THE EFFECT OF BIOCHAR ON IMMOBILIZATION AND PHYTOAVAILABILITY OF CHROMIUM, NICKEL AND LEAD IN SOILS AMENDED WITH SLAG

BY

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DECLARATION

I, Morakene Lambert Letsoalo, Students No: 11640531, hereby declare that this dissertation for Master of Science (MSc.) degree in Agriculture (Soil Science), titled "The effect of biochar on immobilization and phytoavailability of chromium, nickel and lead in soils amended with slag" is my own work and all the sources used or quoted have been indicated and acknowledge by means of reference. This dissertation has not been previously submitted at this university (University of Venda) or any other university.

_________________________ Date: 25 August 2020

Student: M.L Letsoalo

As the candidate's supervisor/co-supervisor, I agree with the submission of this dissertation.

Signature Date: 25 August 2020

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Signature_ Date: 25 August 2020

(Co-supervisor: Prof P Lukhele-Olorunju)

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LIST OF ABBREVIATIONS

AC	Acacia
C	Carbon
Ca	Calcium
CEC	Cation exchange capacity
CH_4	Methane
Cr	Chromium
EC	Electrical conductivity
Fe	Iron
H/C	Hydrogen to carbon ratio
K	Potassium
Ni	Nickel
O/C	Oxygen to carbon ratio
Pb	Lead
PL	Poultry litter

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ABSTRACT

Application of industrial wastes in agricultural fields as sources of plant nutrients has become a common practice in agriculture. In other countries and recently South Africa, steel plant slag (SPS) (an industrial waste) is used in agricultural fields as liming material. Steel plant slag is a potential source of plant nutrients, especially in areas where plant iron chlorosis is a problem. The use of SPS as liming material on agricultural soils seems viable despite the detrimental effects due to heavy metals contained in SPS.

The presence of heavy metals [chromium (Cr), nickel (Ni) and lead (Pb)] in higher concentrations in potential agricultural soils has negative effects to fauna and flora. Naturally soils possess traits that counter the accumulation of heavy metals, for example, texture and pH of a soil can play a role in the adsorption of heavy metals. An alternative strategy would be that capable of enhancing the metal-binding capacity of soil amendments such as biochar. In this study, the aim was to evaluate the effect the different types of texture, the efficacy of different types of biochar and rate of biochar on the concentration of chromium, nickel and lead. The greenhouse pot experiment was therefore conducted to investigate the effect of biochar on the immobilization and phytoavailability of chromium, nickel and lead in soils amended with slag.

A 2 x 2 x 4 factorial experiment was conducted with three factors consisting of two soils (sandy and clay), two biochar and four biochar rates (0 t/ha, 5 t/ha, 10 t/ha, and 20 t/ha). All the soil and biochar were thoroughly mixed. 4 kg of soils was placed in a pot. Slag was then applied to each pot at an amount equivalent to 15 g/kg (75 g per pot). The treatments were replicated four (4) times to give a total of 64 pots and were arranged in a completely randomized design (CRD) in a greenhouse.

The soils were analyzed for texture, exchangeable cations (Mg^{2+}, Ca^{2+}, K^{+}), pH, electrical conductivity and soil aggregates. Slag was analyzed for potassium, phosphorus, pH, electrical conductivity, total nitrogen, chromium, nickel and lead, and biochar was analyzed for organic carbon content, pH, electrical conductivity, calcium, magnesium, potassium and phosphorus.

The data showed that acacia and poultry litter biochar significantly ($P< 0.01$) immobilized and reduced the phytoavailability of Cr, Ni and Pd in the soil. For example, the application of 20 t/ha biochar in this study significantly decreased the concentrations of chromium, nickel and lead. The biochars immobilized the selected heavy metals from a range of 9.3% to 89.9% and reduced the phytoavailability from a range of 0.0248% to 8.1×10^{-8}%. Acacia and poultry litter biochar significantly increased the shoot dry biomass of spinach plant by 1.2% to 85.1%. Following the application of biochar to the soil, electrical conductivity and pH increased significantly (P< 0.01) from 24% to 22% and 11.3% to 44.7% respectively. Application of acacia and poultry litter biochar significantly (P <0.01) increased soil stable aggregates by 14.3% to 43.5%.

Application of acacia and poultry litter biochar reduced the toxicity of heavy metals (chromium, nickel and lead) in spinach by immobilizing and reducing phytoavailability of the heavy metals. Acacia and poultry litter biochar have positive effects on the soil pH, electrical conductivity, plant growth (shoot dry biomass of spinach) and soil stable aggregates.

Keywords: Acacia biochar, poultry biochar, chromium, lead and nickel.

1. INTRODUCTION

1.1 Background

Application of industrial wastes in agricultural fields as sources of plant nutrients has become a common practice in agriculture (Zaier *et al*., 2010). In other countries and recently South Africa, steel plant slag (SPS) (an industrial waste) is used in agricultural fields as liming material. Xian and Qing-Sheng (2006) have also recommended SPS as a potential source of plant nutrients, especially in areas where plant iron chlorosis is a problem. According to Rossouw (2009), the use of SPS as liming material on agricultural soils seems viable although the detrimental effects due to heavy metals contained in SPS on human health and environmental quality need to be considered. Chromium (Cr), nickel (Ni) and lead (Pb) are found in SPS and are reported by Herselman (2007) as having a potential threat to agricultural production systems, whereby Cr, Ni and Pb can adversely affect food quality, crop growth and/or environmental health. On the other hand, Cr and Ni are reported as having the potential to disqualify SPS as an ameliorant of agricultural soil (Rossouw, 2009).

Mobility of heavy metals in soils is the driving force for heavy metal uptake by plants. According to Cao *et al.* (2009), the mobility of heavy metals in the soil and as such, availability of heavy metals to the environment is dependent on the solubility of heavy metals in the soil solution. As a result, non-soluble heavy metals are unlikely to be toxic to plants. On the other hand, Menzies *et al.* (2007) highlighted the fact that availability and toxicity of heavy metals to plants is affected by several factors such as adsorption and desorption potential of heavy metals to the soil surface, precipitation potential of heavy metals and the dissolution potential of minerals containing heavy metals.

Soil acts as a binding agent for heavy metals and the physico-chemical properties of the soil influences the activities and concentrations of heavy metals in the soil solution, thereby directly affecting heavy metals phytoavailability. Layer silicate clays, hydr/oxides minerals and organic matter provide the variable charges responsible for binding heavy metals on the soil solid phase (Carrillo-Gonzalez *et al*., 2006), thereby controlling the availability of heavy metals to plants.

According to Flyhammar and Hakansson (1999) and Dube *et al*. (2001) soil pH, soil redox conditions and the concentration of salts and complexing agents in the soil are the main factors controlling the mobility of heavy metals in the soil. All other factors are either directly or indirectly related to the above-mentioned factors (pH, Redox & complexing agents). Generally, sorption of heavy metals to the soil solid phase is low at soil pH of less than 5.0 and increases as soil pH increases (McLaughlin *et al.*, 2000 & Dube *et al*., 2001). Nonetheless, some heavy metals form oxy-anions in solution (i.e. CrO_4^{2-}) and sorption to the soil solid phase becomes high at low pH. Organic or inorganic ligands can also interact with heavy metals and change prevailing speciation or charge of heavy metals. On the other hand, concentration of heavy metals in the soil solution also plays a role in the selectivity of heavy metal sorption to the soil solid phase. Heavy metal sorption can also be influenced by oxidation-reduction (redox) reactions which affect the formation and reactivity of some soil oxides (i.e. Fe and Mn oxides) responsible for heavy metal sorption. Redox conditions also control the chemical speciation of several heavy metals (i.e. Cr) thus affecting the sorption of that particular heavy metal (Dube *et al*., 2001).

Biochar and other carbonaceous sorbents have been found to reduce the bioavailability of a number of organic contaminants in soils and sediments (Cornelissen *et al*., 2006). Biochar is a fine-grained and porous substance produced by pyrolysis of biomass at low to medium temperature of 450 to 650^{o}C under oxygen –limited conditions (Sohi *et al*., 2010). Since biochar has a porous structure, it contains various functional groups that are effective in the adsorption of heavy metals (Liu *et al.*, 2015).

The effectiveness of biochar is affected by chemical and biological soil properties and this will affect heavy metal redistribution among solid-phase components. The effect of biochar depends on soil types, type of feedstocks used for charring, pyrolysis conditions and the amount of biochar applied to the soil (Park et al., 2011). Biochar decrease metal mobility and bioavailability because of its ability to modify chemical soil properties such as cation exchange capacity and soil pH, providing situations that are suitable for heavy metal immobilization and reducing heavy metal uptake by plants (Al-Wabel., 2015).

1.2 Problem statement

Application of slag as a fertilizer and liming material in agricultural fields is a common practice in South Africa. However, application of slag is normally accompanied by the addition of heavy metals which are toxic when taken up by plants. As a result, it is important and critical to develop approaches or techniques that can immobilize the heavy metals from the soil hence reduce photoavailability of heavy metals to the plants.

1.3 Motivation

In South Africa, the price of synthetic fertilizers is always rising (Farhad *et al*., 2009) which makes it very difficult for small scale and commercial farmers to always afford synthetic fertilizers. Slag is a cheaper source, which can be used as a soil amendment and a source of nutrients. Therefore, if a successful approach or technique is developed to immobilize the heavy metals in slag, it will help in reducing the cost of purchasing lime and fertilizers. In addition this will also help in disposing slag more efficiently with the least or no effect on plants, human health and the environment.

1.4 Objective

1.4.1 Main objective

To evaluate the effect of biochar on immobilization and phytoavalability of Cr, Ni, Pb in soils amended with slag using spinach as a test crop, as well as the effect on soil pH, EC and aggregate stability.

1.4.2 Specific objectives

The specific objectives were to determine

i. soil extractable Cr, Ni and Pb (Immobilization)
ii. concentration of heavy metals (Cr, Ni, and Pb) in spinach (Phytoavailability)
iii. Spinach plant biomass yield
iv. Soil pH, EC
v. Soil aggregate stability.

1.5 Hypotheses

i. The application of biochar in soil amended with slag would immobilize heavy metals.

ii. The application of biochar in soil amended with slag would reduce phytoavailability of heavy metals to the crop (spinach).

iii. The application of biochar in soils amended with slag would have an effect on pH, Electrical Conductivity (EC) and soil aggregate stability.

2. LITERATURE REVIEW

2.1 Chromium and the effect on plant environment

In soils, chromium occurs naturally as chromite ($FeCr_2O_4$) which is insoluble and subsequently immobile (Becquer *et al*., 2003). However, chromium can occur in various forms (oxidation states of -2 to +6) whereby the +3 [Cr(III)] and +6 [Cr(VI)], oxidation states are the most stable forms and are of environmental concern. According to Fendorf (1995), Fendorf and Li (1996) and Becquer *et al*. (2003), the mobility and phytoavailability of chromium depend on the prevailing oxidation state. The trivalent chromium [Cr(III)] occurs in soils as a cation (i.e. $CrOH^{2+}$) and it is easily adsorbed to the soil colloid. As a result, Cr(III) is less mobile and less available to plants. Nonetheless, the presence of manganese oxides in the soil can oxidize Cr(III) to Cr(VI) (the hexavalent form) (Chen *et al*., 2007).

The hexavalent chromium occurs as anion (i.e. $HCrO_4^-$) and is highly mobile and available to plants. However, Cr(VI) can also be reduced by organic matter, Fe(II) and sulfites to Cr(III). Reduction of Cr(VI) by Fe(II) is considered as advantageous over that of organic matter and sulfites because the reduced Cr(III) forms a precipitate which cannot be oxidized back to Cr(VI) by conditions such as the presence of manganese oxide (Chen *et al.*, 2007). Hexavalent chromium can also be adsorbed by hydrous oxides of Al [i.e. $Al(OH)_3$] and Fe [i.e. FeO(OH)] whereby Cr(VI) will form the inner-sphere of the resulting compound (Fendorf, 1995). As a result, chromium will be trapped and become immobile, hence less available to plants. This process makes chromium less accessible to plants.

The reduction in the uptake of essential nutrient elements is the primary negative effect of Cr uptake by plants. Chromium does not possess specific mechanisms for its uptake and therefore, the uptake of this heavy metal (Cr) is through carriers which are also used for the uptake of other elements essential for plant metabolism. As a result, Cr uptake reduces the uptake of other essential elements such as sulphate (Shanker *et al*., 2005). In a study conducted by Sharma *et al.* (1995), grain yield of wheat was severely affected by the presence of Cr(IV) in the soil (Table 1). Maximum grain yield was found at the nil Cr(V1) level and decreased as the supply of chromium increased.

Organic matter plays an important role in the mobilization of Cr in the soil, because the addition of organic compound such as compost or manure can add electrons or act as electron donor to provide energy for the soil micro-organism involved in reducing Cr (VI) to Cr (III), chromium (VI) is mobile while Cr (III) is relatively immobile. The immobile Cr may become available, if decomposition of organic matter can take place. Oxidation and reduction of Cr depend on factors such as aeration, soil moisture content, wetting and drying, microbial activities, pH, and availability of electron donor and acceptors (Banks., *et al*., 2006). Chromium (VI) is highly soluble in water and forms strong divalent anions oxidants (Shanker *et al*., 2005)

Table 1: Effect of varying chromium supply on grain yield of wheat plants (g dry wt/ plant) at 97 days' growth (82 days of chromium exposure) (Sharma *et al.* 1995)

Cr(IV) levels (mM)	0.00	0.05	0.10	0.25	0.5	1.0
Grain yield (g dry wt./plant)	2.11	0.39	0.32	0.19	0.16	00

2.2 Nickel and the effect on plant environment

Nickel commonly exists in soil in several forms such as inorganic crystalline mineral or precipitates, complexed or adsorbed on organic carbon surface or on inorganic cation exchange surface, water soluble, free ion or chelated metal complexes in soil solution (Cempel and Nikel., 2006). In acidic soil, nickel exists in the form of nickelous ions [Ni(II)]. In neutral to slightly alkaline soil, nickel precipitates as nickelous hydroxides [$Ni(OH)_2$], which is a stable compound. In basic conditions, it forms nickelite ion ($HNiO_3$), which is soluble in water. $Ni(O_2H)_6^{2+}$ which is in hydrated form, is the most common form of nickel in soil solutions (Yusuf *et al*., 2011). Nickel in oxidized and alkaline conditions exists as nickel-nickelic oxide (Ni_3O_4) that is stable (Wuana and Okieimen., 2011). Nickel compounds are commonly absorbed to the soil particle, and as a result they are immobile in soil. In acid soil, it becomes mobile. As reported by Pandaa *et al*., (2007), uptake of Ni^{2+} increases with increasing pH up to 5.0 and decreases if the pH goes further up to 8.0. This is as a result of calcium ion (Ca^{2+}), which lowers the adsorption of Ni^{2+} on soils (Yusuf *et al*., 2011).

The uptake of Ni by plants is mainly through the root system via passive diffusion and active transport. The uptake of Ni by plants depends on Ni concentration in the soil or nutrient solution and the variety of plant species. Nickel can as well enter into the plants via leaves. For example, Ni applied on leaves of *Helianthus annus*, 37% of the total amount was translocated to other parts of the plants. Variably, Fe^{3+}> Co^{2+}> Ca^{2+}> Mg^{2+}> NH^{4+}> K^{+} > Na^{+}, have the inhibitory effect on absorption and translocation of Ni^{2+} from roots to shoots. Organic acids and amino acids are potential metal chelators, that likely facilitate Ni translocation through xylem (Yusuf *et al*., 2011).

Toxicity of Ni to plants can lower the rate of physiological/biochemical processes such as accumulation of chlorophyll (chlorosis), photosynthesis and transpiration, which lead to weak plant growth, yield depression and may even be accompanied by reduced nutrient uptake and disorders in plant metabolism (Yang *et al.* 1996 & Baytak and Turker, 2006). According to Yang *et al*. (1996), the presence of high concentration of Ni in the soil can lower the dry matter of both the roots and shoots (Figure 1). Due to the fact that Ni is mobile and that plants take up nickel readily, there is danger of its excessive accumulation in soils and the devaluation of plant products. Excessive accumulation of nickel is a relevant issue particularly in crops used for direct consumption.

According to Michael (1996), nickel (Ni) is not essential for plant growth and development and the level of nickel in soils is generally low, though it can be increased by human activities. Nickel can contaminate soils mainly through industrial processes and waste disposals, especially in areas that are near the processing operations (Michael, 1996).

According to Molas & Baran (2004), Ni is known primarily for its divalent compounds since the most stable oxidation state of the element is +2. However, other compounds which have Ni in the oxidation state between -1 to +4 do exist. The difference in Ni uptake by plants based on the prevailing oxidation state of Ni is not evident (Molas & Baran, 2004). As a result, the variation of the prevailing speciation of Ni in the soil plays a small (if any) role on the availability of Ni to plants.

Nickel occurs both in the form of a free ion and in the form of ionic complexes (combination with organic or inorganic ligands) even in the natural environment. Molas and Baran (2004), assumed that plants absorb Ni in the form of a complex with other ions. However, it was later realized that chelating agents released from the complex might also penetrate the roots (solely) and bring about the thought that Ni was taken up as a complex (as it is in the soil). The uptake of Ni by plants in the form of a free ion was confirmed by the fact that Ni:chelating agent ratio in the plant tissues was different from that in the soil. It is therefore reasonable to believe that the availability of complex-Ni is determined by the stability of the prevailing complex, whereas free ion-Ni is always available (Molas and Baran, 2004).

In trace amounts, Ni is a necessary micronutrient element for animals and higher plants, however, in higher concentrations Ni becomes toxic (Baytak & Turker, 2006). Inhalation of Ni can damage the vessels of the heart muscle, kidneys and central nervous system and reduce the immune capacities of animals. Nickel inhalation may also cause disorders such as eczemas (skin disorder) and allergic inflammation of mucous membranes and lungs (Baytak & Turker, 2006). According to Kabata-Pendias (2004), the risk of a given trace element to the environment is a function of its mobility and phytoavailability.

The movement of Ni in soil can be influenced by soil clay content, surface area, pH and cation exchange capacity (Kabata-Pendias, 2004). Plants absorb less amounts of Ni when the soil has high percentage clay. The complexation (chelation) of Ni in soil by organics can also influence its uptake by plants and its phytotoxicity. The uptake can either be increased or reduced depending on the chemical nature of the chelating agent as well as on the stability of the complex. Soil solutions contain a variety of low molecular weight dissolved organics that come from the breaking down of plant residues and exudation from plant roots. The complexation of Ni by these organic ligands can play an important role in controlling mobility of Ni.

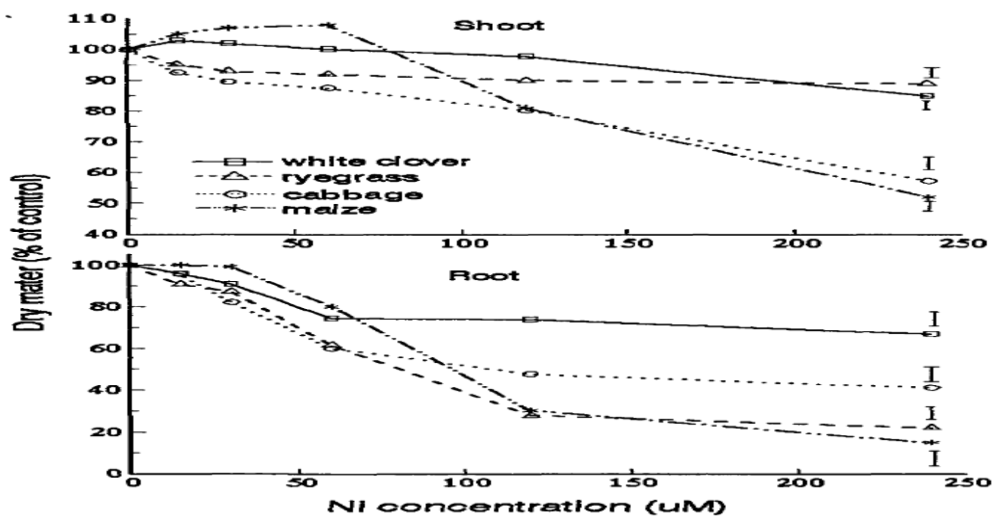

Figure 1: Shoot and root dry matter (DM) of four plant species grown with different levels of Ni in nutrient solution (Yang *et al*., 1996).

2.3 Lead and the effect on plant environment

According to Pitchell *et al*., (1999), lead (Pb) occurs in soils as insoluble precipitation which is unavailable for plant uptake. Lead is one of the heavy metals that keeps on accumulating in soil if present and it does not easily degrade (Kumar and Kumar, 2015). Lead is released in the soil in the form of ionic lead [Pb (II)], lead oxide and hydroxides and lead metal oxyanion complexes. The most common and reactive form of lead is lead (II), and it forms mononuclear and polynuclear oxides and hydroxides. The insoluble Pb compounds are lead phosphates, lead carbonates and lead hydroxides. The ionic compounds of lead (II) are Pb^{2+}, $SO^{2-}{}_4$, whereas Pb (IV) tends to be covalent [tetraethyl lead, $(C_2H_5)_4$]. Lead has limited solubility because of its complexes with organic matter, sorption on oxides and clay, precipitation, carbonates, hydroxides and phosphate (Raymond and Felix, 2011).

Lead (Pb) is regarded primarily as pollutant metal because it is not essential to both plant and animal nutrition (Michael, 1996, Brennan & Shelley, 1999 & Carrillo-Gonzalez *et al*., 2006). However, it can be taken up by plants from the soil and it has toxicity potential (Wang *et al*., 2006). Lead has various oxidation states but the one which is more comfortable (stable) with many environmental conditions is the mobile and toxic Pb^{2+} (Raungsomboom, 2008). For example, at room temperature, lead(IV) Chloride ($PbCl_4$) decomposes to give lead(II) chloride ($PbCl_2$) and chlorine gas (Cl_2), and lead(IV) oxide (PbO_2) decomposes on heating to give lead(II) oxide (PbO) and oxygen (O_2) (Raungsomboom, 2008).

Although Pb^{2+} is the dominant form of lead in the soils, Pb^{4+} can be found occasionally in some highly oxidized soils. Since Pb enters the soil in different forms, its reactions may differ widely from place to place. Once Pb is in the soil, lead may react with anions such as phosphate (PO_4^{3-}), sulphate (SO_4^{2-}) or carbonate ion (CO_3^{2-}) to form less soluble salts such as lead carbonate [$Pb_3(OH)_2(CO_3)_2$] and chloropyromorphite [$Pb_5(PO_4)Cl$] (Yoon, 2005, Chrysochoou *et al.*, 2007 & Cao *et al*., 2009). Soil organic matter (humic and fulvic acids) can also form complexes with Pb which will then be adsorbed onto soil solids and as a result immobilize Pb (Yoon, 2005).

Lead can significantly reduce the growth and development of plants as observed in the experiment performed by Islam *et al.* (2007) (Figure 2). In the same study (Islam *et al.*, 2007) reduction in both plant height and root length was also observed (Figure 3).

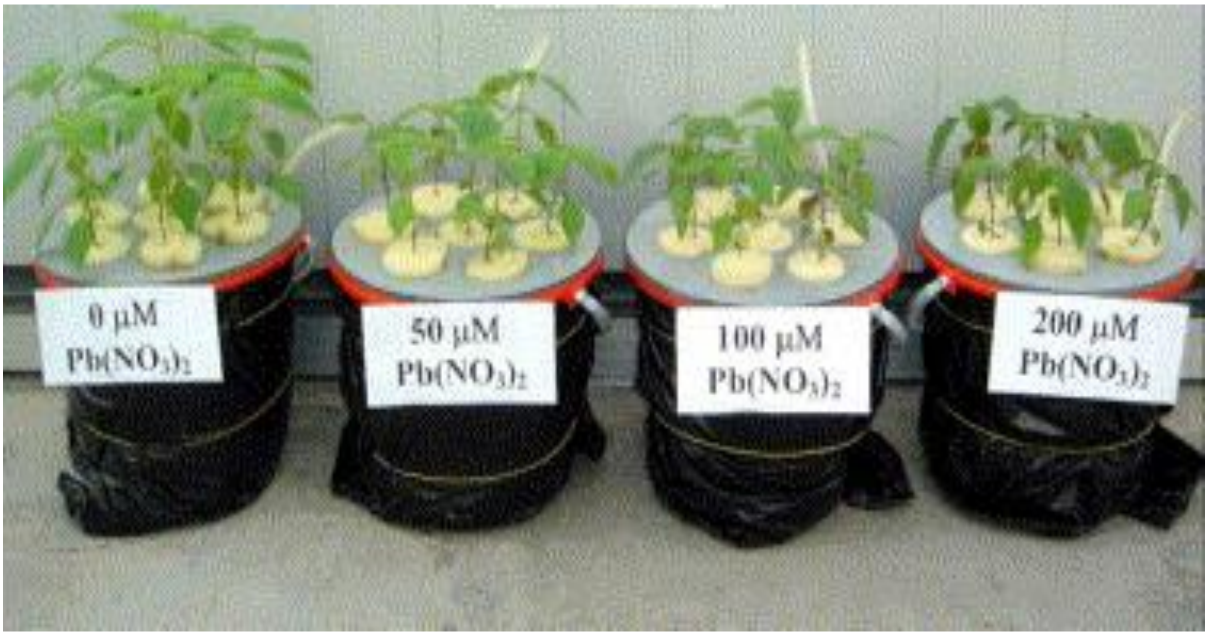

Figure.2: Effects of Pb on the growth of *Elsholtzia argyi* (Islam *et al*., 2007)

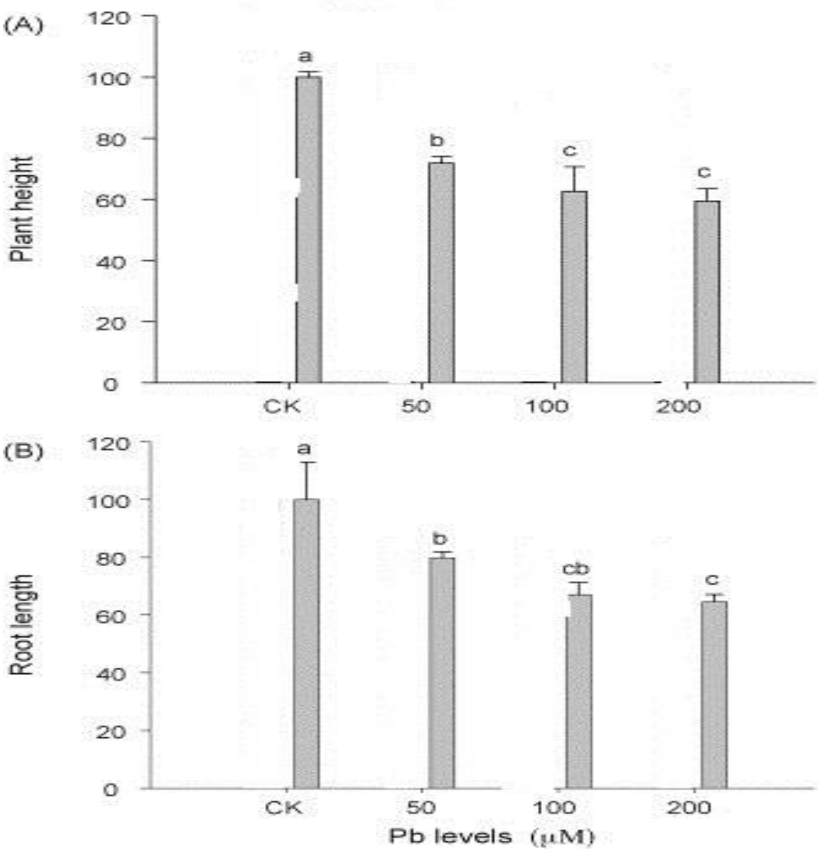

Figure 3: Effect of Pb on the height of plants and length of plant roots (Islam *et al*., 2007)

2.4 Slag use as soil amendment

Slag is a mineral waste resulting from steel industry and is used for civil engineering such as road construction (Ettler et al., 2009) and can also be used as both soil amendment and fertilizer (Torkashvand., 2011). The properties of slag differ according to changes in the process parameters and the physical properties of slag are determined by the process of granulation and blast furnace parameter while the chemical properties are determined by desirable properties of the need (Hiltunen & Hitunen 2004). Large amounts of metallic particles are removed through the use of sieve and magnetic roller during the refining process (Van der Waals & Claassens 2002).

Slag is enriched in toxic elements, in particular heavy metals such as lead (Pb), copper (Cu), Zinc (Zn) and metalloids Arsenic (As) and Antinon (Sb) (Ettler et al., 2004). Slag is also a source of nutrients for plants, such as phosphorous (P), potassium (K), calcium (Ca) magnesium (Mg), iron (Fe) (Torkashvand., 2011) and silicates (Van der Waals & Claassens 2002).

According to Wang *et al*., (2014), slag used as soil amendment can improve rice production (grain yield) and can mitigate CH_4 emission which contributes to greenhouse effect. The increase in rice yield is as a result of availability of inorganic nutrients such as silicon, phosphate, manganese and soluble iron. Slag application increase soil properties such as soil organic carbon, total nitrogen, total phosphorous, total iron, available P_2O_5, available SiO_2 and ferric iron. Torkashvand (2011) reported that the application of slag increased shoot dry matter and uptake of Fe, Mn, K, P uptake in sorghum. Torkashvand (2011), further indicated that slag can be used as a source to correct iron chlorosis in different crops, especially in calcareous soils. For example, the use of slag as a soil amendment in calcareous soils increased the dry matter of sorghum and corrected iron chlorosis (Torkashvand, 2011).

Ning *et al*., (2014), reported that slag applied as soil amendment maintained mesophyll cells and improved brown spot resistance in rice production. Slag as soil amendment increased yield in crops like oats, wheat, corn, soybeans and proved to be a good liming material in the soil. Munn., (2005), and Li *et al*., (2015) found that slag can ameliorate soil acidity better than phosphogypsum because slag can increase pH and base saturation, and it decreases exchangeable acidity of the different segments in the soil profile to different degrees. Li *et al*., (2015), also reported that slag was more effective in suppressing arsenic than calcium hydroxide because slag is able to immobilize arsenic due to its significant increase of Fe concentrations.

Slag is enriched with toxic elements in particular heavy metals (Ettler *et al*, 2004), such as lead, copper, zinc, metalloids arsenic, antimony, chromium and nickel (Van der Waals and Classens., 2002). Ryser and Emerson (2007) reported that heavy metals found in slag can have a strong effect on plant structure such as reducing root tissue density. Reduction of root tissue density leads to plants being susceptible to drought (Ettler *et al*, 2004). Above-ground accumulation of heavy metal in food is one of the most important aspect of food quality assurance and as such, accumulation of heavy metals can have adverse effect on exports and markets in general (Radwan and Salam, 2006). On human beings, heavy metals have adverse effect on kidney, bone disorders, prostate and breast cancer, disturbances of male fertility and pregnancy disorders (Thomas *et al*., 2011). The presence of heavy metal in the soil can also have negative impact on soil enzyme activities such as arylsulfatase, protease, cellulose, dehydrogenase and invertase (Xian *et al*.,2015).

In South Africa, the use of slag has increased in the sugar industry in Kwazulu-Natal province, because slag contain high content of silicate and sugarcane requires high amounts of silicate. Silicate in sugarcane promotes certain insects' resistance (Meyer., 2003). In the Eastern Highveld of South Africa, slag is also used to ameliorate soil acidity. The soils in Eastern Highveld are sensitive to acidification because of sandstone dominated parent material, (Radwan and Salama, 2006). This leads to formation of sandy soils with low buffer capacities, hence the use of slag as agricultural lime in this area. The amount of slag that is sold or used by companies in South Africa is not known and FSSA (Fertilizer Society of South Africa) do not keep statistics of the usage of slag in the agricultural industry (Van der Waals and Claasens, 2002).

2.5 Biochar

2.5.1 What is biochar

Biochar is a natural occurring fine grain and highly porous form of charcoal derived from pyrolysis of biomass under partial or total absence of oxygen (Figure 4) (Cao *et al*., 2009). Biochar differs from charcoal because, charcoal is mainly used for fuel heating process, while biochar is used as soil amendments that improve soil properties (Figure 5) (Lehmann and Joseph., 2009). De Pasquale *et al*., (2012), defined biochar as a carbon rich substance that is created by heating organic matter under low-oxygen conditions and is applied to the soil to improve fertility. Lehmann *et al.*, (2015), described biochar as carbon-rich product of the thermochemical conversion of biomass in an oxygen- limited environment. According to Lehmann and Joseph., (2009), the chemical nature of biochar products depends on pyrolysis condition, type of biomass used. Biochar can be produce by slow pyrolysis and fast pyrolysis (Chowdbury *et al*., 2016). Biochar has a high porosity, has a basic properties with pH less than 7, it can sequestrate carbon in the soil and has long-term carbon storage for many years (Sohi *et al*., 2010). Futher more, according to Brewer *et al* (2009), biochar contains Ca, K, P and Mg.

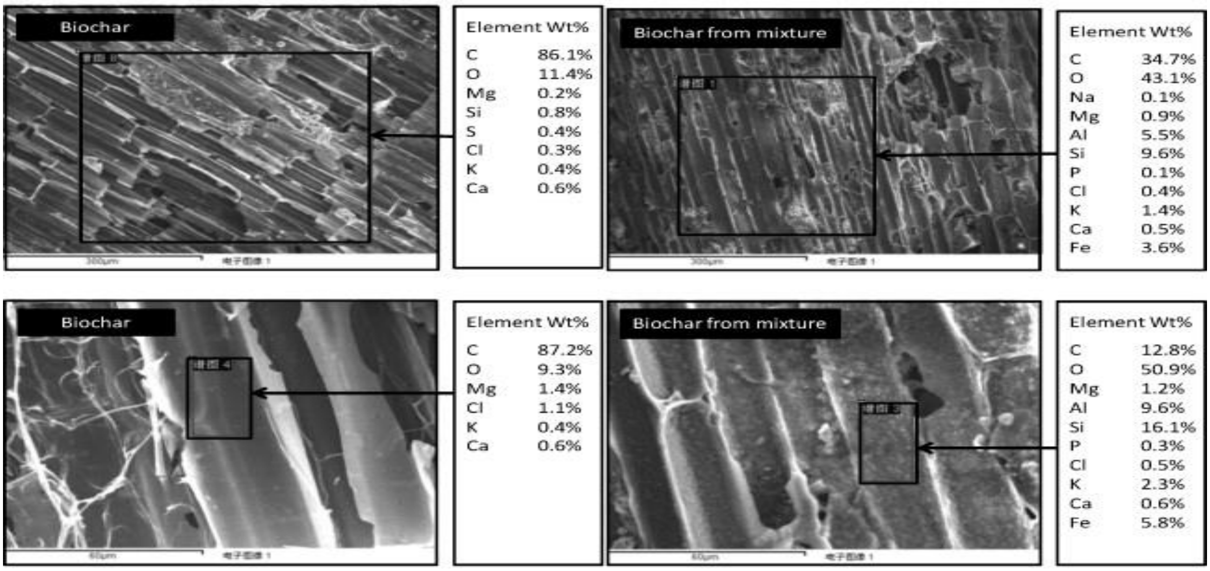

Figure 4: Scanning electron micrographs image and associated energy dispersive X-ray qualification of biochar and biochar separated from mixture (Xu., *et al* 2013)

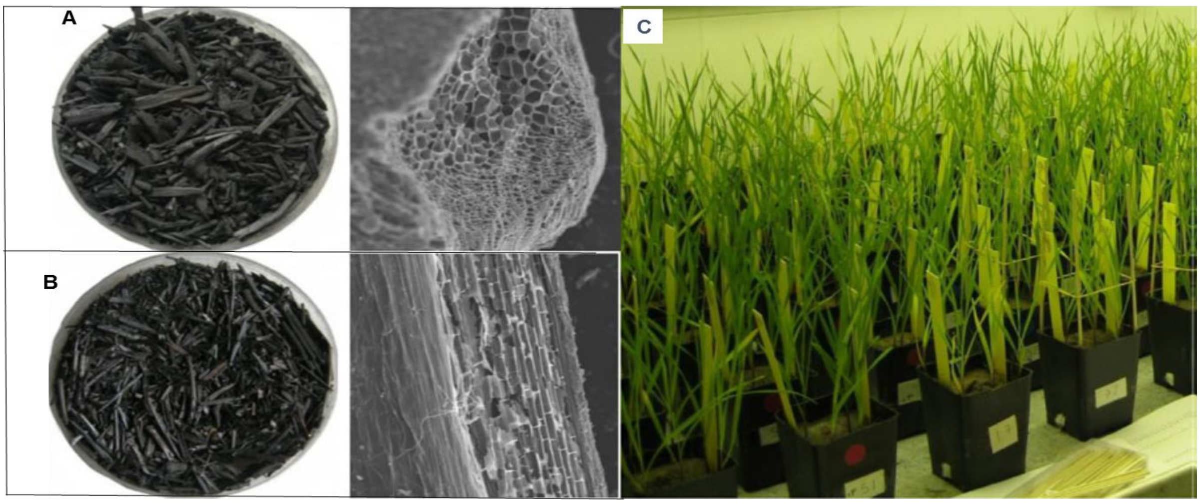

Figure 5: ***A*** Olive tree pruning, ***B*** wheat straw biochar and ***C*** Wheat plants in the growth chamber (Alburquerque *et al*., 2013).

2.5.2 Production of biochar

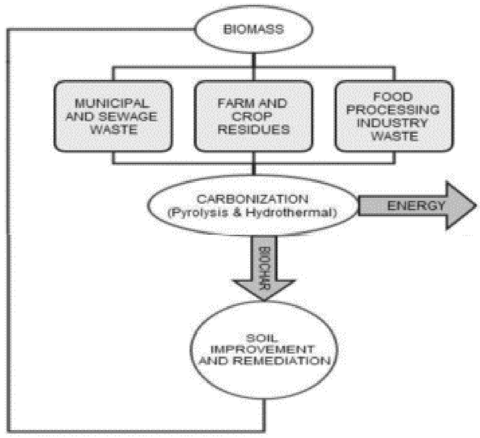

Figure 6: Concept of waste utilization for biochar production, soil improvement and remediation (Parmar *et al*., 2014).

The production methods of biochar are mainly divided into pyrolysis, hydrothermal carbonation and microwave carbonation (Figure 6) (Yang *et al*., 2019). Demirbas (2004) also reported the description of the reaction mechanism of biochar formation as the following three steps:

First step: Biomass $\longrightarrow$ Water + Untreated residue

Second step: Untreated $\longrightarrow$ $(\text{Volatile + Gases})_1 + (\text{Char})_1$

Third step: $(\text{Char})_1$ $\longrightarrow$ $(\text{Volatile + Gases})_2 + (\text{Char})_2$

According to Yang *et al*., (2019), different preparation methods of biochar affects physical and chemical properties such as yield, ash, specific surface area, pore structure, type and number of functional groups and CEC. Biochar can be produced from different feedstock, such as cowpea, peanut, maize, tomato, wheat, oak wood, cornstover and poultry litter (Lehmann *et al.*, 2011), grassland species (forbs, grass and legumes) (Van de Voorde *et al*., 2014) and animal manures (Tang *et al.,* 2013). According to Jeffery *et al* (2011), biochar produced from different feedstock, under the same pyrolysis condition can vary on their impact on crop and soil.

Pyrolysis is the process of thermochemical decomposition of material at elevated temperature in the absence of oxygen (Bridgwater, 2004). Pyrolysis yields variable amount of CO_2, volatile oils tarry oils, H_2, CO, CH_4 and biochar (Tripathi *et al*., 2016). There are slow pyrolysis and fast pyrolysis. The slow pyrolysis takes place at a temperature of 50-300^{o}C (Novotny *et al*., 2015), while fast pyrolysis is heated at high temperature of 300-700^{o}C (Brewer *et al*., 2009). Pyrolysis can impact on the extent of the aromatic structure formation in biochar. According to Joseph *et al* (2010), low aromatic results in high surface functionality compared to material characterized by large aromatic that leads to higher CEC in the soil. Slow pyrolysis has greater aromatic regions than pyrolysis (Brewer *et al*., 2009). The biochar produced from fast pyrolysis have high surface area and porosity and O/C and H/C ratio compared to slow pyrolysis (Dutta *et al*., 2012). Low pyrolysis on the other hand have favorable greater recovery of C and several nutrients such as N, K and S and that are increasingly lost at higher temperature (Keiluweit *et al*., 2010) The increase in temperature during pyrolysis, generally will result in decrease in biochar yield, increase in ash content, increase in CEC and increase in surface area (Meng *et al*., 2013).

According to Parmar *et al*., (2014), the process of thermo-chemical decomposition is done in subcritical aqueous solution it is termed hydrothermal carbonization. Hydrothermal carbonization is a thermo-chemical process where organic matter is converted into carbon rich products (Parmar *et al*., 2014)

2.5.3 Properties of biochar

Biochar contains compounds such as polycyclic aromatic hydrocarbons, cresol, xylenes, formaldehyde, acrolein and toxic carbonyl compounds that contributes to bactericidal or fungal activities (Joseph *et al*., 2010). Joseph *et al*., (2010) also mentioned that porous structure of biochar is likely providing microorganisms with a highly suitable habitat to colonise. Biochar acts as the electronic donor and provides energy source for the soil microorganism involved in reducing heavy metals (Choppala *et al*., 2016).

According to Jeffery *et al* (2011), biochar produced from different feedstock, under the same pyrolysis condition can vary on their impact on crop and soil. This is as a result of differences in chemical composition of the feedstock such as lignin, cellulose content (Streubel *et al* 2011) and carbon from different types of lignocellulosic material (Xie *et al.*, 2015).

Biochar that is produced through fast pyrolysis is found to be more reactive and porous that those from slow pyrolysis (Zanzi *et al*., 1996). The adsorption capacity of biochar to remove the contaminants is attributed by the existence of the functional group (Alkurdi *et al*., 2019).

The functional group such O/C ratio, polarity indices (O + N)/C and zeta potential are important factors in determining the potential of biochar to remove contaminants (Samsuri *et al*., 2013). When protonation of functional groups such as carboxyl, ammine and hydroxyl take place, this will results in overall positive charge on the biochar of negatively charged contaminants such as antimonates (Alkurdi *et al*., 2019).

Biochar's surfaces have different oxygen containing functional groups, such as hydroxyl, anhydride, carboxylic acid, keton, quinone, ether lactone, pyrone, catechol and hydroxyketone. It also contains hydrophilic and hydrophobic sites which are both acidic and basic sites (Mohan *et al*., 2015). Oxygen functional groups allows water penetration into the solid biochar walls. The presence of structural oxygen means that water and dissolved pollutants can penetrate the pore surface and reach large portions of the solid volume (Mohan *et al*., 2015). Carboxyl and hydroxyl groups on biochar are generally the main groups that contribute to the sorbent surface and co-ordinate between heavy metals. Sorption of metal ions generally happens because of biochar surface chemistry and surface area of the sorbent. Heavy metals can be bound to the sorbent surface through functional groups complexation, ion exchange adsorption or co-precipitation with mineral components (Xu et al., 2016).

The morphology of biochar resembles that of the feedstock, for example wood biochar has the exoskeleton of the tracheids, whereas the chicken biochar has a heterogeneous structure (Joseph *et al*., 2010). The pH and EC of the biochar depend on the content and composition of the mineral fraction, which in turn depend on the type of the feedstock used (Singh *et al*., 2010). The nutrient content from biochar depends on the pyrolysis condition and feedstock type (Singh *et al*., 2010). If biochar is produced from low pyrolysis condition, it results in the pores being partially blocked by volatile organic compounds that leads to the decrease in nutrient retention and adsorption capacity (Figueiredu *et al*., 2017). The availability of nitrogen from biochar has been shown to depend on the final temperature of pyrolysis, heating rate and type of the feedstock (Amonette and Joseph, 2009). Fine dust fraction biochar has better sorbent for wide range of trace hydrophobic contaminants than large sized biochar dust particles (Bashir *et al*., 2017). Biochar has high reactive surface that attributes to the presence of wide range of functional groups and some which are pH dependent (Amonette and Joseph, 2009). Aged biochar possesses higher surface area than a fresh biochars (Herath *et al*., 2013).

2.5.4 Benefits of biochar

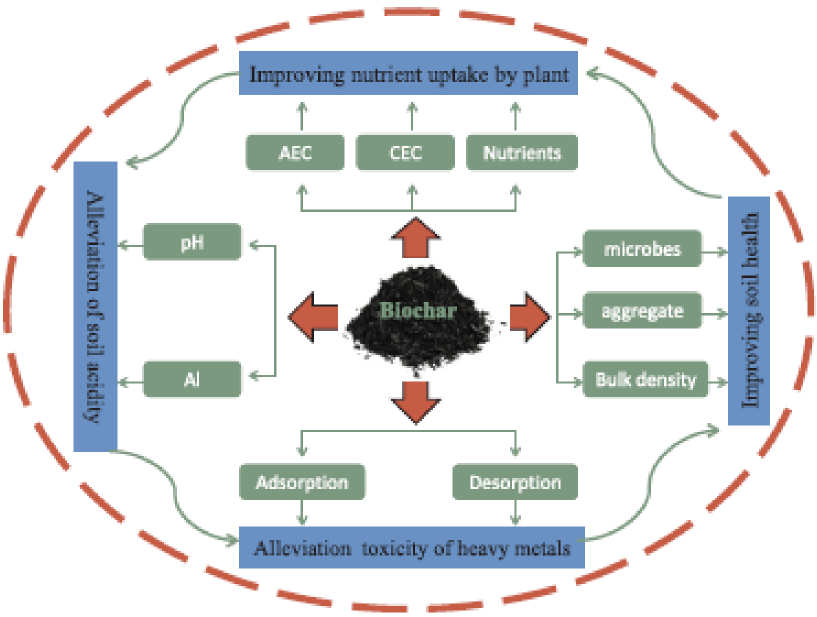

Figure 7: An overview of biochar affecting soil properties for plant production (Shaaban *et al*., 2018).

Biochar plays an important role in soil properties (Figure 7), which positively affects plant growth (Shaaban *et a*l., 2018). Biochar has shown its effectiveness at increasing soil pH (Berek, 2019). According to Fidel *et al*. (2017), the alkalinity of biochar originated from inorganic, organics and low- pK_a organic structure. The basic cations of biochar are in the form of carbonate and oxide (Berek, 2019). The basic cation of biochar attribute to the replacement of the function of lime in producing the ion OH^- to neutralize excess ion H^+ resulting in an increase in soil pH (Berek and Hue, 2016). Increasing soil pH results in reducing the exchangeable Al and Fe which in turn increase P availability in acid soil (Berek, 2019). Increasing soil pH attributes to the alleviation of Fe, Mn and Al toxicity and decreasing the P fixation (Berek and hue, 2016).

Biochar is characterized by micro and macro size particles that compete with clay and metal oxides for microbial attachment and retention (Shaaban *et al*., 2018). According to Toju and Sato (2018), fungi (arbuscular and ectomycorrhizal) play an important role in plant nutrients, which positively affects the plant growth.

Increase in pH influence the CEC in many soils (Slavich *et al*., 2013). Biochar increase the soil pH which subsequently increase CEC and nutrient retention capacity of the soil (Zornoza *et al*., 2016). Carboxyl on biochar surface increase CEC and subsequently decrease nutrient leaching (Zhu *et al*., 2017). The oxygen contained in the functional groups of biochar also contributes to the increase of CEC of soil because of its negative charge surface (Liu *et al*., 2018). Biochar produced by various biomass is a good source of macro nutrients (Ca^{2+}, K^{+} and Mg^{2+}) and micronutrients (Ahmed *et al*., 2016). Application of biochar in the soil has been reported to reduce N loss in the soil (Liu *et al*., 2018). This is subsequently as a result of the increased adsorption of NO_3^- on the functional group (-O-), aromatic ring carboxyl (C=O) and hydroxyl (-OH) (Shaaban *et al*., 2018). Biochar application to the soil, reduced N leaching by holding NO_3^- and thus increasing crop use efficiency (Shaaban *et al*., 2018).

Biochar that is produced through fast pyrolysis is found to be more reactive and porous than those from slow pyrolysis (Zanzi *et al*., 1996). The adsorption ability of biochar to remove the contaminants (heavy metal) is attributed to the existence of the functional groups (Alkurdi *et al*., 2019). The functional group such as O/C ratio, polarity indices (O + N)/C and zeta potential are the important factors in determining the potential of biochar to remove contaminants (Samsuri *et al*., 2013). When protonation of some functional groups such as carboxyl, ammine and hydroxyl take place, it results in overall positive charge on the biochar surface, thus favoring the adsorption of negatively charge contaminants such arsenates and antimonates by the biochar (Alkurdi *et al*., 2019).

The application of biochar in the soil has been shown to modify some chemical properties of the soil, such as CEC and soil acidity (Figure 7), which provides the circumstances for heavy metal immobilization and subsequently reducing their plant availability (Park *et al*., 2011). Biochar has surface functional groups and adsorption sites that could increase the soil CEC and consequently increase the metal exchange capacity of the soil through the formation of complexes with cationic heavy metal (Al-Wabel *et al*., 2015). The immobilization of the heavy metal by biochar, might be possible due to mineral components such as carbonate, silicate and phosphate that results in precipitation of heavy metals (Park *et al*., 2011).

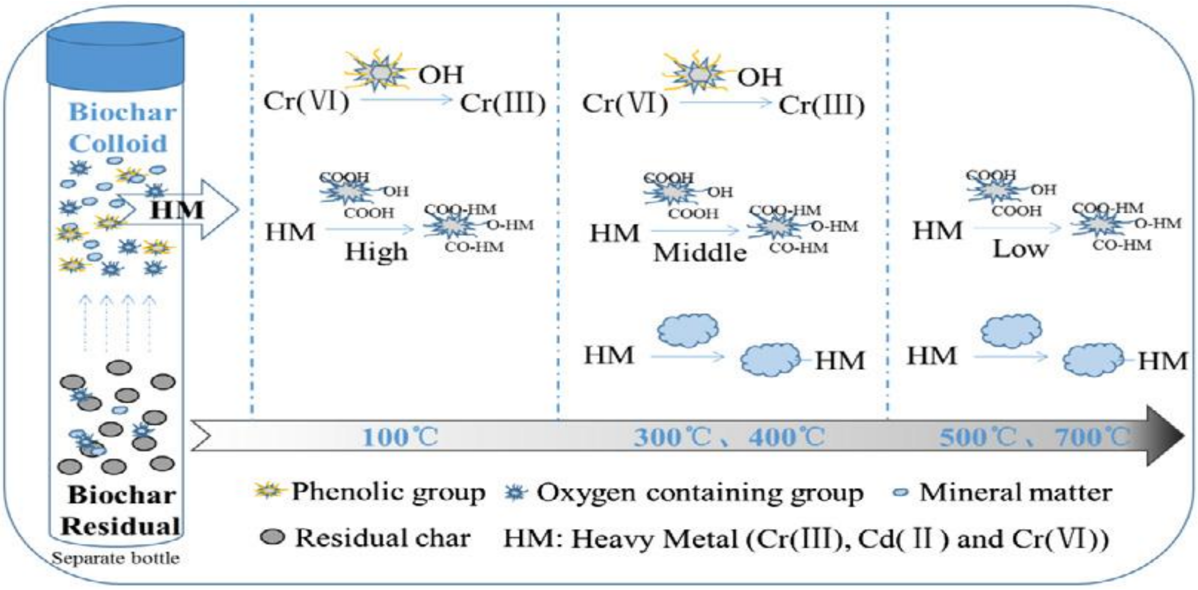

Figure 8: Removal of heavy metals by biochar (Quian *et al.*, 2016)

Biochar has functional groups, especially the O-containing functional groups (Figure 8) such as hydroxyl and carboxylic groups that can adsorb heavy metal through electrostatic interaction (Mukherjee and Lal, 2014). According to Yang *et al*., (2019), electrostatic interaction refers to the electrostatic adsorption between the biochar surface charge and heavy metal ions (Figure 8). When the pH of the soil solution is more than the charge point of the biochar, the negative charge of the biochar surface and the positively charged heavy metals results in electrostatic adsorption (Sohi *et al*., 2010). Some heavy metals such as Pb, Cr and Cu has the competitive adsorption that is influenced by the affinity of metals for biochar surface (Figure 8) (Antonio *et al*., 2014).

Biochar has mineral components such as CO_3^{2-}, PO_4^{3-}, SiO_3^{4-}, Cl^-, SO_4^{2-}, SO_3^{2-} and OH^-, which combine with heavy metal ions to form substance such as metal oxides, metal phosphates and metal carbonates, which promotes the adsorption and immobilization of heavy metals (Yang *et al*., 2019). The adsorption of Cu, Zn and Cd by biochar is mainly attributed by the precipitation of CO_3^{2-} and PO_4^{3-}, and the electron surface complexation through –OH groups (Xu *et al*., 2013). Complexation refers to the interaction between O_2 containing functional groups on the biochar surface and heavy metal to form complexes which could be fixed (Figure 8) (Yang *et al*., 2019). According to Qian and Che (2014), the adsorption of Al by biochar is mainly through the complexation of carboxyl group with $[Al(OH)]^{2+}$ and its monomer surface.

Biochar has surface characteristics such as porosity and large specific surface area that adsorb heavy metal on it surface or diffuse into the micro pores (Yang *et al*., 2019). Heavy metal ions have smaller diameter than the average pores diameter of biochar (Ko *et al*., 2004). The smaller the diameter of the heavy metal, the more the pores of the heavy metal penetrates in to pores of biochar, which results in adsorption capacity (Ngah *et al*., 2008)

Biochar can contribute to the immobilization of heavy metals and adsorption of organic contaminants because of its hydrophobic character and strong affinity of those contaminants of carbon fractions (Koltowski and Oleszczuk, 2016). Zhang *et al*., (2013), suggests that mineral components of biochar such as phosphate and carbonate can play a key role in stabilizing heavy metals in soils because these salts have the ability to precipitate with heavy metals and as a results they can reduce heavy metal availability. The immobilization of heavy metals can be as a result of increase in pH after biochar application, and this can lead to cationic metals on soil particles. Application of biochar in acidic soil can fix heavy metal because of the increase in soil negative charge by the increase in pH (Jiang *et al*., 2015).

Al-Wabel *et al*., (2015), reported that biochar application significantly decreased heavy metal content in maize shoot at soil moisture level of 75% and 100% field capacity. According to the study, heavy metals extracted by NH_4OA_c and AB-DTPA in the soil decreased after biochar application and this seemingly decreased plant content of heavy metals. Park *et al*., *(*2011) also reported that heavy metals (Cd, Cu, Pb) extracted by NH_4NO_3 reduced the concentration in the soil after the application of biochar. The concentration of $CaCl_2$-extractable Cd, Cu Pd and Zn, decrease significantly with an increase of biochar from bamboo and rice straw, when it was determined after a year of incubation. When the results were compared with the control, only coarse and fine straw biochar at 5% addition rate indicate a significant reduction in the concentration of extracted Cd by 17.7 and 25.8%, respectively.

Application of bamboo biochar in the soil reduced the concentration of extractable Cu by 26.8 and 47.7% for coarse treatment, 31.9 and 66.0% for fine treatment, while the application of rice straw biochar showed the highest decrease of extractable Cu (97.3%) and Zn (62.2%), with the 5% application rate for fine particle size (Yang *et al*., 2016).

3. MATERIALS AND METHODS

3.1 Location

The pot experiment was conducted in a greenhouse at ARC (Institute for Tropical and Subtropical Crops) Nelspruit (Latitude-25.451275 and longitude-30.96919 E) Mpumalanga Province.

3.2 Soil sampling and analysis

Two soils (sandy and clay) were taken from uncontaminated site in Mpumalanga Province. Sandy soil was collected from the University of Mpumalanga experimental farm (25°26'11"S 30°58'54'E) and the clay soil was collected from Malelane at (TSB) farm (25°48'31''S 31°54'84'E). Both soils were collected systematically from 12 points in the farms at a depth of 0 to 20 cm with the use of a spade and sampling bag. Soil samples from each site were bulked together and passed through a 2 mm mesh sieve to remove gravel and debris. The clay soil was classified as Hutton soil form and sandy soil as Longlands soil form (Soil Classification Working Group, 1991). Both soil samples were analysed for texture using the hydrometer method (Gee and Bauder, 1986). Exchangeable cations (Mg^{2+}, Ca^{2+}, K^{+}, Na^{+}) were measured using ammonium acetate method (Chapman, 1965). Soil pH and EC was measured in distilled water (1:2.5 soil: solution ratio) using pH meter (Peech, 1965). Soil aggregate stability was determined using the wet sieve technique (Haynes, 1993). Available phosphorus was measured using the Bray 1 procedure (Bray and Kurtz, 1945).

3.3 Biochar analysis

Acacia biochar produced under high temperature (>550 °C) was obtained from commercial supplier [Lanstar (Pty)] in Johannesburg, South Africa. Poultry biochar was produced at the University of Venda under high temperature (>550 °C). Acacia (AC) and poultry litter (PL) were prepared through the pyrolysis method as described by Musulili *et al* (2010). Exchangeable cations (Mg^{2+}, Ca^{2+}, K^{+}, Na^{+}) were measured using ammonium acetate method (Chapman, 1965). Copper, iron, manganese, molybdenum and zinc of the soil were determined by the HF-$HClO_4$-HNO_3 method (Carignan and Tessier, 1988). Available phosphorus was measured using the Bray 1 procedure (Bray and Kurtz, 1945). Soil pH was measured in distilled water (1:2.5 soil: solution ratio) using pH meter (Peech, 1965). Organic C was determined using Walkley and Black wet oxidation method (Soil Survey Laboratory Staff, 1992). Total nitrogen was measured using the Kjeldahl method (Bremner and Breitenbeck, 1983). The surface area of biochar was measured by X-ray photoelectron spectroscopy (XPS). To determine the ash content of biochar, the mass titration method as described by Noh and Schwarz (1990) was used. Moisture content was measured by drying the sample in an oven at 105 ^{o}C for 24 hours.

3.4 Slag analysis

The slag was collected from Columbus Stainless in Middelburg, Mpumalanga Province (25^{o}47'50"S 29^{o}29'29'E). Chromium, lead, nickel, sulphur, manganese and zinc of the soil was determined by the HF-$HClO_4$-HNO_3 method (Carignan and Tessier, 1988) and the samples were analysed using the inductively plasma-optical emission spectrometry (ICP-OES). The same methods for soil analysis as referred to in 3.3 were used to determine Mg^{2+}, Na^{+}, nitrogen, pH and moisture content.

3.5 Experimental set up and design

A 2 x 2 x 4 factorial experiment was conducted with three factors consisting of two soils (sandy and clay), two biochar types (Acacia and Poultry litter) and four biochar rates (0 t/ha, 5 t/ha, 10 t/ha, and 20 t/ha). Four (4) kg of soils was placed in a pot measuring bottom 18 cm, top 22 cm in diameter by 23 cm in height. Plastic saucers were placed underneath the pots to prevent leachate draining from the mixture. Slag was applied to each pot at an amount equivalent to 15 g/kg (75 g per pot). Biochar was then applied at the stated rates. The treatments were replicated four (4) times to give a total of 64 pots and were arranged in a CRD in a greenhouse. The treatments were allowed to equilibrate for one week (7 days). During equilibration process, all pots were adjusted daily to water content of 70% field capacity. Five seedlings of spinach were planted in each pot together with application of 100 kg/N as LAN and 100 kg/P as super phosphate. After two weeks, seedlings were thinned to two plants per pot. The soil water content was adjusted daily to 70% for 45 days.

4. DATA COLLECTION

4.1 Plant tissues

Spinach leaves were harvested after 45 days by gently removing them from the pots, washed with tap water, followed by washing with deionized water (distilled), blotted dry on filter paper and then dried at 70ºC for (48 hours) to determine the dry matter of the plant. The spinach leaves were then ground and passed through 0.5 mm sieve and then digested using H_2SO_4-H_2O_2 (Parkinson and Allen, 1975), and then analyzed for concentration of Cr, Ni and Pb.

The phytoavailability of heavy metals was computed using the following equation 1 (Cao *et al*., 2009)

Phytoavailbility (%) =

$$\frac{\text{heavy metal concentration (mg/kg) in spinach} \times \text{above ground biomass (kg/pot)}}{\text{heavy metal (mg/kg) in soil} \times \text{soil mass (kg/pot)}}$$

………*Equation: 1*

4.2 Soil analysis

At the end of the experiment, soil samples from the pots were air dried and passed through 2 mm sieve. The soil samples were analyzed for soil pH and EC with a glass electrode using a soil to water of 1:1 and soil aggregate stability was determined using the wet sieve technique (Haynes, 1993). Extractable Cr, Ni and Pb were determined by the HF-$HClO_4$-HNO_3 method (Carignan and Tessier, 1988) and measured by inductively-coupled plasma optical emission spectrometry (ICP-OES).

The immobilization of heavy metals was computed using the following equation 2 (Park *et al*., 2011):

Immobilization of heavy metals (%) =

$$\frac{\text{heavy metal for the control} - \text{heavy metal in biochar treated sample}}{\text{heavy metal (control)}} \times 100$$

………… *Equation:2*

4.3. Data analysis

The data were statistically analysed using SAS software (SAS institute, 1999). The data were subjected to one way analysis of variance (ANOVA) to confirm the variability and validity of results and comparisons of means using Least Significant Difference (LSD) test, at $p<0.05$.

5. RESULTS

5.1 Physicochemical properties of soils used in the experiment

The initial physio-chemical properties of sand and clay soils used in the experiment are shown in Table 2. Sandy soil was slightly acidic with the pH of 4.9 and the clay soil with the pH of 5.9. Clay soil has higher P than sand soil and the amount of exchangeable Ca^{2+}, Mg^{2+} and K^{+} were higher in clay than in sand (Table 2).

Table 2: Physical and chemical properties of soils used in the experiment

Parameters	Soil	
	Kranskop (Sand)	**Hutton (Clay)**
pH (H_2O)	4.9	5.9
Sand (%)	90	52
Silt (%)	1	15
Clay (%)	9	33
EC (Ms m^{-1})	27	30.9
Ca (mg/kg)	233	3769
P (mg/kg)	36.7	714
Mg (mg/kg)	51	430
Na (mg/kg)	15	52
K (mg/kg)	42	714

5.2 Chemical composition of the Slag used in the experiment

The chemical composition of the Slag used in the experiment is presented in Table 3. The Slag had very high chromium level of 5748 mg/kg, According to Water Research Commission (1997), in South Africa, the permissible value of chromium is 80 mg/kg in the soil, while internationally in Australia and New Zealand according United State Environmental Protection Agency, (1995) the permissible value of chromium is 50 mg/kg. The lead content of the slag was 118 mg/kg, which is high according to South African permissible value of lead which is 66 mg/kg (Water Research Commission, 1997), and low in Australia and New Zealand, because the permissible value of lead is 300 mg/kg according to United State Environmental Protection Agency, (1995). Nickel was slightly high, because according to Water Research Commission, (1997), the permissible standard value of 50 mg/kg is recommended in South

Africa, while in Australia and New Zealand, the permissible standard value is 60 mg/kg, according to United State Environmental Protection Agency, (1995). The pH of the Slag was 7.4 which is slightly alkaline.

Table 3: Chemical composition of the Slag used in the experiment

Parameters	**Slag**
Chromium (mg/kg)	5748
Nickel (mg/kg)	73
Lead (mg/kg)	118
Magnesium (%)	18
Manganese (mg/kg)	502
Sodium (mg/kg)	820
Zinc (mg/kg)	28
pH	7.4
Moisture (%)	4.9
Nitrogen (%)	0.9

The physical and chemical properties of poultry and acacia biochar used in this experiment is presented in Table 4. The PL and AC biochar had alkaline pH of 10.2 and 9.1, respectively. PL biochar had high fixed organic carbon than AC biochar. PL biochar had high exchangeable cations (Ca^{2+}, Mg^{2+} and K^{+}) than AC. Furthermore, PL biochar had higher P than AC biochar. PL had higher ash content value than AC biochar.

Table 4: Physical and chemical properties of poultry litter and acacia biochar used in the experiment

Parameters	**Biochar feedstocks**	
	Poultry litter (PL)	**Acacia (AC)**
pH	10.2	9.1
Ca (mg/kg)	33	18.5
Mg (mg/kg)	16.5	2.2
K (mg/kg)	40.2	5.1
Na (mg/kg)	10.4	3164.6
S (mg/kg)	5.2	1
P (mg/kg)	25	0.5
Total N (mg/kg)	31.2	6.3
Organic Carbon (g/kg)	730	600
C/N ratio (%)	23.4	95.2
Fe (mg/kg)	6.29	2.04
Mn (mg/kg)	1.17	0.005
Cu (mg/kg)	0.16	0.03
Zn (mg/kg)	1.06	0.03
Mo (mg/kg)	0.01	0.01
Moisture content (mg/kg)	61	70
Ash content (mg/kg)	180	165
Surface area in CO_2 gas (m^2/g)	115.3	908

Mg- Magnesium; Na- Sodium; K- Potassium; S- Sulphur; Fe – Iron; Cu- Copper; Na- Sodium; P-Phosphorus; Mn- Manganese; Zn- Zinc; B- Boron; Mo- Molybdenum; Al- Aluminium

5.3 Effect of soil type, biochar type and application rate on extractable chromium.

Soil type had a significant (P<0.001) effect on extractable chromium concentration in the soil (Table 8). The clay soil had significantly (52%) lower chromium level than the sandy soil. Similarly, biochar type had a significant (P<0.05) effect on extractable chromium concentration in the soil, with the poultry litter biochar application exhibiting significantly lower extractable chromium concentration in the soil compared to the acacia biochar (Table 8). Biochar application rate had a significant (P<0.001) effect on extractable chromium concentration in the soil. Application of 5 t/ha did not have any significant effect on extractable chromium concentration in the soil compared to the control. However, increasing biochar application rate from 10 to 20 t/ha significantly lowered the concentration of extractable chromium in the soil, with the 20 t/ha application having the least extractable chromium concentration.

The interactive effect of soil type x biochar rate on extractable chromium concentration was significant (P<0.05) (Table 8). Increasing biochar application rate from 0 to 20 t/ha lowered the concentration of extractable chromium concentration in both sandy and clay soils (Figure 9). The reduction in the extractable chromium concentration was much greater in the clay soil compared to the sandy soil. A significant (P<0.05) effect of biochar type x biochar application rate on soil extractable chromium was also observed (Table 8). Increasing the application rate of either acacia or poultry litter biochar from 0 to 20 t/ha reduced the amount of extractable chromium in the soil although the reduction was not significantly different between the two biochars at each application rate (Figure 10).

Application of both acacia and poultry litter biochar resulted in significant immobilization of chromium (Table 5). In the sandy soil, application of 5 and 10 t/ha acacia biochar immobilized 5.7 and 3% more chromium, respectively, than the poultry litter biochar. However, at 20 t/ha application, poultry litter biochar immobilized 26.6% more chromium compared to the acacia biochar (Table 5).

In the clay soil, poultry litter biochar consistently immobilized more chromium than acacia biochar at all application rates. The difference ranged from 3% at 5 t/ha to 22% at 20 t/ha biochar application rate.

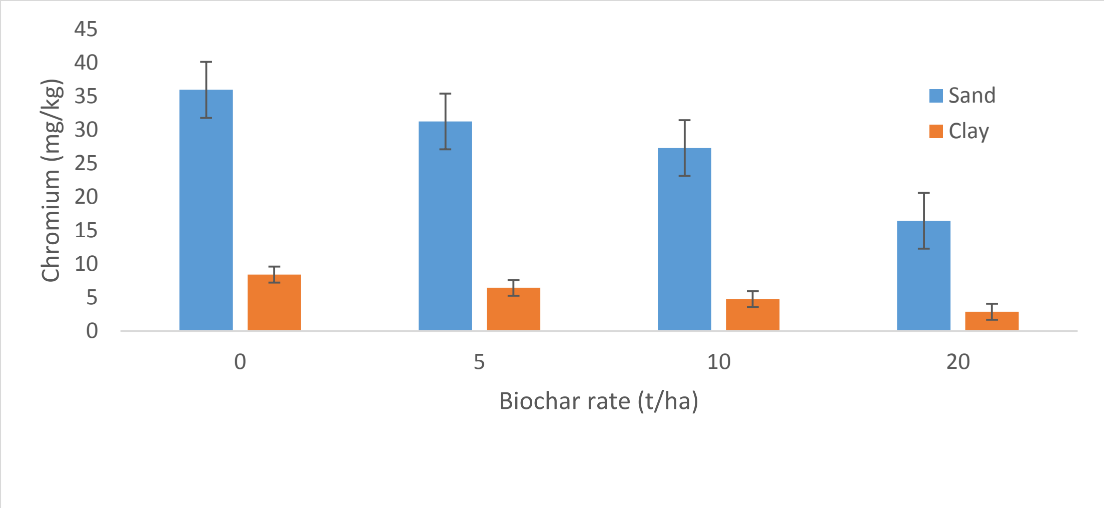

Figure 9: Effect of soil type and biochar rate interaction of chromium in the soil.

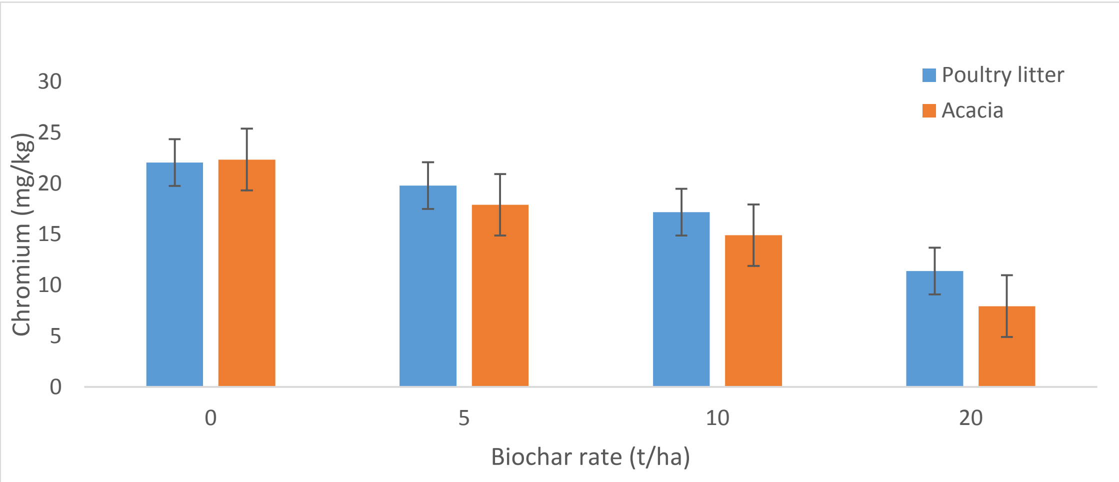

Figure 10: Effect of biochar type and biochar rate interaction of chromium in the soil.

Table 5: The effect of acacia and poultry litter biochar on the immobilization of chromium

		Biochar rate (t/ha)		
		5	10	20
Soil type	**Biochar type**			
Sandy	AC	15%	36.5 %	38.4%
	PL	9.3%	33.5%	65.%
Clay	AC	21.9%	36.5%	52%
	PL	24.9%	47%	74%

5.4 Effect of soil type, biochar type and application rate on extractable nickel.

Soil type had a significant ($p< 0.001$) effect on extractable nickel in the soil (Table 8). The clay soil had significantly (94%) lower nickel level than the sandy soil. Similarly, biochar type had a significant ($p<0.001$) effect on extractable nickel concentration in the soil, with the acacia biochar application exhibiting significantly higher extractable nickel concentration in the soil compared to the poultry litter biochar (Table 8). Biochar application rate had a significant ($p<0.001$) effect on extractable nickel concentration in the soil. Increasing biochar application rate from 0 to 20 t/ha significantly decreased the concentration of the extractable nickel in the soil, with the 20 t/ha biochar application having the least extractable nickel.

The interactive effect of soil type x biochar type on extractable nickel concentration was significant ($p< 0.001$) (Table 8). Extractable nickel concentration was low in clay soil compared to the sandy soil, with the application of either acacia or poultry litter biochar (Figure 11). Poultry litter biochar significantly reduced extractable nickel concentration compared to the acacia biochar in both soils (Figure 11). The interactive effect of soil type x biochar application rate on extractable nickel concentration was significant ($p<0.001$) (Table 8). Extractable nickel concentration was low in the clay soil compared to the sandy soil with the application rate from 0 to 20 t/ha (Figure 12). In both sandy and clay soils the application rate from 0 to 10 t/ha did not have any significant effect on extractable nickel concentration in the soil. However, increasing biochar application rate from 10 to 20 t/ha significantly lowered the concentration of extractable nickel in the soil (Figure 12). A significant ($p<0.001$) effect of biochar type x biochar application rate on extractable nickel concentration was also observed (Table 8). Application rate from 0 to 20 t/ha did not have significant effect on extractable nickel concentration in the soil. However, at 20 t/ha, poultry litter biochar significantly reduced the amount of extractable nickel in the soil compared to acacia biochar (Figure 13).

The interactive effect of soil type x biochar type x biochar rate on extractable nickel concentration was significant ($p<0.001$) (Table 8). Extractable nickel concentration was low in the clay soil compared to the sandy soil with the application of either acacia or poultry litter biochar (Figure 14). Increasing biochar application rate in either sandy or clay soil decreased the amount of extractable nickel in the soil.

The application of both acacia and poultry litter biochar resulted in significant immobilization of nickel (Table 6). In the sandy soil, application of 5, 10 and 20 t/ha poultry litter biochar immobilized 8.9%, 14.3% and 25,4 % more nickel, respectively , than the acacia biochar. In the clay soil, poultry litter biochar consistently immobilized more nickel than acacia biochar at all application rates. The difference ranged from 17.8% at 5 t/ha to 24% at 20 t/ha biochar application rate.

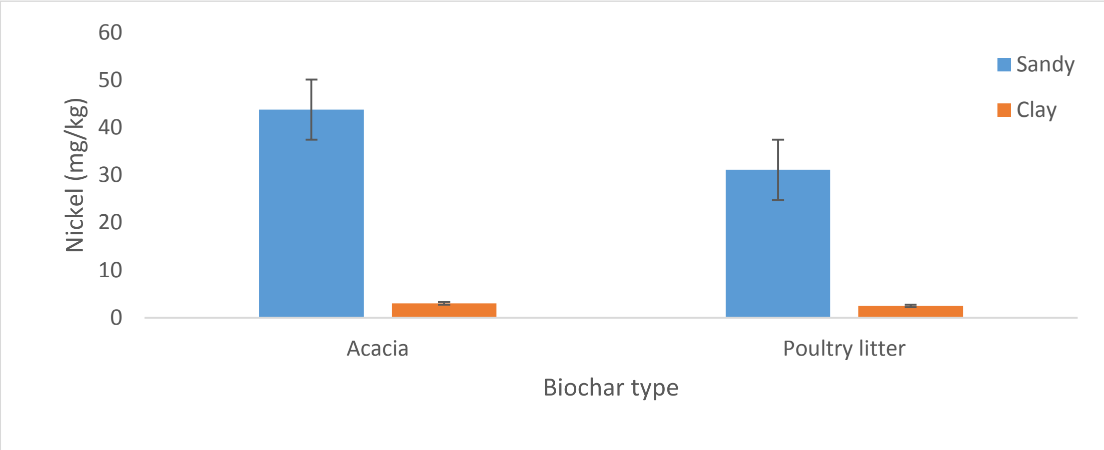

Figure 11: Effect of soil type and biochar type interaction of nickel in the soil

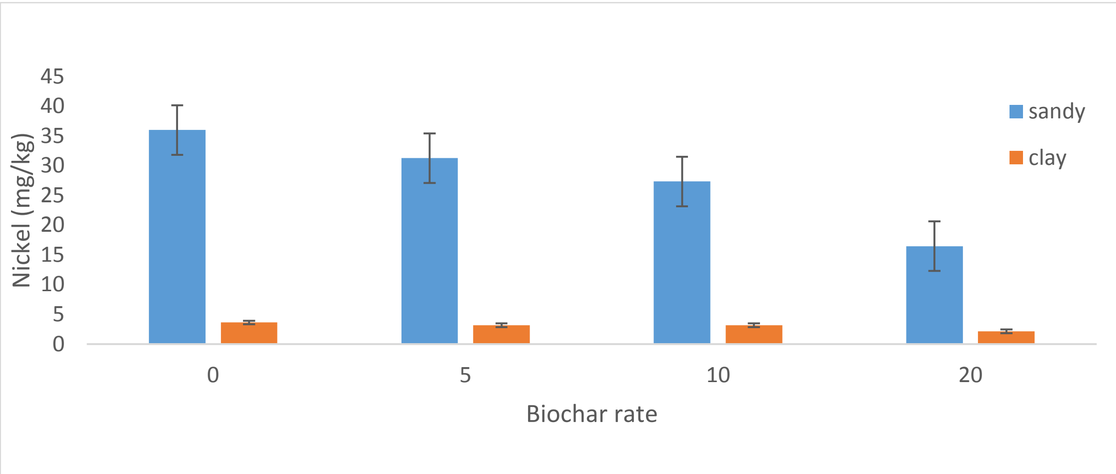

Figure 12: Effect of soil type and biochar rate interactions of nickel in the soil

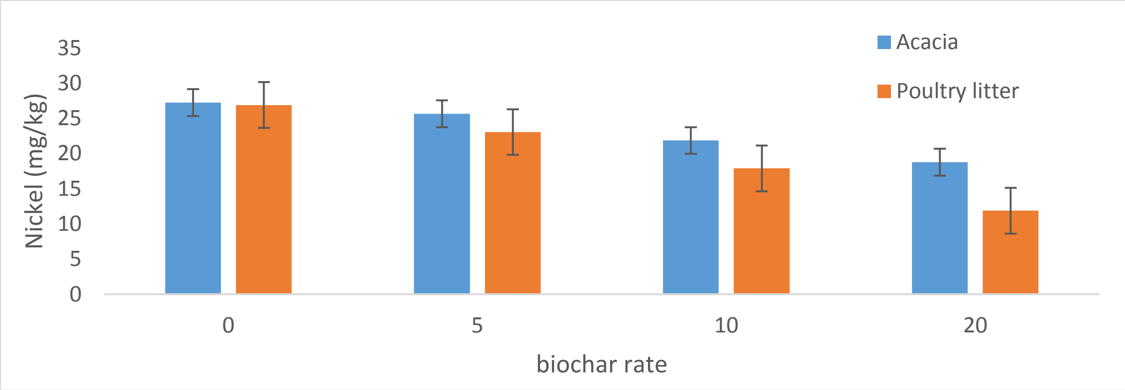

Figure 13: Effect of biochar type and biochar rate interactions of nickel in the soil

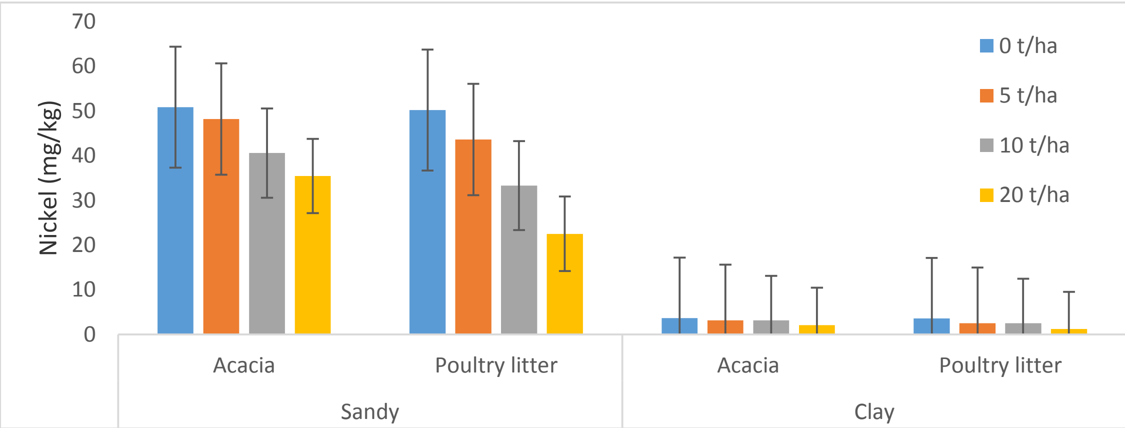

Figure 14: Effect of soil type, biochar type and biochar rate interaction of nickel in the soil.

Table 6: The effect of biochar on the immobilization of nickel

		Biochar rate (t/ha)		
		5	10	20
Soil type	**Biochar type**			
Sandy	AC	5.3%	20.2%	30.3%
	PL	14.2%	34.5%	55.7%
Clay	AC	13.7%	25.3%	41.8%
	PL	31.5%	50.7%	65.8%

5.5 Effect of soil type, biochar type and application rate on extractable lead

Soil type had a significant ($p < 0.001$) effect on extractable lead concentration in the soil (Table 8). The clay soil had significantly (26%) lower extractable lead level than the sandy soil. Similarly, biochar type had a significant ($p < 0.001$) effect on extractable lead concentration in the soil, with the acacia biochar application exhibiting significantly higher extractable lead concentration in the soil compared to the poultry litter biochar (Table 8). Biochar application rate had a significant ($p<0.001$) effect on extractable lead concentration in the soil (Table 8). Increasing biochar application rate from 0 to 20 t/ha, significantly decreased the concentration of the extractable lead in the soil, with the 20 t/ha biochar application having the least extractable lead.

The interactive effect of soil type x biochar type on extractable lead was significant ($p<0.05$) (Table 8). Extractable lead concentration was significantly reduced in the clay soil compared to the sandy soil with the application rate of either acacia or poultry litter biochar (Figure 15). Poultry litter biochar significantly reduced extractable lead concentration compared to the acacia biochar in both soils (Figure 15). The interactive effect of soil type x biochar application rate on extractable lead concentration was significant ($p<0.001$) (Table 8). Increasing biochar application rate from 0 to 20 t/ha reduced the extractable lead concentration in the soil, although the reduction of extractable lead concentration was significant from 0 to 5 t/ha in sandy soil and from 5 t/ha to 10 t/ha in clay soil (Figure 16). A significant effect of biochar type x biochar application rate on extractable lead concentration in the soil was also observed ($p<0.001$) (Table 8). Application rate of biochar from 0 to 20 t/ha reduced the amount of extractable lead in the soil although the reduction was not significantly different between the two biochars at each application rate (Figure 17).

The interactive effect of soil type x biochar type x biochar rate on extractable lead concentration was significant ($P < 0.05$) (Table 8). Extractable lead concentration was low in clay soil compared to the sandy soil with the application of either acacia or poultry litter biochar (Figure 18). There were significant differences in the amounts of extractable lead in both sandy and clay soils with increasing rate of application of either acacia or poultry litter biochar.

Application of both acacia and poultry litter biochar resulted in significant immobilization of lead (Table 7). In the sandy, soil application of 5 t/ha and 10 t/ha acacia biochar immobilized 12% and 8.3% more lead, than the poultry litter biochar. However, at 20 t/ha, poultry litter biochar immobilized 6.3% more lead compared to the acacia biochar (Table 7). In the clay soil, poultry litter biochar consistently immobilized more lead than acacia biochar at all biochar application rates. The difference range from 15.8% at 5 t/ha to 18.5% at 20 t/ha biochar application rate.

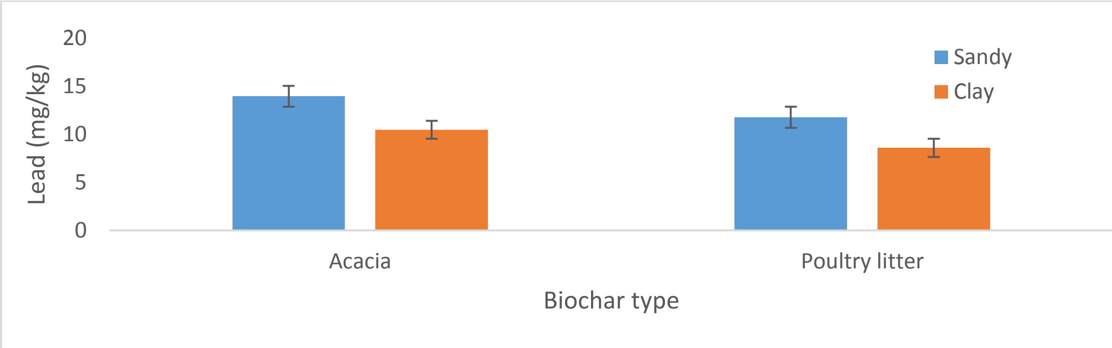

Figure 15: Effect of soil type and biochar type interaction of lead in the soil

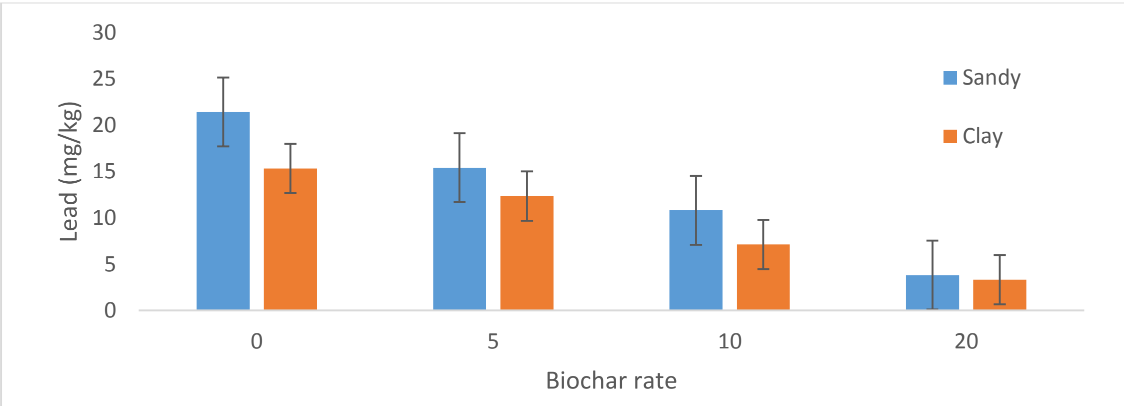

Figure 16: Effect of soil type and biochar rate interaction of lead in the soil

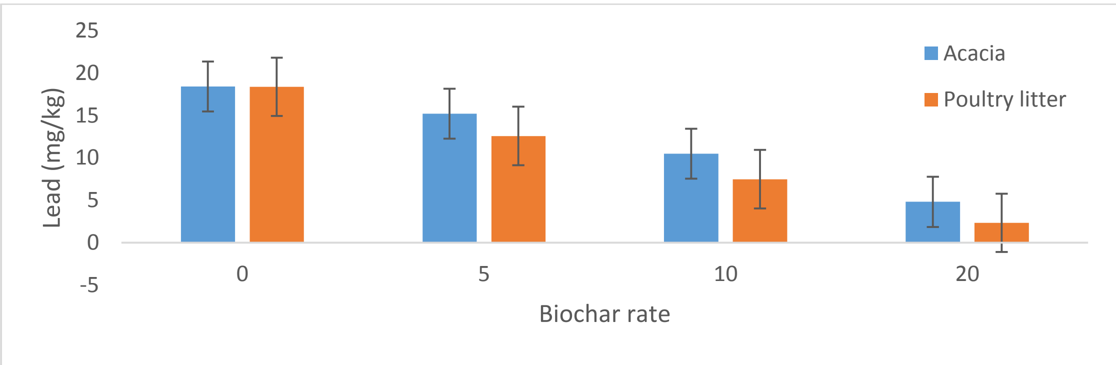

Figure 17: Effect of biochar type and biochar rate interaction of lead in the soil

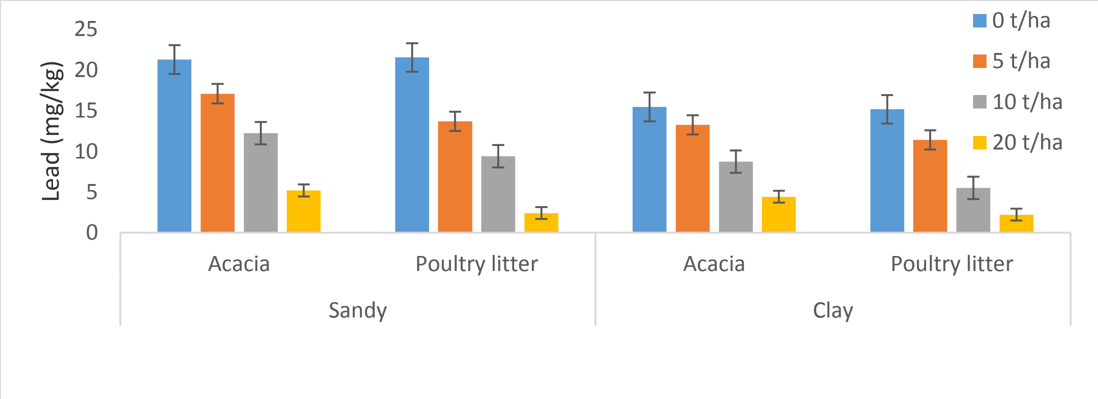

Figure 18: Effect of soil type, biochar type and biochar rate interaction of lead in the soil.

Table 7: The effect of biochar on the immobilization of lead.

		Biochar rate (t/ha)		
		5	10	20
Soil type	**Biochar type**			
Sandy	AC	26.2%	64.3%	83.6%
	PL	14.2%	56%	89.9%
Clay	AC	20.6%	43.6%	71.4%
	PL	36.4%	56%	89.9%

Table 8: Effect of soil type, biochar type and application rate on extractable Cr, Ni and Pb

Parameters	**Chromium**	**Nickel**	**Lead**
	(mg/kg)	(mg/kg)	(mg/kg)
Soil type			
Sandy	100. 22 a	40.59 a	12.89 a
Clay	47.96 b	2.60 b	9.57 b
$LSD_{0.05}$	7.91	0.25	0.33
Biochar type			
Poultry litter	69.56 b	19.87 b	10.26 b
Acacia	78.62 a	23.33 a	12.18 a
$LSD_{0.05}$	7.90	0.25	0.33
Biochar rate (t/ha)			
0	94.28 a	27.08 a	18.38 a
5	88.18 a	24.36 b	13.88 b
10	69.88 b	19.62 c	8.98 c
20	44.00 c	15.34 d	3.65 d
$LSD_{0.05}$	14.82	0.47	0.43
CV %	21.20	2.32	4.07
P value ≤ 0.05			
Soil type (S)	***	***	***
Biochar type (Bt)	*	***	***
Biochar rate (Br)	***	***	***
S × Bt	ns	***	*
S × Br	*	***	***
Bt × Br	*	***	***
S × Bt × Br	ns	***	*

Means with the same letter (s) within a column are not significantly different, *** Significant at P< 0.001; ** Significant at P≤ 0.01**; * Significant at P ≤ 0.05.

5.6 Effect of soil type, biochar type and application rate on chromium concentration in spinach leaves.

Soil type had a significant (p< 0.001) effect on chromium concentration in spinach leaves (Table 12). Spinach grown in the clay soil had significantly (62%) lower lead level than the sandy soil. Similarly, biochar type had a significant ($p < 0.001$) effect on chromium concentration in spinach leaves with the poultry litter biochar application exhibiting significantly lower chromium concentration in spinach leaves (Table 12). Biochar application rate had a significant ($p<0.001$) effect on chromium concentration in spinach leaves (Table 12). Increasing biochar application rate from 0 to 20 t/ha, significantly decreased the chromium concentration in spinach leaves with the 20 t/ha biochar application having the least chromium concentration.

The interactive effect of soil type x biochar type on chromium concentration in spinach leaves was significant ($p<0.001$) (Table 12). Chromium concentration in spinach leaves was significantly reduced in the clay soil compared to the sandy soil with the application of either acacia or poultry litter biochar (Figure 19). Poultry litter biochar significantly reduced chromium concentration in spinach leaves compared to the acacia biochar in both soils (Figure 19). The interactive effect of soil type x biochar application rate on chromium concentration in spinach leaves was significant ($P<0.001$) (Table 12). Increasing biochar application rate from 0 to 20 t/ha lowered the concentration of chromium concentration in spinach leaves, although the reduction was significant on application rate from 0 to 10 t/ha in both soils (Figure 20). The reduction in the chromium concentration in spinach leaves was much greater in the clay soil compared to the sandy soil (Figure 20). A significant ($P<0.001$) effect of biochar type x biochar application rate on chromium concentration in spinach leaves was also observed (Table 12). Increasing the application rate of either acacia or poultry litter biochar from 0 to 20 t/ha reduced the chromium concentration in spinach leaves although the reduction was not significantly different between the two biochars at each application rate (Figure 21).

The interactive effect of soil type x biochar type x biochar rate on chromium concentration in spinach leaves was significant ($P< 0.001$) (Table 12). Concentration of chromium in spinach leaves was low in clay soil compared to the sandy soil with the application of either acacia or poultry litter biochar (Figure 22). Increasing the application rate of either acacia or poultry litter from 0 to 20 t/ha reduced the chromium concentration in spinach leaves in both soils (Figure 22).

Application of both poultry litter and acacia biochar reduced the phytoavailability of chromium to spinach (Table 9). In sandy soil, poultry litter biochar reduced the phytoavailability of Cr by 2.06 and 38.5%, at 10 and 20 t/ha, respectively when compared to 5 t/ha. Acacia biochar reduced the phytoavailability of Cr by 7.6 and 11.3%, at 10 and 20 t/ha respectively when compared at 5 t/ha (Table 9). In clay soil, poultry litter biochar reduced the phytoavailability of Cr by 14.3 and 22.1%, at 10 and 20 t/ha respectively when compared to 5 t/ha. Acacia biochar reduced the phytoavailability of Cr by 1.05 and 10.5%, at 10 and 20 t/ha respectively when compared at 5 t/ha (Table 9).

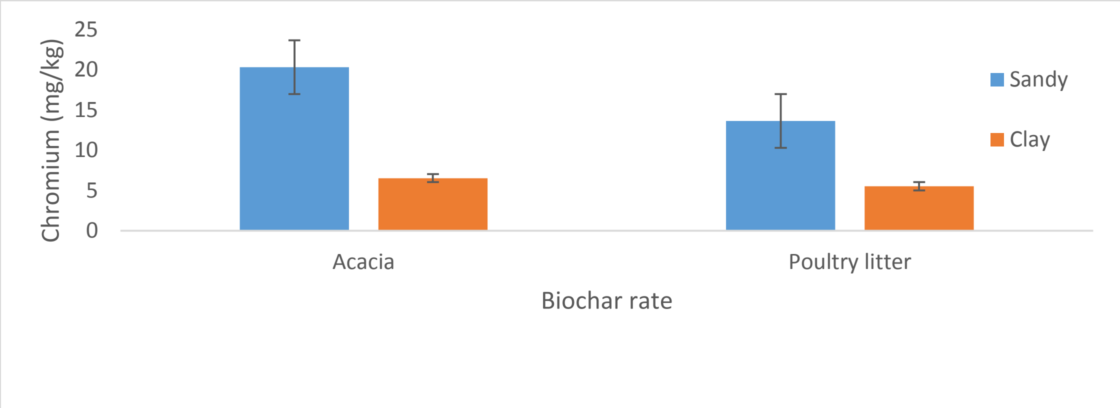

Figure 19: Effect of soil type and biochar type interaction on chromium concentration in spinach leaves

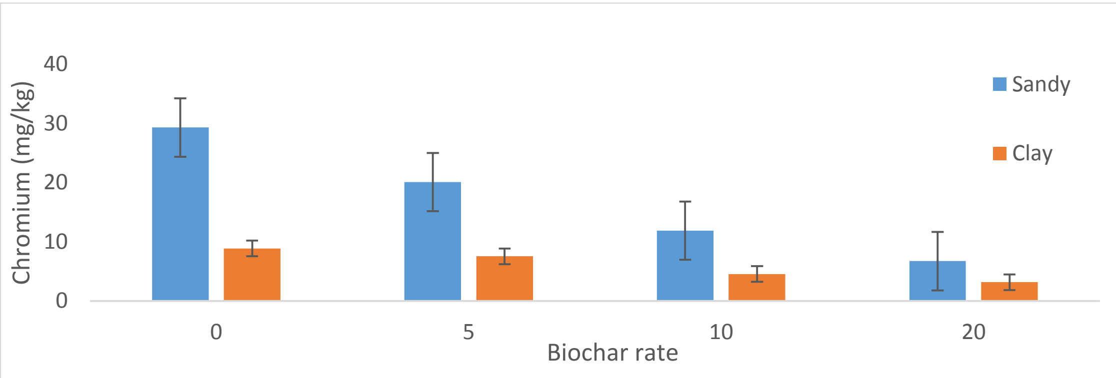

Figure 20: Effect of soil type and biochar rate interaction on chromium concentration in spinach leave

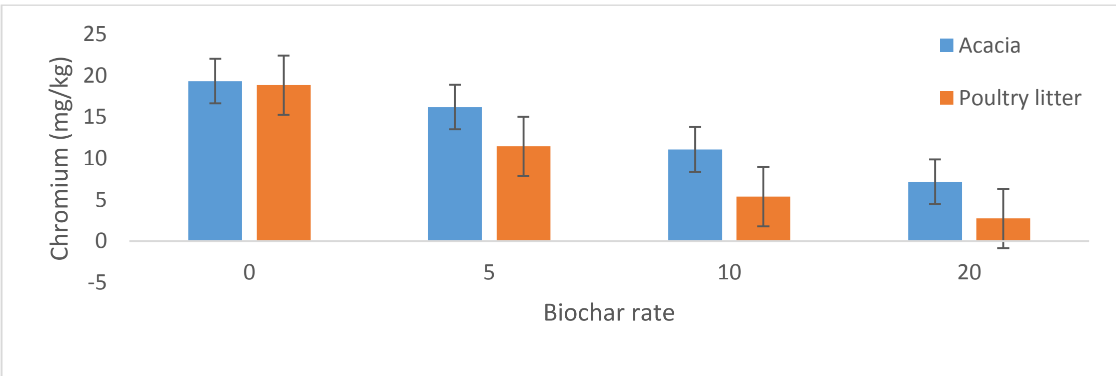

Figure 21: Effect of biochar type and biochar rate interaction on chromium concentration in spinach leaves

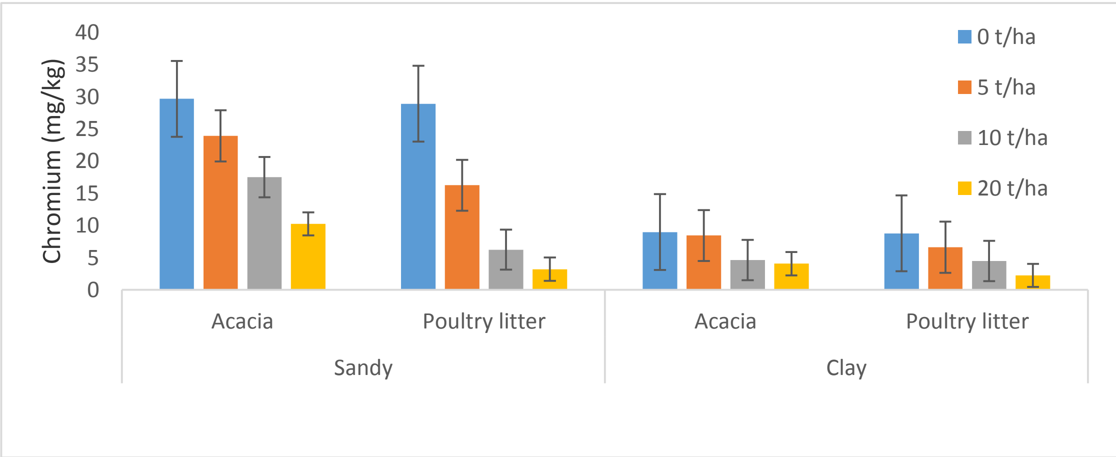

Figure 22: Effect of soil type, biochar type and biochar rate interaction on chromium concentration in spinach leaves.

Table 9: The effect of biochar on the phytoavailability (%) of chromium to spinach

		Biochar rate (t/ha)			
		0	5	10	20
Soil type	**Biochar type**				
Sandy	PL	1.66580	0.00097	0.00095	0.00060
	AC	1.65060	0.00106	0.00098	0.00094
Clay	PL	1.09612	0.00077	0.00066	0.00060
	AC	1.10257	0.00095	0.00094	0.00085

5.7 Effect of soil type, biochar type and application rate on nickel concentration in spinach leaves.

Soil type had a significant ($p < 0.001$) effect on nickel concentration in spinach leaves (Table 12). Spinach grown in the clay soil had significantly (53%) lower nickel level than the sandy soil. Similarly, biochar type had a significant ($p < 0.001$) effect on nickel concentration in spinach , with the poultry litter biochar application exhibiting significantly lower nickel concentration in spinach leaves compared to the acacia biochar (Table 12). Biochar application rate had a significant ($p<0.001$) effect on nickel concentration in spinach leaves (Table 12). Increasing biochar application rate from 0 to 20 t/ha, significantly decreased nickel concentration in spinach leaves, with 20 t/ha biochar application rate having the least nickel concentration in spinach leaves (Table 12).

The interactive effect of soil type x biochar type on nickel concentration in spinach leaves was significant ($p<0.001$) (Table 12). Nickel concentration in spinach leaves was significantly reduced in the clay soil compared to the sandy soil with the application rate of either acacia or poultry litter biochar (Figure 23). Poultry litter biochar significantly reduced nickel concentration in spinach leaves compared to the acacia biochar in both soils (Figure 23). The interactive effect of soil type x biochar application rate on nickel concentration in spinach leaves was significant ($P<0.01$) (Table 12). Increasing biochar application rate from 0 to 20 t/ha significantly lowered the concentration of nickel concentration in spinach leaves (Figure 24). The reduction in the nickel concentration in spinach leaves was much greater in the clay soil compared to the sandy soil (Figure 24). A significant ($P<0.001$) effect of biochar type x biochar application rate on nickel concentration in spinach leaves was also observed (Table 12). Increasing the application rate of either acacia or poultry litter biochar from 0 to 20 t/ha reduced the nickel concentration in spinach leaves, although the reduction was significant with the application rate from 0 to 5 t/ha. (Figure 25).

The interactive effect of soil type x biochar type x biochar rate nickel concentration in spinach leaves was significant ($P< 0.05$) (Table 12). Biochar application rate from 0 to 20 t/ha significantly lowered the nickel concentration in spinach leaves with either acacia or poultry litter biochar in both soils except for the application of 5 t/ha acacia biochar in clay soil which significantly increased the nickel concentration in spinach leaves (Figure 26)

Application of both poultry litter and acacia biochar reduced the phytoavailability of nickel to spinach (Table 10). In sandy soil, poultry litter biochar reduced the phytoavailability of Ni by 22.29 and 30.3%, at 10 and 20 t/ha respectively when compared to 5 t/ha. Acacia biochar reduced the phytoavailability of Ni by 41.5 and 52.0%, at 10 and 20 t/ha respectively when compared to 5 t/ha (Table 10). In clay soil, poultry litter biochar reduced the phytoavailability of Ni by 100 and 100%, at 10 and 20 t/ha, respectively when compared to 5 t/ha. Acacia biochar reduced the phytoavailability of Ni by 82.1 and 100%, at 10 and 20 t/ha, respectively when compared to 5 t/ha (Table 10).

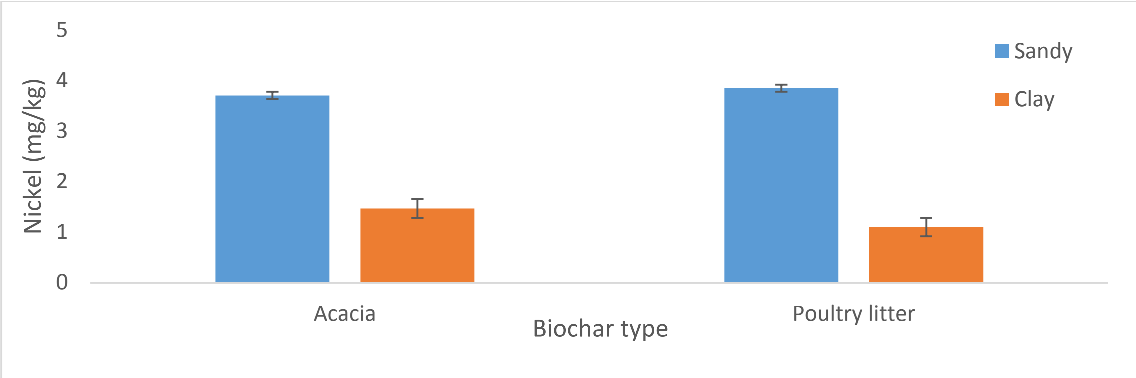

Figure 23: Effect of soil type and biochar type interaction on nickel concentration in spinach leaves.

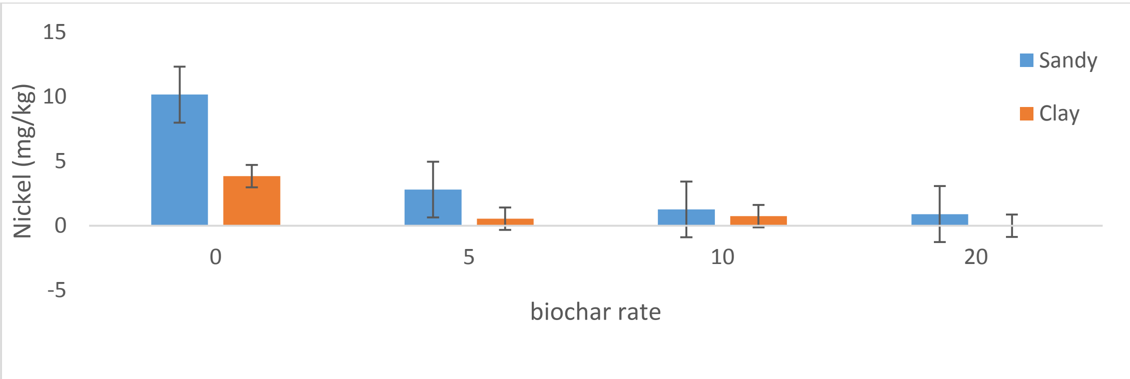

Figure 24: Effect of soil type and biochar rate interaction on nickel concentration in spinach leaves.

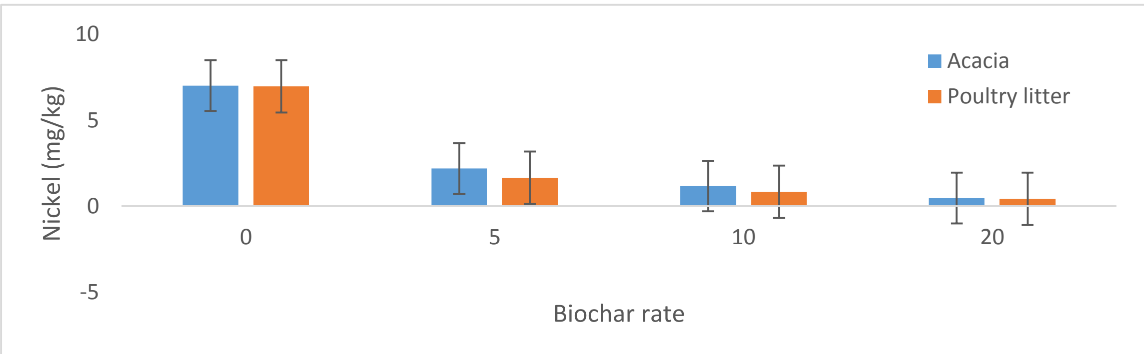

Figure 25: Effect of biochar type and biochar rate interaction on nickel concentration in spinach leaves.

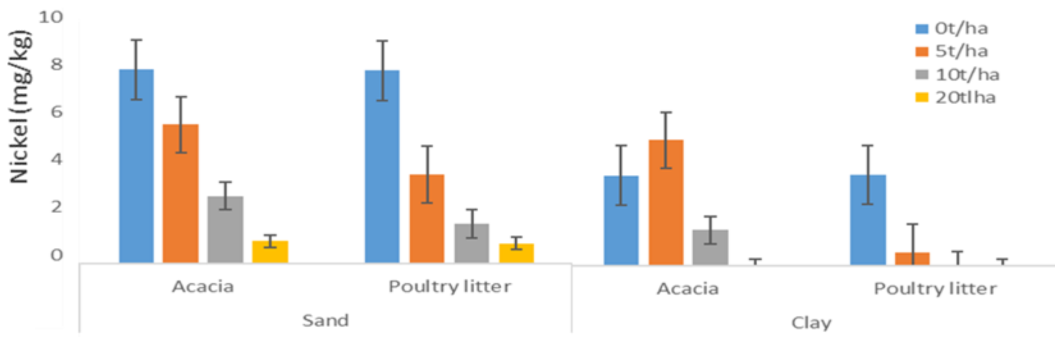

Figure 26: Effect of soil type, biochar type and biochar rate interaction on nickel concentration in spinach leaves.

Table 10: The effect of biochar on the phytoavailability (%) of nickel in spinach leaves.

		Biochar rate (t/ha)			
		0	5	10	20
Soil type	**Biochar type**				
Sandy	PL	0.21875	0.00175	0.00136	0.00122
	AC	0.22288	0.00342	0.00200	0.00164
Clay	PL	0.10339	0.00116	3.44×10^{-7}	8.1×10^{-8}
	AC	0.10168	0.00156	0.00028	1.27×10^{-7}

5.8 Effect of soil type, biochar type and application rate on lead concentration in spinach leaves.

Soil type had a significant ($p< 0.001$) effect on lead concentration in spinach leaves (Table 12). Spinach grown in the clay soil had significantly (34%) lower lead level than the sandy soil. Similarly, biochar type had a significant ($p< 0.001$) effect on lead concentration in spinach leaves with the poultry litter biochar application exhibiting significantly lower lead concentration in spinach leaves (Table 12). Biochar application rate had a significant ($p<0.001$) effect on lead concentration in spinach leaves (Table 12). Increasing biochar application rate from 0 to 20 t/ha, significantly decreased lead concentration in spinach leaves, with 20 t/ha biochar application rate having the least lead concentration in spinach leaves.

The interactive effect of soil type x biochar type on lead concentration in spinach leaves was significant ($p<0.001$) (Table 12). Lead concentration in spinach leaves was significantly reduced in the clay soil compared to the sandy soil with the application rate of either acacia or poultry litter biochar (Figure 27). Poultry litter biochar significantly reduced lead concentration in spinach leaves compared to the acacia biochar in both soils(Figure 27).The interactive effect of soil type x biochar application rate on lead concentration in spinach leaves was significant ($P<0.001$) (Table 12). Increasing biochar application rate from 0 to 20 t/ha significantly lowered the concentration of lead concentration in spinach leaves (Figure 24). The reduction in the lead concentration in spinach leaves was much greater in the clay soil compared to the sandy soil (Figure 28). A significant ($P<0.001$) effect of biochar type x biochar application rate on lead concentration in spinach leaves was also observed (Table 12). Increasing the application rate of either acacia or poultry litter biochar from 0 to 20 t/ha significantly reduced the lead concentration in spinach leaves (Figure 29).

The interactive effect of soil type x biochar type x biochar rate on lead concentration in spinach leaves was significant ($P< 0.001$) (Table 12). Concentration of lead in spinach leaves was low in clay soil compared to the sandy soil with the application of either acacia or poultry litter biochar (Figure 30). Biochar application rate from 0 to 20 t/ha lowered lead concentration in spinach leaves. However, in sandy and clay soil, the application rate of either acacia or poultry litter biochar did not have a significant difference in lead concentration in spinach leaves (Figure 30).

Application of both poultry litter and acacia biochar reduced the phytoavailability of lead in spinach (Table 11). In sandy soil, poultry litter biochar reduced the phytoavailability of lead by 5.8 and 32.2%, at 10 and 20 t/ha, respectively when compared to 5 t/ha. Acacia biochar reduced the phytoavailability of lead by 8.4 and 23.8%, at 10 and 20 t/ha respectively when compared to 5 t/ha (Table 11). In clay soil, poultry litter biochar reduced the phytoavailability of lead by 17.5 and 53.7%, at 10 and 20 t/ha, respectively when compared to 5 t/ha. Acacia biochar reduced the phytoavailability of Pb by 4.70 and 32.2%, at 10 and 20 t/ha, respectively when compared at 5 t/ha (Table 11).

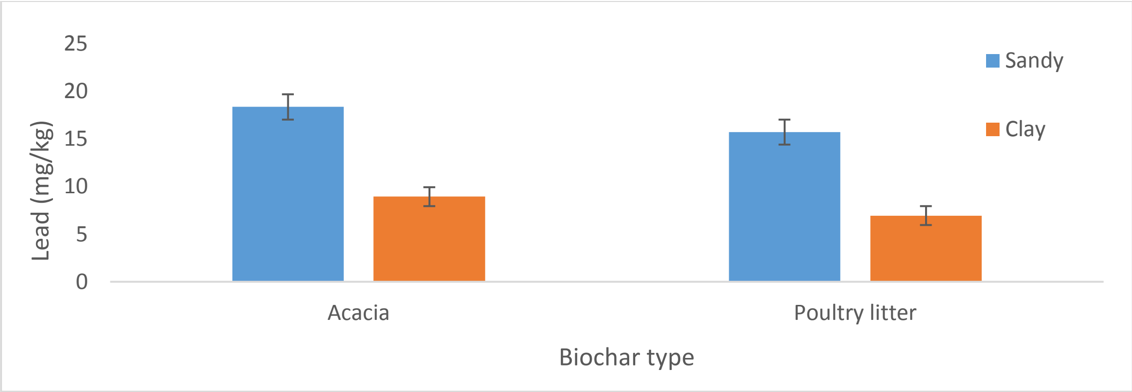

Figure 27: Effect of soil type and biochar type interaction on lead concentration in spinach leaves

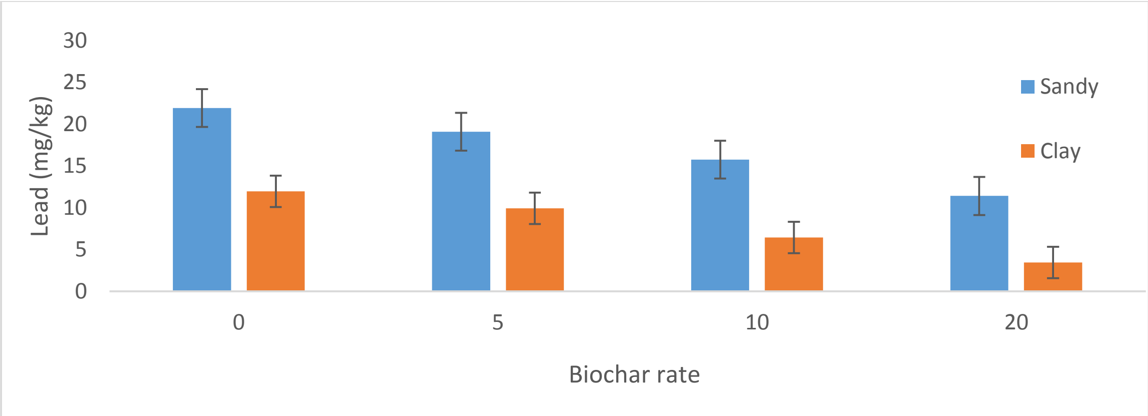

Figure 28: Effect of soil type and biochar rate interaction on lead concentration in spinach leaves

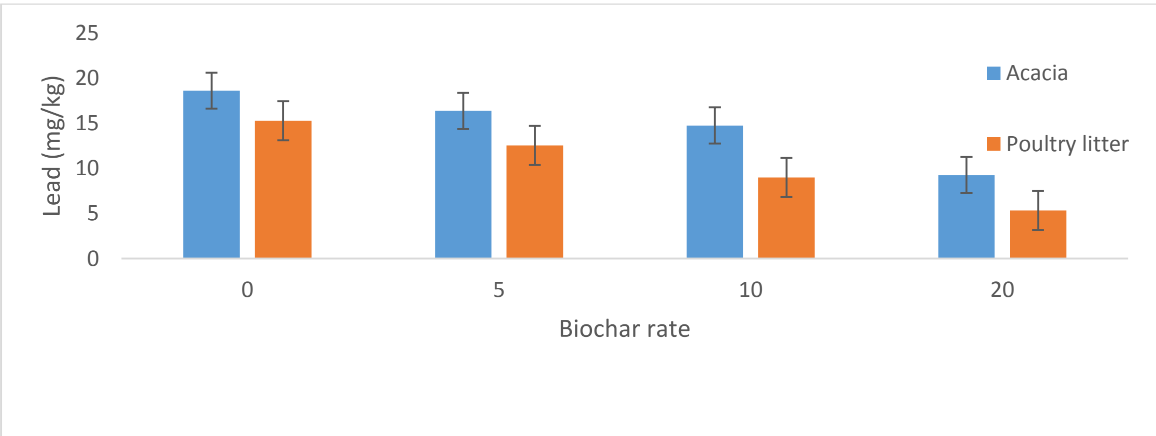

Figure 29: Effect of biochar type and biochar rate interaction on lead concentration in spinach leaves

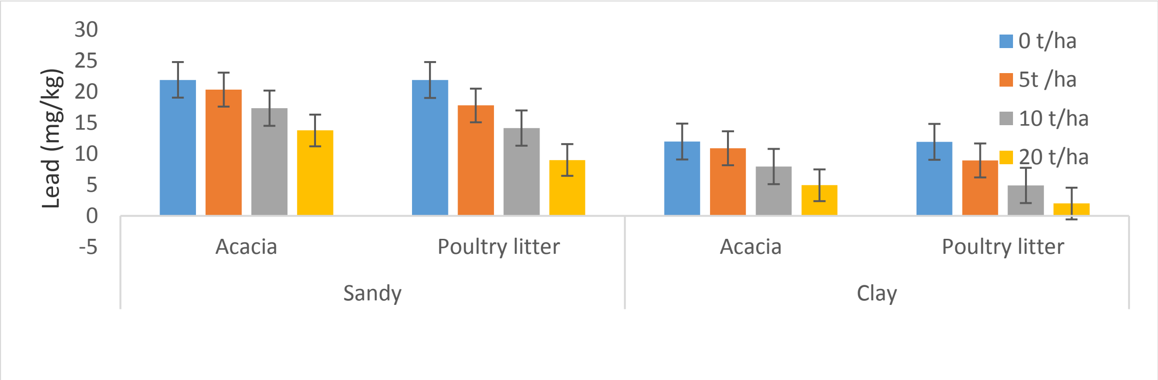

Figure 30: Effect of soil type, biochar type and biochar rate interaction on lead concentration in spinach leaves.

Table 11: The effect of biochar on the phytoavailability (%) of lead to spinach leaves

		Biochar rate (t/ha)			
		0	5	10	20
Soil type	**Biochar type**				
Sandy	PL	1.37506	0.02150	0.02025	0.01460
	AC	1.38993	0.02480	0.02271	0.01891
Clay	PL	1.24024	0.00257	0.00212	0.00119
	AC	1.22093	0.00335	0.00319	0.00227

Table 12: Effect of soil type, biochar type and application rate on concentration of Cr, Ni and Pb in spinach leaves.

Parameters	Chromium (mg/kg)	Nickel (mg/kg)	Lead (mg/kg)
Soil type			
Sand	16.78 a	17.05 a	3.46 a
Clay	6.31 b	7.95 b	2.48 b
$LSD_{0.05}$	0.48	0.20	0.06
Biochar type			
Poultry litter	9.39 b	13.66 a	3.25 b
Acacia	13.69 a	11.34 b	2.48 a
$LSD_{0.05}$	0.48	0.20	0.06
Biochar rate (t/ha)			
0	19.09 a	16.97 a	6.99 a
5	13.81 b	14.51 b	2.87 b
10	8.32 c	11.10 c	1.16 c
20	4.94 d	7.44 d	0.45 d
$LSD_{0.05}$	0.89	0.38	0.10
CV %	8.19	3.21	3.84
P value ≤ 0.05			
Soil type (S)	***	***	***
Biochar type (Bt)	***	***	***
Biochar rate (Br)	***	***	***
S × Bt	***	***	***
S × Br	***	**	***
Bt × Br	***	***	***
S × Bt × Br	***	*	***

Means with the same letter (s) within a column are not significantly different *** Significant at P< 0.001; ** Significant at P≤ 0.01**; * Significant at P ≤ 0.05.

5.9 Effect of soil type, biochar type and application rate on biomass of spinach.

Soil type had a significant ($p< 0.001$) effect spinach biomass (Table 13). The clay soil had significantly (13%) increased spinach biomass than the sandy soil. Similarly, biochar type had a significant ($p< 0.001$) effect on spinach biomass, with the poultry litter biochar application exhibiting significantly higher spinach biomass than acacia biochar (Table 13). Biochar application rate had a significant ($p <0.001$) effect on spinach biomass. The application of biochar from 0 to 20 t/ha significantly increased the spinach biomass (Figure 16).

The interactive effect of soil type x biochar type on spinach biomass was significant ($p<0.001$) (Table 13). Spinach biomass was significantly higher in clay soil compared to the sandy soil, with the application of either acacia or poultry litter biochar (Figure 31). The interactive effect of soil type x biochar application rate on spinach biomass was significant ($p<0.001$) (Table 13). Spinach biomass was high in the clay soil compared to the sandy soil with the application rate from 0 to 20 t/ha (Figure 32). A significant ($p<0.001$) effect of biochar type x biochar application rate on spinach biomass (Table 13). Increasing the application rate of either acacia or poultry litter biochar from 0 to 20 t/ha increased the spinach biomass, although the increase was not significantly different between the two biochars at each application rate (Figure 33).

The interactive effect of soil type x biochar type x biochar rate on spinach biomass was significant on ($P< 0.001$) (Table 13). Spinach biomass was low in sandy soil compared to the clay soil with the application of either acacia or poultry litter biochar (Figure 34). Increasing biochar application rate from 0 to 20 t/ha, significantly increased the spinach biomass with 20 t/ha biochar application having the highest spinach biomass (Figure 34).

Table 13: Effect of soil type, biochar type and application rate on spinach biomass

Parameters	Biomass
	(grams)
Soil type	
Clay	28.17 a
Sand	24.44 b
$LSD_{0.05}$	0.11
Biochar type	
Poultry litter	27.78 a
Acacia	24.83 b
$LSD_{0.05}$	0.11
Biochar rate (t/ha)	
0	21.10 d
5	23.73 c
10	28.19 b
20	32.22 a
$LSD_{0.05}$	0.20
CV %	0.80
P value ≤ 0.05	
Soil type (S)	***
Biochar type (Bt)	***
Biochar rate (Br)	***
S × Bt	***
S × Br	***
Bt × Br	***
S × Bt × Br	***

Means with the same letter (s) within a column are not significantly different *** Significant at P< 0.001; ** Significant at P≤ 0.01**; * Significant at P ≤ 0.05.

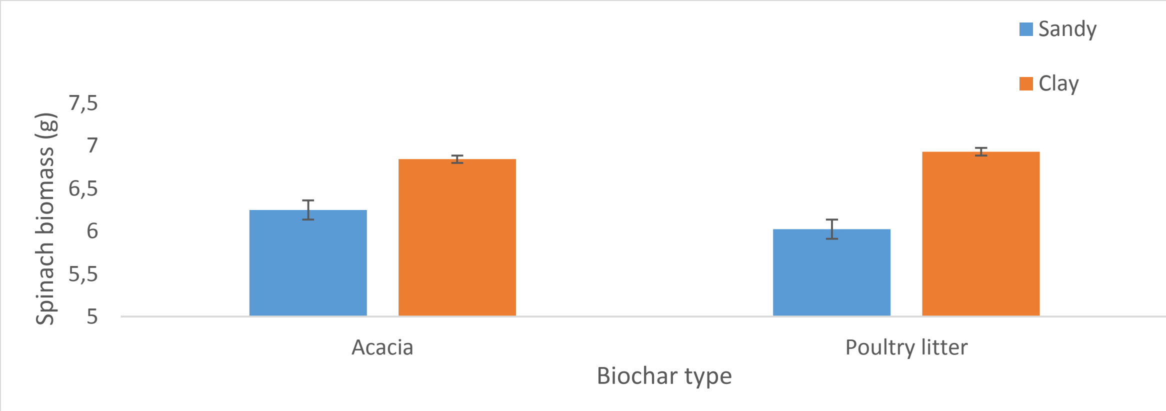

Figure 31: Effect of soil type and biochar type interaction on spinach biomass

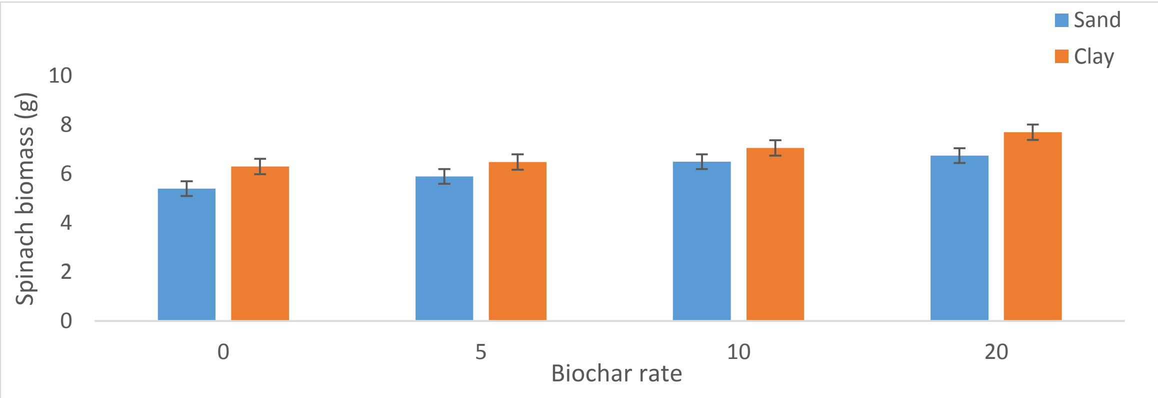

Figure 32: Effect of soil type and biochar rate interaction on spinach biomass

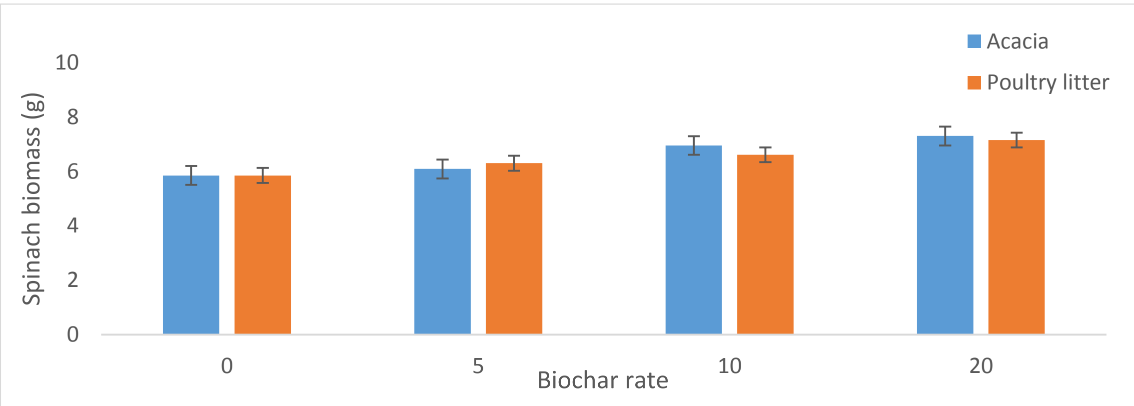

Figure 33: Effect of biochar type and biochar rate interaction on spinach biomass

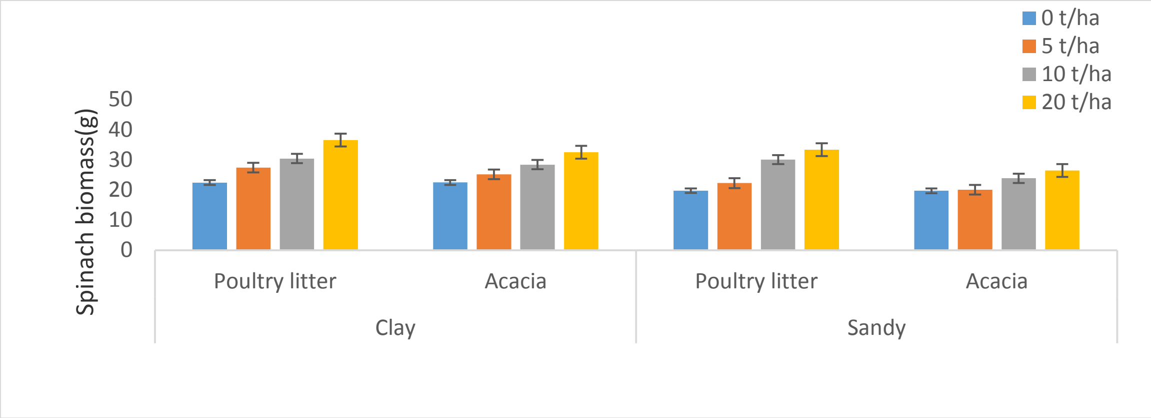

Figure 34: Effect of soil type, biochar type and biochar rate interaction on spinach biomass.

5.10 Effect of soil type, biochar type and application rate electrical conductivity, soil pH and soil stable aggregates

5.10.1 Electrical conductivity

Soil type had a significant ($p< 0.001$) effect on electrical conductivity of the soil (Table 14). The clay soil had significantly (17%) increased electrical conductivity than the sandy soil. Similarly, biochar type had a significant ($p< 0.001$) effect on electrical conductivity of the soil, with the poultry litter biochar application exhibiting significantly higher soil electrical conductivity than acacia biochar (Table 14). Biochar application rate had a significant ($p <0.001$) effect on electrical conductivity of the soil (Table 14). The application of biochar from 0 to 20 t/ha significantly increased the electrical conductivity of the soil (Table 14).

The interactive effect of soil type x biochar type on electrical conductivity of the soil was significant ($p<0.001$) (Table 14). Electrical conductivity was significantly low in sandy soil compared to the clay soil, with the application of either acacia or poultry litter biochar (Figure 35). The interactive effect of soil type x biochar application rate on spinach biomass was significant ($p<0.001$) (Table 14). Electrical conductivity was high in the clay soil compared to the sandy soil, except at 0 and 5 t/ha biochar application rate, although the increase was not significantly different between the two biochars at each application rate (Figure 36). A significant ($p<0.001$) effect of biochar type x biochar application rate on electrical conductivity was also observed (Table 14). Application rate from 0 to 10 t/ha did not have significant effect on electrical conductivity in both soils. However, at the application rate from 10 to 20 t/ha, electrical conductivity increased with the application of either acacia or poultry litter biochar, with the difference being significant at the 20 t/ha application rate (Figure 37).

The interactive effect of soil type x biochar type x biochar rate on electrical conductivity was significant ($P < 0.001$) (Table 14). Electrical conductivity was low in sandy soil compared to the clay soil with either acacia or poultry litter biochar (Figure 37). Increasing biochar application rate from 0 to 20 t/ha, significantly increased electrical conductivity of both soils with the 20 t/ha biochar application having the highest electrical conductivity in both soils (Figure 38)

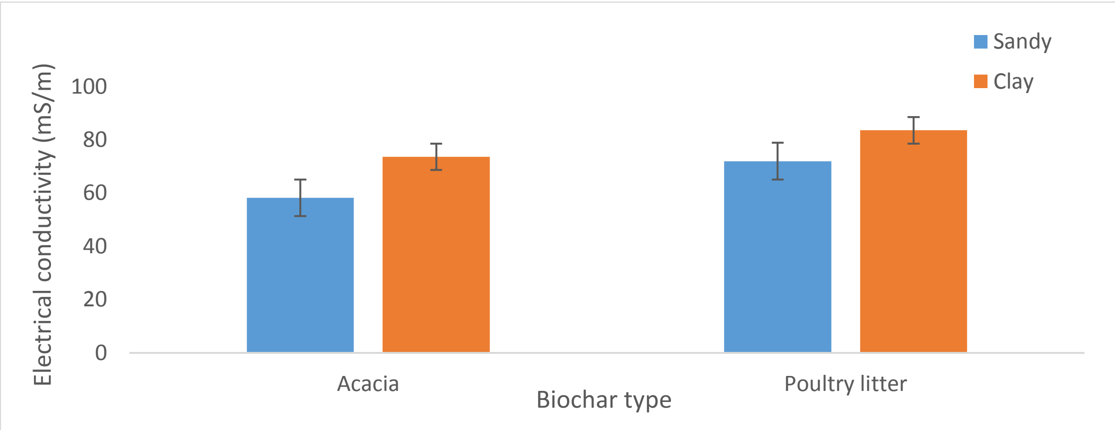

Figure 35: Effect of soil type and biochar type interaction on electrical conductivity

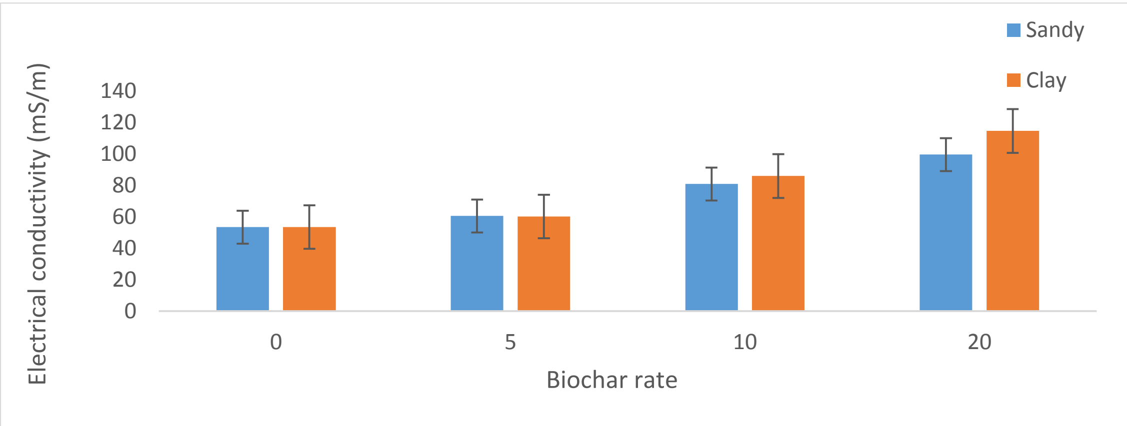

Figure 36: Effect of soil type and biochar rate interaction on electrical conductivity

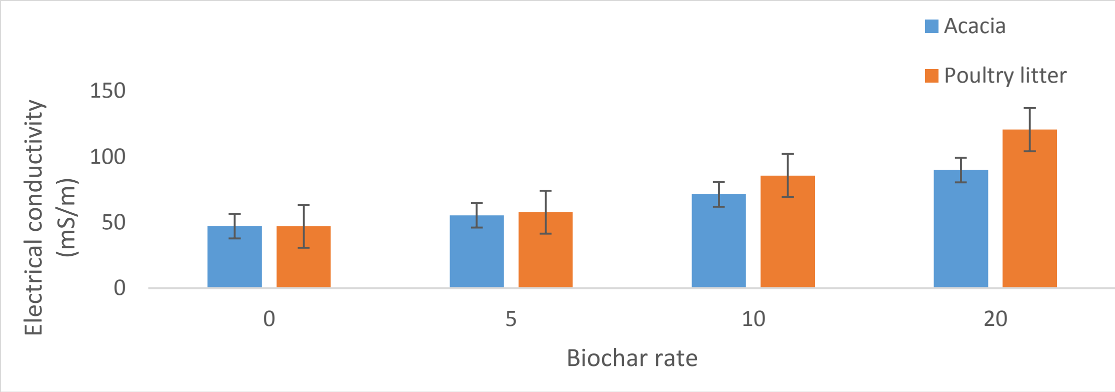

Figure 37: Effect of biochar type and biochar rate interaction on electrical conductivity

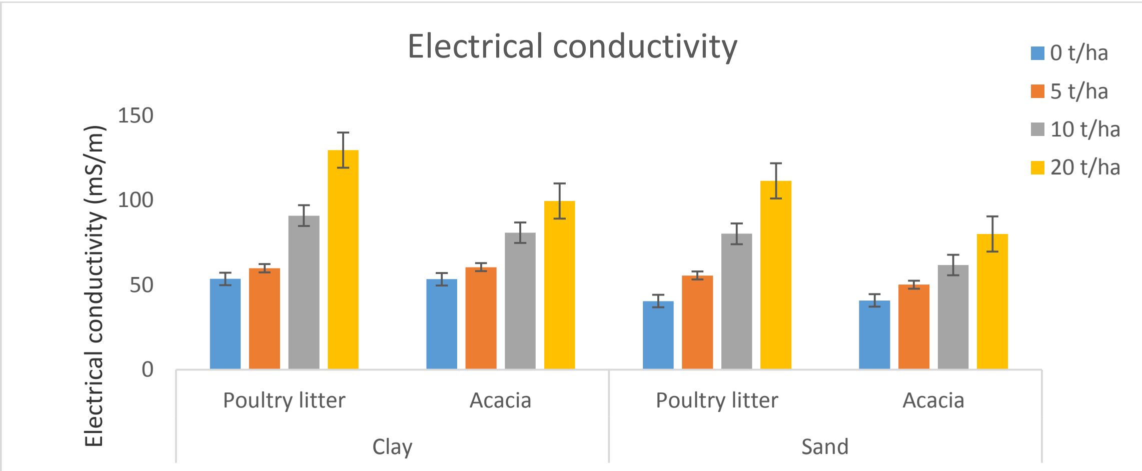

Figure 38: Effect of soil type, biochar type and biochar rate interaction on electrical conductivity.

5.10.2 Soil pH

Soil type had a significant ($p<0.001$) effect on soil pH (Table 14). The clay soil had significantly (9%) increased soil pH than the sandy soil. Similarly, biochar type had a significant ($p< 0.001$) effect on soil pH, with the poultry litter biochar application exhibiting significantly higher soil pH than acacia biochar (Table 14). Biochar application rate had a significant ($p<0.001$) effect on soil pH (Table 14). The application of biochars from 0 to 20 t/ha significantly increased the soil pH (Table 14).

The interactive effect of soil type x biochar type on soil pH was significant ($p< 0.001$) (Table 14). Soil pH was low in sandy soil compared to the clay soil, with the application of either acacia or poultry litter biochar (Figure 39). The interactive effect of soil type x biochar application rate on soil pH was significant ($p<0.001$) (Table 14). Soil pH was low in the sandy soil compared to the clay soil with the application rate from 0 to 20 t/ha (Figure 40). A significant ($p<0.001$) effect of biochar type x biochar application rate on soil pH was also observed (Table 14). Application rate from 0 to 20 t/ha raised the soil pH, although the increase was not significantly different between the two biochars at each application rate (Figure 41).

The interactive effect of soil type x biochar type x biochar rate on soil pH was significant ($P< 0.001$) (Table 14). Soil pH was low in sandy soil compared to clay soil with the application rate of either acacia or poultry litter biochar (Figure 42). Increasing the application rate of either acacia or poultry litter biochar increased soil pH in both soils (Figure 42).

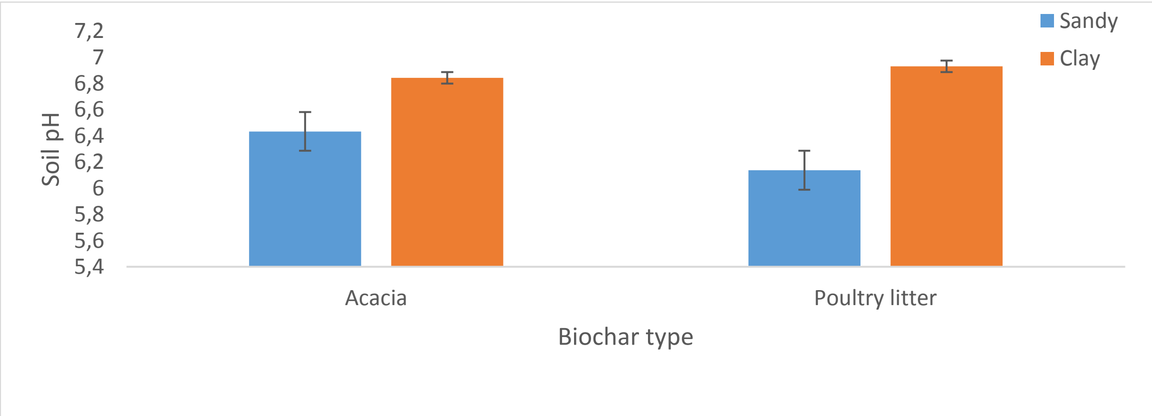

Figure 39: Effect of soil type and biochar type interaction on soil pH.

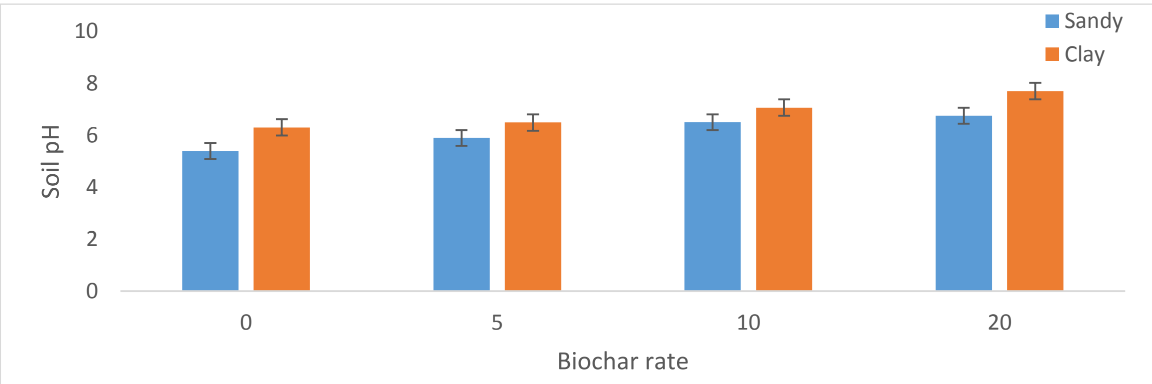

Figure 40: Effect of soil type and biochar rate interaction on soil pH.

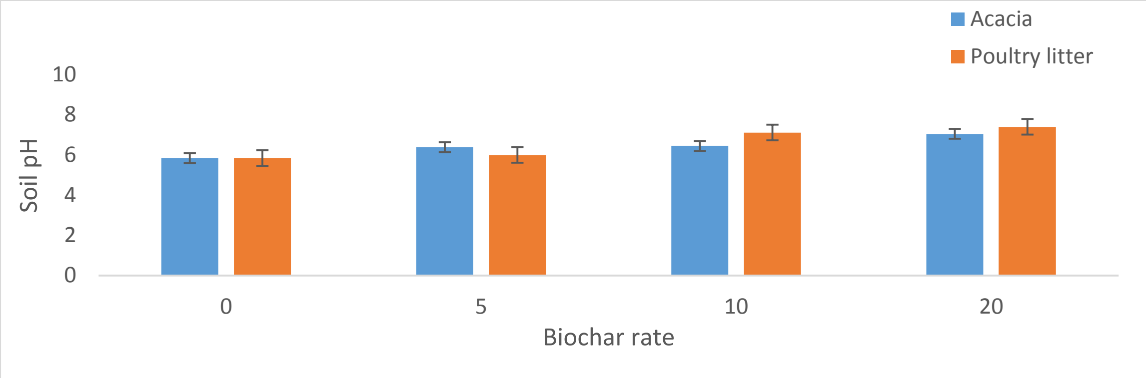

Figure 41: Effect of biochar type and biochar rate interaction on soil pH

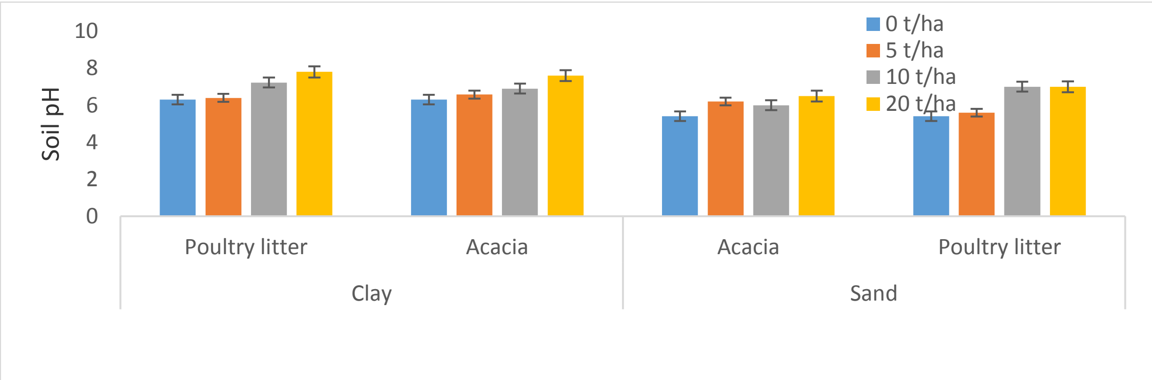

Figure 42: Effect of soil type, biochar type and biochar rate interaction on soil pH

5.10.3 Effect of soil type, biochar type and application rate on soil aggregate stability.

Soil type had a significant ($p<0.001$) effect on soil aggregate stability (Table 14). The clay soil had significantly (55%) increased soil aggregate stability than the sandy soil. Similarly, biochar type had a significant ($p< 0.001$) effect on soil aggregate stability with the poultry litter biochar application exhibiting significantly higher soil aggregate stability than acacia biochar (Table 14). Biochar application rate had a significant ($p<0.001$) effect on soil aggregate stability (Table 14). The application of biochars from 0 to 20 t/ha significantly increased soil aggregate stability (Table 14).

The interactive effect of soil type x biochar type on soil aggregate stability was significant ($p<0.001$) (Table 14). Soil aggregate stability was significantly higher in the clay soil compared to the sandy soil with the application rate of either acacia or poultry litter biochar (Figure 43). Poultry litter biochar significantly increased soil aggregate stability compared to the acacia biochar in both soils (Figure 43). The interactive effect of soil type x biochar application rate on soil aggregate stability was significant ($P<0.001$) (Table 14). Increasing biochar application rate from 0 to 20 t/ha increased the soil aggregate stability. However, the increase was significant with the application rate from 5 to 20 t/ha in both sand and clay soil (Figure 44). A significant (P<0.001) effect of biochar type x biochar application rate on soil aggregate stability was also observed (Table 14). Increasing the application rate of either acacia or poultry litter biochar from 0 to 20 t/ha increased the soil aggregate stability, although the increase was significant with the application rate from 10 to 20 with both acacia and poultry litter biochar (Figure 45).

The second order interaction effect of soil type x biochar type x biochar rate on soil aggregate stability was highly significant (P< 0.001) effect on stable aggregates (Table 14). Application of biochar increased soil aggregates stability, however the increase was significant with the application rate from at 5 t/ha and 10 t/ha with acacia biochar in clay soil (Figure 46). Increasing the application rate of either acacia or poultry litter biochar from 0 to 20 t/ha increased the percent soil stable aggregates in both soils. A significant difference was observed with application rate of poultry litter biochar from 10 to 20 t/ha in the clay soil (Figure 46).

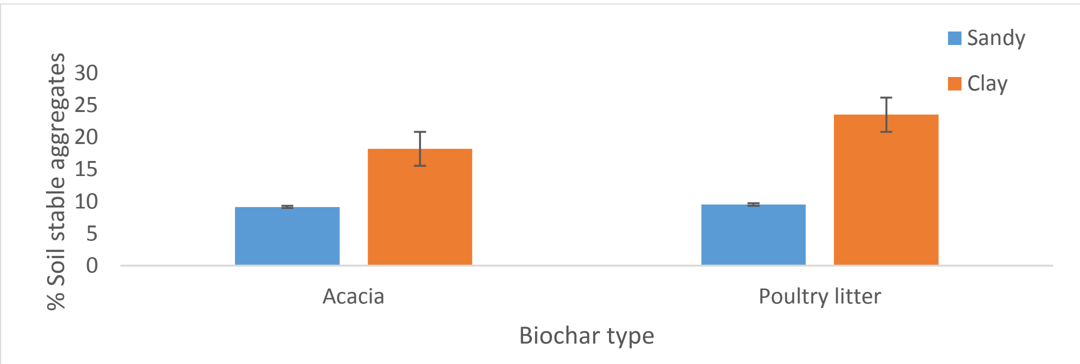

Figure 43: Effect of soil type and biochar type interaction of soil stable aggregate stability

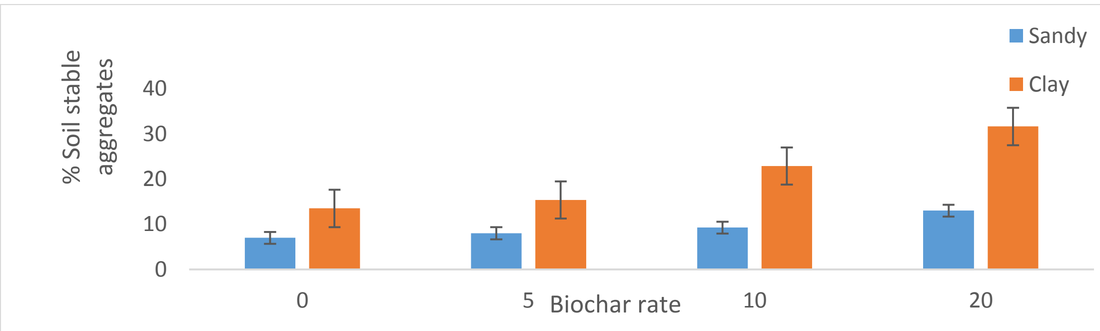

Figure 44: Effect of soil type and biochar rate interaction of soil aggregate stability

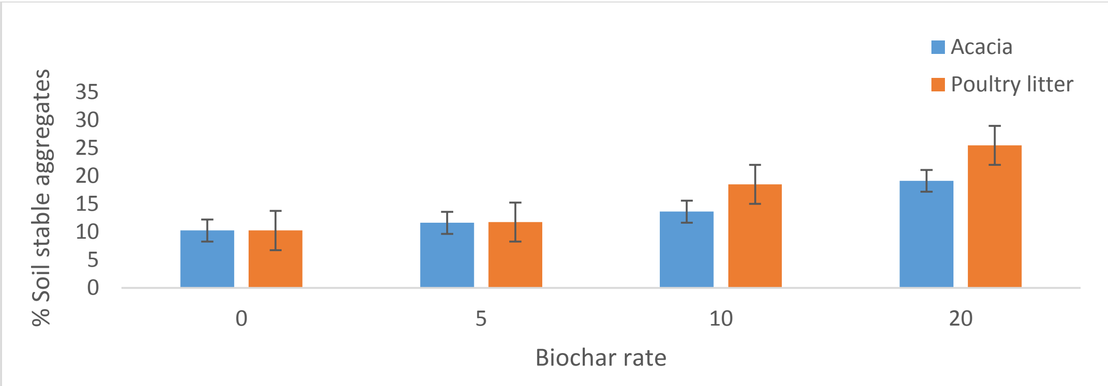

Figure 45: Effect of biochar type and biochar rate interaction of soil aggregate stability

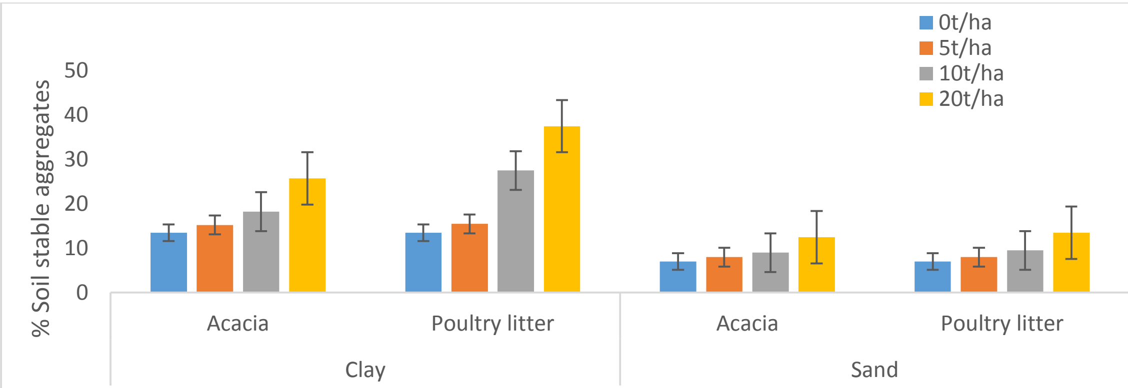

Figure 46: Effect of soil type, biochar type and biochar rate interaction on soil aggregate stability

Table 14: Effect of soil type, biochar type and application rate on electrical conductivity, soil pH and soil aggregate stability

Parameters	**Electrical conductivity** (Ms/m)	**Soil pH (H_20)**	**Soil aggregate stability** (%)
Soil type			
Clay	78.55 a	6.85 a	20.84 a
Sandy	65.10 b	6.21 b	9.31 b
$LSD_{0.05}$	0.63	0.009	0.24
Biochar type			
Poultry litter	77.74 a	6.76 a	16.50 a
Acacia	65.91 b	6.30 b	13.66 b
$LSD_{0.05}$	0.63	0.009	0.24
Biochar rate (t/ha)			
0	47.09 d	5.85 d	10.25 d
5	56.55 c	6.19 c	11.87 c
10	78.47 b	6.78 b	16.06 b
20	105.20 a	7.30 a	22.31 a
$LSD_{0.05}$	1.18	0.02	0.45
P value ≤ 0.05			
Soil type (S)	***	***	***
Biochar type (Bt)	***	***	***
Biochar rate (Br)	***	***	***
S × Bt	***	***	***
S × Br	***	***	***
Bt × Br	***	***	***
S × Bt × Br	***	***	***

Means with the same letter (s) within a column are not significantly different *** Significant at P< 0.001; ** Significant at P≤ 0.01**; * Significant at P ≤ 0.05.

6. DISCUSSION

6.1 Effect of soil type on the concentration of extractable chromium, nickel and lead in soils

Soil texture affects the solubility and therefore concentration of heavy metals in soils mainly through sorption, precipitation and complexation reactions. For example, ions of most heavy metals are most soluble in sandy soils but are largely adsorbed onto clay soils (Rieuwerts et al., 1998). Results of this study showed significantly increased concentrations of chromium, nickel and lead in the sandy soil relative to that in the clay (Table 8). Interestingly, the sandy soil had the least pH, EC, and nutrients (Ca, P, Mg, Na, and K) (Table 2). In general, the immobilisation of heavy metals is partly governed by the chemical properties of soils including soil pH (Lwin et al., 2018). In this study, the sandy soil which had the lowest pH exhibited increased concentration of the selected heavy metals. This was to be expected because according to literature background, a relatively lower soil pH results in increased concentration of heavy metals (Zhang et al., 2017). This could be because, a decrease in soil pH enhances the mobility and solubility of heavy metals and this could explain the increased levels of chromium, nickel and lead as shown in the sandy soil used in this study. Although not tested in this study, low soil pH conditions increased the concentrations of H^+ and Al^{3+} ions which outcompete heavy metals on adsorption sites, resulting in reduced adsorption and therefore increased availability or concentration of heavy metals (Liao et al., 2005). By contrast, the increased soil pH shown by the clay soil could have been conducive for formation of metal hydroxides and carbonate complexes, all which could have resulted in a decrease in the solubility of the selected heavy metals (Chlopecka and Adriano, 1996).

The increased concentration of chromium, nickel and lead in the sandy soil which had the least electrical conductivity is not commonly reported in literature. However, in general, there is an increase in the mobility of heavy metals in soils that contain high levels of electrical conductivity (Salim et al., 2012). The presence of high concentration of Ca in metal-contaminated soils enables it to compete with heavy metals for adsorption sites and this results in lower concentration of metals in such soils (Rieuwerts et al., 1998). This could explain the results of this study that the sandy soil with the least Ca exhibited the highest levels of the selected metals and the clay which had the highest Ca exhibited the least. Lastly, a high concentration of residual or native soil P has been reported to reduce the level of metals in the soil solution through formation of complexes with metals or precipitation of metals or formation of soluble salts between P ions and metals (Rieuwerts et al., 1998). In this study, soil

P was highest in the clay soil (Table 2) and this was associated with the least concentration of chromium, nickel and lead.

6.2 Effect of type of biochar on the concentration of extractable chromium, nickel and lead in soils

The biochar used in this study was produced using different feedstocks namely poultry litter and acacia and each contained different physical and chemical properties. The concentrations of chromium, nickel and lead were markedly enhanced in soil supplied with biochar derived from acacia compared to that made from poultry litter (Table 8). The properties of the biochars listed in Table 4 show that the biochar derived from acacia contained the least Ca, Mg, K, P, N, Fe, Mn, Cu, Zn, B, and Carbon. In addition, it generally had a larger surface area (see Table 4). Interestingly, biochar that has a large surface area increases adsorption especially on its surfaces (Yang et al., 2019) and therefore the concentrations of chromium, nickel and lead were expected to be least in soil treated with the acacia biochar. On the other hand, the relatively higher pH of the biochar derived from poultry litter could have rendered the test heavy metals less soluble, and through the adsorption of especially positively-charged toxic metal ions which could have resulted in their removal from the soil solution (Komkiene and Baltrenaite, 2015). Also, the poultry litter biochar exhibited markedly higher mineral concentrations (Ca, Mg, K, P, N, Fe, Mn, Cu and Zn) (Table 4). According to literature, a higher concentration of mineral nutrients (especially Ca, K and Mg) in biochar provides an opportunity for exchange of heavy metals and therefore results in their increased adsorption in the soil solution (Yang et al., 2015). In this study, this could partly explain the lower concentration of the test metals in soils supplied with poultry litter biochar which by virtue of containing increased concentration of most of the selected mineral nutrients, could have had a higher adsorption capacity compared to acacia-derived biochar which had least nutrient concentrations (Table 8).

6.3 Effect of biochar application rate on the concentration of extractable chromium, nickel and lead in soils

The ability of different types of feedstocks of biochar to extract or reduce the bioavailability of heavy metals from contaminated soils is determined by the rate at which it is supplied (Yang et al., 2016; Alaboudi et al., 2019). For example, the application of 20 t/ha biochar in this study significantly decreased the concentrations of chromium, nickel and lead (Table 8). Interestingly, for most studies involving extracting heavy metals from soil, increasing the rate or the application of the highest rate of biochar resulted in the highest immobilization of heavy metals (Fellet et al., 2011; Alaboudi et al., 2019). By increasing the rate of the poultry litter and acacia-derived biochars that were supplied to the test soil in this study, it could have resulted in increased pH of the soil. It has been shown by numerous studies that an increase in soil pH leads to the formation of metal hydroxides and carbonate complexes and therefore a decrease in the bioavailability of most heavy metals (Chlopecka and Adriano, 1996; Ahmad *et al*., 2012). In addition, most biochars generally contain pH in the alkaline range (including the test biochars) and therefore their application in soil results in a liming effect, which result in reduced bioavailability and mobility of most heavy metals in contaminated soils (Zhang et al., 2013). Lastly, by increasing the pH of contaminated soils through the application of higher rate of acacia of biochar, it can result in the precipitation of some heavy metals (Mousavi et al., 2010).

Whether through the formation of metal hydroxides and carbonates, liming effect, or precipitation, the high pH of the selected biochar could explain the lower concentrations of chromium, nickel and lead which was associated with the application of 20 t/ha, the highest biochar rate in this study. Although this study focussed on bioavailability of heavy metals in the soil, it is important to note that some studies have shown that by increasing the rate of biochar, there is increased uptake/accumulation of some heavy metals including chromium in plant tissue (Alaboudi et al., 2019). Literature background indicates that most biochars exhibit large surfaces and therefore, its application at higher rates increases the surface area which in turn leads to reduced solubility through enhanced adsorption and precipitation of toxic metals in soils (Beesley and Marmiroli, 2011).

6.4 Effect of biochar on immobilization of chromium in the soil

Poultry litter and acacia biochar application immobilized chromium in both sand and clay soil (Table 5). This is in agreement with Choppala *et al*. (2012) and Bolan *et al*. (2003) who found that biochar application in soils contaminated with chromium could significantly immobilize Cr (VI). This could be due to the presence of high carbon content in the biochar.

Chromium is commonly present in the soil, in the oxidized form, trivalent Cr (III) and hexavalent chromium Cr (VI) (Rahman *et al*. 2015). Cr (III) is not mobile and not toxic (Rahman *et al*. 2015). Biochar in the soil reduce Cr (VI) to Cr (III). The addition of Biochar acts as electron donor and provides the energy for the soil microorganism involved in the reduction of Cr. The proposed mechanism for Cr (VI) reduction equation is shown in the following equation:

$$3Fe(II)_{(s)} + Cr(VI)_{(aq)}\ 6OH^{-} \longrightarrow 3Fe(III)OH_{3(s)} + Cr(III)OH_{3(s)}$$

In addition, Cr (VI) can be reduced because of acidic functional groups which reduce Cr (VI) to Cr (III) (Rahman *et al*. 2015).

6.5 Effect of biochar on immobilization of nickel in the soil

The application of poultry litter and acacia biochar immobilized or reduced the concentration of nickel in both clay and sandy soil (Table 6). This is in agreement with the findings of Ehsan *et al*. (2014), Weng *et al*. (2004) and Boostani *et al*. (2019), who reported that biochar immobilized Ni, because of biochar containing organic matter, carbonates, Fe/Mn oxides and residual fractions (Walker *et al*. 2004). In addition, Ehsan *et al.* (2014), found that the increase in Ni immobilization by biochar could be due to O-containing surface fuctional groups that are able to complex metal ions.

According to Weng *et al*. (2004), Ni binds more strongly to clay and iron oxides than soil organic matter and Ni is less depended on soil pH. This agrees with the findings of this study, in which it was found that Ni concentration was reduced in clay soil than in sandy soil. According to Boostani *et al*, (2019), Ni immobilization depends on Fe, Mn and Zn content in the soil. PL had more content of Fe, Mn and Zn than AC. In this study, it was found that PL immobilized or reduced more Ni in the soil than AC. In addition, Ni immobilization could be affected by the ash content of biochar. According to Chen *et al*. (2018), biochar with more ash content tends to immobilize more Ni than the biochar with low ash content. This is in agreement with the findings of this study, whereby it was found that PL biochar which has more ash content, immobilized more Ni than AC biochar with lower ash content.

6.6 Effect of biochar on immobilization of lead in the soil

Poultry litter and acacia biochar application immobilized lead in both sandy and clay soil (Table 7). This is consistent with Wang *et al*. (2017), who reported that Pb adsorption capacity decreases as the application of biochar increases. This happens because at lower pH, the carboxyl and phenolic groups deprotonate and the negative charge of organic functional groups becomes the main site to adsorb Pb (Brady and Weil, 2008). In addition PL biochar immobilized more Pb than AC biochar, because PL biochar has more carbon content than AC biochar (Table 3). According to Cao *et al*. (2009), biochar sorbed Pb through a surface sorption mechanism via co-ordination of Pb d-electron to C=C (μ-electrons) bond and –O-Pb bond. Complexation of metals with ionized O-containing groups (-COO-and –O-) or C=C (μ-electrons) bond has been a major mechanism for metal sorption by AC. Mechanism can be described as follows:

$$>C: + Pb^{2+} \longrightarrow >C:Pb^{2+}$$

$$= C\text{-}COOH + Pb^{2+} + H_2O \longrightarrow =C\text{-}OOPb^{2+} + H_3O^{+}$$

$$= C\text{-}OH + Pb^{2+} + H_2O \longrightarrow =C\text{-}OPb^{2+} + H_3O^{+}$$

(Cao *et al*, 2009)

In addition, the amount of carbonate fraction and non-carbonate fraction determine the degree of lead sorption or immobilization behaviour (Zhou *et al.* 2016). The results obtained in this study is in agreement with those of Shen *et al* (2015) and Lucchini *et al*. (2014) who found that Pb strongly sorbed to the surface of biochar and could complex with the dissolved organic C and HCO_3 contained in biochar, which plays a role in the immobilization of Pb. In addition, Rees *et al*, (2014) found that immobilization of Pb could be directly proportional to the amount of carbon content.

6.7 Effect of type of soil on the accumulation of chromium, nickel and lead in leaves of spinach

Given that soils with different texture exhibit different properties, the properties account for considerable variability in the concentrations of heavy metals that each can retain or release for uptake by plants (Cataldo and Wildung, 1978). Studies have shown that the concentration of heavy metals in plants is largely associated with and affected by their concentrations in the soils they are grown in. Soil pH is one chemical property that could have played a significant role in the release, uptake and accumulation of the selected heavy metals in this study. The clay soil had a higher pH (moderately acidic) (5.9) compared to the sand (very strongly acidic) (4.9) (Table 2). Overall, results of this study revealed the concentrations of chromium, nickel, and lead in leaves of the test spinach were least when grown in the clay soil compared to the sandy soil with the application of either acacia or poultry litter biochar (Figures 21, 26 and 30). In general, clay-textured soils and with a pH that is not strongly acidic generally have an overall better control and therefore slow release of heavy metals (Hooda et al., 1997). In fact, the binding forces of metal ions to soils decrease with increasing pH in contaminated soils. This means that plants grown in such soils show least uptake and accumulation of metal elements and this could explain the low concentration of heavy metals that accumulated in leaves of the test spinach established in the clay soil.

6.8 Effect of type of biochar on the accumulation of chromium, nickel and lead in leaves of spinach

Biochar derived from different sources often show varying chemical and physical properties especially that from plant and animal-based sources. For example, biochar made from plant material contain lower ash content and nutrient concentrations compared to its counterparts derived from manure (Singh et al., 2010; Brewer et al., 2011; Sarkhot et al., 2012). As shown in this study, the leaves of spinach had significantly different concentrations of especially chromium and nickel when grown with acacia and poultry litter biochar. In fact, poultry litter-derived biochar was more effective in reducing the concentrations of Cr and Ni in the leaves of the test spinach. The relatively higher ash content in the poultry litter biochar (180 mg/kg) compared to the acacia-derived biochar (165 mg/kg) could have played a significant role in the sorption of toxic metals (Zhou et al., 2016) through serving as adsorption sites for, in particular the ions of chromium and nickel. Other studies have shown that the immobilisation of heavy metals is enhanced by animal manure-derived biochar that contain a higher ash content (Park et al., 2011; Wang et al., 2017).

6.9 Effect of biochar application rate on the accumulation of chromium, nickel and lead in leaves of spinach

When planted in the sandy soil, spinach showed markedly lower chromium with the application of 20 t/ha acacia biochar while the supply of 5 t/ha poultry litter biochar lowered it in the clay soil (Figure 13). In clay soil, the application of 10 and 20 t/ha acacia biochar significantly lowered the concentration of lead in spinach while the application of 5 t/ha poultry litter biochar significantly lowered the concentration of lead in spinach. In sandy soil, the concentration of lead was reduced by the application of either acacia or poultry litter biochar. Overall, the application of the test biochars reduced the accumulation of chromium and lead in spinach that was grown in clay and sandy soils. Compared to the control, increasing the rates of acacia and poultry litter biochar resulted in the immobilisation of Cr and Pb. Literature background shows that the application and increased rates of biochar reduce the uptake and accumulation of metal ions in plant tissues (Lahori et al., 2017). The markedly decreased accumulation of chromium and lead in leaves of spinach with the application and increased rates of the selected biochars could have been as a result of the ability of the biochar to immobilise these metals and one such mechanism is the liming effect of biochar which increases soil pH and therefore reduces the mobility but enhances immobilisation and uptake of metal ions (Liu et al., 2015). Also, with increased application rates, the increased surface area of biochars improves the ability of biochars to adsorb heavy metals, leading to reduced uptake and accumulation in organs of plants (Yang et al., 2019).

6.10 Effect of biochar on phytoavailability of chromium

Poultry litter and acacia biochar reduced the phytoavailability of Cr in both sandy and clay soils (Table 9). This is in agreement with Choppala *et al*. (2016), who found that the application of biochar has an effect on the photoavailibility of Cr (VI), which is mainly due to the reduction of Cr (VI) toxic to Cr (III) which is less toxic. Arshad *et al*. (2017), also reported that addition of biochar immobilises and reduces the bioavailability of Cr. Park *et al*. (2011), indicated that biochar increased the population of the soil micro-organisms. According to Rahman, *et al.* (2015), micro-organisms resist Cr (VI) by periplasmic biosorption, intracellular bioaccumulation and biotransformation to a less toxic speciation state through direct enzymatic reaction. Application of biochar increased micro-organisms that resulted in the reduction of the phytoavailability of Cr.

6.11 Effect of biochar on phytoavailability of nickel

Poultry litter and acacia biochar reduced the phytoavailability of Ni in both sandy and clay soils (Table 10). This is in agreement with El-Naggar *et al*. (2018) and Shahbaz *et al*. (2018). The reduction of the Ni phytoavailability could be due to the absorption of Ni onto the surface area of biochar (Ramzani *et al*. 2016) through surface precipitation, ion exchange and surface complexion and the high absorption and resultant of Ni, and Ni reduction of solubility in the soil, due to the rise in soil pH (Shahbaz *et al*. 2018). In this experiment poultry litter biochar reduced the phytoavalaibility of Ni than the acacia biochar. This may be because of Fe having antagonistic effect with Ni, which results in the reduction of bioavailability and/ or phytoavailability Ni (Nishida *et al.* 2012). This study is in agreement with Ramzani *et al.* (2016), who reported that Fe affect Ni availability to plants, for example the increase in Fe concentration in the soil, decreases the amount of Ni. In addition the phytoavailability of nickel can be reduced by increasing the pH or Fe can be lowered by increasing pH of the soil. In other words, the increase in soil pH reduces the nickel concentration in the soil and results in reducing the phytoavailability of nickel to the plants.

6.12 Effect of biochar on phytoavailability of lead

Poultry litter and acacia biochar reduced the phytoavailability of Ni in both sandy and clay soils (Table 11). This is consistent with Khan *et al*. (2013) and Bian *et al*. (2014) who found that biochar application, significantly reduced Pb uptake by the plants. This may happen because, biochar contain sulphur that affects the availability of Pb. According to Chao *et al*. (2018), sulphur affects the availability of Pb by affecting the pH, this results in lead forming $Pb_2(SO_4)_2O$ and $Pb_4(CO_3)_2SO_4(OH)_2$, during the adsorption on Pb^{2+} on the biochar, thus reducing the phytoavailability of Pb. Furthermore, sulphur changes the environmental conditions such as pH, oxidation and reduction potential, dissolves oxygen and solution conductivity that affects the phytoavailability of Pb (Wang *et al*. 2015, Mahar *et al*. 2016).

Lead is strongly sorbed to the surface of biochar and could complex with the dissolved organic carbon and HCO_3 contained in the biochar, playing a contributing role in reducing the phytoavailability of Pb (Lucchini *et al*. 2014). Futher more, the increased pH increases the number of negatively charged surface in the soil and the sorption capacity of the soil and Pb, thus resulting in the reduction of phytoavailability of Pb (Zheng *et al*. 2010) In addition, the application of biochar into the soil enhances the transformation of Pb from the acid soluble fraction to the reducible and oxidizable fractions thus reducing phytoavailability of Pb (Liu *et al*. 2015).

The addition of biochar in the soil increased the soil pH (Figure 42) which led to the precipitation of Pb as $Pb_5(PO_4)_3OH$, thus resulting in the reduction of the phytoavailability of Pb (Cao *et al*. 2009) According to Khan *et al.* (2013) and Li *et al.* (2015), the decrease and reduction of the phytoavailability of Pb varies with the type of the feedstock used or from which the biochar are derived. This is in agreement with the finding of this study where it was found that poultry litter biochar reduced the phytoavailability of Pb more than acacia biochar. According to Khan *et al*. (2013), Bian *et al*. (2014), Zheng *et al*. (2010) and Li *et al*. (2015), the decrease in the phytoavailability of Pb varies with the type of the feedstock or from which the biochar is derived. This is in agreement with the finding of this study, where it was found that poultry litter biochar reduced the phytoavailability of Pb than acacia biochar.

6.13 Effect of acacia and poultry litter biochar on electrical conductivity and soil pH

Results of this study revealed that the application of the selected biochars significantly lowered the electrical conductivity in the sandy soil compared to the clay soil (Figure 38). The application of biochar, be it made from plant (acacia biochar) or manure-based sources (poultry litter biochar) has an effect on the electrical conductivity of soils. The effect was significant in both clay and sandy soil (Figure 35). According to research results, the marked reduction of in electrical conductivity of sandy soils is partly caused by the fact that soils that are low in silt are likely to be more hydrologically responsive to biochar application (Sonnie *et al*, 2014). Also, biochars contain salts which promote reduction in EC in sandy soils but increase it in silt and clay soils (Alotaibi and Schoenau, 2019).

Literature has shown that generally, the application of different rates of biochars to soil with different texture results in a direct and most often linear relationship with EC. However, there are few cases where the relationship is inverse or non-existent (Laird and Rogovska , 2016). In this study, increasing the application rate of either acacia or poultry litter biochar in both the clay and sandy soils significantly enhanced the electrical conductivity (Figure 38). This direct interaction shown in this study could mean that the test biochars contained high electrical conductivity and therefore their application and increased rates of application increased the EC (Fernandes et al., 2019)

The pH of soils is one of the most important parameters that are modified by the application of biochar however, the effect is dependent on soil texture. Results of this study show that soil pH was markedly increased in the clay soil compared to sandy soil with the application rate of either acacia or poultry litter biochar (Figure 40). Although both types of soil used in this study were acidic, interestingly, both clay and sand pH were significantly increased by the application of the biochars. The main determining factor in this case was the soil texture and this effect has been shown in other studies that the supply of biochar affects pH especially in soil with different texture (Butnana *et al*., 2015). Interestingly, the increased soil pH as a result of biochar application was shown in the sandy soil which exhibited initial soil pH that was strongly acidic (see Table 2). It is, therefore, possible that the increased soil pH was caused by the application of the biochars in the acidic soil.

6.14 Effect of biochar on soil aggregate stability

Results of this study revealed that the application of the biochars significantly increased the soil aggregate stability in the clay soil compared to the sandy soil (Figure 43 and 46). In both clay and sandy soils, the application of biochar promoted aggregate stability, leading to increased fertility of soils (Pituello et al., 2018). Literature background has shown that biochar can influence soil aggregation through the altering of soil pH which was observed in this study, but also through increased aromaticity of soil organic C pool (Chan et al., 2008; Wang et al., 2017). The effect of biochar on aggregate stability varies with the type of soil. For example, Ouyang and Zhang (2013), showed enhanced formation and stabilization of macroaggregates in sandy loam soil compared to silty clay soil. Similarly, in this study, the observed improved aggregate stability was shown in the clay soil and not the sandy soil. The rate of biochar also has an effect on aggregate stability as shown by the increase with the application of 10 t/ha and 20 t/ha of PL biochar in clay soil. For example, Juriga et al. (2018) found that the application of 20 t/ha biochar markedly increased aggregate stability. According to Juriga et al. (2018), there are three mechanisms that possibly explain the increased aggregate stability in biochar-supplied soils: particles that are present on the surface of biochar are bound by carboxyl and hydroxyl groups; an increase in the hydrophobicity of soil particles by biochar; and the development of soil microorganisms (Glaser et al., 2002; Lehmann et al., 2011).

7. CONCLUSION AND RECOMMENDATIONS

This study investigated the effect of biochar on immobilization and phytoavailability of chromium, nickel and lead. Without exception, biochar significantly immobilized and reduced the phytoavailability of Cr, Pb and Ni. In addition, biochar had an impact on biomass of spinach, electrical conductivity, soil pH and soil aggregate stability. Poultry litter biochar was more effective in immobilizing and reducing phytoavailability of Cr, Pb and Cr than acacia biochar. Therefore, poultry litter can be used to remediate soils contaminated with Cr, Pb and Ni. In addition, poultry litter biochar was more effective in improving biomass of spinach, EC, soil pH and soil aggregate stability than acacia biochar. Therefore, poultry litter biochar can be used to improve the physical and chemical properties of the soil. Overall, the influence of biochar on Cr, Pb, Cr, soil pH, EC, biomass of spinach and soil aggregate stability depends on biochar feedstock used and the application rate. In this experiment, poultry litter was more effective in clay soil and at higher application rate of 20 t/ha. The limitation of this study is that only two types of biochars and one type of slag were used.

Further studies are needed to determine the long-term effect and impact of biochar on immobilization and phytoavailability of Cr, Pb and Ni, and to evaluate the potential effects of toxins present in of biochar and the viability of using biochar as a soil amendment. In addition, research need to be conducted to establish the optimum application rate on sandy soil that can immobilize Cr, Pb and Ni. The findings of this experiment are very encouraging, but they need to be investigated under field conditions that have been contaminated with Cr, Pb and Ni. In addition, studies should be conducted to determine the optimum rate of application of biochar for effective immobilization and reduction in phytoavailability of Cr, Pb and Ni. Lastly the mechanisms involved in immobilizing and reduction in phytoavailability of Cr, Pb and Ni, need to be investigated.

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