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ORIGINAL ARTICLE

Bioavailability and contamination levels of Zn, Pb, and Cd in sandy-loam soils, Botswana

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Abstract
Poultry litter and its biochar has been used recently as alternative effective soil organic amendments due to their significant effect of improving soil properties. However, detailed information on this organic amendment's contribution to the bioavailability of heavy metals in the soil is still lacking. Hence, this study was designed to evaluate the effects of incorporated poultry litter and its biochar on bioavailable Zn, Pb, and Cd concentrations in the soil using various assessment contamination methods. The soil samples treated with poultry litter and its biochar at different application rates of 0, 15, 30, 60, 120 g/kg were collected from a greenhouse pot experiment. The study results indicate a decreasing order in concentrations as follows; Zn (2.04 mg/kg) > Pb (0.22 mg/kg) > Cd (0.02 mg/kg). Geoaccumulation Index (I_{geo}) for Zn was moderate ($0 < I_{geo} < 1$) to heavy contamination ($I_{geo} = 1.00$), while Pb I_{geo} values were within the moderate contamination ($0 < I_{geo} < 1$) in all treated soils. Furthermore, Zn and Pb yielded contamination factor (CF) values within the ranges of low contamination (CF < 1) to moderate contamination (1 < CF < 3), nonetheless Zn exhibited the highest CF compared to Pb and Cd. Higher values of Pollution Load Index (PLI > 1) were observed, indicating the pollution level. Those PLI values point out the need to evaluate bioavailable heavy metals levels rather than total metal concentration for risk assessment of soil contamination by organic amendments.

Keywords Bioavailability · Poultry litter · Biochar · Geoaccumulation index · Contamination factor · Pollution index

Introduction

The bioavailability of heavy metals concentration level in the soil is one of the main factors that need evaluation for successful crop production as it affects soil quality. Soil quality is a significant aspect of effective, intensive, and substantial crop production and higher yields to meet a community's essential demand. Soil plays a vital role in ecological stability, but its quality concerning heavy metal concentrations is likely compromised by several anthropogenic activities (Ngole and Ekosse 2012). Hence, developing and applying reliable policy measures and improved agricultural practices to manage heavy metals (potential toxic elements) in soils and their availability is required. Although there have been some efforts to maintain required levels of metals in soils, it has become a problem worldwide to manage good quality soils due to the accumulation of non-degradable inorganic contaminants at most considerable magnitude in the environment (Kim et al. 2015; Tóth et al. 2016; Alves et al. 2016; Hayyat et al. 2016; Shifaw 2018). These contaminants are referred to as heavy metals, and the most commonly found are; Lead (Pb), Nickel (Ni), Chromium (Cr), Cadmium (Cd), Zinc (Zn), Cobalt (Co), and Copper (Cu) (Wuana and Okieimen 2011; Masindi and Muedi 2018; Selvi et al. 2019).

Heavy metals are characterized by high atomic mass and density of commonly 5 g/cm^3 (Jaishankar et al. 2014; Olalekan et al. 2016; Koller and Saleh 2018). Their rates depend on soils geological characteristics and occur naturally in point sources in limited areas (Alves et al. 2016). Moreover, heavy metals can persist in the soils for a long time as they resist microbial or chemical degradation (Wuana and Okieimen 2011; Hayyat et al. 2016). Heavy metals in soils amended with various wastes are redistributed and transferred with time from the labile forms to the more stable forms (Han et al. 2001; Nzihou and Sharrock 2010; Zheng

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and Zhang 2011). The redistribution processes of metals in the soil depend on their source and waste type. Soil mineralogy and soil pH are crucial as they control metal's coprecipitation and sorption processes in some cases. Other factors influencing soil metal bioavailability are organic matter and clay fractions (Rieuwerts et al. 1998; Han et al. 2001; Angelova et al. 2010). According to Rieuwerts et al. (1998), soil pH governs the concentration of soluble and plant available metals; consequently, metal solubility increases at acidic pH and decreases at higher pH values.

There are several primary sources of heavy metals in agricultural soils, for instance, the use of pesticides, the addition of manure, sewage sludge, and the over-use of agrochemicals (Alves et al. 2016). Apart from agricultural sources, industrial waste, car exhausts, and mines activities (Sun et al. 2018) are potential sources of heavy metals in soils (Choudhury 2015). The concentrations of metals at higher quantities become important environmental pollutants (Alloway 2012; Masindi and Muedi 2018), toxic to the plants, lessening productivity, and posing a dangerous threat to the agro-system (Ozores-Hampton et al. 2005). Once applied to soils, heavy metals may enter the food chain through plants and animals (Thomson 2013; Eze et al. 2018) or contaminate surface and groundwater (Zhang et al. 2013; Santoro et al. 2017).

Along with the soil, sediments and water bodies are also at risk of heavy metal contamination (Gerber et al. 2007; Yabe et al. 2010). For instance, Zinc (Zn) bonds firmly to organic matter (Han et al. 2000) hence it can easily affect the plants at high quantity beyond the optimum requirements. Nevertheless, Zn can negatively influence microorganisms and earthworms' activity, thus retard the breakdown of organic matter (Wuana and Okieimen 2011) necessary for plant growth. As for Lead (Pb), it is a naturally occurring bluish-gray metal usually found as a mineral combined with other elements (Wuana and Okieimen 2011). Ionized Pb can be adsorbed by soil minerals (Nguyen Thi et al. 2018); hence it is usually detected in soils (Alloway 2012). Pb tends to accumulate in aquatic organisms (Eze et al. 2018) and persist in the environment for a long time; therefore, it can contaminate both surface and ground water. In addition, Pb can quickly move from soil to plants through roots absorption, and large amounts can accumulate in their tissues without showing signs hence it is toxic. Similarly, human health is not spared (Santoro et al. 2017), because biomagnifications enter the human body (Alves et al. 2016). In humans, Pb was found to affect the nervous system (Elnazer et al. 2015).

Cadmium (Cd) is a cumulative contaminant, and it can result in various adverse effects in animals (Elnazer et al. 2015). Cd in soils may be found in forms that range from moderately to highly soluble (Elnazer et al. 2015). However, Cd's fate in the soils depends mainly on the relative balance between sorption, leaching, and plant uptake; hence contamination possibilities are higher even at lower concentration rates. Thus, Cd can still be transported by surface runoff to surface waters soluble or precipitated form (Wuana and Okieimen 2011) even at lower concentrations. Moreover, a substantial remediation method is necessary (Hayyat et al. 2016; Igiri et al. 2018; Selvi et al. 2019) to reduce metal solubility and bioavailability in soils (Wuana and Okieimen 2011). Cd has potential toxicity to man associated with allergic dermatitis in humans (Wuana and Okieimen 2011). The relative mobility of cadmium in the soil–plant system (McLaughlin and Singh 1999) may cause pollution in soils, sediments, water and enter the food chain.

Recently, the use of biochar in soils is an effective in situ remediation technique for potential heavy metals and organic pollutants immobilization (Liu et al. 2009) to reduce the concentration in soils (Zhang et al. 2013; Hayyat et al. 2016; Yuan et al. 2019; Antonangelo and Zhang 2020). Biochar refers to a fine-grained and porous substance produced by the slow pyrolysis of biomass at low to medium temperatures (450 to 650 °C) under oxygen-limited conditions (Park et al. 2011; Mulabagal et al. 2017). Different potential feedstock such as agricultural residues, municipal solid waste, anaerobic digestate, and livestock manure are used for biochar production (Duku et al. 2011; Ronsse et al. 2013). The properties of biochar play a major role in determining its effectiveness in terms of improving soil quality. For instance, biochar with large surface area (Zhang et al. 2013) incorporated in the soil lessens metal toxicity by the process of adsorption and desorption (Shaaban et al. 2018). Consequently various functional groups and the highly porous nature have shown biochar beneficial for heavy metals adsorption (Zhao et al. 2019; Wu et al. 2019).Furthermore, biochar with high ash content increases soil pH, a significant number of oxygen functional groups, and phosphorous levels that lead to ligands formation, contributing significantly to heavy metals immobilization (Gondek and Mierzwa-Hersztek 2016). In addition, insoluble metal mineral phosphate and carbonates are formed which decreases metal solubility at high pH. Besides, lower soil pH results to free ions species of metals that are more bioavailable (Olaniran et al. 2013) which can be toxic to the environment. Therefore, it is crucial to choose the right type of biochar to incorporate in soils for reducing metal's toxicity effects on the environment (Lehmann and Joseph 2015; Lima et al. 2015; Beesley et al. 2015) and stabilizing heavy metals (Zhang et al. 2013) concentration levels. Besides reducing metals mobility in contaminated soil (Nartey and Zhao 2014), biochar also enhances soil physicochemical properties and crop yield (Hayyat et al. 2016).

This study aims to evaluate the effects of organic amendments amended in the soil using evaluation contamination methods on the concentration of metals (Zn, Pb, and Cd) bioavailability. According to Ifon et al. (2019), metal mobility in the soil is strongly linked to their bioavailability, because the bioavailable concentrations indicate the risk assessment and classification of polluted soils (Beesley et al. 2015). Furthermore, the metal bioavailability assessment predicts the risk of metal uptake by the plants (Sihag and Lohchab 2017) hence the main thrust of this study is on metal bioavailability.

Materials and methods

Preparation of samples

The soil samples were collected from the greenhouse pot experiment that was carried out from August 2019 to October 2020 at the Botswana University of Agriculture and Natural resources. The pot experiment was conducted by homogenously mixing organic amendments (poultry litter and its biochar) with sandy-loam soil at application rates of 0, 15, 30, 60, and 120 g/kg before saturating the soil to field capacity. Throughout this paper, the phrase soil organic amendment refers to poultry litter and its biochar. The treatments are presented as PL (poultry litter), BC350 (biochar pyrolyzed at a temperature of 350 °C), and BC600 (biochar pyrolyzed at a temperature of 600 °C). The PLBC350 represents a combination of PL and BC350, whereas PLBC600 is PL and BC600 combined. The blended organic amendments were combined in a ratio of 1:1 based on the application rate of pure organic amendments.

The soil textural class was determined by Multisizer (Malvern Instruments, Ltd; Software Version: 3.00) and further soil classification was done using Bouyoucos-Hydrometer method (Bouycous 1951). The soil pH and electrical conductivity were determined in a soil–water extract (Ibitoye 2006) using a digital hand-held pH meter. Potassium dichromate oxidation and titration against ferrous ammonium sulfate (Walkley and Black 1934) method was used to determine organic matter content. The exchangeable cations were determined by ammonium acetate (Gumbara and Darmawan 2019) and their summation was used as cation exchange capacity.

Biochar production

Air-dried 2 mm sieved poultry litter feedstock was used for biochar production. Pyrolysis temperatures of 350 °C and 600 °C were set for biochar production using a crucible muffle furnace (FO310, Yamoto). In-flow of nitrogen gas was run before and during pyrolysis to eliminate oxygen gas in the crucible and from the cubicle. The samples were pyrolyzed for 2 h and left to cool in the furnace after the pyrolysis time elapsed.

Analytical methods and analysis

Digestion reagents and calibration

Initially, glassware was cleaned with phosphate-free soapy water and rinsed with distilled water before soaking overnight in a 10% (w/v) nitric acid solution and finally rinsed thoroughly with deionized water (dH2O). The analytical grade chemical, Ethylene Diamin Tetra-acetic Acid (EDTA), and standard stock solution (Zn, Pb, and Cd) of 1000 mg/L obtained from Merck companies were used. High-purity water (electrical resistivity > 10 MΩ cm) was produced with a Milli-Q system (Millipore, MA, USA). The mixed working standard solution was prepared by serial dilution from a prepared stock solution containing all the metals analyzed in this study, and ultrapure water was used to mark up the solution in 100 ml volumetric flasks. Calibration of the MP-AES was conducted before sample analysis using prepared standards, and all the analyses were run in triplicate for every sample treatment.

Extraction of bioavailable heavy metals in soil

Bioavailable metals in the soil samples were extracted using a 0.005 M Ethylene Diamin Tetra-acetic Acid (EDTA) pH7.0 following a single extraction method at the Department of Environmental science laboratory. The standards, measurements, and testing programme (SM&T) of the European Commission have identified a single extraction method suitable approach (Olaniran et al. 2013) for determining bioavailable heavy metals fractions in soil. A 5 g soil sample was extracted using EDTA by shaking on a mechanical shaker for an hour before centrifugation at 6500rmp for 10 min. After that, the extractant was filtered using Whatman 42 filter paper (Zhang et al. 2010) into 100 ml volumetric flasks. Thereafter, ultra-pure water was used to fill up the filtrates to the mark. A laboratory fridge set to a temperature of 4 °C was used to store prepared solutions in a high-density plastic bottle awaiting metal analysis.

Elemental analysis of samples

The Agilent 4100 Microwave Plasma Atomic Emission Spectrometer (MP-AES) installed with software version 1.5.2.7948 was used to determine concentration levels of bioavailable heavy metals from extracted filtrates at the Department of Medicine in the University of Botswana. MP-AES allows for the analysis of many analytes in a reasonable short time and achieves more comprehensive sample characterization. The standard operating conditions of the instrument were as follows; gas source (Agilent 4107

Nitrogen Generator), auto background correction, 15rmp speed pump, 15 s sample uptake time, 15 s stabilization, sample introduction by Agilent SPS 3, the 30 s rinse time between the samples and calibration coefficient limit of 0.999. The wavelengths used were 213.857 (Zn), 405.781 (Pb), and 228.802 for Cd.

Degree of contamination of bioavailable Zn, Pb, and Cd in soils

The evaluation of soil contamination by Zn, Pb, and Cd was performed after determining metal concentrations levels. The degree of contamination was based on an index of accumulation (I_{geo}) proposed by Muller (1969). I_{geo} has been widely used to assess heavy metals contamination in trace metals pollution in agricultural soils (Rabee et al. 2011; Dotaniya et al. 2018). Besides contamination factor (CF) was also applied to evaluate the degree of metal contamination. CF is a single index, a simple and effective tool in monitoring heavy metal contamination (Shen et al. 2019). Finally, a general level of bioavailable heavy metals in treated soil was assessed by Pollution Load Index (PLI) (Tomlinson et al. 1980; Rabee et al. 2011; Shen et al. 2019). The concentration values of control samples were used as the background values to determine both I_{geo} and CF, the formulas listed below were used to evaluate the degree of contamination, and Table 1 represents the classification levels of I_{geo} and CF.

1. **Geoaccumulation index (I_{geo}) in**

$$I_{geo} = \log_2 \frac{C_m}{1.5 \times B_n}. \quad (1)$$

2. **Pollution Load Index in**

$$PLI = n\sqrt{CF_{Zn} \times CF_{Pb} \times CF_{Cd}}, \quad (2)$$

where C_m is the measured concentration of heavy metal in the soil, B_n is the geochemical background concentration of the same heavy metal, 1.5 = background matrix correction factor due to lithogenic effect, B_m is the local background concentration value of the heavy metal, CF is the contamination factor and n is the number of elements (in this case 3).

Statistical analysis

The data sets were statistically performed using the Statistical Package for the Social Sciences (SPSS) software packages Version 22.0 (SPSS Inc., USA) for mean heavy metal concentrations. The one-way ANOVA (analysis of variance) test was used to determine the statistically significant difference in metals concentration within and between the groups. The means differences were determined using the Tukey (HSD) post hoc test.

Results and discussion

Properties of soil, poultry litter and its biochar

Table 2 summarized the primary characteristics of soil and organic amendments used in this study. The soil was classified as sandy loam (64% sand, 18% clay and, 18% silt) with a slightly acidic pH_{H2O} value of 6.36 and organic matter content of 2.81% (Table 2). The application of organic amendments altered the soil pH as it is shown by a significant increase of pH with organic amendments concentration in all treatments (Table 2). For organic amendments, PL was slightly alkaline with a pH value of 7.77 recorded and BC350 had a substantial pH value of 8.58, whereas BC600 had strongly alkaline pH value of 9.21. A similar trend was displayed by Ebadnejad et al. (2018). Yang et al. (2021) also observed the increase of soil pH from 5.90 to 7.23 with the increase of swine manure biochar application rate. The increase of pH values in biochar may be attributed to high ash content and oxygen functional groups that occur during pyrolysis. The organic matter content determined by the loss of ignition method (Matthiessen

Table 1 Classification of geoaccumulation index and contamination factor values

I_{geo} classes	CF classes
$I_{geo} < 0$—uncontaminated	CF < 1—low contamination
$0 < I_{geo} < 1$—moderately contaminated	1 < CF < 3 moderate contamination
$1 < I_{geo} < 3$—moderately to heavily contaminated	3 < CF < 6 considerable contamination
$3 < I_{geo} < 4$—heavily contaminated	CF > 6—very high contamination
$4 < I_{geo} < 5$—heavily to extremely contaminated	
$I_{geo} > 5$—extremely contaminated	

For PLI, values < 1 indicate no pollution, whereas values > 1 indicate pollution

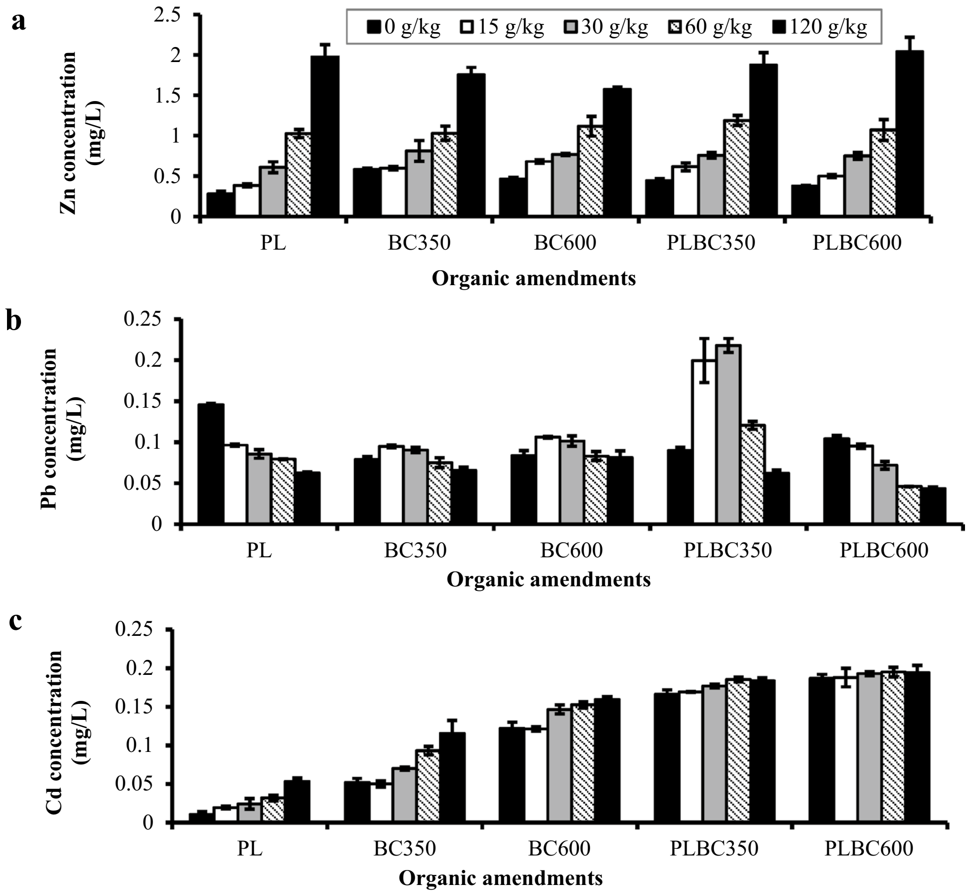

Fig. 1 Concentration levels of the bioavailable Zn (**a**), Pb (**b**), and Cd (**c**) in sandy-loam soils as affected by the application of poultry litter and its biochar at different application rates of 0, 15, 30, 60, and 120 g/kg. Error bars denote the standard error ($p<0.05$), $n=3$

et al. 2005) was 5.69% (PL), 28.57% (BC350), and 23.82% (BC600).

Concentration level of bioavailable Zn, Pb, and Cd (mg/kg) in soils

Analyzing the heavy metals concentration levels should be based on bioavailability (Ravindran et al. 2017) as it removes more significant amounts of heavy metals from the soil (Wang et al. 2006). Bioavailable heavy metals are also considered bio-absorbed or toxic to organisms (Li et al. 2021) hence the need to be considered. Figure 1 summarizes the concentration levels of the bioavailable metal ions (Zn^{2+}, Pb^{2+}, and Cd^{2+}) in experimental sandy-loam soil incorporated with poultry litter and its biochar at different application rates. The control samples (sample with no amendment) exhibited the lowest concentration levels compared to the treated soil samples (Fig. 1). Even though, control samples displayed lower metal bioavailability than treated samples, their concentration levels varied among different organic amendments probably due to metal absorption by Jatropha plants that were grown in the soils. This is because during the experimental growing period some plants lost their leaves which may have affected other chemical processes related to metal absorption hence variations in metal bioavailability. Furthermore, Zn concentration levels were higher (0.28 to 0.58 mg/kg) compared to Pb (0.08 to 0.15 mg/kg) and Cd (0.01 to 0.19 mg/kg) in control samples which indicate that both the soil and the organic amendments contributed to concentration levels of the metals.

In general, Zn increased with the increase of application rate (0.28 ± 0.03 mg/kg to 2.04 ± 0.18 mg/kg), followed by Pb (0.04 ± 0.01 mg/kg to 0.22 ± 0.01 mg/kg) and then Cd (0.01 ± 0.01 mg/kg to 0.20 ± 0.01 mg/kg) (Fig. 1a). A statistically significant effect ($p<0.05$ level) was observed in Zn mean concentrations. The bioavailable Zn values ranged from 0.39 mg/kg to 1.98 mg/kg in PL-treated soils. Bioavailable Zn mean values were significantly higher (2.04 mg/kg) in PLBC600 treated soil (Fig. 1a). The higher Zn concentration values under alkaline conditions (Fig. 2) may be due to the formation of hydroxo-metal complexes as the protons are being replaced, become soluble, and bioavailable (Olaniran et al. 2013). Moreover, the increased Zn content was also observed in the study of Gondek and Mierzwa-Hersztek (2016), this may be due to the considerable charge of easily soluble metal compounds (Gondek and Mierzwa-Hersztek 2016). Furthermore, the results for Zn are consistent with the findings of Shuman (1999), who concluded that organic

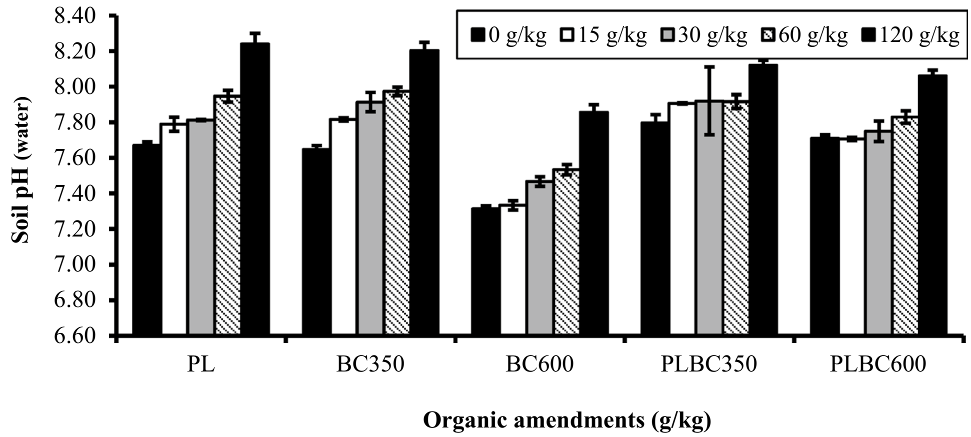

Fig. 2 Soil pH levels in treated sandy-loam soils as affected by the different application rates of poultry litter and its biochar. Error bars denote the standard error ($p < 0.05$), $n = 3$

Table 2 Basic characteristics of samples used for the study

Property	Samples: Soil and Organic amendments				Units
	Soil	PL	BC350	BC600	
pH (D_{H20})	6.36	7.77	8.58	9.21	–
Electrical conductivity	51.0	13.9	365.33	278.67	dS/m
Organic matter	2.81	5.69	28.57	23.82	%
Available P	0.80	33.01	33.37	33.98	–
Exchangeable cations					
K	4.13	760.12	1714.08	855.20	ppm
Ca	6.36	746.81	724.32	748.65	ppm
Mg	4.13	380.86	520.68	367.36	ppm
Na	6.85	127.34	354.75	162.26	ppm

All data values were reported as means ($n = 3$). PL means Poultry Litter, BC350; Biochar produced at a pyrolysis temperature of 350 °C, BC600; Biochar produced at a pyrolysis temperature of 600 °C, while PLBC350 and PLBC600 are a combination of poultry litter with either BC350 or BC600

materials high in Zn could increase bioavailable Zn content in the soil. In this case, poultry litter and its biochar have high doses of organic matter content (Table 2) and liming effect that are also associated with the higher concentration of bioavailable Zn in the soil.

For bioavailable Pb, a statistical difference ($p < 0.05$) was exhibited in all treated samples except for BC600 treated soils ($p > 0.01$) (Fig. 1b). The observed Pb concentration level ranged between 0.04 and 0.22 mg/kg and decreased with the organic amendments application rates (Fig. 1b). The highest bioavailable Pb value exhibited was 0.22 mg/kg Pb at 15 g/kg in PLBC350 treated soils. For soils treated with blended organic amendments, the concentrations varied among the different application rates (Fig. 1b). However, PLBC350 indicated a decreasing concentration rate (0.14 to 0.02 mg/kg) with an increasing application rate as observed in the study by Ihugba et al. (2018). The low concentration of Pb at a higher application rate may be due to the precipitation of abundant phosphate compounds applied to soils (Olaniran et al. 2013) that reduce its availability as insoluble metals phosphate species are formed (Olaniran et al. 2013). Furthermore, Pb's preferential fast adsorption in biochar amended soil, compared with Cd, and Zn could be attributed to Pb's greater affinity for carboxylic and phenolic functional groups (Yang et al. 2021) situated on the surface of oxidized biochar particles (Gondek and Mierzwa-Hersztek 2016; Li et al. 2021). Besides, Alloway (2012) stated that Pb in soils is usually contributed by exhausts from petrol engines in motor vehicles, and this might be the case for Pb being present in poultry litter as the farm is located near a busy main road. The results of this study are constant with the previous studies of Ihugba et al. (2018) and Yang et al. (2021) showing a decrease in Pb concentration levels with increased application rates. In addition, Rostaminia (2018) reported a decrease in Pb concentration values with increased biochar application rate. Ukpe and Chokor (2018) study displayed a Pb value of 1.17 mg/kg that reveals a possibility of poultry birds excreting Pb ions from their system which may have contributed to Pb being detected in poultry litter.

As for bioavailable Cd (Fig. 1c), concentrations levels increased slightly from 0.12 to 0.20 mg/kg with increase in application rates. However, PLBC600 treated soils exhibited the highest bioavailable Cd concentration values (0.20 mg/kg) (Fig. 1c). Park et al. (2011) results have also revealed that extracted Cd reduced (0.73 mg/kg) with the increase of chicken-derived biochar application rates from 0.95 to 0.11 mg/kg. Moreover, a reduction of Cd concentration of 55.5% was also observed by Rostaminia (2018), where chicken-derived biochar was applied in the soil. Similarly, our results show lower Cd concentration level of 0.19 mg/kg (Fig. 1c) compared to Zn. Cd in soil may be found in forms that range from moderately to highly soluble (Elnazer et al. 2015). Therefore, Cd can be transported by surface runoff to surface waters soluble or precipitated form (Wuana and

Okieimen 2011). Hence necessary precautions need to be followed when dealing with the soil.

Abiotic factors including pH, organic matter, clay fractions (Rieuwerts et al. 1998; Angelova et al. 2010) may have contributed to the study's results due to their influence on the availability of heavy metals in soil. According to Rieuwerts et al. (1998), soil pH governs the concentration of soluble and plant available metals; consequently, metal solubility tends to increase at acidic pH and decrease at higher pH values. The remarkable slightly acidic soil pH in control and soils treated with lower application rates of organic amendments (Fig. 2) may have contributed to increased metal solubility. Hence, a lower metal concentration of heavy metals was noticed at lower application rates (Fig. 1). Furthermore, acidic soil pH (Fig. 2) at a lower application rates may have contributed to Pb mobility (Chileshe et al. 2020; Yang et al. 2021), because acidic soil conditions are known to increase the solubility and bioavailability of heavy metals (Uchimiya et al. 2012). In addition, at higher pH, metals tend to form insoluble metal mineral phosphate and carbonates, whereas at low pH, they tend to be found as free ions species or as soluble organometals that are more bioavailable (Olaniran et al. 2013). Increased levels of bioavailable metal concentrations are attributable to higher pH of organic amendments; pH 7.77 (PL), 8.56 (BC350), and 9.76 (BC600). Furthermore, biochar enhance the cation adsorption ability and promoted the precipitation of heavy metals in the forms of oxides, carbonates, hydroxides and phosphates to reduce metal bioavailability via sorption and precipitation reactions (Li et al. 2021).

Along with soil pH, the increase of the organic amendments' application rate may have elevated the organic matter content and concentration of metals in the soil. Olaniran et al. (2013) observed that soils with relatively low organic matter content and a significant quantity of amorphous phase are high sensitivity to contamination by heavy metals and persistence of organic pollutants hence low metal concentration in soils treated with low organic matter content soil amendment. In addition, these study results agree with Uchimiya et al. (2012) where biochar amendments were most effective for decreasing bioavailable Pb and Cd concentrations.

Geoaccumulation Index (I_{geo}) and contamination factor (CF)

Geoaccumulation index (I_{geo}) was used as a measure of soil quality (Fosu-Mensah et al. 2017), as Muller (1969) proposed. The results showed a moderately contaminated ($0 < I_{geo} < 1$) for Zn I_{geo} in all soils except for PL ($I_{geo} = 1.42$) and PLBC600 ($I_{geo} = 1.08$) categorized as moderately to heavily contaminated in all treated soils at an application rate of 120 g/kg (Fig. 3a). Moreover, Zn I_{geo} values consistently increased with the application rate ranging from 0.21 to 1.42, which may be due to the increase of bioavailable Zn with the application rate (Fig. 3a). The highest application rate (120 g/kg) displayed greater I_{geo} values ranging from 0.61 to 1.42 for Zn compared to Pb I_{geo} (0.02 to 0.17) values and Cd I_{geo} (0.21 to 0.99) values. As for Pb, Igeo values decreased with an increase in application rate in the ranges of 0.49 to 0.01 (Fig. 3b).

Pb I_{geo} values were within the moderately contamination ranges ($0 < I_{geo} < 1$) regardless of bioavailable Pb concentration values decrease with application rate. In addition, PLBC350 treated soils displayed the highest level compared to other organic amendments (Fig. 3b). The trend of Pb Igeo values linearly correlates with bioavailable Pb concentration values (Fig. 1). Based on Fig. 3c, the Cd I_{geo} values were within the moderate contaminated category ranging from 0.19 (BC350, 15 g/kg) to 0.99 (PL, 120 g/kg). Moreover, PL and BC350 treated soils revealed higher Cd I_{geo} levels than other organic amendments (Fig. 3c), indicating a possibility of soil contamination by Cd despite lower bioavailable Cd values. Based on CF values (Table 3), bioavailable Zn, Pb, and Cd are categorized in low contamination and large contamination ranges. In addition, Zn exhibited higher CF values (Table 3) ranging in moderate contamination level ($1 < CF < 3$) at all application rates except for an application rate of 120 g/kg (considerable contamination, $3 < CF < 6$). For bioavailable Pb, the CF values ranged from 0.42 to 2.43 in PLBC350 treated soils at an application rate of 30 g/kg and categorized as low to moderate contamination (Table 3). In addition, the Pb CF values decreased with the application rate increase within the PLBC600 treated soils showing the least CF values.

Zn is typically used in poultry feeds as feed additives used in poultry feeds as growth promoters and biocides (Han et al. 2000; Bolan et al. 2010; Kayastha 2014). Other factors that may affect associated concentrations of metals are the birds age, type of the ration, and waste management practices (Irshad et al. 2013). Hence, the increase of bioavailable Zn level, Igeo, and CF values in soil for this study. Zn is considered essential for living organisms (Micó et al. 2006); however, Zn can negatively influence microorganisms and earthworms' activity, thus retard the breakdown of organic matter (Wuana and Okieimen 2011) necessary for plant growth. Pb is a naturally occurring metal usually found as a mineral combined with other elements (Wuana and Okieimen 2011), and ionized Pb can be adsorbed by soil minerals (Nguyen Thi et al. 2018). Hence it is usually detected in soils even though Pb solubility is low in the atmosphere. The decrease of bioavailable Pb, I_{geo}, and CF values may indicate the metals' absorption ability by biochars, especially for BC600 treated soils with

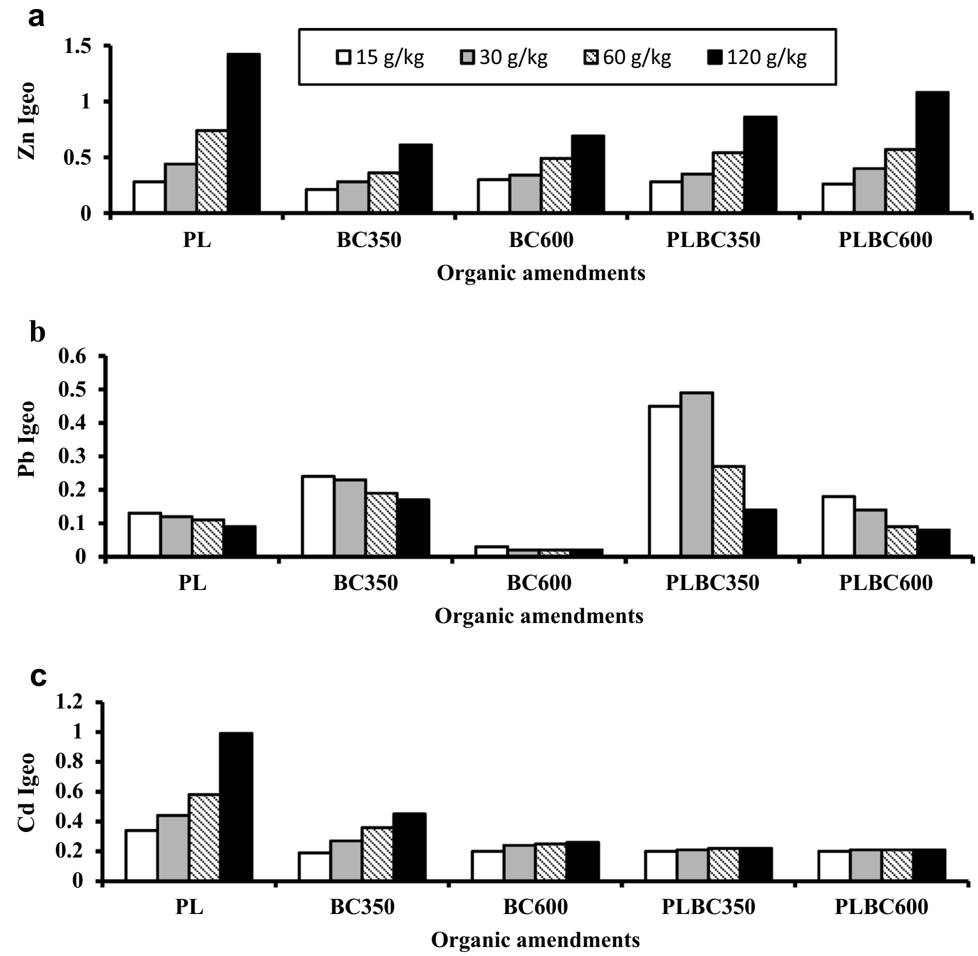

Fig. 3 Geoaccumulation Index (I_{geo}) of Zn (**a**), Pb (**b**), and Cd (**c**) in sandy-loam soils amended with poultry litter and its biochar at different application rates of 15, 30, 60, and 120 g/kg

Table 3 Contamination factor of Zn, Cd, and Pb in sandy soils amended with poultry litter, and it is biochar at different application rates

Element	Rates (g/kg)	PL	BC350	BC600	PLBC350	PLBC600
Zn	15	1.39**	1.03**	1.47**	1.39**	1.32**
	30	2.19**	1.40**	1.66**	1.71**	1.98**
	60	3.68***	1.78**	2.41**	2.68**	2.82**
	120	7.08****	3.02***	3.40***	4.22***	5.37***
Pb	15	0.66*	1.20**	1.27**	2.22**	0.91*
	30	0.59*	1.14**	1.21**	2.43**	0.69*
	60	0.54*	0.95*	0.99*	1.34**	0.44*
	120	0.43*	0.84*	0.97*	0.69*	0.42*
Cd	15	1.84**	0.97*	0.99*	1.02**	1.01**
	30	2.28**	1.35**	1.20**	1.06**	1.03**
	60	3.00***	1.79**	1.25**	1.11**	1.04**
	120	5.03***	2.22**	1.31**	1.10**	1.04**

*Means low contamination; **moderate contamination; ***considerable contamination and ****very high

a more considerable surface area potential for adsorption of metals, such as Pb. As for PL and BC350 that have higher phosphorus concentration, Pb's absorption rate is slightly lower (Fig. 4).

Pollution Index (PLI) of Zn, Cd, and Pb concentrations in the sandy soils

The pollution load index (PLI) was also employed to determine pollution differences and their extent in soils amended

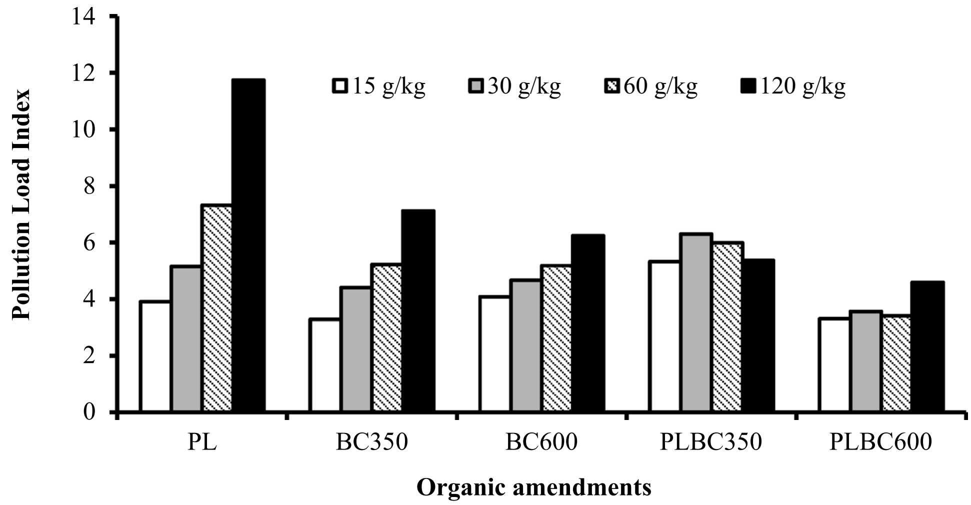

Fig. 4 Pollution Load Index (PLI) of Zn, Cd, and Pb in sandy soils amended with different application rates (15, 30, 60, and 120 g/kg) poultry litter and its biochar

with poultry litter and biochar (Fig. 3). In general, this study's results indicate that PLI Error! Reference source not found. values are higher than 1 (PLI > 1) at all application rates. PLI values (Fig. 4) are in the decreasing order as PLBC350 > PL > BC350 > BC600 > PLBC600. The highest PLI values of 11.74 were recorded from soil treated with PL at 120 g/kg, and the least PLI values are at 15 g/kg in BC350 treated soils (Fig. 3). PLI values being greater than 1 (PLI > 1) means that the presence of these metals in the soil may cause pollution with continuous use, especially at higher application rates of at least 120 g/kg. For an application rate of 15 g/kg, these metals' availability may also cause minimal pollution even though their presences were at lower concentration rates. These results confirmed that the higher the application rate of organic amendments and repeated use, the soil is at risk of pollution with these metals.

Conclusion

The study was carried out to assess the contribution of poultry litter and its biochars on sandy-loam soil heavy metal bioavailability concentrations at different application rates. In addition, risk assessment contamination methods were applied to determine the level of toxicity of heavy metals. In general, the control samples had the lowest concentration rates of the studied bioavailable Zn, Pb and Cd. The mean values of bioavailable Zn, Pb, and Cd in treated soils followed the decreasing order Zn > Pb > Cd with increase in application rates. As for risk assessment contamination, the study I_{geo}, CF and PLI values predict possibility of pollution with increased organic amendments exceeding application rate of 120 g/kg. Despite the increased rate of metal bioavailability concentration with increased application rates for Zn, biochar addition has shown a potential for Pb and Cd remediation. The lower degree of contamination values was mainly observed in soils amended with biochar pyrolyzed at higher temperature (600 °C). Therefore, biochar pyrolyzed at higher temperatures may effectively reduce metals concentration levels in soil compared to raw feedstock and biochar pyrolyzed at lower temperatures (350 °C). However, the use of biochar for immobilization of heavy metal needs further research to identify the optimum temperature suitable for biochar production and further research is suggested for field experiments. In conclusion, caution with the use of the organic feedstock to improve soil quality must be applied, and recommendation policies on the use of biochar are necessary to prevent metals toxicity on the environment. The data of this study would be beneficial for government environmental policy sectors, pollution and waste management authorities.

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Code availability Not applicable.

Declarations

Conflict of interest The authors declare that there is no conflict of interest regarding this paper's publication.

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