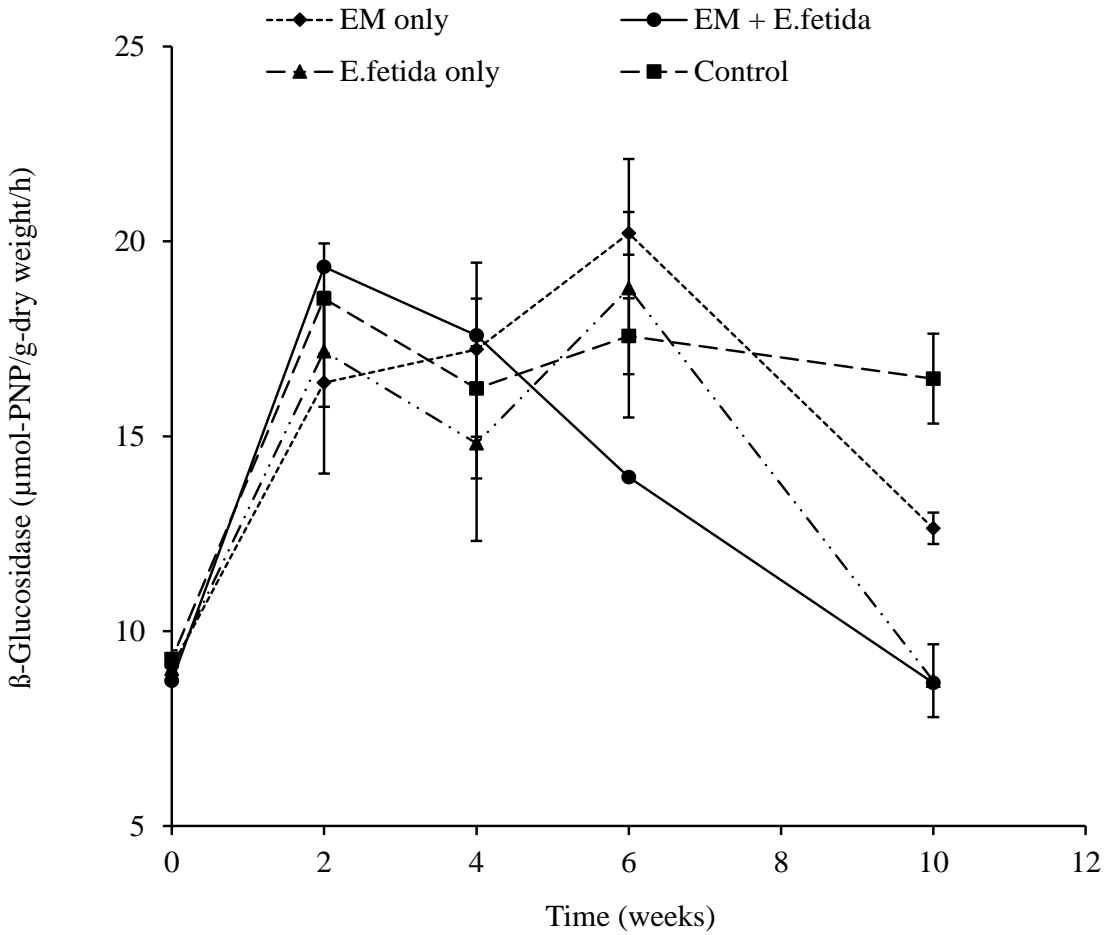
### 6.4.3 Effects of *E. fetida* and EM inoculation on changes in compost enzyme activity

#### *β* - Glucosidase

Except for the *E. fetida* + EM treatment,  $\beta$  – Glucosidase generally increased until 6 weeks and decreased thereafter with varying intensities. The activity of  $\beta$  – Glucosidase varied little from 4 weeks through the 10 weeks of composting (Figure 6.5). It was noteworthy that the activity of  $\beta$  – Glucosidase was not influenced by the presence of EM, but was significantly influenced ( $P = 0.0014$ ; Table 6.4) by the presence of *E. fetida*. After the 10 weeks of vermicomposting, the treatments *E. fetida* + EM and *E. fetida* only showed the lowest activity of  $\beta$  – Glucosidase, which was significantly different from the EM only and control treatments.  $\beta$  – Glucosidase activity was significantly correlated with both C: N ratio, DOC and total bacteria counts in the composts ( $r > 0.5$ ;  $P > 0.001$ ).

**Table 6. 4:** Repeated measures ANOVA for changes in selected biochemical and microbiological parameters following 10 weeks of fly ash vermicomposting.

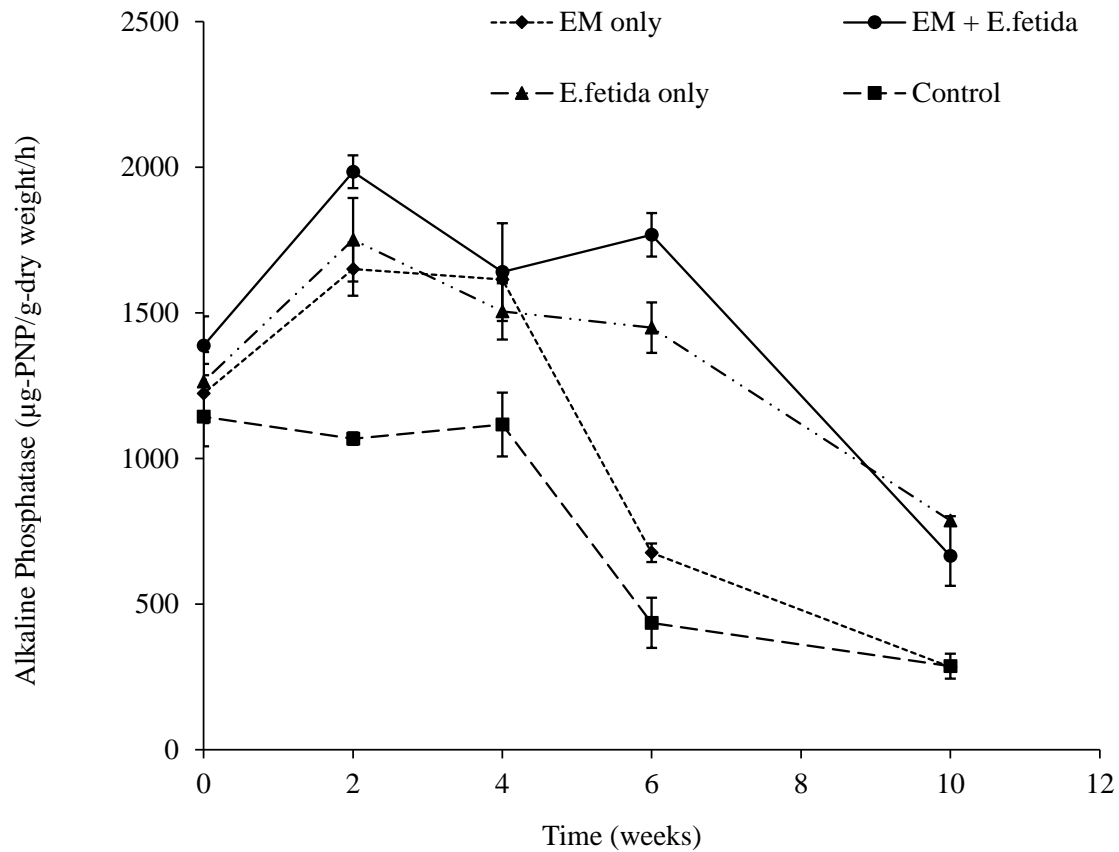
Source of variation		$\beta$ -Glucosidase ( $\mu\text{mol} - \text{PNP/g-dry weight/h}$ )	Alkaline Phosphatase ( $\mu\text{g} - \text{PNP/g-dry weight/h}$ )	Bacteria ( $\log_{10}\text{CFU/g-dry weight}$ )	<i>E. coli</i> ( $\log_{10}\text{CFU/g-dry weight}$ )	Phosphate Solubilizing Bacteria ( $\log_{10}\text{CFU/g-dry weight}$ )	Fungi ( $\log_{10}\text{CFU/g-dry weight}$ )
<i>Between subjects</i>							
EM presence (EM)	$F_{1, 71}$	0.7	142.2	17.7	0.9	2.4	14.4
	<i>P</i>	ns	< 0.0001	0.0056	ns	ns	0.009
<i>E. fetida</i> presence (EP)	$F_{1, 71}$	31.5	781.5	31.6	0.2	216.3	15.9
	<i>P</i>	0.0014	< 0.0001	0.0014	ns	< 0.0001	0.0072
EM $\times$ EP	$F_{3, 71}$	0.7	22.61	51.1	6.5	2.8	2.6
	<i>P</i>	ns	0.0031	0.0004	0.0437	ns	ns
<i>Within subjects</i>							
Time	$F_{5, 71}$	55.7	384.53	38.6	228.5	61.9	133.2
	<i>P</i>	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Time $\times$ EM	$F_{11, 71}$	1.8	22.4	5.6	38.6	2.3	4.5
	<i>P</i>	ns	< 0.0001	0.0032	< 0.0001	ns	0.0287
Time $\times$ EP	$F_{11, 71}$	6.3	82.1	17.7	6.5	4.7	14.8
	<i>P</i>	0.0289	< 0.0001	< 0.0001	0.0025	0.0211	0.0003
Time $\times$ EM $\times$ EP	$F_{23, 71}$	5.0	5.2	3.5	16.7	4.0	4.5
	<i>P</i>	0.0464	0.0179	0.024	< 0.0001	0.0345	0.0295



**Figure 6. 5:** Effects of effective micro-organisms (EM) and *E. fetida* on changes in  $\beta$ -Glucosidase activity during vermicomposting of fly ash – cow dung – waste paper mixtures. Error bars indicate standard deviation.

### *Alkaline phosphatase activity*

Alkaline phosphatase activity showed a much more consistent trend compared to  $\beta$  – Glucosidase. The control treatment had the significantly ( $P < 0.05$ ) lowest levels of alkaline phosphatase activity throughout the 10 weeks of vermicomposting compared to all other treatments (Figure 6.6).



**Figure 6. 6:** Effects of effective micro-organisms (EM) and *E. fetida* on changes in Alkaline Phosphatase activity during vermicomposting of fly ash – cow dung – waste paper mixtures. Error bars indicate standard deviation.

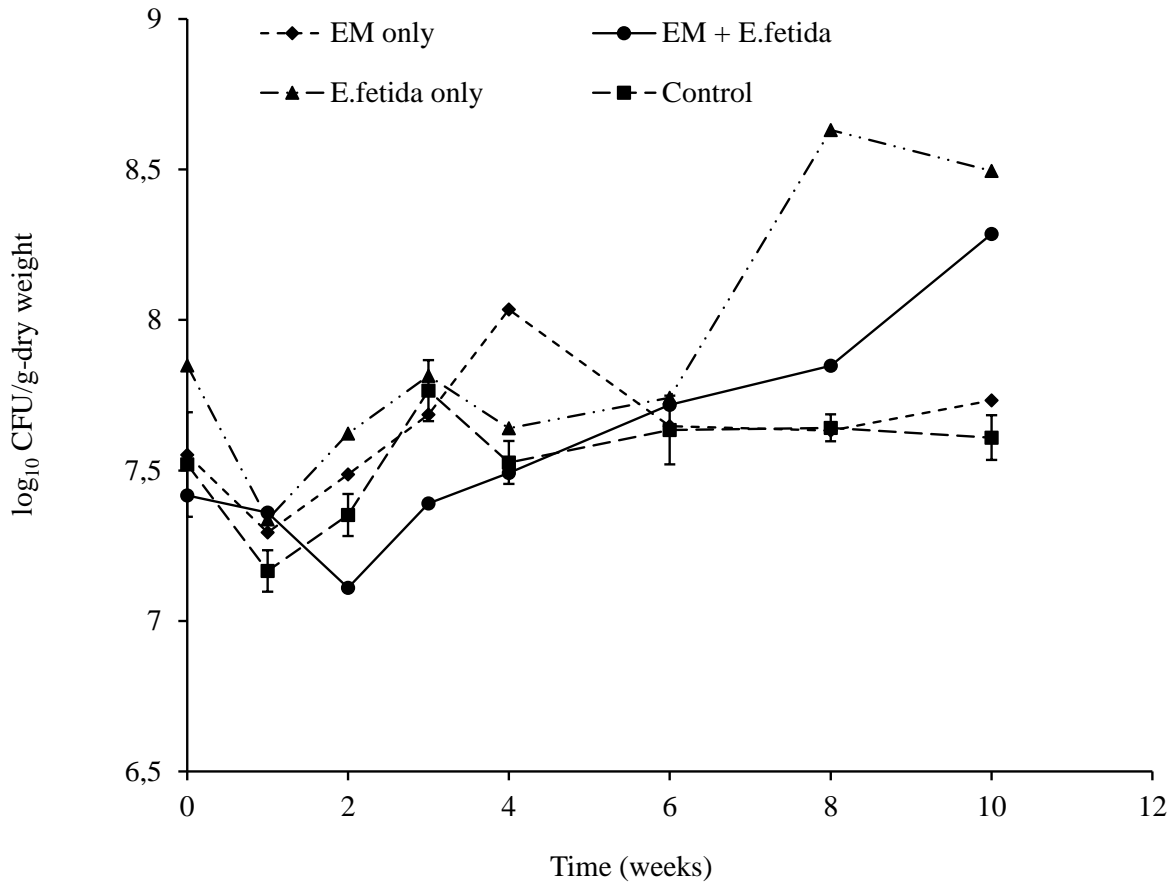
The activity of alkaline phosphatase was significantly influenced ( $P < 0.0001$ ; Table 6.4) by both *E. fetida* presence and EM presence, with the consistently higher alkaline phosphatase activity in the *E. fetida* + EM treatment resulting in a significant interaction ( $P < 0.0031$ ; Table 6.4) between EM inoculation and *E. fetida* presence. The average alkaline phosphatase activity

throughout the 10 weeks of composting was 1453.9; 1340.6; 1087.1 and 823.5  $\mu\text{g} - \text{PNP/g-dry weight/h}$  for the *E. fetida* + EM; *E. fetida* only, EM only and control treatments, respectively, representing an average 76.6%; 62.8% and 32.0% difference respectively, compared to the control. The alkaline phosphatase activity showed no correlation with Olsen P and other parameters measured.

#### **6.4.4 Effects of *E. fetida* and EM inoculation on microbial counts in fly ash vermicompost**

##### ***Total bacteria counts***

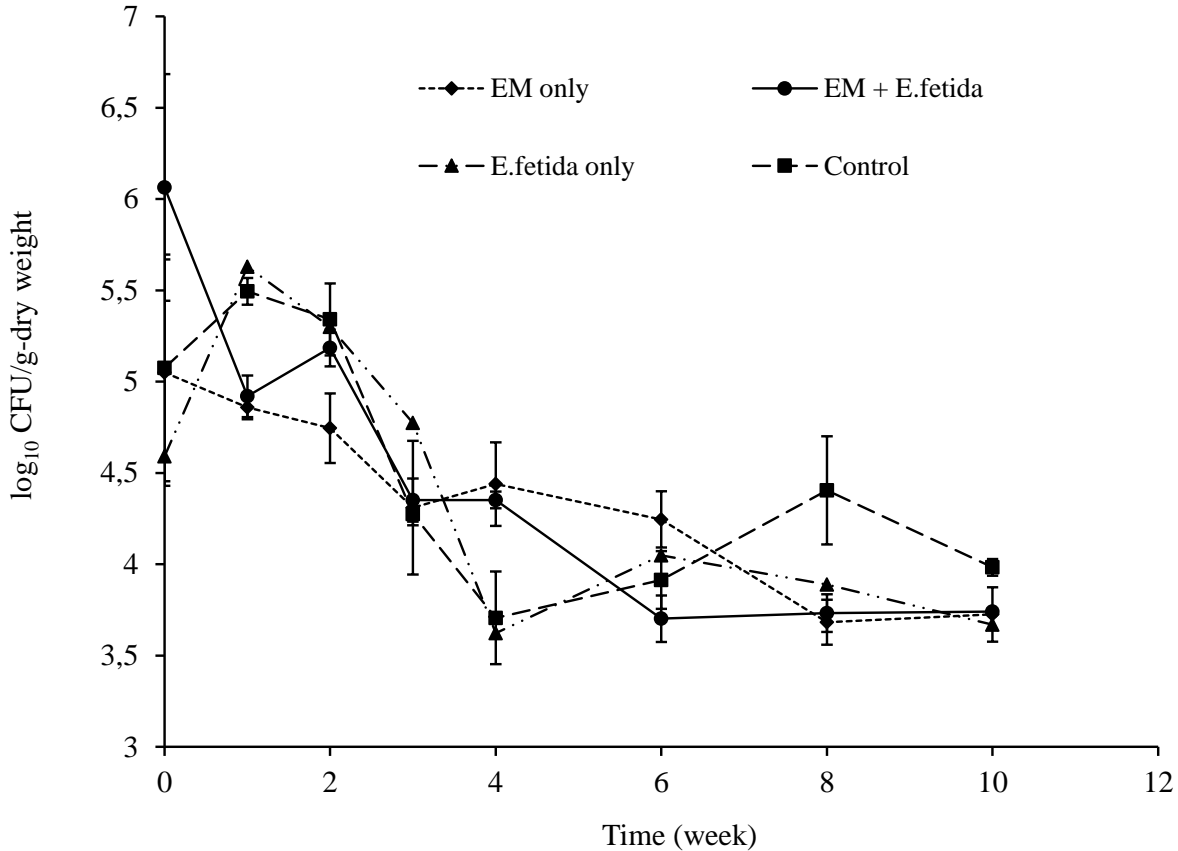
The total bacterial counts were significantly influenced ( $P < 0.005$ ; Table 6.4) by *E. fetida* presence and inoculation with EM with a significant interaction between *E. fetida* presence and EM inoculation ( $P < 0.0004$ ; Table 6.4). From 0 to 6 weeks, total bacterial counts showed a consistent non-significant increase, but beyond 6 weeks, the *E. fetida* and *E. fetida* + EM treatments had the highest total bacterial counts until 10 weeks (Figure 6.7). Bacterial counts showed significant positive correlations with various chemical and biological parameters i.e. Olsen P ( $r = 0.5225$ ;  $P < 0.0001$ ); phosphate solubilizing bacteria ( $r = 0.4494$ ;  $P = 0.0003$ ) and fungi ( $r = 0.5092$ ;  $P < 0.0001$ ).



**Figure 6. 7:** Changes in total Bacteria counts during vermicomposting of fly ash – cow dung – waste paper mixtures with or without effective micro-organisms (EM) and *E. fetida*. Error bars represent standard deviation.

### ***Total E. coli counts***

*E. coli* is an important potential pathogen that is of concern in vermicompost usage in agriculture. There were no significant influences from inoculation with EM or presence of *E. fetida* on total *E. coli* counts (Table 6.4). With no significant differences between treatments throughout the 10 week period, the total *E. coli* counts showed a consistent decrease until 6 weeks and then stabilized thereafter, as indicated by the significant influence ( $P < 0.0001$ ) by time (Figure 6.8; Table 6.4).

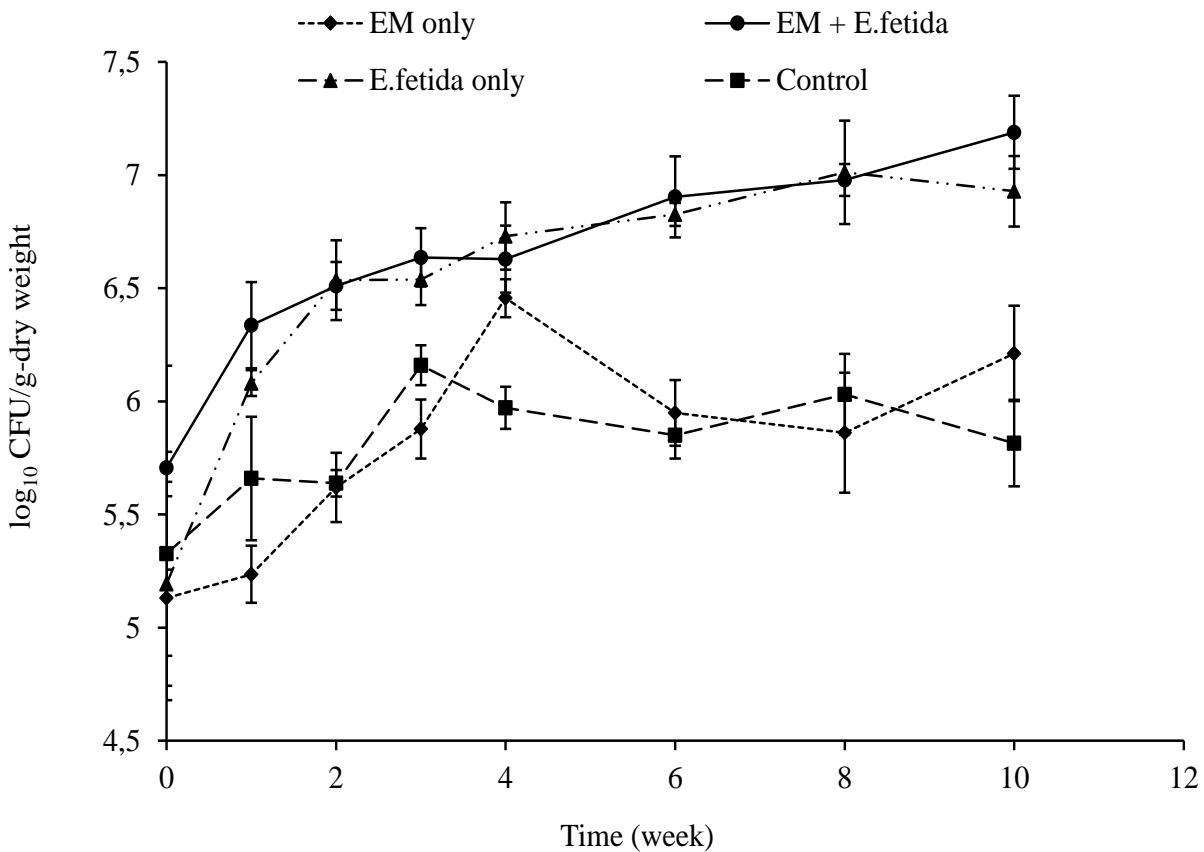


**Figure 6. 8:** Changes in total *E. coli* counts during vermicomposting of fly ash – cow dung – waste paper mixtures with or without effective micro-organisms (EM) and *E. fetida*. Error bars represent standard deviation.

### ***Total phosphate solubilizing bacteria (PSB)***

Total PSB counts were significantly influenced ( $P < 0.0001$ ; Table 6.4) by *E. fetida* presence but not by EM presence. Generally, total PSB increased from 0 to 4 weeks and beyond this, the *E. fetida* + EM and *E. fetida* only treatments showed a continued increase whilst the EM only and control treatments showed a decreased stabilized count of PSB (Figure 6.9). On average, the total

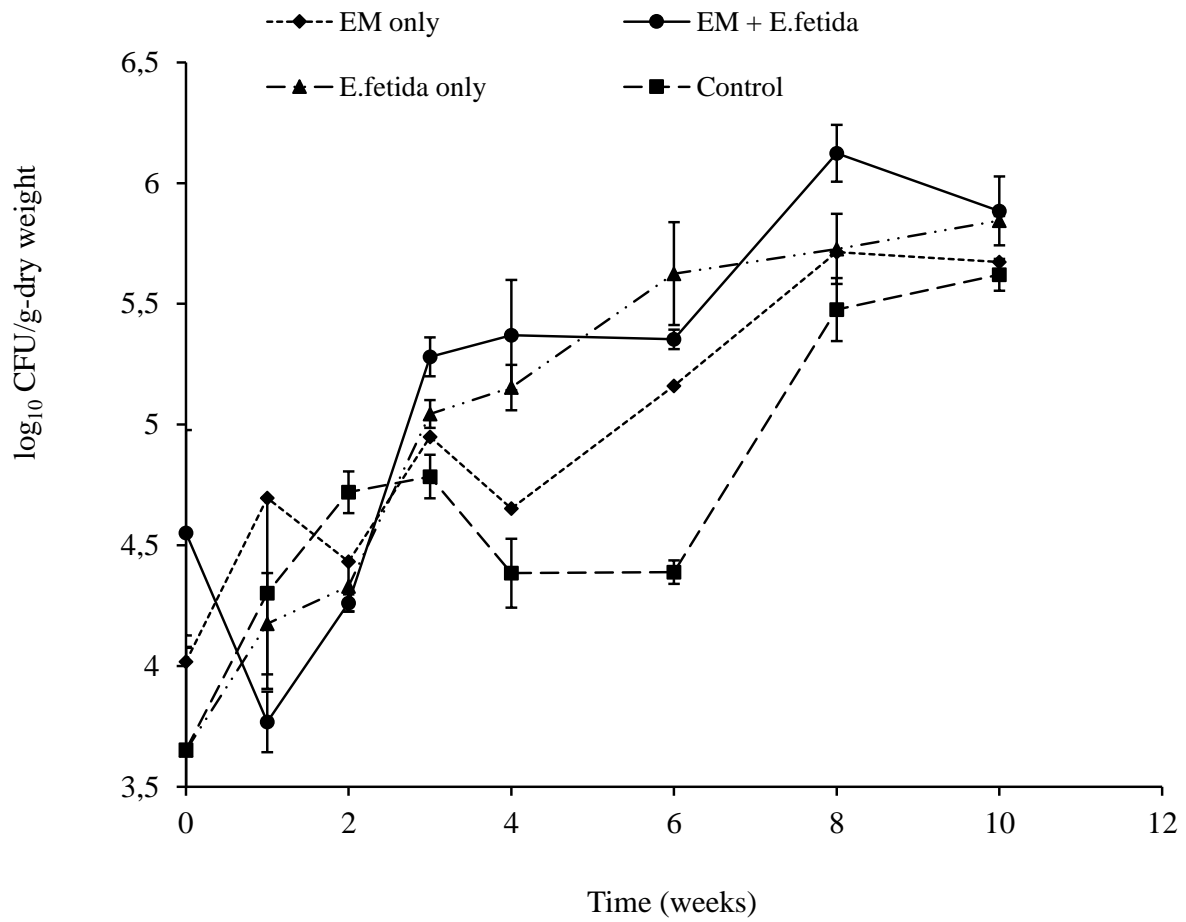
PSB counts for the *E. fetida* + EM and *E. fetida* only treatments showed a non-significantly different total PSB counts of 6.6 and 6.4 log<sub>10</sub> CFU/g- dry weight; whilst the EM and control treatment had another non significantly different average total PSB counts of 5.79 and 5.80 log<sub>10</sub> CFU/g- dry weight. The total PSB counts were significantly correlated to Olsen P ( $r = 0.7358$ ;  $P < 0.0001$ ) and total bacterial counts ( $r = 0.4494$ ;  $P = 0.0003$ ).



**Figure 6. 9:** Changes in total Phosphate Solubilizing Bacteria counts during vermicomposting of fly ash – cow dung – waste paper mixtures with or without effective micro-organisms (EM) and *E. fetida*. Error bars represent standard deviation.

### ***Total fungal counts***

Total fungal counts were significantly influenced by *E. fetida* and EM inoculation ( $P < 0.005$ ; Table 6.4). Generally, total fungi counts showed a consistent almost linear increase across the entire 10 weeks of vermicomposting, with the control treatment having the significantly lowest total fungal counts (Figure 6.10). Total fungal counts showed significant correlations with various chemical and biological parameters i.e. inorganic nitrogen ( $r > 0.6$ ;  $P < 0.0001$ ); Olsen P ( $r = 0.7634$ ;  $P < 0.0001$ ); total bacteria ( $r = 0.5092$ ;  $P < 0.0001$ ) and total PSB ( $r = 0.6947$ ;  $P < 0.0001$ ).



**Figure 6. 10:** Changes in total Fungi counts during vermicomposting of fly ash – cow dung – waste paper mixtures with or without effective micro-organisms (EM) and *E. fetida*. Error bars represent standard deviation.

## 6.5 DISCUSSION

### 6.5.1 Effects of *E. fetida* presence and EM inoculation on changes in chemical parameters of fly ash vermicompost

The technology of Effective micro-organisms (EM) involves use of a liquid microbial inoculant containing a mixture of fungi, bacteria, yeasts and photosynthetic bacteria, with its use in agriculture having been reported to increase plant growth by enhancing organic matter decomposition and nutrient release (Daly and Stewart 1999; Yamada and Xu 2001; Ncube et al. 2011). However, the synergy between EM and other waste decomposers like earthworms is rarely reported (Di-fa et al. 2011). The results of this study indicated that though inoculation with EM resulted in enhanced degradation, its combination with *E. fetida* did not result in significant changes in the C: N ratio when compared to the *E. fetida* only treatment. A C: N ratio of less than 15 has been recommended as representing a mature compost (Bernal et al. 2009). It was, however, noteworthy that inoculation with EM together with *E. fetida* presence resulted in much faster compost maturity, taking around 6 – 7 weeks, slightly faster than *E. fetida* alone. Di-fa et al. (2011) reported similar results, with the interaction between EM and *E. fetida* enhancing organic matter losses and this was attributed to the ability of EM to enhance the living environment for earthworms. The non-significant difference observed between the EM only and control treatment in this study is similar to observations made by Mupondi et al. (2006b) using pine bark, in which they also observed a non-significant difference on changes in C: N ratio between the pine bark alone and pine bark plus EM during composting. It is clear that although micro-organisms are the main agents responsible for organic matter decomposition, earthworms play a much more critical role and their contribution to decomposition may be more direct through their digestive processes (Aira et al. 2006).

A similar trend to that of the C: N data was observed on changes in dissolved organic carbon (DOC), which in this study, rapidly decreased in the *E. fetida* only and *E. fetida* + EM treatments. The decrease in DOC concentration in composts has been observed to be highly correlated to losses in total carbon in composts in which the original solid polymeric material in the compost is initially degraded into soluble organic matter like DOC (Gomez-Brandon et al. 2008). This could explain the positive significant correlation between C: N ratio and DOC observed in this study. Zmora-Nahum et al. (2005) reported that a final DOC concentration of < 4 mg/g signified a mature compost. In our study, the interactions between earthworm activity and EM was also observed on changes in DOC, but the activity of earthworms played a greater role in organic matter turn over as all compost treatments with *E. fetida* resulted in a final DOC concentration way below the 4 mg/g compared to the control and EM alone treatment.

No positive correlations were observed between inorganic nitrogen and total bacteria counts in this study so it is unlikely that the observed increase in inorganic nitrogen (nitrate/nitrite) was due to an increase in nitrogen fixing bacteria. The superiority of the treatments with *E. fetida* in enhancing inorganic N release was possibly a result of the enhanced biodegradation under earthworms which enhanced nitrogen mineralization and its subsequent nitrification. The gut associated processes taking place in the earthworm intestines could thus have been mainly responsible for significant changes in composts under *E. fetida* compared to EM only or control treatments in this study as also reported by Gomez-Brandon and Dominguez (2014). Though positive, the benefits of EM inoculation on increase in nitrate/nitrite within the fly ash vermicomposts were marginal in our study.

Olsen P which represents one of the most important nutrients in fly ash with potential to improve fertilizer value in composts, was observed to significantly increase ( $P < 0.05$ ) in all composts

following the order: *E. fetida* + EM > *E. fetida* only > EM only > control. The influence of EM inoculation was more pronounced on the changes in Olsen P with or without *E. fetida*. It is plausible to attribute the higher Olsen P concentration in the composts to the activity of phosphate solubilizing bacteria (PSB), which showed highly positive significant correlation with Olsen P and the activity of micro-organisms in earthworm gut as also reported by Nair and Okamitsu (2010) and Gomez-Brandon and Dominguez (2014). Busato et al. (2012) working with rock phosphate inoculated with PSB during vermicomposting observed significantly higher levels of both water extractable and resin extractable phosphorus compared to treatments where inoculation had not been done. However, the non-significant influence of EM on total PSB counts suggests that the presence of *E. fetida* resulted in proliferation of PSB in the composts, rather than inoculation with EM. The levels of both inorganic nitrogen and phosphorus showed that it would continue to increase after 10 weeks, so it would be interesting to observe the nutrient release in fly ash vermicompost over a longer period of time which, as reported by Gomez-Brandon and Dominguez (2014) will be predominated by cast associated processes during vermicomposting.

### **6.5.2 Effects of *E. fetida* and EM inoculation on biological properties of fly ash vermicompost**

During vermicomposting, it is well known that most biochemical reactions occurring like C, N and P mineralization are catalysed by both intracellular and extracellular enzymes (Tiquia et al. 2002).  $\beta$ - Glucosidase and alkaline phosphatase, involved in carbon and phosphorus turnover, respectively, during vermicomposting were investigated in this study. The activity of  $\beta$ -Glucosidase in this study showed a characteristic decrease beyond 2 weeks for the *E. fetida* +

EM treatment, representing faster decrease in organic matter. This reflects that this enzyme is of microbial origin as this trend in activity was similar to the microbial biomass observed in Chapter 4. Inoculation with EM to the *E. fetida* seemed to introduce more microbes, which significantly interacted with earthworm activity to result in the faster decrease in C: N ratio (Figure 6.1). Similar decrease in  $\beta$ - Glucosidase was reported by Kayikcioglu and Okur (2011) suggesting that this enzyme is a sensitive indicator of state of organic matter decomposition, so that as organic matter decreases, its activity consequently decreases.

The activity of alkaline phosphatase in this study was found not to be correlated with the release in Olsen phosphorus, contrary to what other researchers have reported (Busato et al 2012; Benitez et al. 1999). The decline observed in alkaline phosphatase with time has been speculated to be due to the feedback inhibition of the enzyme by inorganic phosphate (Gomez-Brandon et al. 2008). The lack of correlation could mean that instead of enhancing phosphatase enzyme activity, phosphate solubilizing bacteria and other microbes may be exuding organic acids which may play a more prominent role in P solubilization compared to the enzyme (Busato et al. 2012). Various micro-organisms have been observed to secrete significant amounts of organic acids like oxalic, citric and tartaric acids with the ability of micro-organisms to solubilize P complexes being attributed mainly to secretion of organic acids (Busato et al. 2012).

During the vermicomposting process, diverse microbes are involved in the stabilization and maturation of the compost (Hachicha et al. 2009). In separate studies involving composting only, Chroni et al. (2009) and Hassen et al. (2001) reported a high resurgence of mesophilic bacteria following the end of the thermophilic stage during composting. The total bacterial counts in our study showed a continued increasing trend throughout the entire 10 week period. This could be

likely due to the proliferation of mesophilic bacteria which may have proliferated in absence of the thermophilic bacteria during vermicomposting.

*E. coli* is one of the most representative bacterium in the group of faecal coliforms, which are potential pathogens when vermicompost is used to produce fresh produce (Hassen et al. 2001). The values of total *E. coli* counts in our study were in the similar range as to those reported by Hassen et al. (2001). It was important to observe that inoculation with EM in compost does not result in an increase in potentially pathogenic *E. coli*, as shown by the non-significant differences observed between the control, *E. fetida* and EM treatments. Vermicomposting has been observed to greatly reduce pathogen populations (Mupondi et al. 2006b); and the results of our study support this idea, meaning EM inoculated vermicomposts can be safely used in agriculture.

The presence of earthworms rather than EM resulted in a significant increase in phosphate solubilizing bacteria (PSB) as observed by the consistently high level in the fly ash vermicompost across 10 weeks. In a similar study but without EM inoculation, Bhattacharya and Chattopadhyay (2002) also reported very high counts of PSB in *E. fetida* treated composts compared to the no earthworm treatments. Hong et al. (2011) attributed this strong interaction between earthworms and PSB to the symbiotic and synergistic relationship between microbes and earthworms. This non-significant influence of EM on the PSB levels in the vermicomposts can mean that EM is devoid of these important phosphorus mineralizing microbes, and creates an opportunity to improve these EM cocktails, especially when P is a significant player in the composting.

The total fungal counts showed an increasing trend with the increase being more evident in the *E. fetida* treatments. In a study using fly ash and vinasse for vermicomposting using *E. fetida*;

Pramanik and Chung (2011) observed a similar increase in total fungal counts. They suggested that not all microbes that pass through earthworm gut are killed, but rather fungi population increases in the material ejected by the earthworms. This is supported by the lower fungal populations observed in this study in the control and EM only treatment where there was no earthworm activity.

## 6.6 CONCLUSIONS

This study has shown that inoculation of fly ash – cow dung – waste paper vermicomposts with effective micro-organisms alone will result in very slow organic matter decomposition not significantly different from no EM inoculation. However, inoculation of EM coupled with *E. fetida* can slightly enhance degradation but not significantly different from *E. fetida* alone and higher rates of EM may need to be considered to achieve a significant difference. Nevertheless, inoculation of EM together with *E. fetida* can result in higher Olsen P release in fly ash vermicomposts, suggesting a synergistic relationship between EM microbes and *E. fetida* in phosphorus mineralization. Furthermore, EM inoculation did not result in increases in phosphate solubilizing bacteria, showing that other microbes than those contained in EM could be involved in the higher Olsen P release observed. In addition, inoculation of fly ash vermicomposts with EM did not increase the potentially pathogenic *E. coli*, but rather the population significantly decreased due to vermicomposting. It would be interesting to create EM microbe cocktails with PSB as a component and observe its interactions with *E. fetida* on Olsen P release during fly ash vermicomposting. Also, with optimization of EM inclusion as an option, the investigation of the fly ash vermicomposting process with EM under industrial conditions may be essential.

## CHAPTER SEVEN

### **7.0 INFLUENCE OF FLY ASH-COW DUNG-WASTE PAPER VERMICOMPOST SUBSTITUTED INTO PINE BARK COMPOST ON MEDIA PHYSICOCHEMICAL PROPERTIES, ORNAMENTAL MARIGOLD (*TAGETES SPP*) GROWTH AND MATURITY**

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This Chapter is based on a manuscript entitled:

Mupambwa HA, Lukashe SN and Mnkeni PNS. Forthcoming. Influence of fly ash-cow dung-waste paper vermicompost substituted into pine bark compost on media physicochemical properties, ornamental marigold (*Tagetes spp*) growth and maturity.

## 7.1 ABSTRACT

This study evaluated the potential of fly ash vermicompost (FA) substitution in to pine bark compost (PB) in improving media physico-chemical properties and the horticultural potential of the resultant media in growing ornamental marigolds (*Tagetes spp*). Fly ash vermicompost was substituted into pine bark compost at 0; 25; 50; 75 and 100% and marigolds flowers planted with or without fertilizer. Fly ash vermicompost substitution up to 50% significantly improved water holding capacity, total porosity and air filled porosity. It also improved pH from 4.52 to a maximum of 8.33 when incorporated up to 75%. However, 100% FA had very poor physical properties which resulted in poor germination of only 61.5% whilst, incorporation of FA up to 75% resulted in significantly high germination percentage above 90% compared to 22.5% for the 100% PB. However, after 4 weeks of growth, seedlings in the 25% and 50% FA substituted media had higher plant height and leaf area. Due to poor performance of the 100% FA and 100% PB treatments, the maturity part of the experiment was carried out using 75%, 50% and 25% FA treatments only. Plant height and leaf area was significantly higher in the 25% FA treatment with and without fertilization, with fertilizer application resulting in even greater differences. The 25% FA treatment also resulted in significantly higher number of flowers and buds compared to the 50% and 75%, despite the higher concentration of essential nutrients in the 50% FA treatment. For effective marigold seedling germination and growth, the 50% FA: 50% PB growing medium is recommended whilst for maturity and flower production, the 25% FA: 75% PB combination is to be preferred as it performed better than all treatments regardless of the nutrient composition of the media.

**Key words:** Horticultural potential; water holding capacity, nutrient content, flower production.

## 7.2 INTRODUCTION

The excessive usage of inorganic fertilizers in agriculture has been observed to adversely affect soil micro-flora, fauna and enzymes that are critical in maintaining soil fertility. In recent times, research has focused on utilization of organic fertilizers like vermicomposts particularly in the horticultural industry, as a source of nutrients (Arancon et al. 2008). These vermicomposts are well stabilized with a desirable balance of essential plant nutrients and humic acids, and have been evaluated as components of traditional planting media (Saranraj and Stella 2012; Arancon et al. 2008). In South Africa, the horticultural industry used to rely on sphagnum peat moss as a horticultural planting media (Mphaphuli et al. 2005). However, regardless of its favorable chemical and physical properties, it's a finite resource and of late there has been a shift towards using abundantly available pine bark as a standard horticultural media (Mupondi et al. 2010). Pine bark has, however, been observed to have some undesirable chemical and physical properties, which makes it a not so suitable horticultural medium (Mupondi et al. 2006a). Pine bark has been observed to have pH that is acidic ranging from 4 – 4.3, suboptimum physical properties like water holding capacity and also being very poor in nutrient supply (Mphaphuli et al. 2005; Mupondi et al. 2010). To counteract these properties, pine bark needs to be mixed with other organic materials that are nutrient rich with desirable physical properties (Mphaphuli et al. 2005).

Several researchers have undertaken studies on how to improve various horticultural planting media using vermicomposts produced from various materials. Atiyeh et al. (2001) substituted a standard planting media (Metro Mix 360) with pig manure vermicompost and observed that tomato seedlings did not grow well in the 100% vermicompost but rather in the 25 – 50% substituted media. Similarly, using the same standard media, Arancon et al. (2004) used food

waste vermicompost and observed highest pepper yields at 40% substitution. Mupondi et al. (2014) using pine bark compost, reported best tomato seedling germination and growth following substitution with 60% cow dung waste paper vermicompost. Most of these studies have confirmed the idea that vermicomposts have significant physical and chemical benefits on plant growth.

Mupambwa and Mnkeni (2015b) established that vermicomposting of fly ash combined with cow dung and waste paper mixture in a ratio of 1: 2 produced a vermicompost with potentially good nutritional properties but with fairly alkaline pH. Fly ash is a product of coal combustion representing more than 70% of the solid residue from coal combustion. South Africa alone produces more than 28 million tons of fly ash annually, of which 95 % of this is not used but is disposed at sites near power stations (Gitari et al. 2010). Due to the processes involved in coal formation, fly ash contains both beneficial plant nutrients like phosphorus, together with potentially toxic heavy metals. Due to the presence of heavy metals in fly ash, it is categorized as an environmental pollutant in South Africa and other countries (Mupambwa et al. 2015). However, vermicomposting of fly ash has been shown to increase the bioavailability of essential plant nutrients like phosphorus, whilst reducing the bioavailability of most heavy metals (Bhattacharya et al. 2012). Fly ash vermicompost can be an important nutrient source in non-edible horticultural crop production, like in the ornamental industry.

Research on the potential of fly ash vermicompost as a substitution of nutrient deficient planting media like pine bark in the production of highly valuable ornamental flowers like marigolds is scarce (Gupta et al. 2014). It was, therefore, of interest to investigate possibility of combining pine bark compost with fly ash vermicompost to produce a growing medium suitable for ornamental plants. It is hoped that the alkaline pH of the fly ash vermicompost will help

counteract the acidity of pine bark compost while the relatively coarse pine bark will improve the porosity of the vermicompost. The objectives of the study were thus (i) to determine the effect of progressive substitution of pine bark compost with fly ash vermicompost on the resultant growing media physical and chemical properties; (ii) to determine the effect of pine bark compost substituted with fly ash vermicompost on the germination and subsequent growth of marigold (*Tagetes spp*) growth and maturity (iii) to evaluate the possibility of optimizing the growth of the marigold flowers grown in the resultant growing media using a water soluble fertilizer.

## **7.3 MATERIALS AND METHODS**

### **7.3.1 Plant media sources and combinations**

The pine bark which had been prepared by Mupondi et al. (2006a) was used in this study. Vermicompost prepared by Mupambwa and Mnkeni (2015b), in which fly ash had been incorporated into cow dung waste paper mixtures at a ratio of 1: 2, was used for the substitution. The cow dung, waste paper and fly ash were sourced from places indicated in Chapter 4 section 4.3.1 with the various chemical properties of the materials highlighted in Table 4.1 of Chapter 4.

### **7.3.2 Treatments and experimental design**

The experiment was carried out in a greenhouse with temperature and humidity control at the University of Fort Hare, Alice campus. The growing media used was the pine bark compost substituted with different proportions of the fly ash vermicompost on a volume to volume basis.

The fly ash vermicompost was substituted into the pine bark at 0%; 25%; 50%; 75% and 100%. These mixtures were evaluated for the various physical and chemical properties as described below. The same combinations were used for the plant growth part of the experiment, in which the experiment was a  $5 \times 2$  factorial arrangement laid down in a completely randomized design with three replications. The two factors were fly ash vermicompost substitution percentage into pine bark compost and fertilizer application at two levels (with or without), giving 10 treatment combinations.

### **7.3.3 Analysis of physical and chemical properties of the growing media**

#### **7.3.3.1 Physical properties**

The physical parameters of the various fly ash vermicompost/pine bark compost mixtures were analyzed as outlined by Atiyeh et al. (2001). Samples from each of the growing media mixtures were wetted thoroughly in bulk batches and placed into containers of known volumes and weights, with a fine mesh cloth attached to the base and allowed to drain overnight. Following the initial drainage, the mixtures within the containers were adjusted so as to be level with the top of the container and further saturated for 48 hours, then allowed to re-drain overnight. After draining, the container's wet mass was measured following which the samples were dried at 60 °C for 96 hrs.

The ash content and organic matter of the samples was determined in samples on known weight that had been further oven dried at 105 °C and then placed in a muffle furnace for 5 hours at 550 °C. The various parameters were determined as shown in the Table 7.1 below.

**Table 7. 1:** Equations used for the calculation of the various physical properties of the resulting media mixtures.

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

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<sup>†</sup> 1.55 and 2.65 are the average particle densities of soil organic and mineral matter, respectively

Source (Atiyeh et al. 2001).

### 7.3.3.2 Chemical properties

#### *pH and Electrical conductivity*

Electrical conductivity and pH were measured potentiometrically in deionized water at a ratio of 1:10 (w/v) (Ndegwa and Thompson 2000). Briefly, a 5g sample of media mixture was mixed with 50 mL of deionized water and the suspension was shaken on a horizontal mechanical shaker at 230 rpm for 30 mins. After 30 mins, pH and EC were determined in the suspension using glass electrode pH and EC meters (Crison Instruments, Spain).

Olsen extractable P was determined according to Schoenau and O'Halloran (2006) and as briefly described in in Chapter 3; Section 3.3.4. Exchangeable ammonium and nitrate and nitrite were

determined according to Maynard et al. (2006). They were extracted using 0.5 M potassium sulphate as described in Chapter 4 section 4.3.8.

#### ***Extractable cations (Mg, Na, K and Ca)***

These were extracted using ammonium acetate solution which had been adjusted to a pH of 7 as described by AgriLASA (2004). Briefly, a 5.0 g sample of the growing media was extracted with 50 mL of the ammonium acetate in a 150ml extracting bottle. The mixture was shaken on a reciprocating shaker for 30 mins at 180 rpm. The suspension was then filtered using Whatman™ No. 42 filter paper. The extractable cations in the filtrate were analyzed using an ICP-OES (Varian Inc. The Netherlands).

### **7.3.4 Establishment of crop, germination and seedling growth**

Marigold (*Tagetes spp* – marigold Afrikaners cv. lemon drop), a commercial ornamental plant, was used in this experiment. Each of the five formulated fly ash vermicompost – pine bark compost growing medium was filled into polystyrene trays with 32 cavities each, with three tray replications for each treatment. To each cavity, two seeds of marigold were sown. The fertilized treatments were separated from the non-fertilized ones to avoid contamination, with fertilizer being applied three times a week. A water soluble fertilizer Multifeed P® recommended for continuous liquid nutrient supply in greenhouse ornamental crops was used for the fertilized treatments and it contained N-19.0% , P-8.2% , K-15.8% , Mg-0.09% , Zn-0.035% , B- 0.1% , Mo – 0.007% , Fe- 0.075% , Mn- 0.03% and Cu-0.0075%. The non-fertilized media were given equivalent quantity of ordinary tap water in order to maintain the same moisture content.

Germination percentage was determined at 7 days after sowing and thinning to one seedling per cavity was done at 14 days after planting. Plant height was measured on five randomly selected plants in each treatment at 2 and 4 weeks after germination (WAG), whilst leaf area was measured at 4 WAG on five randomly selected plants.

Three best performing media based on parameters measured at 4 WAG were selected for continued evaluation to plant maturity. These were 75%FA: 25%PB; 50%FA: 50%PB and 25%FA: 75%PB with and without fertilizer application. Six seedlings were randomly selected from each medium for transplanting as described below. The remaining seedlings from each treatment were dried at 60°C and ground to pass through 1 mm sieve. The ground samples were digested using *aqua regia* (3: 1 (v/v) hydrochloric acid: nitric acid) in a MARS 5 microwave digester (CEM Corporation, Matthews, North Carolina) for analysis of plant tissue P, Ca, Mg, K, and Na content, whilst total nitrogen were determined in the ground samples using the dry combustion method employing a LECO-Truspec C: N auto analyzer (LECO – Corporation 2003).

### **7.3.5 Marigold plant growth experiment**

Seedlings selected for transplanting were transplanted, one seedling per pot, into plant pots containing the same media combination from which they were growing before (75%FA: 25%PB, 50%FA: 50%PB and 25%FA: 75%PB with and without fertilizer application). The seedlings were allowed to grow till maturity with watering and fertilization being managed in the same manner as done during the first four weeks of growth. At 6 and 8 weeks, the plant height, number of buds and number of flowers were determined on the six transplanted seedlings per treatment. At maturity (8 weeks) total leaf area per plant was determined on three randomly

selected plants per each treatment. The plants were harvested at 8 WAG and analyzed for various tissue nutrients contents as described above.

### **7.3.6 Statistical Analysis**

Analysis of variance (ANOVA) for a completely randomized design was done for all parameters determined in the experiment using JMP version 11.0.0 statistical software (SAS Institute, Inc., Cary, North Carolina, USA, 2010). Mean separations were conducted using least significant differences (LSD) at  $P \leq 0.05$  test when ANOVA indicated a significant  $P$ - value.

## **7.4 RESULTS**

### **7.4.1 Effects of fly ash vermicompost substitution on planting media physico-chemical properties**

The results of the physical properties following substitution of fly ash vermicompost into pine bark compost at different levels are shown in Table 7.2. The fly ash vermicompost had the highest bulk density, particle density and water holding capacity whilst it had the lowest total porosity and air filled porosity. Substitution of fly ash vermicompost into pine bark compost resulted in significant ( $P < 0.001$ ) differences being observed on all the physical properties measured. Generally, as the level on fly ash vermicompost substitution into pine bark compost increased, bulk density, particle density and water holding capacity subsequently increased whilst the opposite was observed on total porosity and air filled porosity. The bulk density, particle density and water holding capacity of the 100 % fly ash vermicompost was 2.1; 1.26 and 1.34 times more than that of 100 % pine bark composts, respectively. On the other hand, the total porosity and air filled porosity of the 100 % pine bark compost was 1.15 and 10.59 times more than that of the 100 % fly ash vermicompost, respectively. It was important to note that substitution of fly ash vermicompost into pine bark compost from 25 % to 50 % did not result in significant ( $P < 0.05$ ) differences in all physical properties except for particle density.

The results of the various chemical properties following substitution of pine bark compost with fly ash vermicompost are shown in Table 7.3. The pine bark compost pH was acidic (pH 4.51) whilst that of the fly ash vermicompost was highly alkaline (pH 11.14).

**Table 7. 2:** Selected physical properties of resultant plating media mixtures from pine bark compost substitution with fly ash vermicompost.

Planting media mixtures	Bulk Density	Particle Density	Total Porosity	Water Holding Capacity	Air Filled Porosity
	(g/cm <sup>3</sup> )		%		
100FA	0.630 a <sup>†</sup>	2.333 a	72.996 c	70.017 a	2.979 c
75FA:25PB	0.578 b	2.183 b	73.525 c	66.599 a	6.926 c
50FA:50PB	0.407 c	2.008 c	79.741 b	57.189 b	22.552 b
25FA :75PB:	0.393 c	1.898 d	79.296 b	57.387 b	21.909 b
100PB	0.299 d	1.851 e	83.804 a	52.250 c	31.553 a
<i>P</i> -value	< 0.0001	< 0.0001	< 0.0001	0.0002	< 0.0001
CV (%)	3.96	0.976	1.357	4.319	19.963

FA = fly ash vermicompost; PB = pine bark compost

<sup>†</sup> Means within the same column followed by the same letter are not significantly different at  $P \leq 0.05$

The pine bark compost showed limited buffering capacity, with an increase in the substitution with fly ash vermicompost resulting in an increase in pH as shown by the significant differences (Table 7.3). Substitution with fly ash vermicompost from 25 % up to 75% resulted in a final media pH ranging from 8.33 to 6.68. A significant ( $P < 0.0001$ ) difference was observed between treatments on EC, with the pine bark compost having an EC of 0.36 dS/m whilst the fly ash vermicompost had an EC of 1.47 dS/m. Substitution of fly ash vermicompost did not, however, result in a complementary increase in EC, with no significant differences being observed between 50% fly ash vermicompost and 100% fly ash vermicompost. For all the elements determined in this study, except for nitrate/nitrite, the 100% pine bark compost had the lowest concentrations (Table 7.3). Across the four cations, only Na and K concentrations showed a consistent trend with an increase in concentration as the level of fly ash vermicompost substitution increased resulting in their increase in the planting media mixture. With regards to inorganic nitrogen, the fly ash vermicompost had most of the nitrogen concentrated as ammonium whilst pine bark had high nitrate/nitrite. It was noteworthy that substitution of pine bark with fly ash vermicompost at 50% gave the best inorganic nitrogen concentration in the resultant media which was 2.96 and 1.77 times more than fly ash vermicompost alone and pine bark compost alone, respectively. Total inorganic nitrogen concentration in the media followed the order 50% fly ash vermicompost (FA) > 25 % FA > 75% FA > 100 % pine bark compost > 100% FA. With respect to phosphorus, the 100 FA was highest with 92.8 % more phosphorus compared to the pine bark compost. Substitution of pine bark compost with fly ash vermicompost from 25 % to 75 % resulted in increase in plant available P from 41.5 to 76.2 %.

**Table 7. 3:** Selected chemical properties of resultant plating media mixtures from pine bark compost substitution with fly ash vermicompost.

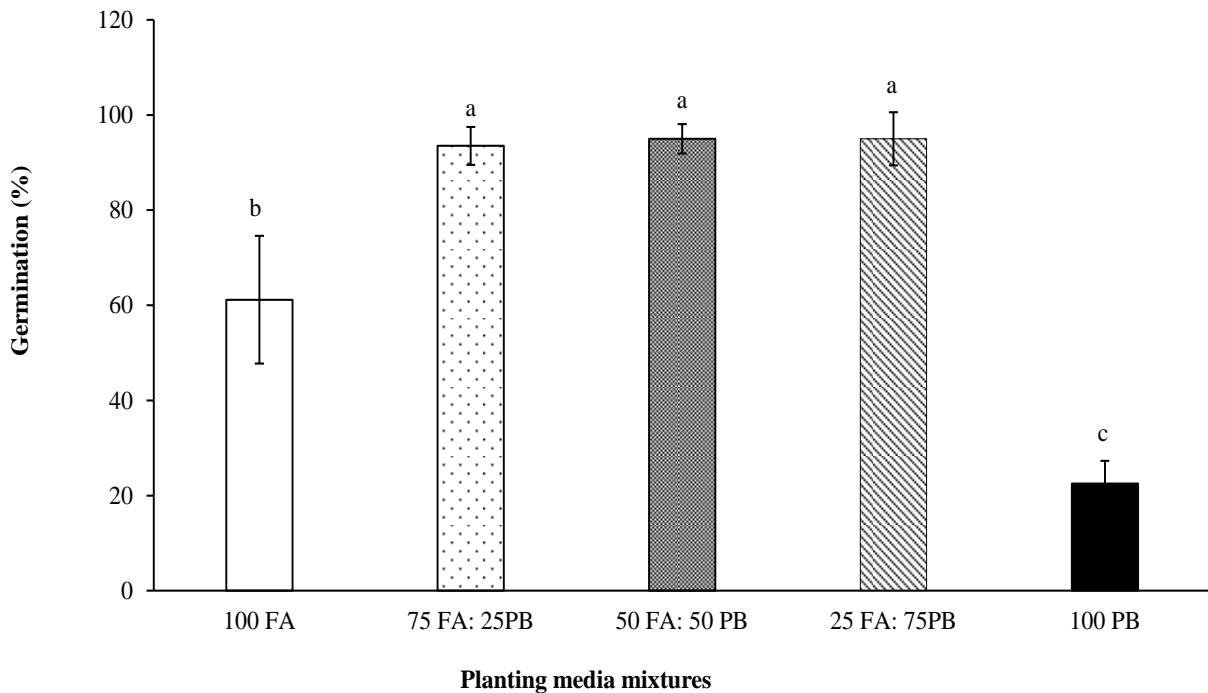
Planting media mixtures	pH	EC dS/m	Ca	K	Mg	Na	NH <sub>4</sub>	NO <sub>2</sub> /NO <sub>3</sub>	Olsen P
	g/kg						mg/kg		
100FA	11.14 a <sup>†</sup>	1.47 a	4.562 bc	4.232 a	2.492 d	0.541 a	61.10a	9.00d	508.59a
75FA:25PB	8.33 b	1.44 a	7.507 a	3.955 b	2.907 c	0.507 b	38.63bc	83.57c	464.78ab
50FA:50PB	7.19 c	1.43 a	6.706 ab	3.613 c	3.170 b	0.424 c	45.77b	162.00a	412.38bc
25FA: 75PB:	6.68 d	1.16 b	7.681 a	3.165 d	3.510 a	0.347 d	25.10cd	160.23a	373.36c
100PB	4.51 e	0.36 c	2.875 c	1.426 e	1.719 e	0.160 e	12.10d	105.5b	263.85d
<i>P</i> -value	<0.0001	<0.0001	0.0038	<0.0001	<0.0001	<0.0001	0.0004	< 0.0001	<0.0001
CV (%)	1.68	2.18	3.10	3.10	2.05	3.23	20.62	10.99	7.35

FA = fly ash vermicompost; PB = pine bark compost

<sup>†</sup> Means within the same column followed by the same letter are not significantly different at  $P \leq 0.05$

### 7.4.2 Effects of fly ash vermicompost and pine bark compost mixtures on marigold seedling germination and early growth

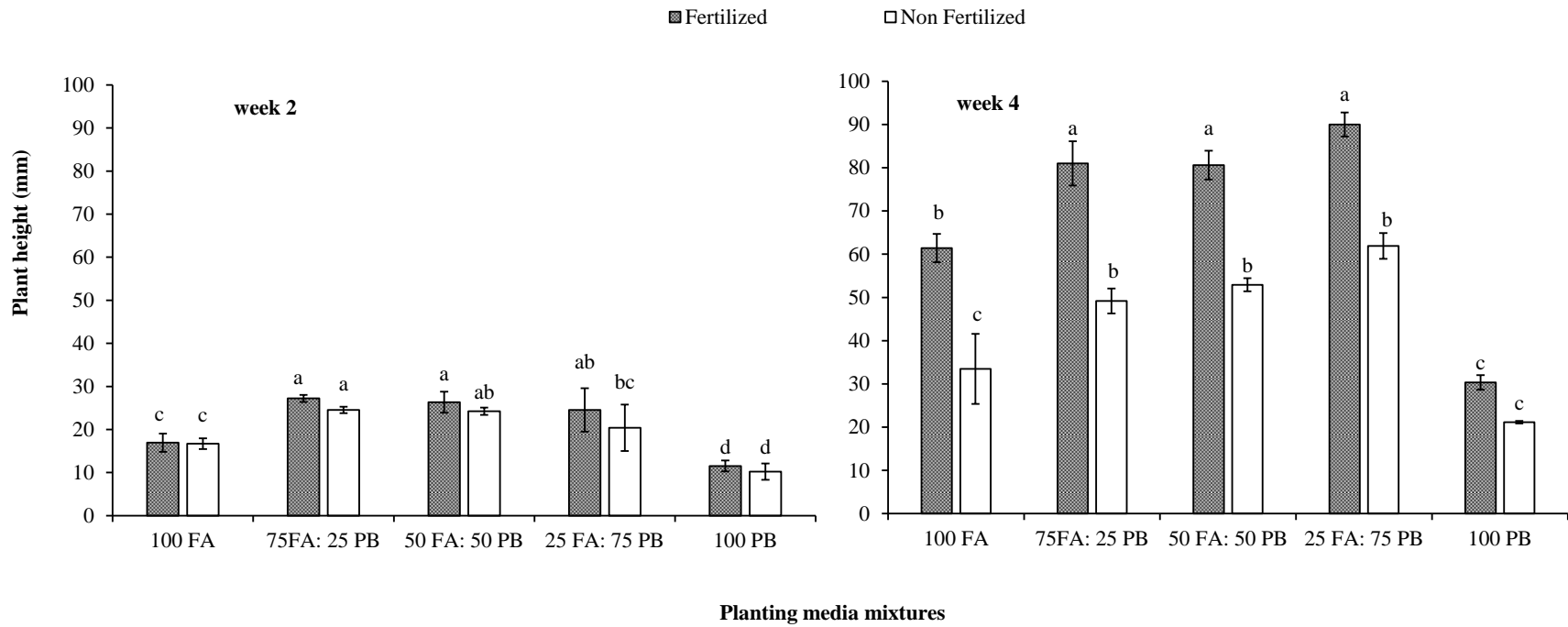
There was a significant ( $P < 0.05$ ) difference on the germination observed after 1 week of planting between the 100 % pine bark compost and the various substituted mixtures (Figure 7.1). The lowest germination percentage of 22.5 % was observed in the pine bark compost followed by the 100 % fly ash vermicompost with 61.2 %.



**Figure 7. 1:** Germination percentage of ornamental marigold seedlings grown in the resultant planting media from pine bark compost substitution with fly ash vermicompost. Error bars represent standard deviation.

There were no significant differences in germination percentages when pine bark compost was substituted with fly ash vermicompost from 75% to 25% which resulted in germination percentage ranging from 93.5% to 95%. It was important to note that substituting pine bark compost with fly ash vermicompost within the range of 75% to 25% resulted in a significant average 4.2 times (319%) increase in percentage germination compared to the pine bark alone. Similarly, substitution of fly ash vermicompost to pine bark from 75% to 25% resulted in an average 1.54 times (54 %) increase in percentage germination of marigold flowers compared to the 100% fly ash vermicompost (Figure 7.1).

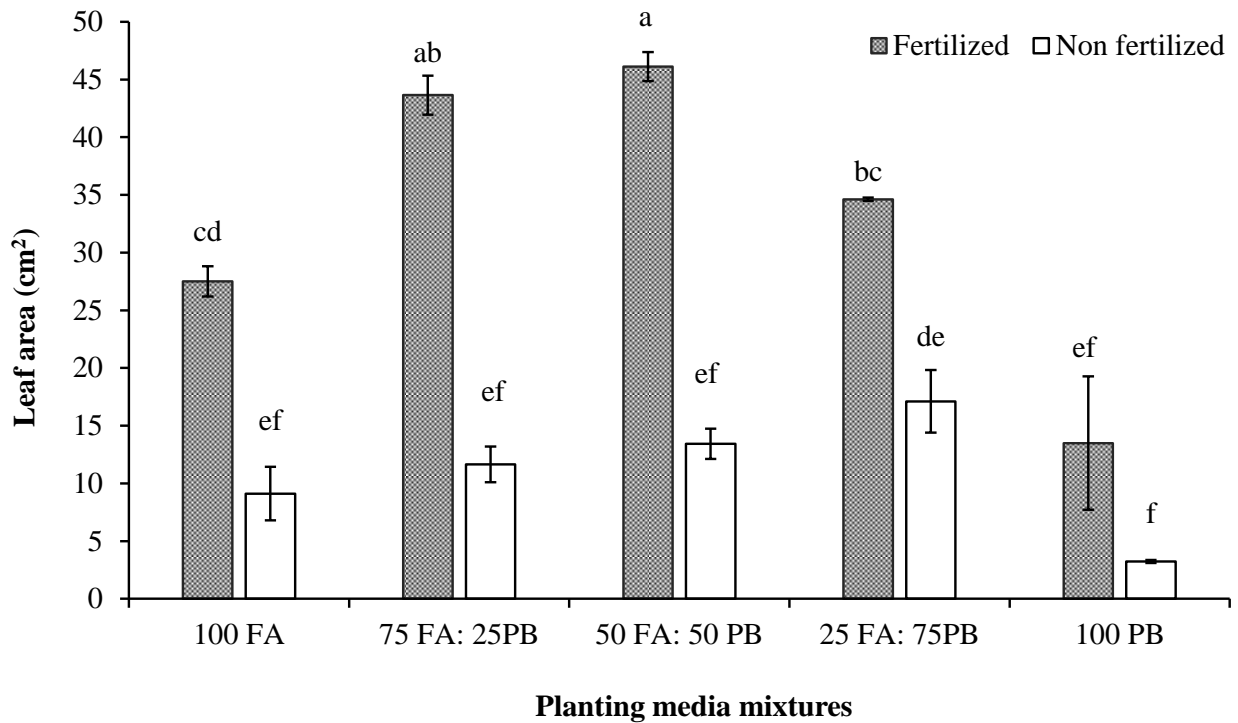
Following the marigold germination, one set of treatments was treated with fertilizer three times a week, whilst the other set was treated with ordinary water during irrigation. Plant height and leaf area was measured with the planting media having a significant effect on both these measured growth parameters (Figure 7.2 and 7.3). At 2 and 4 weeks, the plant heights were highest in the treatments with 75%, 50% and 25% substitution of fly ash vermicompost, with no significant differences being observed between these three treatments. At 2 weeks, fertilization had no significant ( $P < 0.05$ ) effect on plant height. However, substitution of pine bark compost with fly ash vermicompost from 25% up to 75% resulted in an average 2.26 times (125.8%) increase in plant height with or without fertilizer. At 4 weeks, both media mixtures and fertilization significantly affected marigold seedling height. Applying fertilizer to all treatments resulted in an average increase in plant height of 1.57 times (57%) compared to no fertilization.



**Figure 7. 2:** Mean plant heights of ornamental marigold seedlings grown in the resultant planting media from pine bark compost substitution with fly ash vermicompost. Error bars represent standard deviation.

However, the planting media substitutions resulted in a much more significant effect on plant height at 4 weeks, with the substitution of pine bark with fly ash vermicompost from 25% to 75% increasing plant height by an average 2.69 times (170.2%).

Mean leaf area measured at 4 weeks after germination, was also significantly affected by both fertilization and the media type (Figure 7.3).



**Figure 7. 3:** Mean leaf area after 4 weeks growth of ornamental marigold seedlings grown in the resultant planting media from pine bark compost substitution with fly ash vermicompost. Error bars represent standard deviation.

Across all treatments, fertilizer application resulted in the highest leaf area resulting in an average increase in leaf area of 3.12 times (212.6%) compared to the non-fertilized. With fertilizer application, the 50% FA substitution gave the highest leaf area, but without fertilizer application, the 25% FA substitution gave the highest. The treatments 75% FA, 50% FA and 25% FA gave the best results on leaf area with the pine bark alone having the least leaf area.

#### **7.4.3 Effects of fly ash vermicompost and pine bark compost mixtures on marigold seedling nutrient content**

Across all treatments, fertilization had a highly significant ( $P < 0.05$ ) influence on all nutrients measured i.e. P; N; K; Ca; Mg and Na; with the treatments with fertilizer application having the highest nutrient content. Media type also significantly influenced ( $P < 0.05$ ) the nutrient content in the 4 week old marigold seedlings, which resulted in a significant interaction between fertilization and media type (Table 7.4). For all the measured elements except for Na, the 100% pine bark compost had the lowest concentrations when fertilizer was applied to the treatments. The application of fertilizer to the fly ash vermicompost substituted treatments enhanced the efficiency of the uptake of essential nutrients nitrogen, phosphorus and potassium in these treatments. It was also noteworthy that with fertilizer application, the treatment with 50% fly ash vermicompost substitution resulted in the highest tissue content for most of the measured elements.

**Table 7. 4:** Concentration of selected nutrient elements in marigold plant grown in the resultant media mixtures at four weeks.

Planting media mixture	Total elements					
	P	Na	Ca	Mg	K	N
	g/kg					
100 FA	15.42 a	0.26 d	3.08 c	9.25 a	60.64 b	4.89 c
75FA : 25PB	13.77 b	0.41 c	5.56 b	9.34 a	60.51 b	5.12 bc
<b>Fertilized</b> 50 FA : 50PB	15.67 a	0.69 b	7.16 a	9.00 a	68.07 a	5.30 b
25 FA: 75PB	15.61 a	0.84 a	4.82 b	5.61 b	55.88 c	6.52 a
100 PB	12.32 b	0.72 b	2.01 c	4.42 c	18.44 d	nd
100 FA	5.19 b	0.15 c	3.10 b	6.51 a	44.68 a	1.49 a
<b>Non-fertilized</b> 75 FA : 25PB	4.61 b	0.23 c	3.31 b	3.21 b	35.34 bc	0.54 b
50 FA : 50PB	5.13 b	0.42 b	3.62 ab	2.32 c	33.33 c	0.65 b
25FA : 75PB	6.97 a	0.50 ab	4.03 a	2.39 c	37.78 b	1.51 a
100 PB	7.88 a	0.56 a	2.01 c	3.05 b	34.86 bc	nd
<b>ANOVA</b>						
Fertilizer (F)	***	***	***	***	***	***
Treatment (T)	0.0017	***	***	***	***	***
F x T	***	***	***	***	***	0.0002

FA = fly ash vermicompost; PB = pine bark compost; \*\*\* =  $P < 0.0001$ ; nd = no data.

However, this trend was not consistent when fertilizer was not applied to similar treatments, indicating the important interaction between media type and fertilization. In general, comparing the nutrient content averages for the various planting media between fertilized and non – fertilized treatments, it was observed that fertilizer treatments had a significant 144.4 % more phosphorus, 421 % more nitrogen and 41.6 % more potassium compared to the non-fertilized treatments.

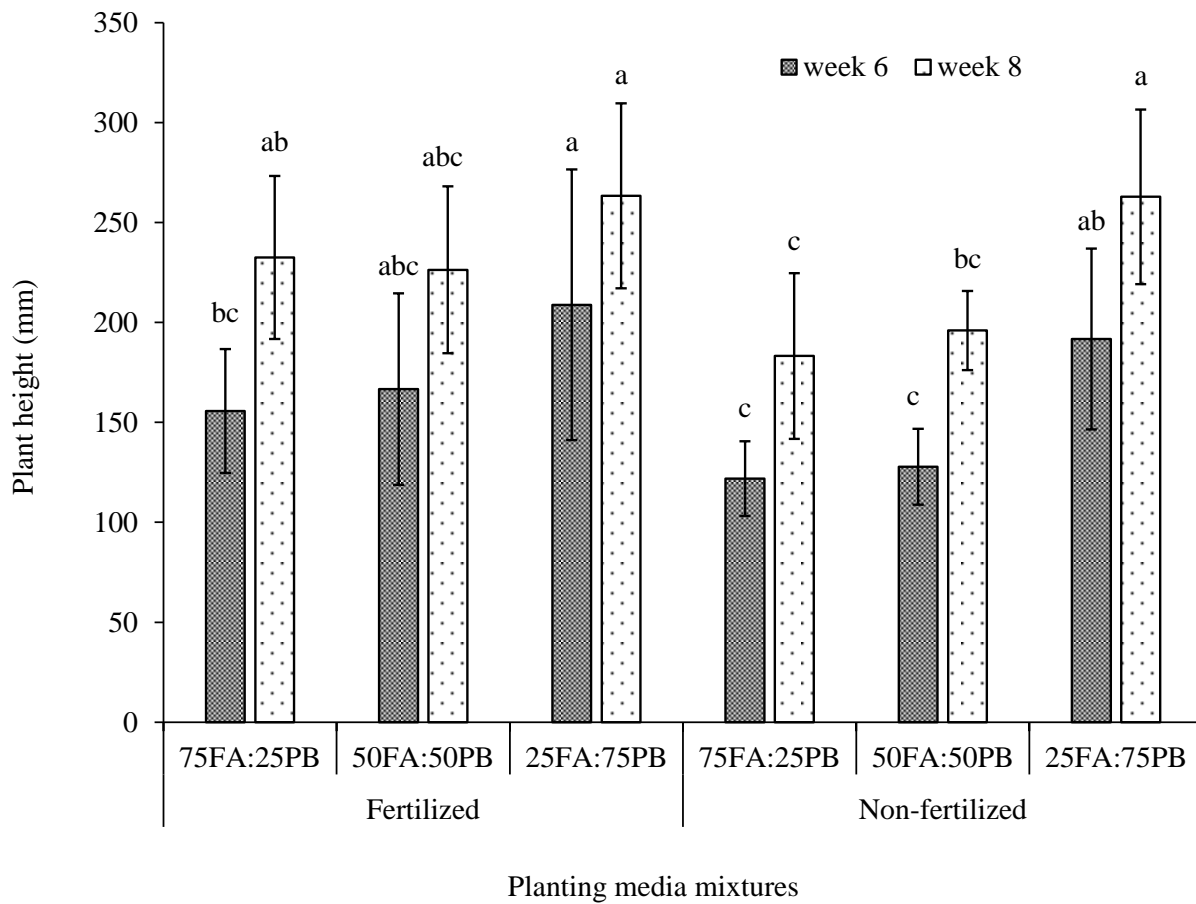
#### **7.4.4 Effects of fly ash vermicompost substituted pine bark compost on marigold growth and maturity**

In this second part of the experiment involving growth and maturity, only the three treatments 75% FA; 50% FA and 25% FA were used based on their performance during the seedling stage. At maturity, all three sources of variation i.e. media type, fertilizer application and time all resulted in significant difference ( $P < 0.05$ ) on plant height, number of buds, number of flowers and leaf area as shown in Table 7.4. Across all measured parameters, planting media effects followed the order 25% FA > 50% FA > 75% FA, with fertilizer application having significantly higher results compared to no fertilizer. It was also important to note that there was no significant interactions between media type  $\times$  fertilizer application; media type  $\times$  fertilizer application  $\times$  time on all measured parameters. However, only significant interaction effects of fertilizer  $\times$  time were observed on number of buds and number of flowers only (Table 7.5).

**Table 7. 5:** Analysis of variance (completely randomized design) for effects of media type, fertilizer application and time on various growth parameters measured at marigold plant maturity.

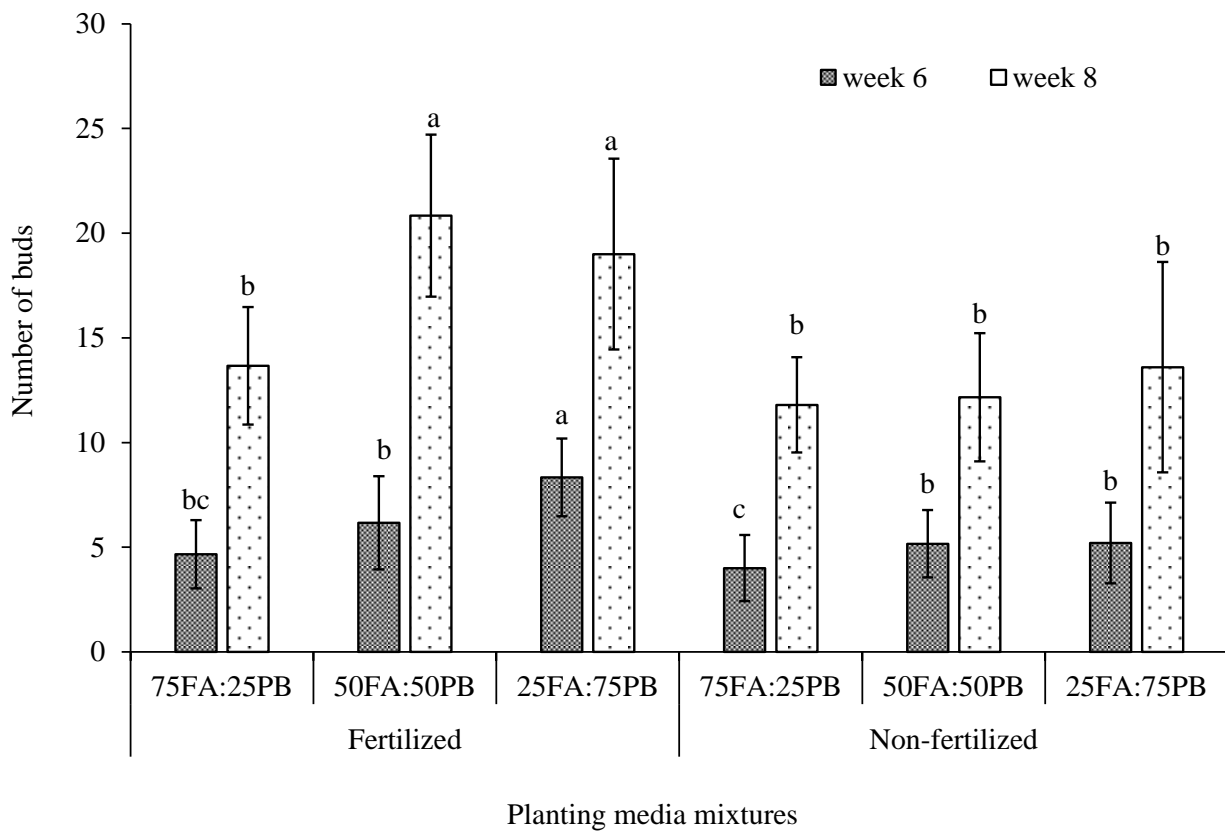
		Plant height	Number of buds	Number of flowers	Leaf area
<b>Source of variation</b>					
Media (M)	<i>F</i>	17.83	9.72	3.74	76.99
	<i>P</i>	< 0.0001	0.0002	0.0299	< 0.0001
Fertilization (F)	<i>F</i>	10.40	31.60	14.01	14.09
	<i>P</i>	0.0021	< 0.0001	0.0004	0.0038
Time (T)	<i>F</i>	55.20	233.76	88.63	-
	<i>P</i>	< 0.0001	< 0.0001	< 0.0001	-
M × F	<i>F</i>	1.28	2.47	0.04	2.08
	<i>P</i>	ns	ns	ns	ns
M × T	<i>F</i>	0.05	0.93	2.17	-
	<i>P</i>	ns	ns	ns	-
F × T	<i>F</i>	0.03	7.56	10.82	-
	<i>P</i>	ns	0.0081	0.0018	-
M × T × F	<i>F</i>	0.30	2.87	0.17	-
	<i>P</i>	ns	ns	ns	-

On average, with or without fertilization, marigold plants grown in the 25% FA media had 33.7% and 29.3% higher plant height compared to the 75% FA and 50% FA, respectively (Figure 7.4). Regardless of media type, application of fertilizer to the fly ash substituted treatments resulted in a 15.7 difference in plant height compared to the non-fertilized treatments (Figure 7.4).

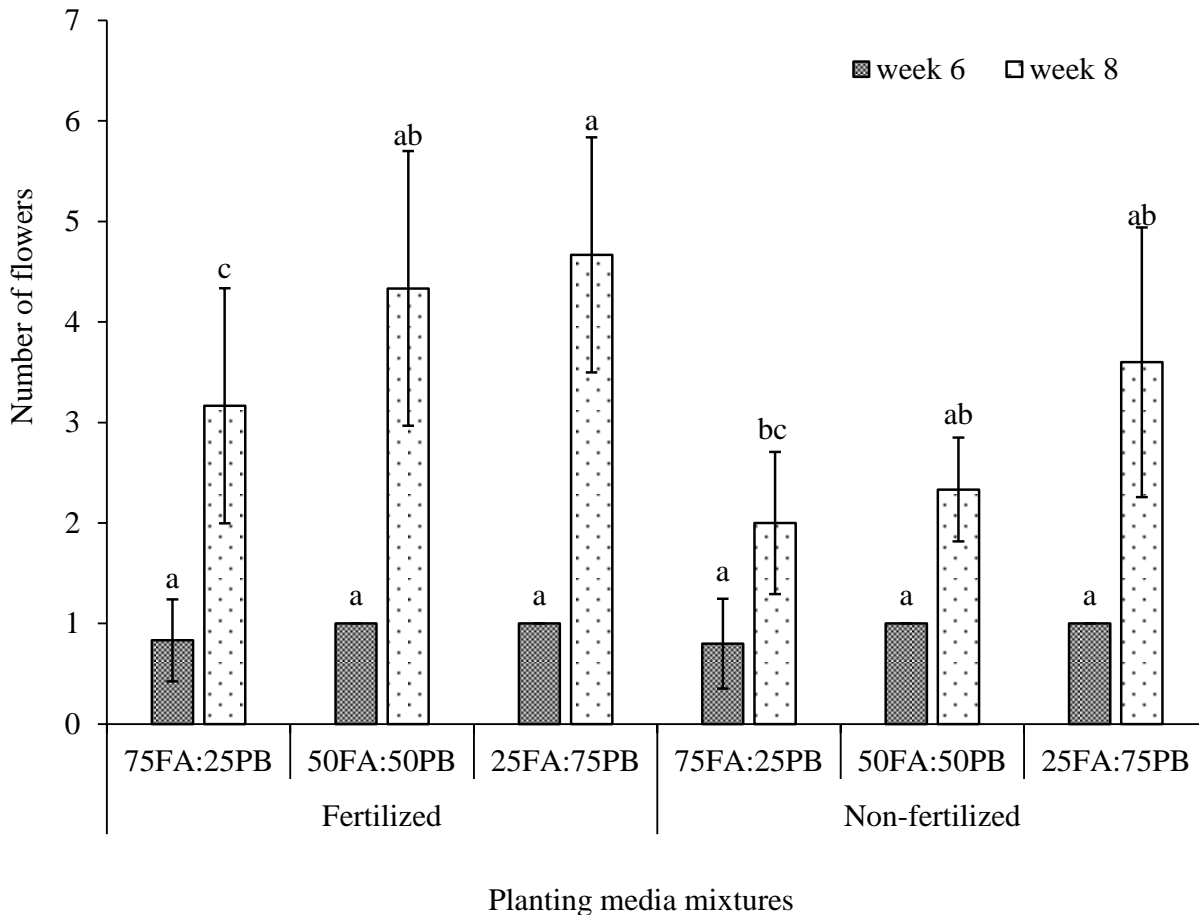


**Figure 7. 4:** Mean heights of the marigold plants grown in the resultant planting media from pine bark compost substitution with fly ash vermicompost. Error bars represent standard deviation.

A trend similar to that of plant height was observed for the number of buds and subsequent flower development, with the 25% FA media having the highest number of buds and flowers, with or without fertilizer application (Figures 7.5 and 7.6). No significant differences ( $P < 0.05$ ) were observed between treatments on number of flowers only at 6 weeks for all treatments with or without fertilizer. For number of buds and flowers at 8 weeks with fertilizer application, no significant differences were observed between the 25% FA and 50% FA treatments.



**Figure 7. 5:** Mean number of buds of the marigold plants grown in the resultant planting media from pine bark compost substitution with fly ash vermicompost. Error bars represent standard deviation.

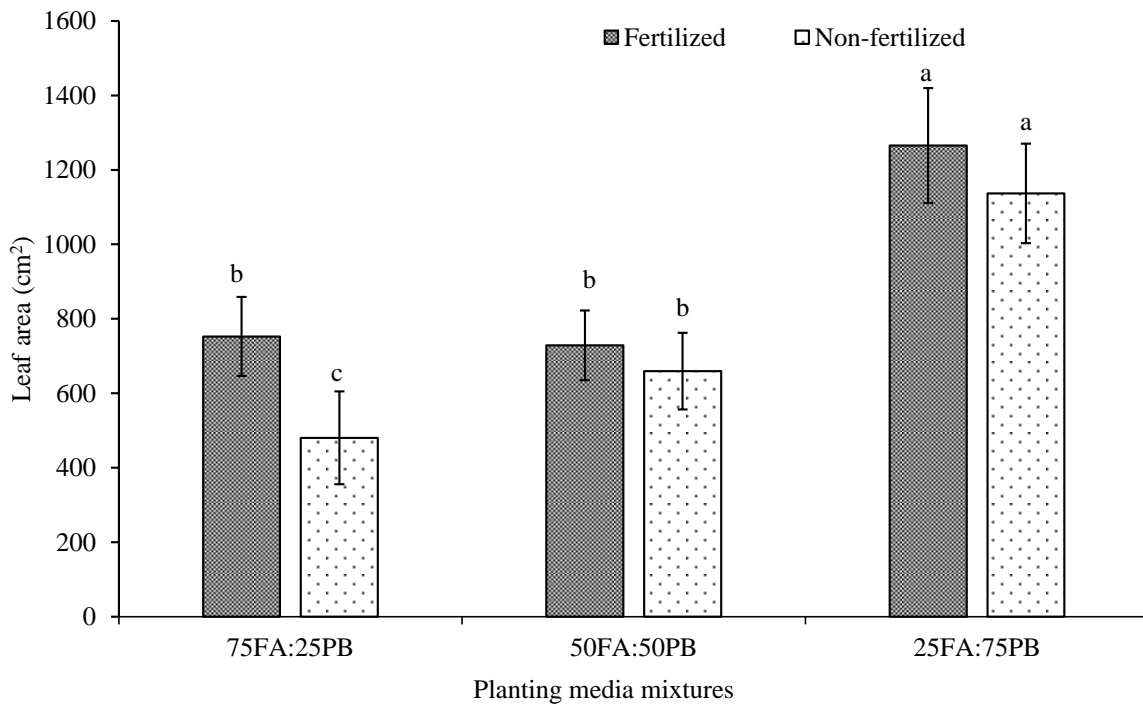


**Figure 7. 6:** Mean number of flowers of the marigold plants grown in the resultant planting media from pine bark compost substitution with fly ash vermicompost. Error bars represent standard deviation.

With fertilizer application at 8 weeks, the 25% FA treatment resulted in a non-significant 7% difference in flower numbers compared to the 50% FA treatment, whilst it resulted in a significant 47.4% difference in number of flowers when compared to the 75% FA treatment. However, without fertilizer application, the effects were more significant with the 25% FA treatment having a significantly ( $P < 0.05$ ) 54.3% and 80% difference in flower numbers

compared to the 50% FA and 75% FA treatments, respectively. Across all treatments, time also significantly affected the number of buds and flowers with counts at 8 weeks having 259.4% and 177% more flowers and buds compared to counts at 6 weeks.

Leaf area at maturity was also significantly affected by both media type and fertilization (Table 7.4). The 25% FA treatment also resulted in the highest leaf area which was significantly different from the 50% FA and 75% FA treatments (Figure 7.7). However, for the 25% FA and 50% FA treatments, no significant differences were observed between fertilized and non-fertilized treatments.



**Figure 7. 7:** Mean leaf area of the marigold plants grown in the resultant planting media from pine bark compost substitution with fly ash vermicompost at eight weeks. Error bars represent standard deviation.

#### **7.4.5 Total nutrient content in marigold plant tissue at maturity**

At maturity, media type resulted in significant differences in all elements measured (P, Na, Mg and N) except for K and Ca (Table 7.6). On the other hand, fertilizer application resulted in significant differences between treatments on other elements except for Na and Ca. Of the three essential macro elements (N P and K), substitution of fly ash at 25% into pine bark compost resulted in significantly higher concentrations of phosphorus and nitrogen, with or without fertilizer. However, for potassium, the 50% FA treatment resulted in a non-significantly higher K concentration in plant tissues with or without fertilizer. As the level of fly ash vermicompost substitution increased from 25% to 75%, the total carbon increased whilst the total nitrogen decreased in plant tissues, with or without fertilization. The 25% FA treatment with fertilizer application resulted in 13.9% and 23.6% difference in N content in plant tissues, whilst without fertilizer application it resulted in a higher 49.6% and 75.1% difference in N content in marigold plant tissues compared to the 50% FA and 75% FA treatments, respectively. A similar trend was also observed on phosphorus with the 25% FA treatment resulting in an average 49.7% and 95.8% difference in plant P tissue content compared to the 50% FA and 75% FA treatments, respectively (Table 7.7). There was also an interaction that was observed between media type and fertilization on the plants ability to absorb P, Ca and N (Table 7.6).

**Table 7. 6:** Concentration of selected elements in marigold plant digests at following growth in fly ash vermicompost substituted media for 8 weeks.

<b>Total elements</b>							
<b>Planting media mixture</b>	<b>P</b>	<b>Na</b>	<b>Ca</b>	<b>Mg</b>	<b>K</b>	<b>N</b>	
	<b>g/kg</b>						<b>%</b>
<b>Fertilized</b>	75FA: 25PB	5.92 b	0.60 b	4.65 a	5.76 a	36.12 b	5.05 c
	50FA : 50PB	6.77 b	1.37 a	4.08 a	5.83 a	43.58 a	5.48 b
	25FA : 75PB	8.16 a	0.11 ab	4.22 a	4.08 b	41.19 ab	6.24 a
<b>Non-fertilized</b>	75FA: 25PB	2.53 c	0.84a	3.86 a	4.81 a	31.45 a	2.41 c
	50FA : 50PB	3.59 b	0.82 a	4.84 a	4.39 a	35.01 a	2.82 b
	25FA : 75PB	6.42 a	1.16 a	4.96 a	4.37 a	30.49 a	4.22 a
<b>ANOVA</b>							
Media (T)	***	0.041	ns	0.0001	ns	***	***
Fertilizer (F)	***	ns	ns	0.0004	0.0007	***	***
T x F	0.0004	ns	0.048	0.0009	ns	0.0020	

## **7.5 DISCUSSION**

### **7.5.1 Influence of fly ash vermicompost substitution into pine bark compost on marigold seedling germination and growth**

The ability to adequately supply water and oxygen are considered as one of the most important physical properties of a planting medium in order to achieve optimum plant growth (Raviv et al. 2002). The physical properties determined in this experiment all affect the media ability to supply water and allow free gaseous exchange. Bulk density, which measures the dry mass per unit volume, was highest in the fly ash vermicompost compared to the pine bark compost. Thus, upon substitution of pine bark compost with fly ash vermicompost, the bulk density of the resultant media mixtures increased which also resulted in changes in macro and micro pore distribution whilst increasing water holding capacity. Atiyeh et al (2001) reported similar results when a commercial planting medium made from vermiculite, Canadian sphagnum peat moss, bark ash and sand was substituted with pig manure vermicompost. An optimized planting medium should not have too high bulk density as this reduces porosity and increases transport cost of the media whilst very low bulk densities have the risk of too much aeration and reduced water holding properties (Atiyeh et al. 2001). According to Edwards et al. (2011) an ideal growing medium for plants should have minimum total porosity of 85%, water holding capacity between 55 – 75% and air filled porosity of between 20 and 30 %.

In this study, the 100% pine bark compost had water holding capacity and air filled porosity outside the recommended range. These results demonstrate that, though amendment with fly ash vermicompost can result in reduced total porosity and air-filled porosity, as observed in the 75% FA substituted medium, the water supply of a medium greatly influences germination of the planted crop. A germinating seed requires mostly water and dissolved

oxygen in the water can be sufficient for the respiration of the germinating seeds. This could explain why the 75% FA, 50% FA and 25% FA substituted media showed no significant differences in germination, which was all above the critical level of 90% (Mupondi et al. 2014). These results are in agreement with several researchers that addition of vermicompost to commercial growing media increases germination (Atiyeh et al. 2000b; Arancon et al, 2008). With regard to media chemistry, pH seemed to play a crucial role in influencing germination, with the highly alkaline pH of the 100% fly ash vermicompost negatively affecting marigold seedling germination, even though it had high water holding capacity. As the marigold seedlings grew, the effects of the various planting media mixtures on growth measured as plant height were more pronounced at 4 weeks than at 2 weeks. This could be likely due to the development of a more effective rooting system to absorb nutrients and photosynthetic system by the crop, which is affected by nutrients such as phosphorus and nitrogen (Arancon et al. 2008).

The pH of the 100% PB and FA may have also significantly affected plant nutrient availability, hence affecting growth. At low pH as observed in the 100% PB, the enhanced mobility of toxic metals has been observed whilst at high pH as observed in the 100% FA, the mobility of essential nutrients is greatly limited, thus creating a toxic or highly nutrient deficit atmosphere for the crop (Brady and Weil 2008). Apart from the pH and nutrient availability, the poor physical properties (porosity and water holding capacity) in the 100% FA and PB mixtures, could have also contributed to the poor seedling growth determined as plant height and leaf area (Atiyeh et al. 2001). Substitution of pine bark compost with fly ash vermicompost also resulted in significant increase in tissue nutrient concentrations. This could be attributed to the increase in water holding capacity and reduction in total porosity

which facilitates adsorption of essential plant nutrients and photosynthetic growth (Ray et al. 2012).

### **7.5.2 Influence of fly ash vermicompost substitution into pine bark compost on marigold growth and maturity**

During the maturity stage of the marigold, as the ratio of fly ash vermicompost increased from 25% to 75%, the plant height significantly decreased. This could be attributed to the increase in pH which consequently affects nutrient mobility in the medium, thus reducing nutrient supply to the growing plant. At 75% FA substitution, the reduced porosity and higher bulk density which greatly affect root growth and proliferation could have contributed to the further reduction in the growth of the plant. Similar results were reported by Atiyeh et al. (2001), in which substitution of a commercial planting medium by up to 25% resulted in the highest shoot growth. Gupta et al. (2014) also attributed this enhanced growth at around 20% vermicompost incorporation to the slow, optimized release of nutrients together with growth hormones which altogether improve plant growth.

A similar trend was observed on the number of buds and flowers and leaf area, which represents the ornamental potential and value of the flower. Incorporation of fly ash vermicompost at 25% again proved superior in improving number of buds, number of flowers and leaf area, compared to the 50% and 75% FA treatments. It was interesting to note that despite the higher concentration of N, P and K in the 50% FA medium compared to the 25% FA, the 25% FA medium still gave the best results. The marigold plants grown in the 25% FA medium seemed to have an optimum supply of essential nutrients, together with other essential growth promoters not investigated in this study. The ability of the plants grown in the 25% FA mixture to grow better was even reflected in the final tissue nutrient content in which the 25% FA showed the highest ability to supply the plant with essential nutrients, particularly phosphorus and nitrogen. Apart from nutrients, it also seems probable that there

are other factors like humic acids, enzymatic activity and presence of beneficial microorganisms which appear to be balanced at 25% substitution ratio, thus resulting in the enhanced growth (Arancon et al. 2003; Gupta et al. 2014). Vermicomposts originating from animal manures and waste paper have been observed to contain large amounts of humic substances, which have been observed to result in growth hormone like behavior (Atiyeh et al. 2002; Bachman and Metzger 2008). However, as the levels of vermicompost increases, the humic acids may exceed a critical value resulting in increased adsorption of cationic nutrients under the high levels of humic colloids, which reduced plant growth even under higher nutrient content as observed (Atiyeh et al. 2002). This may explain the higher marigold growth observed in the 25% FA medium compared to the other treatments, despite their higher nutrient content, making the present results consistent with other researchers (Atiyeh et al. 2000b; Atiyeh et al. 2001; Bachman and Metzger 2008).

## **7.6 CONCLUSION**

Substitution of pine bark compost with fly ash vermicompost, despite its alkaline nature, significantly increased marigold seedling germination and growth. For effective seedling growth, substitution of pine bark compost with 50% fly ash vermicompost proved superior to all treatments. Application of fertilizer to this treatment also showed that it may be possible to reduce the amount of time required for seedlings to reach their transplanting stage, which can save time and resources. For optimum marigold growth and maturity, substitution of pine compost with 25% fly ash vermicompost gave the best results regardless of the nutrition status of the growing media. Lastly, addition of water soluble fertilizer seemed to optimize the growth, development and maturity of ornamental marigolds, giving bigger plants, with greater number of flowers compared to no fertilization.

## **CHAPTER EIGHT**

### **8.0 GENERAL DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS FOR FUTURE STUDIES**

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## 8.1 GENERAL DISCUSSION

In recent years, coal fly ash has become a major solid waste material whose generation is likely to increase especially in developing countries, whose industrialization drive has greatly increased electricity demand. Due to the bulkiness of fly ash, it is usually deposited at sites near power stations, creating a solid waste management challenge (Mupambwa et al. 2015). This is compounded by the presence of a wide range of nutrient elements in fly ash, which originate from the coal, making fly ash a potential metalloid source in the environment. The presence of potentially harmful elements like Cr, Cd, Pb, Ni and others in fly ash has seen it being categorised as an environmental pollutant. Though fly ash contains potentially beneficial nutrient elements like phosphorus, potassium and other micro-elements required for plant growth, the mixture of these beneficial elements with toxic metalloids has overshadowed research on potential benefits of fly ash in agriculture. The other major limitation to fly ash utilization in agriculture has been the very low bioavailability of the essential plant elements in fly ash, though these exist in very high total concentrations (Bhattacharya and Chattopadhyay 2002). Several researchers have shown that the majority of the potentially toxic metalloids in fly ash exist in very low concentrations which are not toxic to plants when fly ash is applied at low rates in soils (Nayak et al. 2014; Pandey et al. 2009; Matsi and Keramidias 1999).

Improving the bioavailability of essential nutrients like phosphorus in fly ash can create an effective opportunity for the management of this solid waste material subsequently creating a cheaper plant nutrient source. This study was undertaken to evaluate the potential phosphorus benefits and metalloid release characteristics when selected alkaline South African fly ashes are used as a liming material in acidic soils. To enhance the nutrient content in fly ash, vermicomposting was suggested as an effective strategy though research on optimization of

this vermicomposting in reference to fly ash is limited. This study thus looked at optimizing the vermicomposting strategy, using *Eisenia fetida* (red wiggler), of fly ash and the potential of inoculating fly ash vermicomposts with microbial cocktails for enhanced biodegradation and nutrient release in fly ash based vermicomposts.

In Chapter 3 of this study, the various chemical properties of eight South African fly ashes were initially characterised together with the potential phosphorus and heavy metal release in an incubation study. The results showed highly significant differences of chemical properties between all the fly ash samples, with all the fly ashes being highly alkaline, confirming the view that these fly ashes are derived from mainly bituminous coals (Gitari et al. 2009). Across all eight fly ash samples, the average phosphorus content was 939.0 mg/kg; of which only 21.3% of this phosphorus was bioavailable for plant uptake, creating an opportunity of technologies like vermicomposting in improving this low nutrient availability (Mupambwa et al. 2015). Subsequently, two of the fly ashes were applied as a liming material in three different acid soils of South Africa with an average pH of 4.9. It was noteworthy that the soil pH of the soils significantly improved following fly ash application to give an average pH of 6.7 after eight weeks, showing a similar ability to calcium carbonate, to function as a liming material. Though fly ash application increased the soils electrical conductivity (EC), the EC values were within the range acceptable for optimum crop growth (Schuman & Sumner 2000). Heavy metal pollution from direct fly ash application has been the major limitation to direct fly ash application to soils. This study supported the view that most heavy metals in fly ash become immobile especially at pH close to neutral, thus limited metalloid release was observed. Even with several researchers observing similar results to ours, of limited bioavailability of heavy metals in fly ash, the lack of clear guidelines on the maximum permissible heavy metals following direct application of fly ash in soils still limits the exploration of fly ash as a soil amendment (Lemly 2014).

### **8.1.1 Fly ash incorporation ratio and earthworm stocking density on fly ash**

#### **biodegradation and nutrient release**

Vermicomposting is a technology that makes us of epigeic earthworm species like *Eisenia fetida*, to enhance microbial activity and thus increase nutrient mineralization during the composting process (Dominguez 2011). Research has been underway to vermicompost inorganic materials like fly ash or rock phosphate to improve the nutrient release and value of the compost (Bhattacharya and Chattopadhyay 2012; 2004; Mupondi 2010, Unoufin and Mnkeni 2014). Fly ash is devoid of carbon and nitrogen which are lost during the combustion process, so fly ash alone has been shown to support minimal microbial activity, which greatly limits nutrient mineralization even when it is applied directly to soils (Bhattacharya and Chattopadhyay 2006). The mixing of inorganic fly ash with organic materials which act as a source of carbon and nitrogen such as cow dung, food waste poultry manure, has been suggested by several researchers during fly ash vermicomposting (Bhattacharya and Chattopadhyay 2002; Gupta et al. 2005; Venkatesh and Eevera 2008; Ram and Masto 2014).

The vermicomposting process has been observed to be a synergistic – symbiotic balanced relationship between earthworms and micro-organisms, in which the balance mainly between carbon and nitrogen plays a critical role in determining the effectiveness of vermicomposting (Ndegwa and Thompson 2000; Dominguez 2011). In a review, Mupambwa et al. (2015) observed that the majority of research on fly ash incorporation during vermicomposting had been done using organic material in which the C: N ratio was not optimised for effective vermicomposting. One material that can be used to adjust the C: N ratio in animal manures is waste paper, which creates another recycling opportunity for the waste paper, which would otherwise be incinerated contributing greenhouse gases. In Chapter 4 of this study, cow dung was adjusted using waste paper to achieve an optimized C: N ratio of 30 for effective vermicomposting, and then fly ash was incorporated at various ratios.

It was noteworthy that the presence of earthworms (*E. fetida*) resulted in a significant enhancement of the composting process, resulting in faster decrease in C: N ratio, and higher humification within the compost. According to Dominguez (2011), earthworms are the crucial drivers of the vermicomposting process, where they are directly involved in fragmentation of organic matter thereby increasing the surface area for enzyme and microbe activity. This study demonstrated the ability of earthworms to enhance organic matter decomposition even under higher levels of highly alkaline fly ash. Incorporation of fly ash up to a ratio of 1: 1 into cow dung waste paper mixtures, with earthworm presence, significantly increased the Olsen extractable P by an average 35%, within the vermicompost. This is far higher than increases reported by Bhattacharya and Chattopadhyay (2002), indicating the effectiveness of vermicomposting at the same incorporation ratio but using a more balanced (C: N) cow dung waste paper mixture as used in this study. Incorporation of fly ash into cow dung waste paper with earthworm presence also resulted in significantly higher humification parameters much more than the cow dung waste paper alone, and this could be due to the influence of the phosphorus, emanating from the fly ash, on earthworm activity (Unuofin and Mkeni). The fly ash incorporation study in Chapter 4 identified the 1: 2 fly ash incorporation ratio as most appropriate for fly ash vermicomposting, contrary to reports by Bhattacharya and Chattopadhyay (2002) and Gupta et al. (2005); of 1:1. The incorporation ratio of 1: 2 resulted in the highest polymerisation index, coupled with the highest release of extractable phosphorus.

Earthworms have been identified to allocate resources either to growth only or both growth and reproduction, depending on the availability of resources, particularly carbon, nitrogen and phosphorus (Aira et al. 2010). However, both growth and reproduction of earthworms are important during growth for the degradation and mineralization processes, especially during vermicomposting of inorganic materials. The other aspect of this study was to then identify

the most appropriate earthworm stocking density in the optimised fly ash – cow dung – waste paper mixtures, as a way of further enhancing both compost degradation and nutritional benefits of fly ash inclusion during composting.

Several researchers have used laboratory based experience in choosing *E. fetida* stocking density during vermicomposting, thus resulting in the use of non-specific, non-optimized stocking density (Bhattacharya et al. 2012; Gupta et al. 2005). In Chapter 5 of this study, various stocking densities were evaluated using the 1:2 fly ash incorporation ratio, based on research done by Unuofin and Mkeni (2014). It was observed that even at lower stocking densities of 12.5 g – worm/ kg material, fly ash vermicompost showed accelerated organic matter losses compared to 0 g – worms/kg, based on changes in C: N ratio and humification properties. This could be explained by very high initial microbial biomass observed where earthworms were included, which enhances organic matter degradation in the first few weeks of vermicomposting consistent with the findings of Gomez-Brandon et al. (2011). Doubling the stocking density from 12.5 to 25 g-worm/kg, however, resulted in a highly significant increase in humification parameters, which was not the case when stocking density was increased beyond 25 g-worm/kg. It was also noteworthy that, for higher phosphorus and inorganic nitrogen release, increase in stocking density significantly influenced mineralization, compared to maturity parameters. This could mean that the organic matter decomposition is mainly associated with the gut associated process which is less sensitive to stocking density, whilst nutrient mineralization is associated with the cast associated processes, which are more dependent on the earthworm activity (Dominguez 2011). A stocking density of 25 g-worm/kg seemed most appropriate to achieve a balance in attaining faster organic matter decomposition and nutrient mineralization in fly ash based vermicomposts.

### **8.1.2 Inoculation of fly ash vermicomposts optimised for incorporation ratio and earthworm stocking density, with Effective micro-organisms**

Earthworms have been shown to play the major role in vermicomposting by increasing surface area and enhancing microbial activity, thus researchers have evaluated the potential of deliberately inoculating composts with specific microbes, with the intention of enhancing biodegradation. In Chapter 6, we used the optimised fly ash incorporation ratio of 1:2 and stocking density of 25 g-worm/kg, to evaluate the potential of using a special group of microbes, known as Effective micro-organisms (EM), in enhancing biodegradation and nutrient release in fly ash vermicomposts. Anecdotal evidence support that inclusion of EM during composting with or without earthworms, resulted in a significant reduction in composting period due to enhanced organic matter loss (Freitag 2000). Though inclusion of EM during vermicomposting of fly ash with *E. fetida* resulted in higher maturation parameters, these were not significantly different from those observed from where there were *E. fetida* alone. It was also noted that EM alone performed non-significantly different from the control with no earthworms. Similar results were reported by Mupondi et al. (2006a) using EM to inoculate pine bark – goat manure composts with no earthworms. The degradation of organic matter measured as change in C: N ratio and dissolved organic matter showed significantly high correlation with  $\beta$ - glucosidase enzyme activity. However, changes in Olsen P were significantly correlated to the total phosphate solubilizing bacteria and not alkaline phosphatase enzyme activity, possibly highlighting the predominance of microbial and organic acids activity in phosphorus mineralization in fly ash vermicompost. Incorporation of EM seemed to have marginal benefits during vermicomposting, and higher inoculation rates or optimized rates for inorganic wastes like fly ash, may need to be considered for significantly different results as recommended by Nair and Okamitsu (2010).

### **8.1.3 Potential of fly ash vermicompost in improving pine bark media physical and chemical properties**

The ultimate goal of the vermicomposting process is to create a compost with potential to improve the nutrient value of traditional potting media, thus creating an artificial soil which can be used in land reclamation or horticultural crop production. Pine bark, the official planting media used in the horticultural industry has been observed to have poor physical properties and nutrient content (Mphaphuli et al. 2005; Mupondi et al. 2006b). Several researchers have observed that vermicomposted material have higher bulk densities and nutrient content, which can be used to improve the major physical properties of traditional planting media (Atiyeh et al. 2000; 2001; Gupta et al. 2014). In Chapter 7 of this study, using the vermicompost from Chapter 4, fly ash vermicompost showed high potential in improving pine bark compost physical and chemical properties when incorporated up to 75%, as evidenced by the high germination percentage and faster plant growth. However, for optimum seedling germination and growth, an inclusion ratio of 50% proved superior whilst for plant growth and maturity, an inclusion ratio of 25% fly ash was superior. Seedling germination and growth have been observed to be greatly influenced by the media's ability to provide water and nutrients only, whilst at growth and maturity, the humic acids also play a critical role by acting as plant growth promoters (Atiyeh et al. 2001). This could explain the differences in optimum inclusion ratios suitable for seedling growth and that of plant growth and maturity. Though fly ash vermicompost without fertilizer application can result in high germination and subsequent growth, with incorporation of fertilizer, a seedling nursery is likely to generate faster maturing seedlings and ultimately bigger plants.

## 8.2 GENERAL CONCLUSIONS

With the major objectives of the study highlighted in the previous section, the findings of this study can conclude that:

1. Though originating from a mostly similar coal type, South African fly ashes vary significantly in elemental concentrations and characterization is essential for customized recommendations to guide utilization in agriculture. However, the fly ashes evaluated here had high total phosphorus, but only a limited percentage is potentially bioavailable.
2. The application of the fly ashes evaluated here as acidic soil ameliorants based on the soils lime requirements does not pose any potential salinity challenge and can provide a cheap liming source and a source of essential phosphorus with minimum risk to soil contamination from metal species. However, changes in pH over time following fly ash application need to be monitored together with the subsequent metalloid release. Technologies that improve the phosphorus bioavailability in fly ash, like vermicomposting, are recommended as they will greatly reduce the amount of fly ash required to enhance the P status of a soil, thus reducing the heavy metal risk.
3. Incorporation of fly ash (FA) into cow dung – waste paper mixtures (CP) at a ratio of 1:2 (FA: CP); during vermicomposting with *E. fetida*, will result in effective biodegradation and phosphorus release. Earthworms like *E. fetida* play a crucial role during vermicomposting of inorganic material like fly ash, compared to normal traditional composting.
4. Biodegradation of fly ash based vermicomposts measured as changes in C: N ratio and humification parameters can be achieved at lower stocking densities of 12.5 g-worm/kg, giving significantly different results compared to where no earthworms

were added. However, vermicomposting fly ash – cow dung – waste paper mixtures at stocking densities of 25 g-worms/kg and above is likely to give a highly matured compost, within a shorter period compared to 12.5 g- worms/kg. A stocking density of 25 g – worm/kg and above also seemed more appropriate for effective nutrient release in fly ash vermicompost. The enzymes  $\beta$  – Glucosidase and FDA are related to microbial biomass and any activity that increases microbial biomass during composting is likely to increase their activity.

5. Effective micro-organisms (EM) inoculation alone with no earthworms, to fly ash based composts results in very slow organic matter degradation with no significant differences compared to where no EM has been added. However, coupled with *E. fetida*, EM inoculation may enhance degradation and nutrient mineralization in fly ash based vermicomposts, but either higher rates or optimized rates specific for fly ash vermicomposts may need to be determined for significant effect of EM during vermicomposting.
6. Fly ash vermicompost (1: 2 FA: CP) can be effective in improving both physical and chemical properties of pine bark compost for more efficient seedling germination and growth. For effective seedling germination, substitution of pine bark with 50% fly ash vermicompost is most ideal, whilst for subsequent seedling growth and maturity, a substitution rate of 25 % with fly ash vermicompost is most ideal. Application of fertilizer to these substituted treatments can result in faster seedling development and higher flower counts on bigger plants, which can increase the profits of seedling growers; compared to where no fertilizer is added in the same combination.

### 8.3 RECCOMENDATIONS FOR FUTURE STUDIES

Since this study was not exhaustive, and based on the present conclusions, the following recommendations are made for future studies.

1. In this study, maturation of composts seemed to be achieved earlier whilst nutrient mineralization seemed to continue beyond the experimental periods. It would be interesting to evaluate the potential maximum mineralizable phosphorus during fly ash vermicomposting by allowing more vermicomposting time even after maturation seemed to have been achieved.
2. Instead of using cow dung, other animal manures like pig and chicken manure may be considered during fly ash vermicomposting with the possibility of creating vermicompost richer in both nitrogen and phosphorus.
3. Optimization of EM inoculation rate may need to be established for effective contribution during fly ash vermicomposting.
4. Instead of EM whose effects are not significantly effective, the use of special inoculants with known targeted microbes like phosphate solubilizing bacteria and nitrifying bacteria may be considered.
5. The heavy metal contribution and bioaccumulation of fly ash and *E. fetida* respectively, needs to be established during fly ash vermicomposting.
6. The fertilizer value of the leachate from fly ash vermicompost may need to be established.
7. Fly ash vermicomposts tend to be alkaline in nature, if alkaline fly ash is used, and it may be interesting to evaluate the possibility of using a mixture of sulphur containing waste mixed with fly ash to neutralize the alkalinity following the vermicomposting process.

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## Elemental composition and release characteristics of some South African fly ashes and their potential for land application

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Eight fly ash samples collected from South African power stations were evaluated for various chemical properties, liming potential and metal species release under incubation. All fly ashes had alkaline pH ranging from 10.97 to 12.75 with much wider variations of electrical conductivity (range 0.46–8.27 dS m<sup>-1</sup>). Their total P content ranged from 553.3 to 1514 mg P kg<sup>-1</sup> and Olsen extractable P from 130 to 345.5 mg P kg<sup>-1</sup>. Application of two of the fly ashes to three different soils showed a high ability to neutralize acidity, resulting in an average of 41% change in pH after 8 weeks of incubation. Across all three soils, the fly ash incorporation increased extractable P content from a P-deficient level to levels above 25 mg P kg<sup>-1</sup> in two of the three soils. Except for Cu, all metal species (Cr, Pb, Ni and Fe) showed significantly ( $P \leq 0.05$ ) low extractability under fly ash treated soils compared to the soil alone control. These results suggest that the South African fly ashes studied are effective liming materials and can provide essential elements such as P with minimum risk of soil contamination from metal species release.

**Keywords:** fly ash; phosphorus release; incubation study; soil contamination; heavy metal species

### Introduction

Throughout the world, the availability of huge coal deposits favour thermal electricity generation as a much cheaper energy option over nuclear or hydro-electricity generation. However, the use of coal combustion for electricity generation results in production of large quantities of fly ash coupled with large quantities of greenhouse gases. Fly ash is the fine airborne solid residue captured from exhausts through electrostatic precipitators during coal combustion and it constitutes approximately 75% of the residues of coal combustion (Haynes 2009). Among the highly industrialized countries like United States, China and India; South Africa ranks highest with more than 95% dependency on coal for energy generation (Seshadri, Bolan, et al. 2010). Coupled with the relatively low calorific value of South African coals, this greatly increases the fly ash residue during coal combustion at power stations (Kruger & Krueger 2005). Globally, fly ash is mainly utilized in the construction industry for road bed material, brick making and cement making with this utilization falling within the 30% range whilst most developing countries only utilize around 5% of the millions of tonnes of fly ash generated each year (Haynes 2009; Gitari et al. 2010). The production of coal combustion products is likely to increase as the generation of energy through coal continues to increase, creating more disposal challenges

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## Optimization of Fly Ash Incorporation into Cow Dung–Waste Paper Mixtures for Enhanced Vermidegradation and Nutrient Release

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### Abstract

This study was conducted to establish an appropriate mixture ratio of fly ash (F) to optimized cow dung–waste paper mixtures (CP) to develop a high-quality vermicompost using earthworms (*Eisenia fetida*). Fly ash was mixed with cow dung–waste paper mixtures at ratios of (F:CP) 1:1, 1:2, 1:3, 2:1, and 3:1 or CP alone and composted for 14 wk. Olsen P, inorganic N ( $\text{NO}_3^-$ ,  $\text{NO}_2^-$ , and  $\text{NH}_4^+$ ), C:N ratio, ash content, microbial biomass C, and humification parameters were measured together with scanning electron micrograph images to determine compost maturity. Based on C:N ratio, the extent of vermicompost maturity of the waste mixtures followed the decreasing order (F:CP) of 1:3 > 1:2 > 1:1 > CP alone > 2:1 > 3:1. Similarly, Olsen P was significantly higher ( $P < 0.05$ ) where earthworms were added. The mean percentage increase in extractable P was in the order CP alone > 1:2 > 1:3 > 1:1 > 2:1 > 3:1, with earthworm addition almost doubling P release across the 1:1, 1:2, and CP alone treatments. Fly ash incorporation enhanced conversion of organic N to the plant-available inorganic forms, with the 1:3 treatment resulting in the highest conversion. Scanning electron micrograph images confirmed the extent of vermicompost maturity reflected by the various humification parameters determined. Fly ash incorporation at the 1:2 ratio proved to be the most appropriate because it allows processing of more fly ash while giving a vermicompost with desirable maturity and nutritional properties.

Coal-fired power stations provide more than 90% of the total electricity generated in South Africa. This trend is likely to increase, as evidenced by the construction of two new high-capacity powered electricity generating stations (the 4700 MW Medupi and 4800 MW Kusile) (Eberhard, 2011). Coal combustion generates large quantities of greenhouse gases together with fly ash. Fly ash, which is the powdery particulate material collected from flue gasses by electrostatic or mechanical devices, forms more than 70% of the solid residue during coal combustion at power stations (Haynes, 2009). Of the more than 28 million t of fly ash generated in South Africa, only around 5% is used in the construction industry, with the rest being disposed of at sites near the power stations.

Fly ash has potential to supply essential plant nutrients in agriculture, as evidenced by the wide range of essential nutrients in fly ash (e.g., P, K, Mg, Ca, and S). Although the levels of total nutrient content of nutrients like P in fly ash are high, the major limitation of direct fly ash utilization in agriculture is its very low plant availability (Bhattacharya and Chattopadhyay, 2006; Basu et al., 2009). This is aggravated by the low microbial activity in fly ash, which further limits mineralization even when fly ash is applied directly to soil (Bhattacharya and Chattopadhyay, 2006). Against this background, several researchers have evaluated the potential of using earthworms during composting to improve the nutrient release in fly ash, thus improving its fertilizer value.

Bhattacharya and Chattopadhyay (2002) evaluated the potential of *Eisenia fetida* in improving the P levels when fly ash was incorporated in cow dung at 25, 50, and 75% levels. The earthworms proved superior in increasing the extractable P levels and P-utilizing bacteria even though the C:N ratio and earthworm stocking density were not optimized for effective vermicomposting. In other studies, the incorporation ratio of fly ash to cow dung has been evaluated, and recommendations have been made after composting under varying C:N ratios of the total material mix, stocking density, and moisture content (Gupta et al., 2005; Bhattacharya and Chattopadhyay, 2006; Ananthakrishnasamy et al., 2009). These parameters have been shown to greatly influence bioconversion effectiveness and nutrient release during vermicomposting of organic and

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Abbreviations: CP, cow dung–waste paper mixtures; F, fly ash; HI, humification index; HR, humification ratio; PI, polymerization index; SEM, scanning electron microscopy.

