UNIVERSITY OF CAPE COAST

USING BIOCHAR AND POULTRY MANURE TO IMPROVE THE FERTILITY OF A HIGHLY WEATHERED TROPICAL SOIL AND YIELD OF LETTUCE

CHRISTIAN ADLER PHARES

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BY

CHRISTIAN ADLER PHARES

THESIS SUBMITTED TO THE DEPARTMENT OF SOIL SCIENCE OF THE SCHOOL OF AGRICULTURE, COLLEGE OF AGRICULTURE AND NATURAL SCIENCES, UNIVERSITY OF CAPE COAST, IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF DOCTOR OF PHILOSOPHY DEGREE IN SOIL SCIENCE

JUNE, 2016

DECLARATION

Candidate's Declaration

I hereby declare that this thesis is the result of my own original research and that no part of it has been presented for another degree in this university or elsewhere.

Candidate's Signature:..... Date: 25/01/17

Name: Christian Adler Phares

Supervisors' Declaration

We hereby declare that the preparation and presentation of the thesis were supervised in accordance with the guidelines on supervision of thesis laid down by the University of Cape Coast.

Principal Supervisor's Signature:..... Date: 26-01-2017

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Name: Dr. Sheila Tagoe

ABSTRACT

The study was undertaken to evaluate the effects of biochar and poultry manure in improving the fertility of a strongly weathered soil and the yield of lettuce. In two separate experiments, three rates (0, 39 and 65 t ha^{-1} per 1 kg soil) of biochar (CCB, CHB and PMB) solely or in combination with poultry manure (0 and 10 t ha^{-1}) were incorporated into pots containing 1 kg soil and arranged in completely randomized design. Biochar and or manure effects on SOC, mineral N, AVP, pH, ECEC, MBC, MBN and MBP were evaluated on days (3, 7, 14 28 and 42) and P solubilizing fungi (PSF) and bacteria (PSB) on day 42. All amendments significantly ($P < 0.05$) increased SOC, pH, ECEC, MBC, MBN and MBP for all sampling periods and PSF and PSB on day 42. Unlike PMB, CCB and CHB amended soils showed no significant differences in mineral N compared with the control by day 42. Available P in CHB and PMB amended soil showed significant ($P < 0.05$) increase at both rates but only significant ($P < 0.05$) at 65 t ha^{-1} for CCB treatments. In experiment three, significant increase in yield and shoot NPK were realized from PMB amended soils but insignificant in CCB and CHB treatments. In all, biochar combined with manure was superior in increasing the concentrations of SOC, NH_4^+-N, NO_3^--N, AVP, pH, ECEC, MBC, MBN MBP, PSF, PSB, shoot NPK and yield of lettuce. In experiment two, ten earthworms were exposed to CCB, CHB and PMB at respective rates of 0, 13, 26, 39, 52, 65, 78, 91, 104, 117, 130, 143 and 156 t ha^{-1}. Significantly inverse relationship was found between biochar rates and earthworm survival. It is therefore recommended that combined biochar and manure is adopted for effective restoration of the fertility of strongly weathered soils in Ghana for lettuce production.

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DEDICATION

To my mum and in memory of my father.

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CHAPTER ONE

INTRODUCTION

Background to the Study

The world Food Programme (WFP) estimated that more than one billion people, approximately 14.3 % of the earth's population are hungry. They therefore reiterated that global food production must increase by 70 to 100 % by the year 2050 to adequately meet global food demand (WFP, 2016).

In developing nations, the realization of food security as highlighted in the Millennium Development Goals remains a great challenge. This is particularly critical in sub-Saharan Africa and Asia where populations are rapidly growing but food production is not keeping pace with it, thus leaving millions hungry and malnourished. According to the World Food Programme (WFP), Ghana is classified as a food-deficit country (WFP, 2016). Early on, Ghana's Ministry of Food and Agriculture (MOFA) had reported that 5 % of the population in Ghana, approximately 1.2 million people, are hungry (MOFA, 2010). One of the key reasons attributed to the food deficit is the low productivity of most agricultural soils.

Examples of such soils (Ultisol and Oxisol) are abundant in the humid and perhumid tropics. These soils cover about 10-15 % of the 23.9 million hectares of potentially arable land in Ghana (Owusu-Bennoah et al., 1997). This group of soils are predominant in the Western and some parts of Ashanti, Brong Ahafo and the Eastern Regions of Ghana where most of the staples of the country are produced. The soils are acidic, with low base saturation and commonly have multiple nutrient deficiencies (nitrogen (N), phosphorus (P), potassium (K), calcium (Ca) and Zinc (Zn)) (Owusu-Bennoah et al., 2000).

Crop production on such soils is seriously constrained, particularly in areas where proper management measures have not been put in place. Management practices to correct this anomaly must therefore be geared towards ameliorating acidity, improving water retention, enhancing nutrient availability and retention, and reducing aluminium toxicity to plant roots and soil biota.

Over the years farmers have employed several strategies to combat the low inherent fertility and its associated Al toxicity of highly weathered tropical soils. Haby (2002) noted that a common treatment to reduce the solubility of aluminum (Al) in acidic soils is to increase the soil pH that is mostly achieved through liming. The ability of liming to increase soil pH and decrease Al, and increase crop yield has received much attention (Haby, 2002; Brown et al., 2008). However, the effect of liming on acid soil is reported to be temporal and has to be repeated over the crop growing seasons (Shamsuddin et al., 1998; Thomas et al., 2003) making it an expensive and uneconomical practice for smallholder farmers to adopt. Eghball et al. (2002) also reported that the application of organic wastes such as poultry manure is key to improving the fertility of low fertile soils by increasing the level of soil organic matter, improving soil microbial diversity, nutrient exchange capacity and increase the water holding capacity of soil (Agbede et al., 2008). Inspite of the advantages derived from using poultry manure, it is rapidly mineralized only within a few cropping seasons (Bol et al., 2000) due to high decomposition resulting from increasing temperature and aeration especially in the tropics. Only a small percentage of the mineralised manure is stabilised in the soil in a long term and majority continually released into the atmosphere

as CO_2. Organic amendments therefore need to be repeated yearly to sustain soil productivity. Another disadvantage is that some investigators have reported that when acid soils were incubated with organic materials, soil pH increased early in the incubation, followed by an apparent decrease later in the incubation. This results from nitrification of NH_4^+ ions and release of H^+ during the mineralization of organic N (Anthony & Franzluebbers, 2003). This characteristic of conferring acidity on soil restrains its use on highly weathered soil characterized by extreme acidity and low fertility.

For the past two decades however, soil management practice that has caught the interest of soil scientists is the use of black carbon (C), increasingly referred to as biochar. The interest in the use of biochar to restore fertility of degraded soils can be traced to the restoration and sustainability of Terra Preta soil. This soil was reported to have contained more charcoal, and was more fertile than surrounding soils (Glaser et al., 2001). Then again, biochar has been shown to correct soil acidity and improve soil fertility (Chan et al., 2008; Steiner et al., 2008).

It is therefore suggestive that the use of biochar may help overcome some of the challenges posed by highly weathered tropical soils for crop production and also limitations associated with the sole use of poultry manure for the restoration of the fertility of such degraded soils. However, research findings on the potential impact of biochar with or without poultry manure on soil fertility and crop yield are limiting hence the need for further research to establish and expand the understanding of the behaviour of these amendments when applied to arable soils.

Statement of the Problem

Although biochar has been demonstrated to enhance soil productivity, various reports on the effects of biochar on soil fertility and crop yield are inconsistent. For instance, Lehmann et al. (2006) reported improvement in soil fertility whereas Gundale and Deluca (2007) and Asai et al. (2009) reported an adverse effect on plant growth.

In addition, biochar effects on soil biota have received much less attention and previous researches have also reported contradicting results. Jin (2010) found greater enhancement of microbial abundance by biochar additions in the rhizosphere. However, Graber et al. (2010) reported an inverse relationship between biochar amendment and microbial abundance. Studies on the effects of biochar amendments on microbial activity have reported enhanced (Luo et al., 2013; Zimmerman et al., 2011) or inhibited (Dempster et al., 2012) activity. Other studies have also reported no effects on soil microbial biomass (Castaldi et al., 2011; Zavalloni et al., 2011) with biochar amendments.

Then again, considering the effect of biochar on macrofauna, Topoliantz and Ponge (2005) reported no pronounced effect of biochar on *Pontoscolex corethrurus* earthworm survival, but Liesch et al, (2010) reported genotoxicity of *Eisenia fetida* earthworm at 10 % poultry litter biochar application to soil.

Variations in observations on the effect of biochar on soil properties and plant growth have been attributed to soil type (Asai et al., 2009; Van Zwieten et al., 2010), varying biochar application rates (Lehmann et al., 2003; Major et al., 2010), quality of biochar resulting from feedstock and pyrolysis

conditions (Blackwell et al., 2009; Gaskin et al., 2010). Hence further work is required to standardise biochar effect on soil fertility in terms of type and quantity of biochar required for a specified soil.

Justification for the Study

Ghana is faced with the problem of increasing food production to meet its ever increasing population due to low inherent fertility of most of its arable lands (Ultisol and Oxisol) as well as conversion of arable lands to human settlements. Ghanaian farmers have used several strategies to improve the fertility of such soils including the application of lime, organic manure and inorganic fertiliser. Liming materials are expensive, depriving most farmers the opportunity to be able to use it. The use of organic manure is hampered by rapid mineralisation, often within one season and are not able to provide required amounts of plant nutrients. The prices of inorganic fertiliser are surging high each day and are not readily available to farmers.

The use of biochar as a soil management option is widely been used worldwide (Lehmann et al., 2006) and has necessitated this research. Biochar feedstock (corn cob, cocoa husk and poultry manure) could be obtained easily from agricultural waste, compared with liming materials and inorganic fertilizers which are both not readily available and also not affordable. Hence, if the limitations posed by nutrient deficient soils could be addressed by using biochar, crop productivity would be significantly increased in the Western, Ashanti, Brong Ahafo and parts of the Eastern Regions; whose arable lands are dominated by highly weathered soils. In addition, conversion of biomass C to biochar C leads to sequestration of about 50 % of the initial C compared to the low amounts (3 %) retained after burning (Lehmann et al., 2006).

Regardless of the benefits derived from biochar, data on the type, optimum application rates, effects on soil fertility and crop yields is still rudimentary in Ghana.

It is therefore important that before large scale deployment of biochar is considered, application rates should be studied in far more detail (Woolf, 2008). It has also been suggested that biochar addition in combination with organic manure could be an alternative to merely adding organic fertilizers, and this could be an important step toward sustainability of soil organic matter (SOM) in tropical soils. This study therefore provided essential information necessary for establishing appropriate application rate of biochar prepared from corn cob, cocoa husk and poultry manure through the evaluation of its impact on the fertility of highly weathered tropical soils when applied solely or combined with poultry manure.

Objectives of the Study

General objective

The main objective of the study was to evaluate the effect of biochar only and in combination with poultry manure on the fertility of a highly weathered tropical soil in Ghana.

Specific objectives of the study

The specific objectives of the study were to evaluate;

1. the effect of biochar and poultry manure on selected chemical properties of a highly weathered tropical soil;
2. the effect of biochar and poultry manure on soil microbial biomass carbon, nitrogen, phosphorus and phosphorus solubilisers;

3. the effect of biochar and poultry manure on earthworm survival and activity; and
4. the effect of biochar and poultry manure on the yield and shoot NPK of lettuce (*Lactuca sativa.* L).

Organisation of the Study

The study consists of eight chapters. Chapter one gives a general overview of the study highlighting certain key aspects of background information that provokes the need for investigating into the topic. Chapter two deals with the review of the literature that is relevant to the study and emphasises scientific facts which are important reference points. The third chapter presents the general methodology on how the research was conducted and this covered description of the study area, materials used in the study and general analytical/laboratory techniques employed. Chapters four, five, six and seven presents the write up on the respective objectives of this study. Key findings made during the study, conclusions and recommendations were summarised in Chapter eight.

CHAPTER TWO

LITERATURE REVIEW

Properties of Strongly Weathered Tropical Soils

The US Department of Agriculture (USDA) soil classification system includes 12 distinct soil orders that are largely defined by the extent of soil weathering. Apart from Gelisols (frozen soil) which is absent, tropical forests contain all of the USDA soil orders. Seven are most common in tropical forests: Alfisols, Entisols, Inceptisols, Mollisols, Oxisols, Ultisols and Vertisols (Palm et al., 2007). Generally, it has been reported that about 43 % of these soils are highly weathered soils and are mainly in the order; Oxisol and Ultisol.

In Ghana, an estimated 10-15 per cent of the 23.9 million hectares of potentially arable land comprises Oxisols and Ultisols (Owusu-Bennoah et al., 1997). Previously, these soils characterized by low acidity (pH < 5) and low nutrient capacity were only visible in the western part and lowland areas of the country, but have now become a nationwide threat. Obiri-Nyarko (2012) explained that most of the soils, particularly those in the south-western parts of Ghana are naturally highly weathered and leached, rendering them acidic and less fertile. Due to the high precipitation and leaching in this agro ecological zone, the leaching of most basic cations; Calcium (Ca^{2+}), Magnesium (Mg^{2+}) Sodium (Na^{+}) and potassium (K^{+}), that would counteract the acidity effects of acidic cations (mainly Hydrogen (H^{+}) and Aluminum (Al^{3+}) are removed from soil. This results in the dominance of acidic cations given rise to soil acidity.

Food crop production in areas covered with these soils (Oxisol and Ultisol) is threatened due to the low soil fertility status and acidic nature of the

soil (Owusu-Bennoah et al., 1997). Then again it has been established that both soil orders commonly have multiple nutrient deficiencies (N, P, K, Ca and Zn) and micro nutrient (Al, Fe, Cu) toxicities are evident.

Highly weathered soils of the tropics (Oxisol and Ultisol) can only be productive if pragmatic management strategies are put in place. Soil management strategies to correct problems posed by these soils must be geared towards enhancing nutrient availability and retention, ameliorate acidity, and reduce aluminum toxicity and improve its overall fertility.

Soil Fertility and its Improvement Methods

Decline in fertility of the soil is a major factor that could result in the reduction in food production which threatens a country's bid to be self-sufficient in food production. Hence, necessary management strategies are required to sustain the soils fertility in congruent with the production goals of a nation. Strategies to correct the limitations associated with the use of Ultisols and Oxisols must be targeted at correcting acidity, increasing the concentrations of essential plants nutrients (NPK) and improving the base saturation.

Over the years strategies adopted have included liming, organic material addition, the use of acid tolerant crops and agroforestry (Brown et al., 2008). The ability of liming to increase soil pH, decrease Al and other heavy metal solubility, and increase crop yield have been documented (Haby, 2002; Brown et al., 2008). Then again, liming has been reported to add two macro nutrients; calcium, and magnesium, to the soil. It also enhances the availability of phosphorus that is added to the soil for plant uptake and growth and increases the availability of nitrogen by hastening the decomposition of organic matter.

In Ghana however, using lime for soil management to remediate the soil acidity and make soils productive is not extensively practiced. Furthermore, it has been found that liming on a highly weathered acid soil is temporal and has to be repeated annually (Shamsuddin et al., 1998). The situation is further aggravated with the high cost involved in transporting liming material. This makes liming as soil management practice very expensive and uneconomical, especially for most Ghanaian smallholder farmers. Then again, over-liming can significantly reduce the bioavailability of micronutrients (Zn, Cu, Fe, Mn and B), which decrease with increasing pH (Fageria et al., 2002). This can produce plant nutrient deficiencies, particularly that of Fe which is made available at medium acidic conditions.

The application of manure have also been employed as a means to minimise the limitations posed by highly weathered soils for crop production in the tropics (Wong & Swift, 2003). The addition of organic materials (crop residues, animal manures, green manures) to highly weathered soils can have a direct effect on soil organic matter (SOM) content, ameliorate Al toxicity, and reduce soil acidity, mainly by complexation (Hue, 1992; Wong & Swift, 2003). Other effects may include improving soil physical characteristics and augmenting microbial activities (Wong & Swift, 2003).

During microbial decomposition of organic materials, organic acid anions (oxalate, citrate and malate) produced are decarboxylated (Yan et al., 1996; Tang et al., 1999). The decarboxylation of organic acid results in the upsurge in hydroxyl ions, consequently causing a rise in pH. Then again, plant materials such as legume residues (soybean, red clover, and acacia) were observed to have a substantially higher total basic cation contents and upon

decomposition releases these elements into solution increasing the pH of the soil (Ano & Agwu, 2007; Agbede et al, 2010).

A study conducted by Arthur (2009) on the changes in soil physico–chemical properties following the application of crop residues on some acidic soils in Ghana found that when maize stover was applied, the pH of the soil was significantly raised from 5.06 to 6.27. It was indicated that the residue on the soil surface, apart from releasing alkali elements into the soil, also protected it from raindrop impact that could lead to erosion and leaching of basic cations. The increase in soil pH is attributed to increase in organic matter and calcium ions released into the soil solution during microbial decarboxylation of manure which is known to buffer change in soil pH (Ano & Agwu, 2007; Agbede et al, 2010).

Apart from direct release of mineral nutrients, poultry manure has been shown to increase soil microbial activity. Irrespective of the good prospects in using manure to improve the fertility of the soil, it is bulky, relatively low in nutrient and must be applied at high rates (Mathew & Karikari, 1995). The application of higher quantities potentially increase human drudgery. However organic manure is easy to access compared with lime. In addition, the benefits of organic amendment are relatively short-lived, especially in the tropics, since decomposition rates are high and the added organic matter is usually mineralized to CO_2 within only a few cropping seasons (Bol et al., 2000).

Acid tolerant crops can be productive when grown on acid soils however this has not been accepted by farmers. Reasons such as the economic importance and domestic consumption of these crop species have been a bother.

The use of agroforestry as a way of rehabilitating highly weathered soils has also received considerable attention. However, competition for water and nutrients during tree establishment phase and long-term organic matter decline has been reported in Africa (Qureshi, 1991). Moreover, it is not economical for farmers with farms that are small in size as land will be too small to allow for the integration of tree species, as the trees will consume most of the land space.

Another key strategy that could be adopted to reduce soil acidity and improve the fertility of nutrient deficient acid soil is the use of inorganic fertilizer. However the use of chemical fertilizers to ameliorate acidity should be done with caution since the addition of nitrogenous fertilizers could also make soil acidic. It is possible to replace nitrogen fertilizers that are acidic (ammonium related fertilizers) with nitrate fertilizers as N source because nitrate gives alkaline effect since it is exchanged by plant roots with bicarbonate and hydroxyl ions. However, these chemical fertilizers (Nitrates) may be scarce and expensive, particularly for the resource poor farmer.

The use of biochar has been proposed to help in restoring the fertility of highly degraded tropical soils. There are however, inconsistent conclusions on the impact of biochar on soil fertility.

Biochar and its Effects on Soil Fertility

The use of biochar is an age old practice. The greatest suggestion that biochar may be beneficial to soil fertility comes from studies of the Amazonian Dark Soils known as Terra Preta and Terra Mulata. Amazonian Dark Soils are prized for their high nutrient levels and high fertility (Lehmann et al., 2003). These soils developed through intense anthropogenic activities

such as biomass-burning and high-intensity nutrient depositions on pre-Columbian Amerindian settlements that transformed the original soils into Fimic Anthrosols throughout the Brazilian Amazon Basin.

Pre-Columbian Amazonians are believed to have used biochar to enhance soil productivity. They produced biochar using agricultural waste – that is covering burning biomass with soil (Solomon et al., 2007) in pits or trenches (Lehmann, 2007). European settlers called it Terra Preta de Indio (Glaser et al., 2002). The term "biochar" was coined by Peter Read in 2009 to describe charcoal used for soil improvement.

Biochar is a product of thermal decomposition of plant or animal biomass produced by pyrolysis (Glaser et al., 2002). Lehmann and Joseph (2009) explained that biochar is a carbon-rich solid material produced by heating biomass in an oxygen-limited environment and is intended to be added to soils as a means to sequester carbon (C) and maintain or improve soil functions. Different materials are used as biomass feedstock for biochar preparation (including wood, crop residues and manures). However the suitability of each feedstock for biochar production and subsequent application to soil is dependent on a number of chemical, physical, environmental, as well as economic and logistical factors (Verheijen et al., 2010).

Biochar Effects on Soil Chemical Properties

It has been suggested that addition of biochar to sandy and nutrient impoverished soils could lead to improvement in soil chemical properties (Glaser et al., 2002).

Biochar and soil pH

Previous studies have demonstrated both an increase and decrease in pH of the soil upon addition of biochar. Uzoma et al. (2011) reported significant increases in pH of a sandy soil with biochar rates of 0, 10, 15 and 20 tons/ha. These rates, respectively, recorded pH values of 6.4, 7.1, 7.3, and 8.4 compared with initial soil pH of 6.4. The increases in pH corresponded linearly with increase in biochar application rates. They explained that the biochar was able to increase soil pH due to high carbonate content or liming effect. On the other hand, Naeem et al. (2014) reported pH reduction to 7.92 when biochar was added to soil with Initial pH of 8.42. The reduction in soil pH might be due to release of protons (H^+) from the exchange sites of biochar and due to the proliferation of acid producing soil microorganisms. They explained that, pH of the soil reduced due to the production of organic acid during the decomposition of organic matter present in the soil and biochar. Similarly Liu and Zhang (2012) observed decreasing pH levels of soils with increasing application of biochar prepared from Chinese pine. They attributed the decrease to the acidic materials produced from the oxidation of biochar and the decomposition of organic matter in the soil. They explained that biochar is not fully inert in soil and can be oxidized, especially at the surface, through chemical and microbial activity (Cheng et al., 2006). The slow oxidization of biochar in soils can produce carboxylic or acidic functional groups (Cheng et al., 2006). Novak et al. (2010) mentioned that in the presence of high organic matter, oxidation of biochar is even enhanced.

Biochar and availability of phosphorus

Biochar has been shown to increase P availability. Biochar may improve available P in soils; both directly through P addition from water-soluble P contained in biochar and/or indirectly through impact on soil chemical, physical and/or biological processes (DeLuca et al., 2009). The changes in soil processes may include modification of soil pH and amelioration of P complexing metals (Al^{3+}, Fe^{3+}, Ca^{2+}) and increase in microorganism activities.

In addition to directly releasing soluble P, biochar can have a high ion exchange capacity (Liang et al., 2006), and may alter P availability by providing anion exchange capacity or by influencing the activity of cations that interact with P. It has been demonstrated that fresh biochar has an abundance of anion exchange capacity in the acid pH range (Cheng et al., 2006), which can initially be in excess of the total cation exchange capacity of the biochar. It is possible that these positive exchange sites compete with Al and Fe oxides (e.g. gibbsite and goethite) for sorption of soluble P, similar to that observed for humic and fulvic acids (Hunt et al., 2007). As biochar ages, the positive exchange sites on biochar surfaces decline and negative charge sites develop (Cheng et al., 2006). The biochemical basis for the high CEC is likely due to the presence of oxidized functional groups (such as carboxyl groups), whose presence are indicated by high O/C ratios on the surface of charred materials following microbial degradation (Liang et al., 2006; Preston & Schmidt, 2006).

Phosphorus availability and recycling may be influenced by the biochar CEC over long timescales and in soils that have inherently low

exchange capacities. By increasing CEC and reducing the presence of free Al^{3+} and Fe^{3+} near root surfaces, biochar may promote the formation and recycling of labile P fractions. Then again, a significant component of the P cycle consists of a series of precipitation reactions that influence the solubility of P, ultimately influencing the quantity of P that is available for uptake and actively recycled between plants and microbes. The degree to which these precipitation reactions occur is strongly influenced by soil pH due to the pH-dependent activities of the ions responsible for precipitation (Al^{3+}, $Fe^{2+,3+}$ and Ca^{2+}). Biochar may influence precipitation of P into these insoluble pools by altering the pH and, thus, the strength of ionic P interactions with Al^{3+}, $Fe^{2+,3+}$ and Ca^{2+} (Lehmann et al., 2003) or by sorbing organic molecules that act as chelates of metal ions that otherwise precipitate P. Biochar is a good surface for sorbing polar or non-polar organic molecules across a wide range of molecular mass (Preston & Schmidt, 2006; Bornermann et al., 2007). Organic molecules involved in chelation of Al^{3+}, Fe^{3+} and Ca^{2+} ions will potentially be sorbed to hydrophobic or charged biochar surfaces. The sorption of chelates may have a positive or negative influence on P solubility.

Van Zwieten et al. (2010) also posited that many biochars have a liming effect, so increased soil pH may increase negative charge which in turn reduces P sorption. The extent of this effect depends on the Acid Neutralizing Capacity (ANC) of biochar. Biochar may also increase microorganism activities through application of C, especially aliphatic C compounds (Zimmermann, 2010). The increase in microbial activities may affect microbial biomass phosphorus (MBP) (Liptzin & Silver, 2009) and phosphatase activity (Trasar-Cepeda et al., 1990) resulting in increased plant

available P. Although higher C/P ratio coupled with an increase in microbial biomass may lead to immobilisation of P.

Some previous studies have investigated potential effects of biochar application on P availability by changing the soil environment for microbial proliferation and activity. Atkinson et al. (2010) found that biochar affected soil P availability and plant uptake of P indirectly by changing the environment to support the proliferation of microbes involved in P solubilisation.

Other works have demonstrated that biochar had limited ability to sorb P (Soinne et al., 2014); instead, biochar can even act as a source of soluble P after application to soil (Parvage et al., 2013). Uzoma et al. (2011), observed that application of cow dung biochar at rates of 0, 10, 15 and 20 tons/ha led to increases in the levels of available P. These application rates resulted in available P of 0.12, 0.15, 0.18 and 0.16 g kg^{-1} for the above biochar rates, respectively; with soil having an initial soil available P of 0.065 g kg^{-1}. They attributed the increases in P availability to high levels of P in the cow dung biochar as well as the increases in soil pH from 6.4 to 8.0, which also led to P availability. Similarly, Zhai et al. (2015) conducted an experiment on short-term effects of maize residue biochar on phosphorus availability in two soils with different phosphorus sorption capacities. They observed an increase of Olsen-P from 3 to 46 mg kg^{-1} in red earth and from 13 to 137 mg kg^{-1} in Fluvo-aquic soil upon the addition of 8 % biochar for 42 days incubation period. They attributed the increase to the high levels of P in ash fraction of biochar.

Biochar and soil nitrogen

Nitrogen is one of the most limiting plant nutrient in most tropical soils. Previous research shows that biochar potentially has the ability to manipulate the rates of N cycling in soil systems by influencing nitrification rates, adsorption of ammonia and increasing NH_4^+ storage by enhancing cation exchange capacity in soils. Its influence on these processes may have further implications in terms of reducing gaseous N losses such as N_2O and NO_3^- leaching. Deluca et al. (2009) postulated that during N mineralisation, biochar increases nitrification rates in natural soils that have very low natural nitrification rates. Conversely, agricultural soils which have already appreciable rates of nitrification, the effect is rather minimal. They further explained that biochar additions to agricultural soils decrease apparent ammonification rates probably due to adsorption of NH_4^+ onto biochar surfaces and subsequently reducing the concentration of NH_4^+ in the soil solution. The effect however is dependent on the type of biochar added and the soil type used.

Further, the incorporation of biochar to soils has been observed in various experiments to reduce ammonium leaching (Lehmann et al., 2003; Major et al., 2009) and in some cases reduce N_2O emission (Spokas & Reicosky, 2009). These mechanisms that lead to reduction in N losses should contribute to increasing N in soils after biochar applications. The above observations were confirmed by Chan et al. (2008) when they observed increasing total N content of an Alfisol with increasing rate of biochar applications. It was revealed in their experiment that the soil with an initial N content of 0.23 % increased to 0.26, 0.28 and 0.33 % with biochar rates of 10,

25 and 50 t ha^{-1}, respectively. In another study, Nelson et al. (2011) studied nitrogen availability in biochar-amended soils where biochar was applied to soils at three rates (0, 2, and 20 g kg^{-1}) in combination with two N rates (0 and 100 mg kg^{-1}) and incubated for 56 days. Biochar application at 20 g kg^{-1} increased NH_4^+-N concentrations by 1.1 to 4.8 mg kg^{-1} during the first 10 days and consistently decreased NO_3-N recovery by 5 to 10 mg kg^{-1} for the duration of the study.

Biochar addition may also enhance the mineralization of N in added organic manure as a result of proliferation of microbes, however not much work have been done to establish this fact. Zackrisson et al. (1996) reported that there is rapid response of the nitrifier community towards addition of biochar to soils. Glaser et al. (2002) explained that due to the high surface area of biochar, it may offer a suitable habitat for the proliferation of microbes. Dempster et al. (2012) found that the addition of a Eucalypt biochar at 25 t ha^{-1} altered the ammonia oxidiser community structure resulting in lower nitrification rates.

Biochar and soil organic carbon

The use of biochar as means for C sequestration requires that SOC mineralization should not be enhanced. So far, the great diversity of biochar in a wide range of different circumstances has not conclusively settled this issue (Wardle et al., 2008; Singh et al., 2010). The application of biochar, although with large C percentage has been suggested to contribute negligibly to SOC concentration due to the recalcitrance of its C to microbial decomposition (Lehmann et al., 2003). However inconsistent conclusions have been drawn regarding the effect of biochar on soil organic carbon content. Biochar may

affect microbial proliferation (Pietikäinen et al., 2000) which may contribute to degradation of biochar and subsequently affect carbon release. Then again, the addition of biochar might cause positive or negative priming stimulating the mineralisation of native soil organic carbon (Kuzyakov et al., 2009). In addition, biochar added to soil could contain an appreciable amount of labile C as well as the degradation of some part of recalcitrant C by microbes (usually about 5 % is degraded) (Brodowski, 2005; Cross & Sohi, 2011). These mechanisms could lead to increment in SOC.

The effect of black carbon (BC) on soil carbon content has been investigated by some researchers (Glaser et al., 2002; Lehmann et al., 2003; Agusalim et al., 2010). Agusalim et al. (2010) observed an increase in soil organic carbon (SOC) upon application of rice husk biochar to rice cropping system in an acid sulphate soil. In their experiment, they observed that a soil with an initial SOC of 0.78 % increased to 4.09 % upon application of 10 tons of rice husk biochar. This represents a percentage increase of 524 % over the unamended soil. Chan et al. (2007) also observed similar trend. In their experiment they observed that a soil with an initial SOC content of 18 g kg^{-1} was increased to 21.6, 27, 43.4 and 64.6 g kg^{-1} with biochar rates of 0, 10, 50 and 100 t ha^{-1}, respectively.

In contrast, Li et al. (2012) reported no significant differences between the control soil and dried cotton stalks biochar applied at 20 g kg^{-1}. The discrepancies in C mineralization of biochar-treated soils are likely to be due to the type of both soil and biochar, the duration of the experiment and the rates of used biochar (Zimmerman et al., 2011).

Biochar and cation exchange capacity

It has been reported that increasing CEC of the soil was related with biochar addition and also correlated with increasing biochar rates. When cow manure biochar was applied to a sandy soil with an initial CEC of 0.71 $cmol_c kg^{-1}$, this was increased to 0.75, 0.92, 1.14, and 1.27 $cmol_c kg^{-1}$ at biochar rates of 0, 10, 15 and 20 t ha^{-1} (Uzoma et al., 2011). The increase in CEC of the soil was attributed to large surface area of the biochar and corresponding negative charges. These results have been confirmed by other workers particularly Chan et al. (2007) who applied green waste biochar to Alfisol. The phenomenon of increase in CEC with biochar incorporation into soils could be due to the high surface negative charge resulting from oxidation of carboxylic and phenolic groups of biochar (Liang et al., 2006).

Effect of Biochar on Soil Biological Properties

The effects of biochar on soil biota have received less attention than its effects on soil chemical properties (Lehmann et al., 2011). In addition the responses of microbial biomass following biochar application to soil are highly variable. Previous studies have reported enhanced (Steinbeiss et al., 2009; Zimmerman et al., 2011) or inhibited microbial activity (Dempster et al., 2012) with biochar amendments. Other studies have reported no effects on soil microbial biomass (Castaldi et al., 2011; Kuzyakov et al., 2009). Lehmann et al. (2011) explained that the effects of biochar on soil biota may be driven by its physical and chemical properties.

Whether the population of microorganisms increases or not, is likely connected to the intrinsic properties of both biochar and the soil. The effects of biochar on soil microbes are dependent on the;

1. labile compounds of biochar; supplying nutrients for the microbes (Lehmann et al., 2011). Biochar has been implicated to stimulate microbial activity as a result of its leachable/labile C fraction and ash content. Depending on the type of biochar, a fraction may be readily leached and therefore mineralizable and in some cases has been shown to stimulate microbial activity and increase abundance (Steiner et al., 2008). In addition, the ash fraction which is a major component of the biochar comprised of minerals that are present as ash inclusions. These minerals include several essential macro- and micro-nutrients for biological uptake and, therefore, represent valuable resources in the soil food web. Then again, biochar-C although largely unavailable to soil microbes is able to cause changes in soil physicochemical properties and the introduction of metabolically available labile-C compounds associated with the biochar may shift the soil microbial community structure.
2. biochar pores and surfaces; providing habitat for microbes and offering physical protection (Pietikäinen et al., 2000) from predators. Lehmann et al. (2011) have suggested that the biochar pores may act as a refuge site or microhabitat for colonizing microbes, where they are protected from being grazed upon by their natural predators or refuge sites for microbes that are less competitive in the soil environment to become established. Biochar high porosity consequently allows it to retain more moisture. An increase in the water holding capacity of biochar may provide surfaces for microbes to colonize. Bacteria may sorb to biochar surfaces, rendering them less susceptible to leaching in soil

(Pietikäinen et al., 2000). This would increase bacterial abundance. The main processes leading to attachment are (1) flocculation, (2) adsorption on surfaces, (3) covalent bonding to carriers, (4) cross-linking of cells, (5) encapsulation in a polymer gel, and (6) entrapment in a matrix.

3. the alkaline nature of biochar which could provide a more chemically favourable environment for microorganisms (Lehmann et al. 2011). One major change that occurs in soil after incorporation of biochar is pH. The changes in soil pH have an immense effect on microbial abundance. Microbial biomass increases with rising pH. This has been shown for a gradient from pH 3.7 to 8.3 (Pietry & Brookes, 2008). It should be noted however that fungal and bacterial populations react differently to changes in pH. Bacteria are likely to increase in abundance with rising pH up to values around 7, whereas, fungi may show no change in total biomass (Rousk et al., 2010), or potentially reduce their growth at higher pH (Rousk et al., 2009).
4. toxic volatile organic compounds (VOCs) inhibiting microbial biomass (Deenik et al., 2010). More recently, compounds inhibiting microbial activity have also been found either on biochar (Deenik et al., 2010) or released after its introduction to soil (Spokas et al., 2010).
5. biochar increasing decomposition of soil OM thereby stimulating microbial activity (Wardle et al., 2008) and
6. biochar sorption and retention of organic C increasing microbial biomass (Lehmann et al., 2011).

Effect of biochar on earthworm survival and activity

Earthworms are believed to have profound direct and indirect impacts on the availability of nutrients, particularly through increased decomposition of plant residues and turnover of soil organic matter. It is therefore indicative that what positively or negatively affects earthworm may indirectly affect soil function and plant growth (Lehmann et al., 2011). Earthworms are also used as a standard test species to investigate the impact of a substance on the soil compartment before approval is given for its application (Van Gestel, 1992).

Meanwhile evidence has shown that some biochars may have negative effects on the soil biota, in particular earthworms (Liesch et al., 2010) resulting in their mortality. For instance, Liesch et al. (2010) studied impact of two biochars (pine chip and poultry litter) on mortality and growth of earthworm (*E. fetida*) in a simulated soil (70 % sand, 20 % kaolin, and 10 % sphagnum peat). Biochar was applied at rates of 5 to 180 Mg ha^{-1}. They found that mortality of earthworms increased with an increasing biochar application rates. Poultry litter biochar was harmful to earthworms at rates above 45 Mg ha^{-1}. In contrast however they observed no difference in survivorship of *E. Fetida* subjected to pine chip biochar and the control treatments even at high rates of application (above 45 Mg ha^{-1}). Liesch et al. (2010) attributed the mortality and reduced growth of earthworms to alterations in soil pH, and ammonia concentration (Liesch et al., 2010). It is well established that earthworms are sensitive to pH (Munnoli & Bhosle, 2009). However, other causes of quick mortality in earthworm studies have been observed by Schmidt et al. (1999). They reported initial mortality of earthworms at seven

days with dried maize residue, which they attributed to potential physical damage arising from the dry material sticking to the earthworm's body.

However, information on the effect of biochar on earthworm population, activity and overall soil function with land application of biochar is limited in the tropics. In addition the effects may be variable depending on type of soil used, the type of biochar, and the application rates (Liesch et al., 2010). It has therefore been suggested that further studies is needed to standardize earthworm studies (Frund et al., 2010). This requires adequate data on biochar properties and information on the environment in which they are to be used.

Biochar effect on soil microbial biomass C, N and P

Soil microbial biomass (SMB) is a measure of the mass of the living component of soil organic matter and at a given point in time. It is a measure of the microbial population density. It consists of organisms having a volume of less than 5×10^3 μm^3 (Brookes, 2001). The soil microbial biomass consists mostly of bacteria and fungi but may also contain lager soil organisms such as algae and protozoans. The microbial biomass typically makes up less than 5 % of total soil organic matter. Recognition of the importance of the microbial biomass has led to the increased interest in measuring the nutrients held in their biomass (Martikainen & Palojarvi, 1990).

During microbial decomposition of SOM and mediation of soil nutrients mineralisation to available forms, it has been noted that these microbes take nutrients from organic materials being decomposed even more than from soil nutrients reservoir (Ocio et al., 1991). Further, Oberson et al. (1997) buttressed this fact indicating that soil microbial biomass is a very

important reservoir of phosphorus, nitrogen and carbon in the soil indicating that microbial biomass is a labile source of C, N and P of SOM and its magnitude directly affects the nutrients flux. The death or turnover of microbial biomass serves as an important and dynamic source of nutrients which are readily available to plants in soil (Smith & Paul, 1990). The release and availability of these immobilised nutrients upon microbial turnover is faster and more readily available than that of plant and animal material. Smith and Paul (1990) mentioned that the turnover time for N immobilized in the microbial biomass was found to be about ten times faster than that derived from plant material.

The use of poultry manure, as suggested by Glaser et al. (2002) to have positive synergy with biochar could increase microbial population. This is because if the soil is amended with material that allows the soil to support a larger microbial population, immobilization will increase for a time until the population reaches a new equilibrium. When biomass is on the rise, the immobilization rate may exceed the mineralization rate, resulting in a net decline of inorganic N and P. Meanwhile the effects of combined application of biochar and poultry manure on microbial biomass C, N and P have not received a considerable attention.

Soil microbial biomass carbon **(SMC)**

A wide range of microbes assimilate carbon; autotrophic organisms assimilate carbon which is obtained from carbon dioxide, heterotrophic organisms assimilate carbon obtained from organic compounds and mixotrophic organisms assimilate carbon from both organic compounds and by fixing carbon dioxide (Paul, 2007). Under aerobic conditions 20-40 % of

the substrate carbon is assimilated and the remainder is released as CO_2. Fungi are more efficient, in their metabolism, since they convert carbon into cell carbon as filaments and release less of CO_2. Range of 30-40 % of the assimilated carbon is used to form new mycelium during the decomposition.

The MBC has been suggested to be a useful tool and a sensitive measure of a change in OM status. Changes in the MBC also provide an early indication of longer term trends in the total OC of soils. It is worth noting that previous studies have used MBC as an indicator to evaluate microbial activity in soils (Chan et al., 2008; Kimetu & Lehmann, 2010).

Conversely, few studies have considered biochar effect on MBC with varied conclusions. Han et al. (2013) investigated the effects of biochar on greenhouse soil. The results indicated that MBC contents with amended treatments were significantly higher than the control treatment at ($P < 0.01$).

In a related study, Dempster et al. (2010) incorporated biochar at 0, 5, and 25 t ha^{-1} into a coarse textured sand in a glasshouse trial. Three nitrogen treatments were added: organic nitrogen, inorganic nitrogen and a control treatment. Post soil analysis revealed that microbial biomass carbon (MBC) decreased with biochar addition ($P < 0.05$). In addition they reported a change in microbial carbon to nitrogen ratio (from 8:1 to 5:1). Other experiments were conducted to study transformation of C, N and P in soils with different pH. Treated soils were incubated at 20-35 °C for 12 months. The results showed that application of biochar increased the accumulation of organic C and microbial biomass C significantly.

Similarly, Jien and Wang (2013) measured the effects of waste wood biochar (*Leucaena leucocephala* (Lam.) de Wit) on the physicochemical and

biological properties of long-term cultivated, acidic Ultisol. This study used three biochar application rates (0 %, 2.5 %, and 5 % (w/w)) incubated for 105 days. The result demonstrated a significant increase in microbial biomass carbon (MBC) from 835 to 1262 mg kg^{-1} at a higher application rate of 5 %. The authors explained that higher MBC content in the biochar-amended soils could be attributed to a transformation of the soil resulting in increased pH (5.0–6.0). The increase in pH of biochar-amended soil created a more suitable environment for the growth of microbes, especially fungal hyphae. This result confirms the submission by Lehmann et al. (2011) who indicated that, biochar porosity served as a habitat for microbes to grow and increase in abundance. The proliferation of microbes leads to higher assimilation of C.

***Soil microbial biomass N* (MBN)**

Nitrogen in soil is one of the major plant nutrients and the most vulnerable to microbial transformations compared to phosphorus and potassium. Organic nitrogen is converted into inorganic forms (NO_3^- and NH_4^+) and the process is referred to as mineralisation. The mineralisation process is mediated by microorganisms. In a review by Hayatsu et al. (2008), denitrifying fungi, nitrifying archaea, anammox bacteria, aerobic denitrifying bacteria and heterotrophic nitrifying microorganisms were reported as key organisms involved in nitrogen cycle. According to Hayatsu et al. (2008), the fate of nitrogen during the transformation process is numerous and includes plant uptake, volatilisation, leaching losses and immobilisation. Of these mechanisms, immobilisation of N by soil microbial biomass converts inorganic N (NH_4^+ and NO_3-) to organic N. In effect, the N becomes temporarily unavailable for plant uptake and also losses through volatilisation,

denitrification and leaching. They further mentioned that, nitrogen is a key building block of protein molecule, hence is a crucial component of the cells of soil microbial biomass/microorganisms. Upon turnover of the microbial biomass, the nitrogen in their bodies may be converted into forms that make up the humus complex or be released as NH_4^+ and NO_3^-.

Soil mineralization and immobilization processes determine the plant-available nitrogen pool. The equilibrium between mineralization and immobilization is influenced by factors such as temperature, moisture, oxygen, microbial populations, and the carbon and nitrogen contents of the organic material (Evangelou, 1998). For instance, net mineralization is generally expected, with relatively low carbon-to-nitrogen ratio (C:N) of organic material. Nitrogen immobilization occurs when C:N exceeds values range above 20:1 to 25:1 as suggested by Burgess et al. (2002).

Meanwhile biochar has been reported as N depleted material having a uniquely high C:N ratio. The C:N ratios of biochar vary widely, between 7 to 400, with a mean of 67 (Lehmann & Joseph, 2009). Based on these values, given the very high C:N ratios, most of the biochars are expected to cause N immobilization and possibly induce N deficiency in plants when solely applied to soils alone. However, there is a degree of uncertainty because Lehmann et al., (2003) reported that although C:N ratios of Terra Preta soils are usually higher than the adjacent Ferralsol, they tend to have higher available N. As the bulk of biochars are made up of biologically recalcitrant organic C, it is expected that N immobilization will be negligible or transient despite the high C:N ratios.

In contrast however, fresh low temperature biochars can contain significant amounts of labile C that can be readily utilised by soil microorganisms (Smith et al., 2010). This temporarily leads to available soil N becoming immobilised. Bruun et al. (2012) produced biochar from wheat straw using slow and fast pyrolysis. Fast pyrolysis resulted in a biochar that contained a labile, un-pyrolysed carbohydrate fraction. When the "slow" and "fast" pyrolyzed biochars were placed in the soil the "fast" biochar resulted in immobilisation of mineral N while the "slow" biochar resulted in net N mineralization over a 65 day period. This is indicative that slow pyrolysis could results in biochar immobilising N in soil preventing them from leaching into the environment. Similarly N immobilization has been confirmed in other studies after addition of fresh biochar leading to decreased N availability (Lehmann et al., 2003; Bridle & Pritchard., 2004).

Other experiments did not show any effect on soil N immobilisation. Dempster et al. (2012) studied soil microbial biomass nitrogen (SMB-N), in soil treated with Eucalyptus biochar (0, 5, or 25 t ha^{-1}) in full factorial combination with nitrogen (N) treatments (organic N, inorganic N, or control) for 10 weeks. The results showed that MBN was unaltered with biochar addition. Similarly, Alburquerque et al. (2013) also studied the effects of two biochar (olive tree biochar and wheat straw biochar) in a controlled growth chamber. The results showed that biochar addition had no significant effect on SMB-N for both biochar although SMB-N was higher for olive tree biochar than wheat straw biochar.

Soil microbial biomass P **(MBP)**

Microbial biomass plays a vital role in phosphorus availability in soil. It helps in the transformation of organic phosphorus in soil to available P through mineralization, and excretion of phosphatase enzymes, phytase, phosphonoacetate hydrolase, D-α-glycerophosphatase and C-P lyase (Oberson et al., 2001).

Phosphorus immobilisation due to microbial biomass is an important mechanism that enhances the availability of P in soil. Immobilisation of P ensures that P is kept in readily mineralizable pool preventing its fixation in insoluble forms. In this process, soil native or added inorganic P is incorporated into living microbial cells and its associated pool of metabolites, and thus becomes temporarily unavailable to plants. Simultaneously, in the mineralization process organic P is converted to inorganic P by microorganisms. Although both processes can take place at the same time, net immobilization or mineralization is decided by the quality of the added organic material, determined by carbon to phosphorus ratio. Addition of organic material in soil with high C:P ratios (> 200) will result in net P immobilization, while the organic materials with low C:P ratios (< 200) lead to net P mineralization (Paul, 2007). Microbial population and activity is stimulated on the addition of any carbon source in the presence of other favorable conditions like optimum moisture, temperature and pH. Therefore, when an organic source is added to the soil, microbial population multiplies due to addition of microorganisms contained in the source, and due to the growth of indigenous soil biota which was previously inactive because of the non-availability of easily decomposable organic carbon. The increased

microbial population will have more demand for P to incorporate it into its living cells and associated metabolites while preventing it to be irreversibly fixed in soil. Such microbially assimilated P is easily hydrolysable and becomes available to the plants on microbial turnover through the process of mineralization (Ayaga et al., 2006; Gichangi et al., 2009).

Another key function of microbial biomass is that it improves the use efficiency of inorganic P fertilizers in soil even if no carbon source is added. The addition of inorganic P stimulates microbial population which was previously inactive due to P deficiency. In this way added inorganic P becomes part of microbial cells or associated metabolites and is prevented from fixation. Microbial biomass P usually constitutes 2-5 % of the total soil P (Takeda et al., 2009). However, the ranges of MBP are variable in different soils. The range of microbial biomass P reported for some productive soils is 0.5-11.9 % of total P (Tate, 1985), whereas in red soils of China narrow range (0.75-8.5 %) has been reported (Wang, 2004). Achat et al. (2010) measured MBP in different soils and reported a range of 0.4-163 $\mu g\ g^{-1}$. In their study, they concluded that soils with high organic matter have high MBP compared with soils with low organic matter. They suggested that microbial biomass can represent 40-53 % and 8-11 % of the total P in litter and mineral soil (0-15 cm) layers, respectively. Brookes (2001) calculated 7 kg P ha^{-1} immobilized in the SMB in an unfertilized 0-10 cm surface soil layer, whereas in soils with high organic matter these concentrations were very high, being 54 and 65 kg P ha^{-1} in woodland and grassland soils, respectively.

Microbial turnover starts immediately after exhaustion of easily decomposable carbon source in soil. Generally, MBP turnover time as

estimated from incubation and field experiments varies and is dependent upon the quality and quantity of the added organic materials, moisture and temperature of the medium (Kouno et al., 2002; Achat et al., 2010). The turnover time estimated with the help of radiotracers ^{32}P and ^{33}P varied from 2 days in grassland to 180 days in soils with diverse cropping systems (Oberson et al., 2001; Oehl et al., 2001). Achat et al. (2010) also observed that 80 % of the MBP pool turned over in just 9 days releasing 8.5, 8.6, and 17.5 kg P ha^{-1} in dunes, dry soils and wet soils, respectively. They assigned the 80 % fast turnover to bacterial species and the remaining 20 % slow turnover (in 200 days) to fungal species.

Biochar addition may influence SMB-P values in soil depending on the biochar feedstock, the pyrolysis condition and the soil type used. Most wood and nut based biochars have extremely high C:P ratios. Conversely, manure, crop, and food-waste biochars have much lower ratios with manure-derived biochars being the most nutrient-rich carbon source (Lehmann & Joseph, 2009). A 42-day incubation experiment was conducted by Zhai et al. (2015) to study how various concentrations of biochar (0, 2, 4, and 8 % soil, w/w) and KH_2PO_4 fertilizer affects soil Olsen-P and soil microbial biomass P (SMB-P). Application of 8 % biochar substantially increased SMB-P from 1 to 9 mg kg^{-1} in Red earth and from 9 to 21 mg kg^{-1} in Fluvo-aquic soil. The increase was mainly due to high concentrations of P in the ash fraction (77 % of total biochar P). Biochar effect on soil Olsen-P and SMB-P increased by higher biochar application rates and by lower P sorption capacity. Zhai et al. (2015) concluded that the increase in SMB-P is likely due to improvement of the soil environment for microbial growth after biochar application or due to the

availability of P from biochar added or low sorption of P. The increase in the abundance of microbes will consequently lead to high assimilation of P.

Effect of biochar on phosphorus solubilising organisms

As plants cannot absorb P in bound form, the P must be converted into available form and this may be accomplished by a group of heterotrophic microorganisms.

Majority of the organisms are bacteria, although several fungi are also known to solubilize phosphates and these are referred to as phosphate solubilizing bacteria (PSB) and phosphate solubilizing fungi (PSF) respectively. Bacteria are more effective in phosphorus solubilisation than fungi (Afzal & Bano, 2008). Among the bacterial genera with this capability are *Azospirillium, Acinobacter, rhizobium, Burkholderia, Citrobacter and Erwinia Klebsellia, Bacillus, Pseudomonas, Erwinia, Agrobacterium, Serratia, Flavobacterium, Enterobacter, Proteus, Micrococcus, Azotobacter, Bradyrhizobium, Salmonella, Alcaligenes, Chromobacterium, Arthrobacter, Streptomyces, Thiobacillus,* and *Escherichia* (Zhao & Lin, 2001). *Penicillium, Aspergillus, Rhizopus, Fusarium*, and *Sclerotium* are the key PSFs. Among the filamentous fungi that solubilize phosphate, the genera *Aspergillus* and *Penicillium* (Fenice et al., 2000; Khan & Khan, 2002) are the most representative although strains of *Trichoderma* and *Rhizoctonia solani* (Jacobs et al., 2002) have also been reported as P solubilizers.

Occurrence of phosphate solubilizing organisms

High proportion of PSM is concentrated in the rhizosphere, and they are metabolically more active than from other sources (Vazquez et al., 2000). The PSB are ubiquitous with variations in form and population in different

soils. Population of PSM depends on the physical and chemical properties, organic matter, P content, and cultural activities of the soil (Kim et al., 1998). Larger populations of PSM are found in agricultural and rangeland soils (Yahya & Azawi, 1998).

Isolation of phosphate solubilizing bacteria from soils, mangrove (Vazquez et al., 2000), rhizosphere (Oliveira et al., 2009), compost of agricultural wastes (rice straw, maize, groundnut, gliricidia leaf, and macrofauna dung) (Hameeda et al., 2008) have been reported. From such studies, various types of phosphate solubilizing bacteria have been successfully identified. Phosphorus solubilising fungi on the other hand have been isolated and characterised in various research findings. Phosphofungi have been isolated from various soils (Pandey et al., 2008). Other sources include sugarcane and sugar beet rhizosphere (Mahamuni et al., 2013), and also rhizosphere soil from banana plants (Reena et al., 2013), and phosphate mines (Wu et al., 2012).

Mechanisms of phosphorus solubilization

McGill and Cole (1981) mentioned that the main P solubilization mechanisms employed by soil microorganisms include:

(1) Release of complexing or mineral dissolving compounds, for example, organic acid anions, siderophores, protons, hydroxyl ions, CO_2;

(2) Liberation of extracellular enzymes (biochemical P mineralization) and

(3) The release of P during substrate degradation (biological P mineralization)

Solubilization of inorganic P occurs mainly by organic acid production by P-solubilizing microorganisms. Among them, gluconic acid seems to be the most frequent acid of mineral phosphate solubilization. It is reported as the

principal organic acid produced by phosphate solubilizing bacteria such as *Pseudomonas sp., Erwinia herbicola* and *Pseudomonas cepacia* (Goldstein, 1994). Another organic acid identified in strains with phosphate-solubilizing ability is 2-ketogluconic acid, which is present in *Rhizobium leguminosarum* (Halder et al., 1990) and *Bacillus firmus* (Banik & Dey, 1982). Strains of *Bacillus liqueniformis* and *Bacillus amyloliquefaciens* were found to produce mixtures of lactic, isovaleric, isobutyric, and acetic acids. Fungal strains are also associated with organic acids. *Aspergillus niger, A. flavus, A. japonica, A. foetidus* and *Penicillium sp* are reported to produce gluconic, succinic, oxalic and citric acids (Maliha et al., 2004).

The production of organic acid results in either by: (i) lowering the pH, or (ii) enhancing chelation of the cations bound to P (iii) competing with P for adsorption sites on the soil (iv) forming soluble complexes with metal ions associated with insoluble P (Ca, Al, Fe) to release P.

Most inorganic P compounds in soil belong to one of the two groups: (i) those in which calcium is the most dominant controlling cation (calcium phosphate) and (ii) those in which iron and aluminium are the controlling cations (iron and aluminium phosphate).

In alkaline soil, phosphate is mainly fixed in the form of calcium phosphates usually under arid and semi-arid region. This includes rock phosphate ores (fluoroapatite, francolite) which contains insoluble inorganic P (Pi) (Goldstein, 2000). Phosphate solubilizing microorganisms could increase the P nutrition of plants through increased solubility of Ca-phosphates (Vassileva et al., 2010) and their solubility increases with a decrease of soil pH. The decerase in pH results from the production of organic acids by PSMs

(Fankem et al., 2006). Microorganisms through secretion of different types of organic acids and pH decrease mechanisms dissociate the bound forms of phosphate ($Ca_3(PO_4)_2$) (Deubel & Merbach, 2005). Then again, acidification of the microbial cell surroundings releases P from apatite by proton substitution / excretion of H^+ or release of Ca^{2+} (Villegas & Fortin, 2002). While, the reverse occurs when uptake of anions exceeds that of cations, with excretion of OH^-/ HCO_3^- exceeding that of H^+. In addition, complexation by microbial carboxylates and phenolic compounds increases the availability of P.

Carboxylates increase P availability by two mechanisms: anion (ligand) exchange and solubilization of Fe and Al. Due to the neutral cytoplasmic pH, carboxylates are released as anions (deprotonated); therefore, they do not decrease the soil pH (Hinsinger., 2001). The carboxylate anions compete with phosphate anions for binding sites, thus releasing P into the soil solution (Gerke, 1994).

In acid soils, solubilization of Fe and Al occurs via proton released by PSMs, in the process decreasing the negative charge of adsorbing surfaces to facilitate the sorption of negatively charged P ions. Proton release also decreases P sorption upon acidification which increases $H_2PO_4^-$ in relation to HPO_4^{2-} having higher affinity to reactive soil surfaces (Whitelaw, 2000). Then again, organic acids, for instance, carboxylic acids produced by PSMs mainly solubilized Al-P and Fe-P through direct dissolution of mineral phosphate as a result of anion exchange of PO_4^{3-} by acid anion, or by chelation of both Fe and Al ions associated with phosphate sorption (Henri et al., 2008). Carboxylic anions replace phosphate on sorption complexes by ligand exchange

(Whitelaw, 2000) and chelate both Fe and Al ions associated with phosphate. Gerke (1994) explained that carboxylates form water-soluble complexes with Fe and Al, thereby decreasing the free Fe and Al ion concentration in the rhizosphere soil solution. This leads to increased solubilization of Fe^{3+} or Al^{3+} and thus release of P bound to Al/Fe oxides or bound to clays and organic matter via Fe/Al bridges (Gerke, 1994).

The solubilisation of organic P on the other hand also plays a major role in phosphorus cycling of a farming system (Khan et al., 2009). Such P can be released from organic compounds in soil by enzymes as described below;

(i) **Non-specific acid phosphatases (NSAPs)**, which dephosphorylate phospho-ester or phosphoanhydride bonds of organic matter. Depending on their pH optima, these enzymes have been divided into acid and alkaline phosphomonoesterases and both can be produced by PSM depending on the external conditions (Jorquera et al., 2008). Although plant roots can produce acid phosphatases they rarely produce large quantities of alkaline phosphatases, suggesting that this is a potential niche for PSM (Criquet et al., 2004). Some evidence suggests that phosphatases of microbial origin possess a greater affinity for organic P compounds than those derived from plant roots (Tarafdar et al., 2001).

(ii) **Phytases**, a group of enzymes causing the release of P from phytate degradation. In its basic form, phytate is the primary source of inositol and the major storage form of P in plant seeds and pollen, and a major component of organic P in soil (Richardson, 1994). Although the ability of plants to obtain P directly from phytate is very limited, the growth and P-nutrition of Arabidopsis plants supplied with phytate was significantly improved when

they were genetically transformed with the phytase gene (phyA) derived from *Aspergillus niger* (Richardson et al., 2001). This led to an increase in P-nutrition to such an extent that the growth and P-content of the plant was equivalent to control plants supplied with inorganic P. Hence microorganisms are in fact a key driver in regulating the mineralization of phytate in soil and their presence within the rhizosphere may compensate for plants inability to otherwise acquire P directly from phytate (Richardson & Simpson, 2011).

(iii) **phosphonatases and C–P lyases;** they cleave the C–P bond of organophosphonates (Rodriguez et al., 2006).

Interactive Effect of Biochar and Poultry Manure on Soil Fertility

Poultry manure acts as storehouse for nutrients (nitrogen, phosphorus, sulphur, boron, zinc), increases cation exchange capacity, provides energy for microorganism activity, increases water-holding capacity, reduces the effect of compaction, buffers the soil against rapid changes in acidity, alkalinity and salinity and stabilizes structure and improves tilth (Magdoff, 1998).

Moreover, it is known to contain the highest nitrogen content among the common farmyard sources of manure such as the dung of cattle, sheep, goat, rabbit and horse. It contains between 2.0 and 3.0 % nitrogen (Agyenim-Boateng et al., 1997). The percentage phosphorus (P) and potassium (K) content of poultry manure are in the ranges (0.5 – 0.8) and (0.4 – 0.5) respectively.

A number of farmers in Ghana have been using it as their major nutrient source (Boateng et al., 2007). Moreover, because of the high cost of inorganic fertilizers, most farmers and vegetable growers have shifted to the use of poultry droppings to fertilize the soil. Masarirambi et al. (2012)

mentioned that chicken manure is natural, locally available and relatively cheap material that the organic vegetable growers can obtain.

Manure has been suggested to be used as a supplement to biochar (Glaser et al., 2002), often characterized by low nutrient levels. The combined effects of biochar and manure have been conducted by few researchers. They reported increased nutrient concentrations, improved physical and biological properties of the soil after the addition of manure and biochar due to additive effect from the two amendments (Chan et al., 2007; Gartler et al., 2013).

Effect of Biochar and Poultry Manure Application on Lettuce Yield

Positive and negative yield responses as a result of biochar application to soils have been reported for a wide range of crops. Yield increment is directly related to nutrients release from biochar material and indirectly to the positive responses due to biochar application either by nutrient savings (in term of fertilizers) or improved fertilizer-use efficiency (higher yield per unit of fertilizer applied). Asai et al. (2009) submitted that biochar amendments have previously been shown to increase crop productivity by improving the physical and biochemical properties of the soil. The variation in crop response is noted to be dependent on the chemical and physical properties of the biochar, rates of application, soil conditions and the type of crop (Yamato et al., 2006; van Zwieten et al., 2010). On the contrary, other authors have reported that the biochar-amended soil did not promote plant yields but rather decreased the productivity.

Combining biochar with manure however have been reported to increase yield. Chan et al. (2007) reported increases in crop yield as a result of combined application of biochar and manure. In an experiment, Gunes et al.

(2014) found that poultry manure and biochar increased dry weight of lettuce. They related increased yield to soil quality improvement, nutrient release into soil solution, increase of beneficial organisms and balanced nutrition of plants

CHAPTER THREE

GENERAL MATERIALS AND METHODS

Preparation and Production of Feedstock and Biochar

Corn cob, cocoa husk and poultry manure were selected for the preparation of biochar. Corn cob and cocoa husk were collected from farms at Jukwa, a farming community in the Central Region of Ghana where majority of farmers engage in corn and cocoa farming. Poultry manure on the other hand was collected from a battery-cage based poultry farm at Saltpond, with no bedding material. The feedstocks were air dried, sorted and crushed. It was then loaded into Lucia biomass pyrolytic stoves – Top Lit-up Draft (Figure 1).

The stove was made of zinc alloy sheet fabricated by the Cape Coast Technical Institute, Cape Coast. The stove consists of combustion chamber, ventilation outlet and a lid (Figure 1). The combustion chamber was filled with fuel materials (dry twigs and candle stick and honey) which were used for lightning purpose. The dry feedstock of corn cob, cocoa husk and poultry manure were placed in the stove. Pieces of the candle were placed on the surface of the materials and a match was lit to start the fire. The lid was placed on the stove, when the fire got intense. The initial yellow colour of the flames was monitored until it started to give off black smoke. Emission of black smoke indicated that charring of the feedstock was assumed to be complete and the fire was put off. The charring process took place at temperatures between 350 °C and 450 °C and residence time of between 30 minutes to 1 hour. The char produced (Figures 3 and 4) was milled (BROOK CROMPTON SERIES 2000 MILL), sieved through a 2 mm sieve and oven-dried at 65 ^{0}C till constant weight.

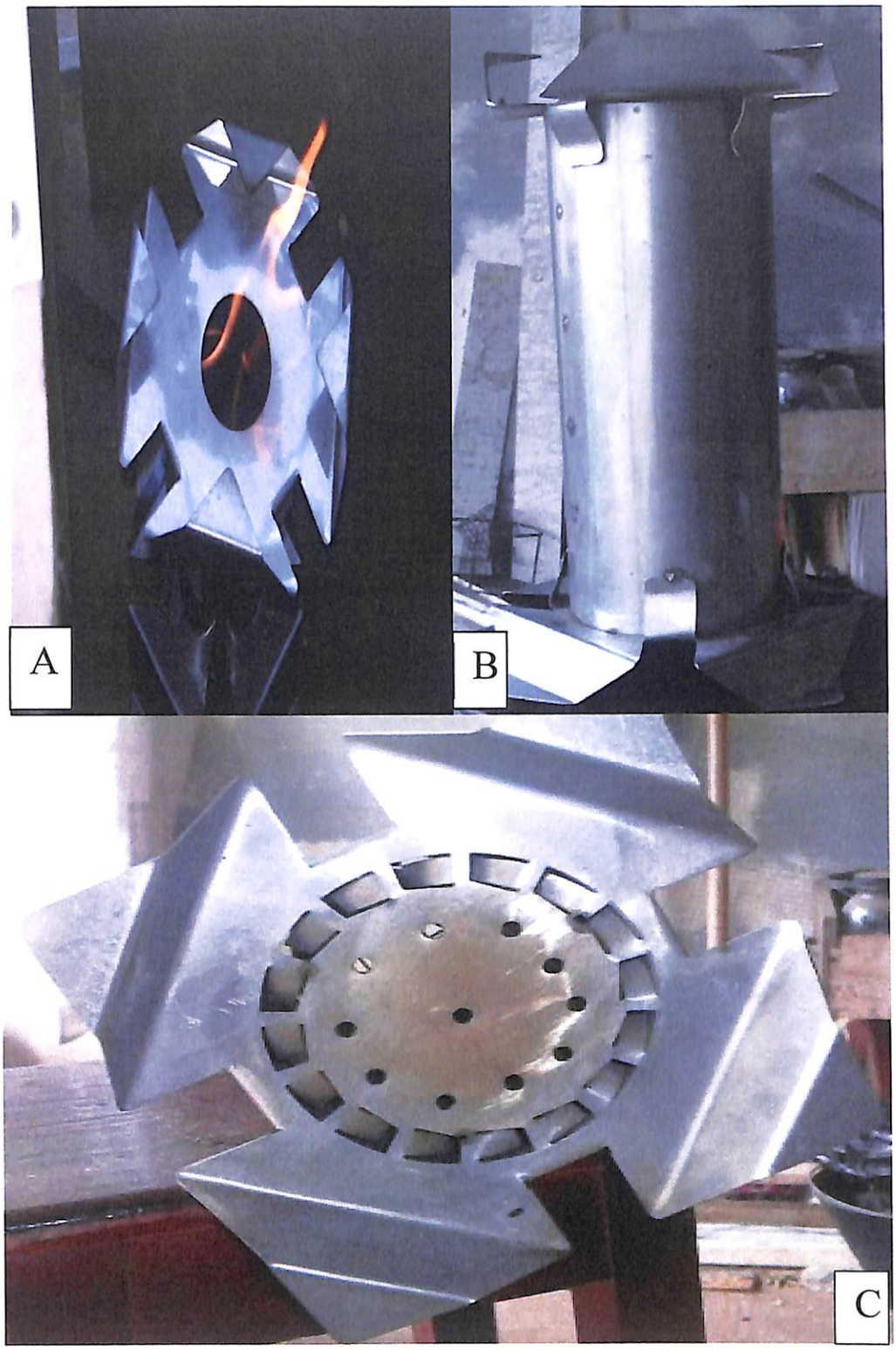

Figure 1: Features of the Lucia biomass stove: (A) stove in use; (B) side view of stove; and (C) stove bottom showing primary air inlet.

Figure 2: Charring of corn cob

Figure 3: Charred corn cob

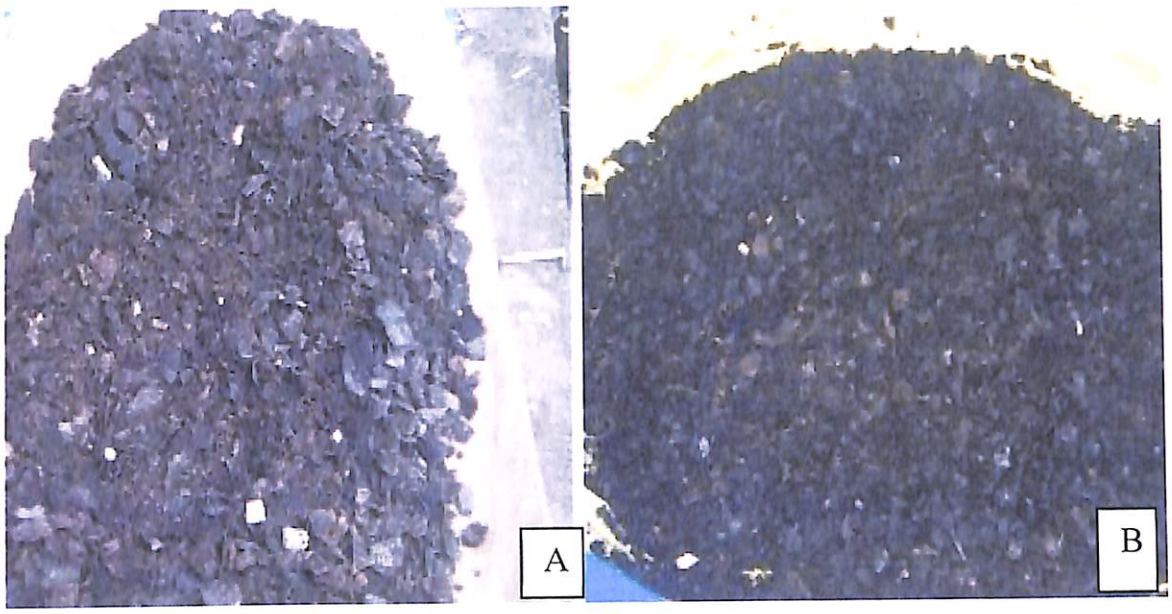

Figure 4: (A) Cocoa husk feedstock; (B) charred cocoa husk

Biochar was analysed for pH, total C, total N, total P, total Ca, total K, total Mg, total Na and selected trace elements (Cu, Zn, Fe and As).

Laboratory Analysis of Biochar

Determination of pH

Five grams of sieved biochar sample was weighed into a centrifuge tube and 25 mL of distilled water added to obtain a biochar-water suspension in a ratio of 1:5. Three replicates of the biochar-water mixture was shaken for 20 minutes using a mechanical shaker. The pH of each suspension was taken using a glass electrode pH meter (Suntex 701 Model pH meter) after calibration using buffer prepared from potassium phthalate, potassium dihydrogen orthophosphate and disodium hydrogen orthophosphate (Rowell, 1994).

Total carbon determination

The ashing method as described by Mclaughlin (2010) was followed to determine the total carbon of the biochar. Five grams of each biochar sample was weighed in triplicates into a pre-weighed porcelain crucible. The crucibles were then placed into a pre-warmed furnace that had its temperature set at 550 °C and ashing left to complete overnight. After cooling, the masses of each crucibles plus ash were weighed and recorded. Measurements were taken in triplicates. Total carbon determination was calculated as follows:

$$\% C = \frac{w2 - w3}{w1 - w3}$$

Where:

W1= wet weight of biochar and porcelain crucible (g)

W2= dry weight of biochar and porcelain crucible (g)

W3= weight of porcelain crucible (g)

Total nitrogen of biochar

The Total Kjeldahl Nitrogen content of the biochar was determined following the method described by Stewarte et al. (1974) employing a steam distillation protocol. About 0.5 g biochar was weighed in triplicate into separate Kjeldahl digestion flasks and digested with concentrated H_2SO_4-H_2O_2 mixture on a Tecator Digestor 2012, by heating vigorously for 2 hours at 360°C. The flasks were removed after a clear digest was obtained and allowed to cool. A blank digest was also prepared. Twenty-five milliliters of the digest each was distilled into a 100 mL conical flask containing 5 mL, 2 % boric acid

indicator. The distillate was titrated against a 1/140 *M* HCl and colour change was observed from green to pinkish end point. The titre values were recorded and used for the calculation. The total N was calculated using the formular below:

$$\% N = (S - B) \times T \times 14 \times 5 \times 100/200$$

Where

S= volume of 0.0071M HCl used for sample titration

B= volume of 0.0071M HCl used for blank titration

T= molarity of HCl

14= atomic weight of nitrogen

5= sample dilution factor

200= sample weight in mg

100= factor for converting N to %

Determination of total phosphorus

Total phosphorus was determined using Ammonium Molybdate-Ascorbic Acid method. The digest prepared from N determination were washed into 100 mL conical flasks. Simultaneously, 5 µg P mL^{-1} (ppm) of standard solution was prepared from a 100 ppm stock solution of P. Approximately 0, 0.1, 0.2, 0.4, 0.6, 0.8, and 1.0 ppm P were prepared from 5 µg P mL^{-1}(ppm) standard solution. Briefly, 0.5, 1, 2, 3, 4 and 5 mL were pippeted into a 25 mL volumetric flask and 4 mL of reagent B (a solution containing ammonium molybdate and potassium antimony tartrate in ascorbic acid solution) was added and made up to 25 mL mark with distilled water. The

solution was allowed to stand for 15 minutes for blue colour development. To ensure homogeneity in treatment, 1 mL of aliquot of digest in the 100 mL conical flask were pipetted into the working standards. For the samples, 1 mL aliquots were pipetted into various 25 mL volumetric flasks and 4 mL of reagent B was added and topped up to 25 mL mark with distilled water. The solutions were allowed to stand for 15 minutes for the development of the blue colour. The readings of the concentrations of phosphorus in both the working standards and samples were done on a spectrophotometer. Before the reading, the spectrophotometer (CECIL CE1021, 1000 SERIES) was warmed up for 20 minutes. It was then calibrated by using the 0 ppm blank standard. Then, the readings of the working standards were taken at 880 nm wavelength. Readings were recorded and graphs of absorbance against working standards generated using Microsoft Office Excel 2013. From the standard P concentrations and following the determination of their respective absorbances, a linear relationship was established. The final concentration of P in the various samples was then calculated using the equation as follows:

ppm of P in biochar =

$$\text{ppm of P in solution} \times \frac{\text{vol of extractant(mL)}}{\text{weight of biochar(g)}} \times \frac{\text{final vol. of aliquot}}{\text{initial vol. of aliquot pipetted}}$$

Determination of Ca, Mg, K and Na in biochar

The flame photometry method was used for the determination of Ca, K and Na in biochar and Mg determined using Atomic Absorption Spectrophotometer (AAS). These elements were determined from the H_2SO_4-H_2O_2 digest prepared for total N determination using flame photometer. Before the flame photometer reading was done, the flame was made to equilibrate for 30 minutes and standards of the elements passed through the flame photometer for calibration. For the samples, approximately 100 mL of digest were then passed through a flame photometer and readings taken in triplicates. The concentration of the elements was determined by flame photometry in triplicates. The final concentration of elements in solution was determined using the formulae below:

$$\% \text{ (Element)} = {}^{C}/_{100} \times \text{wt}$$

Where;

C= concentration of potassium from emission curve

Wt= weight of soil in grammes

Determination of Cu, Zn, Fe, Pb and As

To determine Cu, Zn, Fe, Pb and As, 2 g of samples was weighed into a cylindrical container and 20 mL of 0.1 M HCl solution was added. The suspension was covered and placed on a shaker for 30 minutes and filtered through a Whatmann No. 42 Filter Paper. The filtrate was topped up to the 100 mL mark and aspirated with the standards and blanks of Cu, Zn, Fe, Pb and As. From the aliquots, Cu, Zn, Fe, Pb and As were determined using Spectrophotometer as described by Sabiene et al., (2004). The analyses were

carried out at Soil Science Laboratory, University of Cape Coast and Soil Research Institute, Kwadaso.

Soil Sampling, Preparation and Analysis

The soil used in this study was a highly weathered tropical soil (Table 2), collected from the Agricultural Research Farm, Aiyinasi, in the Western Region of Ghana. It is a typical agricultural soil of the Western region of Ghana and the site has a long history of cropping.

Systematic stratified sampling technique was used to sample the soil. Stratification was based on the slope of the land. The field was partitioned into 4 sub-sites. The area of each sub-site was 50 m^2. Soil samples were taken in a zigzag pattern at a depth of 20 cm from each sub-site. Adequate amount of soil was bagged and sent to the laboratory, air-dried, crushed and sieved through a 2 mm sieve to obtain the fine earth fractions. The fine earth fraction was used for the experimental setups. Initial analysis of the soil was carried out in the laboratory before incubation and pot experiments were done.

Laboratory Analysis of Soil Chemical Properties

The soil chemical properties determined were pH, Total C, total N, available N, total P, extractable P (Bray No.1), exchangeable bases (Ca^{2+}, Mg^{2+}, K^{+} and Na^{+}), exchangeable acidity (Al^{3+} and H^{+}) and effective cation exchange capacity (ECEC).

Determination of soil pH

The Suntex 701 Model pH meter was used to determine the pH of the soil in water. Twenty five (25) mL of distilled water was added to 10 g of the soil samples in a centrifuge tube and shaken on a mechanical shaker to obtain

soil-water suspension in a ratio of 1:2.5. The pH meter was calibrated and pH of the suspension determined.

Determination of total carbon of soil

The ashing method as described by Mclaughlin (2010) was followed to determine the total carbon of the soil. Five grams of each soil sample was weighed in triplicates into a pre-weighed porcelain crucible. The crucibles were then placed into a pre-warmed furnace that had its temperature set at 550 °C and ashing left to complete overnight. After cooling, the masses of each crucible plus soil samples were weighed and recorded. This measurement for each sample was taken in triplicates. Total carbon determination was calculated as follows:

$$\% \, C = \frac{w2 - w3}{w1 - w3}$$

Where:

W1= wet weight of soil and porcelain crucible (g)

W2= dry weight of soil and porcelain crucible (g)

W3= weight of porcelain crucible (g)

Determination of soil total nitrogen

Total nitrogen in the experimental soil sample was determined using Micro-Kjeldahl method according to stewarte et al. (1974). The protocol has been explained in Chapter Three, Page 46.

Determination of NH_4^+ - N and NO_3^- - N

The concentration of NH_4^+ - N and NO_3^- - N were determined using the method described by Rowell (1994). Briefly, 10 g of freshly sampled moist soil was shaken with 40 mL of 2M KCl for 1 hr after which the suspension was filtered through a Whatmann No. 42 Filter Paper. The mineral-N content of this extract was then determined by steam distillation.

To determine NH_4^+ - N, twenty (20) mL of the extract was pippeted into the steam distillation flask with 10 mL of fresh boric acid solution in the receiving flask inserted under the condenser of the steam distillation apparatus. After, a drop of octan-2-ol and 0.5 g of MgO had been added to the extract, steam was passed through the apparatus and 40 mL of the distillate was collected. The NH_4^+ - N receiving flask was removed and retained for titration after the steam line had been disconnected. Another receiving flask was again placed under the condenser for analysis of NO_3^- - N.

For NO_3^- - N determination, half a gram (0.5 g) of Devarda's alloy was added to the extract in the distillation flask and the steam line was immediately reconnected to distil a further 40 mL of distillate. The NO_3^- - N receiving flask was also retained for titration. Each distillate was titrated against 0.01 M HCl using a methyl-red-bromocresol green indicator solution. The procedure also involved carrying out a blank determination. The titre values were recorded and used in the calculation.

Determination of total phosphorus in soil

Total phosphorus was determined using Ammonium Molybdate-Ascorbic Acid method as described in Chapter Three, Page 47.

Determination of available phosphorus in soil

Available phosphorus in the soil samples was determined using Bray 1 method. About 1 g of the air-dry soil sample was weighed into each 50 mL centrifuge tube followed by the addition of 10 mL of Bray 1 extracting solution (15 mL, 1.0 *N* ammonium fluoride (NH_4F) and 25 mL of 0.5 *N* HCl). The tubes were placed on the mechanical shaker for 5 minutes and quantitatively transferred into a 50 mL conical flask fitted with Whatmann No. 42 Filter Paper to leach the soil solution. Two mL aliquot of the filtrate was pipetted into a 25 mL round bottom test tube followed by addition of 4 mL colour forming reagent (reagent B); (a solution containing ammonium molybdate and potassium antimony tartrate in ascorbic acid solution). The resultant solution was then topped up with distilled water to the 25 mL mark and allowed to stand for 10 minutes for colour development. The absorbance of the solution was read on spectrophotometer (CE 1000 series) at 882 nm.

Standard working solutions of P (0, 0.1, 0.2, 0.4, 0.6, 0.8 and 1.0 µg/mL) were prepared from 5 µg P/mL stock solution. The standard solutions were allowed to stand for 15 minutes for the colour to develop and their absorbances read using the spectrophotometer at 882 nm. A calibration curve was obtained by plotting absorbance against concentration for the standard solution. Concentration of P in soil aliquot was calculated using the calibration curve from the formula below:

$$\mu g\ P/g\ soil = \frac{C \times \text{Dilution factor}}{\text{weight of soil (g)}}$$

where:

C = concentration of P obtained from calibration curve (µg/mL);

Determination of soil organic carbon

Soil total organic carbon was determined by wet oxidation using standard laboratory method described by Walkley and Black (1934). Approximately 0.5 g of 2 mm sieved soil samples were weighed in duplicate and then transferred into 500 mL Erlenmeyer flasks. Ten mL potassium dichromate ($K_2Cr_2O_7$) solution was added and the flasks were gently swirled for 30 seconds. After swirling, 20 mL concentrated sulphuric acid (H_2SO_4) was added and swirled for one minute. The flasks were allowed to stand for thirty minutes. The content of each flask was diluted with 200 mL distilled water and swirled to ensure thorough mixing. Ten mL of 85 % orthophosphoric acid was added to the soil solution in the flask followed by 1 mL diphenylamine indicator. The excess Cr_2O_7 was then back titrated with 0.5 M ferrous solution until a green endpoint was reached. A blank titration was also carried out same way. The percentage organic carbon in the soil was calculated using the formula:

$$\%\, O.C = \frac{(B - S) \times Molarity\ of\ Fe^{2+} \times 0.003 \times 100 \times 100}{Weight\ of\ soil\ (g) \times 77}$$

Where:

S= Sample titre value

B= Blank titre value

0.003= 12/4000= milliequivalaent weight of carbon

100/77= the factor which converts the carbon actually oxidized to total carbon

100 = the factor to change from decimal to percent.

Determination of total exchangeable bases

Analyses of total bases have been described in Chapter Three, Page 49, and the exchangeable bases (Ca^{2+}, Mg^{2+}, K^{+} and Na^{+}) were done by the method described by Rowell (1994). Approximately 5 g of sieved soil sample was weighed into 50 mL centrifuge tubes. Twenty mL of ammonium acetate solution was added, shook for 1 hour and allowed to stand overnight. The suspension was transferred into 100 mL conical flasks fitted with Whatmann No. 42 Filter Paper. The soil trapped on the filter paper was successively leached with 20 mL of the ammonium acetate (CH_3COONH_4) solution until 100 mL of the filtrate was obtained. The collected filtrate was used for the determination of Ca^{2+}, Mg^{2+}, Na^{+} and K^{+}.

The Ca^{2+} and Mg^{2+} in the extract were determined using the AAS. Meanwhile K^{+} and Na^{+} concentrations were determined using a flame photometer. The formulae for calculating the various cations are shown below:

$$\text{Exc. Calcium} = \frac{C \times 0.025}{\text{Weight of soil (g)}}$$

$$\text{Exc. Magnesium} = \frac{C \times 0.041}{\text{Weight of soil (g)}}$$

$$\text{Exc. Potassium} = \frac{C \times 0.0256}{\text{Weight of soil (g)}}$$

$$\text{Exc. Sodium} = \frac{C \times 0.44}{\text{Weight of soil (g)}}$$

Where:

Exc = exchangeable

C = concentration from calibration curve

Determination of exchangeable acidity

In the determination of exchangeable acidity, the procedure described by Anderson and Ingram (1993) was followed. A solution of 25 mL of 1.0 *M* KCl was added to 10 g of the soil sample and the suspension stirred and filtered. The soil was then leached with 5 successive 25 mL aliquots of 1.0 *M* KCl. Phenolphthalein indicator was added to the aliquot and titrated with 0.1 *M* NaOH. Colour of the aliquot was observed from colourless to pink. The formular below was used to calculate the final exchangeable acidity:

$$\text{Exc. } (Al^{3+}+H^{+}) = (2 \times T)/Sample\ weight\ (g)$$

Where:

T=titre value (millilitres) of 0.1M NaOH solution

The ECEC was calculated by summing exchangeable bases and exchangeable acidity (Anderson & Ingram, 1993).

Determination of Fe, Cu and Zn

The determination of Fe, Cu and Zn were done following protocol described earlier in Chapter Three, Page 49.

Preparation and Analyses of Poultry Manure

Poultry manure was prepared following the procedure described by Dikinya and Mufwanzala (2010). The Fresh poultry manure without litter (at least two weeks old) was collected from a battery cage chicken production farm in Saltpond, Central Region, Ghana. Samples of manure were removed from the poultry house, pooled, and stored in an airtight container and transported to experimental sites. Manure was air dried at room temperature until constant weight was observed. After drying, the manure was manually

grounded by crushing in a sack bag, sieved through a 2-mm screen, and stored for use in setting up the experiment. Composite sample was kept in polyethylene bag at 4 ^{0}C for laboratory analysis.

The poultry manure was analysed for pH, Total C, total N, total P, K, Ca, Mg, Na, Fe, Cu, Zn, Lead (Pb) and Arsenic (As).

Determination of pH of poultry manure

The pH of the poultry manure was determined using pH meter (manure to water ratio of 1:2.5). The mixture was shaken on a mechanical shaker for 30 minutes after which the pH was measured.

Determination of total carbon in poultry manure

Total C determination followed the Ashing method described by Mclaughlin (2010). This has been described in Chapter Three, Page 51.

Determination of total nitrogen in poultry manure

Determination of total nitrogen in manure followed the Kjeldahl method as described earlier in Chapter Three, Page 46.

Determination of total P in manure

The determination of total P in the manure was by mixed acid digestion procedure as described by Stewarte et al. (1974) outlined in Chapter Three, Page 47.

Determination of total Ca, Mg, K and Na in manure

The laboratory analysis of Ca and Mg were done using AAS whiles K and Na were analysed using flame photometer as described earlier in Chapter Three, Page 49.

Plant Analysis

Five weeks after transplanting, plants were harvested weighed and stored in clean envelopes. The envelopes were kept in an oven at 60 °C till free moisture content of the crop had completely evaporated. Dried plant samples were weighed, ground and analyzed for total N, P and K.

Determination of total P and K in lettuce shoot

The determination of plant total P and K in the plant was carried out by the ashing method as Described by International Institute for Tropical Agriculture (IITA) (1985). Samples were ground and 0.5 g was weighed into crucibles and kept in a furnace overnight at 450 ^{o} C to obtain a greyish white ashes. The samples were allowed to cool and 5 mL of 1 N HNO_3 was added. The mixtures were evaporated to dryness on a hot plate. Samples were then placed in the oven to obtain white ash. After they were allowed to cool, 10 mL of 1 N HCl was added to each sample and filtered into a 50 mL volumetric flask. The flask was topped up to the 50 mL mark with 0.1 N HCl. The filtrate was used for the determination of P using AAS whiles K was determined using flame photometer.

Determination of total N in plant shoot

Determination of total nitrogen in plant followed the Kjeldahl method as described earlier in Chapter Three, Page 46.

Determination of Soil Microbial Biomass Carbon (MBC), Nitrogen (MBN) and Phosphorus (MBP)

Chloroform fumigation and extraction (FE) protocol was adopted as described by Ladd and Amato (1989).

Determination of MBC and MBN

Ten grams moist incubated soil sample, was passed through a 2 mm mesh, into a crucible and placed in a large vacuum desiccator containing boiling chips (Figure 5). The desiccator was lined with Whatmann No. 42 Filter Paper moist paper to help maintain the water content of soils during fumigation. A beaker containing 30 mL alcohol – free chloroform was placed by it. The crucible containing a control sample (10 g) was placed in a separate desiccator without chloroform. The desiccators were covered, sealed and allowed to stand at room temperature for 5 days (Anderson & Ingram, 1993). Evacuation of trapped chloroform was done after incubation using rotary vacuum pump with water pump connected to the desiccators.

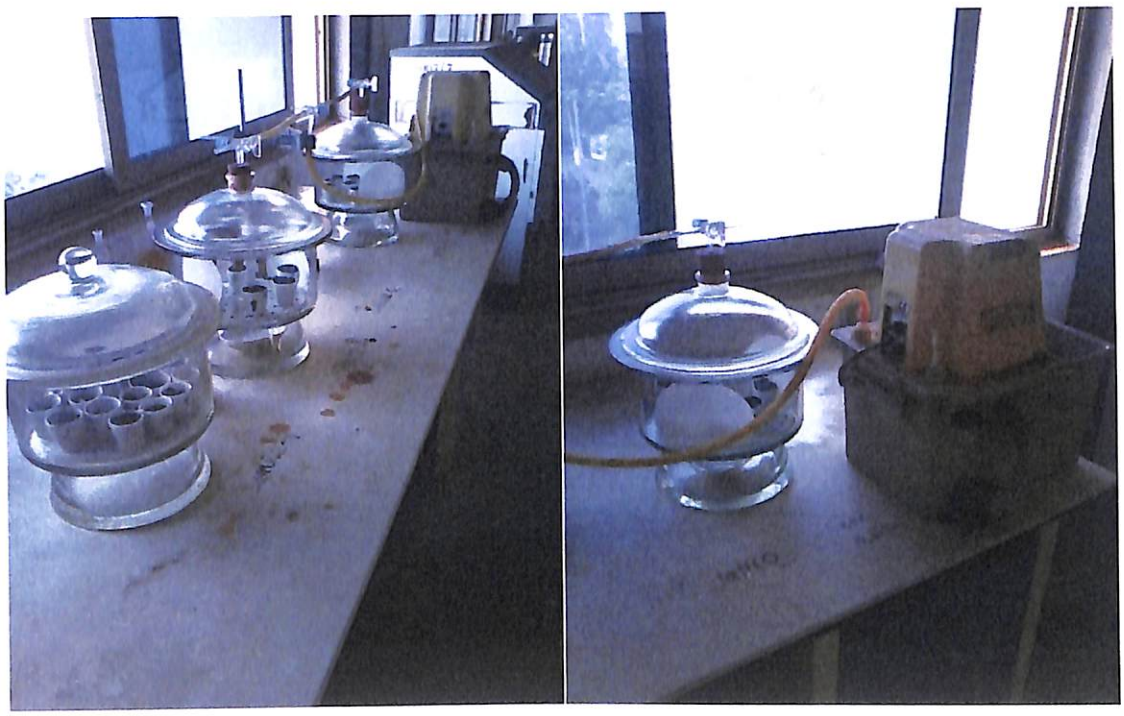

Figure 5: Chloroform fumigation of soil samples

Immediately after fumigation, 50 mL of 0.5 *M* K_2SO_4 solution was added to the soil and shaken. The soil suspension was filtered through a Whatman GF

934-AH filter paper to extract microbial carbon and nitrogen from the lysed microorganisms.

Total nitrogen in the extract was then determined by the Kjeldahl digestion method. The amount of carbon in the extract was determined by wet oxidation procedure described by Walkley and Black (1934). Microbial biomass C and N were calculated from the differences in the amounts of total C and N extracted from fresh soil fumigated with $CHCl_3$ and from the unfumigated control soil. Microbial biomass C and N were calculated using the formulae below;

Microbial biomass C in the soil (MBC):

$$\text{MBC (ug/g soil)} = \frac{\text{CF} - \text{CUF}}{\text{kEC}}$$

Where;

kEC = 0.35 and represents the efficiency of extraction of microbial biomass C (Joergensen, 1995).

Microbial biomass N in the soil (MBN):

$$\text{MBN (ug g} - 1 \text{ soil)} = \frac{\text{NF} - \text{NUF}}{\text{kEN}}$$

where

kEN = 0.5 and represents the efficiency of extraction of microbial biomass N (Joergensen, 1995).

Determination of MBP

Microbial biomass P is calculated from the differences in the amounts of inorganic P (Pi) extracted from fresh soil fumigated (pF) with $CHCl_3$ and from unfumigated soil (upF) (Brookes et al., 1982).

For microbial biomass P analysis, 10 g of field-moist soil was weighed into a crucible and fumigated in a desiccator with 30 mL of alcohol-free chloroform for 5 days. Both fumigated and unfumigated soil samples were shaken with 35 mL Bray's No.1 extracting solution (0.03 M NH_4F + 0.025 M HCl) for 10 minutes and filtered. Correction for adsorption of P to soil colloid during fumigation was made by simultaneously equilibrating unfumigated soil with a series of P containing standard solutions followed by extraction with Bray-1 solution. The amount of chloroform released P was determined by the amount of P added (from standard solutions or microbial lysis) and P extracted by Bray-1 solution (Oberson et al., 1997). The concentration of MBP was determined using the formulae below;

$$\text{MBP (ug/g soil)} = \frac{\text{PF} - \text{PUF}}{\text{kEP}} \times 100/R$$

Where;

kEP = 0.40 and represents the efficiency of extraction of microbial biomass P, and

$$\text{R} = \left(\frac{\text{Pi spiked soil} - \text{soil PUF}}{\text{Pi spike}}\right) \times 100$$

and is the percent recovery of the Pi spike, and Pi spike = 250 *ug* Pi.

Determination of Soil Field Capacity

The field capacity (FC) of the soil sample was determined following procedure described by Anderson and Ingram (1993). For the determination of gravimetric water content at field capacity, a vegetation-free area of 0.5 m × 2 m per plot was covered with a plastic sheet after the soil had drained for 3 days following deep saturation by applied water. Five 0-20 cm depth soil cores were bulked per plot and sub samples of the wet soil weighed. It was then oven-dried at 105 °C for 2 days and the soil reweighed. The gravimetric water content at field capacity (FC) was computed from the relationship:

$$FC\ (\%) = \{(W3 - W2)/(W3 - W1)\} \times 100 \quad \ldots\ldots\ldots\ldots\ldots\ldots\ (5)$$

Where:

W1 = mass (g) of the container

W2 = mass (g) of container and oven-dried soil

W3 = mass (g) of container and wet soil

Soil Microbial Analysis

Soils were analysed for total fungal and total bacterial populations and also for phosphorus solubilising bacteria and fungi.

Soil sampling for microbial analysis

Representative soil samples from each amendment were taken at the end of the experiment using sterile spoons. The soils were stored in sterile zip lock bags and sent to the Molecular Biology and Biotechnology Laboratory, University of Cape Coast for analysis. Soil samples were stored at 4 °C to minimise microbial activity.

Fungal analysis

Fungal analysis was done using soil dilution plate method described by Pages et al. (1982).

Culture media for fungal analysis

General media used for fungal analysis were potato dextrose agar (PDA) and potato dextrose broth (PDB) whiles National Botanical Research Institute's phosphate growth medium (NBRIP) was specifically used to analyze soils for phosphorus solubilizing fungi.

Preparation of PDA and PDB

To prepare PDB, Irish potatoes were peeled and sliced using kitchen knife. Approximately 200 g of the sliced potatoes were weighed on an electronic balance, and boiled in 700 mL distilled water for 30 minutes. The broth was sieved into a 1000 mL measuring cylinder and topped up with distilled water to the 1000 mL mark. The solution was transferred into a flat bottom flask and 20 g of dextrose and agar each were added.

The agar aided in solidifying the medium. No agar was added to the PDB. The solutions were heated in a water bath and swirled gently to obtain a uniform mixture. They were then dispensed into 500 mL conical flasks, corked tightly with cotton wool and aluminium foil. Potato dextrose agar and potato dextrose broth were sterilised by autoclaving at 120 ^{0}C and 0.1 MPa for 15 minutes before use.

Addition of antibiotics

The addition of antibiotics was to inhibit bacterial growth and competition with slow growing fungi in order to increase the chances of isolating fungi. Penicillin G and Streptomycin sulphate antibiotics were added

to the culture media for the isolation of fungi. Penicillin G and Streptomycin sulphate were administered at concentrations of 30 mg mL^{-1} and 133 mg mL^{-1} respectively. It was ensured that antibiotics were filter sterilised before use and media was cooled to about 50 ^0C, before the addition of antibiotics was done.

Isolation of fungi from soil

Approximately 1 g of soil was weighed into sterile test tube containing 9 mL of sterile distilled water and shaken in a vortex for one minute. One millilitre of the agitated solution was taken using a 1 mL pipette into another test tube containing 9 mL of distilled water and shaken with a vortex for a minute. The soil solution was serially diluted up to 10^{-5}.

Aliquot of 1 mL were taken from stock solutions of 10^{-3}, 10^{-4} and 10^{-5} dilutions into a 120 mm Petri dishes. Approximately, 20 mL of the PDA antibiotic mixture was gently poured into the petri dishes containing the aliquot and swirled gently to mix the contents of the plate. Five replications were prepared for each soil sample. Cultures were allowed to solidify under laminar flow hood and incubated at room temperature (25 ± 2) ^0C for 5 days.

Determination of fungal population

Fungal colonies growing on the petri dishes were counted after 5 days using a Quebec colony counter. Colony forming units (cfu) per sample were determined from the formula below and presented as cfu/g of soil (Pages et al., 1982).

$$\text{CFU/g} = \frac{No.of\ colonies \times dilution\ factor}{Dry\ weight\ soil}$$

Identification of fungi

Pure cultures were prepared from fungal colonies. The cultures were preserved in 10 % glycerol at – 20 ^{0}C.

To identify fungal species isolated from soil treatments, the preserved cultures were sub cultured in PDA for 5 days. Identification of the organisms was done using morphological characteristics. Morphological parameters such as colour of the colonies, growth shapes and nutrients were used to identify isolates. Microscopic features used to identify fungal isolates were the reproductive structures such as the spores. Morphological and microscopic examination of the fungal spp were done following the procedures and descriptions made by James and Natalie (2001).

Microscopic observations were done by mounting cultures in lactophenol cotton blue (LPCB) as described by James and Natalie (2001). Lactophenol cotton blue dye was adopted because the lactic acid preserves the fungi structures and cotton blue stains the chitin in the fungal cell walls thereby making the structure more visible.

The identification was achieved by placing a drop of the stain on clean slide. Then with the aid of a sterilised mounting needle, a small portion of the mycelium (mycelial mat) from the fungi cultures was removed and placed on the stained slide. The mycelium was spread very well on the slide using the needle. With the aid of a forcep, a cover slip was gently placed on mycelial mat with little pressure to eliminate air bubbles. The slide was then mounted and observed with x10 and x40 objective lenses respectively. The fungi spp encountered were identified in accordance with Mathur and Kongsdal (2003) and Cheesbrough (2000).

Soil bacterial analysis

Total bacteria in soil was determined using the pour plate technique and the standard plate count (SPC) method (Clesceri et al., 1999). Soil samples were processed in the laboratory for enumeration of viable cell count. Initial isolation was done using nutrient agar media and phosphorus solubilisers were identified using NBRIP media.

Isolation of bacteria from soil

Approximately 10 g of the soil samples were dissolved in 100 mL sterile distilled water and shaken on a vortex for 30 seconds to detach bacterial cells adhered to the soil particles forming soil-water suspension. The suspensions were subjected to sequential dilution up to 10^{-8}. Aliquot (0.1 mL) of serial diluents from 10^{-4} to 10^{-8} were aseptically inoculated onto 20 mL nutrient agar plates after cooling to 50 ^{0}C. Sterile glass spreader was used to spread the culture solution on the media. The samples were incubated at room temperature for 24 to 48 hours.

Estimation of total bacteria population

The number of colonies on the plates were counted and the colony-forming units and recorded as colony-forming units per gram soil (CFU/g) were determined using the formula below;

$$No.of\ viable\ bacteria = \frac{No.of\ colonies\ on\ plates}{volume\ plated} \times \frac{1}{dilution\ factor}$$

Purification and identification of bacterial isolates

Purification of bacterial isolates was done from plates with discrete colonies by sub culturing discrete colonies to obtain pure cultures. Purified isolates were inoculated on nutrient agar slants, labeled and then stored in the refrigerator for further use.

Analysis of phosphorus solubilising organisms

Analysis of phosphorus solubilising bacteria (PSB) and phosphorus solubilising fungi (PSF) were done using the National Botanical Research Institute's phosphate growth medium (NBRIP). Two techniques were observed simultaneously to analyse for the phosphorus solubilizing potential of the isolates. This included the formation of clear halozone on NBRIP agar plates and solubilisation of tricalcium phosphate in NBRIP broth media. Yasser et al. (2014) reported that although some fungi isolates did not show clear zones on selective media, they solubilised appreciable quantities of P in broth media containing tricalcium phosphate.

Briefly, an aliquot (100 µl) from the preserved cultures were plated on NBRIP agar media (pH = 7.0) (contained l^{-1} : glucose, 10 g: $Ca_3(PO_4)_2$, 5 g; $MgCl_2.6H_2O$, 5 g; $MgSO_4.7H_2O$, 0.25 g; KCl, 0.2 g and $(NH_4)_2SO_4$, 0.1 g; Agar, 15 g). The inoculation was accomplished by putting a drop of culture on the NBRIP agar media (drop plate method). The samples were incubated at 28 ± 2 °C in for 7-14 days. Colonies of PSB and PSF showing clear halo zones were noted to have phosphorus solubilising potential.

Quantification of phosphorus solubilized by PSB and PSF

In order to determine the mineral phosphate solubilization activity of PSB and PSF, cultures of the test organisms were added to NBRIP broth.

About 5 % v/v of cultures were inoculated into 100 mL NBRIP culture (pH = 6.5), corked with cotton wool and covered by aluminum foil. No culture was added to the control sample in test tubes. The cultures were incubated for 10 days on rotary shaker (IKA KS 260 basic) at 200 rpm (Muleta et al., 2013). After incubation, the cultures were centrifuged at 10000 rpm for 15 minutes to pellet bacterial cells. Available P was determined by filtering the supernatant through 0.2 μm filter paper. Exactly 2 mL of supernatant was used for available phosphorus determination using Bray 1 extraction method. The amount of P solubilized was determined by deducting the values of soluble P concentration measured in uninoculated control (that is, P released by autoclaving) from P concentration of inoculated media.

Fungal isolates were identified on the basis of colony morphology and microscopic examination (James & Natalie, 2001). Bacteria species were classified as Gram positive ($Gram^{+}$) or Gram negative ($Gram^{-}$) using 3 % KOH.

Statistical Analysis of Data

The results were analysed using statistical products for social scientist (SPSS) for Windows; version 16 (SPSS Inc., Chicago IL, USA). Treatment means were separated using least significant difference (LSD), and treatments effects were declared significant at 1 % and 5 % level of probability. Pearson product-moment correlation analysis was also carried out to establish relationships where necessary.

CHAPTER FOUR

EFFECT OF BIOCHAR AND POULTRY MANURE ON SELECTED CHEMICAL PROPERTIES OF A HIGHLY WEATHERED TROPICAL SOIL

Introduction

Highly weathered tropical soils are known for their low retention capacity of nutrients, high susceptibility to leaching, low fertility and extreme acidity (Van Wambeke, 1992). Crop production on such soils is limited when appropriate management measures have not been put in place. Moreover if external nutrient inputs are not properly managed through synchronization with plant uptake it may lead to environmental pollution.

Sohi et al. (2010) have suggested that adding biochar would potentially increase soil fertility and productivity through reduction in leaching and denitrification in soil. Glaser et al. (2002) has also proposed that due to the nutrient limitations associated with biochar use, it could be co applied with manure for a long term soil fertility management. However, the interactive effect of biochar and organic sources (poultry manure) on soil nutrient mineralization and availability have been limitedly studied (Sohi et al., 2010).

The aim of this work was to evaluate changes in soil organic carbon (SOC) concentration, Bray 1 extractable P, mineral N (NH_4^+ N and NO_3^-N) and ECEC following the application of biochar and manure.

Materials and methods

Feedstock and biochar preparation processes are as described in Chapter Three. The biochar was characterized for pH, total carbon, total nitrogen, total phosphorus, C:N ratio, C:P, total (calcium, magnesium, potassium, sodium, Fe, Cu, Zn, Pb and As), as described earlier in chapter Three of this thesis.

The soil used was analyzed for pH, total carbon, total nitrogen, available P, total P, exchangeable cations (Ca^{2+}, Mg^{2+}, K^{+}, Na^{+}), Exchangeable acidity (Al^{3+} + H^{+}), ECEC, Total (Fe, Cu, Zn) following protocols described earlier in chapter Three of this thesis.

The manure was analyzed for pH, total carbon, total nitrogen, total phosphorus, C:N ratio, total (calcium, magnesium, potassium, sodium, Fe, Cu and Zn).

Experiment One

The effect of different types and fractions of biochar co applied with manure on selected soil chemical properties was investigated in completely randomized design (CRD), with biochar and poultry manure as the experimental factors. This incubation experiment included a total of fourteen completely randomized treatments with six replicates (14×6) kept at the School of Agriculture Teaching and Research Laboratory, University of Cape Coast. Biochar was solely applied to soil at rates of 0, 39 and 65 t ha^{-1}, biochar combined with poultry manure at rates of 0 and 10 t ha^{-1} and poultry manure solely applied at 10 t ha^{-1}.

The treatments were as shown in Table 1.

Table 1: *Treatments used to evaluate the Effect of Biochar and Manure on Soil Chemical Properties*

Code	Treatment
1	Control (no biochar, no poultry manure)
2	39 t ha^{-1} CCB
3	39 t ha^{-1} CHB
4	39 t ha^{-1} PMB
5	65 t ha^{-1} CCB
6	65 t ha^{-1} CHB
7	65 t ha^{-1} PMB
8	10 t ha^{-1} poultry manure
9	39 t ha^{-1} CCB + 10 t ha^{-1} poultry manure
10	39 t ha^{-1} CHB + 10 t ha^{-1} poultry manure
11	39 t ha^{-1} PMB + 10 t ha^{-1} poultry manure
12	65 t ha^{-1} CCB + 10 t ha^{-1} poultry manure
T13	65 t ha^{-1} CHB + 10 t ha^{-1} poultry manure
T14	65 t ha^{-1} PMB + 10 t ha^{-1} poultry manure

CCB: corn cob biochar, CHB: cocoa husk biochar, PMB: poultry manure biochar

The above mentioned amendments were thoroughly mixed with 1 kg equivalent air-dried soil (except the control) and packed into individual plastic cylindrical pots to achieve a bulk density of 1.3 gcm^{-3}. All the pots were then wetted up to 60 % of field capacity using distilled water. The pots were kept at the greenhouse and watered weekly to maintain water content at 60 % of field capacity using distilled water.

Soil analyses

Destructive sampling technique was used to sample soils on the 3rd, 7th, 14th, 28th, and 42nd days after incubation (DAI) and analysed in the laboratory for percent soil organic carbon, mineral N (NH_4^+, NO_3-), available P and ECEC. The laboratory analyses were done following standard procedures as described in Chapter three.

Data analyses

Data was analyzed using SPSS software (version 16.0). The results were presented as mean ± standard deviation (SD). The resulting data were subjected to Post hoc procedure at $P < 0.05$ to separate the means of treatments. Pearson's moment correlation was used to determine how the soil properties were related and also establish the relationship between treatments and soil properties. Results have been presented in Tables.

Results and Discussion

Initial characteristics of soil and amendments

Experimental soil, biochar and poultry manure were initially characterised and the results have been summarized in Tables 2, 3, 4 and 5.

Table 2: *Chemical Properties of Experimental Soil (Mean ± SD)*

Soil properties (0–20 cm)	Mean value	SD (±)
pH ($soil_1$:$water_{2.5}$)	4.17	0.01
Organic Carbon (%)	0.35	0.03
Total N (%)	0.05	0.01
Available P (mg kg^{-1})	1.05	0.001
Total P (%)	0.30	0.02
Exchangeable Ca^{2+} ($cmol_c kg^{-1}$)	0.27	0.002
Exchangeable Mg^{2+} ($cmol_c kg^{-1}$)	0.15	0.002
Exchangeable K^+ ($cmol_c kg^{-1}$)	0.20	0.004
Exchangeable Na^+ ($cmol_c kg^{-1}$)	0.08	0.001
Exchangeable acidity (Al^{3+} +H^+)	1.43	0.008

Table 3: *Physical Properties of Experimental Soil (Mean ± SD)*

Parameter	Mean value	± SD
Particle size distribution		
Sand (%)	92.9	1.2
Silt (%)	2.6	1.0
Clay (%)	4.53	1.2
Bulk density (gcm^{-3})	1.3	0.01

Adapted from Atiah (2012)

Results on the initial properties of the soil used for the experiment have been summarised in Table 2 and 3. The pH of the soil (4.17) was strongly acidic. Soil organic carbon content was low, an indication of low organic matter content and low microbial numbers and low microbial activity

(Rigobelo & Nahas, 2004). Total N was also low due to low organic matter of the soil. Although considerable amount of total P was obtained, available P was very low due to likely complexation reaction with Al and Fe ions in the experimental soil which had a low pH (Brady & Weil, 2007).

Exchangeable cations (Ca^{2+}, Mg^{2+}, K^{+}, Na^{+}) were relatively low which could also be related to the pH of the soil as well as the low organic matter content. Low exchangeable bases could also be due to high leaching of bases and moreover it could be related to low basic cations in parent material from which the experimental soil was formed. Exchangeable Ca^{2+} found in the experimental soil was 0.27 $cmol_c kg^{-1}$ just slightly above the critical level (0.2 $cmolckg^{-1}$) suggested by Rowell (1994). Similarly, exchangeable Mg^{2+} recorded low amounts compared with the critical level (0.10-0.15 $cmol_c kg^{-1}$) required for normal physiological function of crops. Amounts of exchangeable K^{+} and Na^{+} were also low (Rowell, 1994). These properties of the experimental soil require that sustainable corrective measure is embarked on to make it productive. The soil was classified as sandy (Atiah, 2012).

Summarised in Table 4 are the initial chemical composition of the poultry manure.

Table 4: *Properties of Poultry Manure used for the Study (Mean ± SD)*

Parameter	Mean	SD (±)
pH (in water)	7.63	0.01
Total C (%)	33.9	0.47
Total N (%)	3.18	0.09
C:N	10.93	0.26
Total P (%)	1.24	0.02
Ca (%)	3.75	0.06
Mg (%)	1.13	0.06
K (%)	1.36	0.01
Na (%)	0.64	0.001
Fe (%)	0.2467	0.01
Cu (%)	0.49	0.02
Zn (%)	2.29	0.03

The manure was slightly alkaline with a pH of 7.63 similar to that reported by Wortmann and Shapiro (2012). The latter authors reported respective pH range of 6.5 to 8.0 and 6.0 to 8.5. Similarly, Boateng et al. (2006) reported a range of 6.80 to 8.40 with a mean of 7.70. The pH of the poultry manure informed its application to the experimental soil probably because it could augment the pH of the soil. High organic carbon content (33.90 ± 0.47 %) was found. The manure contained high amount of N (3.18 ± 0.09 %) which is comparatively higher than (2.42 %) reported by Boateng et al. (2006). The manure demonstrated C:N ratio of 10.93 ± 0.26 lower than the C:N value (10.9) reported by Adelekan et al. (2010) and the range; 11.30 –

13.80, submitted by Boateng et al. (2006) when they investigated the effect of poultry manure on the yield of maize. The lower C:N ratio has an advantage of preventing microbial immobilisation upon application to the experimental soil. It is also suggestive that it could help in the reduction in N volatilisation when applied together with some biochar which have high carbon but low N content. The Ca, Mg and K content were relatively high with values of 3.75 %, 1.13 % and 1.36 % respectively. Previous research by Adelekan et al. (2010) reported very low calcium concentration of 3.04 mg kg^{-1}. The characteristics of the manure are dependent on the diet fed to the birds, the age of the birds and the age of the manure. Appreciable quantities of Copper, zinc and arsenic were estimated in the manure and the source could be traced to the feed given to the birds. These elements are commonly added to poultry feed in trace amounts as part of the diet to optimize bird growth and performance (Rutherford et al., 2003). The high OC, TN, TP and basic cations coupled with low C:N ratio makes it appropriate to be used in combination with biochar to revamp the productivity of a nutrient depleted soil.

The characteristics of the biochar used in the study are presented in Table 5.

Table 5: *Characteristics of Biochar (Mean ± SD)*

Property	PMB	CCB	CHB
pH ($biochar_1$: $water_5$)	10.14 ± 01	9.57 ± 0.02	10.31 ± 0.01
Total C (%)	48.01 ± 1.86	89.63 ± 2.25	75.80 ± 4.92
Total N (%)	1.90 ± 0.07	0.38 ± 0.045	0.52 ± 0.05
Total P (%)	1.18 ± 0.03	0.21 ± 0.014	0.18 ± 0.020
C:N	25.30	235.88	145.78
C:P	40.68	426.84	421.13
Calcium (g kg^{-1})	18.20 ± 011	4.07 ± 0.015	6.70 ± 0.007
Magnesium (g kg^{-1})	9.55 ± 0.05	3.75 ± 0.384	7.64 ± 0.467
Potassium (g kg^{-1})	11.95 ± 0.02	8.78 ± 0.005	17.06 ± 0.010
Sodium (g kg^{-1})	8.59 ± 0.060	7.36 ± 0.010	9.48 ± 0.026
Iron (%)	0.28 ± 0.01	0.01 ± 0.001	0.011 ± 0.001
Copper (%)	0.01 ± 0.001	< 0.01	<0.01
Zinc (%)	0.03 ± 0.001	< 0.01	< 0.01
Lead (%)	<0.01	< 0.01	< 0.01
Arsenic (%)	0.02 ± 0.001	< 0.01	< 0.01

PMB = poultry manure biochar; CCB = corn cob biochar; CHB = cocoa husk biochar

The pH of all the biochar produced (PMB, CCB and PMB) were highly alkaline with respective pH values of 10.1 ± 01, 9.6 ± 0.02 and 10.3 ± 0.01. The pH of the biochars' produced were similar to the values reported in other studies. For instance the pH of biochar produced from different agricultural feed stock materials at 300 °C to 400 °C by slow pyrolysis method ranged from 7.9 to 11.8 and is all within alkaline range (Kannan et al., 2013).

Chan et al. (2008) also reported pH values of 9.9 and 13 for poultry litter biochar produced at 450 °C and 550 °C, respectively. The high pH is attributable to the feedstock, pyrolysis condition and ash content. The ash content is often dominated by majority of carbonates, specifically calcium, magnesium and potassium carbonates, which resist decomposition even at higher temperatures (750 ^{0}C) (Enders et al., 2012).

High concentration of total C was found in the biochar. Of the three biochar, CCB had the highest total C content of 89.63 ± 2.25 % followed closely by CHB and poultry manure with total C content of 75.80 ± 4.92 % and 48.01 ± 1.86 % respectively. Similar values have been reported by previous researchers (Chan & Xu, 2009; Sun et al., 2014). Chan and Xu (2009) reported total C range between 175 g kg^{-1} to 905 g kg^{-1}. There was marked variation of total N content amongst the three biochar, with PMB having the highest concentration of 1.90 ± 0.07 % compared with CHB and CCB having respective N content of 0.52 ± 0.05 % and 0.38 ± 0.05 %. The highest total P (1.18 ± 0.03 %) concentration was found in PMB followed by CCB (0.21± 0.014 %) and subsequently CHB (0.18± 0.02 %). It is expected that, although high total C of the three biochar was observed, with high C/N and C/P ratios, especially for CCB and CHB, its recalcitrant nature (Lehmann & Joseph, 2009) would prevent mineralisation of N, P and C. The recalcitrant of biochar C results from the conversion of C in feedstock to recalcitrant C in biochar during pyrolysis.

Effect of amendments on soil organic carbon (SOC)

The effect of biochar solely applied and in combination with poultry manure application on SOC (%) is presented in Table 6.

Table 6: *Soil Organic Carbon (%) following application of Soil Amendments*

Treatment	3 Days	7 Days	14 Days	28 Days	42 Days
Control	0.36 (0.02)s	0.37 (0.02)r	0.38 (0.02)r	0.37 (0.03)rs	0.32 (0.01)t
39 t ha^{-1} CCB	0.60 (0.03)op	0.73 (0.02)m	0.73 (0.01)m	0.66 (0.02)no	0.58 (0.03)p
39 t ha^{-1} CHB	0.57 (0.02)pq	0.72 (0.02)m	0.74 (0.01)lm	0.69 (0.02)n	0.63 (0.02)o
39 t ha^{-1} PMB	0.67 (0.02)n	0.68 (0.02)n	0.74 (0.02)lm	0.76(0.02)lm	0.80(0.02)k
65 t ha^{-1} CCB	0.54(0.02)q	0.81(0.03)k	0.83(0.01)jk	0.80(0.02)k	0.76(0.05)lm
65 t ha^{-1} CHB	0.73(0.05)m	0.83(0.02)jk	0.85(0.02)j	0.79(0.03)kl	0.75(0.03)lm
65 t ha^{-1} PMB	0.80(0.03)k	0.87(0.02)ij	0.87(0.02)ij	0.89(0.02)ij	0.89(0.01)i
10 t ha^{-1} poultry manure	0.63(0.02)o	0.67(0.02)n	0.67(0.02)n	0.67(0.02)n	0.68(0.03)n
39 t ha^{-1} CCB + 10 tha^{-1} poultry manure	1.10(0.02)g	1.18(0.02)e	1.20(0.01)de	1.20(0.01)de	1.21(0.02)d
39 t ha^{-1} CHB + 10 tha^{-1} poultry manure	1.10(0.02)g	1.19(0.02)de	1.20(0.02)de	1.21(0.02)d	1.22(0.02)cd
39 t ha^{-1} PMB + 10 tha^{-1} poultry manure	1.14(0.02)f	1.19(0.02)de	1.21(0.02)d	1.22(0.02)cd	1.24(0.01)cd
65 t ha^{-1} CCB + 10 tha^{-1} poultry manure	1.11(0.02)fg	1.18(0.01)e	1.21(0.04)d	1.24(0.01)cd	1.24(0.02)cd
65 t ha^{-1} CHB + 10 tha^{-1} poultry manure	1.14(0.02)f	1.21(0.02)d	1.22(0.02)cd	1.23(0.01)cd	1.24(0.01)cd
65 t ha^{-1} PMB + 10 tha^{-1} poultry manure	1.18(0.02)e	1.28(0.03)b	1.31(0.03)ab	1.31(0.03)ab	1.34(0.03)a

Mean (standard deviation in parenthesis); Means followed by the same letter do not differ significantly from one another ($p \leq 0.05$); CCB - Corn cob biochar, CHB – Cocoa husk biochar, PMB – Poultry manure biochar

The results showed that soil organic carbon (SOC) concentrations in the sole biochar, sole poultry manure and combined biochar with poultry manure increased significantly ($P < 0.05$) above the control throughout the incubation period (Table 6). The increase in SOC varied with type of biochar, biochar rate and time of incubation.

Percent soil organic carbon (SOC) increased in all treatments except the control treatment which is indicative of low organic matter content hence needed to be corrected to make it productive. By the 42nd day, SOC in the control treatment had reduced below the initial concentration measured on the 3rd day of the incubation.

Regarding type of biochar and time effect, the elevation of the SOC concentration in the soil sample amended with PMB was highest by day 42 compared with that of CCB and CHB amended soils. It was observed that CCB and CHB soils demonstrated a rapid increase of SOC in the early stages (3rd and 7th day) which peaked on day 14 and started to decrease till the end of the incubation. On the other hand, PMB showed rapid rise on day 3, and marginal increases were demonstrated for the rest of the incubation. By the 42nd day however, the SOC concentration observed for all three biochar respectively were significantly higher than the control. The higher SOC in PMB amended soil may be as a result of the PMB having more labile C thereby increasing the SOC content compared with CCB and CHB. This was confirmed in a previous study when Singh and Cowie (2014) concluded that manure based biochar increased SOC in soil compared with wood based biochar.

By day 42, PMB applied at 39 and 65 t ha^{-1}, had respectively contributed 150 % and 178.1 % increase in SOC to experimental soil. Similarly CHB increased SOC by 96.9 % and 134.4 % at respective rates of 39 and 65 t ha^{-1} in experimental soils. Then again, CCB amended soils also displayed similar increases of SOC concentration of 81.3 % and 137.5 % at application rates of 39 and 65 t ha^{-1} respectively.

Another observation made was that SOC concentration changed with time. At the early stage of the incubation (day 3 and 7), SOC concentrations increased rapidly in CCB and CHB amended soils and peaked on the 14th day, then started decreasing till the end of the incubation. By the 42nd DAI, SOC concentrations in CCB amended soil was not significantly different compared with the initial concentration measured on the 3rd day. Soil organic carbon in CHB soil on the other hand was significantly different by the 42nd DAI in relation with the initial concentration (3rd day). Similar trend was observed following the application of CCB and CHB at rates of 65 t ha^{-1}. In contrast, the trend of SOC concentration observed in PMB amended soils at 39 and 65 t ha^{-1} showed significant increases till the end of the incubation period with rapid increases in the early stage of the incubation (days 3).

The general increases of SOC in biochar soil revealed in the current study may be as a result of the addition of labile carbon or the mineralisation of recalcitrant C in biochar material applied to soil as well as positive priming effect (Hamer et al., 2004; Kuzyakov et al., 2009). Pietikäinen et al. (2000) reported that biochar may affect microbial proliferation which intend may contribute to degradation of biochar and subsequently affect carbon release. Then again, the addition of biochar might cause positive priming stimulating

the mineralisation of native soil organic carbon (Kuzyakov et al., 2009). In addition, biochar added to soil could contain an appreciable amount of labile C as well as the degradation of some part of recalcitrant C by microbes (usually about 5 % is degraded) (Brodowski, 2005; Cross & Sohi, 2011). These mechanisms might have led to increment in SOC.

The initial rapid increase in SOC (measured on day 3 and 7) upon application of the amendments, could be linked to the proliferation of microbes called r-strategist' microbes that are adapted to respond quickly to newly available C sources, remineralizing soil nutrients and co-metabolizing more refractory organic matter (Kuzyakov et al., 2009). The mineralisation by r-strategist' microbes at the early stages of the incubation coupled with labile C in biochar material and co metabolism, resulted in the rapid rise in SOC at the early stage of the incubation.

As the study progressed, SOC decreased in CCB and CHB soils and could be explained by the exhaustion of readily available C as well as degradable C in biochar probably as a result of microbial assimilation and loss of C in the form of CO_2. Significant microbial biomass C was measured in the current study (Table 12) and could be a probable reason for the decrease in SOC measured in CCB and CHB soils due to increased SOC utilization. Biochar enhances the increase in microbial biomass and this consequently increased the demands on soil organic C. Singh and Cowie (2014) explained that the vast majority of soil microbes require organic carbon compounds to oxidize for energy and to build the organic constituents of their cell bodies. Then again, as mentioned earlier and supported by Singh and Cowie (2014), CCB and CHB used in this study, which are wood base, do not have a high

labile C content and could be a possible cause for the reduction in SOC in such soils with time (Anderson & Domsch, 1989). Meanwhile, PMB which showed consistent increase in the concentration of SOC throughout the experiment could be linked with higher labile C in PMB material increasing the SOC content as compared with low labile C property of CCB and CHB. Singh and Cowie (2014) supported the finding of this study when they also reported that manure based biochar increased SOC in soil compared with wood based biochar.

The SOC content in combined biochar and manure was higher than in sole biochar soil which indicates a more pronounced mineralisation of SOC associated with adding manure. Combining the two materials resulted in additive effect with consequent release of significant amount of SOC. At 39 and 65 t ha^{-1} of biochar rates in combination with poultry manure, SOC increased in all treatments but the increase in combined PMB fractions with poultry manure amended soils were significantly higher ($P < 0.05$) than measured for CCB- and CHB - poultry manure combinations throughout the experiment. At 39 t ha^{-1} in combination with CCB, CHB and PMB, respective SOC concentrations measured were 278.1 %, 281.2 % and 287.5 %. When the rate of biochar was increased to 65 t ha^{-1} in the combination, SOC also increased by 287.5 %, 287.5 % and 318.8 % respectively.

The increase in the concentration of SOC can be attributed to the properties of the manure, biochar and soil used for the study. Each of these entities contributed to the elevated concentration of SOC in the soils through additive effect as well as co metabolism. This result confirms the assertion of Lehmann et al. (2003) that adding manure with biochar would potentially

increase bioavailable C in the soil. The elevation of SOC could also be associated with the increase in microbial biomass as a result of the application of poultry manure and biochar (Table 15). The microbes are added with the addition of the poultry manure. Then again, the decomposition of added poultry manure resulted in availability of higher amounts of mineralizable nitrogen and carbon for microbial utilisation and proliferation (Malik et al., 2013). Biochar on the other hand has been shown by several researchers to have a positive relationship with microbes by protecting microbes or changing the soil environment to aid microbial proliferation (Lehmann et al., 2011). In this study microbial population (both bacteria and fungi) had a strong positive relationship (Appendix D) with SOC (measured on day 42). The increased microbial numbers enhanced microbial decomposition of both poultry manure and biochar; thus resulting in the increased SOC concentration in the biochar-poultry manure amendments. The comparatively higher SOC concentration in combined PMB and manure amended soils above that of CCB- and CHB-manure treated soils in this study could be attributed to presence of more labile carbon in the PMB and manure mixture compared with CCB- and CHB-manure mixture.

The sharp initial SOC increment in the combined treatment could be related to labile C, priming effect and co metabolism due the addition of fresh C. Biochar mineralization has previously been found to be, at times, positively primed by the addition of a labile C source (Hamer et al., 2004), soil humus (Wardle et al., 2008) and whole soil (Kuzyakov et al., 2009). Kuzyakov et al. (2009) observed that the biochar in soil underwent increased decomposition upon the addition of glucose to the soil. This might have happened in the

current study when biochar was applied together with poultry manure leading to the elevation of SOC. Biochar decomposition rates increased due to the availability of easily degradable C-rich substrate. The decomposition of the BC might have resulted from the action of metabolites of the microorganism enhanced by the application of manure. Additionally, Nguyen and Lehmann (2009) reported that higher temperature associated with both manure and biochar also increased biochar oxidation and thus decomposition.

Simultaneously, whiles biochar decomposition could be taken place, manure was as well getting decomposed. The process is referred to as 'co metabolism'. It could be explained that biochar provides habitats for microbes, thereby enhancing microbial activity (Steiner et al., 2011) to decompose poultry manure releasing SOC into immediate soil environment. Adhikari et al. (2009) explained that biochar provides benefits on accelerating composting by acting as a biodegradable carbon and energy source for supporting microbial activity and balancing the initial C:N ratio of the mixture.

The steady rise in SOC in PMB soils and combined biochar and manure soils after the 7^{th} day till the end of the incubation could be related to the reduction in labile C and readily degradable C concentration. This simultaneously triggered the commencement of slow decomposition of recalcitrant C in both biochar and manure used. This explains the slow steady increase in SOC after incubation time. Buttressing this explanation, it has been reported that more complex substrates such as cellulose or straw (Wu et al., 1993; Shen & Bartha, 1997) is known to stimulate K-strategists', microbes. These microbes release extracellular enzymes needed to breakdown complex

biopolymers such as found in biochar consequently releasing it nutrients gradually.

Effect of amendments on net N mineralisation

Soil mineral nitrogen (NH_4^+, NO_3^-) as affected by biochar and/or poultry manure biochar combinations have been summarised in Tables 7 and 8.

Table 7: *Ammonium - N Mineralisation Dynamics in Soil (mg kg^{-1})*

Treatment	3 Days	7 Days	14 Days	28 Days	42 Days
Control	3.39 (0.24)w	3.55 (0.34)w	3.33 (0.12)w	3.30 (0.14)w	3.54 (1.20)w
39 t ha^{-1} CCB	7.40 (0.51)tu	8.26 (0.48)tu	5.87 (0.14)uw	5.75 (0.22)uw	5.29 (0.22)uw
39 t ha^{-1} CHB	9.04 (0.48)tu	9.91 (0.22)st	6.62 (0.51)u	5.67 (0.45)uw	5.15 (0.12)uw
39 t ha^{-1} PMB	11.05 (0.26)rs	12.82 (1.13)qr	13.42 (0.67)qr	13.67 (0.36)qr	14.72 (0.96)qr
65 t ha^{-1} CCB	11.25 (0.63)rs	12.20 (0.40)r	8.39 (0.76)tu	6.23 (1.06)u	5.15 (0.73)uw
65 t ha^{-1} CHB	13.08 (1.10)qr	13.64 (0.86)qr	9.40 (1.02)t	8.32 (0.56)tu	4.57 (0.51)uw
65 t ha^{-1} PMB	14.86 (0.44)q	17.38 (0.50)pq	18.01 (0.38)p	18.58 (1.09)p	19.53 (0.15)p
10 t ha^{-1} poultry manure	8.20 (0.56)tu	10.75 (1.03)s	13.43 (1.29)qr	9.82 (0.64)t	9.05 (0.71)tu
39 t ha^{-1} CCB + 10 tha^{-1} poultry manure	18.99 (0.76)p	30.22 (2.44)n	46.42 (2.28)j	56.27 (1.72)f	59.07 (1.66)e
39 t ha^{-1} CHB + 10 tha^{-1} poultry manure	22.24 (1.35)o	36.82 (1.27)l	44.94 (2.28)j	52.78 (1.43)gh	57.99 (1.96)ef
39 t ha^{-1} PMB + 10 tha^{-1} poultry manure	33.53 (1.48)o	50.09 (2.84)i	70.57 (2.31)c	77.67 (2.09)b	82.60 (3.03)a
65 t ha^{-1} CCB + 10 tha^{-1} poultry manure	23.91 (1.61)o	34.09 (1.81)m	39.16 (2.04)kl	54.44 (0.89)fh	57.10 (5.80)ef
65 t ha^{-1} CHB + 10 tha^{-1} poultry manure	19.56 (1.52)p	29.97 (1.44)n	36.42 (1.13)lm	45.82 (1.69)j	48.90 (3.41)ij
65 t ha^{-1} PMB + 10 tha^{-1} poultry manure	29.83 (2.54)n	41.23 (2.48)k	44.85 (2.39)j	58.51 (1.62)ef	65.08 (2.29)d

Mean (standard deviation in parenthesis); Means followed by the same letter do not differ significantly from one another ($p \leq 0.05$). CCB: corn cob biochar, CHB: cocoa husk biochar, PMB: poultry manure biochar

Table 8: *Nitrate - N Mineralisation Dynamics in Soil (mg kg^{-1})*

Treatment	3 Days	7 Days	14 Days	28 Days	42 Days
Control	2.15 (0.03)s	2.17 (0.05)s	2.21 (0.05)s	2.45 (0.08)s	2.57 (0.07)s
39 t ha^{-1} CCB	2.14 (0.03)s	1.50 (0.03)s	1.44 (0.03)s	1.41 (0.02)s	1.40 (0.02)s
39 t ha^{-1} CHB	3.05 (0.02)s	2.63 (0.02)s	2.82 (0.03)s	1.53 (0.07)s	1.95 (0.03)s
39 t ha^{-1} PMB	6.64 (0.54)rs	13.80 (0.03)q	19.18 (0.04)p	20.70 (0.02)op	20.83 (0.02)op
65 t ha^{-1} CCB	2.41 (0.02)s	2.00 (0.03)s	1.72 (0.03)s	1.26 (0.06)s	1.18 (0.03)s
65 t ha^{-1} CHB	4.82 (0.03)rs	4.69 (0.02)rs	4.21 (0.10)rs	3.72 (0.15)s	2.01 (0.12)s
65 t ha^{-1} PMB	7.50 (0.02)rs	12.47 (0.98)qr	22.61 (1.01)op	23.91 (2.01)o	24.16 (1.00)o
10 t ha^{-1} poultry manure	7.69 (0.98)rs	12.88 (1.02)qr	16.95 (0.94)pq	13.78 (1.19)q	13.34 (0.59)q
39 t ha^{-1} CCB + 10 tha^{-1} poultry manure	8.74 (1.38)r	35.16 (1.45)m	57.07 (1.62)k	72.17 (1.84)i	88.13 (1.23)g
39 t ha^{-1} CHB + 10 tha^{-1} poultry manure	12.00 (0.45)qr	43.91 (1.78)l	73.00 (2.46)i	81.98 (2.58)h	90.42 (2.65)g
39 t ha^{-1} PMB + 10 tha^{-1} poultry manure	16.82 (1.77)pq	64.98 (2.06)j	95.47 (1.85)f	118.31 (3.91)e	126.52 (2.66)d
65 t ha^{-1} CCB + 10 tha^{-1} poultry manure	13.26 (1.66)qr	44.57 (1.91)l	62.31 (0.85)j	95.34 (2.48)f	116.42 (4.06)e
65 t ha^{-1} CHB + 10 tha^{-1} poultry manure	18.90 (1.43)p	66.70 (0.76)j	97.24 (1.28)f	130.58 (3.57)d	141.08 (3.44)c
65 t ha^{-1} PMB + 10 tha^{-1} poultry manure	30.29 (2.50)n	71.87 (1.80)i	130.55 (9.68)d	152.10 (3.25)b	164.82 (2.90)a

Mean ± (Standard error of mean in parenthesis), mean values followed by same letters are not significantly different according to DMRT at $P < 0.05$. CCB: corn cob biochar, CHB: cocoa husk biochar, PMB: poultry manure biochar

As shown in Table 7 and 8, the concentration of mineral N (NH_4^+, NO_3^-) in the unamended soil remained low constantly throughout the incubation period. This could imply that net N mineralisation in soil used for the experiment prior to the addition of biochar and/or poultry manure was minimal. Relatively, the concentration of NH_4^+-N was higher than the NO_3^--N in the control throughout the duration of the experiment which could be due to lower nitrification activity. The minimal net mineralisation in the control soil could be related to low substrate especially C and N originally in the soil (Table 2) with consequent low mineral N released. Then again, the soil used for the experiment was extremely acidic, and this might have resulted in low microbial population in the control soil (Table 15), with consequent slow biological transformation of N and lower mineral N concentration. It has been suggested that the population and activity of microbes involved in the mineralisation of N in soil is affected by pH changes. Soil pH is known to have a considerable effect on the activity and diversity of soil ammonia oxidizers (de Boer & Kowalchuk, 2001) and that the absence of nitrification activity in some highly acidic soils is the result of ammonia oxidising bacteria (AOB) sensitivity to low pH (de Boer & Kowalchuk, 2001). Nicol et al. (2008) reported that AOB abundance decreased significantly with decreasing pH, indicating that pH was an important factor controlling AOB abundance in the soil. The slight steady increase in mineral N (NH_4^+, NO_3^-) observed in the control could be related to the activity of some acid tolerant strains of AOB and to large extent Ammonia oxidising Archaea (AOA) (Yao et al., 2011). Gubry-Rangin, et al. (2010) reported that almost no nitrification could be detected at pH (H_2O) values lower than 4.0.

The addition of sole biochar to soil led to insignificant increases in net ammonification and nitrification in CCB and CHB amended soils. Soil samples that received CCB and CHB showed significantly increased NH_4^+-N concentration up to the 7th day and started decreasing consistently till the 42nd day. By the 42nd day, the concentration of mineral N (NH_4^+, NO_3^-) in soil samples amended with both (CCB, CHB) biochar were respectively not significantly ($P < 0.05$) different from the control. This trend was observed at all application rates (39 and 65 t ha^{-1}) of biochar. More so, NO_3^--N measured in CCB and CHB treated soils at all biochar rates were lower than the control by the 42nd DAI. PMB treated soil on the other hand had significantly elevated concentrations of mineral N (NH_4^+, NO_3^-) than the control as well as CHB and CCB treated soils by day 42 of the experiment. Regarding NO_3^--N, apart from day 3 where NO_3^--N concentrations in PMB treated soils showed no significant differences compared with the control, there were significant increase for the rest of the incubation period (measured on 7 to 42 DAI).

The initial increase of NH_4^+-N could be associated with the addition of fresh N in CCB and CHB material. Cross and Sohi, (2011) submitted that biochar is not biologically inert when added to the soil and follows a biphasic mineralization pattern, with the more labile biochar compounds being mineralized rapidly, after which biochar degradation continues at a much slower rate.

The consistent decrease in NO_3^--N measured in CCB and CHB treated soils, implied less net nitrification activity and evidently it could be seen from Table 7 and 8, that NH_4^+-N concentration was higher relative to NO_3^--N throughout the experiment. The decreased nitrification rate could be attributed

to nitrification inhibitors which slowed the rate of NH_4^+ N mineralization. Biochar has been shown to contain microbially toxic compounds (e.g. polyaromatic hydrocarbons), some of which may inhibit the *Nitrosomonas* bacteria responsible for nitrification (Kim et al. 2003; Clough & Condron, 2010). Clough and Condron (2010) reported nitrification inhibition before 55 days after soil incubation, as weathering of the biochar decreased its ability to inhibit nitrification after the 55th day.

Then again, the decrease in both NH_4^+-N and NO_3^--N concentrations could be attributed to the adsorption of NH_4^+-N or NO_3^--N onto biochar surfaces or loss of mineral N as ammonia gas. When this happen, there is a low NH_4^+ N concentration available for nitrification to take place resulting in the decreased concentration of NH_4^+ N and subsequently NO_3^- N as the study progressed. These explanation is supported by some researchers who indicated that biochar has a strong affinity for NO_3^--N (Mizuta, 2004) and NH_4^+-N (Lehmann et al., 2002) causing a reduction in available N.

More so, the decreasing concentration of mineral N could be due to immobilisation by microbial biomass (Table 13). Zackrisson et al. (1996) explained that there is a rapid response of the nitrifier community toward addition of biochar, with fresh carbon, to soils with low initial nitrification activity. The addition of biochar increased microbial population (Table 15) in this study which might include nitrifying microbes. This confirmed the report of Lehmann et al. (2011) that biochar addition to soil may offer a suitable habitat for the proliferation of microbes. Although some heterotrophic microbes could survive and grow in acidic environments, autotrophic nitrifying bacteria are favoured by less acidic soil conditions (pH > 5.0). This

suggests that the modifying effect of biochar on pH in this experiment possibly stimulated the proliferation of the nitrifier community. Coupled with higher C/N ratio of biochar materials applied, increasing ammonifier and nitrifier population caused immobilisation of mineral N in CCB and CHB amended soils as the days progressed. The C/N ratio was 35.80, 235.88 and 145.78 for PMB, CCB and CHB respectively. In support, Deenik et al. (2009) also posited that an increase in microbial activity due to bioavailable C in biochar and the resultant immobilization of N was a possibility for reduction in mineral N concentrations.

On the other hand, soil samples amended with PMB showed higher mineral N concentration throughout the experiment. This means that there was net nitrification in soils used for this study as a result of application of PMB. The net nitrification rate is as a result of higher N content and relatively lower C/N ratio of the PMB used in this study. It is indicative that as the study progressed, there was availability of N substrate for mineralization to continue till the 42nd day of the experiment, although microbial immobilisation simultaneously took place (Table 13). This gives evidence of the suggestion that biochar prepared from poultry manure can be a source of N required for plant growth (Gaskin et al., 2008; Nelson et al., 2011). Moreover, the result from this study is indicative that the addition of high-N biochar may overcome the problems associated with N immobilization. Although immobilization took place, there was adequate N for mineralization to also take place. The addition of manure based biochar, with high N contents has been found to result in net N mineralization (Schouten et al., 2012; Wang et al., 2012). However, N mineralization rates might be reduced by the adsorption of NH_4^+ or NO_3^- onto

the biochar surface due to increased cation exchange capacity (CEC) or anion exchange capacity (AEC) (Clough & Condron, 2010).

In addition, enhanced native SOM mineralization, called priming effect, due to biochar amendment could just as well explain the higher release of mineral N in case of the PMB addition to soil. Changes in the turnover rate of soil organic matter due to the addition of various organic amendments has been attributed to a priming effect' (Kuzyakov et al., 2000). Upon biochar addition, substrate-induced microbial growth occurs, consequently native soil organic matter is cometabolized (Kuzyakov et al., 2000) or formerly protected soil organic matter could be physically accessed by microorganisms (Schimel et al., 2011). Earlier studies have demonstrated increased soil organic matter mineralization after the addition of isotopically labeled biochars (Luo et al., 2011; Zimmerman et al., 2011). Zimmerman et al. (2011) reported that priming of native soil organic matter in the presence of biochar ranged between 59 and 89 %. The current study did not discriminate between native N and biochar N (isotopic labelling) but based on previous findings it can be concluded that priming effects occurred in the PMB treated soils which might have increased the mineral N concentration in the current study.

Mineral N (NH_4^+ and NO_3-) concentrations of the soil was pronounced following the addition of combined biochar and poultry manure compared with when the two amendments were applied to soil separately. This signifies that the combined biochar and poultry manure stimulated net N mineralisation in nutrient deficient soil used in this study which was superior to applying only biochar to soil without manure. Mineral N concentrations increased regardless of types of biochar and biochar fractions in combination with

poultry manure. The highest mineral N was measured in soil samples that had received combined PMB and poultry manure amendment. Fractions involving CCB combined with poultry manure however recorded relatively the least concentrations of mineral N.

The increases in mineral N could be as a result of the synergistic effect of the two amendments (biochar and manure) applied, with poultry manure having a high concentration of N (Table 4). Upon decomposition of the amendments especially manure, mineral N is released into solution due to higher net ammonification and nitrification. The combined manure and biochar application did not reduce net mineralisation because it is likely that less of NH_4^+ - N released from mineralised manure became bound to the biochar. This was likely due to the saturation of biochar NH_4^+-N binding sites on biochar material by excess NH_4^+-N ammonified, leaving adequate amount to be nitrified. Then again, net N mineralisation could be associated with the increase of manure supplied cations (Mg, Ca, K and Na) in the soil solution (Lentz & Ippolito, 2012) which replaced adsorbed NH_4^+-N at some binding sites on the biochar. The excess, non-sequestered and desorbed NH_4^+-N was then nitrified leading to higher NO_3^--N in soil samples that received combined biochar and manure amendments. The increased net N mineralisation may also be due to regulation of microbial immobilisation as a result of manure addition. It was expected that once biochar was included in the fraction, immobilisation might occur due to the high C/N ratio of the biochar materials used. Although considerable concentration of MBN was estimated in the combined treatment, the addition of poultry manure maximised net N mineralisation to buffer the C/N ratio effect (Tiquia & Tam. 2000).

Immobilisation occurred due to higher availability of N and microbial proliferation. Concurrently the net N mineralisation occurred due to the manure providing excess N and the process is enhanced by the increasing microbial population induced by both biochar and manure application. Lehmann et al. (2011) mentioned that biochar cause the rise in pH of the soil, creating a conducive environment for the proliferation of soil microbes and consequent increase in their metabolic activities. Moreover, the manure used in this study did not contain litter such as sawdust which could have also increased the C/N ratio of the manure consequently resulting in immobilisation which could have delayed the release of mineral N into solution (Hochmuth et al., 2015).

It was also observed that mineral N varied with the fractions of biochar in the combined treatments. Soil samples amended with combined poultry manure and 65 t ha^{-1} CCB or CHB respectively had relatively lower NH_4^+-N concentrations than soils that received same biochar at 39 t ha^{-1}. The reason for this observation maybe due to the fact that higher application rates of biochar resulted in higher NH_4^+-N retention sites in soils that received 65 t ha^{-1} biochar and also enhanced nitrification rates in 65 t ha^{-1} biochar amended soils (Table 7 and 8). Adsorption of NH_4^+-N by biochar has been demonstrated in previous works and increasing amount of biochar addition correlates with higher adsorption (Lehmann et al., 2002; Nelissen et al., 2012). More so, concentrations of NO_3^--N were higher in combined fractions involving 65 t ha^{-1} than in 39 t ha^{-1}. This could mean that as C content of soil increases, due to the increase biochar application rates at (65 t ha^{-1}), the energy and food supply

to the microbes also increased which in turn stimulates their activity to mineralize more N into solution (Abbasi et al., 2007).

The results also showed that net N mineralisation was time dependent in soils that were amended with combined fractions of biochar and manure. It was observed that NH_4^+-N increased rapidly (measured on the 3rd day until the 28th day) but the extent of increase became minimal and steady after the 28th day till the 42nd day. The values obtained implied that decomposable fractions of the added manure and biochar respectively mineralized initially at a fast rate followed by a slow rate mineralisation of the most recalcitrant fraction. This agrees with the submission of Kpomblekou and Genus (2012) that mineralization of organic N added to soils starts -with a rapid mineralization of the easily mineralizable organic N, followed by mineralization of the intermediate fraction, and finally the most resistant organic fraction with increasing incubation time. Buttressing this explanation, Cross and Sohi, (2011) reported that upon biochar addition, the easily mineralizable fractions are broken down rapidly and after it is exhausted, the recalcitrant fractions slowly mineralises. Regarding NO_3^--N, the study demonstrated initially marginal NO_3^--N concentration, measured on day 3 but pronounced and significant increases were recorded from day 7 till the end of the experiment. This was indicative of higher nitrification rate which exceeded rate of ammonification simultaneously in the experimental soil following the application of biochar and poultry manure. Specifically, nitrification rate was observed to be higher in respective fractions of PMB (39 and 65 t ha^{-1}) combined with poultry manure compared to that of combined fractions of CCB and CHB respectively with poultry manure.

From the above results, it is clear that biochar should not be applied solely unless it is manure based, because; CCB and CHB evidently demonstrated lower mineral N. This could affect crop production especially lettuce that is dependent on N for foliar development. It is therefore imperative that such biochar is supplemented with manure since it gave the highest mineral N concentration as observed in this study. Then again, based on the results, it was realised that nitrification rate was high in combined biochar and manure amended soils. In the absence of effective synchronisation with effective plant utilization, it might lead to N losses through increased surface runoff, denitrification and leakage, thereby reducing the soil N concentration and creating environmental issues.

Effect of amendments on soil available P concentration

The mineralisation of phosphorus in amendments are presented in Table 9.

Table 9: *Bray 1 extractable P in soil (mg kg^{-1})*

Treatment	3 Days	7 Days	14 Days	28 Days	42 Days
Control	1.07 (0.01)o	1.08 (0.01)o	1.08 (0.01)o	1.08 (0.01)o	1.08 (0.02)o
39 t ha^{-1} CCB	1.16 (0.04)o	2.38 (0.04)no	2.54 (0.07)no	2.80 (0.11)no	2.87 (0.04)no
39 t ha^{-1} CHB	2.36 (0.09)no	2.82 (0.03)no	4.07 (0.04)mn	4.45 (0.15)mn	4.70 (0.25)mn
39 t ha^{-1} PMB	2.72 (0.06)no	5.84 (0.13)m	6.02 (0.26)m	6.19 (0.20)m	6.44 (0.21)lm
65 t ha^{-1} CCB	1.92 (0.27)no	3.37 (0.16)n	3.60 (0.08)n	4.07 (0.30)mn	4.64 (0.47)mn
65 t ha^{-1} CHB	3.33 (1.35)n	3.96 (0.12)n	4.60 (0.11)mn	4.83 (0.19)mn	4.90 (0.13)mn
65 t ha^{-1} PMB	2.96 (0.06)no	6.68 (0.58)lm	9.27 (0.43)kl	9.33 (0.47)kl	9.77 (0.63)kl
10 t ha^{-1} poultry manure	5.16 (0.35)mn	5.34 (0.52)m	7.10 (0.81)lm	7.96 (0.69)l	8.16 (0.99)l
39 t ha^{-1} CCB + 10 tha^{-1} poultry manure	6.76 (1.34)lm	12.04 (1.25)jk	13.51 (1.72)ij	13.75 (0.35)ij	14.17 (0.75)ij
39 t ha^{-1} CHB + 10 tha^{-1} poultry manure	9.89 (1.05)kl	12.97 (1.58)j	15.98 (1.23)hi	17.22 (1.59)h	17.32 (0.94)h
39 t ha^{-1} PMB + 10 tha^{-1} poultry manure	13.46 (1.16)ij	19.53 (2.14)f	21.39 (3.41)d	23.07 (2.17)d	24.67 (2.17)b
65 t ha^{-1} CCB + 10 tha^{-1} poultry manure	10.27 (1.73)k	14.19 (0.73)ij	14.74 (0.56)ij	15.01 (0.64)i	16.65 (1.70)hi
65 t ha^{-1} CHB + 10 tha^{-1} poultry manure	10.99 (1.43)k	14.32 (0.73)ij	17.82 (1.29)gh	18.19 (0.90)g	18.46 (1.59)g
65 t ha^{-1} PMB + 10 tha^{-1} poultry manure	15.22 (2.08)i	20.78 (1.63)e	24.51 (3.58)c	26.38 (2.66)a	32.47 (2.16)a

Mean (standard deviation in parenthesis); Means followed by the same letter do not differ significantly from one another ($p \leq 0.05$). CCB: corn cob biochar, CHB: cocoa husk biochar, PMB: poultry manure biochar

From Table 9, it would be seen that available phosphorus (AVP) remained constantly low in the unamended (control) soil. The control had initial AVP of 1.07 ± 0.01 mg kg^{-1} which increased to 1.08 ± 0.02 mg kg^{-1} by the 42nd day. The low AVP content could be associated with the low pH of the soil used. According to Brady and Weil (2007), under acid conditions, phosphorus is precipitated as Fe or Al phosphates making it insoluble and unavailable. Then again the low AVP concentration observed could be related to the low organic matter content of the soil used (soil organic carbon content of 0.71 %). Low organic matter content of the soil suggest that the soil contains low organic P. Nelson and Mikkelsen (2008) noted that soil organic matter can be an important source of P for crops. Nelson and Mikkelsen (2008) indicated that soil organic matter contains a variety of organic P compounds, such as inositol phosphate, nucleic acid and phospholipid. Then again, it is indicative that higher organic carbon increases the availability of phosphorus due to chelating of polyvalent cations by organic acids and other decay products. By day 42 of the incubation, the observed small increase in available P concentrations in the control treatment could be explained by the decomposition activity of acid-tolerant microbes (Panhwar et al., 2014).

The results in biochar amended soils showed an increase in AVP concentrations than that of the control. Comparing the types of biochar, availability of P followed a trend of PMB > CHB > CCB. Unlike PMB, the sole CCB and CHB addition respectively resulted in relatively marginal rise in available P. The mineralisation rate of P increased with days of incubation. The trend of increase in AVP concentrations with the days was observed for all biochar used irrespective of application rates. When CCB and CHB were

applied to soil, the rise in AVP concentration was steady throughout the experiment measured on the 3rd. 7th, 14th, 28th and 42nd day. In PMB amended soils however, there was a rapid and high increase on the 3rd, 7th and 14th day followed by slight/steady increases till the 42nd day. By day 42 it was generally observed that the results demonstrated a trend of increasing AVP concentrations with increasing rates of biochar application and days of incubation.

Regarding rates of biochar addition to soil, the increases in the available P concentration in soil treated with 39 t ha^{-1} CCB was not significant ($P < 0.05$) compared with the control throughout the incubation period. Meanwhile when CCB was applied at 65 t ha^{-1} available P concentrations increased significantly compared with the control except that on the 3rd day there was no significant increase compared with that of the control. Upon the application of 39 t ha^{-1} CHB, the increases in the available P on the 3rd and 7th day although were higher than the control they were statistically similar. The addition of 65 t ha^{-1} CHB, contributed to a significant ($P < 0.05$) rise in AVP concentrations above the control. Concurrently PMB applied to soil at both rates (39 and 65 t ha^{-1}) showed higher available P concentration and were significantly higher than the control apart from the rise in AVP on the 3rd day in soils that received 39 t ha^{-1} of PMB.

The marginal increase in AVP concentration in CCB and CHB at 39 t ha^{-1} could be due to immobilisation of AVP (Table 14) by microbes as well as the low P content of the amendment (CCB and CHB). Microbial immobilisation of AVP occurred due to high C/P ratio which was above the critical minimum of 200:1 beyond which P immobilization could take place.

The relative simultaneous increases in AVP with increasing biochar additions (65 t ha^{-1}) recorded in the current study could largely be attributed to equilibrium solution P concentration that might have occurred following the increase in biochar application to soil. After reaching equilibrium, the excess P is released into solution increasing their availability to plants.

More so, biochar addition to soil changes the chemistry of the soil and alters P availability. When biochar is applied to acidic soil it increases the pH of the soil as well as decreases the exchangeable acidity as observed in the current study. This mechanism aids in the precipitation of Al and Fe as $Fe(OH)_3$ and $Al(OH)_3$ in soil, thus increases availability of P in soil whiles decreasing solubility of Al and Fe (Gerke, 1994). A significant positive relationship was found between pH and AVP concentrations in the current study ($p < 0.01$; $r = 0.58$; Appendix A) which is an indication that pH rise influenced the consistent increase over time of AVP in biochar amended soils. This explanation is in line with findings of Chintala et al. (2014) who investigated phosphorus sorption and availability from soil-biochar mixtures. They observed that the incorporation of biochars to acidic soil at 40 g kg^{-1} (4 %) increased the equilibrium solution P concentration (reduced the sorption) and increased the availability of sorbed P.

Then again the increase in AVP concentrations could be associated with labile P fraction in biochar material. The presence of decomposable phosphorus fractions contained in biochar ash influences labile P levels and soil microbial community (Lehmann & Joseph, 2009). This is particular with manure based biochar which has been documented to contain higher labile P. This is confirmed in the current study where PMB amended soils showed

higher AVP concentrations compared with CCB and CHB for all sampling days. Deluca et al. (2009) reported that biochar inherently contains a high content of soluble P salts formed during the charring of organic materials. When biomass are heated, organic C can volatilize at approximately 100 °C, whereas P volatilize at approximately 700 °C (DeLuca et al., 2009). Charring organic materials at 400 °C (employed in this study) can transform organic P to inorganic P, mainly as inorganic orthophosphate and pyrophosphate combined with K, Ca, and Mg in biochar (Qian et al., 2013). So upon the application of biochar, organic P in biochar material is mineralised in addition to the inorganic P, increasing P availability. In addition, biochar addition to soil have been linked with proliferation of microbes; phosphorus solubilising microbes (Table 15). The increase in phosphorus solubilising microbial population correlated positively and significantly with the increased concentration of AVP (measured on 42nd DAI) (phosphorus solubilising bacteria; $p < 0.01$; $r = 0.84$, phosphorus solubilizing fungi; $p < 0.01$; $r = 0.88$) (Appendix D). This implies that biochar addition to soil increased the number of phosphorus solubilisers and consequently contributed to the upsurge in AVP concentrations through their mineralisation activity. These microbes attack biochar and caused mineralisation of remnants organic P which may be part of the recalcitrant fraction held in biochar material consequently releasing inorganic P gradually into solution. Jin et al. (2016) explained that inorganic P availability increased due to the decomposition of some organic P, like monoesters by enhanced phosphomonoesterase activities from manure and biochar addition.

The rate at which AVP was released related with incubation time. The increased AVP concentration on the 3rd, 7th and 14th days may be the direct release of inorganic P into solution. Qian et al. (2013) explained that charring organic materials at 400 °C can transform organic P to inorganic P, mainly as inorganic orthophosphate and pyrophosphate. Labile compounds contained in biochar material decomposed very rapidly within the first months of exposure to soil (Cheng et al., 2006). The steady rise of AVP in the case of CCB and CHB amended soils throughout the experiment could be explained by the gradual release of P from biochar material. Cross and Sohi (2011) explained that labile fractions of biochar rapidly mineralise into solution followed by slow release of more recalcitrant fractions as observed in PMB amended soils.

The combined biochar and poultry manure increased AVP than applying them separately to soils. By the end of the experiment (day 42), AVP concentration in combined biochar (39 and 65 t ha^{-1}) and poultry manure (10 t ha^{-1}) were significantly higher ($P < 0.05$) than the control as well as in soils that received sole biochar application. It was also observed that AVP concentrations increased with increasing fractions of biochar in the set of treatments involving biochar and poultry manure combinations. Generally, it was observed that although all the treatment combinations significantly impacted soil AVP concentrations, the highest availability of P occurred when PMB was combined with poultry manure followed by CHB-poultry manure and CCB-poultry manure combinations in that order for all application rates. The highest value (32.47 ± 2.16 mg kg^{-1}) was observed in treatments of combined 65 t ha^{-1} PMB and 10 t ha^{-1} poultry manure. In addition it was observed that AVP concentrations increased appreciably initially (measured

on days 3 and 7) for CCB-poultry manure and CHB-poultry manure mixtures respectively whiles on days 14, 28 and 42 marginal rise in AVP was recorded. PMB-poultry manure mixture demonstrated high and consistent increases throughout the experiment.

The positive synergy observed could be related to changes in soil pH, direct nutrient addition by the two amendments; biochar and poultry manure, and changes in soil microbial composition.

The major driver controlling the increase in P is the changes in pH. Biochar contains basic cations such as Ca, K, Mg, and Si which can form alkaline oxides or carbonates during the pyrolysis process. Following the release of these oxides into the acidic soil, they can react with the H^+ and Al^{3+}, raise the soil pH, and decrease exchangeable acidity (Novak et al., 2009). This enhances the release of inorganic phosphate ions contained in the biochar material into solution. Moreover, the addition of biochar and manure to soil enhances the proliferation of soil microbes consequently increasing microbial metabolic activities. The increase in microbial activities results in the production of enzymes; for instance by P-solubilising bacteria which releases phosphomoesterase enzymes to help in the mineralisation of organic P from poultry manure (Khan et al., 2009). Kumar et al., (2013) showed that *Bacillus megaterium* isolated from the poultry sample produces higher amount of extra cellular phytase enzyme.

Generally sole biochar application although increased AVP concentrations considerably, soils amended with CCB and CHB at both 39 and 65 t ha^{-1} were below the critical limit for productive agriculture. Soils amended with sole CCB and CHB could still be regarded as deficient in AVP

(Lehmann et al., 2001; Ayeni & Adeleye, 2014). The concentrations demonstrated in PMB soils at both applications (39 and 65 t ha^{-1}) were within the critical range, which suggests that, in considering supply of adequate AVP for optimum crop yield, PMB could also not be solely relied upon for long term and sustainable productivity. Lehmann et al. (2001) submitted a critical range of AVP (Bray's Pl) to be at 5 mg kg^{-1} below which the soil is classified as highly deficient. The Combined biochar and poultry manure proved superior and AVP measured in respective treated soils were above the critical range needed for arable crop production. Combined biochar and poultry manure is therefore recommended to supply adequate AVP for plant nutrition of highly weathered soil. Combined PMB with manure was superior, is recommended, however, CHB and CCB respectively combined with poultry manure could serve as alternative. The ranges of AVP in combined treatments were also found to be below that which could cause any environmental concern.

Effects of amendments on soil pH

Soil pH is an important soil property that affects the nutrient status and growth of most agricultural crops. Lehmann et al. (2006) suggested that biochar can indirectly affect nutrient availability by altering soil pH. Most often biochar has higher pH than soil and can therefore act as a liming agent resulting in an overall increase in soil pH.

Table 10 shows the effect of biochar solely applied or in combination with poultry manure on the pH of the experimental soil.

Table 10: *Effect of Treatments on Soil pH*

Treatment	3 Days	7 Days	14 Days	28 Days	42 Days
Control	4.17 (0.01)a	4.17 (0.01)a	4.16 (0.01)a	4.16 (0.01)a	4.16 (0.01)a
39 t ha^{-1} CCB	5.17 (0.02)e	5.26 (0.01)f	5.34 (0.04)g	5.40 (0.02)h	5.42 (0.01)hi
39 t ha^{-1} CHB	5.48 (0.01)j	5.50 (0.01)jk	5.54 (0.03)k	5.59 (0.04)l	5.62 (0.01)m
39 t ha^{-1} PMB	5.44 (0.01)i	5.67 (0.01)n	5.77 (0.02)p	5.75 (0.01)op	5.74 (0.04)op
65 t ha^{-1} CCB	5.44 (0.03)i	5.73 (0.06)o	5.74 (0.04)o	5.76 (0.01)op	5.79 (0.01)pq
65 t ha^{-1} CHB	5.79 (0.02)pq	5.82 (0.03)qr	5.85 (0.06)r	5.88 (0.01)s	5.91 (0.01)st
65 t ha^{-1} PMB	5.80 (0.05)q	5.96 (0.04)u	6.03 (0.01)v	6.05 (0.01)v	6.04 (0.07)v
10 t ha^{-1} poultry manure	4.65 (0.02)b	4.73 (0.01)cd	4.76 (0.04)d	4.74 (0.01)cd	4.73 (0.02)cd
39 t ha^{-1} CCB + 10 tha^{-1} poultry manure	5.64 (0.01)mn	5.69 (0.02)n	5.74 (0.01)op	5.68 (0.02)n	5.62 (0.02)m
39 t ha^{-1} CHB + 10 tha^{-1} poultry manure	5.82 (0.01)qr	5.83 (0.01)qr	5.83 (0.01)qr	5.80 (0.01)q	5.77 (0.02)p
39 t ha^{-1} PMB + 10 tha^{-1} poultry manure	5.86 (0.01)r	5.88 (0.02)s	5.88 (0.01)s	5.86 (0.01)rs	5.83 (0.01)qr
65 t ha^{-1} CCB + 10 tha^{-1} poultry manure	5.95 (0.01)tu	5.96 (0.01)u	5.96 (0.02)u	5.93 (0.01)t	5.94 (0.01)tu
65 t ha^{-1} CHB + 10 tha^{-1} poultry manure	6.12 (0.01)w	6.13 (0.02)w	6.17 (0.02)x	6.14 (0.01)wx	6.13 (0.01)w
65 t ha^{-1} PMB + 10 tha^{-1} poultry manure	6.21 (0.01)y	6.23 (0.01)yz	6.25 (0.01)z	6.22 (0.01)y	6.17 (0.03)x

Mean (standard deviation in parenthesis); Means followed by the same letter do not differ significantly from one another ($p \leq 0.05$).

CCB: corn cob biochar, CHB: cocoa husk biochar, PMB: poultry manure biochar

The results obtained throughout the 42 days of the experiment showed that all the amendments resulted in significant ($P < 0.05$) increases in soil pH above the control. Meanwhile the pH of the control soil remained acidic throughout the 42 days of the experiment.

The increase in pH of the experimental soil was affected by duration of the experiment, the type and rates of biochar. It would be realized that pH increased sharply initially (Day 3) and thereafter increased steadily (Table 10). By day 42 of the experiment, pH increase measured for all amendments were significantly ($P < 0.05$) higher than measured on the third day. The sharp increase could be related to the immediate decomposition of more readily decomposable fractions of biochar and release of basic cations (Appendix E) which might have contributed to the rise in pH.

There was a drop in pH levels (Day 42) when PMB was applied at 65 t ha^{-1} and also when biochar was combined with poultry manure. More so, apart from treatment that received combined 65 t ha^{-1} PMB and 10 t ha^{-1} manure, all other treatments that recorded drop in pH was insignificant.

Regarding effect of biochar application rate, the pH of the soil increased with increasing rates of biochar application. The trend of increment was; $0 < 39 < 65$ t ha^{-1} for all the three biochar used. It could be observed from Table 5 that, of the three biochar used the highest pH occurred when PMB was applied followed by CHB and lastly CCB at all application rates. By day 42, PMB amended soils demonstrated a pH increase of 5.74 and 6.03, at application rates of 39 and 65 t ha^{-1} respectively. CHB on the other hand increased pH to 5.51 and 5.91 at respective application rates of 39 and 65 t ha^{-1}

^{1}. Similarly, at 39 and 65 t ha^{-1} application rate, CCB increased pH to 5.42 and 5.79 respectively (from initial of 4.17).

The significant rise in pH observed in this study could be attributed to several reasons. One possible reason could be due to the acid neutralising effect of the biochar causing a possible adsorption of cations such as Al^{3+} onto biochar surfaces, consequently, reducing exchangeable acidity (Al^{3+}, H^{+}) of the soil. Then again, all the three biochar used for the study were alkaline due to the appreciable concentrations of basic cations especially Ca, Mg and K (Table 5). The application of biochar to the experimental soil caused a rise in exchangeable Ca^{2+}, Mg^{+} and K^{+} (Appendix E) and this contributed to the rise in the pH of the soil.

The result of the current study is in line with several authors (Chan et al., 2007; Granatstein et al., 2009) who reported rise in soil pH upon application of biochar to soil. In contrast with the finding of the current study, some researchers have found a decrease in the pH of the soil upon biochar application (Naeem et al., 2014; Liu & Zhang, 2012). They ascribed the reduction of soil pH to the release of acidic matter produced from the oxidation of biochar and the decomposition of biochar in soil. The formation of acidic functional groups can neutralize alkalinity, causing a fall in pH values of the soil which didn't happen in the current study because pH increased in all experimental soils.

The effect of sole poultry manure application was observed to have significantly ($P < 0.05$) increased the pH of the soil throughout the experiment and by day 42, pH increased to 4.73 (initial = 4.17). The increase however was significantly ($P < 0.05$) lower compared with soil samples that received sole

biochar or combined biochar and poultry manure amendment. The increase in pH following the application of poultry manure could be as a result of the complexation of Al by decomposition products of organic materials. As organic manures mineralize, calcium ions are released into soil solution. The released basic cations (Ca^{2+}) ions get hydrolysed. The Calcium hydroxide formed reacts with soluble aluminum ions (Al^{3+}) in the soil solution to yield insoluble $Al(OH)_3$. Congruent to the findings of this research, Dikinya and Mufwanzala (2010) observed that application of chicken manure increased pH of amended soil. He explained that the rise in pH was due to ion exchange reactions which occurred when terminal OH of Al or Fe^{2+} hydroxyl oxides are replaced by organic anions which are products of decomposition of manure such as malate, citrate and tartrate.

Soils that received combined biochar and poultry manure recorded higher pH than when these amendments were applied separately. Both amendments contributed to the rise in pH of the soil. Pearson correlation analysis showed positive significant relationship between soil amendments and pH ($P = 0.01$, $r = 0.98$) (Appendix A).

Notably, the highest pH increments were observed for combined amendments of PMB and poultry manure at all levels and compared with combined amendments involving CCB but similar compared with CHB. The pH of the biochar and poultry manure used in this study was high and their application to the experimental soil could have caused the rise in pH. In addition, poultry manure has high CEC and could have influenced the rise in pH of the soil. Rowell (1994) explained that poultry manure upon their incorporation into soil mineralize to release basic cations which displace and

replace H and Al ions at the exchange sites. Then again, as a result of the high carbonate concentration of biochar, it acts as liming material in soils and can raise pH of neutral or acidic soil (Chan et al., 2007). Raison (1979) also explained that the increase in soil pH with the addition of biochar can be attributed to ash accretion as ash residues are generally dominated by carbonates of alkali and alkaline earth metals, sesquioxides, phosphates and small amounts of organic and inorganic N. Depending on the sources of biochar used, basic cations such as Ca, K, Mg, and silicon (Si) can form alkaline oxides or carbonates during the pyrolysis process. Following the release of these oxides into the environment, they can react with the H^+ and Al^{3+}, raise the soil pH, and decrease exchangeable acidity (Novak et al., 2009).

Generally the pH range (5.42 - 6.17) recorded by the 42nd day in this study is promising since it could greatly promote microbial proliferation and increase the availability of plant nutrient elements in soil. Brady and Weil (2007) indicated that soil pH range of 5.5 to 6.5 is appropriate for nutrient availability and decreases the proportion of Al^{3+} and H^+ ions occupying cation exchange sites. This subsequently will increase nutrient availability especially phosphorus and subsequently increase lettuce yield.

Effects of amendments on effective cation exchange capacity

Table 11 gives an indication on how application of the various amendments affected the ECEC of the soil.

Table 11: *Effective Cation Exchange Capacity ($cmol_c kg^{-1}$)*

Treatments	3 Days	7 Days	14 Days	28 Days	42 Days
Control	2.16 (0.04)w	2.14 (0.05)w	2.15 (0.05)w	2.13 (0.05)w	2.12 (0.04)w
39 t ha^{-1} CCB	3.37 (0.03)u	3.49 (0.06)tu	3.58 (0.04)tu	3.59 (0.03)t	3.65 (0.02)st
39 t ha^{-1} CHB	3.47 (0.01)u	3.59 (0.09)t	3.78 (0.06)rs	3.85 (0.09)r	3.96 (0.04)qr
39 t ha^{-1} PMB	3.94 (0.07)qr	4.08 (0.04)pq	4.17 (0.05)p	4.22 (0.06)op	4.30 (0.03)o
65 t ha^{-1} CCB	3.52 (0.04)tu	3.60 (0.05)t	3.73 (0.06)s	3.81 (0.03)rs	3.87 (0.01)r
65 t ha^{-1} CHB	3.69 (0.07)st	3.87 (0.02)r	3.96 (0.08)qr	4.04 (0.04)q	4.08 (0.04)pq
65 t ha^{-1} PMB	4.23 (0.09)op	4.39 (0.06)no	4.46 (0.09)n	4.56 (0.06)mn	4.62 (0.09)m
10 t ha^{-1} poultry manure	3.21 (0.02)v	3.40 (0.11)u	3.46 (0.08)u	3.58 (0.08)tu	3.69 (0.08)st
39 t ha^{-1} CCB + 10 tha^{-1} poultry manure	4.47 (0.01)n	4.72 (0.06)lm	4.85 (0.10)kl	4.96 (0.10)j	5.09 (0.06)i
39 t ha^{-1} CHB + 10 tha^{-1} poultry manure	4.55 (0.12)mn	4.79 (0.06)l	5.09 (0.02)ij	5.31 (0.12)gh	5.42 (0.12)g
39 t ha^{-1} PMB + 10 tha^{-1} poultry manure	5.38 (0.11)g	5.78 (0.11)de	6.04 (0.14)d	6.14 (0.14)d	6.30 (0.02)c
65 t ha^{-1} CCB + 10 tha^{-1} poultry manure	4.92 (0.04)k	5.24 (0.07)h	5.39 (0.03)g	5.50 (0.02)fg	5.60 (0.06)f
65 t ha^{-1} CHB + 10 tha^{-1} poultry manure	4.90 (0.11)k	5.19 (0.11hi)	5.34 (0.08)gh	5.49 (0.08)fg	5.54 (0.05)fg
65 t ha^{-1} PMB + 10 tha^{-1} poultry manure	5.75 (0.09)de	6.06 (0.04)d	6.26 (0.10)c	6.49 (0.18)b	6.71 (0.06)a

Mean (standard deviation in parenthesis); Means followed by the same letter do not differ significantly from one another ($p \leq 0.05$). CCB: corn cob biochar, CHB: cocoa husk biochar, PMB: poultry manure biochar

It could be observed that adding biochar solely or in combination with poultry manure consistently increased ECEC significantly ($P < 0.05$) above the control throughout the experimental period (measured on Days 3, 7, 14, 28 and 42).

The rate of increase was affected by the incubation period. Observably, ECEC increased rapidly in the early phase of the incubation (3^{rd}, 7^{th} and 14^{th} DAI) but the increase became steady after day 14 till the end of the incubation. The rapid increase in the early stages of the experiment can be associated with the release of basic cations from the labile portion of biochar used. Upon the exhaustion of the soluble cations and further gradual breakdown of the recalcitrant portion of biochar resulted in the gradual release of these cations consequently increasing ECEC of the soil (Cross & Sohi, 2011).

Then again, the results showed that ECEC value was affected by the type of biochar and rate of application. Regarding the effectiveness of specific biochar to the elevation of ECEC in the experimental soil, PMB amended soils demonstrated the highest ECEC concentration, followed by CHB and CCB throughout the incubation. The increase in ECEC values in PMB soils is an indication of high concentration of soluble basic cations (Ca, Mg^{2+}, Na^{+} and K^{+}) in the manure based biochar (PMB) used. This clearly confirms the submission of Lehmann et al. (2002) which buttressed the fact that animal based biochar contained more nutrients elements than other biochar prepared from wood or crop residues.

The results as shown in Table 11 demonstrate increase in ECEC with increasing biochar rates throughout the duration of the study. By day 42 of the incubation, the trend of increase followed; $0 < 39 < 65$ t ha^{-1}. However, when

biochar rates were increased from 39 t ha^{-1} to 65 t ha^{-1}, significant increases in ECEC was recorded in treatments that contained CCB and PMB respectively but CHB demonstrated insignificant increase in respective application rates (39 to 65 t ha^{-1}). At the end of the incubation (Day 42) and at application rate of 39 t ha^{-1}, CCB, CHB and PMB recorded an increase of 41.9 %, 46.4 % and 50.7 % respectively above the control soil. When biochar rate was increased to 65 t ha^{-1}, ECEC values in soils amended with CCB, CHB and PMB correspondingly increased by 45.2 %, 48.0 % and 54.1 % above the control.

Generally the increase in ECEC values of biochar soils can been attributed to surface oxidation and creation of carboxylic and phenolic surface functional groups upon biochar application to soil (Liang et al., 2006; Cheng et al., 2006) which causes chelation of Al and Fe. Then again, it could be related to the increase in pH and the elevation of basic cations (Ca^{2+}, Mg^{2+} and K^{-}) in the experimental soil. Pearson correlation indicates a positive correlation between ECEC and Ca ($p < 0.01$; $r = 0.97$), ECEC and Mg ($p < 0.01$; $r = 0.98$) ECEC and K ($p < 0.01$; $r = 0.99$) and ECEC and pH ($p < 0.01$; $r = 0.94$) .The increase in pH resulted in the decline in solubility of Al in soil solution as well as increase in Al chelation with negatively charged surfaces of biochar or soil. Then again, the increases in pH is the cause of the elevated amounts of exchangeable cations especially Ca^{2+}, Mg^{2+} and K+ which reduced exchangeable acidity.

The increases in ECEC of biochar amended soils is similar to the findings of previous studies where they concluded that elevation in ECEC of soil was as a result of increase in basic cations concentrations (Cheng et al., 2006; Van Zwieten et al., 2010; Laird et al., 2010). In contrast, biochar

application failed to produce a significant influence on ECEC (Blackwell et al., 2009).

Similar to the behaviour of sole biochar in experimental soil, combined biochar and manure resulted in higher concentrations of ECEC compared with separate biochar or poultry manure amended soils throughout the experiment. It was demonstrated that ECEC increased sharply at the early stage of the incubation and continue to increase till day 42 of the experiment. By Day 42, respective ECEC values recorded when 10 t ha^{-1} of poultry manure was co applied with 39 t ha^{-1} each of CCB, CHB and PMB were 58.3 %, 60.9 % and 66.3 % over the control. Then again, when the biochar fractions were increased to 65 t ha^{-1} in the combination, ECEC values recorded were 62.1 %, 61.7 % and 68.4 % above the control. This shows that PMB in combination with manure was superior to CCB and CHB combined with manure. Generally it was evident that the combined biochar and manure synergistically increased the pH and basic cations in soil. The compounding effect augmented the ECEC of the soil.

Pearson correlation indicates positive relationship between ECEC and soil amendments ($p < 0.01$; $r = 0.95$). This demonstrates that manure and biochar synergistically increased the pH and basic cations in soil. The increase in ECEC value correlates positively with the increase in Ca, K, Mg of the soil following the application of the combined biochar and manure (Appendix A). Similar results were demonstrated by Inal et al. (2015) in which they reported that biochar and processed poultry manure increased plant nutrient solubility and subsequently ECEC.

Conclusion

At both rates of application (39 and 65 t ha^{-1}), biochar (CCB, CHB and PMB) increased the concentration of SOC, pH and ECEC and were significantly ($P < 0.05$) higher throughout the incubation period compared with the control. Meanwhile regarding the concentration of mineral N, CCB and CHB at both 39 and 65 t ha^{-1} showed significant increases at the early phase of the incubation but by day 42, the concentration of mineral N (NH_4^+, NO_3^-) in CCB and CHB soils were respectively not significantly ($P < 0.05$) higher than that of the control. On the other hand, PMB treated soils demonstrated significantly elevated concentrations of mineral N (NH_4^+, NO_3^-) than the control as well as CHB and CCB treated soils at all rates. More so, AVP concentration in CCB soil were not significant ($P < 0.05$) throughout the incubation period compared with the control at 39 t ha^{-1}, but at 65 t ha^{-1} AVP concentrations increased significantly. At the same time, CHB and PMB showed significant ($P < 0.05$) increases in AVP at both rates above the control with PMB showing superior values.

Combining biochar and poultry manure demonstrated high and consistent increases of SOC, mineral N, AVP, pH and ECEC throughout the experiment and were significantly higher than found in the control and sole applications.

Based on the results of the current study that the use of biochar to improve SOC concentrations is a sustainable energy in any situation to conserve or promote soil health and can be a valuable tool in enhancing fertility of highly depleted tropical soil is used in this study. Improvement in pH and ECEC is a good indication for supporting the growth and development of lettuce. Following the increase in ECEC, the soil nutrient retention capacity

and buffering capacity would be enhanced reducing the leaching potential of basic cations predisposing the soil to low pH. Moreover, increased pH observed is an indication that the biochar can be used as liming material when added to strongly acidic soils thereby leading to reduction in soil acidity and increased nutrient availability.

The use of poultry manure was found to compensate for the low nutrient concentration of biochar especially; CCB and CHB. This enhanced the fertility of nutrient depleted soil by increasing the availability of plant nutrients (mineral N AVP and ECEC) and improving soil health compared to sole biochar application.

CHAPTER FIVE

EFFECTS OF BIOCHAR SOURCE AND POULTRY MANURE ON SOIL MICROBIAL BIOMASS CARBON NITROGEN, PHOSPHORUS AND PHOSPHORUS SOLUBILISERS

Introduction

The effects of biochar on soil biological properties have not received much attention especially regarding its effects on soil macro and microorganisms (Lehmann et al., 2011).

To investigate the effect of biochar on soil microorganisms, microbial biomass C, N and P, were estimated, and phosphorus solubilisers from soils exposed to three sources of biochar (poultry manure biochar (PMB), corn cob biochar (CCB) and cocoa husk biochar CHB) solely applied at varied rates and in combination with poultry manure. According to Gonzalez-Quinones and Carson (2016), an estimate of the weight of C or N in microorganisms can represent the total microbial biomass of the soil.

It has been suggested that due to the high C content of biochar relative to N and P, there could be net immobilisation of N and P when biochar is added to soil; especially to soils low in initial N and C content. Meanwhile Lehmann and Joseph (2009) posited that bulk of biochar C is known to be recalcitrant and therefore undergo slow mineralisation affecting the availability of SOC. In line with this assertion, Brantley et al. (2015) found in their study that, although immobilisation of C and N happened due to the wide C/N ratio of pine wood biochar used, it was not significant. Meanwhile Han et al. (2013) reported significant increases of MBC in soil amended with biochar above the control.

Regarding biochar effect on MBN, previous researchers have reported that biochar application to N depleted soil resulted in N immobilisation by microbial biomass (Novak et al., 2010; Nelson et al., 2011; Bruun et al. 2012) whiles others showed that biochar addition although increased the concentrations of MBN, the values were not significantly different from the control (Alburquerque et al., 2013; Dempster et al., 2012).

Biochar has also been suggested to have effect on microbial abundance. Steiner et al. (2008) explained that biochar has the potential to stimulate microbial activity and increase abundance. Rousk et al. (2010) reported that bacteria are likely to increase in abundance with biochar or potentially dramatically reduce their growth. Jin (2010) found greater enhancement of microbial abundance by biochar additions in the rhizosphere than in bulk soil, whereas Graber et al. (2010) reported the opposite.

The inconsistencies in the results were related to the biochar properties, application rates of biochar and soil characteristics. Then again, literature search shows that very little research has been done on changes in abundance of specific microorganisms for instance phosphorus solubilisers in response to biochar application.

This study therefore evaluated the;

1. impact of biochar and manure on MBC, MBN and MBP.
2. response of phosphate solubilizing fungi and bacteria to biochar applied alone or combined with poultry manure.

Materials and Methods

Experimental setup

Experimental setup have been described in Chapter Four of this Thesis

Soil analyses

To analyze soil for MBC, MBN and MBP, destructive soil sampling technique was used to sample soils on the 3rd, 7th, 14th, 28th and 42nd days after incubation (DAI). Soils were analysed for MBC, MBN and MBP as described in Chapter Three.

Then again, at the end of the incubation period (42 days), soils from each pot were homogenized and sampled to a depth of about 5cm for analysis of PSMs (fungi and bacteria). Prior to soil sampling, it was ensured that moisture content of each treatment was kept at field capacity for about 5 days to stabilize microbial activities (Rowell, 1994). Soil sampling and preparation for microbial analysis, enumeration and identification of phosphorus solubilisers have been described in Chapter Three.

Data Analyses

Data was analysed using statistical products for social scientist (SPSS) (version 16). Data was summarised and presented as means and standard deviations. Test for significant effects ($P < 0.05$) between means of treatment was done using LSD Postdoc procedure. Pearson's moment correlation was used to determine how the soil properties were related and also establish the relationship between treatments and soil properties. Results have been presented in Tables and graphs.

Results and Discussion

Effects of amendments on MBC

The effects of amendments on soil microbial biomass C have been summarised in Table 12.

Table 12: *Effect of Treatments on Microbial Biomass Carbon (mg kg^{-1})*

Treatment	3 Days	7 Days	14 Days	28 Days	42 Days
Control	33.78 (1.78)pq	37.89 (5.48)p	32.67 (2.18)pq	34.87 (1.42)p	25.75 (2.21)q
39 t ha^{-1} CCB	48.08 (2.69)o	62.46 (3.07)no	68.44 (3.58)mn	56.63 (2.74)no	53.33 (3.24)o
39 t ha^{-1} CHB	64.39 (3.63)n	72.98 (2.90)m	73.53 (4.17)m	61.88 (3.69)no	55.25 (5.85)o
39 t ha^{-1} PMB	69.27 (5.71)mn	74.50 (3.82)m	77.13 (2.70)lm	81.76 (4.41)lm	83.52 (3.36)l
65 t ha^{-1} CCB	68.32 (2.95)mn	78.72 (1.63)lm	85.37 (3.65)kl	81.83 (3.78)lm	73.64 (3.92)m
65 t ha^{-1} CHB	68.17 (4.27)mn	84.50 (3.08)kl	91.51 (5.66)kl	83.86 (4.44)kl	78.00 (5.47)lm
65 t ha^{-1} PMB	100.94 (2.17)j	108.67 (4.69)ij	111.19 (3.39)ij	114.71 (3.46)i	115.58 (2.01)hi
10 t ha^{-1} poultry manure	67.48 (1.36)mn	91.89 (1.65)k	109.91 (3.27)ij	113.65 (4.53)i	116.95 (1.82)hi
39 t ha^{-1} CCB + 10 tha^{-1} poultry manure	99.28 (5.47)jk	104.71 (3.45)j	114.88 (5.23)hi	121.13 (4.45)hi	121.99 (4.09)hi
39 t ha^{-1} CHB + 10 tha^{-1} poultry manure	123.16 (4.20)h	132.31 (9.78)g	139.30 (4.20)fg	144.37 (3.78)fg	145.18 (4.28)f
39 t ha^{-1} PMB + 10 tha^{-1} poultry manure	153.81 (8.02)ef	157.80 (4.89)e	160.54 (5.03)de	167.68 (7.76)d	173.49 (5.43)cd
65 t ha^{-1} CCB + 10 tha^{-1} poultry manure	113.57 (9.31)i	136.70 (7.24)g	142.33 (10.50)fg	163.15 (4.61)de	171.50 (8.85)cd
65 t ha^{-1} CHB + 10 tha^{-1} poultry manure	130.76 (6.07)gh	147.54 (7.63)f	165.19 (5.91)de	172.93 (7.08)cd	174.38 (8.89)cd
65 t ha^{-1} PMB + 10 tha^{-1} poultry manure	176.57 (6.34)c	191.87 (7.34)b	202.80 (4.67)a	205.49 (5.48)a	209.88 (3.82)a

Mean (standard deviation in parenthesis); Means followed by the same letter do not differ significantly from one another ($p \leq 0.05$). CCB: corn cob biochar, CHB: cocoa husk biochar, PMB: poultry manure biochar

The control soil showed significantly lower MBC concentration as measured on the 3rd day of the incubation (DAI). By the 7th DAI, it was observed that there was a slight increase in the concentration of MBC in the control treatment. Thereafter, MBC decreased till the end of the incubation (14th, 28th and 42nd DAI). Generally, the low MBC was indicative of low concentration of labile C and N in soil which serves as microbial substrate (Blagodatskaya & Kuzyakov, 2013; Blagodatskaya et al., 2014). The low C and N do not favour microbial growth and proliferation. The slight rise on the 7th DAI however could be as a result of the rewetting of the soil which probably revamped some inactive indigenous microbes (Rowell, 1994) but due to exhaustion of substrates or inadequate substrates (C, N) for microbial assimilation, the microbes become inactive or die leading to the decrease in MBC from the 14th DAI till the end of the incubation. It has been reported that assimilation of C with corresponding respiration slows down very sharply after substrate exhaustion leading to microbial turnover (Blagodatskaya & Kuzyakov, 2013; Blagodatskaya et al., 2014).

The application of amendments resulted in significant ($P < 0.05$) increases in MBC above the control throughout the incubation period (Table 12). Sole biochar application increased MBC and this was evident for all the biochar used (CCB, CHB and PMB). The increases in MBC observed in biochar amended soils were influenced by the type of biochar, application rates and incubation time. Regarding the type of biochar, highest MBC was measured in PMB amended soils followed by CHB and CCB soils. The results obtained also demonstrated that increasing the application rates of biochar resulted in significant increases in the concentrations of MBC in soils with

PMB amended soils demonstrating superior concentrations of MBC at all application rates.

Regarding time effect during incubation, it was realised that MBC increased sharply at the beginning of the incubation, for all three biochar used (measured on 3rd DAI). The increase in MBC was still consistent till it peaked on the 14th DAI and started decreasing steadily (observed on 28th DAI) till the end of the incubation. These fluctuations in MBC concentration were observed in soil samples that received CHB and CCB. In the case of PMB amended soils, MBC increased sharply and consistently till the 14th day. After the 14th day, MBC increased but the increase was steady till the end of the incubation period (measured on 28th and 42nd DAI).

The time effect on the concentration of MBC could be associated with the availability of C in biochar material. Higher concentration of MBC in the initial stages of the incubation for all biochar could be associated with readily available C added from biochar and proliferation of microbes leading to higher microbial assimilation of C (Hopkins & Gregorich, 2005; Lehmann et al., 2011). As the incubation period progressed, there was possibility of exhaustion of labile C, reducing the concentration of SOC, causing microbial turnover. The onset of microbial turnover caused a reduction in MBC, especially in CCB and CHB amended soils. Blagodatskaya and Kuzyakov (2013) explained that when readily available substrate is exhausted microbes become inactive, leading to low respiration and consequently low assimilation of C which might have happened in this study resulting in the low MBC estimated in CCB and CHB soils after the 14th DAI. In the case of PMB, the steady increase after it peaked on the 14th DAI was due to the slow

mineralisation of recalcitrant portion of the biochar which was left after the exhaustion of the more labile portion, usually mineralised slowly by autochthonous microbes (Hopkins & Gregorich, 2005). This resulted in low availability of SOC and caused a steady rise in MBC till the end of the incubation. Lehmann and Joseph (2009) posited that bulk of biochar C is known to be recalcitrant and therefore undergo slow mineralisation affecting the availability of SOC. A class of microbes referred to as autochthonous or K-selected organisms dominate the soil under this condition (Hopkins & Gregorich, 2005). These selected organisms are also few and are more competitive under steady state with low C and nutrient supply in soil. Contrary to the finding of the present study, Brantley et al. (2015) reported in their study that although immobilisation happened due to the wide C/N ratio of pine wood biochar used, it was not significant. The differences in the results obtained could be related to the different biochar and soil used.

The significant increase in MBC in biochar amended soils signifies the impact of biochar on soil microbial community. The addition of biochar is known to improve the soil properties which include increase in pH, increased CEC and carbon availability. This created enabling environment for the proliferation of microbes (Lehmann et al., 2011). Then again, biochar possess key characteristics, such as good porosity which serve as habitat for microbes to grow and increase in abundance. The increases in microbial biomass following the application of biochar resulted in higher demand for energy derived from C resulting in higher assimilation of carbon consequently higher MBC estimated in this study.

Secondly, higher MBC demonstrated in the present study shows that biochar incorporation in the experimental soils appeared to indicate C-limited microbial populations which responded very rapidly to fresh C inputs. Biochar which contains fresh carbon, upon the application to experimental soils seem to activate indigenous microbes which were inactive due to low C and nutrients availability. This group of microbes often called zymogenous or r selected biomass assimilates these available C. More so, the addition of biochar could cause priming of native carbon in soil and a group of microbes called autochthonous or K-selected biomass) assimilates this carbon (Hopkins & Gregorich, 2005). Coupled with higher microbial biomass, there is relative higher MBC in biochar amended soils.

The inclusion of poultry manure increased the concentrations of MBC due to the additive effect from the two amendments. The manure might have undergone higher initial decomposition due to low C/N ratio. This confirms Bitzer and Sims (1988) report that the organic fraction of poultry manure with lower C/N ratio undergoes rapid decomposition. The decomposition of manure made carbon more available for microbial assimilation. Then again, higher MBC could be related to the proliferation of microbes as a result of the application of biochar and manure (Table 15). Biochar and manure addition to soil, improves the soils property and making the soil environment more conducive for microbial habitation and multiplication. An increase in microbial biomass is considered beneficial to the fertility of the soil, while its decline may be considered detrimental because it leads to a decline in soil biological function (Gonzalez-Quiñones et al., 2011).

Effect of amendments on MBN

Presented in Table 13 are the results of the effects of biochar solely applied or in combination with poultry manure on the MBN in soils incubated for 42 days. Data was taken on the 3rd, 7th, 14th, 28th and 42nd day.

Table 13: *Effect of Treatments on Microbial Biomass Nitrogen (mg kg^{-1})*

Treatment	3 Days	7 Days	14 Days	28 Days	42 Days
Control	1.42 (0.05)p	1.16 (0.09)p	1.09 (0.06)p	1.01 (0.03)p	0.96 (0.06)p
39 t ha^{-1} CCB	2.82 (0.31)o	3.66 (1.16)no	4.75 (0.13)mn	4.08 (0.67)no	4.02 (0.08)no
39 t ha^{-1} CHB	3.99 (0.38)no	5.43 (0.24)mn	5.67 (0.14)mn	5.14 (0.06)mn	4.93 (0.06)mn
39 t ha^{-1} PMB	2.57 (0.17)op	3.12 (0.07)o	3.23 (0.07)no	3.26 (0.07)no	3.30 (0.05)no
65 t ha^{-1} CCB	4.11 (0.53)mn	5.39 (0.57)mn	5.60 (0.38)mn	4.87 (0.28)mn	4.54 (0.24)n
65 t ha^{-1} CHB	4.26 (0.43)no	6.84 (0.62)lm	7.46 (0.16)l	5.94 (0.91)m	3.36 (0.48)no
65 t ha^{-1} PMB	2.68 (0.17)op	4.69 (0.36)mn	4.92 (0.22)mn	5.14 (0.13)mn	5.41 (0.36)mn
10 t ha^{-1} poultry manure	2.81 (0.04)op	3.9 (0.05)no	3.96 (0.16)no	3.97 (0.15)no	4.03 (0.12)no
39 t ha^{-1} CCB + 10 tha^{-1} poultry manure	22.78 (1.26)i	24.18 (1.38)h	26.82 (1.26)fg	27.40 (0.95)fg	30.05 (0.63)de
39 t ha^{-1} CHB + 10 tha^{-1} poultry manure	27.09 (1.35)fg	30.87 (1.02)de	30.15 (0.95)de	31.49 (0.59)cd	34.13 (0.65)b
39 t ha^{-1} PMB + 10 tha^{-1} poultry manure	16.83 (0.16)k	17.54 (1.31)k	20.42 (1.01)j	20.82 (0.23)j	21.2 (2.15)j
65 t ha^{-1} CCB + 10 tha^{-1} poultry manure	25.19 (1.39)gh	26.47 (0.78)g	27.92 (0.21)f	29.80 (0.78)e	32.66 (2.48)c
65 t ha^{-1} CHB + 10 tha^{-1} poultry manure	29.35 (1.42)e	32.63 (0.42)c	33.48 (0.71)bc	33.93 (1.20)b	36.09 (1.51)a
65 t ha^{-1} PMB + 10 tha^{-1} poultry manure	18.11 (0.99)k	22.98 (1.02)hi	24.24 (0.32)h	25.72 (0.93)g	26.09 (1.34)g

Mean (standard deviation in parenthesis); Means followed by the same letter do not differ significantly from one another ($p \leq 0.05$).
CCB: corn cob biochar, CHB: cocoa husk biochar, PMB: poultry manure biochar

The application of biochar solely or in combination with poultry manure increased significantly ($P < 0.05$) the levels of MBN above the control. The increase in MBN concentrations in sole biochar amended soils was dependent on biochar type, rates of application and incubation time. At the initial stage of the incubation, concentrations of MBN were highest in CHB amended soils at all application rates followed by CCB and PMB but by the end of the incubation period, PMB demonstrated highest MBN compared with the rest. The results as summarised in Table 13 indicates that MBN increased with increasing biochar application with higher MBN recorded in soils that received 65 t ha^{-1} than that of 39 t ha^{-1}. Incubation time was observed to have had an effect on MBN concentrations. Microbial biomass nitrogen increased rapidly at the early stages (3^{rd} and 7^{th} DAI) and peaked on 14^{th} DAI, thereafter MBN reduced marginally till the 42^{nd} DAI. This trend was observed when CCB and CHB were applied to experimental soil respectively at 39 and 65 t ha^{-1}. Following the application of PMB, sharp rise in MBN concentrations were observed at the initial stages (3^{rd} and 7^{th} DAI) but increased marginally thereafter till the 42^{nd} DAI.

The increases in MBN could be associated directly with the C/N ratio of biochar used in this study. The soil microbial biomass require N in a C/N ratio of about 8:1. Due to the high C/N ratio of biochar used (Table 5), it might have resulted in immobilisation of N especially for CCB and CHB. Although some authors have submitted that biochar is made up of biologically recalcitrant carbon that is not easily mineralized by the soil microbial community (Chan & Xu, 2009, Lehmann et al., 2011). It may however contain some proportion of labile organic components (Lehmann et al., 2011), which

may serve as energy sources for heterotrophs during the initial stages of decomposition of N-poor biochars and, hence, could potentially induce N immobilization in soil in the short term (Lehmann et al., 2006).

The fluctuations in the concentration of MBN with days of incubation that characterised biochar amended soils could be related to the dynamics in microbial proliferation and turnover on one hand, and the availability of substrates in biochar amended soils on the other. It is suggested that upon fresh C addition to soil, there is immediate revamping of microbial life, usually the r-strategist's microbes that depends on fresh C assimilates (Kuzyakov et al., 2009). The increase in microbial numbers at the initial stages might have caused a higher assimilation of available substrates in the biochar. The decreased MBN reported (28^{th} and 42^{nd} DAI) in CCB and CHB amended soils could be as result of exhaustion of available substrates. Biochar used for this study, especially CCB and CHB had low N concentration and might have been exhausted as the incubation period progressed. The exhaustion could be explained by adsorption to biochar surfaces, volatilisation, and microbial assimilation. The reduction in microbial substrates probably resulted in microbial turnover consequently reducing MBN in experimental soil. However, the MBN increased in PMB amended soils till the end of the incubation and could be related to the availability of microbial substrates since PMB used in this study had higher nutrient reserve compared with CCB and CHB.

The immobilisation of N in CCB and CHB amended soils respectively is similar to that reported by Deenik et al. (2010). Deenik et al. (2010) reported that biochar may have stimulated N immobilization in their study

following the addition of high C/N ratio (197:1) biochar. Other previous researchers have also demonstrated in their work that biochar application to N depleted soil could result in decrease in N availability caused by initial N immobilisation by microbial biomass (Novak et al., 2010; Nelson et al., 2011; Bruun et al., 2012). The increase in N immobilisation in biochar amended soils does not cause the loss in soil N but rather prevents losses of N through volatilisation, leaching and denitrification. The immobilised N is retained temporarily in microbial tissue as organic N. Upon microbial turnover the organic N held in their bodies may be converted into forms that makes up the humus complex or released as NH_4^+ or NO_3^-. In contrast to the findings of the current study, some previous studies showed that biochar addition although increased the concentrations of MBN, the values were not significantly different from the control (Alburquerque et al., 2013; Dempster et al., 2012). They attributed the results to the less degradable compounds (especially C) in the biochar material used.

Higher effects on MBN was obtained when biochar and poultry manure were applied together to experimental soils. This varied with biochar type and rates of application. Combined fractions that had CHB added (both 39 and 65 t ha^{-1}) showed MBN values that were higher compared with other combined fractions involving CCB and PMB.

The high MBN could be associated with high availability of N in soil introduced from the addition of manure. The high availability of this N substrate consequently resulted in higher assimilation, causing the elevation of MBN concentration. Concurrently, the increase in MBN concentrations could be associated with the proliferation of microbes that resulted from the addition

of both biochar and manure to the soil (Table 15). The initial rise on the 3rd and 7th day could be attributed to the rapid revamping of microbial life as a result of the creation of conducive environment for their growth courtesy biochar and manure application. The increased microbial biomass resulted in higher N assimilation (Blagodatskaya & Kuzyakov, 2013).

Effect of amendments on MBP

This section presents the results of the effect of addition of amendments on the concentration of MBP to experimental soil. The results are as shown in Table 14.

Table 14: *Effect of Treatments on Microbial Biomass Phosphorus (mg kg^{-1})*

Treatment	3 Days	7 Days	14 Days	28 Days	42 Days
Control	0.92 (0.042)n	0.81 (0.025)n	0.76 (0.035)n	0.74 (0.03)n	0.69 (0.20)n
39 t ha^{-1} CCB	1.65 (0.021)m	1.87 (0.045)lm	1.95 (0.032)lm	2.12 (0.040)lm	1.71 (0.032)m
39 t ha^{-1} CHB	1.85 (0.080)m	2.19 (0.021)lm	2.49 (0.110)lm	1.91 (0.12)lm	2.04 (0.066)lm
39 t ha^{-1} PMB	1.24 (0.095)mn	1.66 (0.137)m	1.98 (0.117)lm	1.82 (0.031)m	1.88 (0.066)lm
65 t ha^{-1} CCB	1.55 (0.080)mn	2.12 (0.035)lm	2.27 (0.040)lm	2.14 (0.030)lm	2.02 (0.060)lm
65 t ha^{-1} CHB	1.92 (0.066)lm	2.28 (0.031)lm	2.55 (0.070)l	2.06 (0.047)lm	1.81 (0.087)m
65 t ha^{-1} PMB	1.41 (0.045)mn	1.70 (0.035)m	1.74 (0.031)m	1.83 (0.032)m	1.82 (0.026)m
10 t ha^{-1} poultry manure	3.54 (0.170)k	3.92 (0.216)k	4.16 (0.141)k	4.95 (0.245)j	5.41 (0.173)j
39 t ha^{-1} CCB + 10 tha^{-1} poultry manure	7.34 (0.550)i	10.11 (0.258)g	10.82 (0.406)fg	11.14 (0.225)f	11.64 (0.290)ef
39 t ha^{-1} CHB + 10 tha^{-1} poultry manure	8.41 (0.312)h	12.01 (0.550)e	14.04 (0.763)c	14.41 (1.208)c	14.69 (0.727)bc
39 t ha^{-1} PMB + 10 tha^{-1} poultry manure	4.86 (0.165)j	6.95 (0.261)i	7.50 (0.181)i	7.87 (0.065)hi	7.96 (0.325)hi
65 t ha^{-1} CCB + 10 tha^{-1} poultry manure	4.87 (0.387)j	10.33 (0.490)g	11.23 (0.730)f	11.91 (0.708)ef	13.17 (1.941)d
65 t ha^{-1} CHB + 10 tha^{-1} poultry manure	7.27 (0.319)i	10.43 (0.423)g	14.69 (0.494)bc	15.30 (0.486)b	16.14 (0.523)a
65 t ha^{-1} PMB + 10 tha^{-1} poultry manure	5.03 (0.080)j	7.53 (0.360)i	7.93 (0.106)hi	8.14 (0.095)hi	8.42 (0.170)h

Mean (standard deviation in parenthesis); Means followed by the same letter do not differ significantly from one another ($p \leq 0.05$). CCB: corn cob biochar, CHB: cocoa husk biochar, PMB: poultry manure biochar

The MBP concentration in the control soil remained significantly ($P < 0.05$) lower than soils that were amended with biochar solely or in combination with poultry manure. Microbial biomass P decreased throughout the incubation period in the control which is attributable to the low microbial population and low microbial substrate in control soil. The soil is acidic and might have created an unfavourable environment for microbial survival and proliferation except for some few acid tolerant species. More so, the soil had low carbon content hence resulted in lower availability of microbial substrate for assimilation. As microbial substrate reduces, microbial turnover commences and this results in the reduction of microbial numbers hence the lower concentration of MBP in the control (Blagodatskaya & Kuzyakov, 2013).

The addition of Biochar increased the concentrations of MBP in soil. The concentration of MBP also increased when rates of biochar application were increased but the differences were statistically not significant ($P < 0.05$). Regarding incubation time, initial sharp increase (3^{rd}, 7^{th} and 14^{th} DAI) were observed and thereafter the concentration of MBP fluctuated with no regular pattern. By the 42^{nd} DAI, all biochar amended soils demonstrated significantly higher MBP concentrations above the control.

The increased MBP concentration in CCB and CHB amended soils could be associated with high C/P ratio of these biochar materials. The application of soil amendments with higher C/P ratio greater than 200:1, could result in P immobilisation and from Table 5, the C/P ratio of CCB and CHB were higher than this threshold ratio required to initiate P mineralisation. Then again, the increased amount of MBP could be related to the availability of

microbial substrate introduced by the application of biochar. The availability of substrate helps in microbial proliferation and increased microbial activity and subsequently resulting in higher assimilation of P. Moreover, biochar application increases pH of the soil. The rise in pH of the soil creates favourable environment for promoting the proliferation of microbes and making native P available. The increased microbial biomass eventually leads to higher P immobilisation (Blagodatskaya & Kuzyakov, 2013).

Upon the application of combined fractions of biochar and poultry manure, MBP significantly ($P < 0.05$) increased above that recorded in sole biochar or manure amended soils. The trend observed shows poultry manure together with CHB amended soils having highest MBP concentration than the rest of the combined fractions. When both amendments were applied together, MBP increased sharply at the initial stages (3rd and 7th DAI) but steadily thereafter (14th, 28th and 42nd DAI). The increase in the concentrations of MBP could be attributed to the availability of microbial substrate enhancing the assimilation of P. Poultry manure added had low C/P ratio, hence mineralised rapidly releasing more available P for microbial assimilation (Bitzer & Sims, 1988). The increases in MBP is promoted by higher microbial biomass as a result of the synergistic effect of both biochar and manure addition. The addition of inorganic P contained in biochar and manure could stimulate indigenous microbial population which was previously inactive due to the unavailability of readily decomposable organic carbon and with P deficiency. Then again, the amendments could contain microbes which were added to experimental soil upon their application to the soil. More so, properties of the biochar and manure improve the soil environment for microbial growth and

activity. The increased microbial population will mean a higher demand for P to be incorporated into their cells. The immobilised P is temporarily unavailable and upon microbial turnover, the P becomes available for plant uptake.

The increase in MBP found in this study is similar with the submissions of Zhai et al. (2015). They reported that MBP increased in biochar amended soils and attributed the increase to the improvement of the soil environment for microbial growth or due to the availability of P (Anderson et al., 2011; Lehmann et al., 2011).

Effect of Biochar and Manure on Phosphate Solubilizing Microbes

The compositions of fungi and bacteria in incubated soil have been summarized in Table 15. Biochar solely applied, or combined with poultry manure influenced soil microbial community. Fungi and bacteria biomass significantly ($P < 0.05$) increased in all treatments above the control by the end of the incubation period. In CCB amended soils, fungal and bacteria biomass increased with increasing biochar rates. The increase in the respective biomass when biochar rates were increased from 39 t ha^{-1} to 65 t ha^{-1} were not significant for both the biomass of fungi and bacteria. In CHB amended soils, both fungal and bacterial biomass significantly ($P < 0.05$) increased in soils amended with 39 t ha^{-1} biochar rates but at 69 t ha^{-1}, fungi biomass slightly decreased whiles bacteria population increased.

Table 15: *Fungal and Bacteria Biomass as Affected by Biochar and Manure Amendments*

Treatments	Fungal colony count (cfu×$10^{-5}g^{-1}$)	Bacteria colony count (cfu×$10^{-7}g^{-1}$)
Control	2.33 (0.38) f	6 (1.00)e
39 t ha^{-1} CCB	7.70 (1.45)e	30.3 (2.60)cd
39 t ha^{-1} CHB	8.60 (0.67)e	31.7 (5.61)cd
39 t ha^{-1} PMB	8.00 (1.50)e	42.3 (4.10)cd
65 t ha^{-1} CCB	10.00 (1.15)de	33.0 (6.56)cd
65 t ha^{-1} CHB	8.30 (1.45)e	34.7 (3.28)cd
65 t ha^{-1} PMB	12.30 (1.76)d	47.3 (4.86)c
10 t ha^{-1} poultry manure	6.33 (0.88)e	22.0 (4.62)d
39 t ha^{-1} CCB + 10 t ha^{-1} poultry manure	16.00 (1.53)bc	105.3 (5.49)b
39 t ha^{-1} CHB + 10 t ha^{-1} poultry manure	17.33 (2.03)bc	101.0 (5.29)b
39 t ha^{-1} PMB + 10 t ha^{-1} poultry manure	20.67 (1.76)ab	137.3 (16.83)a
65 t ha^{-1} CCB + 10 t ha^{-1} poultry manure	18.00 (2.08)b	129.7 (13.30)a
65 t ha^{-1} CHB + 10 t ha^{-1} poultry manure	23.00 (1.15)a	109.0 (6.60)b
65 t ha^{-1} PMB + 10 t ha^{-1} poultry manure	23.00 (2.65)a	96.7 (11.61)b

Values are expressed as mean± SEM. Different letters following the data in the same column denote significance ($P < 0.05$).
CCB: corn cob biochar, CHB: cocoa husk biochar, PMB: poultry manure biochar

Notwithstanding, the mean counts were significantly ($P < 0.05$) higher than the control. When PMB was increased from 39 to 69 t ha^{-1}, a significant increase was recorded for fungal biomass but bacterial biomass recorded an insignificant increase.

The increase in fungal and bacterial biomass could be associated with the effect of biochar on the properties of soil which influenced the microbial community. The current study showed an improvement in soil properties. Soil properties respectively correlated positively with fungal and bacterial biomass (Appendix D). Evidently, the soil used in this study was strongly acidic (pH = 4.17) and the application of biochar increased the pH significantly. pH correlated positively with fungi ($p < 0.01$; $r = 0.69$), bacteria ($p < 0.01$; $r = 0.60$). This might have resulted in the increase of microbial biomass. Although it has been shown that not all microorganisms react similarly to a pH increase, fungi and some bacteria species dominate in acidic soils, whereas actinomycetes avoid this environment and prefer soils with high pH values (Giri et al., 2005).

Apart from pH, soil organic carbon and other soil properties might have contributed to the increase in microbial numbers. Soil organic carbon, available P, ECEC and mineral N were all found to be positively correlated with both increase in fungal and bacterial biomass (Appendix D). Lehmann et al. (2011) noted that biochar addition changes the soil environment making it favourable for microbial proliferation. Increase in water holding capacity associated with biochar application increases the soils suitability as microbial habitat (Glaser et al., 2002). Especially in sandy soils, the biochar microspore's and surface structure cause a potential water retention effect.

Then again, in case of soil dehydration, biochar can offer refuge areas for microorganisms (Schimel et al., 2007) because the pores might contain film of moisture conducive for microbial growth. Furthermore, the pores in biochar can be valuable microhabitats for microorganisms which could act as a safe refuge from predators (Pietikäinen et al., 2000). Then again, the sorption of easily degradable organic compounds, dissolved organic carbon (DOC) and chemisorption of ammonium (NH_4^+) (Anderson et al., 2011) at biochar surfaces due to the presence of functional groups, could indicate its suitability as a favourable habitat (Pietikäinen et al., 2000). In contrast with some suggestions that biochar could affect microbial biomass negatively, due to the presence of volatile organic compounds (VOCs) in the labile fractions (Lehmann et al., 2011, Deenik et al., 2010, Kloss et al., 2013), this didn't happen in the current study. It might be suggestive that the rates used were probably appropriate to sustain, and improve microbial proliferation and their activity and did not cause the mortality of these microbes. In a recent study, Khan et al. (2014) found that biochar exerted a negative effect on the abundance and proliferation of soil microorganisms. They linked the reduced microbial biomass to the high C/N ratio (up to 400), of the biochar causing rapid mineralization of labile carbon leading to reduced soil nitrogen. As a result, availability of total N and C decreased due to microbial assimilation. In the current study, decrease in C and N concentration occurred in CCB and CHB amended soils and might have caused microbial turnover, the estimated number was still higher than that of the control. The finding in the present study is confirmed by previous authors. They posited that the addition of biochar changes physical and chemical parameters of the soil which indirectly

cause shifts in microbial abundance (Pietikäinen et al., 2000; Kolb et al., 2009; Liang et al., 2010) and structure (Lehmann et al., 2011; Glaser & Birk, 2012; Watzinger et al., 2014).

The combined effect of biochar and manure application elevated the fungal and bacterial biomass significantly above the sole biochar amendment and the control soils respectively. The increased in microbial biomass could be related to the addition of microbially available carbon in the poultry manure. This explanation is consistent with the submissions made by previous researchers that when microbially available carbon sources (e.g. plant residues or vegetable oil) are added to the soil, microorganisms tend to react by increasing their biomass (Stemmer et al., 2007; Mellendorf et al., 2010). Then again, the addition of the manure and biochar was synergistic and this enhanced the availability of nutrient (N, P and other cations) which improved the soil environment consequently leading to the microbial proliferation. Generally, bacterial biomass dominated in all the treatments over fungal biomass with higher biomass observed in combined manure and biochar treatments.

Determination of phosphorus solubilizing potential of microbes

Phosphorus solubilizing potential of fungi and bacteria isolates were determined on NBRIP agar and solubilization efficiency estimated in NBRIP broth (Nautiyal, 1999). The formation of clear (halo) zone on agar was used as a determinant of phosphorus solubilization capacity and the estimated concentration of available P in the broth was used as index for solubilization efficiency of the isolates (Nautiyal, 1999).

Identification of fungi and their phosphorus solubilizing capacity

Fungal isolates were identified on the basis of their morphological and microscopic features. A total of ten fungi strains were isolated from all amended-soils. Eight of the isolates were identified to belong to five genera (Figure 6 to 15). Identified strains were in the genus *Aspergillus* (*A. flavus, A. niger*), *Fusarium (sp1, sp2)*, *Penicillium (sp1, sp2)*, *Colletotrichum, Phytophthora spp.*

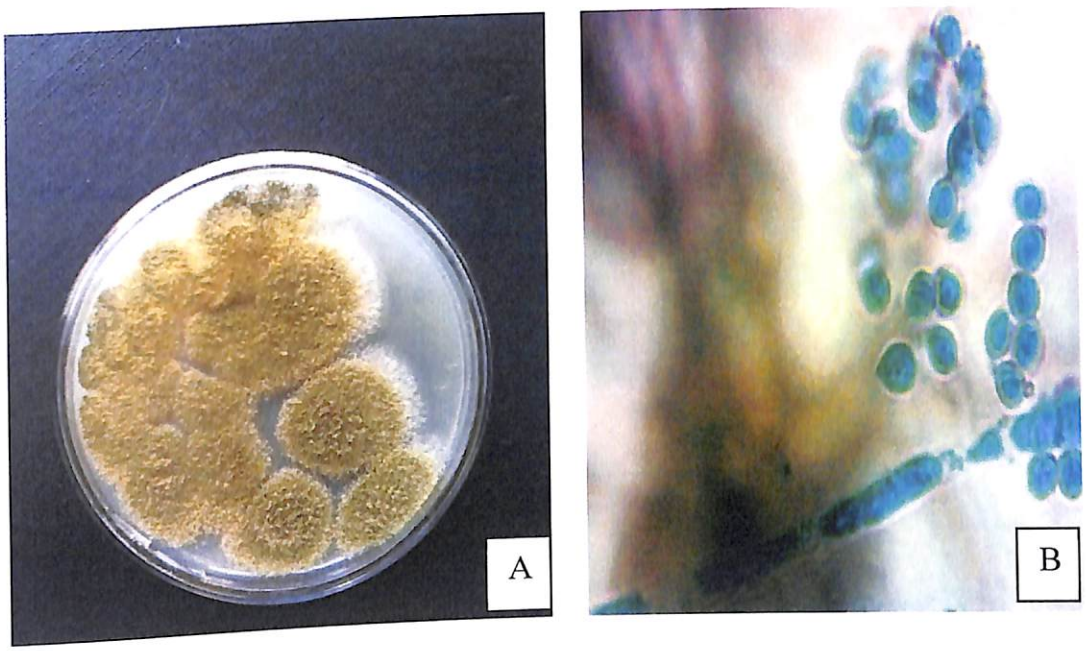

Figure 6: *A. flavus* (A) Colonies (B) Spores (magnification ×40)

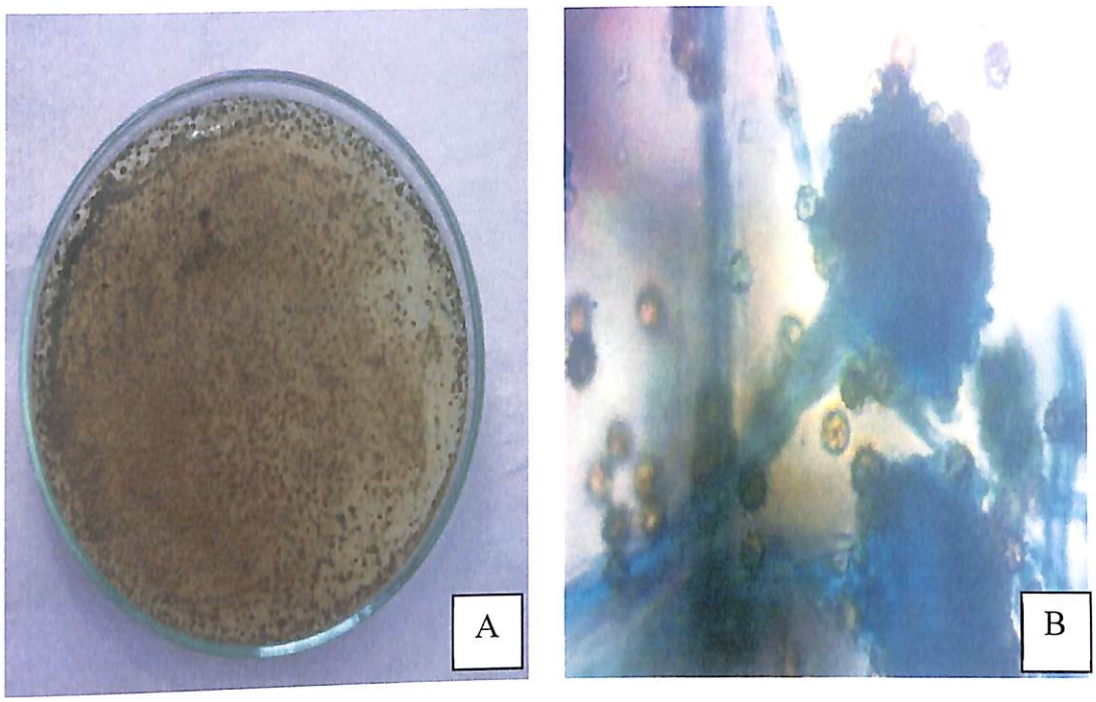

Figure 7: *A. niger* (A) Colonies (B) Spores (magnification ×40)

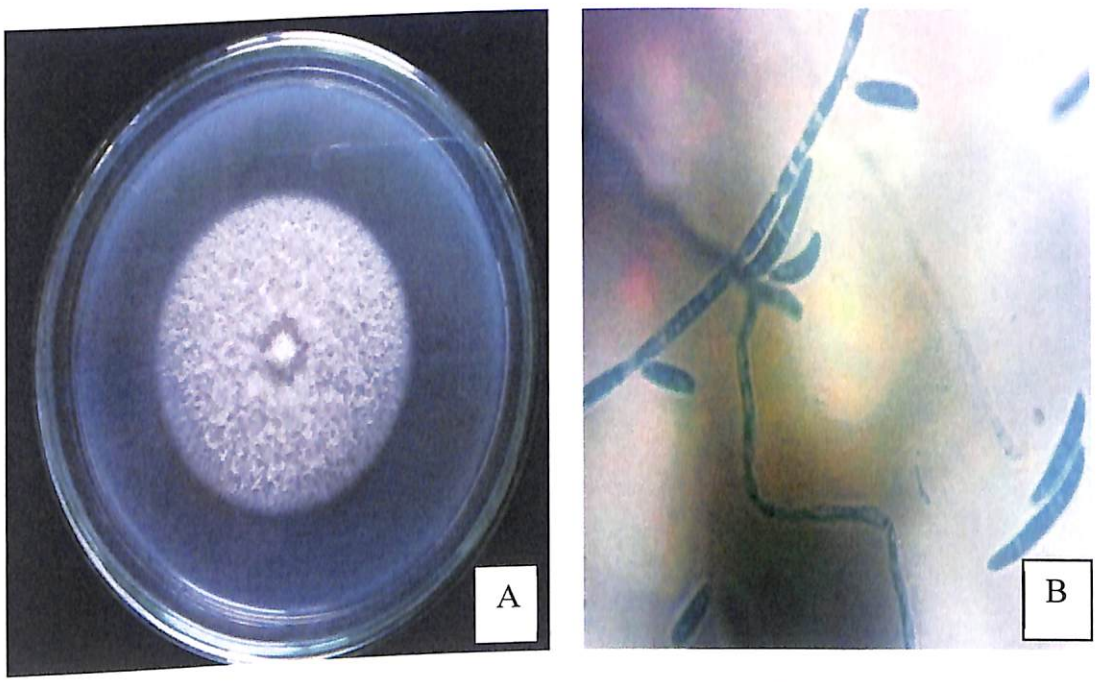

Figure 8: *Fusarium sp 1* (A) colony (B) Spores (magnification ×40)

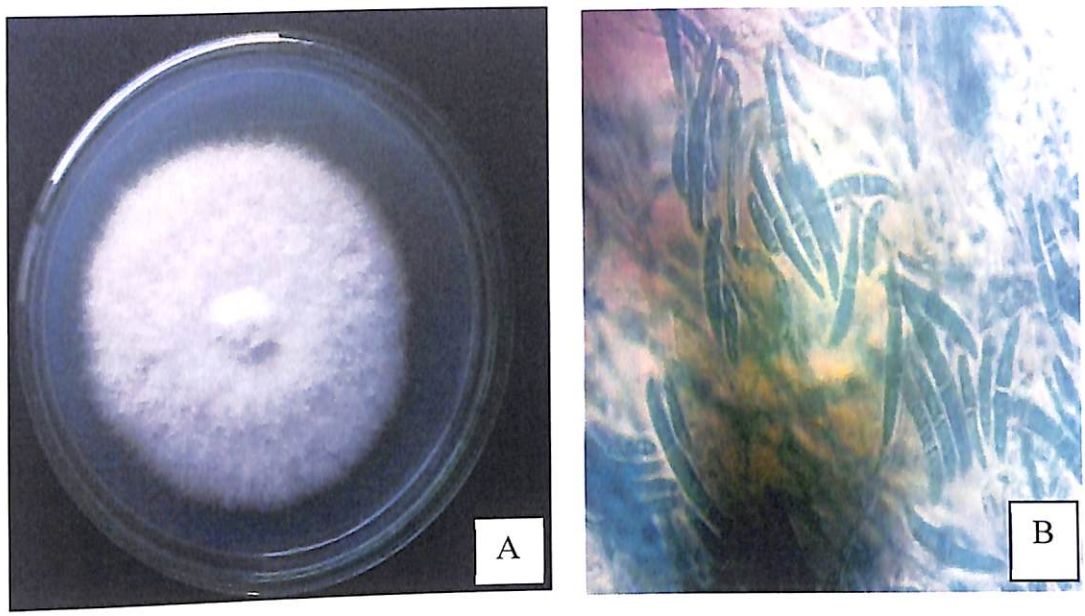

Figure 9: *Fusarium sp 2* (A) Colony (B) Spores (magnification ×40)

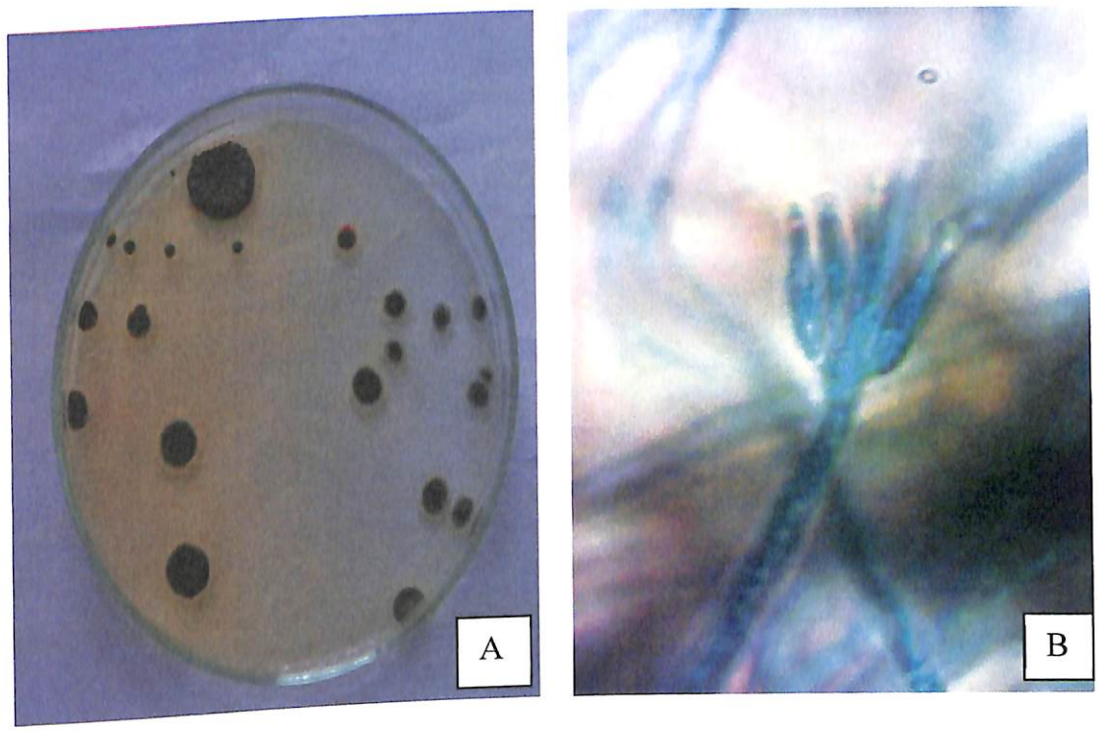

Figure 10: *Penicillium sp 1* (A) Colonies (B) Spores (magnification ×40)

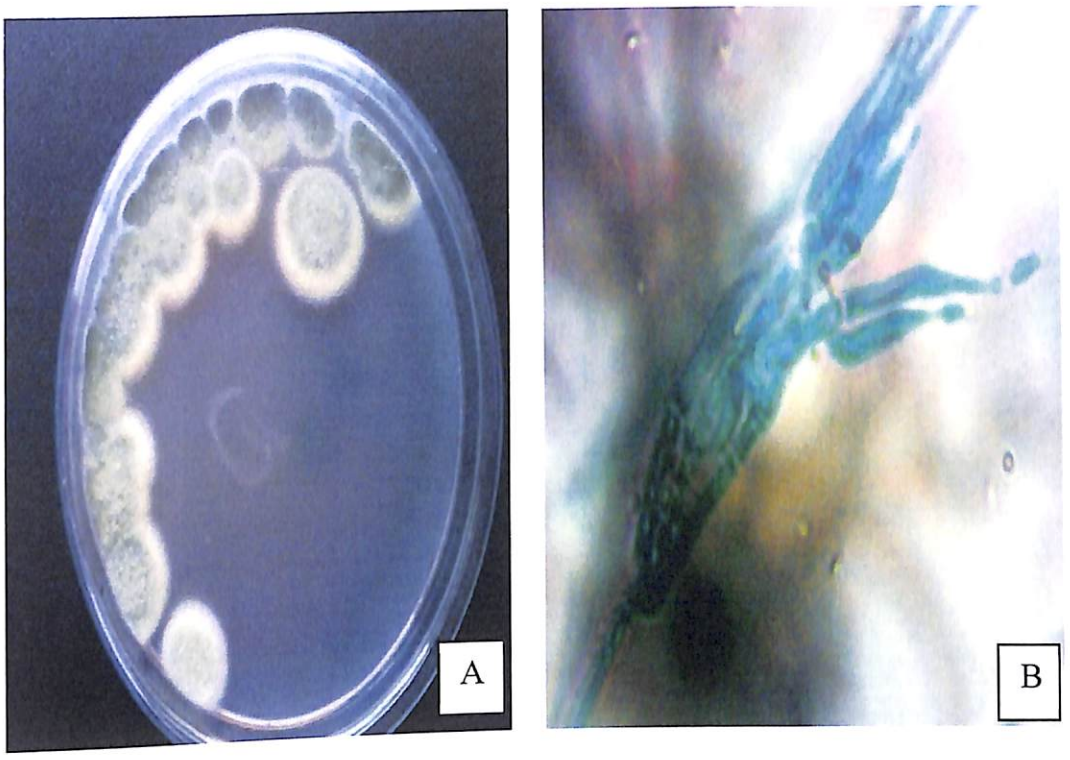

Figure 11: *Penicillium sp 2* (A) Colonies (B) Spores (magnification ×40)

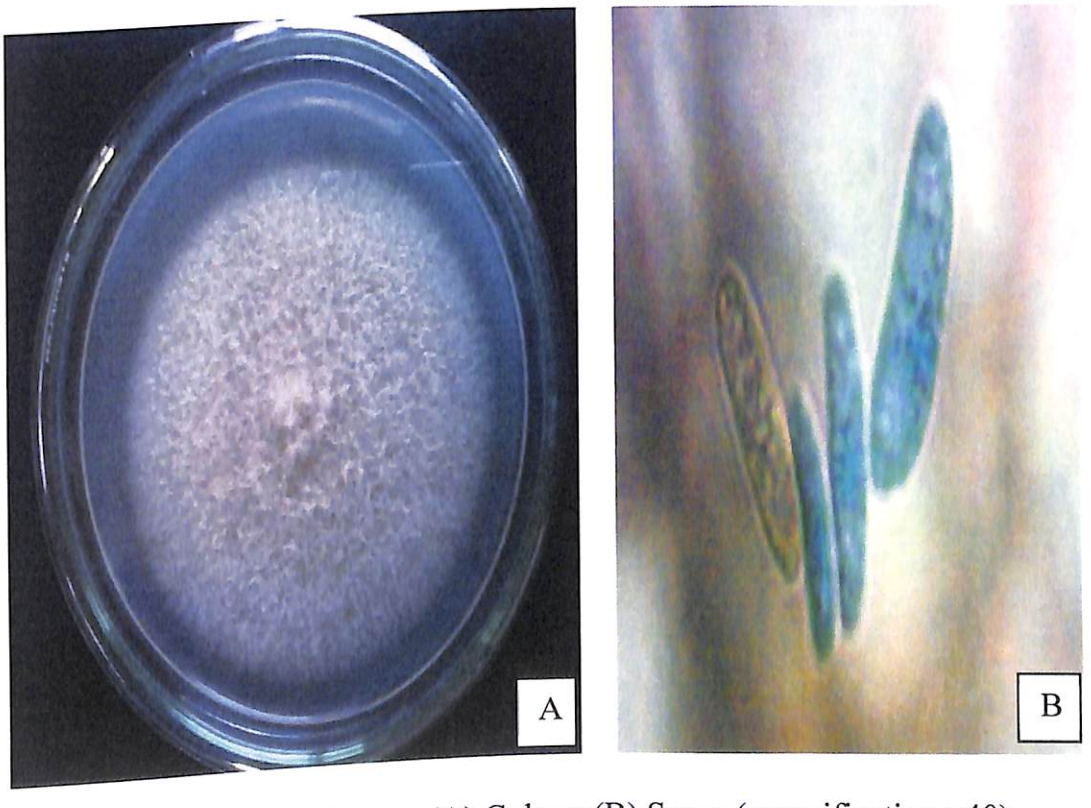

Figure 12: *Colletotrichum sp* (A) Colony (B) Spore (magnification ×40)

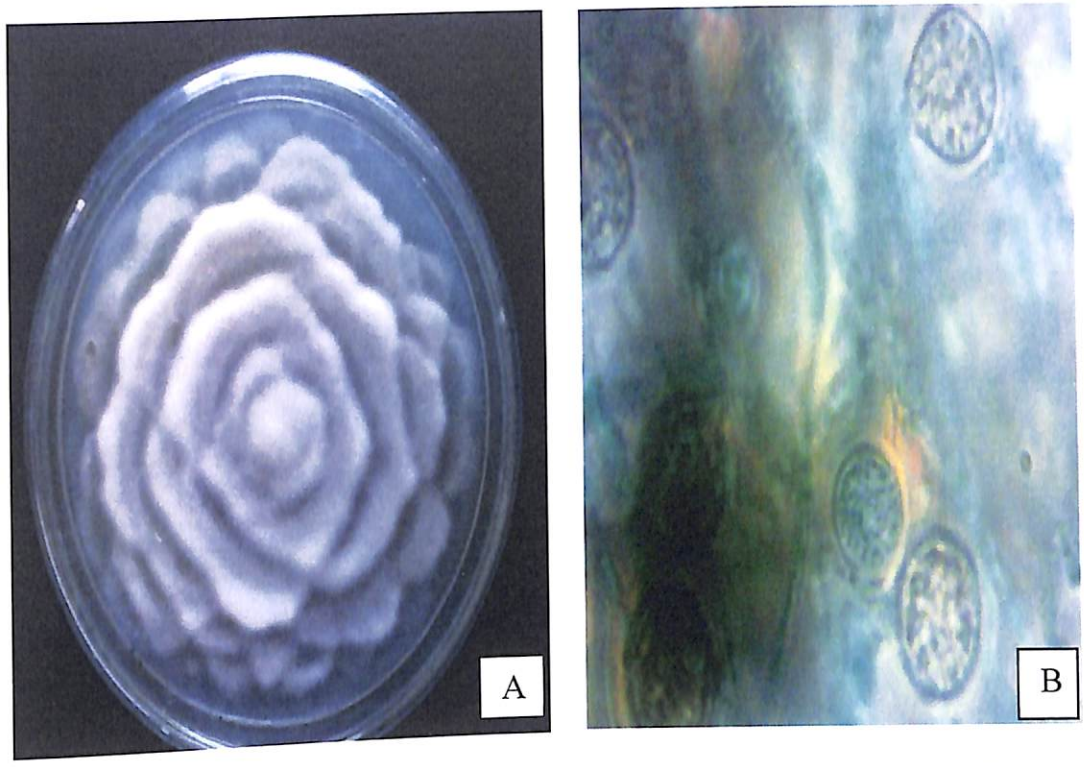

Figure 13: *Phytophthora sp* (A) Colony (B) Spores (magnification ×40)

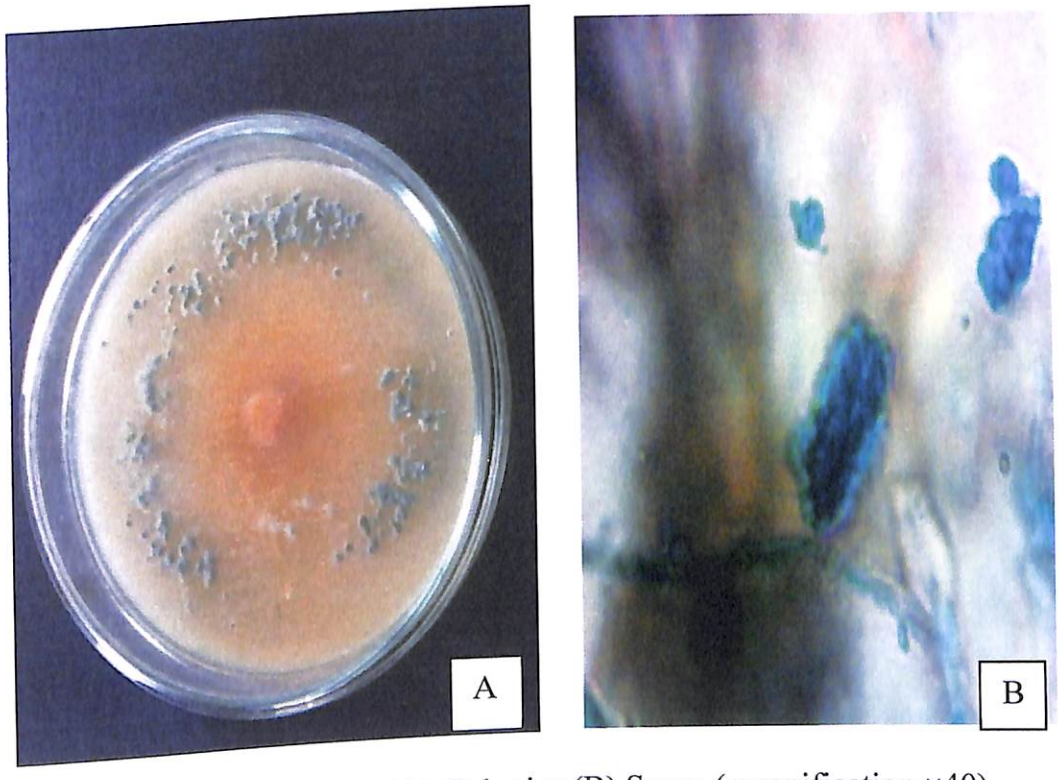

Figure 14: Unidentified 1 (A) Colonies (B) Spore (magnification ×40)

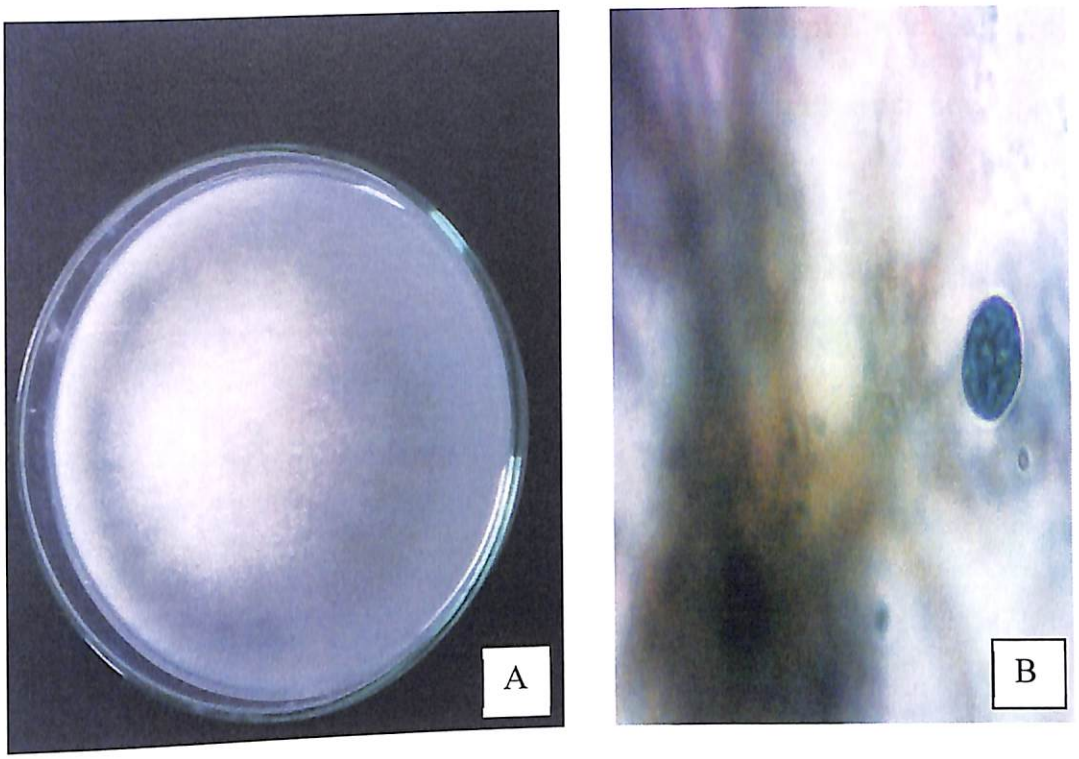

Figure 15: Unidentified 2 (A) Colony (B) Spore (magnification ×40)

It was observed that the isolates showed zones of clearance when cultured on NBRIP agar. Figure 16 is showing *A. flavus* growth on NBRIP agar.

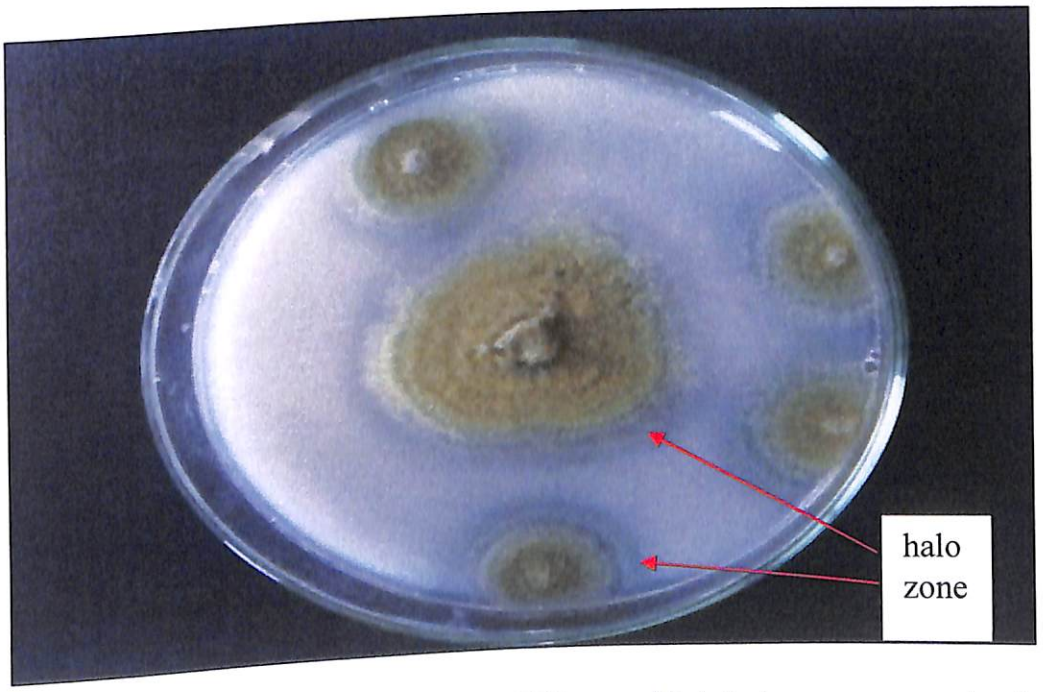

Figure 16: *A. flavus* growing on NBRIP agar. Note halo zones around colonies

Isolates that showed clear zones around their colonies were cultured in NBRIP broth to determine their phosphorus solubilizing efficiency (Figure 17).

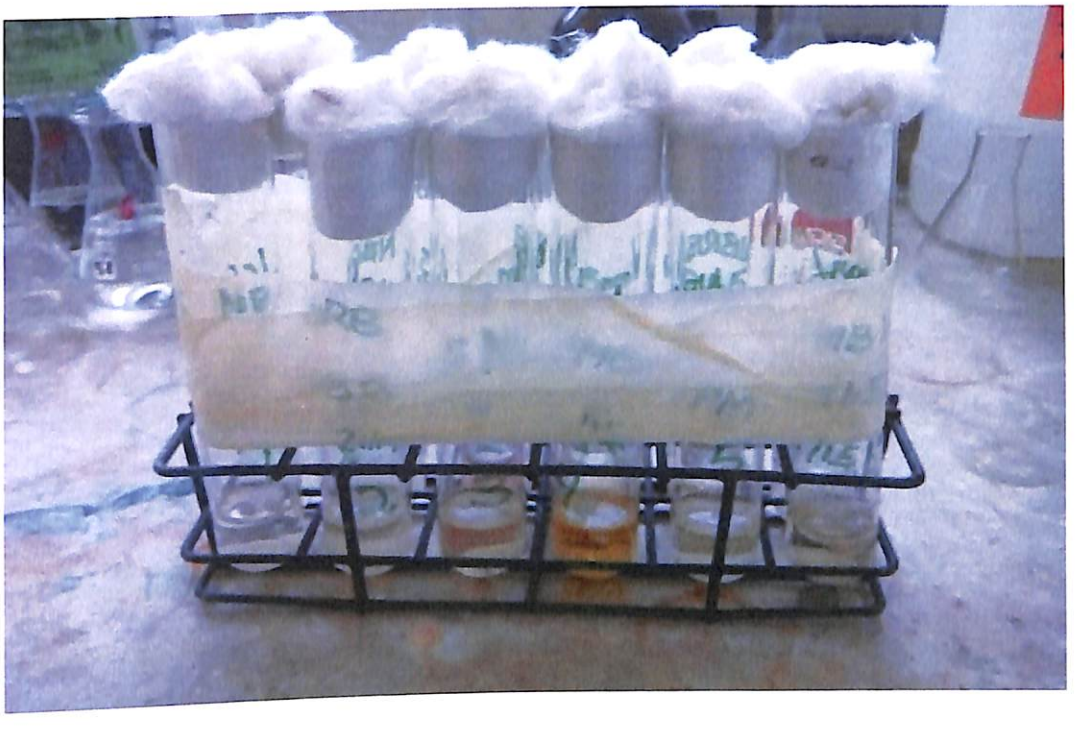

Figure 17: NBRIP broth inoculated with fungal species

Estimated solubilized P in NBRIP broth as a result of inoculating it with isolates of fungi were as shown in Figure 18.

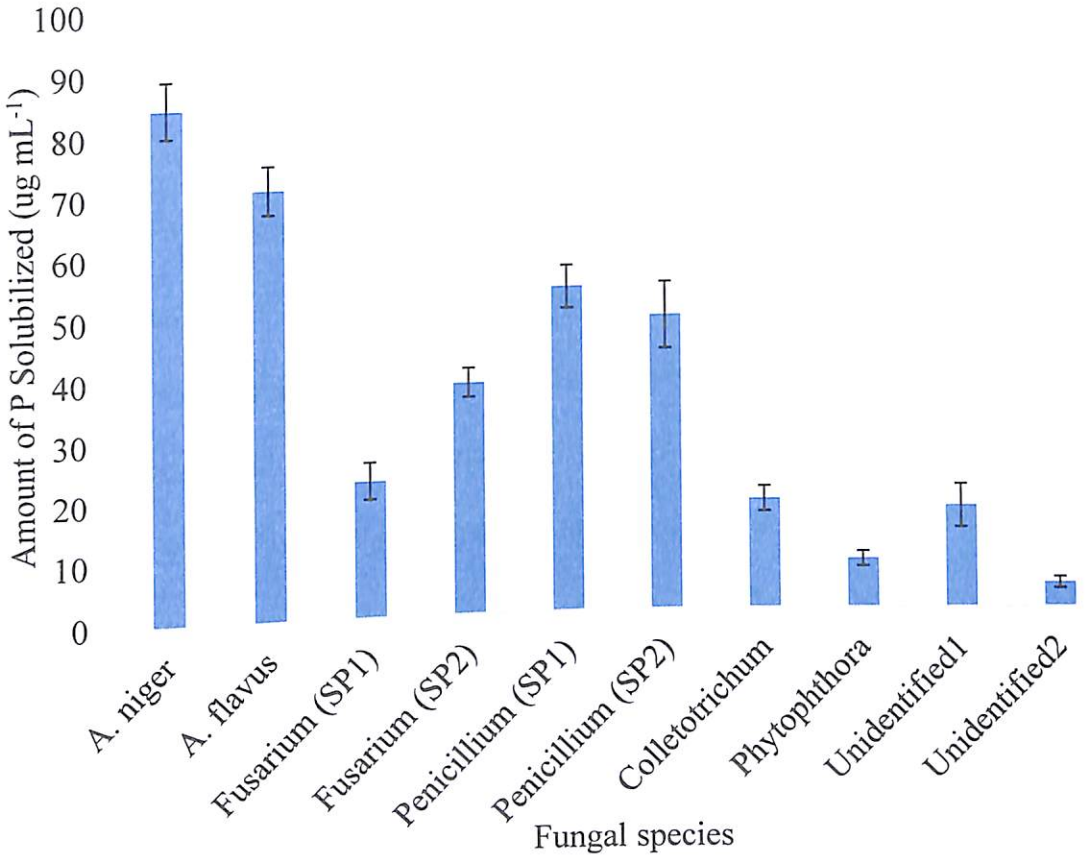

Figure 18: Amount of phosphorus solubilized by different fungal species in NBRIP broth media (ug mL^{-1})

It was observed that all the isolates solubilized P when inoculated in NBRIP broth. The estimation of P solubility efficiency in NBRIP broth showed that *A. niger* had the highest solubility ability recording P concentration of 84.67 ± 4.63 ug mL^{-1} in the broth culture. This was followed by *A. flavus* and *Penicillium (SP1) and (SP2)*, recording 71.08 ± 4.0, 53.87 ± 3.49 and 48.93 ± 5.43 ug mL^{-1} respectively. The other fungal isolates solubilized tricalcium phosphate (TCP) in NBRIP but not as efficient as estimated for *Penicillium* and *Aspergillus*. *Fusarium (sp1, sp2)* and *Colletotrichum* respectively solubilized 38.54 ± 2.42, 22.68 ± 3.13 ug mL^{-1} of P. Meanwhile *Phytophthora* isolated in this study solubilized the least concentration of P in NBRIP broth media.

Phosphorus solubilizing potential of *Aspergillus niger* and *flavus* have also been documented in previous studies. This was confirmed in a research where *Aspergillus* species isolated solubilized the highest concentration of P. In addition, Silva and Vidor (2001) and Yu et al. (2005), also reported high solubilization of TCP in liquid culture by *A. niger* and *Penicillium oxalicum*. In the current study, *Penicillium* species solubilized high amount of P in NBRIP broth and confirmed report of earlier study that *Penicillium* was superior to other fungi in phosphate solubilization (Gupta et al., 2007). The finding in this study is also supported by Salih et al. (1989) who also observed higher P solubilization potential of *Penicillium spp* compared to *Aspergillus spp*. Yadav *et al.* (2011) however reported higher P solubilization potential of *Aspergillus* species compared to *Penicillium spp*.

Fusarium have been reported in some studies to possess the potential for solubilizing phosphate (Srivastav et al., 2004; Akintokun et al., 2007; Kannahi & Umaragini, 2013). *Fusarium* increased phosphate solubilization significantly by increasing activities of acid phosphatase and alkaline phosphatase with a concurrent decrease in TCP concentration in the culture medium (Radhakrishnan et al., 2015). Similarly the ability of *Colletotrichum* to solubilize P unlike *Aspergillus* and *Penicillium* have been explored by few researchers as having the capacity to solubilize P (Tarafdar & Gharu, 2006) where it catalyzes the release of inorganic P from organic P compounds such as inositol hexaphosphate (Yadav & Tarafdar, 2011). No studies were found on P solubilizing potential of *Phytophthora spp*. Further studies is required to ascertain the P solubilizing potential of *Phytophthora spp*. This would help ascertain its importance in solubilizing P in soil apart from being an agent of

plant diseases. Results obtained from in this work however show that *Phytophthora spp* is a weak P solubiliser (Figure 18).

It is well known that phosphate solubilizing microorganisms in soil solubilized insoluble phosphates mainly by secreting acids into the medium (Dave & Patel, 2003, Chung et al., 2005). The organisms isolated in this study might have used the same mechanism. *Penicillium* and *Fusarium* have been documented to produce lactic acid, maleic, acetic, gluconic acid (Akintokun et al., 2007). In addition, *A. niger* and *A. flavus* produce oxalic, gluconic, succinic and citric acids (Maliha et al., 2004).

The high solubility of P by *Aspergillus* and *Penicillium* signifies that P held in insoluble forms such as tricalcium phosphate $(Ca_3PO_4)_2$, aluminium phosphate (Al_3PO_4), iron phosphate (Fe_3PO_4) can be converted to soluble P by these organisms in the soil ecosystems (Khan et al., 2013; Sharma et al., 2013). The upsurge in the biomass of P solubilisers (Table 15) coupled with other related factors like increase in pH and basic cations could be possible reasons for the increase in AVP observed in the current study.

Identification of bacteria and their phosphorus solubilizing capacity

Bacteria isolated from amended soil were categorized into two groups based on the reaction with 3 % potassium hydroxide (KOH) (string method) solution.

Eight bacterial species comprising six gram negatives (PSB1N, PSB2N, PSB3N, PSB4N, PSB5N and PSB6N) and two gram positives (PSB7P and PSB8N), were isolated. The number of bacterial species that showed positive and negative reactions with KOH are presented in Figure 19.

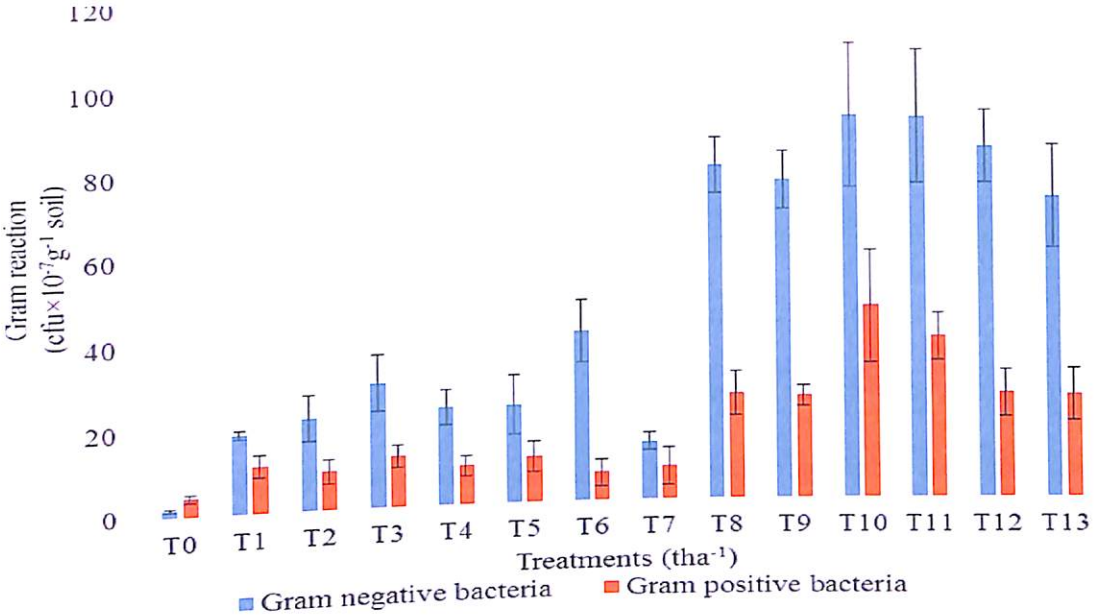

Figure 19: Distribution of bacteria based on KOH reaction

It was observed that both strains of bacteria were responsive to the application of amendments causing significant ($P < 0.05$) increases in their numbers compared with the control (T0). Apart from the control where Gram positive bacteria were dominant, the addition of the amendments (T1 to T13) resulted in a shift in bacteria community in favour of Gram negative bacteria. The increase observed for both strains of bacteria (gram negative and positive) in the current study conforms to that reported by other authors (e.g Prayogo et al., 2014; Paz-Ferreiro et al., 2015).

Increase in the numbers of both Gram negative and positive bacteria could be explained by the reactions of these two bacteria groups upon biochar application. It has been reported that Gram positive bacteria is stimulated by the presence of recalcitrant organic compounds, in this case recalcitrant biochar C might cause the proliferation of Gram positive strains. On the other hand Gram negative bacteria have been associated with the labile C compounds (Treseder et al., 2011) possibly added by biochar material.

Although the number of both strains of bacteria increased, above the control, Gram negative bacteria were dominant in all the treatments.

The dominance of Gram negative bacteria found in the current study could be due to the nature of biochar and the changes caused to soil properties as result of the biochar and manure amendments. Increase in pH of the amended soils affected the composition of both bacterial groups; (Gram negative bacteria: $p < 0.01$, $r = 0.60$) and Gram positive bacteria: $p < 0.01$, $r = 0.42$). In acidic soil with low C and N contents, gram positive strains dominated (as observed in the control soil) but as pH increased, there was a shift of bacteria community in favour of Gram negative bacteria. The pH rise might have increased the effectiveness of sugars and amino acids to stimulate the proliferation of Gram negative bacteria compared with Gram positive bacteria (Cong et al., 2014). The findings in the current research is congruent to that reported by Watzinger et al. (2014) who reported higher population of Gram negative bacteria upon application of willow biochar to a Planosol. The effects of biochar were mainly attributed to an increase in the pH of the Planosol. Then again, it would be plausible to link the upsurge in the numbers of gram negative bacteria to high C input in sole biochar treatment and the high nutrients input (C, N, P, cations and micro nutrients) in combined treatments (Stark et al., 2007). Pearson correlation revealed a significant positive relationship between increasing microbial numbers and soil properties (Appendix D).

In line with this explanation, Cong et al. (2014) found that the proportions of fungi and Gram negative bacteria increased when high carbon material was introduced into the soil, while the proportion of Gram positive

bacteria was reduced. Nitrogen availability, and with greater contents of total and labile soil C have been linked with higher negative bacteria population (Salinas et al., 2011; Zimmermann et al., 2012). Several studies have shown that Gram negative bacteria, which are sensitive to oligotrophic conditions (Esperschütz et al., 2009) are often stimulated by added organic matter resulting in a low Gram-positive/Gram negative bacteria ratio (Larkin et al., 2006; Buyer et al., 2010). This was also very evident when biochar combined with manure treatment resulted in relatively high composition of Gram negative compared with Gram positive bacteria. Stark et al. (2007) explained that organic amendments significantly improved the soil fertility status, which enhanced the microbial diversity, biomass and activity. The dominance of gram negative bacteria and the lower gram-positive/gram negative bacteria ratio is indicative of better soil nutrition (Rajendran et al., 1997).

Further observation indicates that all the isolates showed a clear zone when cultured on NBRIP agar indicating P solubility potential of these species (Figure 20).

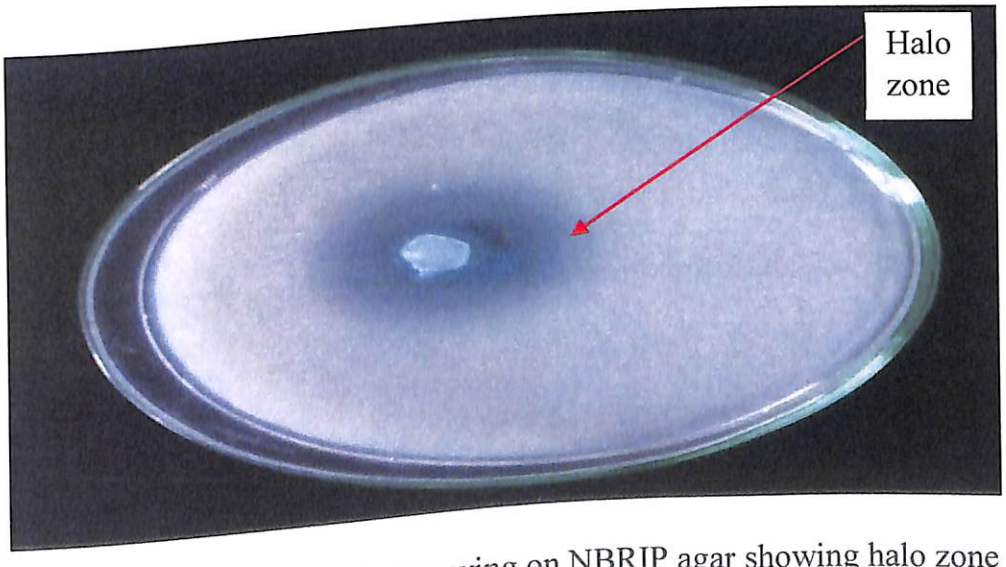

Figure 20: Bacteria species growing on NBRIP agar showing halo zone

In addition, when the isolated bacteria species were cultured in NBRIP broth for 10 days, they solubilized TCP and the results have been shown in Figure 21.

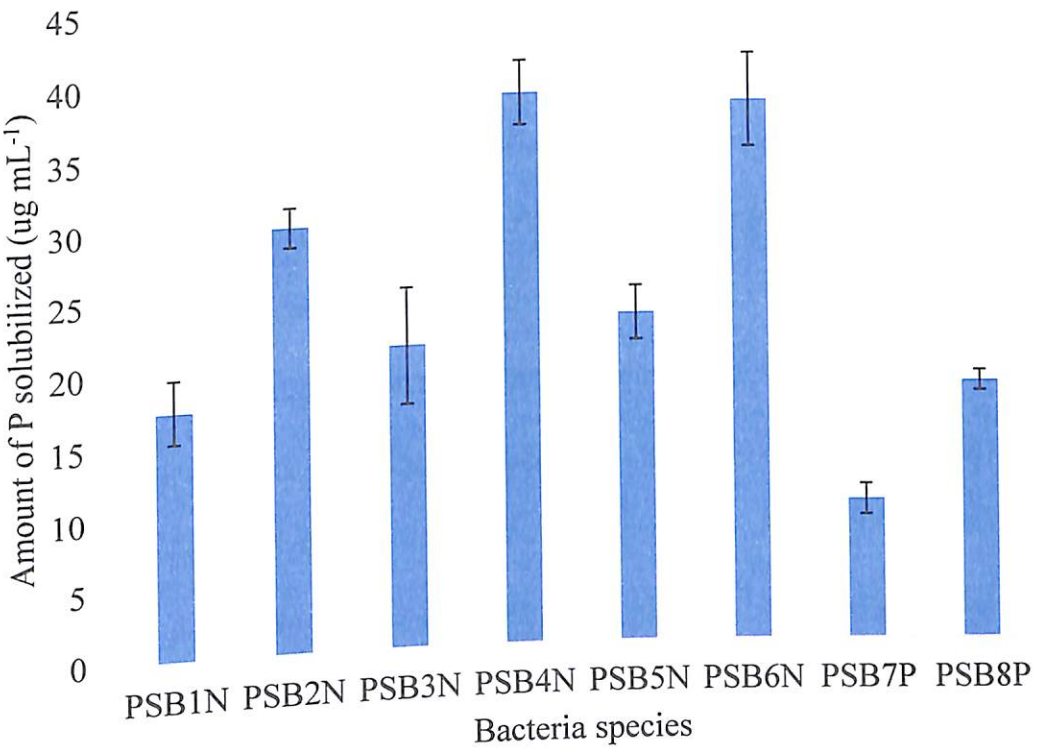

Figure 21: Amount of phosphorus solubilized by soil bacteria in NBRIP broth (ug mL-1). N = Gram negative, P = Gram positive species

It was indicative that all the isolates solubilized P, with gram negative bacteria species showing a higher solubilization potential. Bacterial species (PSB4N) showed higher solubility efficiency in NBRIP broth with soluble P concentration of 38.97 ug mL^{-1}. This was followed by PSB6N, PSB2N, PSB5N, PSB3N and PSB1N with respective P concentrations of 38.01, 30.08, 23.18, 21.35 and 17.10 ug mL^{-1} (Figure 21). On the other hand, PSB7P and PSB8P respectively solubilized 10.00 and 18.25 ug mL^{-1}.

The ability of both Gram negative and Gram positive to solubilize P have been reported in related studies (Kundu et al., 2009; Tilak et al., 2005). The results also demonstrated that Gram negative bacteria solubilized higher amounts of P compared with the Gram positive bacteria. This finding is encouraging because it has been posited that Gram negative bacteria dominates the rhizosphere accounting for 90 % of bacteria biomass (Midekssa et al., 2015; Muleta et al., 2009), therefore their ability to solubilize P will be an advantage in replenishing P deficient soils.

Generally, fungi showed higher potential of solubilizing P than bacteria which contrast the findings of a related study where bacteria were found to be more active than fungi in conversion of insoluble P to soluble P (Alam et al., 2002). In consistent with the findings of the current research, Seshachala et al. (2012) found that fungi have been more efficient in solubilizing phosphates than bacterial species. It was explained that P-solubilizing fungi do not lose the P dissolving activity upon repeated sub culturing under laboratory conditions as occurs with the P-solubilizing bacteria (Pandey et al., 2008). In addition, fungi present other characteristics, such as a wide range of tolerance for temperature, pH and salt concentration (Pandey et al., 2008) and production of phytohormone or siderophore (Vassileva et al., 2010). Further, P-solubilizing fungi produce more acids than bacteria and consequently exhibit greater P-solubilizing activity (Venkateswarlu et al., 1984).

Conclusion

The application of biochar solely or combined with poultry manure resulted in significant ($P < 0.05$) increases in MBC, MBN and MBP above the

control throughout the incubation period (7, 14, 28 and 42 DAI). Then again, co application of biochar and poultry manure yielded higher concentrations of MBC, MBN and MBP above sole biochar application.

The increases in MBC, MBN and MBP observed in soils varied with biochar (type of biochar, application rates and incubation period. Microbial biomass C was highest in PMB amended soils followed by CHB and CCB amended soils. Considering MBN, CHB treated soils produced the highest MBN followed by CCB and PMB which is indicative of the effect of high C/N ratio. Increasing the application rates of biochar resulted in significant increases in the concentrations of MBN, MBC and MBP above the control. The trend observed in the values were $0 < 39 < 65$ t ha^{-1}.

The study also revealed that PSF and PSB significantly increased above the control upon sole application of biochar or biochar co applied with poultry manure. Moreover, combined biochar and poultry manure increased both PSF and PSB numbers significantly compared with when they were applied separately.

CHAPTER SIX

EFFECT OF BIOCHAR ON EARTHWORM SURVIVAL AND ACTIVITY

Introduction

Earthworms are highly recognised in many cultural settings because of the role they play in the fertility improvement of the soil (Ampofo, 2007). This implies that any disturbance; positively or negatively that affects earthworm may indirectly affect soil function and plant growth. Moreover, earthworms are used as a standard test species to investigate the impact of a substance on the soil properties before approval is given for its application (Van Gestel, 1992).

Since the upsurge in the use of biochar as a soil amendment, evidence has shown that some biochars' may have negative effects on the earthworms (Liesch et al., 2010) resulting in their reduced growth and mortality. The negative impact was attributed to alterations in soil pH and ammonia concentration (Leisch et al., 2010). Other studies have attributed causes of earthworm mortality to potential physical damage arising from the biochar sticking to the earthworm's body (Schmidt et al., 1999).

However, information on the effect of biochar on earthworm population, activity and overall soil function following the application of biochar is limited in the tropics. In addition, the effects may be variable depending on type of soil used, the type of biochar, and the application rates (Leisch et al., 2010). It has therefore been suggested that further research is needed to standardize earthworm studies (Frund et al., 2010). This requires adequate data on biochar properties and information on the environment in

which they are to be used. This would help to develop appropriate recommendations for the application of biochar. This study therefore evaluated the impact of different types of biochar on survival of tropical earthworm (*Eisenia fetida*) and also to identify the appropriate rates for some biochar application to soil.

Materials and Methods

The study area has been described in chapter Three. Earthworms were collected from the same sampling sites in order to reduce variability in biotype. They were sampled from subsurface of soil litter and within plantain farm after rainfall. The site chosen for earthworm collection was to ensure that there was adequate moisture in the soil where earthworms inhabit. According to Munnoli et al. (2010) earthworms lose a lot of water and their survival and activities are suppressed when they are exposed to dry environment. They often do well at moisture contents optimum range of 50-90 %.

Preliminary sampling was carried out to identify the earthworm species available at the selected sampling site. The most common earthworms identified were *Eudrilus eugeniae and Eisenia fetida*. However *E. fetida* was used for this study (Figure 22) following the guideline by the Organization for Economic Cooperation and Development (OECD) for earthworm toxicity test. In addition it was recommended as the test species because it is able to survive laboratory conditions. It has also been used extensively for toxicity and bioaccumulation studies of a variety of compounds (Byung-Tae, 2008; Abbiramy et al., 2013).

Live earthworm sampling was done by digging and hand-sorting. A soil core, to a depth of 10 cm, was collected and washed in perforated plastic

bowls to collect the worms. Earthworms with well developed clitellum were sampled and used for biochar toxicity incubation study at the School of Agriculture Teaching and Research farm, University of Cape Coast.

Figure 22: *Eisenia fetida* earthworms used for the experiment

Worms used for the study did not differ considerably in size and had a relatively homogeneous age structure (measured by weights). In the laboratory, the worms were kept in plastic pot, about 5 L, which was half filled with a mixture of the experimental soil, supplemented with dry leaves and moistened with distilled water as described by Terhivuo and Saura (1993). The earthworms were kept in the pots for a minimum of ten days in order to allow them to adapt to experimental conditions. The adaptation incubation showed that *E. fetidia* was tolerant of this prepared soil substrate.

Experiment two

A 42 day incubated, controlled experiment using three types of biochar (PMB, CCB and CHB) was setup in 1.5L cylindrical containers at the University of Cape Coast Research and Teaching Farm. About 1kg of the experimental soil was measured and used for the pot experiment. Three biochar types each were added at the rates of 0 t ha^{-1}, 13 t ha^{-1}, 26 t ha^{-1}, 39 t ha^{-1}, 52 t ha^{-1}, 65 t ha^{-1}, 78 t ha^{-1}, 91 t ha^{-1}, 104 t ha^{-1}, 117 t ha^{-1}, 130 t ha^{-1}, 143 t ha^{-1} and 156 t ha^{-1} and mixed with the soil before packed into experimental pots. During potting, moist loose shredded papers were placed in the pots to serve as beddings for the worms and poultry manure was used as food substrate. Ten sub-adult of *E. fetida* earthworms, with average weight range of 0.55 – 0.60 g, were introduced into each pot and monitored for survival. Each treatment had seven replications. The pots containing the earthworms were then kept in the greenhouse and moisture content of the mesocosm maintained between 50–70 % during the study by watering the contents of the pots every 3 days.

Data was collected on earthworm survival after days; 3, 7, 14, 28 and 42 of exposure to the biochar. The mortality of earthworms was assessed following OECD 207 Test Guideline. The LC_{50} in this Test Guideline highlights the median lethal concentration i.e. that concentration of the test substance which kills 50% of the test animals within the test period.

Surviving earthworms were counted and recorded on each monitoring day. For each sampling day, soil sample from each pot was carefully poured unto a tray and soils separated and earthworms counted. After the counting, the soils with the earthworms were returned into the experimental pots. During

monitoring and especially on the 3rd day of incubation, dead earthworms found at the surface of soil in pots were noted and removed. In addition, as dead tissue decomposes rapidly in soil, earthworms not found were assumed to have died during the incubation period. More so, an earthworm was judged to be dead if it did not respond to stimulus with a blunt probe.

Earthworm activity was assessed through the amount of cast produced at the top 5-10 cm of the soil surface. The cast were collected and oven dried at 105 ^{0}C till constant weight was obtained and results expressed as grams per kilogram oven dry weight of cast.

Data analyses

Data was analysed using statistical products for social scientist (SPSS) (version 16). Descriptive data was generated using SPSS and exported into excel to plot bar graphs. Mean comparism was carried and significant differences estimated using standard error of the mean. Pearson's moment correlation was used to determine how the biochar treatments related with mortality rates. Results have been presented in graphs and tables.

Results and Discussion

Earthworm survival after exposure to biochar

Presented in Figure 23 to 27 are the number of earthworms that survived on the 3rd, 7th, 14th, 28th and 42nd day of the incubation experiment following the exposure to biochar.

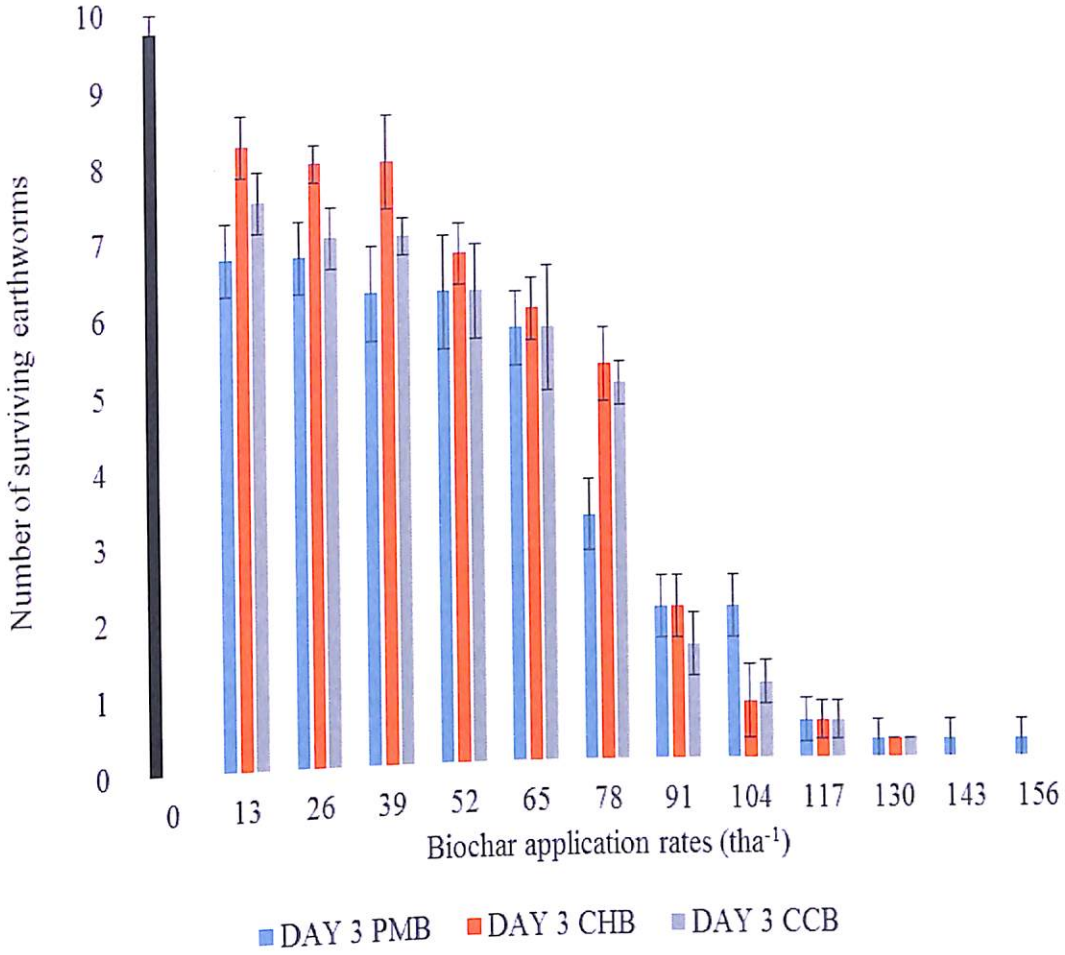

Figure 23: Mean earthworm survival on the 3rd day

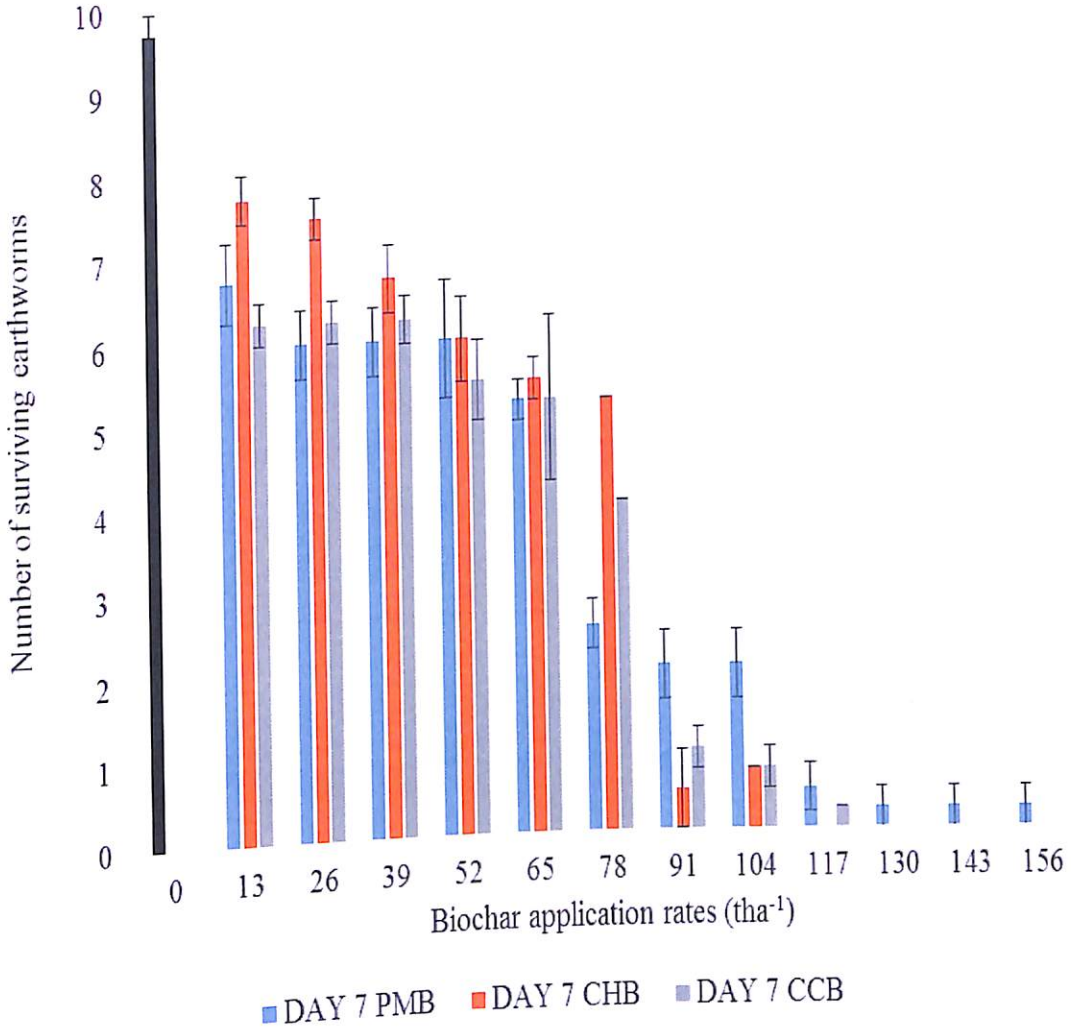

Figure 24: Mean earthworm survival on the 7th day

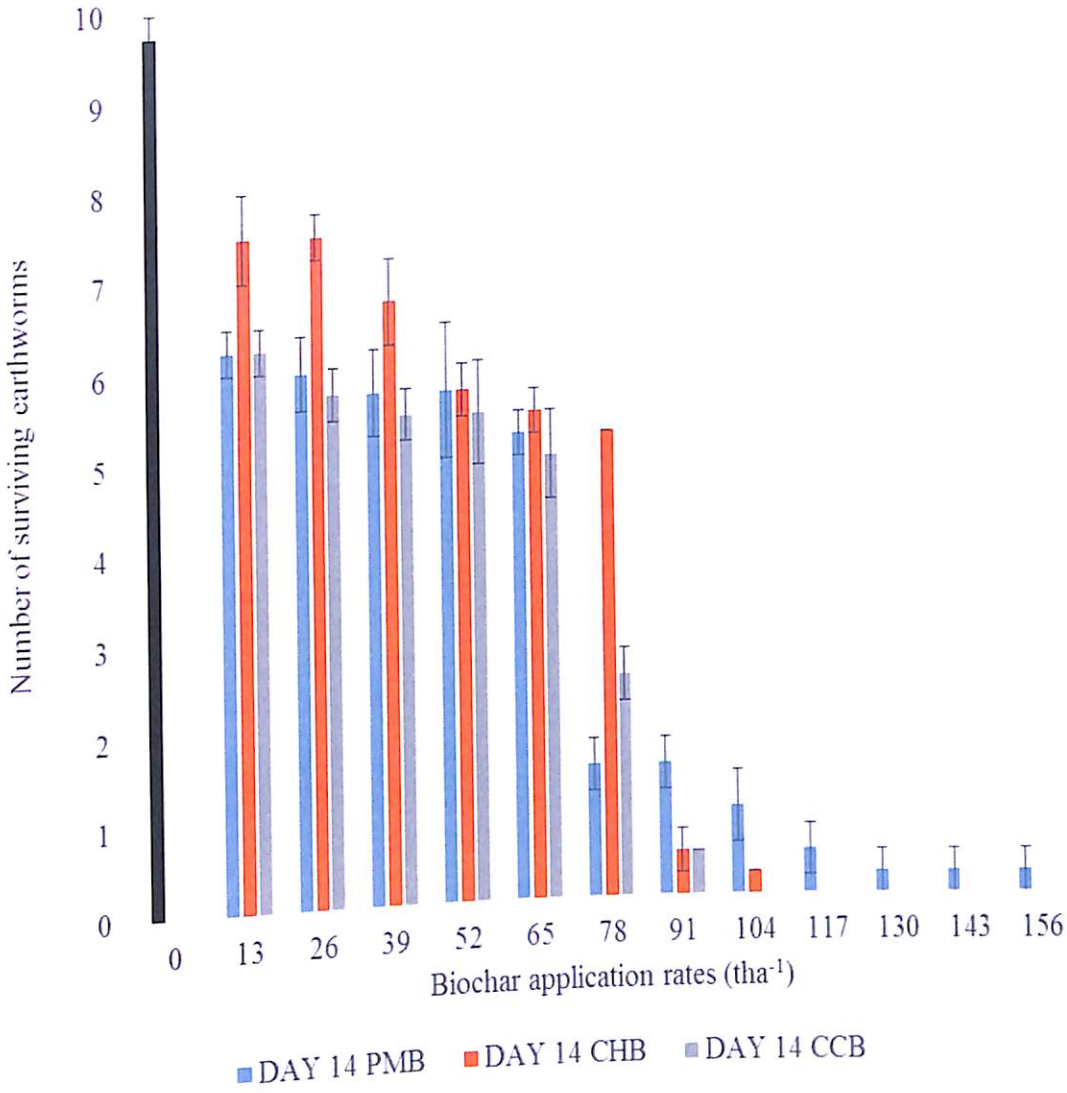

Figure 25: Mean earthworm survival on the 14th day

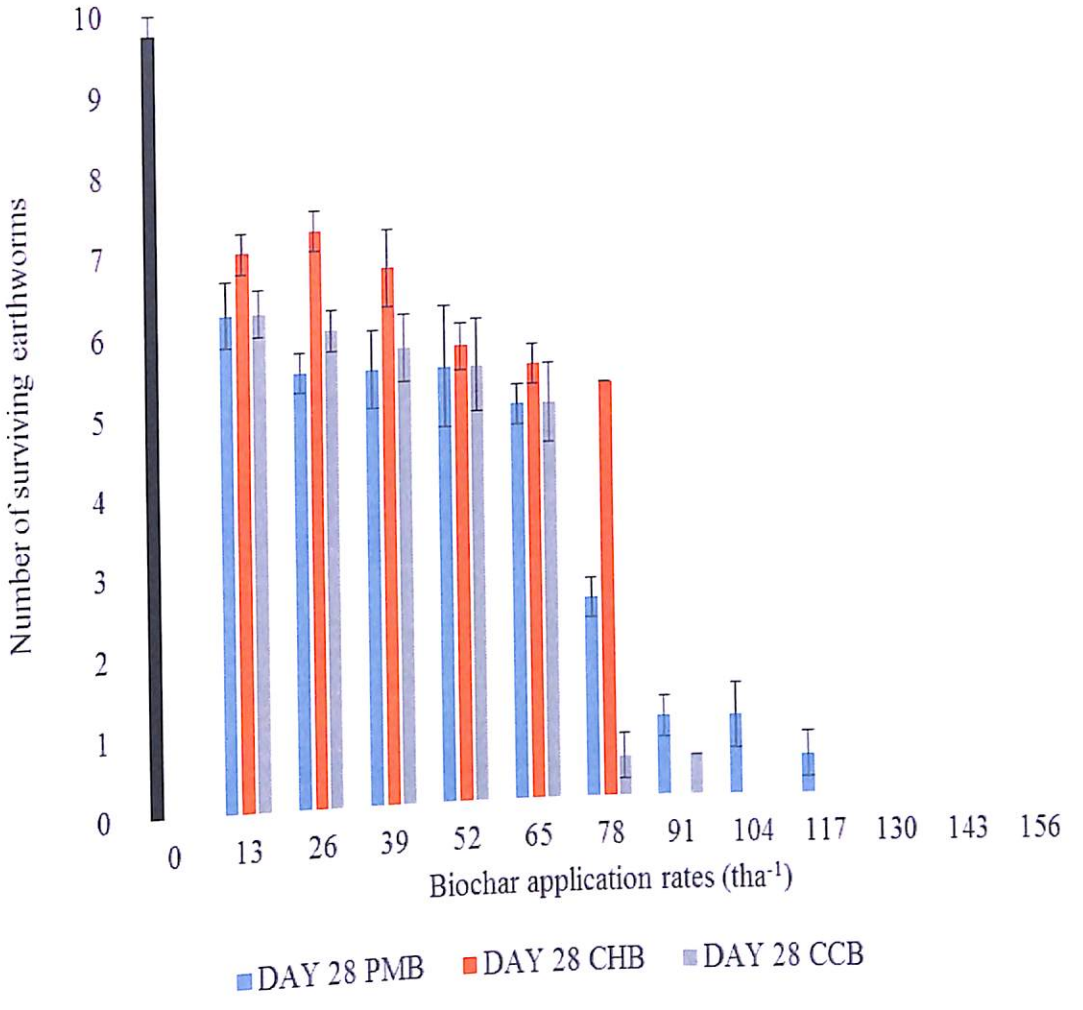

Figure 26: Mean earthworm survival on the 28th day

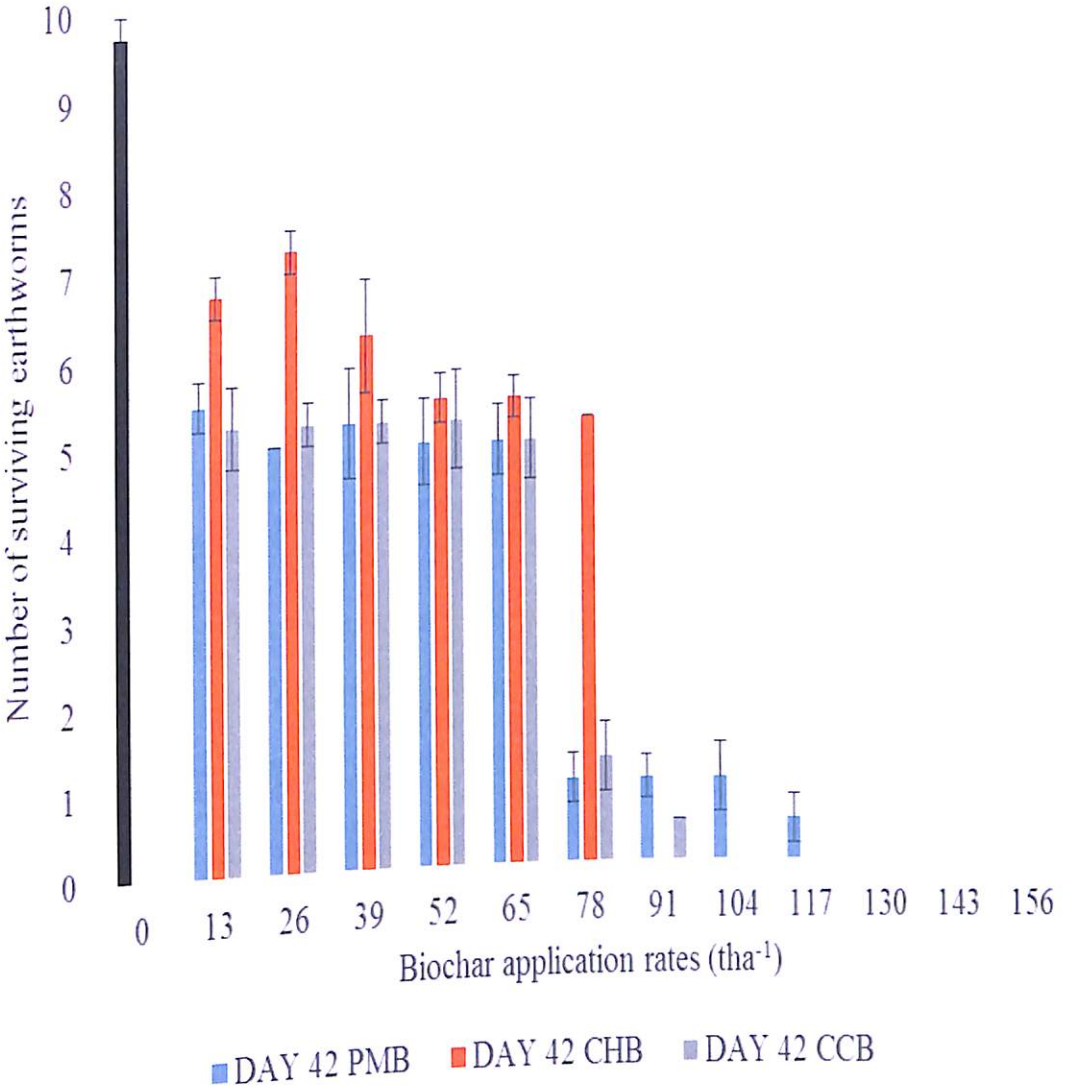

Figure 27: Mean earthworm survival on the 42nd day

Generally, earthworm survival was dependent on the type of biochar present, application rates and time of exposure. Treatment that received no biochar recorded significantly higher survival ($P < 0.05$; $N = 9.75 \pm 0.50$) compared with all other treatments. As can be observed (Figures 23 to 27) most earthworms' death occurred in the first 3 days of the incubation in all treatments. The dead earthworms were found on the soil surface by day 3 and were subsequently removed (Figure 28 and 29). Surviving earthworms were seen burrowing into soil (Figure 30 and 31).

In addition, earthworm survival reduced with increasing biochar application rates. Statistically significant ($P < 0.05$) and inverse relationship was found between biochar application rates and earthworm survival as indicated by Pearson moment correlation analysis (Appendix C).

The use of CCB revealed that survival of earthworms; that is above 50 % was observed at lower biochar application rates of between 13 to 65 t ha^{-1}. Exceeding 65 t ha^{-1} of biochar application, earthworm survival reduced below 50 % and at application rates of 143 and 156 t ha^{-1}, all earthworms were dead by the third day. More so, on day 3, at application rates of 13 t ha^{-1}, mean survival (7.5 ± 0.58) recorded were not significantly different from survival at 26 t ha^{-1} (7 ± 0.82) and 39 t ha^{-1} (7.0 ± 0.82) respectively. However there were significant differences ($P < 0.05$) in survival rates at 13 t ha^{-1} compared with that from 52 t ha^{-1} to 156 t ha^{-1}. On subsequent days of the incubation (7, 14, 28 and 42), it was observed that treatments with CCB rates between 13 to 65 t ha^{-1} lost between 1-2 earthworms, yet had survival rates above 50 %. By day 42 significant differences were observed for mean survival at 13 t ha^{-1} compared with 26, 39, 52 and 65 t ha^{-1} respectively. No significant differences were observed for survival at biochar rates of 26, 39, 52 and 65 t ha^{-1} respectively.

Appreciable effect on earthworm survival was realized by the 3rd day following the application of CHB and the effect was pronounced compared with other days of the incubation. Below 78 t ha^{-1} of CHB application, earthworm survival was above 50 % and at CHB rates of 143 and 156 t ha^{-1}, all earthworms were observed to be dead (3rd DAI). As shown in Figure 23 to 27, few earthworms died on subsequent days of the incubation similar to what

happened in CCB amended soils indicating adaptation to the amendments. Mean survival at 13 t ha^{-1} was statistically not significant ($P < 0.05$) compared with earthworm survival at biochar rates of 26 and 39 t ha^{-1}. Similarly there were no significant differences for survival rates at 39, 52 and 65 t ha^{-1}. Meanwhile by day 42, 50 % survival was observed at 78 t ha^{-1} and all earthworms in soils treated with CHB at rates of between 91 to 156 t ha^{-1} were dead.

In PMB treatments, rates that had survival above 50% included 13, 26, 39 52 and 65 t ha^{-1}. Above 65 t ha^{-1} of PMB rates, earthworm survival reduced below 50 %. Subsequent days of incubation recorded some number of deaths in PMB amended soils though not pronounced compared with what happened on the 3rd day. By the 42nd day of the incubation, more than 50 % death of the earthworm population was recorded at rates exceeding 65 t ha^{-1} PMB rates.

Comparing the effect of individual biochar, generally, it was observed that CHB recorded the highest survival rates for all application rates whiles both PMB and CCB demonstrated 50 % survival at rates of 65 t ha^{-1}. CHB recorded survival rate less than 50 % only after application rates above 78 t ha^{-1}.

The rapid death of the earthworm at higher rates (on the 3rd and 7th day) could be associated with the physical damage caused to the earthworm by biochar. Characteristically, it was seen that biochar was stuck to the body of the dead earthworms, trapped on the soil surface. The sticking nature of the biochar prevented the earthworm from penetrating the soil. It has been suggested that biochar is kept wet before or immediately after its application to soil to climinate the propensity of biochar to stick to the body of the

earthworm (Li et al., 2011). This suggestion was observed in this study; however, earthworms were seen dead on the surface of the soil in mesocosm with wet biochar stuck to their body at higher concentrations. It could be therefore he deduced that prewetting biochar prior to application could reduce negative impact only at a lower biochar rates which is evident from the results of the current study.

The increasing mortality observed in the current study as a result of increasing biochar application rates is similar to the submissions of Liesch et al. (2010). In their study, they reported that, mortality of earthworms increased with an increasing biochar application rates involving poultry litter biochar. On the contrary, they observed no difference in survivorship of *E. Fetida* subjected to pine chip biochar even at high rates of application (above 45 $Mgha^{-1}$). The findings of the current study also contrast the results of experiments with *P. corethrurus* that showed 100 % survival to powdered charcoal/soil mixtures in mesocosms using natural char and native soil (Topoliantz & Ponge, 2003).

The high mortality caused by biochar could be attributed to one of such reasons. The toxicity of animal manures to earthworms has been attributed to ammonia or ammonia salt contents (Curry, 2004). It has been reported that poultry litter biochar contains high N and consequently might result in the production of high ammonium concentration through mineralization. Some portion of this ammonium may be ammonium salts that decompose to ammonia with sufficient moisture (Cantrell et al., 2007). Although earthworms excrete nitrogenous wastes in the form of ammonia or urea, other nitrogenous compounds and ammonium salts, particularly ammonium chloride,

ammonium citrate or glutamic acid, can be toxic to the earthworm. This assertion is in line with the submission by Liesch et al. (2010) where they also attributed the mortality and reduced growth of earthworms to alterations in soil ammonia concentration. The death of earthworm following exposure to CCB and CHB could be attributed to osmotic shock. The osmotic shock resulted from the potential of biochar to absorb water from the body of the earthworms. Biochar have a high water-holding capacity and when in contact with earthworm may cause desiccation. Although soil-biochar mixture was prewetteed it couldn't prevent the biochar from sticking to the body of the earthworms, especially at increasing biochar rates. Similar conclusions were drawn by Liesch et al. (2010) who associated earthworm's death to osmotic shock.

Then again, the death of the earthworms could be attributed to the elemental composition of the biochar. Biochar prepared from manure (PMB) could contain appreciable levels of potentially toxic elements, such as Arsenic, that can be preserved in the production of low-temperature biochars (350 - 400 ^{0}C) (Arai et al., 2003). Although PMB used in this study contained trace amounts of metals and other micronutrients, including Na, Mg, Cu, Fe, Zn and As (Table 5), high concentrations of these micronutrients may affect earthworm survivorship, growth and activity (Lukkari et al., 2005; Arai et al., 2003). However, the estimated concentration of these elements in the present study were below reported toxic concentrations and unlikely to have contributed to earthworm death. The marginal levels of these metals, the presence of ammonia, high pH and salinity, in PMB may have jointly contributed to the mortality of the earthworms. Last but not least, biochar has

been implicated to have contained polynuclear aromatic hydrocarbons (PAHs) (Naphtahalene and Phenanthrene) which are known toxins to soil biota including earthworm (Alburquerque et al., 2015). The current study did not estimate the levels of these PAHs but could be related to the cause of death of earthworms in this study.

The finding in the current study is also confirmed in a related study which revealed that after 42 days of earthworm exposure to biochar, toxic effects on earthworms were observed at application rates (100 t ha^{-1}) that are generally considered beneficial for most crops (Malev et al., 2015). Apart from day 3 where most deaths occurred, there was insignificant reduction in mortality rate with incubation time and it is consistent with the submission of Weyers and Spokas (2011) that, biochars negative effects on earthworms may reduce with time.

Figure 28: Dead earthworms on disturbed soil surface (at higher biochar rates)

Figure 29: Dead earthworms on undisturbed soil surface (at higher biochar rates)

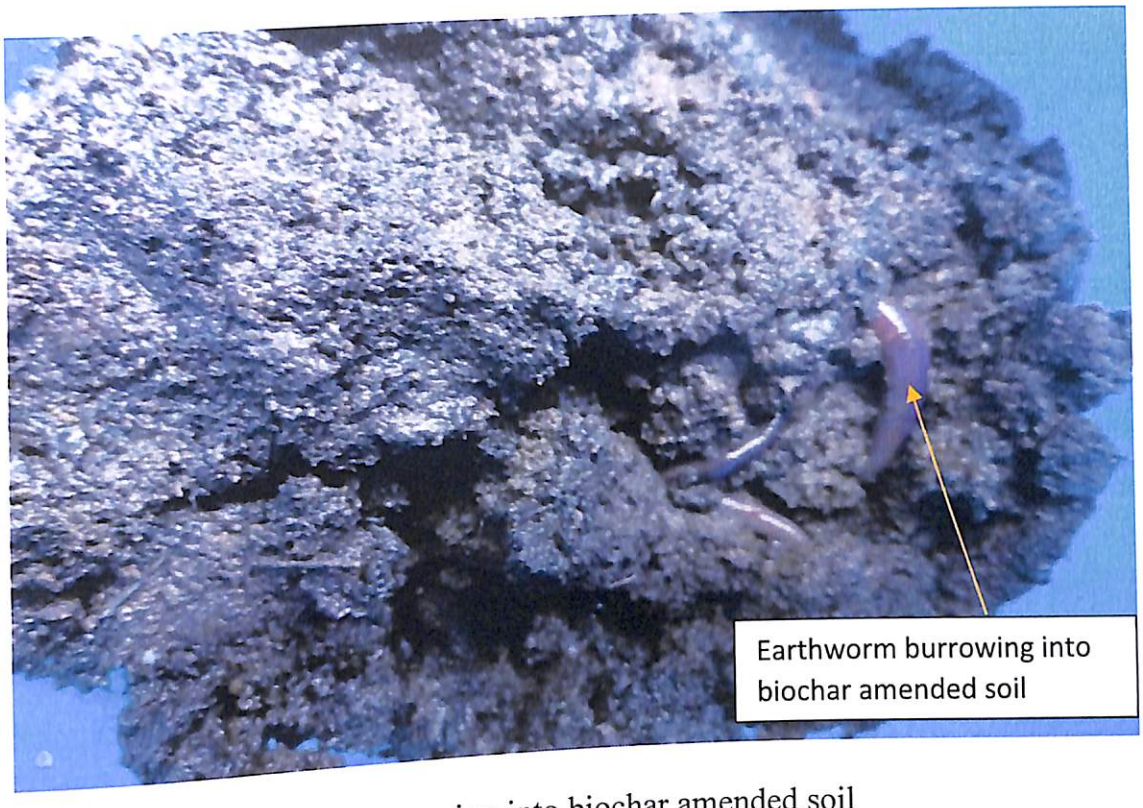

Figure 30: Earthworms burrowing into biochar amended soil

Figure 31: Live earthworms in biochar amended soil

Figure 32: Earthworm cast in biochar amended soil

Figure 33: Earthworm casts in control soil

Earthworm activities in biochar amended soils

Estimation of weight of cast produced (Table 16) is used as an indicator of earthworm activity. The results of the cast produced (g kg^{-1}) after 42 days of earthworm incubation have been summarized in Table 16.

Table 16: *Weight of Earthworm Cast 42 DAI (g kg^{-1}) (Mean ± SD)*

Treatments (t ha^{-1})	CHB	CCB	PMB
0	368.3 (35.8)a	368.3 (35.8)a	368.3 (35.)a
13	147.0 (14.5)b	143.3(11.0)b	84.0 (10.5)de
26	110.7 (7.1)c	102.5 (7.0)c	72.3 (5.1)e
39	83.0 (12.5)de	105.7 (9.7)c	71.3 (9.3)e
52	88.0 (11.8)d	83.7 (9.5)de	45.3 (8.5)f
65	98.0 (12.1)cd	68.67 (11.0)e	36.0 (6.3)f
78	44.0 (9.9)f	43.0 (14.5)f	32.5 (10.6)f
91	0 (0.0)h	40 (8.5)f	32 (7.6)f
104	0 (0.0)h	0 (0.0)h	25.3 (9.5)g
117	0 (0.0)h	0 (0.0)h	21.3 (3.1)g
130	0 (0.0)h	0 (0.0)h	10.3 (1.5)g
143	0 (0.0)h	0 (0.0)h	9.3 (2.3)h
156	0 (0.0)h	0 (0.0)h	9.7 (5.0)h

Treatments that are not followed by the same letter differ significantly from one another ($P < 0.05$).

Earthworm casts were present in all treatments except for the treatments that recorded 100 % mortality. This implied earthworm ingested biochar-soil mixture contrasts the report of earlier studies that, earthworms

significantly avoided biochar-soil mixture (Li et al., 2011; Malev et al., 2015). Li et al. (2011) reported that earthworm avoided apple wood chip biochar-soil mixture. Malev et al. (2015) studied the effect of two biochars produced at low temperature from wine tree cuttings (WTB) and a commercial low tar hardwood lump charcoal (HLB). Their study showed that earthworms avoided biochar-treated soil (48-h exposure) with rates higher than 16 t ha^{-1} for HLB and 64 t ha^{-1} for WTB.

It was also observed that cast production varied with types of biochar and number of surviving earthworms. It was demonstrated that the control had significantly ($P < 0.05$) higher casts (368.3±35.8 g kg^{-1}) produced compared with all other treatments. Apart from CCB recording statistically significant ($P < 0.05$) higher weight of cast at 26 t ha^{-1} biochar rates, CHB recorded the highest casting activity in respect to other biochar rates. Casting activity was lowest in PMB-soil mixture at all application rates.

Casting activity was significantly higher in CHB amended soils at biochar rates of 13, 26 and 52 t ha^{-1} (Table 16) respectively but statistically not significant ($P < 0.05$) compared with the amount produced in CCB-soil mixture at same biochar rates. The cast produced for CCB and CHB were significantly higher compared with amount of cast produced in PMB amendment at 13 and 26 t ha^{-1} only.

The increased higher casting activity recorded for the control could probably be as a result of low nutrient quality of the soil used. It is known that turnover of the soil by earthworm increases when the quality of soil organic matter is low (Flegel & Schrader, 2000). This difference in cast production can be considered as a compensatory mechanism, where the higher ingestion rate

of the control soil compensated for its low nutrient content. On the other hand, PMB soil mixture was the least to be ingested and this could be attributed to the high nutrient content of the PMB as well as increased microbial population in the treatments (Table 15).

Ingestion of biochar soil-mixture is a positive attribute in that the earthworm could carry the mixture in their gut where microbes in the gut mineralize the biochar or could be agents of transport of biochar within the soil profile. Similar findings were reported by Topoliantz and Ponge (2005) using a peregrine tropical endogeic earthworm species, *Pontoscolex corethrurus*. Their study demonstrated that earthworm ingested biochar particles in microcosm experiments. They showed that earthworms evidently could grind the material and mix it into the soil. They also indicated that earthworm preferred soil with biochar over soil alone. Van Zwieten et al. (2010) also showed that earthworms clearly preferred biochar amended soil over the controls. The reason for earthworm ingesting soil-biochar mixture is probably because earthworm grinding to feed on microbes and microbial metabolites (Lavelle, 1988) which are more abundant on biochar surfaces. Topoliantz and Ponge (2003) also proposed that earthworm ingestion may favour microbes on which earthworms depend for enzymatic digestion. It has also been suggested that earthworm ingest charcoal for its detoxifying and liming effects (Zackrisson et al., 1996); and its improvement of microbial communities could favour the production of earthworm's digestive enzymes of bacterial origin (Lattaud et al., 1999). The ingested matter as shown in the cast produced (black and brown cast deposition; (Figure 32), underlines the importance of earthworm for bioturbation (Garcia & Fragoso, 2002). More

especially, by ingesting charcoal and incorporating it to the soil matrix. Based on the findings, *Eisenia fetida* could be integrated with appropriate biochar rate to promote sustainable agriculture and improving the fertility of highly weathered tropical soil.

Conclusion

Significant ($P < 0.05$) and inverse relationship was found between biochar application rates and earthworm survival. Fifty percent survival of earthworm was observed at respective biochar application rates below 65 t ha^{-1} for CCB and PMB and that of CHB was 78 t ha^{-1}. This implicates the recommendation of biochar application rates that have been suggested by previous researchers for the production of crops; sometimes at or above 100 t ha^{-1}.

CHAPTER SEVEN

YIELD AND NPK CONTENT OF LETTUCE GROWN ON A HIGHLY WEATHERED TROPICAL SOIL AMENDED WITH BIOCHAR AND POULTRY MANURE

Introduction

It has been postulated that the application of biochar (Lehmann et al., 2003) or in combination with organic fertilizers (Glaser, 2007) can help restore the productivity and increase yield of nutrient deficient soils. There is, however, considerable variation in plant responses to biochar due to differences in biochar type, biochar rates, soil and type of crop.

Some results indicated biochar amendment improved crop yield (Baronti et al., 2010). On the contrary, other authors have reported that the biochar-amended soil did not promote plant yields and even decreased the productivity (Rajkovich et al., (2012). Sohi et al. (2010) in their review mentioned that the effect of biochar on crop yield is dependent on the characteristics of soil, type of biochar and rates of biochar applied. This is the reason why researchers obtained different results in their experiments hence the need to further investigate biochar effects on crop production under specific site conditions.

Apart from the yield, the concentrations of plant tissue (NPK) grown on biochar amended soils are often ignored. One key reason to investigate the crops quality is to know the right biochar application rate in combination with either inorganic or organic amendment without compromising the quality of the yield. If the right application of biochar or the right quantity of manure is not applied it could lead to excessive accumulation of N, P and K in the plant

tissues and the environment which can be detrimental to human health (Ikemoto et al., 2002; Sharifi et al., 2011). Hoque et al. (2010) indicated that commercial lettuce production requires adequate uptake of NPK to provide high-quality postharvest attributes needed for longer shelf life. For instance high N in lettuce generally leads to storage disorders and the potential for rapid postharvest decay.

Biochar application does not only affect soil properties or the plant biomass but also the mineral composition (Van Zwieten et al., 2010; Rajkovich et al., 2012) of crops. Van Zwieten et al. (2010) showed in their study that, addition of biochar to ferrosol did not provide significant increases in N uptake. This study was conducted therefore to evaluate how biochar applied solely or combined with poultry manure affects yield and postharvest quality of lettuce in terms of NPK concentration in shoot. It also evaluated how the yield correlates with some soil properties.

Materials and Methods

The location of the study has been described earlier in Chapter Three of this thesis. Biochar feedstock and characteristics were as described earlier in Chapter Three of this thesis. The soil used in this study has earlier been described under chapter Three of this thesis.

Experiment three

The experimental setup has been described in Chapter Four of this thesis. Lettuce seedlings were transplanted into pots containing biochar-soil/biochar-soil manure- mixture.

Plant analysis

The plants were harvested at 5 weeks after transplanting (WAT). Analysis for dry matter yield, total N, P and K were done according to protocol described in Chapter Three

Data Analysis

Statistical analysis was performed to compare variations in DW as well as plant NPK for biochar and combined biochar and manure treatments using the SPSS package (Version 16). Data was exported to Microsoft Excel, 2010 and error bars generated to separate the means. Pearson product-moment correlation analysis was also carried out to establish the relationships between soil properties that had effect on yield and tissue NPK concentrations. Results of statistical analyses have been presented in graphs and tables.

Results and Discussion

Results on the yield and shoot NPK concentrations have been presented in Figure 34 to 37.

Yield of lettuce

The yield estimated five weeks after transplanting (WAT) are shown in Figure 34.

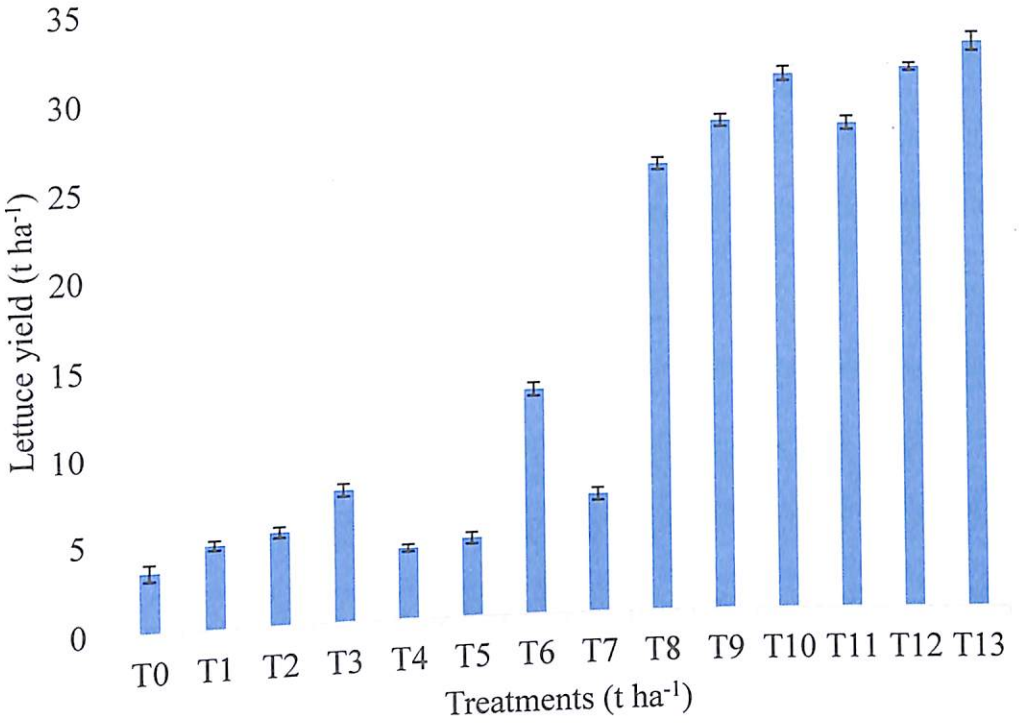

Figure 34: Treatments effect on yield of lettuce (t ha^{-1})

At 39 t ha^{-1} of biochar application rate, yield increased significantly ($P < 0.05$) by 29.4 % for CCB (T1) and 36.9 % for CHB (T2) above the control (T0) (3.37 ± 0.5 t ha^{-1}). Interestingly, when the rate of biochar application was increased from 39 to 65 t ha^{-1}, yield declined by 12.1 % and 11.7 %, less the yield at 39 t ha^{-1} respectively for CCB and CHB treatments. In contrast, the application of PMB (T3, T6) significantly increased yield above that of the control and sole CCB, CHB amended soils. Unlike CHB and CCB, a trend of increasing yield was observed with increasing PMB application rates. At PMB rate (T3) of 39 t ha^{-1}, yield increased by 55.7 %, significantly above the control and when PMB rate (T6) was increased to 65 t ha^{-1} there was a corresponding yield increased by 18.5 % above the yield obtained at 39 t ha^{-1}.

The control soil had low yield due to the properties of the soil used which couldn't have supported the growth and yield of the lettuce plant. The

soil used was strongly acidic (4.17). Pearson correlation analysis established that pH had a positive relationship with yield of lettuce ($r = 0.512$, $p = 0.001$). This means that as pH moved from strongly acidic state towards near neutral, yield correspondingly increased. The growth of lettuce is affected upon exposure to low pH and this caused physiological dysfunction in its growth and subsequent yield. Lettuce is often grown on neutral sandy-loam soils and pH > 5.5. Moreover the acidity that characterized the soil used for the experiment affected the availability of major plant nutrients required and consequently the uptake of right amount of nutrient for the optimum growth of the lettuce plant and subsequent yield. For instance, there was low availability of P probably as a result of complexation with Al and Fe making them unavailable. Organic carbon content was low in the control soil which is an indication of low soil organic matter. Magdoff and Weil (2004) explained that when organic matter decomposes, it releases nutrients to add to the nutrient pool in soil. Conclusively, soil with low organic matter cannot support optimum plant growth because it has low concentration of nutrients. Microbial activity is slow due to the sensitivity to acidic medium by higher proportion of microbes. Microbial activity helps in the breakdown of organic matter and mineralization of soil nutrients.

As shown for sole CCB and CHB, yield declined when biochar rates were increased from 39 to 65 t ha^{-1}. Reduction in yield associated with sole CCB and CHB applications as observed in this study could be as a result of the influence of amendments on the availability of essential nutrients. Pearson correlation shows a positive and significant relationship between amendments and yield ($p < 0.01$; $r = 0.94$), signifying that the higher the nutrient supplying

capacity of treatments, the higher its capacity to influence yield. For instance, in the soil the reduction in the concentration of mineral N through adsorption to biochar surfaces and immobilization by microbes might affect the supply of N to plants and that might have occurred in CHB and CCB soils. Nitrogen is a component of chlorophyll and therefore essential for photosynthesis. It is also the basic element of plant and animal proteins, including the genetic material DNA and RNA, and is important in periods of rapid plant growth. The influence of poultry manure on yield was significant.

The results showed that the sole addition of poultry manure (T7) to the soil used in this experiment produced significantly higher ($P < 0.05$) yield than sole application of CCB and CHB. Meanwhile the increase in yield following the application of poultry manure was significantly ($P < 0.05$) lower than lettuce yield when PMB was applied at rates of 39 and 65 t ha^{-1} as well yield observed for combined biochar and poultry manure treatment. Poultry manure upon preliminary analysis contained higher concentrations of essential plant nutrients (NPK). Although when poultry manure was applied solely, the pH of the medium was increased, the increase observed did not fall within the range at which soil nutrients availability could be enhanced. In addition, at a lower pH, it's not only soil nutrients especially phosphorus whose availability is influenced but also plants ability to absorb available nutrients from the soil are affected (Beegle & Durst, 2014). Several other authors have indicated that at pH less than 5.5, Al toxicity is the main stress factor for plants and limits crop yield. Further, they explained that micro nutrient toxicity is imminent at the lower pH, hence might have accounted for the lower yield realised.

Significant increase of yield was observed in combined biochar and poultry manure amendments (T8 to T13) comparatively more than observed for sole biochar. As biochar rate increased in the combined fraction, yield also increased correspondingly for all biochar used for the study. But then the most increased in yield was observed when PMB (at both 39 and 65 t ha^{-1}) was applied together with 10 t ha^{-1} poultry manure. Treatments involving mixture of 65 t ha^{-1} in combination with 10 t ha^{-1} poultry manure gave yield of 32.6 $\pm$0.14 t ha^{-1} which happened to be the highest of all the treatments. On the other hand the integration of CCB and CHB respectively with poultry manure produced significant yield of lettuce more than the control but this was not as expressed for PMB and poultry manure mixtures.

The increase in yield could be associated with the increase in plant nutrient elements in the soil as a result of the application of both manure and PMB which had higher nutrients reserves. This assertion is supported by Yan et al. (2007) that, poultry manure is the richest animal manure and supply higher concentrations of N, P and K to plants. The additive effect of the biochar especially PMB and the poultry manure might have influenced the higher yield obtained from this treatment involving combined biochar and manure treatment. According to Major et al. (2010), biochar also helps improve the efficiency of fertilizers that are applied to the soil by enhancing nutrient mineralization and improving plant growth whiles retaining nutrients in the soil. Therefore, except biochar of manure origin, when it is applied alone, it has little benefit to plants. Then again the increase in dry natter yield could possibly be that upon the addition of a mixture of biochar and poultry manure amendments to the soil, the biochar improved the soil physical,

chemical and microbial environments' for enhanced absorption of nutrients (from the manure amendment) by the lettuce plants. The enhanced absorption of nutrients could be associated with improved myccorhizal association with the roots of the plants resulting from the addition of biochar to the soil, especially in sole biochar amended soils. Pearson moment correlation showed that the addition of biochar and manure increased soil basic cations as well. There was a positive relationship between soil exchangeable cations and yield (Appendix B) which implies that yield was enhanced as a result of the increase in these cations in the experimental soil. Generally the combined biochar and manure improved the yield of lettuce which was above the worlds' average of 20 t ha^{-1} (Grubben & Denton, 2004).

NPK contents of lettuce

The total N, P and K contents in lettuce shoot were estimated, five weeks after transplanting (WAT) and the results are presented in Figures 35, 36 and 37 respectively.

Figure 35 represents the mean concentration of N (g kg^{-1}) in lettuce shoot 5 WAT as influenced by amendments.

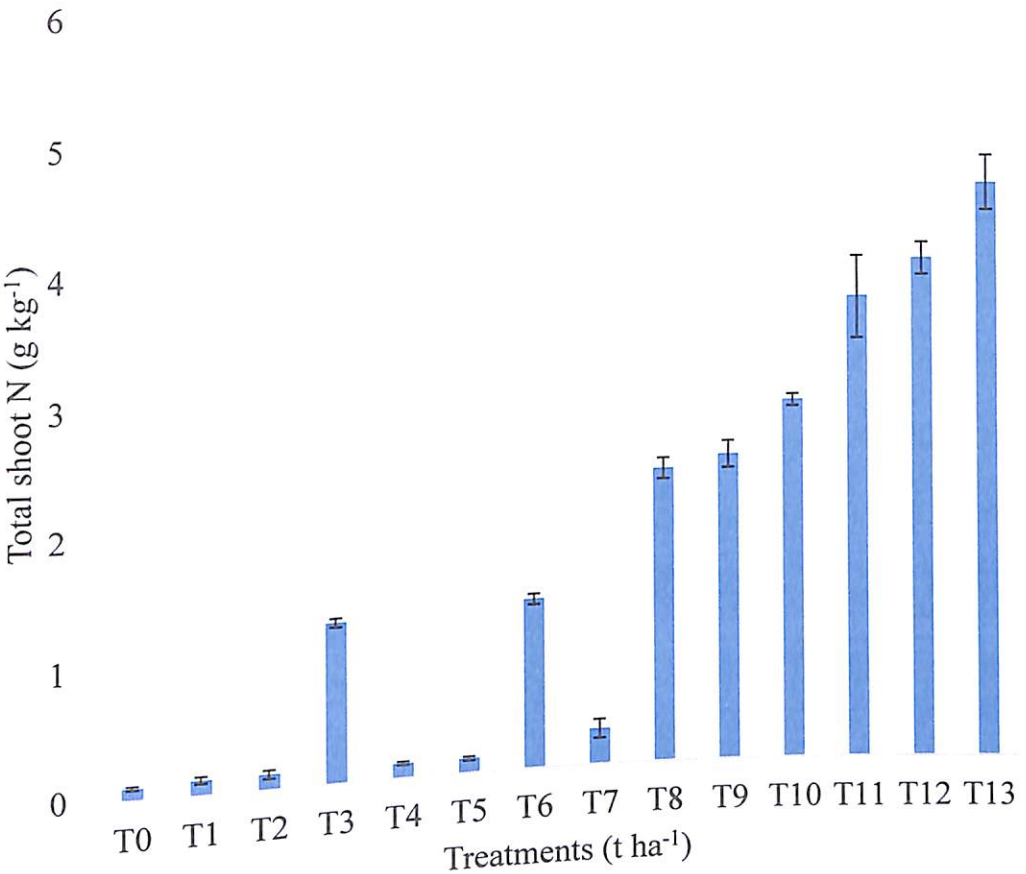

Figure 35: Treatments effect on total shoot N content of lettuce (g kg^{-1})

Total N in lettuce ranged from 0.09 to 4.55 (g kg^{-1}) with the least recorded in the control treatment (T0) and the highest was found in combined PMB and manure mixture. The N concentration of lettuce grown on control soil was significantly lower than all treatments except plants harvested from soils that had received sole CCB (T1, T4) and CHB (T2, T5) at respective rates of 39 and 65 t ha^{-1}. At 39 t ha^{-1} CCB rates, shoot N increased by 26.47 % and CHB by 29.69 % but when biochar rates increased to 65 t ha^{-1} shoot N was less than that recorded at 39 t ha^{-1} but above the control (0.09±0.02 g kg^{-1}). Both CCB and CHB recorded shoot N concentrations rise of 25.08 % and 21.44 % respectively at 65 t ha^{-1}.

Lettuce harvested from PMB (at both 39 and 65 t ha^{-1}) (T3, T5) treated soil on the other hand, contained respectively, significant concentration of shoot N above the control as well as CCB and CHB soil. Then again, significantly ($p \leq 0.05$), pronounced levels of shoot N was observed in plants harvested from combined treatments of biochar and manure (T8 to T13) than that from the separate treatments of biochar and poultry manure. The highest shoot N however was found in plants from treatments amended with combined PMB and poultry manure and was significantly different from that observed in combined fractions involving CCB- and CHB- poultry manure mixtures. There were no significant differences between shoot N observed in plants from CCB and CHB – poultry manure mixture amended soils.

Generally, it was revealed that shoot N concentration was dependent on the soil mineral N concentration. This was confirmed when a correlation analysis using Pearson product-moment correlation coefficient showed a strong positive correlation between soil N and lettuce shoot N ($p < 0.01$; $r = 0.94$; Appendix B). Base on the correlation results and mineralization data, it could be explained that the low shoot N concentration from plants grown on sole CCB and CHB treated soils is as a result of the low mineral N concentration in soil. The lower soil concentration resulted in lower uptake of N by the lettuce plant. This explanation is also confirmed in the results obtained from plants grown on PMB amended soils where plant N was higher due to the availability of higher concentration of mineral N in the soil.

Shoot N concentrations from CCB and CHB grown plants were not significantly different from each other and similar to plant N from control treatment. Similar results have been reported by Van Zwieten et al. (2010)

where it was shown in their study that addition of biochar to ferrosol did not provide significant increases in N uptake. In contrast to the finding of this study, other authors have reported that N uptake in biochar amended soils were significantly different from the control with plants showing higher shoot N concentration. Regarding the decrease in shoot N with increasing biochar rates (from 39 to 65t ha^{-1}) observed in CCB and CHB amended plants, similar results were found by Ali et al. (2015). He reported that wheat straw N-uptake increased as biochar application rate increased from 0 to 25 ton ha^{-1} but further increasing biochar application rate to 50 ton ha^{-1} reduced straw N content of wheat. Similarly Rajkovich et al. (2012) also found low foliar N concentrations and low N uptake and also found that higher application rate resulted in lowest shoot N concentrations.

The elevated shoot N found in crops grown on PMB as well as combined biochar and poultry manure soils suggestive is congruent with that submitted by Rajkovich et al. (2012) who found increased total N uptake after application of low-temperature biochar made from poultry manure and this increased with greater application rates while N uptake decreased for biochars made from all other feedstocks (food waste, hazelnut shells, corn stover, oak). Then again the positive synergy in combining biochar and manure resulted in highest utilization of N by plants indicated in Figure 35. It could also be explained by the fact that the combination also improved soil properties, creating an enabling root environment for N uptake. Similar results have been reported by other researchers where it was indicated that biochar enhanced N uptake and efficiency in soils amended with inorganic fertilizer (Van Zwieten et al., 2010).

The phosphorus concentrations of the lettuce plant 5 WAT as influenced by amendments has been displayed in Figure 36.

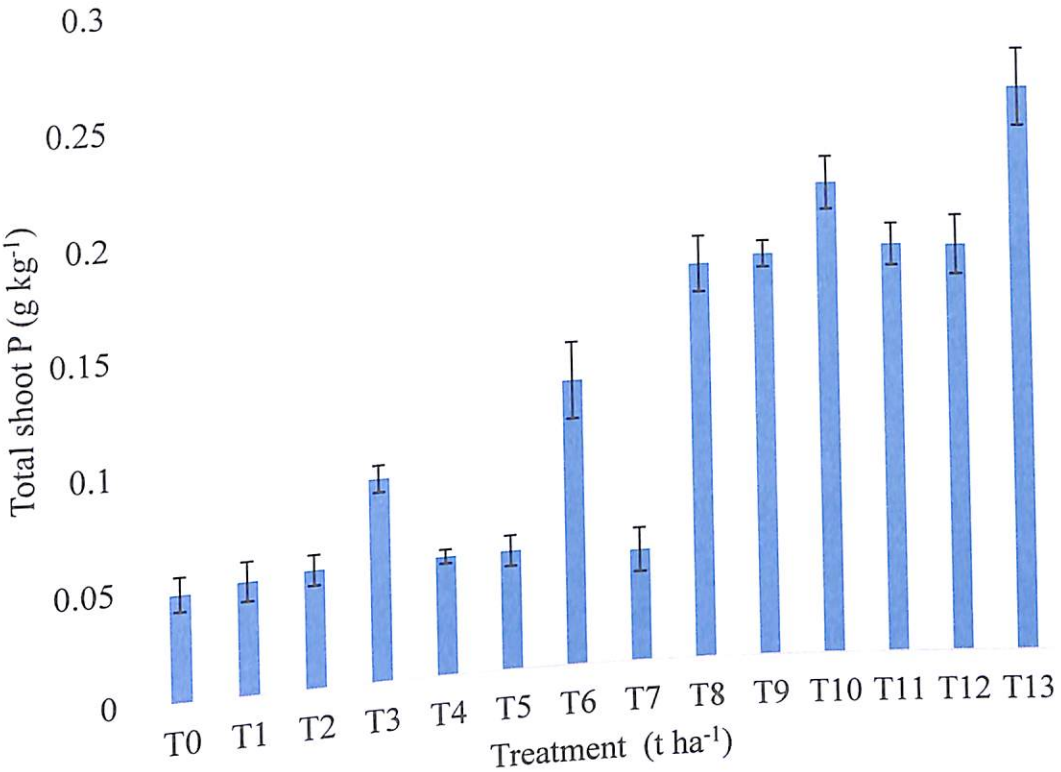

Figure 36: Treatments effect on total shoot P content of lettuce (g kg^{-1})

At the end of the study, shoot P ranged from 0. 048 to 0.25 g kg^{-1}. The lowest shoot concentration of 0.048 mg kg^{-1} was recorded in the control (T0) and the highest (0.252 g kg^{-1}) found in the combined biochar and poultry manure amendment.

Shoot P concentrations in sole biochar; that is in CCB (T1, T4) and CHB (T2, T5) amended soils although increased, they were not statistically different ($P < 0.05$) compared with control. Then again, increasing CCB and CHB rates did not cause any significant change ($P < 0.05$) in lettuce P content. Upon the application of 39 t ha^{-1} CCB (T1) the results showed 6.25 % rise of shoot P above the control whiles CHB (T2) applied at same rate also resulted

in increased shoot P concentration by 10.42 %. When CCB (T4) and CHB (T5) rates were increased to 65 t ha^{-1} respectively, P concentrations increased correspondingly to 11.04 % and 11.25 %. In contrast, shoot P of lettuce plants from PMB (T3, T6) amended soils revealed significantly ($P < 0.05$) highest shoot P concentration above the control and significantly increased with increasing PMB concentrations.

The results show significantly higher shoot P values recorded in lettuce plants grown on soils that received combined biochar and poultry manure amendments (T8 to T13) compared with plants from sole biochar treated soil as well as the control. When biochar rates were increased (from 39 to 65 t ha^{-1}) for each biochar in the fractions of biochar and poultry manure, respective changes in lettuce P were not significantly different. However, combination involving PMB and poultry manure recorded the highest lettuce P among all the treatments and the shoot P was significantly higher than all other treatments. The concentration of plant P could be related to the P concentration of the soil. Pearson correlation showed a strongly positive relationship between soil available P and plant P ($p < 0.01$; $r = 0.92$; Appendix B). This correlation value shows that increasing concentrations of available P will directly result in the elevated concentrations in plant P due to higher uptake.

The uptake of P by plant could be related to the impact of biochar and/or manure in creating a conducive environment for the proliferation of microbes; among which are phosphorus solubilizing bacteria (PSB) and fungi (PSF) (Table 15). It could be explained that the addition of biochar alters soil physico-chemical properties that lead to increases in soil nutrient availability

and increases in root colonization by mycorrhizal fungi (Matsubara et al., 2002; Yamato et al., 2006). Mycorrhizal fungi, growing in association with root cells and extending up to several centimeters into the soil, helps transfer P to the root for absorption. The explanation is supported by Conversa et al. (2015) who reported higher leaf P concentration upon biochar addition and related it to *mycorrhizal* colonization which improved plant P acquisition. In support of this explanation, Hammer et al. (2014) reported that *Arbuscular mycorrhizae* fungal hyphae access microsites within biochar, that are too small for most plant roots to enter, and may mediate plant P uptake from the biochar. *Mycorrhizal* fungi colonize the roots of > 90 % of plant species to the mutual benefit of both the plant host and fungus. The most common are the *arbuscular mycorrhizae*, which are formed by the majority of crop and horticultural plants, including lettuce (Baslam et al., 2011).

The total K content of lettuce as influenced by the amendments were also observed and the results have been displayed Figure 37.

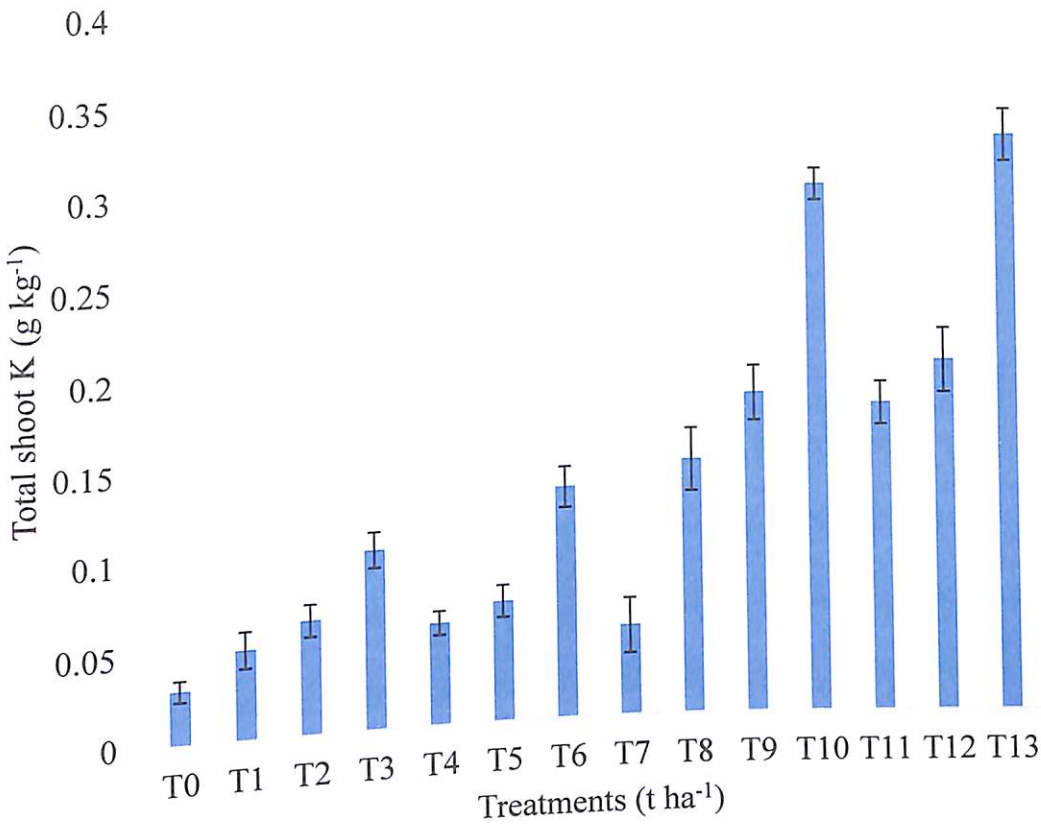

Figure 37: Treatments effect on total shoot K content of lettuce (g kg^{-1})

Potassium concentration in plants shoot grown on control soil (T0) had K concentration significantly ($P < 0.05$) lower than all treatments. When biochar rates were increased from 39 and 65 t ha^{-1}, no significant differences were observed in plant K concentrations from both CCB (T1, T4) and CHB (T2, T5) amended soils. In PMB soils (T3, T6), plant showed significantly ($P < 0.05$) higher shoot K concentrations even at increasing biochar rates. These were significantly higher at each rates compared respectively with CCB and CHB. The most increases in shoot K were observed in plants from treatments involving varying fractions of biochar mixed with poultry manure (T8 to T13). Amongst the combined treatments of biochar and poultry manure, PMB mixed with poultry manure produced lettuce with higher K concentration.

Differences in the concentrations of shoot K, related with the potassium content of the soil ($P < 0.05$, $r = 0.791$; Appendix B). An indication that, as K increased in the soils following the amendments, shoot K also increased correspondingly in lettuce plants. The relatively low plant K realized from the analysis of plant material grown on CCB and CHB amended soils correspond with the concentrations of K in the soils. In support of this explanation, Nigussie et al. (2012) reported a significant increase in plant K in biochar amended soil more than in the control and attributed the increase to higher concentration of K in biochar and subsequently in soil. Gaskin et al. (2010) also reported an increase in plant K upon the addition of peanut hull biochar. Similarly to the findings of the current, the addition of biochar to soils increased above ground productivity, crop yield, soil microbial biomass, rhizobia nodulation, plant K shoot concentration (Biederman & Harpole, 2013). Plant K concentrations were also increased than the control when soils were amended with poultry manure, biochar, and their P-enriched forms (Gunes et al., 2014).

Conclusion

For yield and NPK accumulation of lettuce, biochar combined with manure showed significantly positive effect compared with sole biochar treatment. Nevertheless, PMB also showed positive impact in increasing yield and NPK content hence could be considered.

CHAPTER EIGHT

GENERAL SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

General summary

To overcome the limitations posed by strongly weathered soils for crop production and achieve long term soil fertility maintenance, the use of biochar solely or in combination with manure have been suggested (Lehmann et al., 2006). However previous researchers have reported both positive and negative effect of biochar on soil properties and crop yield. Further work is therefore required to standardise biochar effect on soil fertility in terms of type and quantity of biochar required for a specified soil.

This study was therefore undertaken using biochar prepared from corn cob, cocoa husk and poultry manure solely or in combination with manure to improve the fertility of highly weathered soil. The objectives were to evaluate;

1. the effects of biochar and poultry manure on selected chemical properties of highly weathered tropical soil;
2. the effects of biochar and poultry manure on soil microbial biomass carbon, nitrogen, phosphorus and phosphorus solubilisers;
3. the effects of biochar and poultry manure on earthworm survival and activity; and
4. the impact of biochar and poultry manure on the yield and shoot NPK of lettuce (*Lactuca sativa.* L).

Three experiments were setup to find results to the stated objectives.

Experiment one was designed to give information on biochar and manure effect on selected soil chemical properties, microbial biomass C, N

and P, as well as phosphorus solubilisers. Biochar was solely applied to soil at rates of 0, 39 and 65 t ha^{-1} per 1 kg soil or combined with poultry manure at rates of 0 and 10 t ha^{-1} using completely randomized design. The experiment was without a test crop. Destructive soil sampling technique was used to sample soils on days 3, 7, 14, 28 and 42 after amendments and analyzed for percent SOC, mineral N (NH_4^+, NO_3^-), AVP, pH, exchangeable cations (Ca^{2+}, Mg^{2+}, K^+, Na^+), exchangeable acidity and ECEC. On the 42nd day, soil samples were taken and analyze for P solubilizing bacteria and fungi as affected by the biochar and/or manure.

Experiment two evaluated the effect of biochar on the survival and activity of earthworm. Ten earthworms (*Eisenia fetida)* were exposed to biochar prepared from CCB, CHB and PMB at respective rates of 0, 13, 26, 39, 52, 65, 78, 91, 104, 117, 130, 143 and 156 t ha^{-1}. Data was collected on earthworm survival after day 3, 7, 14, 28 and 42 of exposure to the biochar and earthworm activity assessed through the amount of cast produced at the top 5-10 cm of the soil surface.

Experiment three was conducted to evaluate the effect of biochar and poultry manure on yield and shoot NPK concentration of lettuce. Soils were amended with biochar only at rates of 0, 39 and 65 t ha^{-1} per 1 kg soil or combined with poultry manure at rates of 0 and 10 t ha^{-1} using completely randomized design. Lettuce was transferred into pots 8 days after emergence and harvested 5 weeks after transplanting. Yield of lettuce was estimated and shoot N, P and K concentration was also analysed.

All data was analyzed using the SPSS package (Version 16). Treatment means were separated using least significant difference (LSD), and

treatments effects were declared significant at 1 % and 5 % level of probability. Pearson product-moment correlation analysis was also carried out to establish relationships where necessary.

Conclusions

Based on the results of the study, the following findings were established;

1. All amendments significantly ($P < 0.05$) increased SOC, pH, ECEC, MBC, MBN and MBP for all sampling periods.
2. Fungi and bacteria biomass increased with increasing CCB rates (increased from 39 to 65 t ha^{-1}). In CHB amended soils, both fungi and bacteria biomass increased in soils amended with 39 t ha^{-1} but at 65 t ha^{-1}, fungi biomass slightly decreased whiles bacteria population increased when compared with the biomass observed at 39 t ha^{-1}. When PMB was increased from 39 to 65 t ha^{-1}, significant increase was recorded for fungi biomass but bacteria biomass recorded a non significant increase.
3. Unlike PMB, CCB and CHB soils showed no significant differences in mineral N compared with the control by day 42.
4. Available P in CHB and PMB amended soil showed significant ($P < 0.05$) increases at both rates but only significant ($P < 0.05$) at 65 t ha^{-1} for CCB treatments.
5. Regarding biochar and manure effects on yield and shoot N, P and K, significant increases in yield and shoot NPK were realized from PMB amended soils but insignificant in CCB and CHB treatments. Yield decline was obtained when CCB and CHB respectively were increased from 39 to 65 t ha^{-1}.

6. In all, biochar combined with manure was superior in increasing the concentrations of SOC, NH_4^+-N, NO_3^--N, AVP, pH, ECEC, MBC, MBN MBP, PSF, PSB, shoot NPK and yield of lettuce compared with separate applications of biochar and manure.
7. Statistically significant ($P < 0.05$) and inverse relationship was found between biochar application rates and earthworm survival. In addition, fifty percent survival of earthworm was observed at respective biochar application rates below 65 t ha^{-1} for CCB and PMB and that of CHB was 78 t ha^{-1}.

Recommendations

1. Survival of earthworms were negatively affected by all biochar used in this study especially at higher application rates. The application of CCB, CHB and PMB is recommended at rates not exceeding 65 t ha^{-1} for CCB and PMB and 78 t ha^{-1} for CHB.
2. Sole application of CCB and CHB to highly weathered tropical soil is not recommended; this is because it resulted in higher MBC, MBN and MBP and concurrently failed to improve soil fertility in terms of the availability of major nutrients (N, P and K), needed to make the soil productive. This was translated in the low yield and insignificant shoot N, P and K content obtained from lettuce grown on CCB and CHB amended soils. The application of PMB; manure based biochar, on the other hand could be used without external inputs since it improved soil fertility, enhanced biological properties of the soil, increased yield and shoot N, P and K in the current study.

3. However, combined application of biochar and manure is recommended because it helped modified the soil environment consequently increasing the soils' fertility and improving yield and shoot N, P and K of lettuce.
4. Further study could be done over a longer period to ascertain the interaction of biochar and soil on the measured parameters; and if possible translate it to the field.
5. Future research on this study could also target the evolution of gases implicated in global warming; such as CO_2, CH_4 and various derivatives of nitrogen oxides in both short and long term following biochar addition. This is because; the increase in the concentration of SOC especially in PMB and in the combined treatments could result in CO_2 evolution. When this happens, the underlying idea of using biochar to sequester carbon and mitigate climate change will be defeated.
6. Future research into cost-benefit analysis of biochar deployment in Ghana's agriculture must be considered.

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Correlation (r) matrix for soil properties

	Treatment	pH	AVP	NH4	NO3	SOC	ECEC	Ca	Mg	K	Na	ExcA
Treatment												
pH	.983**											
	0.000											
AVP	.839**	.804**										
	0.000	0.000										
NH4	.660**	.641**	.855**									
	0.000	0.000	0.000									
NO3	.677**	.605**	.869**	.847**								
	0.000	0.000	0.000	0.000								
SOC	.947**	.974**	.827**	.743**	.653**							
	0.000	0.000	0.000	0.000	0.000							
ECEC	.948**	.936**	.933**	.824**	.763**	.946**						
	0.000	0.000	0.000	0.000	0.000	0.000						
Ca	.928**	.922**	.928**	.775**	.750**	.914**	.973**					
	0.000	0.000	0.000	0.000	0.000	0.000	0.000					
Mg	.942**	.940**	.881**	.818**	.726**	.960**	.980**	.924**				
	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000				
K	.956**	.950**	.900**	.790**	.715**	.957**	.990**	.944**	.981**			
	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000			
Na	.696**	.632**	.841**	.789**	.767**	.674**	.816**	.731**	.778**	.801**		
	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000		
ExcA	-.919**	-.942**	-.814**	-.644**	-.624**	-.917**	-.905**	-.941**	-.888**	-.895**	-.583**	
	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	

**. Correlation is significant at the 0.01 level (2-tailed).

Correlation for selected soil properties, yield and shoot N, P, K

	Treatments	pH	AVP	OC	Ca	Mg	K	Na	ExchA	ECEC	TN	TP
Treatments		.609**	.916**	.859**	.909**	.801**	.897**	.712**	-.552**	.905**	.927**	.909**
		0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
yield	.942**	.512**	.945**	.808**	.904**	.818**	.968**	.677**	-.392*	.978**	.973**	.963**
	0.000	0.001	0.000	0.000	0.000	0.000	0.000	0.00	0.01	0.000	0.000	0.000
TYN	.927**	.548**	.919**	.795**	.870**	.874**	.934**	.580**	-.355*	.952**	.942**	.935**
	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.021	0.000	0.000	0.000
TYK	.834**	.600**	.951**	.794**	.865**	.772**	.905**	.518**	-.427**	.905**	.903**	.895**
	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.005	0.000	0.000	0.000
TYP	.910**	.553**	.924**	.842**	.907**	.793**	.943**	.593**	-.380*	.963**	.946**	.958**
	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.013	0.000	0.000	0.000

**. Correlation is significant at the 0.01 level (2-tailed).

*. Correlation is significant at the 0.05 level (2-tailed).

APPENDIX C

Correlation between biochar and earthworm survival

	Survival3	Survival7	Surival14	Survival28	Survival42
CCB	-.945**	-.934**	-.925**	-.922**	-.897**
	.000	.000	.000	.000	.000
CHB	-.954**	-.946**	-.937**	-.933**	-.928**
	.000	.000	.000	.000	.000
PMB	-.937**	-.938**	-.918**	-.902**	-.879**
	.000	.000	.000	.000	.000

APPENDIX D

Correlation for soil properties and microbial biomass

	Total bacteria population	Total fungi population	Gram negative bacteria	Gram positive bacteria
Treatments	.825**	.868**	.809**	.642**
	.000	.000	.000	.000
pH	.598**	.692**	.604**	.423**
	.000	.000	.000	.005
AVP	.842**	.883**	.820**	.669**
	.000	.000	.000	.000
SOC	.756**	.797**	.739**	.594**
	.000	.000	.000	.000
ECEC	.917**	.898**	.893**	.730**
	.000	.000	.000	.000
Mineral N	.911**	.894**	.877**	.748**
	.000	.000	.000	.000

APPENDIX E

Exchangeable bases

Exchangeable Calcium

Treatments	3 Days	7 Days	14 Days	28 Days	42 Days
T0	0.290	0.263	0.283	0.283	0.257
	(0.020)	(0.025)	(0.021)	(0.031)	(0.025)
T1	1.033	1.113	1.197	1.193	1.230
	(0.021)	(0.032)	(0.025)	(0.025)	(0.010)
T2	1.043	1.093	1.217	1.230	1.243
	(0.025)	(0.021)	(0.040)	(0.026)	(0.025)
T3	1.230	1.290	1.317	1.333	1.343
	(0.010)	(0.026)	(0.030)	(0.031)	(0.025)
T4	1.117	1.143	1.247	1.280	1.293
	(0.021)	(0.040)	(0.032)	(0.0265)	(0.021)
T5	1.167	1.227	1.263	1.280	1.300
	(0.031)	(0.025)	(0.025)	(0.026)	(0.026)
T6	1.323	1.413	1.423	1.443	1.457
	(0.031)	(0.025)	(0.032)	(0.015)	(0.015)
T7	1.197	1.227	1.257	1.283	1.237
	(0.031)	(0.042)	(0.031)	(0.015)	0.102)
T8	1.250	1.340	1.380	1.407	1.480
	(0.030)	(0.030)	(0.030)	(0.031)	(0.030)
T9	1.510	1.593	1.707	1.847	1.910
	(0.036)	(0.025)	(0.061)	(0.040)	(0.069)
T10	1.807	1.863	2.013	2.073	2.140
	(0.040)	(0.047)	(0.045)	(0.083)	(0.036)
T11	1.283	1.430	1.473	1.500	1.645
	(0.035)	(0.030)	(0.035)	(0.036)	(0.061)
T12	1.617	1.737	1.800	1.937	1.950
	(0.061)	(0.085)	(0.108)	(0.038)	(0.042)
T13	2.073	2.133	2.183	2.260	2.310
	(0.038)	(0.031)	(0.051)	(0.056)	(0.052)

Exchangeable Magnesium

Treatments	3 Days	7 Days	14 Days	28 Days	42 Days
T0	0.137	0.147	0.147	0.127	0.147
	(0.015)	(0.025)	(0.006)	(0.035)	(0.006)
T1	0.503	0.520	0.527	0.533	0.547
	(0.025)	(0.030)	(0.023)	(0.021)	(0.015)
T2	0.530	0.543	0.553	0.597	0.650
	(0.026)	(0.025)	(0.015)	(0.081)	(0.040)
T3	0.650	0.673	0.730	0.737	0.773
	(0.020)	(0.015)	(0.053)	(0.031)	(0.035)
T4	0.543	0.597	0.617	0.633	0.647
	(0.035)	(0.012)	(0.031)	(0.021)	(0.015)
T5	0.603	0.677	0.687	0.737	0.750
	(0.031)	(0.021)	(0.021)	(0.031)	(0.030)
T6	0.767	0.797	0.827	0.860	0.853
	(0.025)	(0.025)	(0.010)	(0.035)	(0.035)
T7	0.377	0.440	0.463	0.503	0.573
	(0.030)	(0.062)	(0.012)	(0.031)	(0.035)
T8	0.887	1.003	1.030	1.083	1.113
	(0.040)	(0.031)	(0.046)	(0.040)	(0.025)
T9	0.827	0.943	1.067	1.103	1.140
	(0.096)	(0.064)	(0.091)	(0.071)	(0.056)
T10	1.103	1.213	1.263	1.327	1.370
	(0.050)	(0.040)	(0.047)	(0.045)	(0.053)
T11	1.180	1.213	1.203	1.240	1.238
	(0.010)	(0.035)	(0.032)	(0.010)	(0.031)
T12	0.960	1.067	1.143	1.177	1.170
	(0.072)	(0.042)	(0.035)	(0.032)	(0.014)
T13	1.120	1.223	1.277	1.350	1.400
	(0.046)	(0.045)	(0.050)	(0.030)	(0.017)

Exchangeable Potassium

Treatments	3 Days	7 Days	14 Days	28 Days	42 Days
T0	0.233	0.223	0.200	0.183	0.180
	(0.015)	(0.012)	(0.026)	(0.021)	(0.026)
T1	0.597	0.627	0.640	0.653	0.663
	(0.025)	(0.015)	(0.010)	(0.015)	(0.021)
T2	0.777	0.827	0.880	0.900	0.947
	(0.031)	(0.068)	(0.030)	(0.046)	(0.035)
T3	0.853	0.887	0.903	0.937	0.977
	(0.038)	(0.040)	(0.035)	(0.031)	(0.035)
T4	0.647	0.657	0.667	0.690	0.713
	(0.025)	(0.021)	(0.015)	(0.020)	(0.031)
T5	0.810	0.870	0.897	0.920	0.937
	(0.030)	(0.040)	(0.050)	(0.046)	(0.031)
T6	0.977	1.013	1.037	1.103	1.137
	(0.040)	(0.032)	(0.015)	(0.035)	(0.010)
T7	0.343	0.390	0.403	0.443	0.500
	(0.045)	(0.056)	(0.061)	(0.045)	(0.030)
T8	1.050	1.080	1.117	1.133	1.143
	(0.030)	(0.030)	(0.025)	(0.021)	(0.021)
T9	1.057	1.093	1.110	1.133	1.140
	(0.042)	(0.021)	(0.036)	(0.031)	(0.026)
T10	1.217	1.343	1.423	1.440	1.467
	(0.035)	(0.025)	(0.042)	(0.046)	(0.025)
T11	1.223	1.267	1.317	1.340	1.350
	(0.038)	(0.015)	(0.031)	(0.036)	(0.034)
T12	1.113	1.177	1.207	1.220	1.275
	(0.035)	(0.031)	(0.031)	(0.020)	(0.007)
T13	1.327	1.477	1.487	1.557	1.640
	(0.045)	(0.061)	(0.032)	(0.061)	(0.062)

Exchangeable Sodium

Treatments	3 Days	7 Days	14 Days	28 Days	42 Days
T0	0.073	0.070	0.073	0.080	0.070
	(0.007)	(0.005)	(0.012)	(0.009)	(0.016)
T1	0.100	0.107	0.103	0.107	0.113
	(0.025)	(0.015)	(0.006)	(0.015)	(0.015)
T2	0.060	0.077	0.093	0.110	0.110
	(0.009)	(0.002)	(0.015)	(0.005)	(0.002)
T3	0.067	0.093	0.100	0.117	0.113
	(0.008)	(0.007)	(0.003)	(0.010)	(0.015)
T4	0.107	0.113	0.123	0.137	0.153
	(0.012)	(0.021)	(0.008)	(0.007)	(0.010)
T5	0.060	0.080	0.107	0.120	0.127
	(0.002)	(0.004)	(0.002)	(0.004)	(0.007)
T6	0.073	0.080	0.090	0.097	0.143
	(0.009)	(0.005)	(0.004)	(0.007)	(0.008)
T7	0.087	0.107	0.110	0.107	0.113
	(0.005)	0.009)	(0.003)	(0.008)	(0.012)
T8	0.123	0.150	0.190	0.207	0.210
	(0.021)	(0.026)	(0.020)	(0.035)	(0.040)
T9	0.100	0.110	0.137	0.153	0.160
	(0.030)	(0.020)	(0.025)	(0.012)	(0.010)
T10	0.173	0.230	0.257	0.270	0.273
	(0.025)	(0.021)	(0.031)	(0.035)	(0.018)
T11	0.147	0.203	0.227	0.237	0.228
	(.035)	(0.031)	(0.015)	(0.032)	(0.054)
T12	0.137	0.153	0.153	0.163	0.180
	(0.015)	(0.020)	(0.031)	(0.010)	(0.028)
T13	0.197	0.223	0.300	0.300	0.337
	(0.035)	(0.015)	(0.036)	(0.026)	(0.025)

Exchangeable Acidity

Treatments	3 Days	7 Days	14 Days	28 Days	42 Days
T0	1.427	1.433	1.450	1.453	1.467
	(0.025)	(0.035)	(0.036)	(0.015)	(0.025)
T1	1.137	1.120	1.117	1.107	1.093
	(0.015)	(0.010)	(0.012)	(0.015)	(0.021)
T2	1.063	1.053	1.033	1.013	1.007
	(0.021)	(0.021)	(0.006)	(0.015)	(0.010)
T3	1.143	1.140	1.117	1.097	1.097
	(0.035)	(0.026)	(0.021)	(0.035)	(0.015)
T4	1.103	1.090	1.077	1.070	1.063
	(0.021)	(0.026)	(0.051)	(0.020)	(0.025)
T5	1.047	1.017	1.007	0.987	0.967
	(0.021)	(0.021)	(0.010)	(0.051)	(0.008)
T6	1.093	1.083	1.087	1.057	1.033
	(0.025)	(0.023)	(0.031)	(0.080)	(0.012)
T7	1.210	1.233	1.227	1.240	1.267
	(0.018)	(0.031)	(0.046)	(0.054)	(0.016)
T8	1.163	1.150	1.133	1.133	1.143
T9	1.077	1.127	1.083	1.030	1.050
	(0.058)	(0.015)		(0.022)	(0.027)
T10	1.060	1.050	1.067	1.070	1.070
	(0.046)	(0.012)	(0.040)	(0.030)	(0.009)
T11	1.083	1.130	1.173	1.183	1.138
	(0.025)	(0.011)	(0.024)	(0.038)	(0.017)
T12	1.070	1.057	1.033	0.997	0.965
	(0.051)	(0.022)	(0.008)	(0.030)	(0.010)
T13	1.030	1.003	1.013	1.020	1.020
	(0.031)	(0.007)	(0.017)	(0.015)	(0.020)