UNIVERSITY FOR DEVELOPMENT STUDIES

FACULTY OF AGRICULTURE

DEPARTMENT OF BIOTECHNOLOGY

BIOCHAR AND WASTEWATER USE IN URBAN AGRICULTURE: SOIL HEALTH AND PATHOGENIC PROPERTIES

BY

ASIRIFI ISAAC

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BY

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(BSc. Agriculture Technology)

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2017

DECLARATION

I, Asirifi Isaac hereby declare that this submission presented to the Department of Biotechnology in partial fulfillment of the requirements for the Award of Master of Philosophy Degree in Biotechnology, is a true account of my own work except for the references that have been duely acknowledged.

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ABSTRACT

The ability of biochar and its potentials as a powerful soil enhancer that holds carbon and makes soils more fertile is well known. Likewise, the use of wastewater in agriculture has received the world's greatest attention due to the global water crisis. Wastewater is nutrient-rich and its organic matter content serves as a soil conditioner and humus replenisher. This study therefore investigated the effect of biochar and wastewater in improving soil health and fertility. Not compromising on health risk of farmers and consumers, the pathogenic properties of the soil and vegetables under cultivation were assessed. The study was conducted in 2 × 4 factorial experimental laid outs in Randomized Complete Block Design with four (4) blocks and 4 replications. The treatment included application of biochar at 20 tha^{-1}, NPK at 212.5 $kgha^{-1}$, Biochar at 20 Tha^{-1} + NPK at 212.5 $kgha^{-1}$ and a control. Each irrigated with either wastewater or domestic piped water. *Amaranthus* spp. was used as a test vegetable crop in two cropping seasons (dry and wet). Standard laboratory analyses procedures were adopted for the microbial, chemical and enzymatic analysis. The study revealed significantly higher concentration of Fe and Al amongst the other elements analysed and these ranged 70044 – 86100 mg/l and 22064-26519 mg/l respectively. However, all other heavy metals analysed were lower than the WHO recommended standards. Wastewater application increased the absolute values of the macro-nutrients concentration with only phosphorus, carbon and nitrogen being significantly *($P < 0.01$)* different. Amongst all the substrates used to induce the soil, citric acid had higher microbial respiration rate followed by glucose, alanine and basal respectively. All enzymes assayed in the soil were significantly different *($P < 0.01$)* with wastewater plots recording the highest values. Feacal coliform and *E. coli* counts on soil and vegetables in both seasons exceeded the WHO limits. *Salmonella* spp. was negative on all vegetables in the two seasons except NPK and Biochar+NPK plots irrigated with wastewater in the dry season. Helminths eggs population was to be detected high in wastewater irrigated plots than piped water. However, no helminths egg was detected on the vegetables in the wet seasons regardless of the type of irrigation water. Identified helminth eggs were *Ascaris lumbricoides*, *Strongyloides* spp., *Trichuris trichura*, *Schistosoma mansoni* of which the major population was *Ascaris lumbricoides.* The study concluded that wastewater and biochar improves the physical, chemical and biological qualities of soil and should be encouraged. Yet, there is the need to reduce the potential health risks from the wastewater through further research.

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DEDICATION

I dedicate this work to my parents, Mr. and Mrs. Osei, my wife, Selina, my pillars, Mrs. Elsie Abankwah and Steffen Werner.

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LIST OF ACRONYMS AND ABBREVIATIONS

AMC	Amido-4- methylcoumarin
BPW	Buffered peptone water
CEC	Cation Exchange Capacity
ECC	*E. coli* and Coliforms
ICP-OES	Inductively Coupled Plasma Optical Emission Spectrometry
IWMI	International Water Management Institute
MSRV	Modified semisolid Rappaport-Vassiliadis
MUF	Methylumbelliferone
NAG	N-acetyl-b-D-glucosamine
NPK	Nitrogen Phosphorus and Potassium
PAWC	Plant available water content
PBS	Phosphate Buffered Saline
SHC	Saturated Hydraulic Conductivity
SIR	Substrate Induced Respiration
SOM	Soil Organic Matter
SSA	Sub Saharan Africa
STEC	Shiga toxin Producing *E. coli*
UN	United Nation
WHO	World Health Organization
XLD	Xylose-Lysine Deoxycholate Agar
BAM	Bacteriological Analytical Manual
UNEP	United Nations Environmental Programme
US-EPA	United States - Environmental Protection Agency
CFU	Colony Forming Unit
FAO	Food and Agriculture Organization
PPM	Parts Per Million

CHAPTER ONE

INTRODUCTION

1.1 Background

Urbanization coupled with population growth and increase in demand for food especially perishable vegetables in major Ghanaian cities has resulted in increased demand for agricultural resources such as land and water. Irrigated urban farming in Ghana is intensive and is on-going, hence significant amount of nutrients are exported from the soil with each harvest and therefore needs corresponding external inputs (Drechsel and Zimmerman, 2005). However, soil amendment practices such as biochar incorporation, wastewater irrigation and fertilizer application management contain factors that may potentially affect soil microorganisms and the enzymes they produce which has direct or indirect effect on soil and human health indicator parameters.

Biochar, a product of the pyrolysis of organic material, has received wide attention as a means to improve soil fertility and crop productivity, absorb pollutants in soil, and sequester carbon to mitigate climate change whilst simultaneously improving soil properties and functions (Jeffery *et al*., 2011). The addition of biochar to soil will alter the soil's chemical, physical and biological properties (Lehmann *et al.,* 2011). The net effect on the soil properties will depend on the interaction of the biochar with the physicochemical characteristics of the soil (Verheijen *et al.*, 2010). Biochar is highly porous, thus its application to soil is considered to improve a range of soil physical properties including; total porosity, pore-size distribution, soil density, soil moisture content, water holding capacity or plant available water content (PAWC) and infiltration or hydraulic conductivity (Zwieten *et al*., 2012). Distinctive properties of biochar, including

high water-holding capacity, large surface area, cation exchange capacity, elemental composition and pore size/volume have impacts on soil, especially on the microbial communities and enzymatic activities in terms of composition and abundance (Ennis *et al*., 2012).

Microbially-produced extracellular enzymes are important for decomposition of organic matter and cycling of nutrients for microbial as well as plant uptake. Biochar, with its capacity to absorb a wide range of organic and inorganic molecules, may affect enzymes by sorbing them and/or their substrates (Bailey *et al*., 2010; Jin, 2010; Lehmann *et al*., 2011). The enzymatic activity in the soil is mainly of microbial origin, being derived from intracellular, cell-associated or free enzymes. A unique balance of chemical, physical, and biological (including microbial enzyme activities) components contribute to maintaining soil health. A definition of soil health based on this concept would encompass only a small fraction of the many roles soil play (Singh *et al*., 1999). Soil health is the net result of on-going conservation and degradation processes, depending highly on the biological component of the soil ecosystem, and influences plant health, environmental health, food safety, and quality (Halvorson *et al*., 1997). Deterioration of soil, and thereby soil health, is of concern for human, animal, and plant health because air, groundwater, and surface water consumed by humans, can be adversely affected by mismanaged and contaminated soil (Singer and Ewing, 2000). A better understanding of the function of this soil enzyme activity in maintaining the soil health will provide a unique opportunity for an integrated biological assessment of soils due to their crucial role in several soil biological activities, their ease of measurement, and their rapid response to changes in soil management.

Industrial, agricultural and domestic effluents such as biosolids and wastewater are either dumped on land or used for irrigation and fertilization purposes, which creates both opportunities and problems (Yadav *et al*., 2000). The reuse of wastewater for irrigation as a fertilizer source is a common and popular practice, especially in urban and peri-urban areas of Africa. The use of wastewater for irrigation is a positive way to dispose of urban sewage water rather than dumping it in water bodies (Bogner *et al*., 2007). This water contains a lot of nutrients and can serve as alternative water source in arid and semi-arid areas. The water and nutrient value of wastewater can be important resources for farmers. Additionally, wastewater flows are often consistent across seasons, and offer a reliable source of water throughout the year, especially in times of drought (Gleick, 2000). However untreated wastewater which is used for irrigation may contain concentration of helminth eggs and pathogenic bacteria that exceeds far the WHO guidelines (IWMI, 2000; Amoah, 2014). This poses a high potential health risk to both farmers and crop consumers. Farmers specifically in Africa are at high risk, because they have intensive contact with wastewater, as they do most of the fieldwork manually and barefoot. Also crop consumers are at high risk, because vegetables grown in the wastewater irrigated fields are usually eaten uncooked or may cause cross contamination in the immediate environment before the "must cook" vegetable enter the cooking pot. Furthermore, wastewater can transport different types of heavy metal contaminants which through irrigation may accumulate in soil (Ramírez *et al*., 2012) and have detrimental effect on human health. Metals such as Fe, Cr, Zn, Pb, Ni, Cd and Cu are abundant in wastewater and are on the top list of potential contaminants accumulating due to wastewater irrigation (Aleem *et al*., 2003, Hayat *et al*., 2002).

1.2 Problem Statement and Justification

The slow economic growth and high poverty level prevailing in the three regions of Northern Ghana is directly linked to the underdeveloped agricultural sector mainly due to several problems such erratic rainfall and poor soils (Ekekpi and Kombiok, 2008). Further analysis of Northern Ghana agricultural sector problems indicates that poor soils result in low crop yields which are negatively affecting the development of agriculture (Drechsel and Zimmerman, 2005). Soil amendment practices such as biochar incorporation and wastewater irrigation is a potential agricultural invention to transform the small scale farming system and reduce poverty in Northern Ghana.

Several research works on biochar applications have indicated its agronomic benefits, particularly on nutrient use efficiency, crop yield and reduced leaching losses (Major *et al*., 2010) and pollutant immobilization (Beesley *et al*., 2011). Biochar may provide appropriate conditions for beneficial soil microbes, such as nitrogen-fixing rhyzobia, mycorrhizal fungi and enzymes whose activities is very sensitive to both natural and anthropogenic disturbances in the soil which may serve as an indicator of a fertile soil (Knowles *et al*., 2011).

Wastewater can be considered as both a resource and a problem. Though the actual composition of wastewater may differ from community to community, all municipal wastewater contain the following broad groupings of constituents: organic matter nutrients (nitrogen, phosphorus and potassium), inorganic matter (dissolved minerals), toxic chemicals and pathogens. Several studies have reported increase in the yield of crops irrigated with wastewater (Intizar *et al*., 2002, Cordell *et al.,* 2011, Kanyoka and Eshtawi, 2012). Despite the obvious benefits of wastewater irrigation, the human and environmental health is of much concern. An estimated 90 per cent of all wastewater in developing

countries is discharged untreated directly into rivers, lakes or the oceans (Santamaria and Toranzos, 2003). Enteric pathogens, usually bacteria, viruses, protozoa and helminthes are reported to be associated with wastewater which is conveyed to the soil and vegetables (Santamaria and Toranzos, 2003). This poses high risk to farm workers and to consumers of food products irrigated with wastewater (Kalavrouziotis *et al*., 2008). The extent of the pollution increases if the vegetable's edible plant parts are near the ground due to the possibility of splashing (Minhas and Samra, 2004). The impact on health varies depending on location and type of contaminant, however bacteria and intestinal worm infestations have been shown to pose the greatest risk (Drechsel *et al.*, 2010).

It is hypothesized that biochar could positively enhance the activities of soil microbial communities including the pathogens introduced by the wastewater by providing a habitat where bacteria and fungi could escape from predators (Thies and Rillig, 2009). Although there are a lot of comprehensive studies on biochar and wastewater use in agriculture, little information is gathered on the effect of wastewater biochar combination on urban and peri-urban agriculture. It is therefore important to quantify the combined effect of biochar and wastewater in relation to soil physicochemical, biological and pathogenic properties of the vegetables, as they offer information for the assessment of soil and human health.

1.3 General Objective

The main objective of the study was to assess the effect of biochar and wastewater on physicochemical and biological properties of soil and vegetables in irrigated urban agriculture.

1.3.1 Specific Objectives

- To quantify the level of enzymatic activities in soil after treatment with biochar and wastewater.
- To determine the effect of biochar and wastewater on soil microbial respiration (community structure).
- To gather information on the availability of microbial biomass carbon and hot water extractable carbon in soil after one year of biochar wastewater application.
- To isolate and characterize faecal coliform and pathogenic bacteria from soil and vegetables treated with biochar and wastewater.
- To trace the presence and abundance of helminth eggs in the soil and vegetables under the treatment of wastewater and biochar

CHAPTER TWO

LITERATURE REVIEW

2.1 Biochar

The term biochar has risen to describe the type of charcoal that results from the thermal treatments of organic feedstock such as crop waste, wood shavings, municipal waste or manure in an oxygen-limited environment called pyrolysis (Bridgwater, 2003). It is distinguished from charcoal and similar materials by its intended use as a soil amendment while charcoal is known by its use as fuel (Lehmann and Joseph, 2009). Otherwise there is no difference between charcoal carbon and biochar carbon (Tenenbaum, 2009). The quality of biochar is dependent on feedstock and the conversion process used (Anon, 2012) which affects its characterization and usability (Reddy *et al*., 2011).

By charring the organic material between the temperatures 300 to 1000 °C under low (preferably zero) oxygen, much of the carbon becomes "fixed" into a more stable form when it is applied to soil. Biochar has distinguishing properties of high stability in the environment (Nguyen *et al*., 2008). It has been estimated to have a mean resident time of 10,000 years in the soil (Swift, 2001). An example of the stability of biochar is the Terra Preta soils found in the Amazon Basin, South America. An indigenous practice by the Indians over 2000 years ago, added organic wastes to the soil which due to the anaerobic conditions turned into stable forms. These soils are still very fertile and suitable for agriculture (International Biochar Initiative, 2007). Due to the large amounts of biochar incorporated into its soils, this soils still remain highly fertile despite centuries of leaching from heavy tropical rains.

2.1.1 Biochar Production Condition

The quality of biochar and its potential application to agricultural soil or carbon sequestration is highly affected by process temperature and the type of organic material used as feedstock (Gaskin *et al*., 2008). Different elements such as lignin, hemi-celluloses and cellulose are degraded at different production temperatures. All organic materials start to undergo thermal decomposition at temperatures above 120 ˚C. Biochar produced under low temperature conditions (< 300- 400 ˚C) has a low surface area and are only partly carbonized, whereas higher temperatures (400- 600 ˚C) increases the porosity (Lehmann and Joseph, 2009). The active surface area is enhanced by high temperature conditions, while cation exchange capacity (CEC) is decreased as a result of the loss of functional groups (Gou and Rockstraw, 2007). Elemental composition of biochar as reported by Bruun *et al.* (2011) includes carbon (> 60 %), nitrogen, hydrogen, and some lower nutrient element (K, Ca, Na, Mg, and Si). Increases in pyrolysis temperature from 300 to 800 °C, was also observed to increase the carbon content at the expense of nitrogen and hydrogen content (Lehmann and Joseph, 2009).

2.1.2 Sources of Biochar

Biochars can be produced from many different organic materials and under different conditions resulting in products of varying properties (Guerrero *et al*., 2005). It can be produced from a wide range of biomass sources, for example, woods and barks, agricultural wastes such as corn cobs and tea waste (Demirbas 2004; Ioannidou and Zabaniotou, 2007), greenwaste (Chan *et al*., 2007), animal manures and other waste products (Downie *et al*., 2007; Chan *et al*., 2008; Lima *et al*., 2008). It can also be produced from poultry litter (Revel *et al*., 2012), sewage sludge (Khan *et al*., 2013), rice-

husk (Carter *et al*., 2013; Lu *et al*. 2014), wheat straw (Junma *et al*., 2014) and several other materials.

2.1.3 Biochar Properties

Biochar is a light weight, highly porous material with high carbon content (Downie *et al*., 2009). The combined heterogeneity of the feedstock and the wide range of chemical reactions which occur during processing, give rise to a biochar product with a unique set of structural and chemical characteristics (Antal and Gronli, 2003; Demirbas, 2004). According to Sohi *et al*. (2009), it is important that the properties of biochar such as pH, volatile compound content, ash content, water holding capacity, bulk density, pore volume, and specific surface area measured so as to have an assessment for the agronomical use of the material. The structural and chemical composition of biochar is highly heterogeneous, with the exception of pH, which is typically > 7 (Lehmann and Joseph, 2009). Some properties are general throughout all biochars, including the high C content and degree of aromaticity, partially explaining the high levels of biochar's inherent recalcitrance (Downie *et al*., 2009). Carbon, volatile matter, mineral matter (ash) and moisture are generally regarded as its major constituents (Antal and Gronli, 2003). According to Sohi *et al*. (2009), the high carbon contents and strong aromatic structure of biochar largely account for its chemical stability. Total carbon content in biochar has been found to range between 172 - 905 g kg^{-1}, although organic carbon often accounts for <500 g kg^{-1}, as reviewed by Chan and Xu (2009) for a variety of source materials. Total N varies between 1.8 and 56.4 g kg^{-1}, depending on the feedstock (Chan and Xu, 2009).

The ash content of biochar is dependent on the ash content of the biomass feedstock. Grass, grain husks, straw residues and manures generally produce biochar with high ash

contents, in contrast to that from woody feedstocks (Demirbas, 2004). Despite the seemingly high, N content in biochar, the nutrient may not be necessarily beneficial to crops, since N is mostly present in an unavailable form (generally N contents < 2 mg kg^{-1}) (Chan and Xu, 2009).

Total P and total K in biochar range broadly according to feedstock, with values between 2.7 – 48.0 and 1.0 - 58.0 g kg^{-1}, respectively (Chan and Xu, 2009). Interestingly, total ranges of N, P and K in biochar are wider than those reported in the literature for typical organic fertilizers (Demirbas, 2004). Most minerals within the ash fraction of biochar are thought to occur as discrete associations independent of the carbon matrix, with the exception of K and Ca (Amonette and Joseph, 2009). Typically, each mineral association comprises more than one type of mineral (Amonette and Joseph, 2009).

Biochar pores are classified into three categories (Downie *et al*., 2009), according to their internal diameters (ID): macrospores (ID > 50 nm), mesopores (2 nm < ID <50 nm) and micropores (ID < 2 nm). These categories are orders of magnitude different to the standard categories for pore sizes in soil science. The elementary porosity and structure of the biomass feedstock is retained in the biochar product formed (Downie *et al*., 2009).

2.1.4 Biochar as Soil Amendment

The long residence time of biochar in the soil with other positive properties provided the awareness that biochar can be used to improve soil quality and sequester carbon from the atmosphere which is apparent in the terra preta (Lehmann *et al*., 2006). Hence, a number of agronomic benefits have been reported with biochar application to cropping soil, particularly, where soil fertility and productivity is an issue (Novak *et al*., 2009). Addition

of biochar to the soil causes changes to soils ranging from chemical, physical and biological effects (Woods *et al*., 2006). Many field trials have been carried out in tropical or semi-tropical areas with acidic soils and it has been hypothesized that the improved crop yield seen in these soils may be at least partially attributed to a liming effect (Jeffrey *et al*., 2011). The International Biochar Initiative (IBI, 2013) stated that biochar can be used as a product for its own sake or as an ingredient in a blended product, with a range of applications: as agent for soil improvement, to improve resource use efficiency, remediation and/or protection against particular environmental pollution, and as a strategy for greenhouse gas emission reduction.

2.1.5 Biochar Effect on Soil Chemical Properties

Chemically, biochar increases soil pH (Chan *et al*., 2007; Novak *et al*., 2009; Laird *et al*., 2010; Van Zwieten *et al*., 2010; Peng *et al*., 2011) thus reducing lime requirements and increases cation exchange capacity (CEC) (Chan *et al*., 2007; Laird *et al*., 2010; Novak *et al*., 2009; Peng *et al*., 2011). Steiner *et al*. (2008), established that biochar can operate as an absorber lowering N leaching and increasing N use efficiency. Nitrogen use efficiency is of great importance, especially to sustain future population growth. Biochar also performs bioremediation functions through reduced mobility of heavy metals and organic soil contaminants such as insecticides (Hilber *et al*., 2009). High levels of CECs are due to high charge density per unit surface of organic matter which equates with a greater degree of oxidation, or high in surface charge area for cation adsorption and/ or amalgamation of both (Atkinson *et al*., 2010). Biochar can reduce soil acidity and increase concentration of essential elements such as Ca, Mg and K and decrease Al availability (Deenik *et al*., 2011)

while high-surface-area biochar can improve soil water retention capacity (Gray *et al*., 2014).

2.1.6 Biochar Effect on Soil Properties

Soil physical conditions have a direct effect on soil productivity for crop production by determining water holding capacity, aeration and soil strength limitations for root activity (Benjamin *et al*., 2003). Biochar additions to soils that are impermeable increases porosit69y, through the nature of its particle size and shape, and since biochar particularly have porous internal structure (Laird *et al*., 2010). Herath *et al*. (2013), reported overall increased in porosity of soil by the application of biochar, but this increases in porosity was dependent on type of biochar used and soil type where biochar was applied. Besides, increased soil porosity increases the surface area of soil (Jessica and Peter, 2011), enhances of soil water permeability and saturated hydraulic conductivity (SHC) (Asai *et al*., 2009), changes soil bulk density (ρb) (Laird *et al*., 2010) and alters aggregate stability of the soil (Peng *et al*., 2011). Laird *et al*. (2010) reported that the biochar amended soil retained 15 % more moisture contents as compared to controlled treatment. According to Mukherjee *et al*. (2013), biochar application decreased the soil bulk density because porosity of biochar is very high and when it is used in soil it significantly decrease bulk density by increasing the pore volume.

2.1.6.1 Biochar Effect on Soil Biological Properties

Soils can be observed as complex communities of organisms which are repeatedly shifting in response to soil characteristics and climatic and management factors, especially the addition of organic matter (Thies and Rillig, 2009). Though biochar effects on soil biological processes are not well understood (Lehmann *et al*., 2011), biochar amendments

have been shown to increase microbial biomass due to the presence of labile C fractions and un-pyrolysed feedstocks (Bruun *et al*., 2011; Luo *et al*., 2013). Contrarily, other studies have reported that biochar has no effect on soil microbial biomass (Castaldi *et al*., 2011) as a result of its recalcitrance (Kuzyakov *et al*., 2009). Dempster *et al*. (2012), reported that biochar amendments reduced soil microbial biomass induced by a toxicity effect whiles Lehmann *et al*. (2011), concluded that biochar application rates and soil type also affected or response to soil microbial biomass.

Explanations for soil microbial biomass change in response to additions of biochar include enhanced available soil nutrients (P, Ca and K), adsorption of toxic compounds and improved soil water and pH status, all of which can influence the activity of soil micro-organisms (Lehmann *et al*., 2011). The internal porosity of biochars may help soil micro-organisms avoid grazers (Pietikäinen *et al*., 2000) and store C substrates and mineral nutrients (Saito and Muramoto, 2002; Warnock *et al*., 2007). Also, some researches have suggested that changes in soil microbial community composition may occur due to biochar as observed in Amazonian Dark Earths (Terra Preta) (Steiner *et al*., 2008). These soils have greater microbial biomass, and in some cases, greater diversity than the surrounding area (Kim *et al*., 2007).

2.1.7 Effect of Biochar Application on Agronomic Parameters

Application of biochar affects soil fertility in many ways; it either adds nutrients by itself or makes them more available for plant uptake (Lehmann *et al*., 2011). Moreover, the large surface area results in increased CEC, which may prevent nutrient leaching (Lehmann and Joseph, 2009). A growing number of field and pot trials have assessed the impact of biochar on crop yield. The findings of these trials have been highly variable and have

ranged from no difference in yield over the control to doubling crop productivity as a result of biochar addition. Lehmann *et al.* (2003), reported a significant decrease in leaching of applied fertilizers after biochar addition. Further, improved plant uptake of P, K and Ca was observed. By increasing CEC, applied fertilizers can be adsorbed to the surface area and thereby used more efficiently by plants (Steinbeiss *et al*., 2009). Similar observation was made by Steiner *et al.* (2007), who reported a doubling up of maize grain yield on the plot used with the combination of NPK fertilizer with biochar compared to use of NPK alone.

Asai *et al*. (2009), studied the effects of biochar application on rice yields (*Oryza sativa* L.) and selected plant traits. The following were found: biochar improved the response to N fertilizer treatments; biochar application led to higher grain yields; improved xylem sap flow; reduced leaf chlorophyll concentration and concluded that biochar application is highly dependent on soil fertility and fertilizer management. Oguntunde *et al*. (2004), conducted an experiment on biochar site and adjacent fields and found out that there were significant differences between the biochar site and the adjacent fields. Grain and biomass yield of maize increased by 91% and 44% respectively. So far, studies have not shown any severe negative results from biochar amendments to agricultural soils.

2.2 Enzymatic Activities in Soil

Soil is a living system containing several free enzymes, immobilized extracellular enzymes and enzymes within the microbial cell (Stanislaw, 2008). The enzymes include a wide spectrum of oxidoreductases, transferases, hydrolases and lyases. Each of them plays a key biochemical function in the overall process of material and energy conversion (Gu *et al*., 2009). Enzymes present in soil are similar to enzymes in plant and animal systems (Singh

and Kumar, 2008). Soil enzymes mainly originate from soil microorganisms, which can indicate microbial activities in soil environment. Soil enzymes are continuously playing an important role in maintaining soil ecology, physical and chemical properties, fertility and soil health. These enzymes play key biochemical functions in the overall process of organic matter decomposition in the soil system (Sinsabaugh *et al*., 1991). Findings from soil enzyme studies have been the basis for the development of conceptual models that provide a more understanding of the key processes linking microbial populations and nutrient dynamics (Schimel and Weintraub, 2003). Soil enzymes are the direct mediators for biological catabolism of soil organic and mineral components. Thus, these catalysts provide a meaningful assessment of reaction rates for important soil processes. Soil enzymes are the mediators and catalysts of important soil functions that include decomposition of organic inputs, transformation of native soil organic matter, release of inorganic nutrients for plant growth, N_2 fixation, nitrification, denitrification and detoxification of xenobiotics (Dick, 1997). In addition, soil enzymes have a crucial role in C (β-glucosidase and β-galactosidase), N (urease), P (phosphatase), and S (sulphatase) cycle (Karaca *et al*., 2011). Soil enzymes are synthesized by microorganisms and act as biological catalysts to facilitate different reactions and metabolic processes to decompose organic pollutants and produce essential compounds for both microorganisms and plants (Moreno *et al*., 2003).

2.3 Some Selected Enzymes in the Soil

2.3.1 Dehydrogenase

Soil dehydrogenases (EC 1.1.1.) are the major type of the oxidoreductase enzymes class (Gu *et al*., 2009). Dehydrogenases are the top essential enzymes in the soil environment

and are used as an indicator of overall soil microbial activity in the soil (Salazar *et al*., 2011). This is because they occur intracellular in all living microbial cells (Zhao *et al*., 2010; Yuan and Yue, 2012) and are tightly linked with microbial oxidoreduction processes (Moeskops *et al*., 2010). Dehydrogenases play an important role in the biological oxidation of soil organic matter by transferring hydrogen from organic substrates to inorganic acceptors (Zhang *et al*., 2010). Specifically, some dehydrogenase transfer hydrogen to either nicotinamide adenine dinucleotide (NAD) or nicotinamide adenine dinucleotide phosphate (NADP) (Subhani *et al*., 2001). Several soil factors stimulate the activities of dehydrogenase. Such factors include availability of soil moisture (Geisseler *et al*., 2011), soil aeration state thus redox potential and oxygen diffusion rate (Brzezińska *et al*., 2001; Wolińska and Bennicelli, 2010), organic matter content (Fontaine *et al*., 2003), pH and temperature (Moeskops *et al*., 2010). Season of the year and heavy metal accumulation is also reported to have effect on dehydrogenase activity (Pan and Yu, 2011; Yuan and Yue, 2012)

2.3.2 Glucosidase

Naturally, due to the wide variety of glycosidic bonds, there is a wide diversity of enzymes that are known as glucosidases (Daroit, 2007). Generally, the name glycosidase has been used to describe a group of enzymes that catalyze the hydrolysis of various glycosides playing a vital role in carbon cycling (Eivazi and Tabatabai, 1988). Glucosidases in the soil are produced from plants, and mostly from micro-organisms acting as the catalysts in the hydrolysis of maltose and cellobiose. Their catalytic activity results in a final product that serves as an important source of energy to microorganisms (Matsuoka, 2006).

2.3.3 Glucosaminidase (Chitinase)

Chitin degradation is an important feature in both carbon and nitrogen recycling in soil and is primarily a microbial process (Gooday, 1990). Chitinolytic activity may consist of several enzymes hydrolyzing L-1,4-bonds between N-acetyl-glucosamine residues: N-acetyl-b-D-glucosaminidase (EC 3.2.1.30), sometimes referred to as NAGase, is an enzyme that hydrolyzes N-acetyl-b-D-glucosamine (NAG) residues from the terminal non-reducing ends of chitooligosaccharides (Tronsmo and Harman, 1993). It is not surprising that chitinolytic enzymes are widely distributed in nature. They are found in bacteria, fungi, plants and invertebrates such as protozoans, arachnids, insects, crustaceans and nematodes (Trudel and Asselin, 1989). The substrates for this enzyme include chitobiose and higher analogs and glycoproteins. In humans, the same enzyme is found in lysosomes and also cleaves the amino sugar N-acetyl-b-D-galactosamine. Activities of b-glucosaminidase might also be involved in biological control of plant pathogens. Purified b-glucosaminidase from *Trichoderma* spp. demonstrated antifungal activities against several plant fungal pathogens (Lorito *et al*., 1994).

2.3.4 Arginine and Tyrosine Aminopeptidase

Proteins are the largest source of organic nitrogen. In soils, Abe and Watanabe (2004) found that peptide N accounted for 66 – 90 % of the N content. Aminopeptidases catalyze the hydrolysis of amino acid residues from the amino terminus of proteins and peptides. Aminopeptidases are widely distributed in microorganisms in the soil. Amendment studies in the soil show that aminopeptidase activity can be depressed by additions of nitrate, ammonium, and amino acids and induced by protein addition (Foreman *et al*., 1998).

2.3.5 Cellulase

Cellulase catalyses hydrolysis of cellulose to D-glucose (Hussain *et al*., 2009). Cellulose is the most abundant structural polysaccharide of plant cell walls with β-1, 4 -glucosidic linkages and represents almost 50 % of the biomass synthesized by photosynthetic fixation of CO_2 (Eriksson *et al*., 1990) of at least three enzymes (Joachim and Patrick, 2008). They include: endo-1,4-β-glucanase which attacks the cellulose chains at random, exo-1,4-β-glucanase which removes glucose or cellobiose from the non-reducing end of the cellulose chains, and β-D-glucosidase which hydrolyses cellobiose and other water soluble cellodextrins to glucose. Cellulases activity in agricultural soils are affected by several abiotic factors. These include temperature, soil pH, water and oxygen contents. The chemical structure of organic matter and its location in the soil profile horizon (Deng and Tabatabai, 1994; Alf and Nannipieri, 1995), quality of organic matter/plant debris and soil mineral elements (Sinsabaugh and Linkins, 1989; Deng and Tabatabai, 1994) and the trace elements from fungicides can also affect the performance of cellulose activities (Deng and Tabatabai, 1994; Arinze and Yubedee, 2000).

2.3.6 Phosphatases

Phosphatases are important enzymes that are involved in the hydrolysis of phosphorus cycle in the soil (Chodak and Niklinska, 2012). These enzymes are of great agronomic significance because they hydrolyze the compound of organic phosphorus and transform the phosphorus into inorganic form, which can be taken up by plants and microorganisms (Rejsek *et al*., 2012). Generally, plants acquire this element through the uptake of soluble inorganic forms (Hedley *et al*., 1982). After uptake as $H_2PO_4^-$ it remains as inorganic phosphate (Pi) or is esterified to a carbon chain as a phosphate ester or attached to another

phosphate by the energy rich pyrophosphate bond (e.g. ATP). Exchanges between Pi and the P in the ester or pyrophosphate bond are very high. Phosphatases can also be subdivided according to their regulation (e.g. calmodulin), the requirements of metal cations for their activity and sensitivity to various phosphatase inhibitors.

It has been shown that the activities of phosphatases (like those of many hydrolases) depend on several factors such as soil properties, soil organism interactions, plant cover, water inputs and the presence of inhibitors and activators (Rejsek *et al*., 2012; Speir and Ross, 1978).

It has been shown that the P contained within microbial biomass can constitute a significant component of total soil P, with values in the range of 1 – 10 % being common for agricultural soils (Richardson, 1994). Microbial P is influenced by numerous factors (soil moisture and temperature, management practices, etc.). In some increased drought areas, such as the sub-Saharan Africa, and guinea savanna zone, dry conditions lead to a great degree of P limitation to plant growth because of a decrease in P supply through impairing soil phosphatase activity (Garcia *et al*., 2002; Sardans and Penuelas, 2005).

Therefore changes to global precipitation have potential influence on P cycling dynamics, since soil moisture is a key factor of controlling P availability in soils (Houghton *et al*., 2001). Through several processes, including affecting mineralization processes, influencing P demand for plant growth and impacting microbial activity (Leiros *et al*., 1999; Raghothama, 1999; Grierson and Adams, 2000; Sardans *et al*., 2007).

2.3.7 Sulfatase

Sulfatase enzymes play an important role in transformation of organic S into inorganic S, which is subsequently made available for plant utilization (Schnug and Haneklaus, 1998).

The enzyme catalyses the hydrolysis of organic sulfate ester (Kertesz and Mirleau, 2004) and is typically widespread in the soils (Gupta *et al*., 1993; Ganeshamurthy *et al*., 1995). Sulphatases are secreted mainly by bacteria into the external environment as a response to sulphur limitation (McGill and Colle, 1981). The release of sulphate from soluble and insoluble sulphate esters in the soil is affected by various environmental factors such as heavy metal pollution (Tyler, 1981; Kucharski, *et al*., 2011), pH changes in the soil solution (Acosta-Martinez and Tabatabai, 2000), organic matter content and its type (Sarathchandra and Perrott, 1981; Dalal, 1982) and the concentration of organic sulphate esters in the soil (Dodgson and Rose, 1976).

2.3.8 Amylase

Amylase is a starch hydrolyzing enzyme (Ross, 1976) and it is known to be constituted by a-amylase and b-amylase (King, 1967; Thoma *et al*., 1971). The a-amylases are synthesized by plants, animals, and micro-organisms, whereas, b-amylase is synthesized mainly by plants (Pazur 1965; Thoma *et al*., 1971). It plays an essential role in the breakdown of starch, which converts starch-like substrates to glucose and/or oligosaccharides and β-amylase, which converts starch to maltose (Thoma *et al*., 1971). The roles and activities of α-amylase and b-amylase enzymes may be influenced by different factors ranging from cultural practices, type of vegetation, environment and soil types (Pancholy and Rice, 1973; Ross, 1976).

Table 2.1: Soil Enzymes as Indicators of Soil Health

Soil Enzyme	Enzyme Reaction	Indicator
Dehydrogenase	Electron transport system	Microbial activity
Beta-glucosidase	Cellobiose hydrolysis	C-cycling
Cellulase	Cellulose hydrolysis	C-cycling
Phenol oxidase	Lignin hydrolysis	C-cycling
Urease	Urea hydrolysis	N- cycling
Amidase	N-mineralisation	N- cycling
Phosphatase	Release of PO_4	P- cycling
Arylsulphatase	Release of SO_4	S- cycling

Source: National Environmental Research Institute (NERI) Technical Report No. 388, 2002

2.4 Microbial Respiration in Soil

Soil respiration, which is the biological oxidation of organic matter to CO_2 by aerobic organisms, notably microorganisms, occupies a key position in the C cycle of all terrestrial ecosystems. Yiqi and Xuhui (2006) defined soil respiration as the production of carbon dioxide by organisms and the root. Soil microbial respiration is a central process in the terrestrial carbon (C) cycle. It is an important indicator of soil health because it shows the level of microbial activity, soil organic matter (SOM) content and its decomposition (Giardina and Ryan, 2002). Components of soil respiration are identified into two major categories: autotrophic and heterotrophic respiration. The autotrophic components are the metabolic respiration of live root, associated mycorrhiza, and symbiotic N fixing nodules. The heterotrophic respiration is from microbial decomposition of root exudates in

rhizosphere, above-ground and below ground litter, and SOM (Coleman *et al*., 2004). Soil organisms such as bacteria, fungi, protozoa, and algae are responsible for soil respiration (Buscot, 2005). The metabolic activities of soil microorganisms can be quantified by measuring CO_2 production and/or O_2 consumption. Soil respiration can be determined by either CO_2 production or O_2 consumption (NERI Technical Report No. 388, 2002). Soil is mainly involved in ecosystem carbon balance, nutrient cycling, regional and global carbon cycling, climate change, and carbon storage processes.

2.4.1 Factors Affecting Microbial Respiration

Soil respiration is affected by many factors, such as substrate supply, temperature, moisture, oxygen, nitrogen, soil texture and pH value. Soil respiration increases with soil moisture up to the level where pores are filled with too much water limiting oxygen availability which interferes with soil organisms' ability to respire (Silver *et al*., 1999). When soil water content exceeds optimal conditions, soil respiration is depressed due to limitation of oxygen (O_2). Soil O_2 environment becomes a main limiting factor of soil respiration in wetlands, flooding areas, and rain forests (Crawford, 1992). Soil texture is related to porosity, which in turn determines soil water-holding capacity, water movement and gas diffusion in the soil, and ultimately its long-term fertility. Thus, soil texture influences soil respiration mainly through its effects on soil porosity, moisture, and fertility (Dilustro *et al*., 2005).

Most of the known bacterial species grow within the pH range of 4 to 9 (Mendham *et al*., 2002). The fungi are moderately acidophilic, with a pH range of 4 to 6. Thus, soil pH has a marked effect on the growth and proliferation of soil microbes as well as soil respiration. Soils with pH 3.0 produce 2 to 12 times less CO_2 than the soils at pH 4.0 (Sitaula *et al*.,

1995), due to the adverse effect of low pH on soil microbial activity. Nitrogen directly affects respiration in several ways. Respiration generates energy to support root nitrogen uptake and assimilation. High nitrogen content is generally associated with high growth rates, leading to high growth respiration. Thus, differences in nitrogen availability and changes in its deposition rate can alter root respiration rates. (Aber *et al*., 1989) or global change (Cohen and Pastor, 1991).

2.4.2 Substrate induced respiration (SIR)

The technique of substrate induced respiration (SIR) has been designed to evaluate the degree of respiration in the soil due to the employment of a substrate such as glucose, amino acids, alanine and glutamic acid (Eswaran, 2003). The SIR method uses the soil organism's physiological respiration reactions to substrate addition, such as CO_2 production or O_2 consumption, as a means of quantifying microbial activities in soils (Garland *et al*., 2003). This method was developed by Anderson and Domsch (1978) to provide a quick estimate of living microorganism carbon biomass in soils. One of the perceived advantages of SIR is the measurement of the contribution of bacterial and fungal biomass to substrate-induced CO_2 respiration through coupling with antibiotics (Beare *et al*., 1990). Several analytical devices and methods can be used to determine SIR such as Gas chromatography, the Warburg device, infrared gas analyzers, and the Wosthoff CO_2 analyzer (Sparling, 1995). However, many characterizations suffer from common limitations (long gas analysis, large volumes required and complex set-up).

The MicroResp™ method is an easy substrate-induced respiration method that overcomes these limitations (Campbell *et al*., 2003), and offers a wide range of applications (Ben Sassi *et al*., 2012; Tlili *et al*., 2011): it couples the microplate format of the Biolog™ test

restricted to cultivable microorganisms (Garland and Mills, 1991; Stefanowicz, 2006) with the measurement of CO_2 air fraction according to the work of Rowell (1995) on indicator dyes in agar gel. In each closed well of a 96-well microplate, moist soil with or without C substrates is incubated for 6 h in the presence of an agar gel carrying cresol red as indicator dye (Campbell *et al*., 2003). The method only takes into account CO_2 in the well air space, which is assumed to be of microbial origin (Campbell *et al*., 2003).

2.5 Microbial Biomass Carbon

Soil microbial biomass represents the fraction of the soil responsible for the energy and nutrient cycling and the regulation of organic matter transformation (Gregorich *et al*., 1994; Turco *et al*., 1994). A number of studies have reported a close relationship between soil microbial biomass, decomposition rate and N-mineralisation (Smith and Paul 1990; Carter *et al*., 1999). Microbial biomass has also been shown to correlate positively with grain yield in organic, but not in conventional farming (Mäder *et al*., 2001). Finally, soil microbial biomass contributes to soil structure and soil stabilization (Fließbach *et al*., 2000; Smith and Paul 1990). Soil microbial biomass has also been recommended as indicators of soil organic carbon (Carter *et al*., 1999).

Microbial biomass is an important constituent of the soil biological fertility, involved in the biogeochemical cycle of nutrients and carbon. In addition, it is an important reservoir of nutrients in ecosystems. Soil microorganisms immobilize carbon and nitrogen by forming new biomass using the energy they obtain from oxidation of carbon sources through respiration, or inorganic chemical reactions (Chen *et al*., 2003). Therefore, more microbial biomass can stock and cycle more nutrients (Gregorich *et al*., 1994), improving the sustainability of an ecosystem (Kaschuk *et al*., 2010)

2.6 Wastewater Reuse in Irrigated Agriculture

Wastewater reuse is not a new concept although recently, it is receiving a great importance because of the global water crisis (Corcoran *et al*., 2010). The world's water resources will not change, but the human population and its demands on supply are growing rapidly. Meeting these demands will require wise investment in how we use and reuse our water (UN Water, 2010). The World Water Forum meeting in March 2012 echoed the problems and the need to bring wastewater to the fore in world water politics and described the existing situation. Notably, wastewater is used in agriculture for irrigation but it can be used for other purposes including; industrial processes, firefighting, aquaculture, domestic use and wetland (Bogner *et al*., 2007). The reuse is conventional and economically viable, particular in agricultural purposes (Kanyoka and Eshtawi, 2012). This offers an opportunity for increasing food and environmental security, avoiding direct pollution of rivers, canals and surface water; conserving water and nutrients, thereby reducing the need for chemical fertilizer, and disposing of municipal wastewater in a low-cost, sanitary way (WHO, 2006). Wastewater is not only a fertilizer. Its organic matter content, which serves as a soil conditioner and humus replenisher, – an asset not shared by chemical fertilizers – is of agricultural importance. Wastewater is nutrient-rich (Cordell *et al.,* 2011). Phosphorus, for example, is essential to all life and is a key component of fertilizers. The main source of phosphorus (phosphate rock) is non-renewable and is becoming increasingly expensive. Human faeces, however, contains about 0.5 % phosphorus by weight and recovery/reuse could improve phosphorus security and reduce environmental pollution (Cordell *et al.,* 2011). These attributes tend to overshadow the health and environmental risks associated with wastewater use. Though pervasive, this practice is

largely unregulated in low income countries, and the costs and benefits are poorly understood (Scott *et al*., 2004).

Almost 100 % of wastewater used for agricultural purposes in most developing countries are untreated especially in Sub-Saharan Africa (SSA), due to the unavailability of treatment facilities (WHO 2000). In many developing countries, as a result of rapid urbanization and the absence of wastewater treatment facilities, urban farmers often use wastewater either directly from sewage drains or indirectly through wastewater-polluted irrigation water. The scarcity of water resources has increasingly rendered recycled wastewater a valid substitute resource (Srinivasan *et al*., 2009). Therefore wastewater use needs to be better incorporated into the overall management of water resources (WHO, 2006).

2.7 Microbial Contaminants of Wastewater

Wastewater use in agriculture has substantial benefits, but can also pose substantial risks to public health, especially when untreated wastewater is used for crop irrigation. Farmers often have no alternative but to use untreated wastewater because there is no wastewater treatment and freshwater is either unavailable or too expensive (ETWWA, 2010). It is therefore very important to be mindful of the health hazards that may result from the reuse of wastewater in irrigated farming, despite the potential benefits. Although many potential pathogens could be associated with the contamination of wastewater, only representative indicators associated with human and animal contamination are used as a means to detect such pollution (Barrell *et al.*, 2000). These pathogens include bacteria, fungi, viruses, protozoa and nematodes (Asano and Cortuvo, 2004). The numbers and types of pathogens found in wastewater vary both spatially and temporally depending on season, water use,

economic status of the population, disease incidence in the population producing the wastewater, awareness of personal hygiene, and quality of water or food consumed (WHO, 2006). Most pathogenic microbial agents found in wastewater are enteric in origin i.e. they are excreted in faecal matter, contaminate the environment and enter new hosts through ingestion (Toze, 1997). These microbes get into the environment through the faeces of infected hosts and can enter surface water through run-off from soil and other land surfaces, direct defecation into water, and contamination with sewage effluent (Feachem *et al*., 1983).

2.8 Commonly Detected Microbial Indicators in Wastewater

Microbiological examination and monitoring is commonly used worldwide to ensure the safety of a range of water sources whereby contamination with human and animal excreta could pose serious risks. Many potential pathogens could be associated with contaminated water. However, it is both time consuming and expensive to test for all possible pathogens present hence, representative indicators associated with human and animal contamination are used as a means to detect such pollution.

2.8.1 Total and Faecal Coliform

Guidelines for drinking-water quality published by WHO in 2008 defined total coliforms as all those aerobic or facultative anaerobic, gram-negative, non-spore-forming, oxidase-negative, rod-shaped bacteria which have the ability to ferment lactose with gas and acid formation within 48 h at 35 °C whilst faecal coliforms have been defined as those coliforms which can proliferate at an elevated temperature of 44.5 °C. The total coliform group includes those micro-organisms that can survive and proliferate within the water environment and includes several species of the *Enterobacteriaceae* family (Grabow,

2001). Faecal coliforms are the group of the total coliforms that are considered to be present specifically in the faeces of humans and animals. Because the origins of faecal coliforms are more specific than the origins of the more general total coliform group of bacteria, faecal coliforms are considered a more accurate indication of animal or human waste than the total coliforms (Ashbolt, 2004).

2.8.2 *Escherichia coli (E. coli)*

Escherichia coli (E. coli) are the major species in the fecal coliform group. It is regarded as one of the first microorganisms of choice in water quality monitoring programs and serves as the primary indicator for water contaminated with faecal matter due to their prevalence in the gut of warm-blooded animals as well as high numbers excreted in both human and animal faeces (Grabow 2001). Enteropathogenic *E. coli* (EPEC) have been primarily associated with outbreaks of infantile gastroenteritis whilst enteroinvasive *E. coli* (EIEC) are known to produce dysentery by a mechanism similar to *Shigella* sp. causing severe bloody diarrhoea whilst enterotoxigenic *E. coli* (ETEC) are known to possess a heat-labile enterotoxin similar to the cholera (WHO, 2011). EHEC has been implicated in a range of foodborne-related outbreaks since 1983 with one of the largest European outbreaks occurring recently in 2011, caused by the *Shiga toxin-producing E. coli* (STEC) O104:H4 (Soon *et al.,* 2014). Typhoid fever, foodborne cholera, and those caused by pathogenic E. coli, are much more common to low-income countries (WHO, 2015). Most infections caused by *E. coli* O157 result from the consumption of food and water contaminated with faecal matter of infected animals (Smith *et al.,* 2014). Saba *et al.* (2015), discovered high rate of pathogenic *E. coli* in the dung of free-ranged cattle that roam on the streets of

Tamale in the Northern region of Ghana which could end up in the streams or dugouts from which people fetch water for domestic use (drinking, cooking, washing etc.).

2.8.3 *Salmonella*

Salmonella sp. belong to the family *Enterobacteriaceae* and are defined as motile, gram-negative bacilli that are oxidase negative, catalase positive and may utilize citrate as a sole carbon source (Cabral, 2010). They are generally transmitted via the faecal-oral route, with infections being characterized by mild to full blown diarrhoea, nausea, vomiting, septicaemia, typhoid and enteric fever (WHO, 2008). *Salmonella* is the most common aetiological agents associated to fresh produce related infections (Heaton and Jones, 2007) and its main habitat is the intestinal tract of both humans and animals. These pathogens are not known to multiply significantly in the environment, however previous studies have shown prolonged periods of survival in both water and soil environments, provided external temperature, humidity and pH conditions are favourable (Cabral, 2010). *Salmonella* is a major cause of morbidity and mortality in both humans and animals. *Salmonella* remains a major public health problem in many parts of various developing countries including Ghana (Saba *et al.*, 2013). Studies in Northern Ghana have shown the presence of *Salmonella* from outpatients and in-patients departments of hospitals as well as open defecation zones (Saba *et al.*, 2013), irrigated urban vegetables (Cobbina *et al.* 2013) and meat products (Adzitey *et al.*, 2014).

2.9 Helminths

Helminths exist in three different kinds, namely: plathelminths or flat worms, nemathelminths or non-segmented round worms, and annelida or segmented round worms. Those infecting humans through wastewater, sludge or faecal sludge belong only to the

first two groups. They vary in sizes from 1 mm to several metres in length; thus, they are not microorganisms although their eggs are microscopic. Helminth eggs are discharged to the environment in faeces and the oral faecal route is the main dissemination pathway of the disease. These eggs are not normally infective and to become so infective they need to develop a larva (embryonated egg). Some of the helminth parasites require an intermediate host for development prior to becoming infectious for humans (Toze, 1997). These parasites that are of significant health risk, include round worm *(Ascaris lumbricoides*), the hook worm *(Ancylostoma duodenale* or *Necator americanus*), the causative agent of strongyloidiasis *(Strongyloides stercoralis*), and the whip worm (*Trichuris trichiura*) (Bethony *et al*., 2003).

Intestinal nematodes are the greatest health risk involved in the use of untreated wastewater in agriculture (Murray, 2013). The incidences of such infections are relatively low in the developed nations compared to that of the tropical developing countries (Khuroo, 1996), where many areas are endemic with a high proportion of the population as healthy carriers and continuously contaminate the environment and spreading disease. Helminth infection levels are particulary endemic where human faecal matter is used as a fertilizer for growing vegetables (Khuroo, 1996). The type of helminth infection is dependent on environmental and socio-economic conditions (Toze, 1997). Helminth eggs in the soil require moist conditions for embryonation of the eggs over a period of five to ten days before they are able to cause infection (Toze, 1997). Following embryonation, however, the eggs can remain infectious in the contaminated soil for up to ten years (Khuroo, 1996). This means that any soils which have been in contact with recycled waters

contaminated with faecal material could be considered as potential long-term sources of these parasites (Ellis *et al*., 1993).

2.10 Health Risks of Wastewater

The use of wastewater in agriculture can be risky from a health point of view, mainly due to its harmful constituents such as pathogens, thus disease-causing organisms that are usually present in untreated or partially treated wastewater. The detection of pathogens in wastewater irrigated crops and soil, indicates potential environmental and health menace to occupationally exposed farmers and consumers of the contaminated crops. WHO-FAO (2006), estimated that 10 per cent of the world's population relies on food grown with contaminated wastewater. It can be seen that many of the pathogens present in wastewater cause gastroenteritis and it has been estimated that, globally, 1.45 million people per year die as a result of water related illness each year, 58 % of which is caused by inadequate water, sanitation and hygiene, 43 % of the deaths occur in children aged five and below (Corcoran *et al.*, 2010). Diarrhoea, ascariasis, salmonellosis and schistosomiasis are major wastewater-related diseases which have high risk of causing death.

The closer the farmers and consumers are to the source of pollution, the more vulnerable they are. Hence, consumers and communities living around agricultural regions where untreated wastewater is used are particularly exposed to the risks (Corcoran *et al*., 2010).

2.11 Environmental Impacts of Wastewater

Sewage effluents from municipal origin are rich in organic matter and also contain appreciable amounts of major and micro-nutrients (Pescod, 1992; Brar *et al*., 2000). However, these chemical constituents may affect public health and/or environmental

integrity (Assadian *et al*., 2005). Micro-nutrient concentration levels may be very high in the municipal wastewater, contributing to surface water eutrophication and accumulation of organic matter in the soil (Rattan *et al*., 2005). The wastewater may also contain significant quantities of toxic metals (Som *et al*., 1994; Yadav *et al*., 2002) and therefore its long-term use may result in toxic accumulation of heavy metals with unfavourable effects on plant growth (Rattan *et al*., 2005). Farming products produced in regions of high micro-nutrient and heavy metal content may have adverse effects on human health due to the high level of these metals in the edible plant part (Tiller, 1986; Mapanda *et al*., 2005).

Elevated amounts of faecal and aggregate coliform levels have additionally been accounted for from zones where crisp vegetables are developed (Mapanda *et al*., 2005).

Obuobie *et al.* (2006) found that for the Tamale Metropolitan area, Zagyuri community presented a high level of contaminated wastewater used for dry season vegetable production. High levels of faecal and total coliform levels have also been reported from areas where fresh vegetables are cultivated (Amoah *et al.*, 2008).

2.12 Heavy Metals

The term “heavy metals” refers to metals and metalloids having densities greater than 5 gcm^{-3} and is usually associated with pollution and toxicity although some of these elements (essential metals) are required by organisms at low concentrations (Adriano, 2001). For example, zinc (Zn) is the component of a variety of enzymes (dehydrogenases, proteinases, peptidases) but is also involved in the metabolism of carbohydrates, proteins, phosphate, auxins, RNA and ribosome formation in plants (Kabata-Pendias and Pendias, 2001; Mengel and Kirkby, 1982). Copper (Cu) contributes to several physiological processes in

plants (photosynthesis, respiration, carbohydrate distribution, nitrogen and cell wall metabolism, seed production) including also disease resistance (Kabata-Pendias and Pendias, 2001). The good functioning of the metabolisms of humans and bacteria is also dependent on these two metals (Adriano, 2001; Blencowe and Morby, 2003; Cavet *et al*., 2003). On the contrary, cadmium (Cd) is not involved in any known biological processes (non-essential metal) and may be quite toxic as it is accumulated by organisms. It is known to disturb enzyme activities, to inhibit the DNA-mediated transformation in micro-organisms, to interfere in the symbiosis between microbes and plants, as well as to increase plant predisposition to fungal invasion (Kabata-Pendias and Pendias, 2001). In humans, it may promote several disorders in the metabolism of Ca and vitamin D leading to bone degeneration and kidney damage (Adriano, 2001).

Heavy metals in soils may be of natural or anthropogenic origin. Natural sources include vulcanic emissions, aeolian dusts and weathering of rocks (Ernst, 1998). However, the major contamination is usually of anthropogenic origin due to mining and smeltering, application of metal-containing pesticides and fertilizers in agriculture, combustion of fossil fuel, waste disposal, as well as military activities (Alloway, 1995). Cu, Ni, Cd, Zn, Cr and Pb are the most important inorganic soil pollutants. Because soils have limited capacity to inactivate or to attenuate these inputs, heavy metals can persist in soil for very long times, with little decrease in their biological potency (Brookes, 1995).

The excessive uptake of heavy metals by animals and humans is the result of the successive accumulation of these elements in the food chain, the starting point being the contamination of the soil (Gremion, 2003).

2.13 Soil Macronutrients

According to Young (1989), a large quantity of nutrients or elements is believed to circulate between plants and the soil annually in forest ecosystems. It is observed that this circulation provides a closed cycle thereby providing equilibrium in the whole system. Brady and Weil (1999) stated that potassium (K) is known to activate over 80 different enzymes responsible for such plant processes as energy metabolism, starch synthesis, nitrate reduction, photosynthesis, and sugar degradation. As a component in plant cytoplasmic solution, potassium plays a critical role in lowering cellular osmotic potentials, thereby reducing the loss of water from leaf stomata and increasing the ability of root cell to take up water from the soil. As a result of the functions of potassium, a good supply of this element promotes the production of plump grains and large tubers (Young, 1989). Good potassium nutrition is linked to improved drought tolerance, better resistance to certain fungal diseases and greater tolerance of insect pests. Potassium also enhances the quality of flowers, fruits and vegetables by improving flavour, colour and strengthening stems (Brady and Weil, 1999).

Sodium is involved in the regeneration of phosphoenolpyruvate in CAM and C4 plants. It can also substitute for potassium in some circumstances. It can stimulate the growth - increase leaf area and stomata, as well as improves the water balance of plants. Sodium also improves crop quality e.g. improves the taste of carrots by increasing sucrose (Havlin *et al.*, 2005). The preferred concentration of Na in the soil for plants growth ranges between 1-1000 mg/kg (Rai, 1977).

Calcium is used in large amounts by plants second to N and K (Brady and Weil, 2008). It is a major component of the middle lamella (Ca-pectates) of the cell wall. It strengthens the

cell walls, is involved in cell elongation and division, membrane permeability, and activation of several critical enzymes (Brady and Weil, 2008). It is important in N metabolism and protein formation by enhancing NO_3^- uptake and it is also important in translocation of carbohydrates and other nutrients (Havlin *et al*., 2005). In accordance with its functions, calcium influences crop and food quality. Calcium is less mobile such that its influence on crop quality is easily noted with foliar application. Seven fold calcium foliar application also improved some fruit quality characteristics of 'Sinap Orlovskij' apple such as fruit calcium content (high quality) increased by 50-150mg/kg and decreased bitter pit incidence (poor quality) by two times as compared with the control in Lithuania (Launaskas and Kvikliene, 2006). Levels of exchangeable calcium together with pH helps to determine which specific organisms thrive in a particular soil. Although in any chemical condition found in soils some bacterial species will thrive, high calcium and near- neutral pH generally result in the largest, most diverse bacterial populations (Brady and Weil, 2008). Low pH allows fungi to become dominant. The effect of pH and calcium helps explain why fungi tend to dominate in forested soils, while bacterial biomass generally exceeds fungal biomass in most sub-humid to semi-arid prairie and rangeland soils (Brady and Weil, 1999). The preferred concentration of Ca in the soil for plant growth ranges between 20-100 mg/kg (Cakmak and Yazici, 2010).

Magnesium is another secondary nutrient element. It is important as a primary constituent of chlorophyll and as a structural component of ribosomes, it helps in their configuration for protein synthesis (Havlin *et al*., 2005). Brady and Weil (1999), stated that like calcium, important ways by which available magnesium are supplied to the soil are by lime and fertilizer applications and also by plant residues and manures. The preferred concentration

of Mg in the soil for plant growth ranges between 10-40 mg/kg (Hermans *et al*., 2004). It is also required for maximum activity of almost all phosphorylating enzymes in carbohydrate metabolism. Usually, the first things to be noticed due to influence of Mg are chlorophyll level, photosynthesis (photosynthetic CO_2 fixation), and protein synthesis, however, recently, distribution of carbohydrates among shoot and root organs have been reported as well (Cakmak and Yazici, 2010). These in turn affect quality of plant product depending on which part is used for food by humans or animals. A four-fold increase of sucrose in leaves of Mg-deficient sugar beets compared to the Mg-adequate sugar beet plants was reported and this affected quality of Mg-deficient sugar beets (Hermans *et al*., 2004). This was attributed to inhibition of sucrose/sugar distribution from leaves to root organs in the Mg-deficient plants.

2.14 Micronutrients in Soil

Soil fertility experts define trace elements as those elements that are essential to plant growth in small amounts, but toxic to plants at higher concentrations (Tisdale *et al*., 1993). Interest in trace elements has increased because of the widespread trace element contamination caused by industrial processing. The manufacture of many goods requires the use of trace elements such as V, Co, Cu, Pb, Cd, Ni, Hg, As, and Se, so these elements have become common in industrial wastes and in some cases end up in the environment (Allard and Whitney 1994). Much of the research conducted by soil scientists for fertility purposes has been centered on deficiencies that limit plant growth and the essentiality of trace elements in the plant life cycle (Tisdale *et al.*, 1993). A wide variation in soil content has been noted in soil fertility research. Certain trace elements are toxic to humans and other animals at elevated concentrations. However, some trace elements are required by

humans and other animals for healthy function of the body (Smith and Huyck, 1999). Adriano (1986) stated the factors that determine the fate of trace elements in soil to be soil pH, CEC, anion exchange capacity, organic matter content, clay content and type, oxide content and type, and redox potential.

CHAPTER THREE

MATERIALS AND METHODS

3.0 Study Area

The experiment was a field based type carried out under both rain-fed cropping season (August and September) and irrigated cropping season (January and February). The study was performed at the Urban Food Plus Central Experimental Field located at Zagyuri (9°28'28.94"N, 0°50'53.64"W). Zagyuri is near the Kamina Barracks in the northern part of Tamale Metropolis, one of the major vegetable growing sites in urban Tamale. The area experiences one rainy season in a year which usually occurs from April to October. The annual mean rainfall is about 1,000 mm and the mean monthly temperature is between 17 and 40 ° C. The farming site has a total area of 12 ha. The water source for irrigation is an untreated wastewater from broken sewage pipes at the Kamina Military Barracks and surrounding communities.

Figure 3.1: Aerial View of the Experimental Site

3.1 Experimental Design and Material

The study was 2 x 4 factorial experiment laid out in Randomized Complete Block Design (RCBD) with four (4) blocks and four (4) replications. A total of 32 plots were used. The size of each plot was 2 m x 4 m separated by an alley of 0.5 m between plots. The experiment consists of two factors; irrigation water quality and soil amendment type. The soil amendments applied in the study include; rice husk biochar (20 tha^{-1}), NPK (212.5 $kgha^{-1}$) as normal agricultural practice by farmers, combine treatment of biochar (20 tha^{-1}) and NPK (212.5 $kgha^{-1}$) and control plot. The irrigation water quality involved wastewater sourced from the broken sewer in the study area and domestic piped water sourced from Ghana Water Company. Before the commencement of this experiment, the field had been in existence for one year and used to study the yield effect on the following test crops in the order of Maize, Lettuce, Cabbage, Amaranths, Lettuce, Amaranths, Ayoyo, Ayoyo, Amaranths, Ayoyo, Resselle, Lettuce, Carrots respectively.

3.2 Agronomic Practices of Amaranth

Rice husk biochar (20 tha^{-1}) was incorporated into the soil and mixed uniformly. Amaranth seeds were planted by broadcasting. NPK fertilizer (212.5 $kgha^{-1}$) was applied to the respective plots also by broadcasting method after 7 days of emergence. The respective treatment plots received an average of 7.79 mm depth of the irrigation water daily during the dry season. This was based on farmer's prescription although soil moisture content was intermittently checked to avoid excess irrigation. However, the experiment was subjected to rain-fed during the wet seasons. Soil moisture contents were checked after every rainfall and rain gauge used to check the amount of rainfall. Weeds were controlled manually by hand picking to avoid crop-weed competition. This was done anytime there was weed

growth. The harvesting was done after four (4) weeks of planting. The cutting method of harvesting was used, which was performed at 20 cm height above the ground level.

3.3 Soil Physicochemical and Enzymatic Analysis

3.3.1 Soil Sampling

Soil samples were collected within the depth 0-20 cm from the experimental site from six different locations within each plot by using an auger. The samples were then air dried under room temperature and sieved with 2 mm sieve to remove plant debris. Two hundred grams (200 g) of the sieved soil were packaged and sent for analysis at Ruhr University of Bochum, Germany.

3.3.2 pH and Electrical Conductivity

The pH and electrical conductivity of the soil were determined by using 1:5 of soil to 0.01 M calcium to chloride ratio. Five grams (5 g) of soil was weighed into a 50 ml glass beaker and 25 ml 0.01M $CaCl_2$ added. The suspension was stirred with a glass rod and allowed to settle for one hour. The pH and electrical conductivity meters were calibrated with their appropriate buffer by the manufacturer's instructions before the reading was taken. Buffer solution of pH 7 and 4 were used for the pH meter and solution of 2 mS for the electrical conductivity.

3.3.3 Carbon to Nitrogen (C: N) ratio

The soil samples for C: N ratio determination was first ground to a fine size of 0.02 mm using a ball mill (Pulverisette 7, Fritsch GmbH, Idar-Oberstein). Approximately 1 g of soil sample and a standard of 250 mg aspartic acid were weighed into stainless steel crucibles. The measurement was performed by C/N analyzer (Vario MAX cube, Elementar, Hanau).

The C/N analyzer works on the principle of combustion, gas separation and gas detection. The samples were first combusted at a high temperature between 950 to 1200 °C. Gas separation followed, where helium or carbon dioxide carrier gas pushes combustion gases through the analyzer. Carbon, hydrogen and sulfur combustion gases are trapped in separate columns then subsequently released while Nitrogen gas flows directly into the columns. Gas detection was made by thermal conductivity detector utilizing a Wheatstone bridge circuit to compare the relative thermal conductivity differences between the carrizer gas and the analyte gas.

3.3.4 Elemental Analysis Soil

Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES) was used for the soil elemental analysis. The elements measured include carbon (C), nitrogen (N), phosphorus (P), potassium (K), sodium (Na), Titanium (Ti), Beryllium (Be) copper (Cu), zinc (Zn), mercury (Hg), cobalt (Co), lead (Pb), cadmium (Cd), magnesium (Mg), calcium (Ca), iron (Fe), nickel (Ni), selenium (Se), chromium (Cr), vanadium (V) and aluminum (Al). Prior to the ICP-OES the soil was microwave digested using concentrated nitric acid for 15 minutes. About 0.25 g of the ground soil sample was weighed out in an inert teflon microwave vessel. Ten (10) milliliters concentrated nitric acid was then added to the soil in a fume hood chamber, the mixture was allowed to stay overnight. The vessels were firmly closed and fitted into the microwave (MARS exprexx, CEM, Kamp-Lintford) according to the manufacture's instruction. The microwave was programmed to operate under the temperature of 120 °C, power of 1600 w and time of 15 minutes. The digested mixture was allowed to normalize to room temperature before 10 ml of water was added to the content in the vessel. Cellulose membrane filter paper of 0.2 microns was used to filter the digested

samples before ICP-OES reading. The ICP-OES makes use of electromagnetic ratio emitted from the excited atoms molecules, and their ionized form for the identification of the elements contained in a sample with the help of a computer software.

3.3.5 Hot Water Extractable Carbon

The soil sample was pre-incubated for a week at 60 % water holding capacity prior to the analysis. Thereafter, 2 g of the pre-incubated soil was weighed into 50 ml inert centrifuge plastic tubes. An amount of 20 ml deionized water was added to the centrifuge tube containing the soil samples. The tubes were placed in a water bath at a temperature of 70 °C for 16 hours. The suspension was centrifuged (Beckman J2-HC) at a speed of 1000 rpm for 15 minutes. The supernatant was carefully decanted and filtered through 0.45µm membrane paper. DIMATOC 2000 C Analyzer (Dimatec) was used to measure the total organic carbon (TOC) of the solution to represent the hot water extractible carbon. The autosampler DIMATOC 2000 measures TOC on the principle of thermal-catalytic oxidation with subsequent nondispersive (infrared) NDIR detection, results are accessed with the help of a computer software.

3.4 Microbial Biomass Carbon

The one week pre-incubated soil sample at 60 % water holding capacity at a temperature of 25 °C was used for the microbial carbon analysis. The pre incubation was done to allow the microbial activities to equilibrate after the initial disturbance. Ten (10) grams of duplicate sub-samples from each type of soil was placed in two individual 50 ml glass bottle. Samples designated for fumigation were placed in vacuum desiccators. The desiccator was prepared with paper towels moistened with deionized water, placed at the bottom where a glass with soda lime is kept to help maintain the water content of soils

during fumigation. Approximately 25 ml chloroform ($CHCl_3$) was filled in a glass beaker and placed in the desiccator. The desiccators were sealed, placed in a laboratory hood, and evacuated, to allow the chloroform to boil for approximately 30 minutes. The desiccator was kept in the dark for 24 hours after the evacuation for incubation. The beaker containing the residual chloroform was taken out from the desiccator after the 24 hours of storage in the dark. The desiccator containing the samples was again subjected to eight minutes evacuation under water jet pressure after the 24 hour storage to remove traces of chloroform. The samples were then removed from the desiccator and extracted with 40 ml 0.05 M potassium sulphate ($K2SO_4$). Bottles were shaken for 30 min on a horizontal shaker (Edmund Bahler GmbH) at a speed of 200 rev min^{-1} and supernatants were filtered through a Whatman (GF/A513-5204) filter. The filtrates were measured for microbial biomass carbon with DIMATOC 2000 analyser.

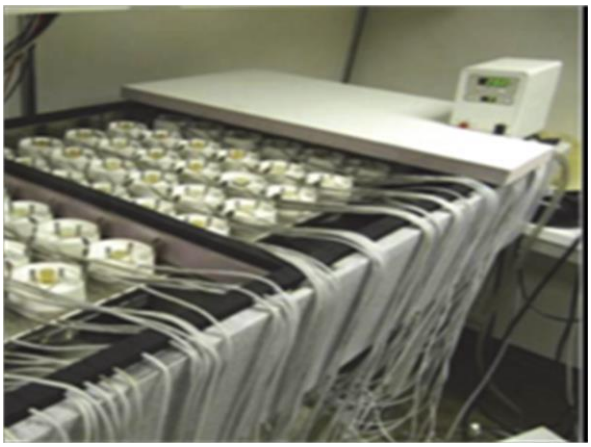
The non-fumigated samples were directly extracted with 40 ml 0.05M potassium sulphate ($K2SO_4$) solution by shaking for 30 minutes on a speed of 200 rev min^{-1}. The samples were filtered and filtrate analyzed with DIMATOC 2000 C analyzer.

3.5 Soil Microbial Basal Respiration

Air dried soil samples where rewetted to 60 % water holding capacity prior to the pre incubation and respiration measurement. The soil microbial respiration was measured by Respicond IV device (Nordgren Innovations, Djäkneboda). The Respicond respirometer consists of 96 individual experimental cells that are placed in a water bath with adjustable temperature for soil respiration. Each cell is made of vessel into which the soil sample was placed. The lid of the vessel involves a smaller jar attached to it, into which an alkali trap (KOH) solution and platinum electrodes is inserted prior to analysis. The CO_2 evolved

from the soil sample was captured by the KOH solution. The absorbed CO_2 was determined by measuring changes in conductivity of the KOH solution. Data from each vessel was collected and analyzed using the software supplied with the Respicond VI. The absorption of CO_2 by the KOH solution is chemically represented as

$KOH \rightarrow CO_2 + KHCO_3$, $KHCO_3 \rightarrow K_3OH + K_2CO_3 + H_3O$ …… Equation 3.1



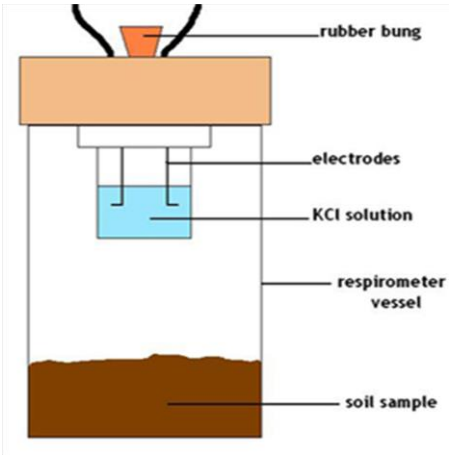


Figure 3.2: Ninty Six (96) Unit Respirometer

3.6 Substrate Induced Respiration

The substrate induced respiration was determined using the MicroResp system as described by Campbell *et al.* (2003). The micro plate-based MSIR system measures the respired carbon (C) released from soil. MicroResp makes use of a deep well plastic plates with 96 wells for samples and a silicon rubber seal with an air hole for each well, allowing the carbon dioxide (CO_2) to pass from a deep well plate to the detection plate on top of the seal. The detection plate contains an agar with indicator dye and bicarbonate which changes colour with change in pH when CO_2 reacts with bicarbonate.

The 96 well detection micro plates containing agar with indicator dye and bicarbonate were prepared a week before they were used for the respiration. The detection agar plate

was prepared with an indicator dye, cresol red (12.5 ppm, wt/wt), potassium chloride (150 mM), and sodium bicarbonate (2.5 mM) in 1% dissolved agar. Using automated microplate pipette, 150 µl was carefully dispensed in well of the detection plate avoiding bubbles. The plates were then stored to equilibrate in plastic bags with wet paper towels and soda lime to ensure that they do not desiccate or react with atmospheric CO_2.

Three hundred (300 mg) milligrams of pre-incubated soil samples were loaded into the 96 deep well plates in a replicate of four. The deep well plates were tapped about five times to ensure the sticking soil particles were concentrated at the bottom of the well. The weight of soil was recorded, and the packing density was calculated. Alanine, citric acid and glucose were used as carbon source test for the respiration with distilled water as basal or control test. The choice of the carbon source was based on their relevant to the soil as plant root exudates and that they can be dissolved in water. Each carbon source was dissolved in deionized water and prepared as a stock solution at a concentration designed to deliver 30 mg of C g of soil water in each deep well when 25 µl was dispensed.

Just before incubation, the plate containing the agar was read with an absorbance (Ab_{590nm}) microplate reader. The deep well and the agar plates were then sealed together with a silicone rubber gasket with interconnecting holes. The setup was firmly clamped to avoid the occurrence of gas exchange. Colorimetric measure of the detection plate was re-read immediately after 6 h of incubation at 25°C with a microplate reader (Infinite 200 Pro, TEKAN, Switzerland) at 590 nm.

3.7 Enzymes Assays

Soil enzyme assays performed in the laboratory were useful for assessing microbial community functions (Wallenstein and Weintraub, 2008). This function was central to the

processing, stabilization, and destabilization of soil organic matter and nutrient cycling in terrestrial ecosystems (Burn sand Dick 2002). In this study, ten different enzymes were assayed according to the method described by Marx *et al.,* (2001). The general concept of the fluorescence enzyme assay is that synthetic substrates bound with a fluorescent dye, are added to soil samples. During enzyme-catalyzed substrate degradation, the bond breaks between the fluorescent dye and the substrate. The fluorescent dye liberated from the substrate is consequently used as an indirect assessment of enzyme activity and can be quantified using a microplate reader to detect the fluorescence intensity of the dye.

The potential activity of the soil enzymes where assessed using Methylumbelliferone (MUF)-and Amido-4- methylcoumarin (AMC) labeled substrate analogues (Sigma-Aldrich CO) with MES monohydrate buffer salt (Sigma-Aldrich), TRIZMA Buffered Saline (Sigma-Aldrich CO). The substrates were dissolved in 300 µl dimethyl sulfoxide (DMSO) and brought to a final volume of 10 ml with sterile deionized water for a 10 mM stock solution. The substrate preparation for each enzyme is summarize in the Table 3.1.

Table 3.1: Preparation of Enzymes Substrates

Substrate	Abreviation	Buffer	Enzyme
4-Methylumbelliferyl-α-D-Glucoside	α-Glu	MES	α-Glucosidase
4-Methylumbelliferyl-Phosphate	Pho	MES	Phosphatase
4-Methylumbelliferyl-β-D-Glucopyranoside	β-Glu	MES	β-D-Glucopyranosidase
4-Methylumbelliferyl-β-D-Xylopyranoside	β-Xyl	MES	β-Xylosidase
Sulfate	Sul	MES	Sulfatesterase
4-Methylumbelliferyl-β-D-Cellobioside	β-Cello	MES	β-D-cellubiosidase
4-Methylumbelliferyl-N-Acetyl-β-D-Glucosaminide	N-Acet	MES	N-acetyl-glucosaminidase
L-Leucine-7-amido-4-methylcoumarin	L-Leu	TRIZMA	Leucine-Aminopeptidase
L-Tyrosine-7-amido-4-methylcoumarin	L-Tyr	TRIZMA	Tyrosine-Aminopeptidase
L-Argenine-7-amido-4-methylcoumarin	L-Arg	TRIZMA	Argenine-Aminopeptidase

Prior to the enzyme activity analysis, the pre-incubated sub-samples that were stored frozen at -20 °C were thawed to 4 °C. Approximately 1 g of the thawed samples was weighed into sterile glass beaker. A 50 ml portion of sterile water was then dispersed to the soil in the beaker and stirred with ultrasonic disaggregator at 300 rpm for 5 min. The soil

suspension was continuously stirred while an aliquot of 50 µl was transferred into a black micro-titer plate in four replicates. Then 50 µl of autoclaved buffer (0.1 M MES-buffer or 0.05 M Trizma-buffer) was added to the slurry. Thereafter, 100 µl of the respective 1 mM substrate containing the fluorescent compounds 4-methylumbelliferone (4-MUF, for ß-glucosidase, ß-xylosidase, α-glucosidase, β-D-cellubiosidase N-acetyl-glucosaminidase, Sulfatase and phosphatase) or 7-amino-4-methylcoumarin (7-AMC, for L-leucine aminopeptidase, L-Leucine aminotyrosinase and L- Leucineaminoarginase) were added to the soil suspension. Further, 10 µM standards (4-MUF standard, 7-AMC standard) were added to the soil suspension with buffer to obtain final concentrations of 0, 100, 200, 500, 800 and 1200 pmol $well^{-1}$. Wells without soil suspension were used as control for autocleavage of substrates. Micro-titer plates were incubated in the dark for 180 min at 30˚C. Fluorescence was measured after 30, 60, 90, 150 and 180 min with 360 nm excitation and 460 nm emissions using a microplate reader (Infinite 200 Pro, TEKAN). The quantitative enzymatic activities (nmol) after blank subtraction were calculated based on standard curves of MUF and AMC.

3.8 Dehydrogenase Activity

Dehydrogenase activity was measured according to von Mersi and Schinner (1991) using 2 (p-iodophenyl)-3-(p-nitrophenyl)-5-phenyl tetrazolium chloride (iodonitrotetrazolium chloride, INT) as the substrate and 1M TRIS buffer. The 4 % INT substrate was prepared by dissolving 400 mg INT in 100 ml of sterile water using ultrasonic bath for approximately 10 minutes because it is less soluble. The solution was prepared fresh each day and stored in the dark for accuracy. A 30.28 g hydroxymethyl aminomethan was dissolved in 250 ml sterile with HCl to produce 1 M TRIS buffer of pH 7.

One gram (1 g) of pre-incubated sample of 60 % water holding capacity was weighed into 15 ml plastic test tube. To every tube 1 ml of 0.2 % INT (2 : 1 ratio of 0.4 INT and TRIS buffer) was added. For the blank samples only 1 ml of TRIS buffer was added without mixing it with INT solution. The tubes were sealed with screw caps, shaken and incubated for 2 hours at 40 ˚C. After the incubation period, the enzymatic reaction was terminated by the addition of 10 ml methanol to the tubes. The samples were again kept in the dark for additional 1 hour with vigorous vortexing after every 20 minutes. Subsequently, the samples were centrifuged for 10 min at 300 rpm. By using automated micro pipette, 260 µl of the filtrates was transferred into 96 well microplates. The absorption of the filtrates were measured by microplate reader (Infinite 200 Pro, TEKAN) at an excitation of 460 nm.

3.9 Determination of Helminth Eggs in Irrigation Water, Soil, and Vegetables

The population and identification the types of helminth egg in irrigation water, soil, and vegetables were determined using the flotation and sedimentation method which is a modified US-EPA method by Schwartzbrod (1998). A chart for diagnosis of intestinal parasites by WHO (1994) was used for the identification of the helminths which were captured by camera microscope (OMAX 3.0 MP).

3.9.1 Preparation of Reagents

Zinc sulphate solution of about 1.3 specific gravity was prepared by completely dissolving 373 g of zinc sulphate in 1 litre of deionized water. Acid/alcohol buffer solution was also prepared by mixing 5.16 ml H_2SO_4 to 350 ml of ethanol and then topped up with deionized water to produce 1 litre of the solution.

3.9.2 Helminth Eggs Determination Procedure

Two litres of the irrigation water samples were allowed to settle overnight, enabling helminths eggs to settle under their own weight. Much of the supernatant as possible was sucked up and the sediment transferred into 15 ml centrifuge tubes. The 2-litre containers were rinsed 2-3 times with deionized water and then were transferred into another set of centrifuge tubes. The tubes were then centrifuged at 1,450 rpm for three minutes. The sediments in the centrifuge tubes for each sample were pooled into one centrifuge tube and centrifuged again at 1,450 rpm for another three minutes.

The supernatant was carefully decanted and the deposit was re-suspended in about 150 ml $ZnSO_4$ solution. The mixture was homogenized with a spatula and centrifuged at 1,450 rpm. At a density of 1.3 ($ZnSO_4$), all helminth eggs float leaving other sediments at the bottom of the centrifuge tube. The $ZnSO_4$ supernatant was poured into a 2-litre flask and diluted with at least one litre of water. This was allowed between 18 to 24 hours for the eggs to settle again. As much supernatant as possible was sucked up and the deposit was re-suspended by shaking. The re-suspended deposit was transferred into centrifuge tubes. The 2-litre container was rinsed 2-3 times with deionized water and the rinsed water added to the centrifuged tubes and centrifuged at 1600 rpm for three minutes. The deposit was pooled into one tube and centrifuged again at the same speed and for the same period of time.

The deposit was then re-suspended in acid/alcohol (H_2SO_4 + C_2H_5OH). Ethyl ether was added after sucking much of the supernatant. The mixture was shaken and the centrifuge tube occasionally opened to let out gas before centrifuged at 2200 rpm for three minutes. After the centrifugation, a diphasic (lipophilic and aqueous phase representing the ethyl

ether and acid/alcohol, respectively) solution was formed. With a micropipette, as much of the supernatant as possible was sucked up leaving about 1 ml of deposit. The deposit was observed on a cell under the microscope (x100) and the eggs counted.

3.9.3 Preparation of Vegetables for Helminth Eggs Determination

Determination of helminth eggs on vegetables sample requires the pre-treatment of the vegetable samples so as to release the micro-organisms into a sterile diluent. Hundred grams (100 g) fresh weight of the vegetable was washed in 1 litre of distilled water with 5 ml of tween twenty (20). The vegetables were rinsed with distilled water and the washing solution made up to at least 2 litres. The washed water was analyzed for helminth egg as described in procedure 3.9.2.

3.9.4 Preparation of Soil for Helminth Eggs Determination

Pre-treating soil samples for helminths eggs requires blending of the samples at high speed to cause the release of the ova from the soil particles. A 20 g (dry weight) portion of soil samples was blended at high speed in 200 ml distilled water for about 1 minute and the volume of the mixture was further increased to 2 litres. This mixture was allowed to settle and the number of helminth eggs determined as described in procedure 3.9.2. The number of helminth eggs was expressed per gram soil (oven-dried weight basis).

3.10 Sampling for Microbial Analysis

Vegetable (Amaranthus), irrigation water and soil were analyzed for microbial contamination in the two major seasons (dry and wet season). The pathogenic indicators that ware analyzed includes Feacal coliform, *E. coli Salmonella* and Shiga Toxin Producing *E. coli* (STEC). Consumable portion of the vegetables were aseptically collected

from ten plants randomly from each plot. Irrigation water (wastewater and domestic piped water) was also sampled into sterile bottles. Soil for microbial analysis was sampled from ten (10) locations within the top 5 cm of the soil, which was pooled together to make a composite sample for each plot. Each sample was properly identified, labeled, placed separately in a sterile plastic bag and transported to the laboratory in an ice box where they were prepared for bacteriological examination.

3.11 Media Used and Preparation

All media used were of analytical reagent grade and were prepared according to the manufacturers' instructions. The media used in this study includes CHROMagar *E. Coli* and Coliform (ECC), CHROMagar STEC, Modified semi-solid Rappaport-Vassiliadis (MSRV), Xylose-Lysine Deoxycholate Agar (XLD), buffered peptone water (BPW) and Phosphate Buffered Saline (PBS).

An amount of 32.8 g CHROMagar ECC, 30.8 g CHROMagar STEC, 31.6 g MSRV and 56.68 g XLD media were separately dispersed into 1 L of sterile distilled water. Each content was brought to boil (100 °C) with continues stirring and agitation using magnetic stirrer. The dissolved media were not autoclaved as prescribed by the manufacturers because they are susceptible to overheating. They were allowed to cool to room temperature. About 15 ml of the respective media were dispersed in sterile 90 mm Ø petri dishes and allowed to solidify under room temperature prior to their usage.

Buffered peptone water (BPW) was made by dissolving 20 g of the medium base in 1 liter purified water, mixed thoroughly and autoclaved at 121°C for 15 minutes. After autoclaving, the broth was cooled to room temperature and stored at 4 ºC for use.

Phosphate Buffered Saline (PBS) was prepared by dissolving 8 g of NaCl, 0.2 g of KCl, 1.44 g of Na_2HPO_4 and 0.24 g of KH_2PO_4 in 1 L of sterile distilled water. The pH was re-adjusted to 7 after which it was autoclaved at 121°C for 15 minutes.

3.12 Sample Preparations and Plating for Faecal Coliform, *E. coli* and STEC Isolation

About 20 g of vegetables was weighed into 180 ml of phosphate-buffered saline and rinsed vigorously. Further tenfold serial dilution was made and triplicate tubes. In the case of the soil sample, 10 g phosphate-buffered saline and stirred vigorously. Irrigation water samples were also serially diluted before inoculation and incubated. Aliquots (0.5 mL) of each sample were pipetted on plates containing CHROMagar ECC solidified medium while plate with CHROMagar STEC were inoculated without further diluting the homogenated inoculum. Sterile glass bead were used to evenly spread the aliquots on the solidified surface before they were incubated at 37 °C for 24 hours. Results were read base on their appearance in terms of colour differences as a result of their biochemical properties.

3.13 Sample Preparations and Isolation of *Salmonella*

Salmonella isolation was based on the method published by Food and Drugs Authority (FDA) Bacteriological Analytical Manual (BAM, 2014). It typically involves pre-enrichment of the sample in non-selective media (Peptone water) to recover sub-lethally injured cells due to heat, cold or osmotic shock. Next, primary enrichment cultures are inoculated into secondary selective enrichment media (MRSV) before it is transferred to a selective media (XLD).

An amount of 25 g of vegetable and soil sample was weighed into separate sterile incubation bags. In similar type bags, 25 ml of irrigation water was measured into them. Thereafter, 225 ml of non-selective nutrient broth, buffered peptone water was added to the samples. The content was carefully agitated to allow uniform mixture of sample and the medium. The samples were then incubated at 37 °C for 24 hours.

The pre enriched samples were mixed thoroughly and 3 drops of the mixed broth culture were inoculated at separate spots on an MSRV plate. The plates were again incubated at 42 °C for 18 hours. Growth of migrated cells which appeared as gray-white, turbid zone extending out from the inoculated drops are presumptively positive for motile *Salmonella*.

Presumptive *Salmonella* colonies were transferred to XLD agar plate by streaking for confirmation. The plates were again incubated for a period of 24 hours at 42 °C. *Salmonella* cells were observed by change in colour of both the colonies and in the medium from red to yellow as well as hydrogen sulfide production conditions results in colonies with black-center**.**

3.14 Data Handling and Analysis

The results were analysed with GENSTAT version 13. All data were double-keyed and cross tabulated to ensure the accuracy of the entries made. Total and faecal coliform populations were normalized by log transformation before analysis of variance (ANOVA). The t-test (both one sample and two independent samples) was used to test significance of difference between mean faecal coliform levels on vegetables and in irrigation water from different sources and the recommended standards. ANOVA was used to compare faecal

coliform levels on different crops as well as helminth eggs in the different treatment plots. Unless otherwise stated, results of analysis are quoted at $p < 0.05$ level of significance.

CHAPTER FOUR

RESULTS

4.1 Summary of Irrigation Water Quality

Table 4.1 displays the quality of water (piped and wastewater) used for irrigation in the study. The feacal coliform and *E. coli* loads were indicated to be higher in the wastewater than that of the piped water. Both water qualities revealed the presence of *Salmonella* spp. in both seasons. *Shiga toxin* producing *E. coli* was detected only in the wastewater. Helminths eggs incidence were more abundant in the wastewater than the piped water.

Table 4.1: Pathogenic Quality of Irrigation Water

Pathogens	Dry Season		Wet Season	
	Piped water	Wastewater	Piped water	Wastewater
Feacal Coliform (Log CFU)	4.07^{a}	7.38^{c}	4.01^{a}	6.50^{bc}
E. coli (Log CFU)	1.76^{a}	2.09^{bc}	1.62^{a}	2.24^{c}
Salmonella spp.	Present	Present	Present	Present
STEC	Present	Present	present	Absent
Helminth eggs/litre	6^{a}	25^{b}	4^{a}	19^{b}

STEC: Shiga toxin producing *E. coli*

4.2 Microbial Contamination of Vegetables

The results of microbial contamination of vegetables are presented in Table 4.2. Microbial concentration on the vegetables was seen to be more pronounced in the dry season than in the wet season. The treatment effect was more evident in the dry season as compared to the wet season. Obviously from Table 4.2, wastewater irrigated vegetables recorded high microbial concentrations in all the treatments than the piped water. The highest CFU of

faecal coliform and *E. coli* were recorded in biochar + NPK under wastewater irrigation in dry season with the values of 6.96 CFU/100g and 0.84 CFU/100g respectively. This was followed by NPK with wastewater at 6.33 CFU/100g for faecal coliform and 0.84 CFU/100g for *E. coli* in the dry season. The control plots under piped water recorded the lowest counts for both faecal coliform and *E. coli* in the two seasons.

As seen from the Table 4.2, *Salmonella* spp. was detected only in dry season on vegetables under NPK and Biochar+NPK plot with wastewater irrigation whereas all other treatments were negative for *Salmonella* spp. In the case of STEC, none of the treatments was detected positive in the two seasons.

Table 4.2: Microbial Quality of Vegetable

Treatments		Dry season				Wet season			
		Feacal coliform (CFU/g)	*E. coli* (CFU/g)	*Salmo-nella* spp	STEC	Feacal coliform (CFU/g)	*E. coli* (CFU/g)	*Salmo-nella* spp	STEC
Piped water	Control	3.48^{c}	0.54^{c}	-	-	3.12^{c}	0.51^{c}	-	-
	Biochar	3.32^{c}	0.52^{c}	-	-	3.15^{c}	0.51^{c}	-	-
	NPK	3.56^{c}	0.55^{c}	-	-	3.16^{c}	0.49^{c}	-	-
	Biochar+NPK	5.10^{b}	0.71^{ab}	-	-	3.15^{c}	0.56^{c}	-	-
Waste water	Control	5.79^{a}	0.76^{ab}	-	-	3.83^{ab}	0.65^{b}	-	-
	Biochar	5.49^{bc}	0.74^{ab}	-	-	3.96^{a}	0.61^{b}	-	-
	NPK	6.33^{a}	0.80^{a}	+	-	3.56^{b}	0.65^{b}	-	-
	Biochar+NPK	6.96^{a}	0.84^{a}	+	-	3.92^{a}	0.70^{a}	-	-
LSD		**0.48**	**0.22**			**0.65**	**0.11**		
F pr.		**<.001**	**<.001**			**<.001**	**<.001**		

4.3 Pathogenic Contamination of Irrigated Soil

Microbial concentration in the irrigated soil in both the dry and wet season is presented in Table 4.3. Prevalence of feacal coliform and *E. coli* counts as well as the incidence of

Salmonella spp. and STEC was more elevated in the wastewater irrigated plots than irrigation plots where domestic piped water was used. Averagely the contamination indicators were higher in the wet season compared to the dry season. Biochar+NPK treated soil under wastewater irrigation was positive for *Salmonella* spp. and STEC in both seasons. The highest count for faecal coliform in the wet season was 5.03 CFU/g and dry season was 4.86 CFU/g while *E. coli* recorded 1.83 log CFU and 1.01 log CFU in wet and dry seasons respectively. The highest *E.coli* load was seen in NPK treated soil with wastewater irrigation which also recorded positive for *Salmonella* spp. in both season and STEC in only dry season. However there were no detectable STEC in the piped water irrigated soil in both seasons.

Table 4.3: Microbial Contamination of Irrigated Soil

Treatments		Dry season				Wet season			
		Feacal coliform (CFU/g)	***E.coli* (CFU/g)**	***Salmo-nella* spp**	**STEC**	**Feacal coliform (CFU/g)**	***E.coli* (CFU/g)**	***Salmo-nella* spp**	**STEC**
Piped water	**Control**	2.45^{c}	0.40^{bc}	-	-	3.23^{c}	1.08^{c}	-	-
	Biochar	3.22^{bc}	0.18^{c}	-	-	3.21^{c}	0.96^{c}	-	-
	NPK	2.68^{c}	0.24^{c}	-	-	3.08^{c}	1.21^{b}	+	-
	Biochar+NPK	3.31^{bc}	0.54^{bc}	-	-	3.67^{c}	1.14^{b}	-	-
Waste water	**Control**	4.04^{b}	0.97^{a}	-	-	4.51^{b}	1.78^{ab}	+	-
	Biochar	4.03^{b}	1.01^{a}	+	-	4.54^{b}	1.76^{ab}	-	-
	NPK	4.14^{b}	0.85^{b}	+	-	4.49^{b}	1.83^{a}	+	+
	Biochar+NPK	4.86^{a}	1.01^{a}	+	-	5.03^{a}	2.05^{a}	+	+
LSD.		**0.42**	**0.28**			**0.47**	**0.26**		
F pr.		**0.01**	**0.01**			**0.01**	**0.01**		

4.4 Helminth Egg Concentration in Vegetables and Soil

The occurrence of helminth eggs was more obvious in soil than the vegetables. Averagely, wet seasons recorded high values of helminths concentration than the dry seasons. Biochar+NPK soil under wastewater irrigation recorded the highest count of helminths eggs of 23 egg 10 g^{-1} dry weight in the wet season and 20 eggs 10 g^{-1} dry weight in the dry season. As presented in Table 4.4 vegetables in the wet season recorded no helminth count regardless of the treatment.

Table 4.4: Helminths Egg Loads in Irrigated Soil and Vegetable

Treatments		Helminths Eggs			
		Dry season		Wet season	
		Soil ($10g^{-1}$ dry weight)	Vegetable ($100g^{-1}$ wet weight.)	Soil ($10g^{-1}$ dry weight)	Vegetable ($100g^{-1}$ wet weight)
Piped water	Control	3.33^{a}	0	5^{b}	0
	Biochar	5^{b}	0	1.67^{a}	0
	NPK	3.33^{a}	0	3.33^{ab}	0
	Biochar + NPK	5^{ab}	1.67^{a}	10^{c}	0
Waste water	Control	6.66^{ab}	1.67^{a}	13.33^{cd}	0
	Biochar	11.66^{c}	0	16.67^{d}	0
	NPK	11.66^{c}	0	20^{e}	0
	Biochar + NPK	16.67^{d}	3.33^{b}	23.33^{e}	0

4.5 Trace Elements Concentrations in Irrigated Soil

Differences in all heavy metal concentrations in the soil under study were not pronounced, with the exception of Titanium (Ti) which was highly significant *($P<0.01$)*. From Table 4.5, the concentrations of all the metals were generally low the WHO (2006) recommended standard with the exception of Ti which was about three times higher than the recommended values in the various treatments. The heavy metal concentrations among all treatments ranged between 0.01 mg/l and 0.93 mg/l. Averagely, the lowest concentrated metal was Cobalt (Co), at 0.01 mg/l and the highest being Titanium (Ti) at 0.93 mg/l.

Control plots irrigated with piped water recorded the highest values in Titanium (Ti), Cobalt (Co), Chromium (Cr), Mercury (Hg), Selenium (Se) and Vanadium (V). There was a reduction in most of the metal concentrations with respect to biochar treated plots irrigated with piped water, but the trend reversed in the case of biochar plots under wastewater irrigation.

Table 4.5: Heavy Metal Concentration of One Year Irrigated Soil

	Piped water				Wastewater				Statistics		
Metal	Control	Biochar	NPK	Biochar + NPK	Control	Biochar	NPK	Biochar + NPK	LSD	F pr.	WHO (2006) Std.
Be	0.11	0.11	0.11	0.10	0.10	0.11	0.11	0.10	0.031	0.825	0.2
Cd	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.003	0.569	4
Co	0.14	0.12	0.12	0.10	0.09	0.10	0.11	0.09	0.054	0.555	
Cr	0.28	0.24	0.24	0.23	0.25	0.28	0.25	0.25	0.040	0.157	100
Cu	0.05	0.07	0.04	0.04	0.04	0.05	0.06	0.04	0.028	0.129	140
Hg	0.12	0.11	0.11	0.11	0.11	0.11	0.11	0.11	0.012	0.546	7
Ni	0.06	0.06	0.06	0.11	0.05	0.06	0.06	0.06	0.060	0.516	107
Pb	0.18	0.18	0.19	0.17	0.17	0.17	0.17	0.15	0.018	0.930	84
Se	0.23	0.21	0.23	0.20	0.20	0.22	0.21	0.20	0.063	0.925	6
Ti	0.93^{a}	0.63^{b}	0.63^{b}	0.61^{b}	0.82^{a}	0.89^{a}	0.65^{b}	0.89^{a}	0.066	0.001*	0.3
V	0.41	0.38	0.40	0.36	0.36	0.41	0.39	0.32	0.101	0.600	47
Zn	0.23	0.34	0.23	0.41	0.30	0.32	0.21	0.25	0.101	0.132	

(*) Represents metal with significant difference.

4.6 Major Elements

Phosphorus was significantly different *(P < 0.01)* with the highest concentrations observed in NPK treated plots. However there was no significant difference *(P > 0.05)* in Potassium

(K), Sodium (Na), Magnesium (Mg), Calcium (Ca), Aluminium (Al) and Iron (Fe). Biochar plots had high concentration of phosphorus as compared to control plots in both waste and piped water treatments. The highest Fe was recorded in Biochar plots irrigated with wastewater, Al in NPK treated plots irrigated with piped water, Ca in Biochar plots under piped water irrigation, Mg in control plots with piped water irrigation, Na in NPK plots with wastewater, K in control plots with piped water irrigation and P recorded in NPK plots under wastewater irrigation.

Table 4.6: Major Elements of One Year Irrigated Soil

	Piped Water				Wastewater				Statistics	
Element	Control	Biochar	NPK	Biochar + NPK	Control	Biochar	NPK	Biochar + NPK	LSD	F pr.
Fe	71981	72181	84556	71750	81856	86100	82219	70044	21062.2	0.86
Al	24254	22064	24556	22648	26519	24499	22666	24274	5992.7	0.49
Ca	7.33	8.24	7.58	7.35	7.28	7.67	7.58	6.53	3.066	0.98
Mg	5.59	5.55	5.72	5.62	6.15	5.97	5.61	5.41	2.539	0.999
Na	2.89	2.73	2.67	2.45	2.98	2.8	3.54	3.5	1.097	0.409
K	1.667	1.762	1.885	1.842	1.955	1.772	1.847	1.905	0.4302	0.793
P	1.72^{d}	2.18^{bcd}	2.50^{ab}	2.44^{abc}	1.94^{cd}	2.11^{bcd}	2.91^{a}	2.58^{bc}	0.312	0.01*

(*) Represents element with significant difference

4.7 Carbon Content of Soil

The treatment applications at the end of the experiment improved the soil inherent carbon which was recorded as 0.3%. The content of soil carbon under the various treatments is presented in Figure 4.1. There was a highly significant effect *(P < 0.01)* generally across all treatments with respect to the carbon concentration. However there was no significant difference *(P > 0.05)* in biochar treated soil, similar observation was made in treatment

without biochar. The highest carbon percentages are seen in biochar treatments. Generally, wastewater irrigated plots recorded higher carbon concentration than piped water irrigated plots. Non biochar treated plot had closely similar values in terms of percentage carbon concentration.

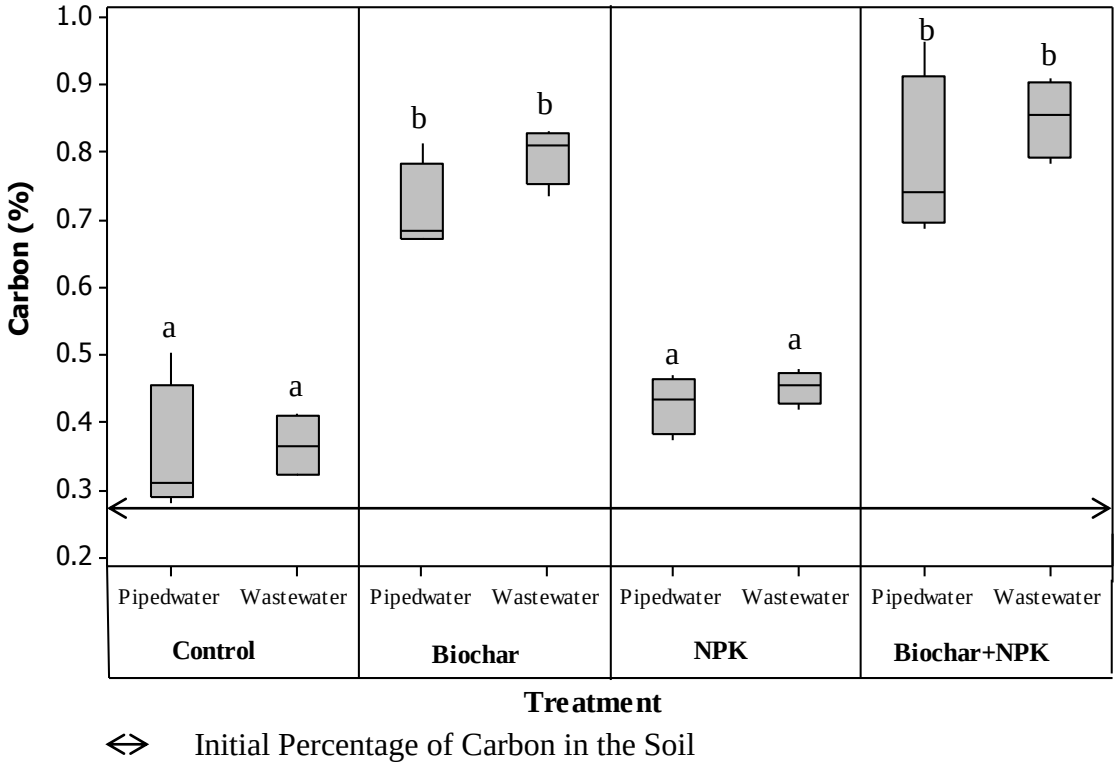


Figure 4.1: Box Plot of Percentage Carbon of One Year irrigated Soil.

4.8 Nitrogen Content

The percentage concentration of nitrogen was not significantly different *(P > 0.05)* across the treatments. Wastewater treatments improved nitrogen content compared to piped water irrigation. The highest nitrogen was recorded in treatment involving Biochar+NPK under wastewater irrigation. Irrigation with piped water least improved the soil nitrogen, which was however higher than the initial nitrogen of the soil before the start of the experiment.

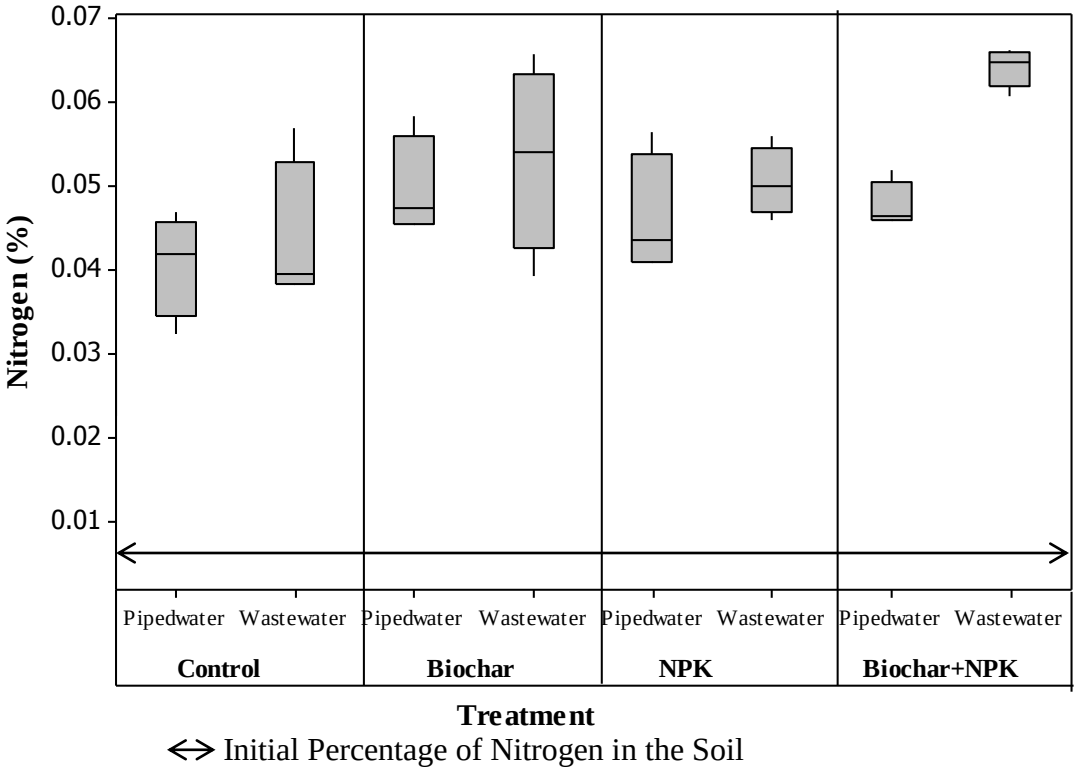


Figure 4.2: Box Plot of Percentage Nitrogen of One Year irrigated Soil.

4.9 Hot Water Extractable Soil Carbon.

Hot water extractable carbon per kilogram of soil was affected significantly *(P < 0.01)* by the treatments. Treatments involving the use of biochar recorded the highest levels of hot water extractable carbon. Combined treatment of biochar and NPK irrigated with wastewater had the highest record of hot water extractable carbon with the value of 223.4 $mgkg^{-1}$ whiles the least value was recorded in the control plots irrigated with piped water with value 166.5 $mgkg^{-1}$.

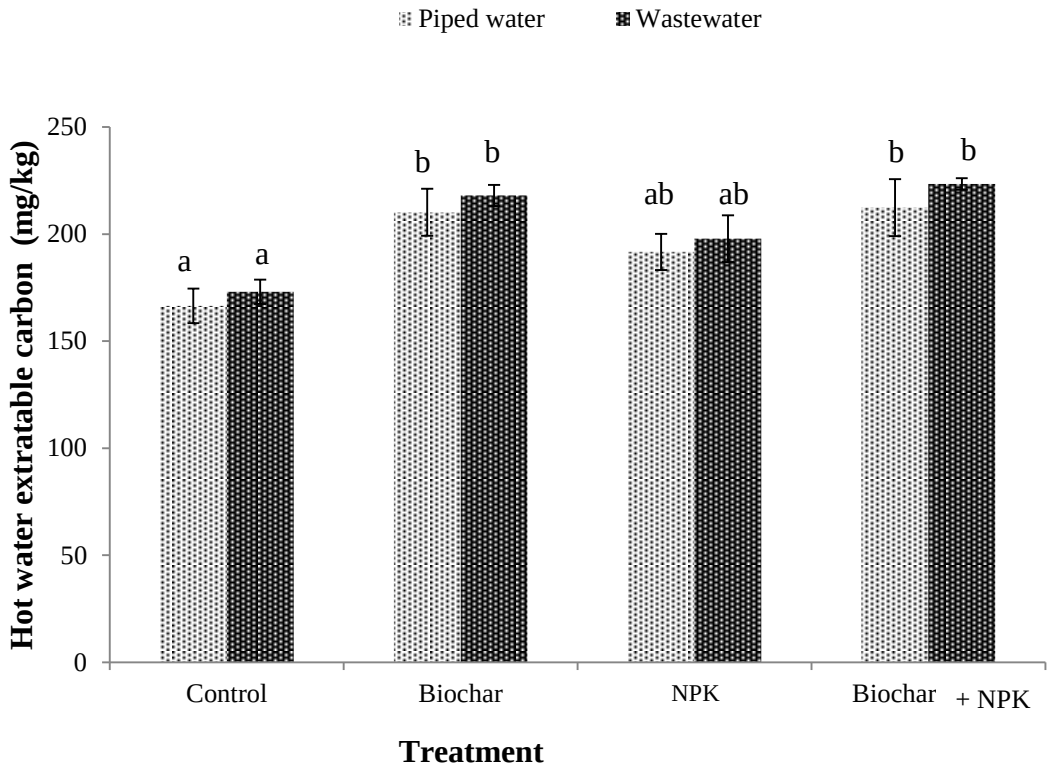


Figure 4.3: **Hot Water Extractable Carbon per Soil Total Carbon.**

4.10 Microbial Biomass Carbon

The amount of microbial biomass carbon to soil total carbon was observed to be high in biochar treated plots although there was no significant difference *(P > 0.05)* among the various treatments. Averagely, wastewater irrigation gave higher amount of microbial biomass carbon per total carbon content compared to piped water irrigation. The highest was recorded in combined biochar and NPK under wastewater treatment as 2.24 µg Cmik/g soil followed by biochar + NPK under piped water irrigation at 1.97 µg Cmic/g soil and 1.92 µg Cmic/g soil under wastewater. The lowest concentration was observed in piped water control plots (0.95 µg Cmic/g soil).

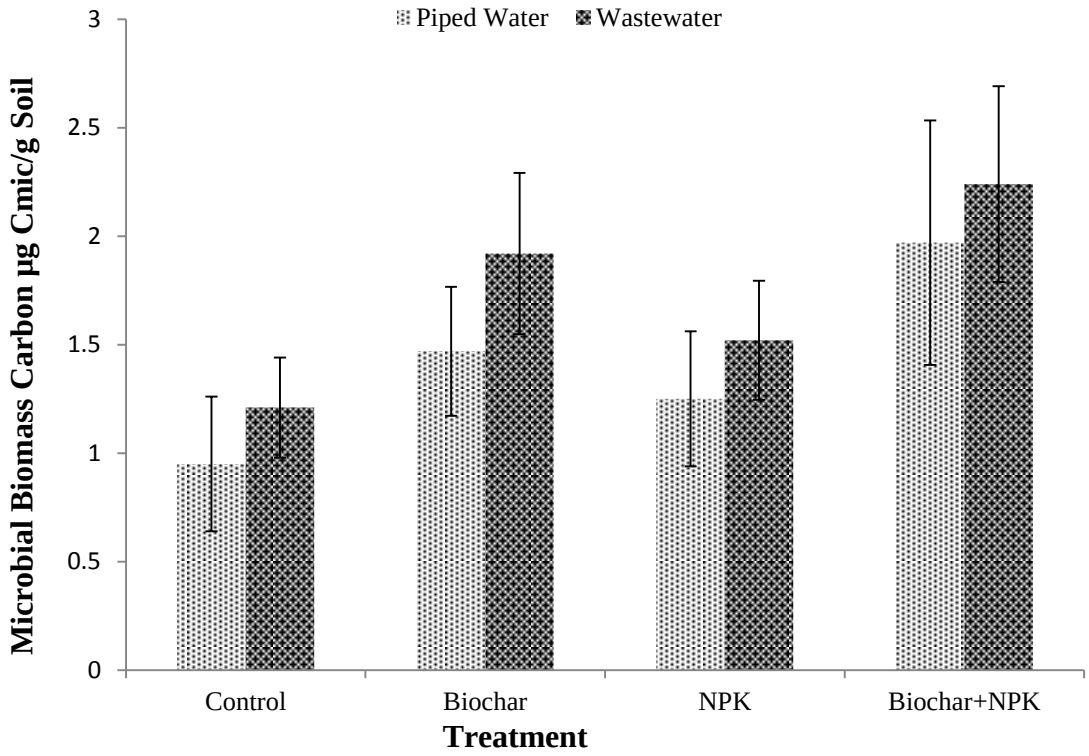


Figure 4.4: Microbial Biomass Carbon per Soil Total Carbon (µg Cmic/g soil).

4.11 Substrate Induced Respiration

Microbial respiration to alanine, citric acid, glucose and water (basal) as induced substrates are presented in Figure 4.5. Significant ($P<0.05$) observation was generally made in glucose and basal respiration. However, the treatment had no significant *($P > 0.05$)* effect on both alanine and citric acid respiration. As shown in Figure 4.5, microbial respiration among all treatments was highest in citric acid, followed by glucose, alanine with glucose having the least respiration rate. The rate of microbial respiration to the various substrates used was affected by water quality. Respiration was seen to be more effective in piped water treated plots as compared to wastewater irrigated plots among all the treatments.

Statistically, significant impact was observed in the response of the soil to basal respiration. The highest mean 0.778 µg $C\text{-}CO_2.g^{-1}.h^{-1}$ was recorded in NPK treated plots

with piped water irrigation followed by NPK+Biochar combination piped water irrigation (0.709). Amongst the wastewater irrigated plots, NPK treatment recorded the highest value of 0.551 µg $C\text{-}CO_2.g^{-1}.h^{-1}$ which was lower than the least value recorded for piped water irrigation plots treatments.

The highest mean rates of respirations with alanine were revealed to be greater in biochar under both water quality treatments but slightly higher in wastewater irrigated plots. This was followed by NPK and control treatment with domestic piped water irrigation with the values of 0.97 and 0.96 µg $C\text{-}CO_2.g^{-1}.h^{-1}$ respectively. Alanine respiration was least observed in wastewater irrigated with combined treatment of biochar and NPK at 0.787 µg $C\text{-}CO_2.g^{-1}.h^{-1}$.

Microbial respiration to glucose in the biochar treated soil was revealed to be more effective regardless of the water quality. Piped water biochar had the highest and wastewater recorded the second highest value of 1.817 and 1.727 µg $C\text{-}CO_2.g^{-1}.h^{-1}$ respectively. Similarly, control plots under both water qualities respired effectively to glucose than treatments with NPK incorporation.

As seen in Figure 4.5, respirational response to citric acid was high as compared to the other substrates used in the experiment. Although there was no significant difference *($P > 0.05$)* among the various treatments, NPK treated plots under piped water and wastewater irrigation had the highest respective values of 2.63 and 2.47 µg $C\text{-}CO_2.g^{-1}.h^{-1}$. Also, biochar-NPK combination under both water qualities respired more actively than only biochar treated plots.

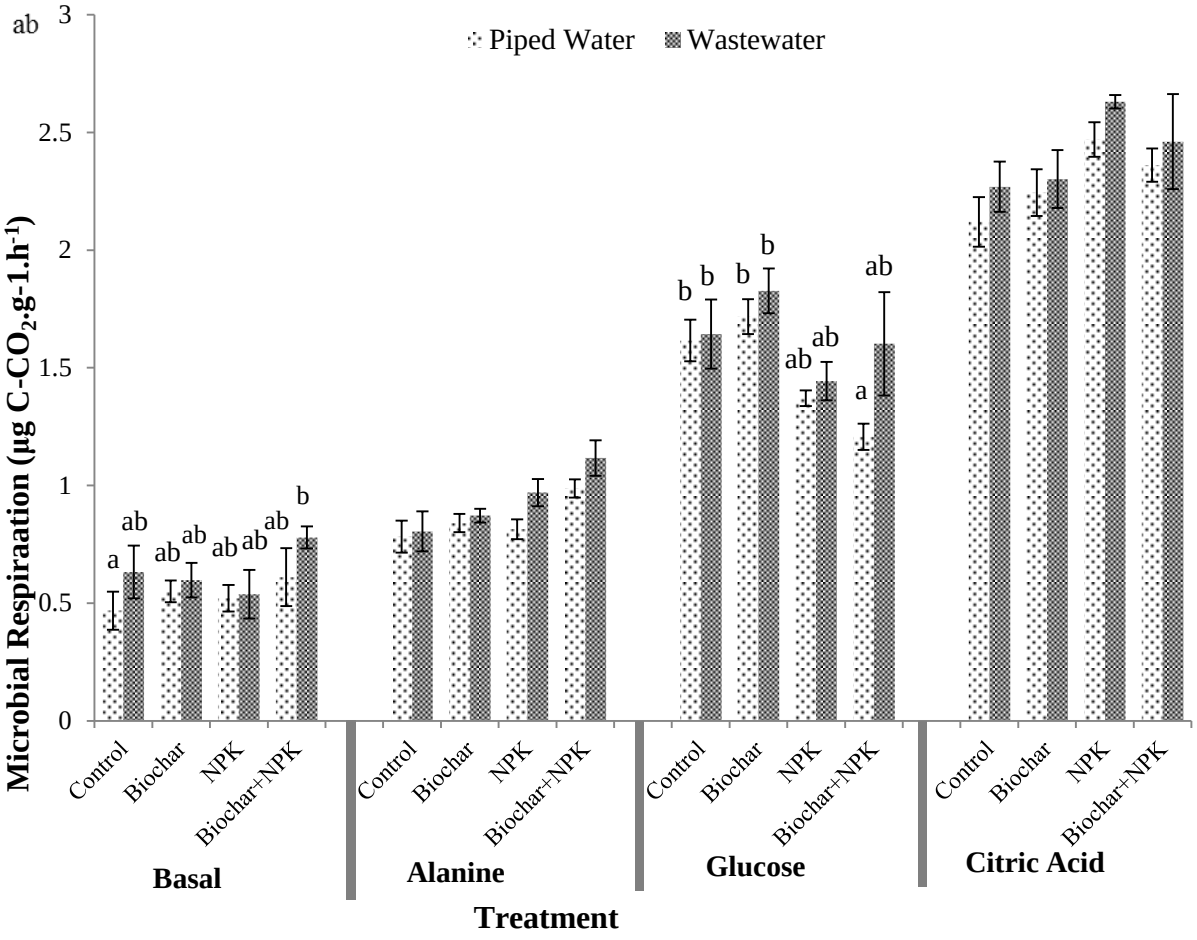


Figure 4.5: Substrate Induced Respiration (µg C-CO2.g^{-1}.h^{-1}).

4.12 Enzyme Activity in the Soil

The eleven soil enzymes studied in the experiment are listed in Table 4.7. As shown in the Table 4.7 the treatment had highly significant impact *(P < 0.001)* on ten out of the eleven enzymes activities with the exception of arginine-aminopeptidase which was significant at *(P < 0.01)*. Generally observed from the Table 4.7, wastewater irrigated plots had higher activities in all the studied enzymes across all treatments. The highest average response of enzymes activities was observed in the arginine-aminopeptidase (695.38 nmol product g^{-1} dry soil) whiles the least response was observed in β-xylosidase (14.58 nmol product g^{-1} dry soil).

Table 4.7: Enzymatic Activities of One Year Irrigated Soil

		Piped Water + Control	Wastewater + Control	Piped Water + Biochar	Wastewater + Biochar	Piped Water + NPK	Wastewater + NPK	Piped Water + Biochar+NPK	Wastewater + Biochar+NPK	Lsd	F pro
		nmol product g-1 dry soil									
De		15^{c}	16.5^{ab}	14.58^{ab}	18.27^{a}	14.18^{ab}	15.5^{ab}	12.04^{a}	18.21^{b}	3.623	<.001*
α-I		17^{abc}	23.9^{ab}	15.1^{bc}	15^{bc}	6.4^{c}	11.7^{c}	8.2^{c}	37^{a}	13.07	<.001*
β-I		19^{b}	25.5^{a}	13.76^{c}	12.11^{cd}	8.04^{e}	10.11^{de}	8.13^{e}	19.95^{b}	3.51	<.001*
N-	idase	6.7^{c}	10.2^{c}	60.9^{b}	134^{a}	16^{c}	119.6^{a}	14.5^{c}	8.9^{c}	37.77	<.001*
Su		7^{c}	9^{c}	564^{b}	1143^{a}	273^{bc}	1219^{a}	280^{bc}	295^{bc}	462.4	<.001*
Ph		15.95^{c}	17.39^{c}	34.32^{b}	39.05^{b}	1.63^{d}	38.73^{b}	5.68^{d}	114.97^{a}	3.394	<001*
β-I	e	235.6^{b}	254.1^{b}	132.8^{c}	101.6^{c}	2.1^{d}	95.8^{c}	3.4^{d}	1180.3^{a}	40.63	<.001*
β-I		1.7^{c}	9.3^{c}	130.2^{a}	144.8^{a}	29.4^{c}	65.9^{b}	4.5^{c}	33.8^{bc}	35.76	<.001*
Le	e	3.4^{e}	6.3^{e}	140.5^{b}	227.3^{a}	24.6^{de}	208.8^{a}	38.7^{d}	92.5^{c}	22.48	<.001*
Ty	e	44.5^{cd}	179.2^{a}	84.9^{bc}	60.6^{bc}	13.4^{d}	68.8^{bc}	47.1^{cd}	108.8^{b}	50.91	<.001*
Ar	e	39^{cd}	323^{cd}	427^{cd}	1243^{a}	497^{cd}	1216^{ab}	707^{bc}	1111^{ab}	678.3	<.007*

(*) Represents enzymes with significant difference

4.12.1 Dehydrogenase

Potential dehydrogenase activities was seen to be more effective in biochar which had 18.27 nmol product g^{-1} dry soil followed by biochar + NPK of 18.21nmol product g^{-1} dry soil and control of 16.5 nmol product g^{-1} dry soil all under wastewater irrigation. However the least dehydrogenase activities was recorded in same treatments but under piped water irrigation, biochar 14.18 nmol product g^{-1} dry soil and biochar + NPK 12.04 nmol product g^{-1} dry soil. The average dehydrogenase activity across the treatment was observed to be 15 nmol product g^{-1} dry soil.

4.12.2 Alpha-Glucosidase

The mean values for α-glucosidase under wastewater and piped water irrigations were 21.9 and 11.7 nmol product g^{-1} dry soil respectively. The activity was seen to be more pronounced in biochar + NPK treatment with wastewater quality at 37 nmol product g^{-1} dry soil. The control wastewater and piped water plots followed with 23.9 and 17 nmol product g^{-1} dry soil respectively. Biochar treatments recorded low α-glycosidase activity as compared to non biochar plots but the situation was different in NPK treatments.

4.12.3 Beta -Xylosidase

Wastewater control and biochar + NPK recorded the highest values of 25.58 and 19.95 nmol product g^{-1} dry soil for β-Xylosidase activities in the soil. NPK under piped water irrigation had the least activity of 8.04 nmol product g^{-1} dry soil which is far below the mean β-Xylosidase activities of 14.56 nmol product g^{-1} dry soil across the various treatments.

4.12.4 N-acetylglucosaminidase

N-acetylglucosaminidase was more active in biochar amended plots in both water qualities. Biochar in the wastewater category had the greatest influence having 134 nmol product g^{-1} dry soil of N-acetylglucosaminidase activities followed by NPK at 119 nmol product g^{-1} dry soil. Biochar in the piped water category also recorded the highest value of 60.9 nmol product g^{-1} dry soil and the third highest across all treatments. Among all the treatments, the control under piped water irrigation had the least impact 6.7 nmol product g^{-1} dry soil.

4.12.5 Sulfatesterase

Averagely, sulfatesterase activities were seen to be the second most active soil enzyme revealed in the study Table 4.7. Wastewater-NPK, wastewater – biochar, piped water - biochar had the respective highest values of 1219, 1143 and 564 nmol product g^{-1} dry soil. Control treatment under both water qualities was seen to have low readings of 7 and 9 nmol product g^{-1} dry soil respectively.

4.12.6 Phosphatase

Both biochar incorporation and wastewater irrigation had a significant impact on phosphatase activities in the soil. The effect was more pronounced in biochar + NPK combination with wastewater irrigation (114.98 nmol product g^{-1} dry soil). Wastewater biochar, wastewater NPK and piped water biochar recorded 39.05, 38.73 and 34.38 7 nmol product g^{-1} dry soil respectively without significant difference among them. NPK treatment irrigated with piped water as seen from Table 4.7 performed poorly, recording 1.67 nmol product g^{-1} dry soil with regards to soil phosphatase activities.

4.12.7 Beta-glucopyranosidase

Aside biochar + NPK under wastewater irrigation that recorded the highest value of 1180 nmol product g^{-1} dry soil, the control treatments were more improved in terms of β-glucopyranosidase activities in the soil. Piped water control plots had 235.6 nmol product g^{-1} dry soil as compared to its biochar treatment of 132.8 nmol product g^{-1} dry soil and wastewater control recorded 253.1 nmol product g^{-1} dry soil compared to 101.6 nmol product g^{-1} dry soil of biochar. Biochar + NPK and NPK with piped water irrigation attained the least values of 2.4 and 2.1 nmol product g^{-1} dry soil respectively.

4.12.8 Beta-cellubiosidase

B-cellubiosidase was effectively influenced by biochar incorporation. Biochar under both water qualities accomplished the highest β-cellubiosidase activities of 144.8 and 130.2 respectively with no significant difference between the two. NPK plots irrigated with wastewater and combined application of NPK and biochar follow the highest values with 65.9 and 33.8 nmol product g^{-1} dry soil respectively. β-cellubiosidase activity was least in piped water control treatment at 1.7 nmol product g^{-1} dry soil.

4.12.9 Leucine aminopeptidase

Biochar under wastewater management exhibited the greatest activity of leucine aminopeptidase of 227.3 nmol product g^{-1} dry soil. NPK plots irrigated with wastewater gave the second highest values of 208.8 nmol product g^{-1} dry soil and was followed by biochar plots irrigated with piped water with the value of 140.5 nmol product g^{-1} dry soil.

The control treatments in both water managements recorded the least activities of 6.3 and 3.4 nmol product g^{-1} dry soil.

4.12.10 Tyrosine aminopeptidase

The activities of tyrosine aminopeptidase were high in control and biochar + NPK thus 179.2 and 108.8 4 nmol product g^{-1} dry soil with wastewater irrigation. These were followed by NPK plots irrigated with wastewater and biochar plots irrigated piped water with the values of 84.9 and 68.8 nmol product g^{-1} dry soil respectively. Control and NPK plots under wastewater irrigation were least in tyrosine aminopeptidase performance among the various treatments 44.5 and 13.4 nmol product g^{-1} dry soil.

4.12.11 Arginine-aminopeptidase

Arginine-aminopeptidase recorded the highest mean of enzymes activity among all the various enzymes analyzed in the experiment of 695.38 4 nmol product g^{-1} dry soil. Biochar and NPK amended plots which received wastewater irrigation had the most intense effect on arginine-aminopeptidase recording 1243 and 1216 nmol product g^{-1} dry soil respectively. Biochar + NPK combination under wastewater and piped water managements had the values of 1111 and 707 nmol product g^{-1} dry soil respectively. Arginine aminopeptidase activities were seen to be less effective in the control plots in both wastewater and piped water managements.

CHAPTER FIVE

DISCUSSION

5.1 Microbial Contamination of Irrigation Water

Health risk posed by pathogens is among the main disadvantages of using untreated wastewater especially for irrigation on vegetable farms. The quality of water is based on the amount of indicator micro-organisms found in the water (Okafo *et al.,* 2003). The water quality assessment in this study focused on feacal coliform concentration, *E. coli* loads, presence of *Salmonella* spp., Shiga toxin producing *Escherichia coli* and helminths eggs. The results from the study indicate that wastewater used for irrigation in the study which is also used for vegetable farming at Zagyuri is contaminated with enteric bacteria high above WHO (2006) recommended value of log 3 CFU/100 ml for feacal coliform, 30 for *E. coli*, zero counts for *Salmonella* spp., Shiga toxin producing *E. coli* and helminths eggs. The dissolution of human excreta from the communities probably contributed to the levels of contaminations in the wastewater. These results correspond to earlier finding by Amoah *et al*. (2008) with similar reports from Tamale and other major cities in Ghana. Influence of seasonal variation (dry and wet) caused about 6.3 % reduction in feacal coliform load from log 7.38 CFU/100 ml in dry season to 6.50 CFU/100 ml in the wet season. Rainfall inputs can dilute surface water and decrease the indicator concentration

(McLain and Williams, 2008). Pachepsky and Shelton (2011) reported of increased pathogen loads in the wet seasons as a result of surface runoff into waterways. Although *E. coli* counts were above the WHO (2006) standards, generally its contamination level was appreciably low considering the source of the irrigation water. These *E. coli* count could possibly be due to the phenomenon of pathogen death through natural UV (Tyrrel *et al*., 2006). The presence of Shiga toxin producing *E. coli* (STEC) in wastewater detected in both seasons can be linked to studies conducted by Saba *et al.* (2015), which investigated the prevalence of *Shiga Toxin producing E. coli* in Tamale Metropolis.The author reported about 44 % and 42 % of STEC positive incidents in Tamale teaching hospital and cattle faeces.

Concentration of helminth in the domestic piped water used for irrigation and wastewater exceeded the WHO recommended standard of less than 1 egg L^{-1} for unrestricted irrigation (WHO, 2006). *Ascaris lumbricoides, Schistosoma* spp and *Strongyloides stercoralis* were the main eggs detected in the irrigation water. About 75 % of identified eggs were *Ascaris lumbricoides* confirming the studies of Abagale *et al.* (2013) who investigated the types and seasonal diversity of helminth eggs in wastewater used for peri-urban vegetable crop production in tamale metropolis and recorded predominant number of *Ascaris lumbricoides*.

Pipe water in the study recorded low levels of contaminations although they were above WHO standards as seen from Table 4.1. This result contradicts the findings of Amoah *et al*. (2008), who reported less or no microbial count in piped water in Kumasi and Dzowulu in Ghana. The piped water in the study was treated as according to farmer's practice which involves the use of watering cans. The practice requires temporal storage of the water in tanks usually unprotected open storage tanks before use for irrigation. WHO (2006) stated that one of the factors influencing the microbial quality of farm produce is the type of irrigation method used. Other studies by Keraita *et al.* (2008) confirmed that irrigation by sprinkling especially by the use of watering cans increases microbial contamination compared to drip irrigation.

5.2 Microbial Quality of Vegetable (*Amaranthus* spp.*)*

This study was conducted at the farm level to compare the pathogenic risk of *Amaranthus* spp under wastewater and piped water irrigation application on varied soil treatments in two major seasons (dry and wet). Vegetables under wastewater irrigation were significantly higher in microbial contaminations as compared to piped water applied plots (Table 4.2). The major source of coliform contamination according to Okafo *et al.* (2003) when vegetables are grown is probably the irrigation water. This high pathogen loads on amaranthus has earlier been reported by Cobbina *et al.* (2013) who recorded high levels of pathogen above International Commission on Microbiological Specifications for foods

(ICMSF) recommended level. The result revealed that microbial concentration on amaranth is dependent on the season (Table 4.2). The significant reduction in pathogen concentration as well as zero record of *Salmonella* spp. during the wet season is attributed to the excessive rainfall that may have washed the pathogens off the vegetables surfaces. Vegetables with larger leaf surface area are more exposed to microbial contamination from irrigation water especially when overhead irrigation techniques are used as reported by Amoah *et al*. (2005). This reason could have contributed to the elevated loads of pathogens and the presence of *Salmonella* spp. on the amaranth grown on Biochar + NPK and NPK soil amended plots which had higher above ground biomass compared to other treatments as a result of improved soil nutrients.

E. coli O157 has been recognized as the leading cause of human food borne diseases throughout the world with deadly complications such as hemolytic uremic syndrome (WHO, 2006). It has been recognized that vegetables can be an important vehicles for transmitting *E. coli* to humans (Khatib and Khawaja, 2015). Although *E. coli* was detected on the vegetables in this study, none of the vegetable samples from all the treatments were detected positive for *E. coli* O157 in the two seasons. This result contradicts that of Kabiru *et al.* (2015) who recorded STEC positive on lettuce irrigated with abattoir effluent.

5.3 Microbial Quality of Soil

The potentials of pathogenic contaminations to soil with regards to wastewater application is of concern, due to their ability to cause high risk of enteric disease outbreaks to farmers and consumers (Mara *et al.*, 2007). The current study focused on the combined effect of

irrigation water quality and biochar on soil pathogen concentration in dry and wet season of amaranth production. The slightly acidic nature of the soil observed in this study which ranged between pH 4.35 to 5.61 could have promoted the survival of the bacteria in the soil. The concentrations of faecal coliform and *E. coli* in the soil increased with the application of wastewater as compared to pipe irrigated plots. Increase in the soil organic matter content as a result of wastewater irrigation has a positive impact also on soil retention ability of irrigation water borne pathogens as reported by Landa–Cansigno *et al.* (2013) who made similar observations. This is due to the high affinity of micro-organism to the organic domain of soil (La Para *et al*., 2011). As indicated on Table 4.3, biochar treated soil assumed the highest microbial concentrations. This has been reported in earlier investigations that the soil microbial activity has increased with biochar additions (Steiner *et al*., 2008; Kolb *et al.,* 2009). This confirms the hypothesis that biochar can provide refuge for micro-organisms due to its porous nature (Peitikainen *et al.,* 2000) and as well feed them with sorbed nutrients from the environments (Atkinson *et al.,* 2010). High moisture content detected in the wet season ranging between 22.1 vmc and 47.5 vmc as compared to 10.1 to 17.9 in the dry season might have provided conducive environment for microbial growth and proliferation of microbial numbers in the wet season. This effect might have also accounted for the comparatively higher pathogen loads in biochar+NPK treated plot under both water qualities due to its organic matter content which helps to maintain soil moisture for microbial growth. No *Salmonella* spp. was detectable in the piped water irrigated fields in both seasons but was present in all wastewater plots in both seasons. Amoah (2005) also reported of the presence of *Salmolalla* in wastewater irrigated

plots and not plots irrigated with piped water. Armon *et al.* (2002) stated that microbial quality of irrigated soil depends on the source and type of the irrigation water. Although *E. coli* was detected in all the soil samples studied, only biochar+NPK combination and NPK in the wet season recorded STEC positive. This could be attributed to the availability of suitable conditions like moisture, pH and reduced sun intensity and temperature as a result of adequate above ground biomass.

5.4 Helminths Eggs Load in the Soil and on Vegetables

The soils used in this study were all found to be polluted with helminth eggs regardless of the amendments applied or the quality of irrigation water. However, the prevalence was significantly higher in wastewater irrigated soil compared to piped water (Table 4.4). A study by Amoah (2014) reported of similar findings of helminths contamination in urban vegetable production in Kumasi, Ghana. The results of the study show that the farm workers and their families have a high risk of helminth infection from contact with the soil due to their usual habit of working bare footed and unprotected (WHO, 2006; Blumenthal *et al*., 2000). The risk of exposure to helminths in the soil tends to be severe in the wet season compared to the dry season. Biochar and NPK treated soils provide good conditions for the survival of eggs especially under wastewater irrigation, hence recording the highest mean count of helminth eggs in the study. *Ascaris lumbricoides, Strongyloides stercoralis, Trichuris trichura, Schistosoma mansoni* were the identified helminths in the soil confirming the importance of the parasite as a health hazard in wastewater used for irrigation. *Ascaris lumbricoides* was the most predominant species with about 94 % of the

total population probably due to its high persistence and survival time in the soil. Abagale *et al*. (2013) and Amoah *et al.* (2006) reported of the prevalence of *Ascaris* in irrigation water and vegetables in urban agriculture in Ghana. *Ascaris* according to WHO (2001) occurs with greatest frequency in tropical and sub-tropical region where inadequate sanitation prevails especially in the case of using wastewater for agricultural activities.

Unlike the soil, amaranthus vegetable in this study recorded less helminth eggs count. This can be attributed to the height of amaranth plant which aids to reduce water splashes from the soil. Amaranthus in the dry season had few count of helminths averaging 2 egg/g, slightly above the WHO standard of < 1egg/g of vegetables. Amaranthus is eaten cooked and therefore does not pose risk considering the numbers counted, although it has the possibility of causing cross contamination in the kitchen. In the wet seasons where there was rainfall, no helminths eggs were detected on the amaranthus across all the treatments hence making it safer for consumption. The zero helminth record of amaranth during the wet season may be due to the excessive washing of Amaranthus leaves by rain water.

5.5 Microbial Respiration

The rate of CO_2 emission by micro-organisms in soil is essential as it plays a role in global carbon cycling as well as other nutrient cycles (Anderson, 1982). Laboratory induced basal, amino acid, sugar and carboxylic based substrates were used in the study. Specifically, the degree of release of CO_2 through respiration in the soil was evaluated by SIR method using water, alanine, and glucose and citric acid substrates as carbon of source. The magnitude of CO_2 emission by various treatments responded to the addition of

carbon nutrient sources. Increased microbial respiratory activities were observed in piped water irrigated plots compared to wastewater plots despite high microbial biomass in the wastewater treated plots. A similar report was made by Marschner *et al.* (2003) who stated that, the low microbial activities in the wastewater may have been limited by the availability of easily degradable substrate.

The result revealed different level of CO_2 emission in increasing order of basal, alanine, glucose and citric acid respectively. As reported by several researchers (Abubakari *et al*., 2012; Adu, 1995; Adongo *et al.,* 2015; Buri *et al*., 2006)) and has also been confirmed in the study, the soil in the Savanna zone in the northern part of Ghana is acidic. Strobel (2001) did record exceptional amount of citric acid in the soil, many of which were associated with low pH soils. Degradation of organic acids such as citric acid in the soil has been proven to be rapid (Jones *et al*., 2003 and Fujil *et al.,* 2012), and can contribute to about 20 to 70 % of respirable carbon. This acidic condition thereby describes the native micro-organism in the soil to be acid loving and hence respires profoundly to citric acid substrates than the others. As observed from Figure 4.5 the highest respiratory activity occurred in NPK treated soil at 2.63 and 2.47 μg $C\text{-}CO_2.g^{-1}.h^{-1}$ in piped and wastewater respectively for citric acid. Biochar + NPK combination in piped and wastewater followed the highest values recorded in the study corresponding to increase in the soil pH. This might be due to antagonistic reaction between the NPK and biochar with nutrient variation. Biochar treated plots although has higher pH than the control respired more effectively to citric acid. This may be due to the additional source of carbon in the biochar treated soil unlike the control. Fujil *et al.,* (2012) similarly observed CO_2 production in grassland and

hardwood forest soils of about 91 % and 82 % respectively above controls upon the addition citric acid.

Biochar amended soil sample was revealed to have high rate of microbial respiration when induced with glucose in both water qualities. Similar report was made by Jeffrey *et al.* (2011), who studied the effect of biochar on soil respiration and mentioned of an increase in CO_2 production with increased biochar additions. Biochar provides significant amounts of labile C that is readily available to soil micro-organisms for use as an energy source therefore giving the high values of CO_2 evolution. The respiratory activity in the control was not significantly different although it was less as compared with biochar soil considering the application rate. The rate of biochar application was also commented on by Jeffrey *et al.* (2011), who recorded no significant difference from the lowest rate of application and the control. The result indicates that biochar+NPK combination and NPK plots had reduced emission of carbon dioxide when induced with glucose. The use of inorganic fertilizers has been found to cause a reduction in microbial biomass C (Wang *et al.,* 2008). Similarly Dinesh *et al.* (2012) also reported of reductions in soil respiration with inorganic fertilizer to the soil. Biochar in conjunction with nutrient addition by fertilizer may reduce microbial reproduction in soil (Steiner *et al.,* 2009) and this also agreed with the biochar+NPK soil treatments in the current study.

Alanine induced microbial respiration although comparatively low to citric acid and glucose respiration emitted more CO_2 than the basal respiration. Carbon mineralization of induced alanine was detected to be more active in NPK+ biochar combined plots more especially in the piped water compared to wastewater irrigation at 1.117 and 0.987 µg C-

$CO_2.g^{-1}.h^{-1}$ respectively. It can be mentioned that, the high microbial population, C: N ratio, moisture content and slightly low pH stimulated the levels of amino acids (alanine) mineralization in the biochar+NPK combined soil. Although not significantly different, biochar amended soil samples were higher than that of the unamended soil sample. Laboratory basal microbial respirations assumed a different trend with an elevated respirational rate in NPK and NPK+biochar under piped water regime compared to other treatments. This could be due to the inorganic N being supplied by the addition of NPK which may have enhanced the increased CO_2 emission in the soil and provision of C substrate by biochar that stimulates the activities of microbial community. The inorganic fertilizer associated with NPK amendments might have additionally improved the activities of beneficial bacteria involved in carbon and nitrogen cycling. Wastewater application as a treatment on the other hand recorded the least CO_2 emission due to slow priming effect of the wastewater as well as its SOM content. Biochar amendment improved the rate of microbial respiration which might be due to additional carbon supply in the biochar samples.

5.6 Enzyme Activity of Soil

In this study, dehydrogenase, ß-glucosidase, ß-xylosidase, α-glucosidase, β-D-cellubiosidase for carbon cycling, for nitrogen L-leucine aminopeptidase, L-Leucine aminotyrosinase and L-Leucine aminoarginase while C:N cycling was tested by N-acetyl-glucosaminidase. Phosphatase and sulfatase were used to assess phosphorus and sulphur cycling respectively. The results revealed significant variation in the various group of

enzymes under all the treatments. The study demonstrated that wastewater managed soil exhibited greater exo-enzyme activities than the piped water: agreeing with Melero *et al*. (2006) and Benitez *et al.* (2006). Evidently, dehydrogenase activity, an enzyme that solitary functions inside the cell was seen to be enhanced in the wastewater irrigated plot compared to piped water plots. The accumulated dissolved organic matter via wastewater irrigation (Scholl *et al.,* 1990) might have enhanced the proliferation of bacteria hence the increased level of dehydrogenase in wastewater irrigated soil. Yuan and Yue (2012), made similar observations and stated that the higher the organic matter, the higher the microbial biomass, and hence the more dehydrogenase activities in the soil. The improved performance of the dehydrogenase activities in biochar treated soil can be attributed to the porous nature of the biochar that supports the growth and the survival of micro-organisms in the soil (Steiner *et al*., 2010). The inorganic NPK fertilizer had less effect on dehydrogenase activity causing insignificant reduction in the level of dehydrogenase activities in the soil as reported by Romero *et al*. (2010).

Among the enzymes responsible for C cycling, β-glucosidase was the most common and predominant more especially in biochar+NPK [1180.3 nmol/ (gTR*h)] under wastewater treatments considering its organic matter content. The β-glucosidase enzyme plays a major role in the degradation of soil organic matter and plant residues. It catalyzes the hydrolysis of β-d-glucopyranosidase in the final, rate-limiting step in the degradation of cellulose, the most abundant polysaccharide on the earth, providing simple sugars for the soil microbial population (Acosta-Martínez *et al.,* 2000; Green *et al.,* 2007). Other studies have also proved the high stability properties of β-glucosidase (Renella *et al*., 2002). Interestingly

Biochar+NPK amendment with piped water as well as NPK at both water application recorded the least activities of 3.4, 95.8 and 2.1 respectively even far below the control plots which had 235.6 and 254.1 nmol/(gTR*h) for piped and wastewater. B-glucosidase as reported by several scientist is an enzyme that is very sensitive to changes in pH and soil management (Acosta-Martı´nez and Tabatabai, 2000; Madejon *et al*., 2001). Although biochar caused reduction in β-glucosidase activities in both water situations, the difference was not significant agreeing with the findings of Bailey *et al.* (2010). Biochar effect on β-glucosidase on this study also conforms to the findings of Jin (2010) who showed increased in β-glucosidase activity for C cycling.

The activity of α-glucosidase was significantly low when compared with that of β-glucosidase although they followed similar trend in their response to treatment. Alpha glucosidase cleaves starch, mostly maltose into simple sugar. These functions according to previous studies can be achieved by several mechanisms. This describes the lower level of activity of α-glucosidase observed in this study. Like β-glucosidase, the highest potential α-glucosidase activity was observed more profoundly in the wastewater treated plots. The highest potential activity was detected at the combined treatment of biochar, NPK and wastewater at 37 nmol/(gTR*h). This can be accounted for by the presence of high microbial biomass and sufficient soil organic carbon content. NPK under both water applications recording the least performance like it was in the β-glucosidase correlating with reduced levels of pH. Biochar inputting has little effect on soil α-glucosidase. Thies and Rilling (2009), related the slight reduction of biochar to α-glucosidase activity to masking of enzymes, rather than sorption of substrates. Decomposition of cellulose

fragments to release glucose as well as soluble carbons is related to β-cellobiosidase activity. In this study, the activity of these enzymes was realized to be prominent in biochar amended soil both under waste and piped water irrigation at the level of 144.8 and 130.2 nmol/(gTR*h) respectively as presented in table (4.7). This finding is in contrast to a report by Bailey *et al.* (2010) and Jin (2010). Although during pyrolysis hemicellulose, lignin and cellulose are thermally transformed, causing the release of gases and volatiles, leaving behind a carbon-rich material (McLaughlin *et al.*, 2009). Allison *et al.* (2014), stated the variations in responsiveness of β-cellobiosidase to their target substrates (cellulose). Cellulose degradation is not exclusively related to extra-and intracellular cellulase activity but also related to the activity of other hydrolytic as well as oxidative enzymes (Pandiyan *et al*., 2002). This might be the reason for the unpattern treatment effect on β-cellobiosidase in the study.

Like other C cycling enzymes, β-xylosidase activities were more elevated in biochar+NPK soil treated wastewater which was about 15 folds compared to the control. Elzobair (2013). made similar observation where manure and biochar + manure increased the potential activity of β-xylosidase 4.7-fold and 5.6-fold, respectively, related the findings to an increased microbial abundance and provision of optimal pH. Similarly, biochar in this study had significant influence on the activity of β-xylosidase in both water qualities. The increase is likely related to the sorption of substrates or products presumably due to biochar porosity and reactive surface area corresponding to the finding of Jindo *et al.* (2012). The impact of NPK involving inorganic fertilizer caused about 3 folds reduction in the activity of β-xylosidase more especially in the piped water plots agreeing with Miao-

zhen *et al.* (2015). Correlating these results with the soil pH and SOM confirms that soil enzymes are mainly a function of the amount of substrate available (Kandeler *et al*., 1999) and of the microbial biomass present to potentially synthesize at the suitable pH for maximum operation.

Phosphatase, an enzyme responsible for the breakdown of phosphorus to labile phosphate (German *et al*., 2011) was noted to be more active in biochar-NPK combination under wastewater treatment. The significantly low activity of phosphates in the corresponding piped water treatment (Table 4.7) indicates the improved organic matter content of the wastewater and its useful contribution to soil enzymatic activities. The increased performance of phosphatase activity with combined application of wastewater, biochar and inorganic fertilizer (NPK) may be attributed to the increasing population of micro-organisms due to increased availability of substrate through organic matter by the wastewater which is in agreement with a report made by Rani *et al.* (2014). NPK treatment had contradicting actions in the two water qualities. Phosphatase in the wastewater treated plots obeyed the resource limitation model, thus the activity was positively affected by the presence of substrate it can degrade which can possibly be found in the wastewater. Alternatively, the presence of inorganic fertilizer in the NPK treatments which have been reported to increase phosphatase activities (Kiran *et al.,* 2005; Rani *et al*., 2014) rather caused less activity in the piped water applied plots. The availability of phosphorus in the soil regulated the activities of phosphates conforming to the substrate stimulation model of enzyme activities.

N-acetylglucosaminidase (NAG), an enzyme responsible for the release of N-acetyl-D-Glucosamine from the terminal non-reducing ends of chitooligosaccharide compounds was investigated to represent the degradation of C/N in the soil. Like all the other enzymes, activities positively correlated with the SOM. Wastewater-NPK and wastewater-biochar improved NAG of about ten folds higher than the control, which may probably be due to the abundance of fungal population and the presence of chitin in the soil. A number of soil-borne micro-organisms produce chitin in their cell walls, membrane and spores (Gooday 1990). Most fungi species, more especially *Aspergillus* and *Trichoderma* genera are the most commonly studied among the micro-organisms. As reported by Kishore *et al.* (2005), microbial NPK activities submits to the substrate stimulating model of enzyme activities because its levels and performance are elevated upon detecting chitin-containing material.

Organosulphur compounds found in the soil are not directly available to plant for use thereby causing sulphur limitations in the soil. Arylsulfatases, an extracellular enzyme secreted by microorganisms is responsible for hydrolysis of sulphate esters ($R–O–SO_3$) into phenols (R-OH) and sulfate sulfur (SO_4^{-2}) of the sulphate esters especially in sulphur limited soil (Pierzynski *et al.*, 2004). Critical look at Table 4.7 shows the positive effect of biochar on arylsulfatases production in the soil. The supply of carbon through the addition of biochar might have caused the significant increase in the arylsulfatases production. The current result in this study is comparable to the work of Naidu *et al*. (2001) who reported that, the presence of carbon and sulphur availability controls the production level of arylsulfatases. As reported by several researchers (Gianfreda and Bollag, 1996; Calderon *et al*., 2000; Drijber *et al*., 2000; Nannipieri *et al*., 2002 Gil-Sotres *et al.*, 2005) there are

always contradictory act with regards to the response on enzymes to nutrient addition and organic amendments. The high nutrients and organic matter content of the wastewater caused an increase in the arylsulfatase production compared to their piped water treatment which could be as a result of greater microbial biomass. The combination of organic fertilizer contained in the wastewater and the inorganic fertilizer (NPK) treatment produced the highest activity of arylsulfatase in the study. This supports the finding of Gagnon *et al.* (1999), who reported of high influence of wastewater on arylsulfatase and related their findings to high microbial biomass production. Combining biochar and wastewater was expected to trigger arylsulfatase more actively than their individual application but the result showed contrary to that effect. This reduction may be likely due to the presence of some inhibitory effect of trace elements which may be present as well as feedback response to its production.

The rate of nitrogen mineralisation of the soil under study was determined using the microbial extracellular enzyme activities of leucine aminopeptidase, tyrosine aminopeptidase and arginine aminopeptidase as indicators. These groups of enzymes responded differently to biochar and the two water quality treatments as well as inorganic fertilizer. The aminopeptidase enzymatic response to wastewater treatment which is also regarded as organic fertilizer was high compared to the piped water plots. The levels increased with increase in soil organic matter with positive correlation to nitrogen composition. Wastewater application enriched organic matter content of the soil which in turn had an influence on the activities of leucine, tyrosine and arginine aminopeptidase activities. Similar results were also reported by Liang *et al.* (2014), who linked the greater

enzyme activity levels to higher organic matter contents and improved microbial activity. Soil organic matter is known to promote the immobilization of inhabitance like Mg^{2+} by adsorption or through the formation of complexes and also stimulate the proliferation of degrading organisms (Lin *et al.,* 2004). Addition of biochar in combination with the different water qualities were about 100 folds higher in the amino acid synthesis than the unamended control with the exception of tyrosine aminopeptidase under wastewater irrigation application. Biochar has been proved to have nutrient retention ability and can sorb about 5 mg NH^{+} from solutions (Sarkhot *et al.,* 2013). NPK treatments, involving the repeated application of NPK in the various cropping seasons, stimulated the hydrolysis of argenine and leucine but had retarded impact on tyrosine compared to the control. Deamination of tyrosine was the least among the analyzed amino acids across the various treatments in the current study. Stumpe *et al.* (2013), reported of similar observation in enzymatic hydrolysis of amino acid in liquid manure and ranked them in the order leucine > argenine > tyrosine. These findings may be explained by the formation of phenylethylamine (tyramine poisoning) during tyrosine decarboxylation (Dai *et al.,* 2012). This could undertake oxidative deamination catalyzed by microbial amine oxidases resulting in phenylpyruvate and its phenolic derivatives through hydroxylation of the aromatic ring. Interestingly, treatment of biochar and NPK reduces the tested aminopeptidases activities as compared to the individual application in wastewater treatment and vice versa in piped water qualities. The inconsistencies observed in this study may be related to the resource limitation model and substrate stimulation model of enzyme activities where addition of nutrient may halt the actions of enzymes involved in

attaining the added nutrient (Sinsabaugh and Moorhead, 1994). Alternatively in the substrate stimulation model, stimulations of enzyme activity by the addition of its substrate could arise either from specific microbes increasing their production of a particular enzyme or from a shift in the composition of the microbial community taxa to better able to use the added substrate.

5.7 Carbon Concentration of Soil

Biochar incorporation into the soil significantly influenced the carbon pool in the soil during the study. Biochar in its natural form contains high amount of organic carbon which is the reason for the high amounts of carbon in the biochar amended plots compared to the control. Several studies (Downie *et al*., 2009; Lehmann and Joseph, 2009; Chan and Xu, 2009; Amonette and Joseph, 2009) suggest that the carbon components in biochar is recalcitrant in soils, it has about 10-1,000 times residence times longer than of most soil organic matter. The sequestration abilities of biochar may have also contributed to the high percentage of carbon in the biochar amended soil. Similar reports have been made by several scientists although the carbon level may vary based on the feedstock used for the biochar production (Lehmann *et al*., 2006; Reddy *et al*., 2011). Wastewater irrigation on the other hand has been reported to increase soil organic matter content through the booster effect by increased microbial activities introduced or native soil organic matter accounting to the improved carbon content in wastewater irrigated plots compared to the piped water irrigation.

5.8 Nitrogen Concentration of Soil

Nitrogen is among the common and abundant compound in plant and soil. It plays a vital role in plant growth and development processes including cell division, transport and catalysis of biochemical reaction. Nitrogen in the soil can be in organic or inorganic form (Schulten and Schnitzer, 1997). Application of wastewater caused an increase in the nitrogen concentration in all the treatments compared to the piped water irrigation as shown on Figure 4.2. The enrichment of soil nitrogen through wastewater irrigation has been reported by several researchers (Drangert, 1998; Van der Hoek *et al.,* 2002; Keraita *et al.,* 2008). Organic matter composition of wastewater contains nutrient similar to the formulated fertilizer. Jiménez (1995) estimated about 16-62 kg organic nitrogen from every 1000 m^3 of wastewater irrigation to each hectare of land. Other studies have proved that a majority of the nitrogen in wastewater is in the form of organic nitrogen and ammonium (Mathews *et al.,* 2001). Biochar application had insignificant positive impact on the soil nitrogen compositions. This increment can be attributed to the sorption ability of biochar to sorb nutrients which in turn help to reduce nutrient lost. The combined effect of NPK and wastewater on nitrogen concentration was observed slightly above the control treatments with less effect. This could either be as a result of high rate of plant uptake or leaching in the forms of nitrate (Von Wirén *et al.,* 1997). However, biochar in combination with wastewater and NPK had a significant positive impact with the highest nitrogen percentage. This finding further proves that biochar has the sorbing ability of nitrogen fertilizers and can maintain nitrate concentration in the soil by reducing their nitrification.

5.9 Hot Water Extractable Carbon of Soil

Hot water extractable carbon, an extent of labile carbon substance was essentially influenced by wastewater irrigation and biochar addition. The higher values recorded in wastewater irrigated plots (Figure 4.3) may be related to the additional introduction of easily decomposable organic matter to the soil. It is well documented that, wastewater irrigation increases the soil microbial population and also enhanced the activities of the native micro-organism (Marschner *et al.*, 2003; Stumpe *et al.*, 2013). The elevated performance of microbial activities is a delicate measure of organic matter decomposition giving rise to high levels of soil organic carbon as well as hot water extractible carbon. Observed hot water extractable carbon substances in this study positively correlated with the measure of soil organic carbon as well as total soil carbon. Mañas *et al.* (2009) and Muamar *et al.* (2014) have also reported of comparable findings concerning increased soil organic carbon and hot water extraction carbon as an after effect of wastewater irrigation linking their result to higher content of microbial, especially fungal and bacterial biomass. Changes (about 12 %) in hot water extractable carbon by biochar can be connected with the organic carbon portion of biochar. Application of NPK fertilizer caused about 7 to 10 % increase in hot water extractable carbon in both water qualities. However, combined effect of wastewater, NPK and biochar recorded the highest labile hot water extractable carbon compared to the control treatment. This finding is in agreement with the investigations of Tomáš (2008) who reported of elevated amounts of hot water extractable carbon upon the blend of NPK and organic compost relating the finding to improved organic matter content by the treatment.

5.10 Microbial Biomass Carbon of Soil

The microbial biomass of soil is perceived as a petulant indicator of quality soil. Its information is essential for supportable soil management. In this study, biochar incorporation demonstrated an enhanced microbial biomass carbon in both water qualities; however wastewater caused between 10 and 12 % increase in the respective amendments. Microbial biomass carbon is emphatically identified with organic matter substance of soil. The general agreement is that biochar is moderately inert and in this way adds to inherent soil organic matter. Wastewater application to soil is then again very much noted in soil fertility taking into account the supply of organic matter. Soil with high organic matter inputs and accessible soil organic matter has a tendency to have higher microbial biomass since they are favoured energy source for micro-organism (Landgrade *et al*., 2002). This therefore explains the highest values recorded in the combined wastewater and biochar amended soil than the control. On the other hand, NPK application increased the content of microbial biomass C compared to control although not statistically significant. These results are similar to the results of Tomáš (2008).

5.11 Macronutrients

Mineral nourishment is a vital variable impacting the development of plants. Following one year of wastewater irrigation, an increased in K, P, Na, Mg, Ca, Al and Fe was realized. The general enhancement of the soil macro-nutrient in the study can be attributed to the source, quality and type of the wastewater or to the short time of irrigation. Urban wastewater is chiefly made of particulate and dissolved organic matter, and inorganic

substances (e.g., P, K, Na, Ca, Mg, Cl and Al) (Rizzo *et al.,* 2013; Varela and Manaia, 2013). The statistical indifference in response of soil macronutrient to wastewater against irrigation has been highlighted by Runbin *et al.* (2010), who reported of non-statistical improvement in soil K, Na, Ca, and Mg within a year of wastewater treatment. On the other hand, studies conducted on long period irrigation with wastewater, however, have reported substantial accumulations of the nutrient in the soil, remarkably in the subsurface layers of the soils (Lado *et al.,* 2012; Bame *et al.,* 2014).

Wastewater in combination with biochar enhanced the macro-nutrients concentrations in the study. The improvement can be linked to report by Chan *et al.* (2007), who stated that biochars can comprise of variable concentrations of alkalinity that is directly added into the soil as Ca, Mg, K and Na oxides, hydroxides and carbonates. Making reference to Skodras *et al.* (2006), rice husk biochar contains 1800, 530, 1600, 130, 9100, 340, 220,000 mg/kg of Si, Ca, Fe, Mg, Na, K and P respectively which could be the reason for the improved concentration in the biochar treated soil irrespective of the water quality. A great part of the positive yield reactions from biochar application reported cannot be directly credited to the supplement substance of the biochars, however rather to the adsorption impact of expanding nutrient use efficiency. Inorganic fertilizer NPK addition to the soil similarly enriched the macronutrient when applied alone and also when it was in combination with biochar. The combined treatment effect of biochar and NPK especially with wastewater irrigation had positive influence on soil the macro-nutrients. The effect was more pronounced in phosphorus (P) which has been a limiting nutrient in the soil of Northern Ghana. This current result further confirms that, biochar-induced treatment with additional

treatment sources like wastewater, manure and inorganic fertilizer improves the soil nutrients. The impact is more purported in a long term study although increment is still observed in a short term study. This finding is in agreement with the report of Okonwu and Mensah (2012), who observed improved content of N, P, K, Ca, Na and Mg in a combined biochar and NPK application.

5.12 Heavy Metals Concentration in the Soil

Heavy metals or trace elements present in soil are important components of many enzymes and proteins needed for plant growth and development but excess may cause reduction in plant growth and subsequently harmful to human health (Nicholls and Mal, 2003). The heavy metals in the study revealed varied concentration with no statistical difference except for titanium. The wastewater from Zagyuri used in the study has a record of low heavy metal concentration and this was indicated by Abagale *et al*. (2013), to be as a result of the source being domestic. This explains the low levels of heavy metal concentration even below WHO (2006) and FAO (2003) standards for agricultural soil. The fact that heavy metals occur naturally in the soil environment from the pedogenetic processes of weathering of parent materials may have accounted for the values recorded. Biochar effect on heavy metal concentration was not obvious as compared to other report on the heavy metal remediation abilities of biochar. The effect of biochar on metal remediation differs with the types of biochar products, the application rate as well as types of heavy metals (Beesley *et al.,* 2011). The utilization of inorganic NPK fertilizer had no effect on heavy metal concentration accumulated in the soil. This finding contradicts the report by

Pierzynski et *al.* (2000). Raven *et al.* (1998) also made similar observation and reported that inorganic fertilizer has the ability to increase the heavy metal element of soil, in such case; the application rate ought to be high and more intensive.

CHAPTER SIX

CONCLUSION AND RECOMMENDATION

6.1 Conclusion

The study showed that irrigation with wastewater improves the nutrition quality of soil compared to piped water. Irrigation water source caused an increase in microbial respiration activities, enhanced enzymatic performance, improved microbial biomass carbon, labile carbon and nitrogen concentrations of the soil.

The inclusion of biochar (20 Tha^{-1}) under both water qualities improved the soil quality although the difference was not significant. The peak of the above soil health indicators was observed under the combine treatment of wastewater, biochar and NPK which led to improved soil fertility.

Significantly higher pathogen contaminations occurred in wastewater irrigated vegetables and soil far above WHO (2006) recommended values for faecal coliforms, *E. coli* and *Salmonella* unlike piped water irrigated plots which had relatively lower concentration although slightly above WHO standards. Shiga toxin producing *E. coli* was not detected on Amaranthus vegetable in both cropping seasons. The study also confirmed higher pollution of helminth eggs in the soil than the vegetables. *Ascaris lumbricoides* was the most predominant species, being about 94 % of the total population detected. The study further revealed higher risk of pathogen contamination of soil and vegetable in the dry season compared to the wet season. The study has also confirmed that pathogenic properties of irrigation water has direct link with the pathogenic properties of the soil and vegetables under cultivation.

However, heavy metal accumulation in wastewater irrigated soil was generally lower than FAO levels defined as impeding to plant growth and development.

6.2 Recommendation

Considering the contribution of wastewater to soil fertility, the practice of wastewater irrigation should not be condemned but rather be encouraged. However holistic approach and methods should be adopted to reduce the health risk of farmers and consumers through formal and informal education by the government and health workers.

Again different rates of biochar should further be investigated; combining it with compost instead of NPK. Farmers are therefore advised to always be in protective clothing to reduce the risk of contamination. Also irrigating the vegetables with piped water few days before harvesting is expected to help reduce pathogen loads on the vegetables.

References

Abagale F. K., Kyei-Baffour N., Ofori E. and Mensah E. (2013). Types and Seasonal Diversity of Helminth Eggs in Wastewater Used For Peri-Urban Vegetable Crop Production in Tamale Metropolis, Ghana. *International Journal of Current Research* 5(11): pp.3354-3359.

Abe T. and Watanabe A. (2004). X-ray photoelectron spectroscopy of nitrogen functional groups in soil humic acids. *Soil Science* 169:35–43.

Abubakari A. H., Nyarko G., Yidana J. A., Mahunu G. K., Abagale F. K., Quainoo A., Chimsah F. and Avornyo V. (2012). Comparative studies of soil characteristics in Shea parklands of Ghana. *Journal of Soil Science and Environmental Management* 3(4), pp.84-90.

Acosta-Martínez V. and Tabatabai M. A. (2000). Enzyme activities in a limed agricultural soil. *Biology and Fertility of Soils* 31: 85-91.

Adongo T. A., Abagale F. K. and Kranjac-Berisavljevic G. (2015). Soil Quality of Irrigable Lands of Irrigation Schemes in Northern Ghana. *International Journal of Innovative Science, Engineering & Technology* 2:8 Pp. 314-326.

Adriano, D. C. (1986). Trace elements in the terrestrial environment. Springer-Verlag, New York.

Adriano, D. C. (2001). Trace Elements in Terrestrial Environments. *Biogeochemistry, Bioavailability and Risks of Metals.* Springer-Verlag, New York.

Adu, S. V. (1995). Soils of the Nasia River Basin, Northern Region, Ghana. Memoir No. 11, SRI, Kumasi.

Adzitey F., Abdul-Aziz A. and Moses O. (2014). Microbial quality of beef in the Yendi Municipality of Ghana. *Global Journal of Animal Scientific Research* 2: 10-17.

Aleem A., Isar J. and Malik A. (2003). Impact of Long–Term Application of Industrial Wastewater on the Emergence of Resistance Traits in Azotobacter chroococcum Isolated from Rhizospheric Soil. *Bioresource Technology* 86 (1) 7–13.

Alf K. and Nannipieri P. (1995). *Cellulase activity, methods in applied soil microbiology and biochemistry*. Academic, London.

Allard G. O. and Whitney J. A. (1994). Geology of the Inner Piedmont, Carolina terrane, and Modoc zone in Northeast Georgia. Project Report 20. Georgia Department of Natural Resources, Environmental Protection Division, Georgia Geologic Survey, Atlanta.

Allison S. D., Chacon S. S. and German D. P., (2014). Substrate concentration constraints on microbial decomposition. *Soil Biology & Biochemistry* 79 43-49.

Alloway B. J. (1995). *Soil processes and the behaviour of heavy metals*. pp. 11-37. In: Heavy metals in soils. Alloway, B. (Eds). Chapman and Hall, New York.

Amoah P, Drechsel P. and Abaidoo R. C. (2005). Irrigation urban vegetable production in Ghana: sources of pathogen contamination and health risk elimination. *Irrigation and Drainage* 54: 49–61.

Amoah P. (2008). Wastewater Irrigated Vegetable Production: Contamination pathway for health risk reduction in Accra, Kumasi and Tamale – Ghana. A Thesis submitted to the Department of Theoretical and Applied Biology, Kwame Nkrumah University of Science and Technology

Amonette J. and Joseph, S. (2009). Characteristics of biochar e micro-chemical properties. In: Lehmann, J., Joseph, S. (Eds.), Biochar for Environmental Management: Science and Technology. *Earthscan*, London, pp. 13-32.

Anderson J. P. E and Domsch K. H. (1978). A physiological method for the quantitative measurement of microbial biomass in soils. *Soil Biology and Biochemistry*, 10: 215-221.

Anderson J. P. E. (1982). *Soil respiration*, p. 833–871. In A. L. Page, R. H. Miller, and D. R. Keeney (ed.), Methods of soil analysis, part 2: chemical and microbiological properties. Madison, New York, N.Y.

Angulo F. J. Tippen S., Sharp D. J., Payne B. J., Collier, C., Hill J. E., Barett T. J., Clark R..M., Geldrich E. E. and Donnell H. D. (1997). A community waterborne outbreak of salmonellosis and the effectiveness of a boil water order. *American Journal Public of Health*, 87, 580–584.

Antal M. J. and Grønli M. (2003). The art, science and technology of charcoal production. *Industrial and Engineering Chemistry Research*, 42: 1619-1640.

Arinze A. E. and Yubedee A. G. (2000). Effect of fungicides on Fusarium grain rot and enzyme production in maize (Zea mays L.). *Global Journal of Applied Science* 6:629–634.

Armon R., Gold D., Brodsky M. and Oron G. (2002). Surface and subsurface irrigation with effluents of different qualities and prescence of Cryptosporidium oocytes in soils and on crops. *Water Science and Technology* 46, 115–122.

Asai H., Samson, B. K., Stephan H. M., Songyikhangsuthor K., Homma K., Kiyono Y., Inoue Y., Shiraiwa T. and Horie T. (2009). Biochar amendment techniques for upland rice production in Northern Laos 1. Soil physical properties, leaf SPAD and grain yield. *Field Crop Research,* 111: 81 – 84.

Asano T. and Curtivo J. (2004). Groundwater recharge with reclaimed municipal wastewater: Health and regulatory considerations. *Water Resources.* 38:1941 – 1951.

Ashbolt N. J. (2004). Microbial contamination of drinking water and disease outcomes in developing regions. *Toxicology,* 198, 229–238.

Assadian N. W., Di Giovanni G. D., Enciso J., Iglesias J. and Lindemann W. (2005). The transport of waterborne solutes and bacteriophage in soil subirrigated with a wastewater blend. Agriculture, *Ecosystems and Environment* 111:279 – 291.

Atkinson C. J., Fitzgerald J. D. and Hipps N. A. (2010). Potential mechanisms for achieving agricultural benefits from biochar application to temperate soils: a review. *Plant and Soil,* 337: 1– 18.

Bacteriological Analytical Manual Book (2014). U.S Food and Drugs Administration, New Hampshire Avenue 1-888-INFO-FDA (1-888-463-6332).

Bailey V. L., Fansler S. J., Smith J. L. and Bolton H. (2010). Reconciling apparent variability in effects of biochar amendment on soil enzyme activities by assay optimization. *Soil Biology and Biochemistry*, 43(2), 296-301.

Bame I. B., Hughes J. C., Titshall L. W. and Buckley C. A. (2014). The effect of irrigation with anaerobic baffled reactor effluent on nutrient availability, soil properties and maize growth. *Agricultural Water Management*, 134, p.50-59.

Barrell R.. A. E., Hunter P. R. and Nichols G. (2000). Microbiological standards for water and their relationship to health risk. *Communicable Disease and Public Health*, 3, 8–13.

Beare M. .H., Neely C. L., Coleman D. C. and Hargrove W. L. (1990). A substrate-induced respiration (SIR) method for measurement of fungal and bacterial biomass on plant residues. *Soil Biology & Biochemistry* 22, 585 - 594.

Beesley L., Moreno-Jiménez E., Gomez-Eyles J. L., Harris E., Robinson B. and Sizmur T. (2011). A Review of Biochars' Potential Role in the Remediation, Revegetation and Restoration of Contaminated Soils. *Environmental Pollution*, 159, 3269-3282.

Ben Sassi M., Dolinger J., Renault P., Tlili A. and Bérard A. (2012). The FungiResp method: an application of the MicroResp™ method to assess fungi in microbial communities as soil biological indicators. *Ecological Indicators* 23, 482–490.

Benitez E., Nogales R., Campos M. and Ruano F. (2006). Biochemical variability of oliveorchard soils under different management systems. *Applied Soil Ecology* 32,221–231.

Benjamin J. G., Nielsen D. C. and Vigil M. F. (2003). Quantifying effects of soil conditions on plant growth and crop production. *Geoderma*. 116: 137-148.

Bethony J, Brooker S, Albonico M, Geiger S. M. and Loukas A. (2006). Soiltransmitted helminth infections: ascariasis, trichuriasis, and hookworm. *Lancet* 367: 1521–1532.

Blencowe D. K. and Morby A. P. (2003). Zn (II) metabolism in prokaryotes. FEMS *Microbiology Reviews*, 27: 291-311.

Blumenthal U. J., Peasey A., Ruiz-Palacios G. and Mara D. D. (2000). Guidelines for wastewater reuse in agriculture and aquaculture: recommended revisions based on new research evidence (WELL Study Task No: 68 Part 1). Water and Environmental Health at London and Loughborough, London School of Hygiene and Tropical Medicine, London.

Brady, N. C. Weil R. R. (2002). *The Nature and Properties of Soils*. (13 Eds.), Pearson Education Inc, USA, 823pp.

Brandy N. C., Weil R.R. (1999). The nature and propreties of soils. (12th edition). Prentice Hall Inc., New Jersey, pp: 860.

Brar M. S., Mahli S. S., Singh A. P., Arora C. L. and Gill K. S. (2000). Sewer water irrigation effects on some potentially toxic trace elements in soil and potato plants in northwestern India. *Can J Soil Sci,* 80:465 – 71. In Ensink JHJ, van der Hoek W, Matsuno Y, Munir S and Aslam M. R. (2003). The use of untreated wastewater in peri-urban agriculture in Pakistan: risks and opportunities. IWMI Research Report No. 64, International Water Management Institute, Colombo, Sri Lanka.

Bridgwater A. V. (2003). 'Renewable fuels and chemicals by thermal processing of biomass', *Chemical Engineering Journal*, 9(1), pp87–102.

Stumpe B., Werner S., Jung R., Heinze S., Jüschke E., Strippel C. and Marschner B. (2012). Organic carbon dynamics and enzyme activities in agricultural soils amended with biogas slurry, liquid manure and sewage sludge. *Agricultural Sciences* (3): 1, 104-113.

Brookes P. C. (1995). The use of microbial parameters in monitoring soil pollution by heavy metals. *Biology and Fertility of Soils* 19, 269-279.

Bruun E. W., Hauggaard-Nielsen, H., Ibrahim N., Egsgaard H., Ambus P., Jensen P. A. and Dam-Johansen K. (2011). Influence of fast pyrolysis temperature on biochar labile fraction and short-term carbon loss in a loamy soil. *Biomass and Bioenergy*, 35(3), 1182-1189.

Brzezińska M. Stępniewski W. Stępniewska, Z. and Przywara G. (2001). Effect of Oxygen Deficiency On Soil Dehydrogenase Activity In a Pot Experiment With Triticale CV. Jago *Vegetation. International Agrophysics*, 15, pp. 145-149.

Buri M. M., Issaka R. N. and Wakatsuki, T. (2006). Selected lowland soils in Ghana: Nutrient levels and distribution as influenced by agro-ecology. Proceedings of the International Conference and the 17th and 18th Annual General Meetings of the Soil Science Society of Ghana. Pp 149-162.

Buscot F. (2005). What are soils? In: Buscot F, Varma S, eds. Micro-organisms in soils: roles in genesis and functions. Heidelberg, Germany: *Springer-Verlag*, 3–18.

Cabral J. P. S. (2010). Water microbiology. Bacterial pathogens and water. *International Journal of Environmental Research and Public Health*, 7, 3657–3703.

Cakmak I. and Yazici A. M. (2010). Magnesium: A forgotten element in crop production. *Better Crops* 94 (2): 1-3.

Calderon J. F., Jackson L. E, Scow K. M. and Rolston D. E. (2000). Microbial responses to simulated tillage in cultivated and uncultivated soils. *Soil Biology and Biochemistry* 32:1547–1559.

Calderon J. F., Jackson L. E., Scow K. M., Rolston D. E. (2000). Microbial responses to simulated tillage in cultivated and uncultivated soils. *Soil Biology Biochemistry* 32:1547–1559.

Campbell C.D., Chapman S. J., Cameron, C. M., Davidson, M. S. and Potts, J. M., (2003). A rapid microtiter plate method to measure carbon dioxide evolved from carbon substrate amendments so as to determine the physiological profiles of soil microbial communities by using whole soil. *Applied and Environmental Microbiology* 69, 3593–3599.

Carter S., Shackley S., Sohi S., Suy T. B. and Haefele S. (2013). The impact of biochar application on soil properties and plant growth of pot grown lettuce (Lactuca sativa) and cabbage (Brassica chinensis), *Agronomy*, 32, pp. 404-18.

Castaldi S., Riondino M., Baronti S., Esposito F. R, Marzaioli R., Rufigliano F.A., Vaccari F. P. and Miglietta F. (2011). Impact of biochar application to a Mediterranean wheat crop on soil microbial activity and greenhouse gas effluxes', *Chemosphere*, vol. 85, pp. 1464-71.

Cavet J. S., Borrelly G. P. M. and Robinson N. J. (2003). Zn, Cu and Co in cyanobacteria: selective control of metal availability. *FEMS Microbiology Reviews*, 27: 165-181.

Chan K., Dorahy C. and Tyler S. (2007). Determining the agronomic value of composts produced from garden organics from metropolitan areas of New South Wales, Australia, *Australian Journal of Experimental Agriculture*, 47(11), p. 1377.

Chan, K, Van Zwieten, L, Meszaros, I, Downie, A and Joseph, S (2008). Agronomic values of greenwaste biochar as a soil amendment, *Soil Research*, (45) 8, pp. 629-34.

Chan, K. Y. and Xu Z. (2009). Biochar: nutrient properties and their enhancement, in J Lehmann and S Joseph (eds), Biochar for environmental management: Science and technology, *Earthscan*, London, pp. 67-84.

Chen, G., Zhu, H. and Zhang Y. (2003). Soil microbial activities and carbon and nitrogen fixation. *Research in Microbiology* 154, 393–398.

Chodak M. and Niklinska M. (2012). Development of microbial biomass and enzyme activities in the mine soil. *Polish Journal of Environmental Studies.* 21 (3), 569.

Cobbina S. J., Kotochi M. C., Korese J. K., and Akrong M. O. (2013). Microbial Contamination in Vegetables at the Farm Gate Due to Irrigation with Wastewater in the Tamale Metropolis of Northern Ghana. *Journal of Environmental Protection*, 4, 676-682.

Cohen Y. and Pastor J. P. (1991). The responses of a forest model to serial correlations of global warming. Ecol. 72: Currie, R.G. and O'Brien, D.P. 1988. Periodic 18.6-year and Eco~. 60: 849-872. 600-605. Mod. 49: 229-266. 1161 -1 165.

Coleman D. C., Crossley J. D. A. and Hendrix P. F. (2004). *Fundamentals of soil ecology*. London: Academic Press.

Corcoran, E., C. Nellemann E., Baker R.. Bos D., Osborn H. and Savelli P. (2010). Sick Water? The central role of wastewater management in sustainable development. A Rapid Response Assessment. United Nations Environment Programme, UN-HABITAT, GRID-Arendal.

Cordell D., Rosemarin A., Schröder, J. J. and Smit A. L., (2011). Towards global phosphorus security: A systems framework for phosphorus recovery and reuse options. *Chemosphere*, 84: 747-758.

Crawford R. M. M. and Brändle R. (1996). Oxygen deprivation stress in a changing environment. *Journal of Experimental Botany*. 47:145–159.

Dai Z. L., Li X. L., Xi P. B., Zhang J., Wu G. and Zhu W.Y. (2012). Metabolism of select amino acids in bacteria from the pig small intestine. *Amino Acids*. 42(5):1597-1608.

Dalal R. C. (1982). Effect of plant growth and addition of plant residues on the phosphatase activity in soil. *Plant Soil.* 66: 265-269.

Daroit D. J. (2007). Caracterização de uma beta glucosidase de Monoscuspurpureus. Programa de pós-graduação microbiologia agrícola e do meio ambiente da Universidade Federal do Rio Grande do Sul – UFRS.

Deenik, J. A., Diarra G., Uehara S., Campbell Y. Sumiyoshi M. and Antal J. (2011). Charcoal ash and volatile matter effects on soil properties and plant growth in an acid Ultisol. *Soil Science*, 176 (2011), pp. 336–345.

Demirbas A. (2004). Effects of temperature and particle size on bio-char yield from pyrolysis of agricultural residues. *Journal of Analytical and Applied Pyrolysis*, vol. 72, no. 2, pp. 243-8.

Dempster D. N., Gleeson D. B., Solaiman Z. M., Jones D. L. and Murphy D. V. (2012). Decreased soil microbial biomass and nitrogen mineralisation with Eucalyptus biochar addition to a coarse textured soil. *Plant and Soil* 354, 311 – 324.

Deng S. P and Tabatabai M. A. (1994). Cellulase activity of soils. *Soil Biol Biochem* 26:1347–1354.

Dick R. P. (1997). Soil enzyme activities as integrative indicators of soil health. In: Pankhurst CE, Doube BM, Gupta VVSR (eds) *Biological indicators of soil health*. CABI, Wellingford, pp 121–156 549-563.

Dilustro J. J., Collins B., Duncan L. and Crawford, C. (2005). Moisture and soil texture effects on soil CO_2 efflux components in southeastern mixed pine forests. Forest Ecol. Manage. 204, 85–95.

Dinesh R., Anandaraj M., Srinivasan V. and Hamza S. (2012). Engineered nanoparticles in the soil and their potential implications to microbial activity. *Geoderma* 173–174, 19–27.

Dodgson K. S. and Rose F. A (1976). Sulfohydrolases. In: Metabolism of Sulfur Compounds Vol. 7, *Metabolic Pathways* (Greenberg DM. Ed.), pp. 359-431. Academic Press, London.

Downie A., Crosky A. and Munroe P. (2009). Physical properties of biochar. In 'Biochar for environmental management : *Science and technology*.' (Eds J Lehmann and S Joseph) pp.13-32. *Earthscan*: London ; Sterling, VA, USA.

Downie A., Klatt P. and Munroe P. (2007). Slow pyrolysis: Australian demonstration plant successful on multi-feedstocks, in Bioenergy 2007 Conference, Jyvaskyla, Finland, pp. 225-57.

Drangert J. O. (1998). 'Fighting the urine blindness to provide more sanitation options. *Water South Africa*, vol 24, no 2, pp157–64.

Drechsel P. and Zimmerman U. (2005). Factors influencing the intensification of farming systems and soil-nutrient management in the rural-urban continuum of SW Ghana. *Journal of Plant Nutrition and Soil Science* 168, 694–702.

Drechsel P, C. A. Scott L. Raschid-Sally M. Redwood and Bahri A. (2010). Wastewater Irrigation and Health. Assessing and Mitigating Risk in Low-Income Countries, IWMI-IDRC *Earthscan*. 432pp.

Drijber R. A., Doran J. W., Parkhurst A. M. and Lyon D. J. (2000). Changes in soil microbial community structure with tillage under long-term wheat-fallow management. *Soil Biology and Biochemistry* 32:1419–1430.

Drijber R. A., Doran J. W., Parkhurst A. M. and Lyon D. J. (2000). Changes in soil microbial community structure with tillage under long-term wheat-fallow management. *Soil Biology and Biochemistry* 32:1419–1430.

Eivazi F. and Tabatabai M. A. (1988). Glucosidases and galactosidases in soils. *Soil Biology and Biochemistry* 20:601–606.

Ekekpi G. K and Kombiok J. M. (2008). Report on baseline study in three target districts of conservation agriculture project in Northern Ghana. Care International Report.

Ellis K. V., Rodrigues P. C. C. and Gomez C. L. (1993). Parasite ova and cysts in waste stabilization ponds. *Water Research*. 27:1455-1460.

Elzobair K. (2013). Biochar Effects on Soil Microbial Communities and Resistance of Enzymes to Stress Department of Soil and Crop Sciences. Thesis Colorado State University

Energy Transport and Water Department Water Anchor (ETWWA) (2010). Improving Wastewater Use in Agriculture: An Emerging Priority.

Ennis C. J. (2012). Biochar: carbon sequestration, land remediation and impacts on soil microbiology, *Critical Reviews in Environmental Science and Technology*, 42 (22), pp.2311-2364.

Eriksson K. E. L., Blancbette R. A. and Ander P. (1990). Biodegration of cellulose. In: Eriksson K. E. L., Blanchette R. A, Ander P. (eds*) Microbial and enzymatic degradation of wood and wood components*. Springer, New York, pp 89–180.

Ernst, W.H.O. (1998). The origin and ecology of contaminated, stabilized and non-pristine soils (Chapter 2). pp. 17-29. In: *Metal-contaminated soil*. Vangronsveld, J. and Cunningham, S.D. (Eds). Springer, New York.

Eswaran H. Beinroth F. H and Reich P. F. (2003). A global assessment of land quality, in Land Quality, Agriculture, and food security, Biophysical process and economic choices at local, regional, and global scales Wiebe, K (ed.). pp 111-132.

ETWWA (Energy Transport and Water Department Water Anchor) (2010). Improving Water Management in Rainfed Agriculture: Issues and Options in Water Constrained Production Systems.

FAO. (2003). Review of World Water Resources by Country. Water Reports No. 23.

Feachem R. G., Bradley D. J., Garelick H. and Mara D. D. (1983). *Sanitation and Disease: Health Aspects of Excreta and Wastewater Management.* John Wiley, Chicester.

Fließbach A., Mäder, P. and Niggli U. (2000). Mineralization and microbial assimilation of 14C-labeled straw in soils of organic and conventional agricultural systems. *Soil Biology & Biochemistry* 32: 1031-1039.

Fontaine S., Mariotti A. and Abbadie L. (2003). The priming effect of organic matter: a question of microbial competition. *Soil Biology and Biochemistry* 35, 837–843.

Foreman C. M, Franchini P. and Sinsabaugh R. L. (1998). The trophic dynamics of riverine bacterioplankton: relationships among substrate availability, ectoenzyme kinetics and growth. *Limnology and Oceanography* 43:1344–52.

Fujii K., Aoki M. and Kitayama K. (2012). Biodegradation of low molecular weight organic acids in rhizosphere soils from a tropical montane rain forest. *Soil Biology and Biochemistry.* 47, 142-148.

Gagnon B., Lalande R.. Simard R. and Roy M. (1999). Soil enzyme activities following paper sludge addition in a winter cabbage-sweet corn rotation *Canadian Journal of Soil Science* 91-97.

Ganeshamurthy A. M., Singh G. and Singh N. T. (1995). Sulphur status and response of rice to sulphur on some soils of Andaman and Nicobar Islands. *Journal of the Indian Society of Soil Science* 43:637–641

Garcia C., Hernandez, T., Roldan A. and Martin A. (2002). Effect of plant cover decline on chemical and microbiological parameters under Mediterranean climate, *Soil Biology and Biochemistry* 34, 635–642.

Garland J. L. and Mills A. L. (1991). Classification and characterization of heterotrophic microbial communities on the basis of patterns of community-level sole-carbon-source utilization. *Applied and Environmental Microbiology* 57, 2351–2359.

Garland J. L., Roberts M. S., Levine L. H. and Mills A. L. (2003). Community-level physiological profiling performed with an oxygen-sensitive fluorophore in a microtiter plate. *Applied and Environmental Microbiology* 69, 2994–2998.

Gaskin J. W., Steiner C., Harris K., Das K. C. and Bibens B. (2008). Effect of low-temperature pyrolysis conditions on biochar for agricultural use. *Transactions of the ASABE*, 51: 2061-2069.

Geisseler D., Horwath W. R. and Scow K. M. (2011). Soil moisture and plant residue addition interact in their effect on extracellular enzyme activity. *Pedobiologia* 54, 71–78.

German D. P., Weintraub M. N., Stuart A. G., Lauber C. L., Zachary L. R. and Allison S. D. (2011). Optimization of hydrolytic and oxidative enzyme methods for ecosystem studies Review *Soil Biology & Biochemistry* 43 1387-1397.

Gianfreda L. and Bollag J. M (1996). Influence of natural and anthropogenic factors on enzyme activity in soil. In: Stotzky G, Bollag JM (eds) Soil biochemistry. Marcel Dekker, New York, pp 123–194.

Giardina C. P. and Ryan M. G. (2002). Total belowground carbon allocation in a fast growing Eucalyptus plantation estimated using a carbon balance approach. *Ecosystems* 5, 487–499.

Gil-Sotres F., Trasar-Cepeda C., Leiros M. C. and Seoane S. (2005). Different approaches to evaluating soil quality using biochemical properties. Soil Biology and Biochemistry 37:877–887.

Gleick P. H. (2000). The Worlds Water 2000-2001: *The Biennial Report on Freshwater Resources*. Island Press. Washington DC, 315pp.

Gooday G. W. (1990). Physiology of microbial degradation of chitin and chitosan. *Biodegradation*, 1, 177–190.

Gooday G. W. (1990). *The ecology of chitin degradation. In: Advances in Microbial Ecology*. Plenum Press, New York pp. 387-430.

Grabow W. (2001). Bacteriophages: Update on application as models for viruses in water. *Water Sa*, 27, 251–268.

Gray C. L. and Lewis O. T. (2014). Do riparian forest fragments provide ecosystem services or disservices in surrounding oil palm plantations? *Basic and Applied Ecology* 15 pp. 693–700.

Green V. S., Stott D. E., Cruz J. C., and Curi N., (2007). Tillage impacts on soil biological activity and aggregation in a Brazilian Cerrado Oxisol. *Soil and Tillage Research* 92:114–121.

Gregorich E. G., Carter M. R., Angers D. A., Monreal C. M. and Ellert B. H. (1994). Towards a minimum data set to assess soil organic matter quality in agricultural soils. *Canadian Journal of Soil Science* 74, 367–385.

Gremion F. (2003). Analysis of Microbial Community Structures and Functions In Heavy Metal-Contaminated Soils Using Molecular Methods. *Soil Biology and Biochem*istry 34, 643–642

Grierson P. F. and Adams, M. A. (2000). Plant species affect acid phosphatase, ergosterol and microbial P in a Jarrah (Eucalyptus marginata Donn ex Sm.) forest in south-western Australia, *Soil Biology and Biochemistry* 32, 1817–1827.

Guerrero - Gonzalez M., Azcon-Aguilar C., Mooney M., Valderas A., MacDiarmid C. W. Eide D. J. and Ferrol N. (2005). Characterization of a Glomus intraradices gene encoding a putative Zn transporter of the cation diffusion facilitator family, *Fungal Genetics and Biology*, vol. 42, no. 2, pp. 130-40.

Guo R., Li G., Jiang T., Schuchardt F., Chen T., Zhao Y. and Shen Y. (2012). Effect of aeration rate, C/N ratio and moisture content on the stability and maturity of compost. *Bioresource Technology, 112*, 171-178.

Guo Y. and Rockstraw D. A. (2007). Physicochemical properties of carbons prepared from pecan shell by phosphoric acid activation. *Bioresource Technology*, 98, 1513-1521.

Gupta V. V. S. R., Farrell R. E. and Germida J. J. (1993). Activity of arylsuphatases in Saskatchewan soils. *Canadian Journal of Soil Science* 73:341–347.

Halvorson J. J, Smith J. L and Papendick R. I (1997). Issues of scale for evaluating soil quality. *Journal of Soils and Water Conservation* 52:26–30

Havlin J. L., Beaton J. D., Tisdale S. L. and Nelson W. L. (2005). Soil Fertility and Fertilizers: An Introduction to Nutrient Management. 7th (ed.). Pearson Prentice Hall. New Jersey.

Heaton, J. C., and Jones, K. (2007). Microbial contamination of fruit and vegetables and the behavior of enteropathogens in the phyllosphere: A review. *Journal of Applied Microbiology*, 104(3), 613–626.

Hedley M. J., Stewart J. W. B. and Chauhan B. S., (1982). Changes in inorganic and organic soilphosphorus fractions induced by cultivation practices and by laboratory incubations. *Soil Science Society of America Journal* 46:970-976.

Herath H. M. S. K., Arbestain, M. C. and Hedley M. (2013). Effect of biochar on soil physical properties in two contrasting soils: An Alfisol and an Andisol. J. *Geoderma* 209-210 (2013). pp. 188-197.

Hermans C, Johnson GN, Strasser RJ and N Verbruggen (2004). Physiological characterization of magnesium deficiency in sugar beet: acclimation to low magnesium differentially affects photosystem I and II. *Plants*.; 220: 344- 355.

Hilber I., Wyss G. S., Mäder P., Bucheli T.D., Meier I., Vogt, L.,and Schulin R. (2009). Influence of activated charcoal amendment to contaminated soil on dieldrin and nutrient uptake by cucumbers. *Environment Pollution*, 157: 2224 – 2230.

Houghton J. T., Ding Y., Griggs D. J., Noguer M., van der Linden P. J., Dai X. Maskell K., and Johnson C. A. (2001). Climate Change *The scientific basis, Contribution of working Group I to the third assessment report of the intergovernmental panel on climate change*, Cambridge University Press, Cambridge, UK, 142–145.

Hussain J., Khan A.L., Rehman N., Zainullah Hussain S.T., Khan F. and Shinwari Z. K. (2009). Proximate and nutrient analysis of selected medicinal plant species of Pakistan. *Pakistan J.Nut.*, 8 (1): 620 -624.

International Biochar Initiative, (2007). Conference to review Pregress Achieved in the field of biochar production and utilization. New South Wales Australia.

Intizar H., Liqa R., Munir A. H., Fuard M. and Wim H (2002). Wastewater use in Agriculture: Review of Impacts and Methodological Issues in Valuing Impacts IWMI working paper.

Ioannidou O. and Zabaniotou A. (2007), Agricultural residues as precursors for activated carbon production--A review, *Renewable and Sustainable Energy Reviews*, 11 (9) pp. 1966-2005.

Jeffery S., Verheijen F. G. A., van der Velde M. and Bastos A. C. (2011). A quantitative review of the effects of biochar application to soils on crop productivity using meta-analysis. Agriculture, *Ecosystems and Environment 144*: 175-187.

Jessica S. and Peter S. (2011). Biochar: Implications for aagriculture productivity. Technical Report 11.06. Department of agriculture, fisheries and forestry. ABERE

Jiménez B. (1995). Wastewater Reuse to Increase Soil Productivity. *Water Science and Technology* 32(12) 173–180.

Jin H. (2010). Characterization of microbial life colorizing biochar and biochar-amended soils. PhD Dissertation, Cornell University, Ithaca, NY.

Jindo K., Sánchez M., M. A., Hernández T., García, C., Furukawa T., Matsumoto K. and Bastida F. (2012). Biochar influences the microbial community structure during manure composting with agricultural wastes. *Science of the Total Environment*, 416, 476-481.

Joachim H. J. R. Makoi and Patrick A. N (2008). Review Selected soil enzymes: Examples of their potential roles in the ecosystem. *African Journal of Biotechnology* Vol. 7 (3), pp. 181-191.

Jones D. L., Dennis P. G., Owen A. G., Hees P. A. W. (2003). Organic acid behavior in soil misconception and knowledge caps. *Plant and Soil*. 248, 31-41.

Junma S., Bingchen W., Gang X. and Hongbo S. (2014). Effects of wheat straw biochar on carbon mineralization and guidance for large scale soil quality improvement in the coastal wetland, *Ecological Engineering*, vol. 62, pp. 43-47.

Kabata-Pendias A. and Pendias H. (2001). *Trace elements in soils and plants*. CRC Press, London.

Kabiru L. M., Bello M., Kabir J., Grande L. and Morabito S. (2015). Detection of Pathogenic *Escherichia coli* in Samples Collected at an Abattoir in Zaria, Nigeria and at Different Points in the Surrounding Environment . *International Journal of Environmental Research and Public Health* 12, 679-691.

Kalavrouziotis I. K., Robolas P., Koukoulakis P. H. and Papadopoulos A. H. (2008). Effects of municipal reclaimed wastewater on the macro- and micro-elements status of soil and of Brassica oleracea var. Italica, and B. oleracea var. Gemmifera.

Kandeler E., Tscherko D., and Spiegel H. (1999). Long-term monitoring of microbial biomass, N mineralisation and enzyme activities of a Chernozem under different tillage management. *Biology and Fertility of Soils* 28:343-351.

Kanyoka P., and Eshtawi T. (2012). Analyzing the trade-offs of wastewater re-use in agriculture: An Analytical framework. Center for Development Research, Interdisciplinary Term Paper, University of Bonn, November.

Karaca A., Cetin S. C., Turgay O. C. and Kizilkaya R. (2011). SoilEnzymes as Indication of Soil Quality. *Soil Enzymology*, 78-3-642.

Kaschuk K., Alberton O., Hungria and M., (2010). Three decades of soil microbial biomass studies in Brazilian ecosystems: lessons learned about soil quality and indications for improving sustainability. *Soil Biology and Biochemistry* 42, 1–13.

Keraita B., Jiménez B. and Drechsel P. (2008). Extent and Implications of Agricultural Reuse of Untreated, Partially Treated and Diluted Wastewater in Developing Countries. CAB reviews: Perspectives in Agriculture, *Veterinary Science, Nutrition and Natural Resources* 3(58) 1–15.

Kertesz M. A. and Mirleauy P. (2004). *The role of soil microbes in plant sulphur nutrition School of Biological Sciences*, University of Manchester, 1.800 Stopford Building, Oxford Road.

Khan S, Chao, C, Waqas M. and Zhu Y. G. (2013). Sewage sludge biochar influence upon rice (Oryza sativa L) yield, metal bioaccumulation and greenhouse gas emissions from acidic paddy soil, *Environmental Science and Technology* 47 (15), pp. 8624-32.

Khuroo M. S. (1996). Ascariasis. *Gastroenterology Clinics of North America*. 25:553-557.

Kim J. S., Sparovek S., Longo R. M., De Melo W. J. and Crowley D. (2007). Bacterial diversity of terra preta and pristine forest soil from the Western Amazon. Soil *Biology and Biochemistry* 39, 648-690.

King N. J. (1967). Glucoamylase of Coniophora cerebella. *Biochemistry Journal* 105:577-583.

Kiran B., Qudrat U. K., Rubina N. M., Jamil K., Obaid U. S., Aneela B. and Asif L. (2005). Integrated Effect of Organic Manures and Inorganic Fertilizers on Soil Phosphatase Activity and Yield of Maize-Spinach Cropping System. Thesis Gomal University.

Kishore G. K., Pande S. and Podile A. R. (2005). Chitin-supplemented foliar application of Serratia marcescens GPS 5 improves control of late leaf spot disease of groundnut by activating defense related enzymes. *Journal of Phytopathology* 153, 169–173.

Knowles O. A., Robinson B. H., Contangelo A. and Clucas L. (2011). Biochar for Mitigation of Nitrate Leaching from Soil Amended with Biosolids. *Science of the Total Environment*, 409, 3206-3210.

Kolb S. E., Fermanich K. J. and Dornbush M. E. (2009). Effect of charcoal quantity on microbi*al* biomass and activity in temperate soils. *Soil Science Society of America Journal* 73, 1173-1181.

Kucharski F., Kang I. S., Farneti R. and Feudale L., (2011). 'Tropical Pacific response to 20th century Atlantic warming.' *Geophysical Research Letters* 38, L03702.

Kuzyakov Y., Subbotina I., Chen H., Bogomolova I. and Xu X., (2009). Black carbon decomposition and incorporation into microbial biomass estimated by 14C labeling. *Soil Biology and Biochemistry* 41, 210e219.

Lado M., Bar-Tal A., Azenkot A., Assouline S., Ravina I., Erner Y., Fine P., Dasberg, S. and Ben-Hur M. (2012). Changes in chemical properties of semiarid soils under long-term secondary treated wastewater irrigation. *Soil Science Society of America Journal* 76, p.1358- 1369.

Laird D., Fleming P., Wang B., Horton R. and Karlen D. (2010). Biochar impact on nutrient leaching from a Midwestern agricultural soil. *Geoderma* 158, 436e442.

Landa–Cansigno O., Durán–Álvarez J. C. and Jiménez B. (2013). Retention of Escherichia coli, Giardia lamblia Cysts and Ascaris lumbricoides Eggs in Agricultural Soils Irrigated by Untreated Wastewater. *Journal of Environmental Management* 128 22–29.

Launaskas J and Kvikliene N (2006). Effect of calcium foliar application on some fruit quality characteristics of 'Sinap Orovskij' apple. *Agronomy Research* 4(1): 31-36.

Lehmann J., da Silva J. P., Steiner C., Nehls T., Zech W. and Glaser B. (2003). Nutrient availability and leaching in an archaeological Anthrosol and a Ferralsol of the Central Amazon basin: fertilizer, manure and charcoal amendments. *Plant and Soil* 249, 343e357.

Lehmann J., Gaunt J. and Rondon M. (2006). Bio-char sequestration in terrestrial ecosystems e a review. Mitigation and Adaptation Strategies for Global Change 11, 403-427.

Lehmann J. and Joseph S. (2009). Biochar for environmental management: an introduction.In: Lehmann J., Joseph, S. (Eds.), Biochar for Environmental Management:Science and Technology. *Earthscan*, London, pp. 1-12.

Lehmann J., Rillig M. C., Thies J., Masiello C. A., Hockaday W. C. and Crowley D. (2011). Biochar effects on soil biota–a review. *Soil Biology and Biochemistry* 43(9), 1812-1836.

Leiros M. C., Trasar-Cepeda C., Garcia-Fernandez, F. and Gil- Sotres F. (1999). Defining the validity of a biochemical index of soil quality. *Bioogy and. Fertility of Soils* 30, 140–146.

Liang C. H., Yin Y. and Chen Q. (2014): Dynamics of soil organic carbon fractions and aggregates in vegetable cropping systems. Pedosphere 24: 605–612.

Lima I. M., McAloon A. and Boateng A. A. (2008). Activated carbon from broiler litter: process description and cost of production, *Biomass and Bioenergy*, vol. 32, no. 6, pp. 568-72.

Lin H., Decuypere E. and, Buyse J. (2004). Oxidative stress induced by corticosterone administration in broiler chickens (Gallus gallus domesticus) Short-term effect. *Comparative Biochemistry and Physiology* 139 745–751.

Lorito M., Hayes C. K., Di Pietro A., Woo S. L. and Harman G. E. (1994). Purification, characterization, and synergistic activity of a glucan 1,3-b-glucosidase and an N-acetyl-b-glucosaminidase from Trichoderma harzianum. *Phytopathology* 84, 398-405.

Lu S. G., Sun F. F. and Zong Y. T. (2014). Effect of rice husk biochar and coal fly ash on some physical properties of expansive clayey soil (vertisol), *Catena* 114, pp. 37-44.

Luo, L. F., Hou, C. C., and Yang, W. X. (2013). Nuclear factors: roles related to mitochondrial deafness. Gene 520, 79–89. doi: 10.1016/j.gene.2013.03.041.

Madejon E, Burgos P, Lo´pez R, Cabrera F. (2001). Soil enzymatic response to addition of heavy metals with organic residues. *Biology and Fertility* of *Soils* 34: 144150.

Madejón E., López R., Murillo J. M. and Cabrera F. (2001). Agricultural use of three (sugarbeet) vinasse composts: effect on crop and on chemical properties of a soil of the Guadalquivir River Valley (SW Spain). *Agriculture Ecosystems and Environment*, 84: 55–67.

Major J., Rondon M., Molina D, Riha S. J. and Lehmann J. (2010). 'Maize yield and nutrition during 4 years after biochar application to a Colombian savanna oxisol', *Plant and Soil* (333) 1-2, pp. 117-28.

Mañas P., Castro E., de Las and Heras J. (2009). Irrigation with treated wastewater: effects on soil, lettuce (Lactuca sativa L.) crop and dynamics of microorganisms. *Journal of Environmental Science and Health* 44(12):1261-73.

Mapanda F., Mangwayana E. N., Nyamangara J. and Giller KE (2005). The effect of long-term irrigation using wastewater on heavy metal contents of soils under vegetables in Harare, Zimbabwe. *Agriculture, Ecosystems and Environment* 107:151-165.

Mara D. D., Sleigh P. A., Blumenthal U. J. and Carr R. M. (2007). Health Risks in Wastewater Irrigation: Comparing Estimates from Quantitative Microbial Risk Analyses and Epidemiological Studies. *Journal of Water and Health* 5(1) 39–50.

Marschner P. Kandeler E. and Marschner B. (2003). Structure and function of the soil microbial community of a long-term fertilizer experiment. *Soil Biology and. Biochemistry* 35, 453-461.

Marx M. C., Wood M. and Jarvis S. C. (2001). A microplate fluorimetric assay for the study of enzyme diversity in soils. *Soil Biology and Biochemistry* 33, 1633–1640.

Mathews M. C., Frate C., Harter T. and Sather S., (2001). Lagoon Water Composition, Sampling and Field Analysis, University of California Cooperative Extension.

Matsuoka M. (2006). Atributos biológicos de solos cultivados com videira na Região da Serra Gaucha. Universidade Federal do Rio Grande do Sul. Porto Alegre/RS. (Dissertação de mestrado)

McGill W. B. and Colle C. V. (1981). Comparative aspects of cycling of organic C. N. S. and P through soil organic matter. *Geoderma* 26:267–286.

McLain, J. E. T. and Williams, C. F. (2008). Seasonal variation in accurate identification of Escherichia coli within a constructed wetland receiving tertiary-treated municipal effluent. *Water Reseasch* 42, 4041–4048.

McLaughlin H., Anderson P. S., Shields F. E. and Reed T. B. (2009*). All bio-chars are not created equal, and how to tell them apart,* Proceedings, North American Biochar Conference, Boulder, Colorado.

Melero S., Porras J. C., Herencia J. F. and Madejon E. (2006). Chemical and biochemical properties in a silty loam soil under conventional and organic management. *Soil Tillage Research* 90, 162–170.

Mendham D. S, O'Connell A. M. and Grove T. S (2002). Organic matter characteristics under native forest, log-term pasture, and recent conversion to Eucalyptus plantations in Western Australia: microbial biomass, soil respiration, and permanganate oxidation. *Australian Journal of Soil Research* 40: 859–872.

Mengel K. and Kirkby, E. A. (1982). Principles of plant nutrition. International Potash Institute, Bern, Switzerland.

Miao-zhen F., Chang Y., Fen-liang F., A-lin S., Bo-ren W., Dong-chu L. and Yong-chao L. (2015). Effects of different long-term fertilization on the activities of enzymes related to carbon, nitrogen, and phosphorus cycles in a red soil. *Chinese Journal of Applied Ecology* 26 (3): 833-838.

Minhas, P. S. and Samra, J. S. (2004). Wastewater use in peri-urban agriculture: impacts and opportunities. Bulletin No. 1/2004, 78p. Central Soil Salinity Research Institute, Karnal.

Moeskops B., Sukristiyonubowo B. D. and Sleutel S. (2010). Soil microbial communities and activities under intensive organic and conventional vegetable farming in West Java, Indonesia. *Applied Soil Ecology* 45, 112–120.

Moreno J. L., Garcła C. and Hernandez T, (2003). Toxic effect of cadmium and nickel on soil enzymes and the influence of adding sewage sludge. *European Journal of Soil Science* 54: 377–386.

Muamar A. Jaboobi L., M'hamed T., Shawqi E., Abdellah E., Abdelmajid Z. and Mohammed B. (2014). Assessment of the impact of wastewater use on soil properties *Journal of Materials and Environmental Science* 5 (3) 747-752.

Mukherjee A. and Lal R. (2013). Biochar impacts on soil physical properties and greenhouse gas emissions. *Agronomy* 3:313–339.

Murray C. J., Vos T., Lozano R., Naghavi M. and Flaxman A. D. (2013). Disability-adjusted life years (DALYs) for 291 diseases and injuries in 21 regions, 1990–2010: a

systematic analysis for the Global Burden of Disease Study 2010. *Lanc*et 380: 2197–2223.

Naidu R., Krishnamurti G. S. R., Wenzel W., Megharaj M. and Bolan N. S. (2001). Heavy Metal Interactions in Soils and Implications for Soil A Tox Hazard Subst Environ Eng.

Nannipieri P., Kandeler E. and Ruggiero P. (2002). Enzyme activities and microbiological and biochemical processes in soil. In: Burns RG, Dick RP (eds) Enzymes in the environment. Activity, ecology and applications. Marcel Dekker, New York, pp 1–33.

National Environmental Research Institute (NERI) (2002). Microorganisms as indicators of soil health. Technical report no. 388, Denmark.

Nguyen B., Lehmann J., Kinyangi J., Smernik R., Riha S. J. and Engelhard M. H. (2008). Long-term black carbon dynamics in cultivated soil. *Biogeochemistry* 89, 295e308.

Nicholls A. M. and Mal T. K. (2003). Effects of lead and copper exposure on growth of an invasive weed, Lythrum salicaria L. (Purple Loosestrife). *Ohio Journal of Science*, 103: 129–133.

Novak J. M. (2009). Impact of biochar amendment on fertility of a southeastern Coastal Plain soil. *Soil Science* 174(2), p.105.

Obuobie E., Keraita B., Danso G. Amoah P., Cofie O., Raschid-Sally L. and Drechsel P. (2006). Irrigated urban vegetable production in Ghana: Characteristics, benefits and risks. IWMI-RUAF-IDRC-CPWF, Accra, Ghana MI, pp 150.

Oguntunde P.,G., Fosu M, Ajayi A.,E., van de Giesen N. (2004). Effect of charcoal production on maize yield, chemical properties and texture of soil. *Biology and Fertility of Soils*. 39: 295-299.

Okafo C. N., Umoh V. J. and Galadima M. (2003). Occurrence of pathogens on vegetables harvested from soils irrigated with contaminated streams. *The Science of the Total Environment* 311:49–56.

Okonwu K. and Mensah S. I. (2012). Effects of NPK (15:15:15) Fertilizer on Some Growth Indices of Pumpkin. *Asian Journal of Agricultural Research*, 6: 137-143.

Pachepsky Y. and Shelton D. (2011). Escherichia coli and fecal coliforms in freshwater and estuarine sediments. *Critical Reviews in Environmental Science and Technology* 41, 1067–1110.

Pancholy S. K and Rice E. L. (1973). Soil enzymes in relation to old field succession; amylase, cellulose, invertase, dehydrogenase and urease. *Soil Science Society of America Journal* 37: 47-50.

Pandiyan T., Martínez Rivas O., Orozco Mez J., Burillo A. G., Martínez C. M. A. (2002). Comparison of methods for the photochemical degradation of chlorophenols. *J Photoch Photobiol A* 146: 149–155.

Pazur J. H. (1965). *Enzymes in the synthesis and hydrolysis of starch. In: Starch: Chemistry and Technology*. Vol. 1 Fundamental aspects (Whistler R, Paschall EF Eds.) pp. 133-175. Academic press, New York.

Peng, X., Ye, L. L., Wang, C. H., Zhou, H. and Sun B. (2011). Temperature and duration dependent rice straw-derived biochar: Characteristics and its effects on soil properties of an Ultisol in southern China. *Soil and Tillage Research*, 112.

Pescod M. B. (1992). Wastewater treatment and use in agriculture. Bull FAO; 47:125 [Rome].

Pierzynski G. M., Sims J. T., and Vance G. F. (2000). *Soils and Environmental Quality*, (2nd Eds.) CRC Press, London, UK.

Pierzynski G. M., Sims J. T., Vance G. F. (2004). Soils and Environmental Quality. CRC Press, Boca Raton.

Pietikäinen J., Kiikkilä O. and Fritze H. (2000) Charcoal as a habitat for microbes and its effect on the microbial community of the underlying humus. Oikos 89, 231-242.

Raghothama K. G. (1999). Phosphate acquisition, *Annual Review of Plant Physiology* 50, 665–693.

Rai M. M. (1977). Principles of Soil Science.4th Edition, Macmillan Publishers India Limited.pp 218-278.

Ramírez K. S., Craine J. M. and Fierer N. (2012). Consistent effects of nitrogen amendments on soil microbial communities and processes across biomes. *Global Change Biology* 18, 1918–1927.

Rani U., Padmaja G. and Chandrasekhar P. (2014). Integrated Effect Of Organic Manures And Inorganic Fertilizers On Soil Phosphatase Activity And Yield Of Maize-Spinach Cropping System *Agricultural Science Digest* 34 (1) : 31 – 35.

Rattan R. K., Datta S. P., Chhokar P. K., Suribabu K. and Singh A. K. (2005). Long-term impact of irrigation with sewage effluents on heavy metal content in soils, crops and groundwater – a case study. *Agriculture, Ecosystems & Environment* 109: 310-322.

Raven P. H., Berg L. R., and Johnson G. B. (1998). Environment, Saunders College Publishing, (2nd Eds.), New York, NY, USA.

Reddy G. B., Ro K., Lima I. M. and Mahajan D. (2011). Adsorption Characteristics Of Biochar And Applications, Farm Pilot Project Coordination, Inc., Chesapeake Bay Regional Technology Summit, Westin, Annapolis, MD.

Rejsek K., Vronnova V., Pavelka M., Formanek P. (2012). Acid phosphomonoesterase (E.C. 3.1.3.2.) location in the soil. *Journal of Plant Nutrition and Soil Science* 175, 196.

Renella G., Landi L. and Nannipieri P. (2002). Hydrolase activities during and after the chloroform fumigation of soil as affected by protease activity. *Soil Biology and Biochemistry* 34:51–60

Revel K. T., Maquire R. O. and Agblevor F. A., (2012). Field trials with poultry litter biochar and its effects on forages, green peppers and soil properties, *Soil Science* 177 (10), pp. 573-79.

Rizzo L., Fiorentino A. and Anselmo A. (2013). Advanced treatment of urban wastewater by UV radiation: effect on antibiotics and antibioticresistant E. coli strains. *Chemosphere* 92:171–176.

Rizzo L., Manaia C., Merlin C., Schwartz T., Dagot C. and Ploy M. C. (2013). Urban wastewater treatment plants as hotspots for antibiotic resistant bacteria and genes spread into the environment: a review. *Science of The Total Environment* 447, 345–360.

Romero E., Fernandez-Bayo J., Diaz J. and Nogales R. (2010). Enzyme Activities and Diuron Persistence In Soil Amended With Vermicompost Derived From Spent Grape Marc and Treated With Urea. *Applied Soil Ecology* 44, pp. 198-204.

Ross D. J. (1976). Invertase and amylase activities in ryegrass and white clover plants and their relationships with activities in soils under pasture. *Soil Biology and Biochemistry* 8: 351-356.

Rowell M. J. (1995). Colorimetric method for CO_2 measurement in soils. *Soil Biology and Biochemistry* 27, 373–375.

Runbin D., Christopher D. S. and Clifford B. F. (2010). Short-Term Effects of Wastewater Land Application on Soil Chemical Properties. *Water, Air, and Soil Pollution* 211:165–176.

Saba C. K. S., Escudero J. A., Herrera-León S., Porrero M. C., Suárez M., Domínguez L., Demuyakor B. and Gonzalez-Zorn B. (2013). First identification of *Salmonella Urbana* and *Salmonella Ouakam* in humans in Africa. *The Journal of Infection in Developing Countries* 7(10):691-695.

Saba C. K. S., Eric Y. and Adzitey F. (2015). Prevalence of *Escherichia coli* and Shiga Toxin Producing *Escherichia coli* in Cattle Faeces and Raw Cow Milk Sold in the Tamale Metropolis, Ghana. *Journal of Dairy, Veterinary & Animal Research* 2(5): 00052.

Saito M. and Marumoto, T. (2002). Inoculation with arbuscular mycorrhizal fungi: the status quo in Japan and the future prospects. *Plant and Soil* 244, 273-279.

Salazar S., Sánchez L. E., Alvarez J., Valverde A., Galindo P., and Igual J. M. (2011). Correlation among soil enzyme activities under different forest system management practices. *Ecological Engineering* 37: 1123-1131.

Santamaria J. and Toranzos G. A. (2003). Enteric pathogens and soil: a short review. *International Microbiology* 6: 5-9.

Sarathchandra S. U. and Perrott K. W. (1981). Determination of phosphatase and arylsulphatase activity in soils. *Soil Biology and Biochemistry* 13:543–545.

Sardans J. and Penuelas J. (2005). Drought decreases soil enzyme activity in a Mediterranean Quercus ilex L. forest, *Soil Biology Biochemistry*., 37, 455–461.

Sardans J., Penuelas J. and Estiarte M. (2007). Seasonal patterns of rootsurface phosphatase activities in a Mediterranean shrubland. Responses to experimental warming and drought, *Biology and Fertility of Soils*, 43, 779–786.

Sarkhot D. V., Ghezzehei T. A. and Berhe A. A. (2013). Biochar for nutrient recapture from dairy wastewater: recovery of major nutrients, *Journal of Environmental Quality* 42, 1545–1554.

Schimel J. P. and Weintraub M. N. (2003). The implications of exoenzyme activity on microbial carbon and nitrogen limitation in soil: a theoretical model. *Soil Biology and Biochemistry* 35 pp 152-161.

Schnug E. and Haneklaus S. (1998). Diagnosis of sulphur nutrition. In: Schnug E, ed. Sulphur in agroecosystems. *Dordrecht: Kluwer*, 1–38.

Scholl M. A., Mills A. L., Herman J. S. and Hornberger G. M. (1990). The Influence of Mineralogy and Solution Chemistry on the Attachment of Bacteria to Representative Aquifer Materials. *Journal of Contaminant Hydrology* 6(4) 321–336.

Schulten H. R. and Schnitzer M. (1997). The chemistry of soil organic nitrogen: a review. *Biology and Fertility of Soils* 26(1), 1-15.

Scott C. A., Faruqui N. I. and Raschid-Sally, L. (2004). Wastewater use in irrigated agriculture: management challenges developing countries. In Scott CA, F aruqui NI, Raschid-Sally L, (eds) *Wastewater Use in Irrigated Agriculture: Confronting the Livelihood and Environmental Realities*. CABI Publishing, Wallingford. p 1-10.

Silver W. L., Lugo A. E. and M. Keller (1999). Soil oxygen availability and biogeochemistry along rainfall and topographic gradients in upland wet tropical forest soils. *Biogeochemistry* 56(1), 30-45.

Singer M. J. and Ewing S. (2000). Soil quality. In: Sumner ME (ed) Handbook of soil science. CRC, *Boca Raton,* FL, pp 271–298.

Singh D. K. and Kumar S. (2008). Nitrate reductase, arginine deaminase and dehydrogenase activities in natural soil (with forest) and in cotton soil after acetamiprid treatment. *Chemosphere* 71, 412.

Singh P. P., Shin Y. C., Park C. S. and Chung Y. R. (1999). Biological control of Fusarium wilt of cucumber by chitinolytic bacteria. *Phytopathology* 89:92–99.

Sinsabaugh R. L., Antibus R. K. and Linkins A. E. (1991). An enzymic approach to the analysis of microbial activity during plant litter decomposition. *Agriculture, Ecosystems & Environment* 34:43–54.

Sinsabaugh R. L. and Linkins A. E. (1989). Natural disturbance and the activity of Trichoderma viride cellulase complex. *Soil Biology and Biochemistry* 21:835–839.

Sitaula B. K., Bakken L. R. and Abrahamsen G. (1995). "N-fertilization and Soil Acidification Effects on N2O and CO2 Emission from Temperate Pine Forest Soil." *Soil Biology and Biochemistry* 27 (11): 1401–1408.

Skodras G., Natas P., Basinas P. and Sakellaropoulos G. P. (2006). Effects of Pyrolysis Temperature, Residence Time on the Reactivity of Clean Coals Produced from Poor Quality Coals. *Global NEST Journal* 8: 89-94.

Smith J. L. and Paul E. A. (1990). *The significance of soil microbial biomass estimations*. In: Bollag JM, Stotzky G (ed) *Soil Biochemistry*, 6. York, pp. 357-396.

Smith J. L., Fratamico P. M. and Gunther N.W. 4th (2014). Shiga toxin-producing Escherichia coli. *Advances in applied microbiology Journal* 86: 145-97.

Smith K. S. and Huyck H. L. O. (1999). An overview of the abundance, relative mobility, bioavailability, and human toxicity of metals. p. 29-70. In G.S. Plumlee and M.J. Logsdon (eds.). The environmental geochemistry of mineral deposits, Part A: Process, techniques, and health issues. Society of Economic Geologists, Inc., Chelsea, Michigan.

Sohi S., Lopez-Capel E., Krull E. and Bol R. (2009). Biochar, climate change and soil: A review to guide future research., CSIRO Land and Water Science Report Rep. No. 05/09, Geln Osmond, Australia.

Som S., Gupta S. K. and Banerjee S. K. (1994). Assessment of quality of sewage effluents from Howrah sewage treatment plant. *Journal of the Indian Society of Soil Science* 42: 571-575.

Soon, J. M. Seaman, P. and Baines R. N. (2013). Escherichia coli O104:H4 outbreak from sprouted seeds. *International Journal of Hygiene and Environmental Health* 216, 346–354.

Sparling, G. P. (1995). The substrate induced respiration method, pp. 397–404. In K. Alef and P. Nannipieri (ed.), *Methods in applied soil microbiology and biochemistry*. Academic Press, London, United Kingdom.

Speir T. W and Ross D. J. (1978). Soil phosphatase and sulphatase. In: Burns RG (ed) Soil enzymes. Academic, London, UK, pp 197–250.

Srinivasan, J. T. and Reddy V. R. (2009). Impact of irrigation water quality on human health: A case study in India. *Ecological Economics* 68, 2800–2807.

Stanislaw K. and Symannovic B. (2012). Enzymatic activity of soil after applying various waste organic material, ash and fertilizers. *Polish Journal of Environmental Studies* 21(6) 1635-1641.

Stefanowicz A. (2006). The Biolog plates technique as a tool in ecological studies of microbial communities. *Polish Journal of Environmental Studies* 15, 669–676.

Steinbeiss S., Gleixner G. and Antonietti M., (2009). Effect of biochar amendment on soil carbon balance and soil microbial activity. *Soil Biology and Biochemistry*, 41(6), pp.1301–1310.

Steiner C., (2007). Soil Charcoal Amendments Maintain Soil Fertility and Establish a Carbon Sink– Research and Prospects. *Soil Ecology Research Developments* ISBN 978-1-60021-971-9

Steiner C., Das, K. C., Garcia M., Förster B. and Zech W. (2008). Charcoal and smoke extract stimulate the soil microbial community in a highly weathered xanthic Ferralsol. *Pedobiologia* 51, 359 – 366.

Steiner C., Das K.C., Melear N. and Lakly, D., (2010). Reducing Nitrogen Loss during Poultry Litter Composting Using Biochar. *Journal of Environmental Quality* 39: 1236–1242

Strobel B. W. (2001). Influence of vegetation on low-molecularweight carboxylic acids in soil solution - a review. *Geoderma* 99(3): 169-198.

Subhani A., Liano M., Huang C. Y. and Xie Z. M. (2001). Impact of some agronomic practices on paddy field soil health under varied ecological condition: influence of soil moisture. *Pedosphere* 11, 38–48.

Swift R. S. (2001). Sequestration of carbon by soil. *Soil science*. 166, 858-871

Tenenbaum D. J. (2009). Carbon Mitigation from the Ground Up. *Environmental Health Perspectives* 117(2), pp. A70-A73.

Thies J.E. and Rillig M., (2009). Characteristics of biochar: biological properties. In: Lehmann, J., Joseph, S. (Eds.), *Biochar for Environmental Management: Science and Technology*. *Earthscan*, London, pp. 85e105.

Thoma J. A, Spradlin J. E. and Dygert S. (1971). Plant and animal amylases. In: The Enzymes (Boyer PD Ed.) 5: 115-189.

Tiller K. G. (1986). Essential and toxic heavy metals in soils and their ecological relevance. Trans. XIII Congr. *International Union of Soil Sciences* 5: 106-111.

Tisdale S. L., Nelson W. L., Beaton J. D., and Havlin J. L. (1993). Soil fertility and fertilizers. 5th edition. Macmillan Publishing Company, NY.

Tlili A. Marechal M., Montuelle B., Volat B., Dorigo U. and Bérard A. (2011). Use of the Microresp™ method to assess pollution-induced community tolerance for lotic biofilms. *Environmental Pollution* 159, 18–24.

Tomáš Šimon (2008). The Influence of Long-term Organic and Mineral Fertilization on Soil Organic Matter Crop Research Institute, Prague-Ruzyně, Czech Republic

Toze S. (1997). Microbial pathogens in wastewater, Literature Review for Urban Water Systems.Multi-divisional Research Program. CSIRO Land and Water Technical report No 1/97.

Tronsmo A. and Harman, G. E. (1993). Detection and quantification of N-acetyl-beta-D-glucosaminidase, chitobiosidase, and endochitinase in solutions and on gels. *Analytical Biochemistry* 208, 74-79.

Trudel, J. and Asselin A. (1989). Detection of chitinase activity after polyacrylamide gel electrophoresis. *Analytical Biochemistry* 178, 362-366.

Turco R. F., Kennedy A. C. and Jawson M. D. (1994). *Microbial indicators of soil*

Tyler G. (1981). Heavy metals in soil biology and biochemistry. In: Paul EA, Ladd JN (eds) *Soil biochemistry* 5, pp 371–414

Tyrrel S. F. and Quinton J. N. (2003). Overland flow transport of pathogens from agricultural land receiving faecal wastes, *Applied Microbiology*, 94: 87s-93s.

Tyrrel S. F., Knox, J. W., and Weatherhead, E. K. (2006). Microbiological water quality requirements for salad irrigation In the United Kingdom. *Journal of Food Protection* 69, 2029–2035.

United Nations (2010). Resolution adopted by the General Assembly, The human right to water and sanitation, A/RES/64/292

Van der Hoek W., Ul–Hassan M., Ensink J. H. J., Feenstra S., Raschid–Sally L., Munir S., Aslam R., Ali N., Hussain R. and Matsuno Y. (2002). Urban Wastewater: A Valuable Resource for Agriculture. Colombo: International Water Management Institute Research;

Varela A. R. and Manaia, C. M., (2013). Human health implications of clinically relevant bacteria in wastewater habitats. *Environmental Science and Pollution Research.* 20, 3550–3569.

Verheijen V., Zwieten L. V. Singh B. P. and Cox J. (2012). Chapter four: Biochar effects on soil properties. In: J Cox (ed) *Biochar in Horticulture: Prospects for the Use of Biochar in Australian Horticulture*. Horticulture Australia, NSW Department of Primary Industries.

Verheijen F. G. A., Jeffery S., Bastos A. C., van der Velde, M. and Diafas, I. (2009). Biochar Application to Soils - A Critical Scientific Review of Effects on Soil Properties, Processes and Functions. EUR 24099 EN, Office for the Official Publications of the European Communities, Luxembourg, 149pp.

Von Wirén N., Gazzarrini S. and Frommer W. B. (1997). Regulation of mineral nitrogen uptake in plants. *Plant Soil* 196, 191–199. .

Von Wiren, N., Roemheld, V., Morel, J .L., Guckert A. and Marschner, H. (1993). Influence of microorganisms on iron acquisition in maize. *Soil Biology and Biochemistry* 25, 371 - 376.

Wallenstein M. D. and Weintraub M. N. (2008). Emerging tools for measuring and modeling the in situ activity of soil extracellular enzymes. *Soil Biology & Biochemistry* 40, 2098-2106.

Wang Q. K., Wang S. L. and Liu, Y. X. (2008). Responses to N and P fertilization in a young *Eucalyptus dunnii* plantation: microbial properties, enzyme activities and dissolved organic matter. *Applied Soil Ecology* 40, 484–490.

Warnock, D. D., Lehmann, J., Kuyper, T.W., Rillig, M.C., (2007). Mycorrhizal responses to biochar in soil-concepts and mechanisms. *Plant and Soil* 300, 9-20.

WHO (1994). Bench Aids for the Diagnosis of Intestinal Parasites. World Health Organization, Geneva. ISBN 92 4 154476 7.

WHO (2015). global estimates of foodborne diseases Dutch National Institute for Public Health and the Environment, Amsterdam.

WHO (2008). Guidelines for Drinking-water Quality. Third edition

WHO (2009). 'Quantifying Environmental Health Impacts',

WHO, (2000). Global Water Supply and Sanitation Assessment 2000 Report.World Health Organization (WHO) United Nations Children‟s Fund (UNICEF), Geneva and New York.

WHO, (2006). Guidelines for the safe use of wastewater, excreta and grey water: Wastewater use in agriculture (Volume 2). Report of a WHO Scientific Group. WHO Technical Report Series 778. World Health Organization, Geneva, Switzerland, pp74-219.

Wolińska, A. (2010). Dehydrogenase Activity of Soil Microorganisms and Oxygen Availability During Reoxidation Process of The Selected Mineral Soils From Poland. *Acta Agrophysica*, 180, pp. 1-88

Woods W. I., Falcao N. P. S. and W. G. Teixeira, (2006). Biochar Trials Aim to Enrich Soil for Smallholders, *Nature*, 443 (7108), 144–144.

World Health Organisation (WHO), (2006). WHO Guidelines for the Safe Use of Wastewater, Excreta and Greywater. *Policy and Regulatory Aspects.* Vol. 1.

World Health Organization (2008). Guidelines for Drinking-water Quality (Electronic Resource): Incorporating 1st and 2nd Addenda, Volume 1, Recommendations, 3rd ed. Geneva, Switzerland.

World Health Organization (2011). Guidelines for Drinking-Water Quality, 4th ed.; WHO: Geneva, Switzerland,;ISBN:978-92-4-154815-1.

Yadav R. K., Goyal B., Sharma R..K., Dubey S.K. and Minhas P.S. (2002). Post-irrigation impact of domestic sewage effluent on composition of soils, crops and ground water - a case study, *Environment International* 28 481.

Yiqi L. and Xuhui Z. (2006). Soil Respiration and the Environment Academic Press is an imprint of Elsevier.

Young .C, Juang T. and Guo H. (1989). 'The effect of inoculation with vesicular-arbuscular mycorrhizal fungi on soybean yield and mineral phosphorus utilization in subtropical-tropical soils', *Plant and Soil*, vol. 95, no. 2, pp. 245-53.

Yuan, B. and Yue, D. (2012). Soil Microbial and Enzymatic Activities Across a Chronosequence of Chinese Pine Plantation Development On The Loess Plateau of China. *Pedosphere*, 22, pp. 1-12

Zachary L. R. and Steven D. A. (2011). Optimization of hydrolytic and oxidative enzyme methods for ecosystem studies. *Soil Biology and Biochemistry* 43 (2011) 1387-1397

Zavalloni C., Alberti G., Biasiol S., Vedove G.D., Fornasier, F., Liu J. and Peressotti, A. (2011). Microbial mineralization of biochar and wheat straw mixture in soil: A short-term study. *Applied Soil Ecology* 2011, 50, 45–51.

Zhang A., Liu, Y, Pan G., Hussain, Q, Li L., Zheng J. and Zhang, X (2012). Effect of biochar amendment on maize yield and greenhouse gas emissions from a soil organic carbon poor calcareous loamy soil from Central China Plain', *Plant and Soil*, 351 (2) pp. 263-75.

Zhao Y., Wang, S. X., Nielsen, C. P., Li, X. H., and Hao, J. M. (2010). Establishment of database of emission factors for atmospheric pollutants from Chinese coal-fired power plants, *Atmospheric Environment* 44, 1515–1523,.

Zwieten L. V, Singh BP, Cox J (2012). Chapter four: Biochar effects on soil properties. In: J Cox (ed) *Biochar in Horticulture: Prospects for the Use of Biochar in Australian Horticulture*. Horticulture Australia, NSW Department of Primary Industries.

Appendices

Appendix 1: Analysis of variance, concentration of aluminum in soil.

Variate: Al					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	7	229.65	32.81	0.49	0.835
Residual	24	1618.17	67.42		
Total	31	1847.82			

Appendix 2: Analysis of variance, concentration of calcium in soil.

Variate: Ca					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	7	6.47	0.924	0.21	0.98
Residual	24	105.961	4.415		
Total	31	112.431			

Appendix 3: Analysis of variance, concentration of iron in soil.

Variate: Fe					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	7	4997.4	713.9	0.86	0.553
Residual	24	19995.4	833.1		
Total	31	24992.8			

Appendix 4: Analysis of variance, concentration of potassium in soil.

Variate: K					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	7	0.33045	0.04721	0.54	0.793
Residual	24	2.08507	0.08688		
Total	31	2.41552			

Appendix 5: Analysis of variance, concentration of magnesium in soil.

Variate: Mg					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	7	1.631	0.233	0.08	0.999
Residual	24	72.62	3.026		
Total	31	74.251			

Appendix 6: Analysis of variance, concentration of sodium in soil.

Variate: Na					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	7	4.2507	0.6072	1.08	0.409
Residual	24	13.557	0.5649		
Total	31	17.8077			

Appendix 7: Analysis of variance, concentration of phosphorus in soil.

Variate: P					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	7	3.74295	0.53471	11.73	<.001
Residual	24	1.0944	0.0456		
Total	31	4.83735			

Appendix 8: Analysis of variance, β-glucopyranosidase activities in soil.

Variate: β-glucopyranosidase					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	7	4190140	598591.4	772.18	<.001
Residual	24	18604.6	775.2		
Total	31	4208744			

Appendix 9: Analysis of variance, α-glucosidase activities in soil.

Variate: α-glucosidase					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	7	3606.25	515.18	6.42	<.001
Residual	24	1925.88	80.25		
Total	31	5532.13			

Appendix 10: Analysis of variance, β-xylosidase activities in soil.

Variate: β-xylosidase					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	7	1122.142	160.306	27.71	<.001
Residual	24	138.857	5.786		
Total	31	1260.999			

Appendix 11: Analysis of variance, β-cellubiosidase activities in soil.

Variate: β-cellubiosidase					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	7	89481	12783	21.29	<.001
Residual	24	14408.5	600.4		
Total	31	103889.4			

Appendix 12: Analysis of variance, Arg-aminopeptidase activities in soil.

Variate: Arg-Aminopeptidase					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	7	5703826	814832	3.77	0.007
Residual	24	5184812	216034		
Total	31	10888638			

Appendix 13: Analysis of variance, dehydogenase activities in soil.

Variate: Dehydogenase					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	7	958.769	136.967	22.22	<.001
Residual	24	147.923	6.163		
Total	31	1106.692			

Appendix 14: Analysis of variance, Leu-aminopeptidase activities in soil.

Variate: Leu-Aminopeptidase					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	7	227487.8	32498.3	136.99	<.001
Residual	24	5693.6	237.2		
Total	31	233181.5			

Appendix 15: Analysis of variance, N-acetylglucosaminidase activities in soil.

Variate: N-acetylglucosaminidase					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	7	77881.6	11125.9	16.61	<.001
Residual	24	16071.3	669.6		
Total	31	93953			

Appendix 16: Analysis of variance, phosphatase activities in soil.

Variate: Phosphatase					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	7	36212.96	5173.28	224.6	<.001
Residual	24	552.79	23.03		
Total	31	36765.76			

Appendix 17: Analysis of variance, sulfatesterase activities in soil.

Variate: Sulfatesterase					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	7	6214952	887850	8.84	<.001
Residual	24	2409306	100388		
Total	31	8624258			

Appendix 18: Analysis of variance, Tyr-aminopeptidase activities in soil.

Variate: Tyr-Aminopeptidase					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	7	71316	10188	8.37	<.001
Residual	24	29212	1217		
Total	31	100527			

Appendix 19: Analysis of variance, concentration of carbon in soil.

Variate: Carbon					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	7	240.631	34.376	21.28	<.001
Residual	24	38.777	1.616		
Total	31	279.408			

Appendix 20: Analysis of variance, concentration of nitrogen in soil.

Variate: Nitrogen					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	7	0.001197	0.000171	2.19	0.072
Residual	24	0.001875	7.81E-05		
Total	31	0.003072			

Appendix 21: Analysis of variance, alanine respiration in soil.

Variate: Alanine					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	7	0.27566	0.03938	1.29	0.298
Residual	24	0.73374	0.03057		
Total	31	1.0094			

Appendix 22: Analysis of variance, basal respiration in soil.

Variate: Basal					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	7	0.80037	0.11434	4.19	0.004
Residual	24	0.65449	0.02727		
Total	31	1.45486			

Appendix 23: Analysis of variance, citric acid respiration in soil.

Variate: Citric Acid					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	7	0.63215	0.09031	1.2	0.338
Residual	24	1.80032	0.07501		
Total	31	2.43247			

Appendix 24: Analysis of variance, glucose respiration in soil.

Variate: Glucose					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	7	1.12302	0.16043	3.11	0.018
Residual	24	1.23866	0.05161		
Total	31	2.36169			

Appendix 25: Analysis of variance, *E. coli* counts of soil in dry season.

Variate: *E.coli*_Soil_dry					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	7	1.58E+08	22512352	23.38	<.001
Residual	24	23113611	963067		
Total	31	1.81E+08			

Appendix 26: Analysis of variance, *E. coli* counts of soil in wet season.

Variate: *E. coli*_Soil_wet					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	7	41643	5949	145.84	<.001
Residual	24	979	40.79		
Total	31	42622			

Appendix 27: Analysis of variance, *E. coli* count of Amaranthus in dry season.

Variate: *E.coli*_Veg_dry					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	7	1222244	174606	13.96	<.001
Residual	24	300261	12511		
Total	31	1522505			

Appendix 28: Analysis of variance, *E. coli* count of Amaranthus in wet season.

Variate: E_coli_Veg_wet					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	7	1526	218	90.21	<.001
Residual	24	58	2.417		
Total	31	1584			

Appendix 29: Analysis of variance, faecal coliform counts of soil in dry season.

Variate: FC_Soil_dry					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	7	2.22E+09	3.17E+08	11.69	<.001
Residual	24	6.51E+08	2.71E+07		
Total	31	2.87E+09			

Appendix 30: Analysis of variance, faecal coliform counts of soil in wet season.

Variate: FC_Soil_wet					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	7	5.38E+08	76835087	14.14	<.001
Residual	24	1.3E+08	5433792		
Total	31	6.68E+08			

Appendix 31: Analysis of variance, faecal coliform counts of Amaranthus in dry season.

Variate: FC_Veg_dry					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	7	1.39E+14	1.99E+13	4.41	0.003
Residual	24	1.08E+14	4.50E+12		
Total	31	2.47E+14			

Appendix 32: Analysis of variance, faecal coliform counts of Amaranthus in wet season.

Variate: FC_Veg_wet					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	7	26662348	3808907	66.52	<.001
Residual	24	1374215	57259		
Total	31	28036564			

Appendix 33: Analysis of variance, concentration of beryllium in soil.

Variate: Be					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	7	0.001529	0.000218	0.5	0.825
Residual	24	0.010481	0.000437		
Total	31	0.01201			

Appendix 34: Analysis of variance, concentration of cadmium in soil.

Variate: Cd					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	7	2.60E-05	3.71E-06	0.84	0.569
Residual	24	1.07E-04	4.45E-06		
Total	31	1.33E-04			

Appendix 35: Analysis of variance, concentration of cobalt in soil.

Variate: Co					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	7	0.008076	0.001154	0.85	0.555
Residual	24	0.032429	0.001351		
Total	31	0.040505			

Appendix 36: Analysis of variance, concentration of chromium in soil.

Variate: Cr					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	7	0.008925	0.001275	1.7	0.157
Residual	24	0.018009	0.00075		
Total	31	0.026934			

Appendix 37: Analysis of variance, concentration of copper in soil.

Variate: Cu					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	7	0.004628	0.000661	1.82	0.129
Residual	24	0.008717	0.000363		
Total	31	0.013344			

Appendix 38: Analysis of variance, concentration of mercury in soil.

Variate: Hg					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	7	0.000421	6.01E-05	0.87	0.546
Residual	24	0.001664	6.93E-05		
Total	31	0.002085			

Appendix 39: Analysis of variance, concentration of nickel in soil.

Variate: Ni					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	7	0.010723	0.001532	0.91	0.516
Residual	24	0.040465	0.001686		
Total	31	0.051188			

Appendix 40: Analysis of variance, concentration of lead in soil.

Variate: Pb					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	7	0.003124	0.000446	0.33	0.93
Residual	24	0.031995	0.001333		
Total	31	0.035119			

Appendix 41: Analysis of variance, concentration of selenium in soil.

Variate: Se					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	7	0.004488	0.000641	0.34	0.925
Residual	24	0.044785	0.001866		
Total	31	0.049273			

Appendix 42: Analysis of variance, concentration of titanium in soil.

Variate: Ti					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	7	0.531811	0.075973	8.79	<.001
Residual	24	0.207408	0.008642		
Total	31	0.739218			

Appendix 43: Analysis of variance, concentration of vanadium in soil.

Variate: V					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	7	0.026502	0.003786	0.79	0.6
Residual	24	0.114595	0.004775		
Total	31	0.141097			

Appendix 44: Analysis of variance, concentration of zinc in soil.

Variate: Zn					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	7	0.12826	0.01832	1.81	0.132
Residual	24	0.24297	0.01012		
Total	31	0.37123			

Appendix 45: Analysis of variance, concentration hot water extractible carbon in soil.

Variate: mg_HWC_kgsoil					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	7	7266.1	1038	3.35	0.012
Residual	24	7425.6	309.4		
Total	31	14691.7