


Effects of Chicken Manure and Chicken Manure-Derived Biochar on the Bioavailability and Concentration of Lead (Pb) in Two Brassica Vegetables

Moses Mulenga

¹University of Zambia, School of Agricultural Sciences and Hokkaido University, Japan.

*Corresponding author: Moses Mulenga, University of Zambia.

Submitted: 10 January 2023 Accepted: 17 January 2023 Published: 21 January 2023

 <https://doi.org/10.63620/MKJAEES.2023.1007>

Citation: Mulenga, M., Mukumbuta, I., Chishala, B., & Uchida, Y. (2022). Effects of chicken manure and chicken manure-derived biochar on the bioavailability and concentration of Lead (Pb) in two Brassica vegetables. *J of Agri Earth & Environmental Sciences*, 2(1), 01-08.

Abstract

Lead (Pb) contamination has been widely reported in Kabwe town soils due to mining activities. The bioavailability and excessive amounts of Pb in soils are toxic to both plants and microorganisms. Manure and biochar have been reported to immobilize heavy metals in soil. This study, thus evaluated the effects of chicken manure and chicken manure-derived biochar on the bioavailability of Pb in the soil and its uptake by plants in polluted garden soils of Kabwe.

Pots containing Pb contaminated soil with five treatments of manure and biochar in the following proportions: CT (un-amended polluted soil, control); 2% and 4% of CM (chicken manure); 2% of and 4% of CMB (chicken manure-derived biochar) were used in this study. The manure and biochar were applied as percentage of total mass of soil in the pot. Brassica napus (rape) was planted and grown in the soils under greenhouse conditions for nine weeks. After harvesting the rape, Brassica rapa (Chinese cabbage) was planted in the same pots and grown for eight weeks. The planting of Chinese cabbage was done to ascertain the residual effects of the amendments. Soil pH and bioavailable Pb was measured six times during the experiment and after the harvest, Pb concentration in plant tissues were determined using Atomic Absorption Spectroscopy (AAS - Z series 2010).

Concentrations of bioavailable Pb were higher in the amended soils of 2 % and 4% CM (18.90 ± 1.5 ; 21.18 ± 2.3 mg Pb/kg), 2% and 4% CMB (29.16 ± 2.4 ; 41.15 ± 2.6 mg Pb/kg) compared to CT (18.36 ± 1.6 mg Pb/kg). Despite higher bioavailable Pb in the manure and biochar amended soils; the concentration of Pb in both rape and Chinese cabbage tissues was lower than that in un-amended polluted soil (CT). Concentration of Pb in rape from 2% and 4% CM were less than detection limit, 2 % and 4% CMB were 12.79 ± 2.49 mg Pb/kg and 8.4 ± 0.20 mg Pb/kg while concentration of Pb in rape from CT was 35.13 ± 13.72 mg Pb/kg. In Chinese cabbage, all amendments had Pb concentrations in plant tissues less than detection limit while the value in CT was 8.13 ± 0.97 mg Pb/kg.

The experiment showed that, amendment application with increase in dose application results to reduced Pb uptake by the plants and concentration, despite increased Pb mobilization in soil. It is recommended that these amendments can be used for remediating Pb contaminated soils, as they seem to reduce Pb concentrations in plant tissues.

Keywords: Contaminated lead (Pb) soil, bioavailability, remediation, amendments.

Introduction

Globally, many studies have been conducted on the health risks of Pb once consumed. Pb exposure to the environment can influence the neurologic system, kidneys, and blood circulation in humans, especially in children, infants, and foetuses [1]. Other studies have revealed that, elevated lead (Pb) in the environment can result in decreased growth and reproduction in plants and animals. In view of the aforesaid, growing of vegetables on contaminated soils with Pb has negative effects on human health.

The sources of lead (Pb) to ecosystems include direct discharge of waste streams to water bodies and mining. This problem of Pb pollution to the environment has affected even most African countries and Zambia inclusively.

Lead (Pb) pollution from the Mine in Kabwe town has resulted in contamination of soils leading to most backyard gardeners with no option but to grow their vegetable crops on the same soils. Lead (Pb) polluted soils severely affect plant growth parameters by influencing physico-biochemical processes, oxida-

cation exchange capacity and some metalloids.

Chicken manure – derived biochar

The chicken manure-derived biochar was made using the pit method under limited oxygen conditions (Figure 2), and then, the charred materials were ground using a mortar and pestle and sieved through a 2 mm sieve. The chicken manure – derived biochar was equally characterised for pH, total Pb concentration, electrical conductivity, organic carbon, cation exchange capacity and some metalloids.



Figure 2: Showing pit used for making chicken manure – derived biochar (left) and chicken manure-derived biochar (right).

Experimental Design and treatments

The complete randomized design (CRD) was employed in this study. A pot experiment was conducted in a greenhouse at the University of Zambia. It was set up with 4 replications. The amendments application rates were calculated as percentage of mass of the soil in the pot. Chicken manure and Chicken manure-derived biochar were applied at rates of 2% and 4% of the soil mass.

For the pot experiment 2500g soil was used and the manure and biochar amendments were set up with a control, meaning, CT- no amendment was applied, 2% of 2500g of soil of which calculated 50g of Chicken manure and Chicken manure-derived biochar was applied (CM2% and CMB2%), 4% of 2500g of soil of which calculated 100g of chicken manure and chicken manure-derived biochar was applied (CM4% and CMB4%) as shown in Table 1 below. The treatments below were repeated with 200g soil for destructive sampling with similar application rates for analysis of soil pH and bioavailable Pb. All treatments were replicated 4 times.

Table 1: Treatment set up showing application rates calculated by percentage of mass of the soil in the pots.

Treatment	Mass of soil (g)	Mass of amendment applied in grams
Control(CT)	2500	0
CM2%	2500	50
CM4%	2500	100
CMB2%	2500	50
CMB4%	2500	100

CT is un-amended polluted soil; CM2% and CM4% are chicken manure treatments at 2% and 4% respectively; CMB2% and CMB4% is chicken manure-derived biochar treatments at 2% and 4 % respectively.

Planting, irrigation and crop management

Five (5) seeds of each variety of vegetable, that is, rape (Brassica

napus) and Chinese cabbage (Brassica rapa) were planted. After germination, two healthy seedlings per pot were maintained after thinning. Tap water was used to irrigate the treatments as needed.

The rape (Brassica napus) was sown on 12th March, 2020 a day after soil-amendments mixture preparations. It was then harvested on 13th May, 2020 (harvested after 9 weeks). Thereafter, the pots were left for five weeks without any plant planted in them. This soil incubation was done to determine the residue effects of the amendments on bioavailable Pb in the soil. Incubation of soil samples is used to measure mineralization [11]. The second crop plant, Chinese cabbage (Brassica rapa), was planted on 21st June, 2020 and was harvested on 11th August, 2020 (8 weeks after). Chinese cabbage was chosen as a second crop, because it is also a Brassica vegetable. It has ability to accumulate Pb in its edible parts. It is equally most grown vegetable in Kabwe town. Management practices were the same for both the first and second crops.

Soil and amendment analysis Soil analysis

During the experiment, soil samples were collected six times for analysis of bioavailable Pb and soil pH. Bioavailable Pb was extracted in DTPA – TEA (Diethylene Triamine Penta Acetic Acid, Triethanolamine) buffered at pH 7.3 according to [12]. Ten grams of soil was weighed and 20 ml of DTPA-TEA solution added. The mixture was shaken for two hours using the mechanical shaker. The suspensions were filtered by gravity through the filter paper. Then filtrates were analyzed for Pb concentration using Flame Atomic absorption spectroscopy (FAAS - Z series 2010). To determine soil pH, 25 ml of CaCl₂ was added to each ten grams of soil and shaken for thirty minutes using the mechanical shaker. pH was determined using a soil pH meter. The electrical conductivity for soil was determined by adding 25 ml of deionized water to ten grams of each sample. The mixture shaken for one hour using mechanical shaker. The filtrates analyzed for electrical conductivity using electrical conductivity meter.

Organic carbon was determined by Walkley and Black method. One gram of air dry soil into a 250 cm³ conical flask to which ten centimetre cubic of 1N K₂Cr₂O₇ (with a pipette) was added. Then rapidly 20 cm³ of concentrated H₂SO₄ was added and was directed into the stream suspension (automatic pipette was used).

Cation exchange capacity was determined using distillation method. Ten millilitres of leached solution (25 ml 1N NaCl solution in a 50mls volumetric flask) was pipetted. Magnesium oxide (MgO) was added as a catalyst. Distillation was done for five minutes while collecting NH₄⁺ in 20 ml boric acid indicator. Thereafter, the indicator was titrated with 0.1 N HCl solution from green to purple and then, meq CEC/100 g soil was calculated.

Ammonium and nitrate was extracted using 2M KCl. Ten grams of soil sample was weighed into conical flask and added to 100 ml 2M KCl solution. Then the mixture had shaken for 30 minutes. Thereafter, filtered the solution and the clear filtrate taken for analysis within 24 hours.

The available phosphorous in the soil was determined using

0.5M NaHCO₃ (pH 5.8) as extracting solution. The soil texture of the soil sample was analyzed using Hydrometer method.

Amendments analysis

The electrical conductivity for amendments (chicken manure and chicken manure – derived biochar) was determined by adding 25ml of deionized water to ten grams of each sample and shaken for one hour. The filtrates analyzed for electrical conductivity using electrical conductivity meter. To determine the pH for amendments, 25 ml of deionized water was added to each ten grams of amendments and shaken for thirty minutes using the mechanical shaker. The pH reading was taken directly from the suspensions of chicken manure and chicken manure-derived biochar. At least 4 replicates were used for each analysis.

For the amendments, the organic carbon, cation exchange capacity and available phosphorous was determined using the same methods outlined in soil analysis. While for the metallic elements in amendments, the samples were weighed 10 g each and put into 250 cm³ Erlenmeyer flask. Fifty centimetre cubic of NH₄OAc, pH 7.0 was added. Shaken for 30 minutes on a shaker. The suspension was filtered using number forty two filter paper, and then potassium, calcium, manganese and iron among others were measured in the filtrate.

Lead (Pb) determination in soil and plants

To determine total Pb concentrations in soils, 0.05g of soil was weighed and placed in test tubes to which 5 ml 30% nitric acid (HNO₃) and 1 ml hydrogen peroxide (H₂O₂) were added. The mixture was digested for 45 minutes using the Speedwave digester (Berghof, Germany). Thereafter filled up to 15 ml with deionized water in a 15 ml transparent bottle.

To determine Pb concentrations in the plants, the plants were oven dried for 72 hours at 70°C then ground with pestle and mortar. Pb concentrations in plant samples was determined by weighing 0.05g ground plant sample to which 5 ml 30% nitric acid (HNO₃) and 1 ml hydrogen peroxide (H₂O₂) were added. The samples were then digested using the Speedwave digester (Berghof, Germany) digester for 45 minutes. Thereafter filled up to 10 ml with deionized water in a 15 ml transparent bottle.

Post-harvest soil was collected and bioavailable Pb determined in 20 ml of DTPA – TEA as described in soil analysis. In both plant and soil, Pb concentrations were analyzed using Flame Atomic absorption spectroscopy (FAAS-Z series 2010).

Statistical Analysis

All experiments were conducted with four replicates. The data were statistically analyzed using the one-way analysis of variance (ANOVA) test using Graphpad Prism software 9.0.0. Tukey's multiple comparison tests were conducted at significance level $p < 0.05$ to separate the statistically significant treatment means. Pearson correlation was used to determine the relationship be-

tween soil pH and bioavailable Pb in soil. Other calculations and graphs were prepared using Microsoft Excel 2010.

Results and Discussions

Soil and amendment properties

The texture of the soil used in the experiment was sand clay loam and the soil was alkaline. The pH values of chicken manure and chicken manure– derived biochar was 7.37 ± 0.07 and 10.26 ± 0.01 , respectively, which showed an increase in pH by approximately 42.9% when chicken manure was pyrolyzed. These results are in agreement with the results of Chan et al. and Cely et al. who reported an increase in pH after manure pyrolysis [13,5]. Total lead Pb in the soil was 1849 ± 246 mg Pb/kg which is above the WHO recommended threshold of 100 mg/kg and the FAO threshold of 150 mg/kg respectively [14,15]. Generally, the soil was contaminated with Pb as evidenced by the total Pb of 1849 ± 246 mg Pb/kg detected compared to recommended soil concentration of 100 mg Pb/kg and 150 mg Pb/kg by WHO and FAO, respectively.

Effects of chicken manure and chicken manure- derived biochar on soil pH

The chicken manure reduced the soil pH compared to the control. The soil pH was observed to be lower in CM4% than CM2% (Figure 3). This could be attributed to less base-forming ions released in the soil in the pot amended with CM4%, this was also observed by McCauley et al in their study. In most cases, application of chicken manure to the soil results in soil pH increase because of the presence of basic cations in the chicken manure released upon microbial decarboxylation [16,17]. In this study, the pH for soil-chicken manure mixture was found to be slightly neutral to alkaline which is in agreement with the results by Dikinya and Mufwanzala [4].

However, the addition of chicken manure-derived biochar application rates increased the pH of the soil (Figure 3). The increase in soil pH in pots amended with chicken manure-derived biochar was attributed to free basic cations present in biochar such as calcium (Ca), magnesium (Mg) and potassium (K) which are readily released into the

soil solution resulting in net increase of soil pH as also observed in the study by Pocknee and Sumner [18]. The substantial pH increase or response with increasing application rate of chicken manure- derived biochar was also observed in this study which is in agreement with other studies when biochar is added to soil [19,20]. The pH for soil-amendments mixture was found to be alkaline.

The average pH values were 7.35 ± 0.36 , 7.13 ± 0.23 , 8.01 ± 0.23 , 8.27 ± 0.14 and 7.72 ± 0.29 for CM2%, CM4%, CMB2%, CMB4% and CT treatments respectively. There was significance difference among mean soil pH values ($P < 0.05$).

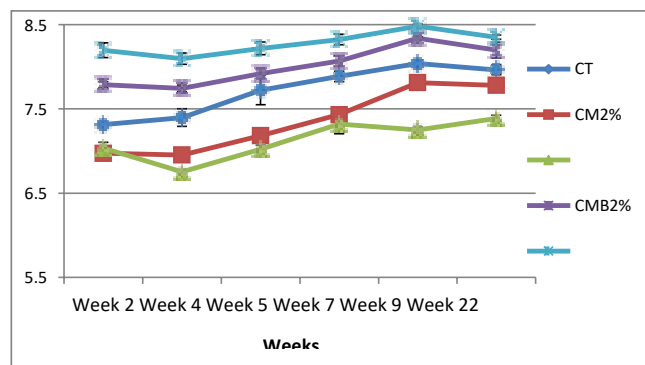


Figure 3: pH of soil from different treatments over time. Data is mean \pm SD (n=4). Error bars represents standard deviation

Effects of chicken manure and chicken manure- derived biochar on bioavailable Pb in the soil

The mean bioavailable Pb concentration was highest in CMB4% with concentration of 41.15 ± 2.6 mg Pb/kg and followed by CMB2% with 29.16 ± 2.4 mg Pb/kg. The Pb concentration in CM4% was 21.18 ± 2.3 mg Pb/kg, 18.90 ± 1.5 mg Pb/kg in CM2% treatment compared to the un-amended control (CT) with 18.36 ± 1.6 mg Pb/kg (Figure 4). Krol, et al. observed that Pb concentration in the soil was higher in alkaline conditions [21]. This agrees with results of the chicken-manure derived biochar treatments in this study. The increase in bioavailable Pb in amended soils could attributed to cation competition on adsorption sites of chicken manure and chicken manure-derived biochar which resulted to Pb ions being released into the soil solution as also observed by Bloomfield. Bloomfield, (1981) further stated that, microbial action due to decomposition process result to mobilization of Pb from carbonate and oxide forms by aerobic decomposition as may be the case in this study [22]. This led to more bioavailable Pb in the soil of the amended pots. Many studies have revealed that low pH (acidic conditions) results to high mobility and subsequently high concentrations of Pb in the soil while high pH (alkaline conditions) results to low mobility and low concentrations of Pb in the soil [23,24].

However, the application of amendments to the soil influences the formation of soluble complexes and can result in solubilisation and mobilization of Pb or soluble organo-metal complexes which accounts for majority metal content in the solution [22,25,26]. Rieuwerts et al. explains that bioavailability of metals in soils depends to a large extent on their distribution between the solid and solution phases which results in mobilisation of metals [27].

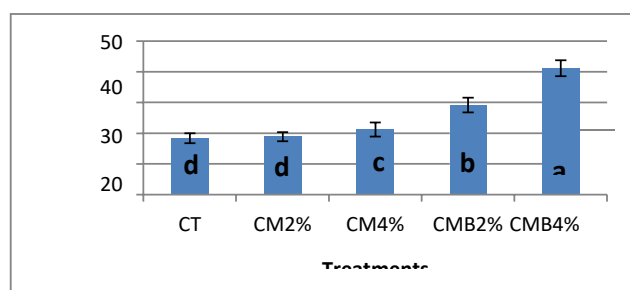


Figure 4: Mean concentration of bioavailable Pb in soil with different treatments. Data is mean \pm SEM (n=4). Bars with different letters are significantly different ($p < 0.05$) while those with similar letters are statistically non-significant according to Tukey's multiple comparison tests.

Soil pH influences the bioavailability of heavy metals in the soil, therefore, the current study evaluated the effect of pH change after application of the chicken manure and chicken manure-derived biochar. Figure 5 shows that bioavailable Pb in the soil decreased with increasing pH between soil pH 6.5 and 7.5 but after pH 7.5 the bioavailable Pb increased as the pH increases, representing a “V-

shape” relationship between soil pH and bioavailable Pb concentrations. Similar results were observed by Krol et al. and Jorris et al [21,28]. The behaviour of Pb in alkaline conditions in this study is related to amphoteric character of heavy metals i.e. representing a “V-shape” and such behaviour is as the result of the formation of hydroxyl complexes [21]. Cui et al. explains that Pb mobilization can occur in either acidic or alkaline conditions but at pH 7 (neutral) very little Pb is likely to be found in solution due to precipitation and sorption as observed in this study [29].

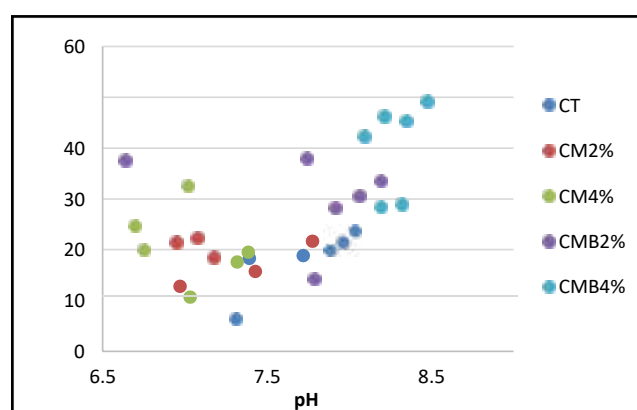


Figure 5: Graph showing distribution of bioavailable Pb as a function of pH.

Effects of chicken manure and chicken manure- derived biochar on the concentration of Pb in rape and Chinese cabbage tissues

The mean Pb concentration in rape tissues was 35.13 ± 13.72 in the CT, CMB2%- 12.79 ± 2.49 and CMB4%- 8.4 ± 0.20 mg/kg, respectively (Figure 6). The Pb concentration in rape tissues was below detection in the manure-amended treatments (CM2% and CM4%). It was also observed that increase in dose application of chicken manure- derived biochar from 2% to 4% resulted in the reduction of Pb concentration in rape tissues from 12.79 ± 2.49 to 8.4 ± 0.20 mg/kg. According to Rinklebe, the reduction in Pb concentration in plant tissues in pots amended with CMB2% and CMB4% could be related to soil activities in the soil in which increased complexing agents

(amendments) resulted to mobilization of adsorbed metals leading to more complexation, cation exchange competition and Pb ion speciation in soil solution [25]. These activities in the soil resulted in reduced plant uptake by disrupting the pathway. Violate et al. observed that mobility and plant uptake of metals depend not only on its activity in the solution but also in the relation existing between solution ions and solid phase, which may be the case in this study [30].

The reduction in plant available Pb uptake in manure amended

pots compared to control was a result of complexation, presence and concentration of dissolved chicken manure, cation exchange competition, Pb ion speciation and distribution too in the soil. Large number of studies found that biochar and manure can decrease the mobility, leachability and availability of the metals in contaminated soils, reduce plant uptake [31,32].

Generally, reduction in plant uptake was observed with increased dose application which is an indicator that with increased dose application of chicken manure-derived biochar amendments to the soil, Pb concentration in the plants can be reduced equally.

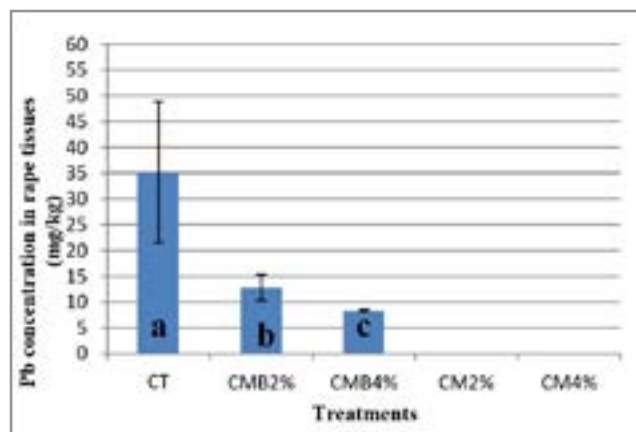


Figure 6: Pb concentration in rape tissues from different treatments. Data presented as mean±SEM, (n=4). Treatments with different letters are significantly different ($p < 0.05$) according to Tukey's multiple comparison tests

In Chinese cabbage tissues, the concentration of Pb were below the detection limit in pots with amended soils while in the CT it was 8.13 ± 0.97 mg Pb/kg (Figure 7). There was a reduction in Pb concentration in Chinese cabbage tissues in the CT (8.13 ± 0.97 mg Pb/kg) compared to rape tissues (35.13 ± 13.72 mg Pb/kg). This could be due to most of the bioavailable Pb were taken up by rape as observed in the results presented and shown in Figure 6 and 7. The other factor could be due to leaching of Pb in the pots. The maximum permissible concentration of Pb in vegetables is 0.3 mg/kg (WHO/FAO), therefore the concentration of Pb in the biochar amended treatments was higher than the permissible not suitable for human consumption.

The low Pb concentration in Chinese cabbage tissues in pots amended with manure and manure- derived biochar (Figure 7) was the result of increased complexation and Pb speciation or distribution processes in the soil overtime. This led to the reduction in uptake and concentration of Pb in Chinese cabbage tissues. Further, the results observed in Chinese cabbage validates or ascertains that both manure and biochar has significant role in reducing Pb concentration in plants and this could depend on the amount of amendments applied to the soil.

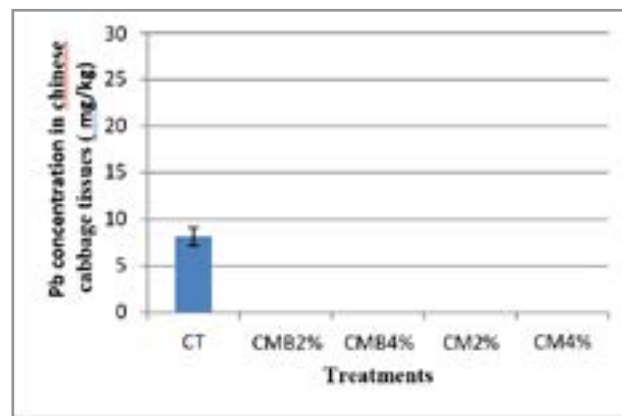


Figure 7: Pb concentration in Chinese cabbage tissues from different treatments. Data presented as mean±SEM. (n=4).

Generally, amendments increased bioavailable Pb in the soil but reduced Pb concentration in the plant

tissues. Similar results were observed by Violate et al. (2010). According to Krol et al. (2020) and Joris et al. (2004), under alkaline conditions Pb reacts with organic matter to form hydroxyl complexes which disrupts the movement of Pb from soil to the plant. This could be the reason for the observed contradicting results between the bioavailable Pb in the soil and concentration of Pb in the plant tissues in this current study. The results in this study are contradictory to other studies that show the application of chicken manure and biochar immobilizing Pb in the soil [31,32].

Conclusion

Chicken manure and chicken manure – derived biochar application to Pb contaminated soils led to increased bioavailable Pb in the soil, but reduced the plant Pb concentration in plants. The experiment showed that, amendment application with increase in dose application results to reduced Pb concentration in plants, despite increased Pb mobilization in soil.

Recommendation

Chicken manure and chicken manure-derived biochar application might be recommended as a cheap management method for remediating contaminated Pb soils because they reduced plant uptake and concentration of Pb in plant tissues. Though the WHO/FAO permissible vegetable Pb concentration is lower than what was investigated in this study, still it has given the indicator of the effects of amendments when applied to Pb polluted soils.

This research also observed the increase in Pb concentrations in the soil when amendments were applied. More research must be conducted on the scientific cause of this, because this research was limited to evaluate the effects of amendments to minimize the uptake and concentration of Pb in plant tissues and avoid associated health risks.

In view of the aforesaid, the findings of this research will help gardeners or farmers in surrounding areas of Zinc and Lead Mine in Kabwe to use these amendments and grow their crops on these polluted soils and reduce on the associated health risks.

Acknowledgements

I would like to express my sincere gratitude to God Almighty who granted me sufficient blessings to go through the process of research and writing this research.

It was not an easy task to come up with research. Therefore, my heartfelt gratitude goes to Dr. Ikabongo Mukumbuta for his invaluable guidance, criticism and promptness in attending to my need during the research period. Definitely, his contributions to the completion of this work is priceless. His comments were always straight forward to the point and useful in steering the work into the right direction despite distance constraints.

Further, I would also like to acknowledge the contributions of all the University of Zambia – Institute of Distance Education lecturers whose contribution through their various courses in Masters in Sustainable Land and Environmental Management has made it possible to produce this work. Dr. Benson Chishala, Dr. Hendrix Chalwe, Dr. Yengwe, Prof. Yoshitaka Uchida, among others deserves special mention for their insightful comments and commitments.

My sincere gratitude goes to my family for their moral support.

I also extend my heartfelt appreciation to Mr. G. Zyambo of KAMPAI laboratory – School of Veterinary, UNZA, who provided laboratory information I wanted during the time I was carrying out my experiments. God richly bless you all.

References

1. Guo, G. M., Lei, Y., Wang, B., & Song, J. Y. (2018). Accumulation of As, Cd, and Pb in sixteen wheat cultivars grown in contaminated soils and associated health risk assessment. *International Journal of Environmental Research and Public Health*, 15, 2601.
2. Kribek, B., Nyambe, I., Majer, V., Knesl, I., Mihaljevic, M., et al. (2019). Soil contamination near the Kabwe Pb-Zn smelter in Zambia: Environmental impacts and remediation measures proposal. *Journal of Geochemical Exploration*, 197, 159–173.
3. Imasiku, A. N., & Chirwa, M. (2013). Impacts of past mining on the ecosystem and on human health in Kabwe Town, Zambia. *Workshop on Environmental and Health Impacts of Mining Activities in the Sub-Saharan African Countries*, 33.
4. Dikinya, O., & Mufwanzala, N. (2010). Chicken manure enhanced soil fertility and productivity: Effects of application rates. *Journal of Soil Science and Environmental Management*, 1, 46–54.
5. Cely, P., Gasco, G., Paz-Ferreiro, J., & Mendez, A. (2015). Agronomic properties of biochars from different manure wastes. *Journal of Analytical and Applied Pyrolysis*, 111, 173–182.
6. Bolan, N. S., & Duraisamy, V. (2003). Role of inorganic and organic soil amendments on immobilisation and phytoavailability of heavy metals: A review involving specific case studies. *Soil Research*, 41, 533–555.
7. Bernal, M. P., Clemente, R., & Walker, D. J. (2006). The role of organic amendment in the bioremediation of heavy metal-polluted soils. *Environmental Research at the Leading Edge*, 1–57.
8. Mourato, M. P., Moreira, I. N., Leitao, I., Pinto, F. R., Sales, J. R., et al. (2015). Effects of heavy metals in plants of the genus *Brassica*. *International Journal of Molecular Sciences*, 16, 17975–17998.
9. Kribek, B., Nyambe, I. A., Njamu, F., Chitwa, G., & Ziwa, G. (2010). Assessment of impacts of mining and mineral processing on the environment and human health in selected regions of the Central and Copperbelt Provinces of Zambia, 127.
10. Mbuki, R., & Mbewe, G. (2017). Phytoremediation of lead and zinc metals using sunflower: Sampled from an abandoned mine tailing dump site in Kabwe, Zambia. *The International Journal of Multi-Disciplinary Research*, 1–11.
11. Shan, S., Devens, H., Fahey, T. J., Yanai, R. D., & Fisk, M. C. (2022). Fine root growth increases in response to nitrogen addition in phosphorus-limited northern hardwood forests. *Ecosystems*, 25(7), 1589–1600.
12. Lindsay, W., & Norvell, W. (1978). Development of DT-PA-TEA soil test for zinc, iron, manganese and copper. *Soil Science Society of America Journal*, 42, 421–428.
13. Chan, K. Y., Van, Z. L., Meszaros, I., Downie, D., & Joseph, S. (2008). Using poultry litter biochars as soil amendments. *Soil Research*, 46, 437–444.
14. Mamtaz, R., & Chowdhury, H. (2006). Leaching characteristics of solid waste at an urban solid waste dumping site. *Journal of Civil Engineering*, 34, 71–79.
15. Batjes, N. H. (2005). ISRIC-WISE global data set of derived soil properties on a 0.5 x 0.5-degree grid. ISRIC-World Soil Information, Wageningen, the Netherlands. Report, 8, 20.
16. McCauley, A., Jones, C., & Jacobsen, J. (2009). Soil pH and organic matter. *Nutrient Management Module*, 8, 1–12.
17. Natsher, M., & Schwetmann, N. (1991). Proton buffering in organic horizons of acid forest soils. *Geoderma*, 48, 93–106.
18. Pocknee, S., & Sumner, A. (1997). Cation and nitrogen contents of each of organic matter determine its liming potential. *Soil Science Society of America Journal*, 61, 86–96.
19. Tang, J., Zhu, W., Kookana, R., & Katayama, A. (2018). Characteristics of biochar and its application in remediation of contaminated soil. *Journal of Bioscience and Bioengineering*, 116, 653–659.
20. Thies, J. E., & Rillig, M. C. (2009). Characteristics of biochar: Biological properties. In Lehmann, J., & Joseph, S. (Eds.), *Biochar for environmental management: Science and technology* (pp. 85–95). Earthscan.
21. Krol, A., Mizerna, K., & Bozym, M. (2020). Assessment of pH-dependent release and mobility of heavy metals from metallurgical slag. *Journal of Hazardous Materials*, 384, 121502.
22. Bloomfield, C. (1981). The translocation of metals in soils. In Greenland, D. J., & Hayes, M. H. B. (Eds.), *The Chemistry of Soil Processes*.
23. Tills, A. R., & Alloway, B. J. (1983). The speciation of lead in soil solution from very polluted soils. *Environmental Technology*, 4, 529–534.
24. Chuan, M. C., Shu, G. Y., & Liu, J. C. (1996). Solubility of heavy metals in a contaminated soil: Effects of redox potential and pH. *Water, Air, and Soil Pollution*, 90, 543–556.
25. Rinklebe, J., Shaheen, S. M., & Frohne, T. (2016). Amendment of biochar reduces the release of toxic elements under dynamic redox conditions in a contaminated floodplain soil. *Chemosphere*, 142, 41–47.
26. Dermatas, D., Shen, G., Chrysoschoou, M., Grubb, D. G., Menounou, N., et al. (2006). Speciation versus TCLP release in army firing range soils. *Journal of Hazardous Materials*, 136, 34–36.
27. Rieuwerts, J. S., Thornton, I., Farago, M. E., & Ashmore, M. R. (1998). Factors influencing metal bioavailability in soils: Pre-

liminary investigations for the development of a critical loads approach for metals. *Chemical Speciation & Bioavailability*, 10, 61–75.

28. Joris, J. D., Johannes, C. L., & Rob, N. J. C. (2004). Leaching of heavy metals from contaminated soils: An experimental and modeling study. *Environmental Science & Technology*, 38, 16.

29. Cui, Y., Chen, J., Zhang, Y., Peng, D., & Huang, T., et al. (2019). pH-dependent leaching characteristics of major and toxic elements from red mud. *International Journal of Environmental Research and Public Health*, 16, 2046.

30. Violante, A., Cozzolino, V., Perelomov, L., Caporale, A. G., & Pigna, M. (2010). Mobility and bioavailability of metals and metalloids in soil environment. *Journal of Soil Science and Plant*

Nutrition, 10, 268–292.

31. Beckers, F., Awad, Y. M., Beiyuan, J., Tsang, D. C. W., Ok, Y. S., & Rinklebe, J. (2019). Impact of biochar on immobilisation, methylation and ethylation of mercury under dynamic redox conditions in a contaminated floodplain soil. *Environment International*, 127, 276–290.

32. Mwilola, P. N., Mukumbuta, I., Shitumbanuma, V., Chishala, B. H., Yoshitaka, U., et al. (2020). Lead, zinc and cadmium accumulation, and associated health risks, in maize grown near the Kabwe Mine in Zambia in response to organic and inorganic soil amendments. *International Journal of Environmental Research and Public Health*, 17, 9038.