



Significant reduction of blood and tissue lead and cadmium concentrations in free-range and broiler chickens through soil remediation with biochar, phosphates, and calcined dolomite: Implications for public health and food safety

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ABSTRACT

Food safety is increasingly threatened by environmental metal contaminants such as lead (Pb) and cadmium (Cd), which accumulate in animals, including poultry and poultry products. Consumption of these contaminated products could pose a health risk to humans, which could manifest itself in the form of neurological, renal, or developmental disorders. This concern is particularly critical in mining-affected regions such as Kabwe in Zambia. The current study evaluated the efficacy of four soil amendments: biochar (BC), triple superphosphate (TSP), phosphoric acid (PA), and calcined dolomite (CD) in reducing toxic metal accumulation in chicken tissues raised on Pb and Cd-contaminated soils. Using *ex-situ* and *in-vivo* approaches, chickens were raised on both amended and non-amended soils, and chicken organ samples were analyzed using inductively coupled plasma mass spectrometry. Biochar was the most effective amendment, significantly reducing ($p < 0.05$) both Pb (by 73.6 to 90.3 % and 63.2 to 89.8 % in free-range and broiler chickens, respectively) and Cd (by 56.8 to 89.7 % and 50.2 to 77.5 %) accumulation in various organs compared to the positive control (PC) group. Importantly, the levels of essential trace elements such as copper and zinc remained stable across treatments, maintaining the nutritional quality of poultry meat. These findings highlight the potential of cost-effective and accessible soil amendments, particularly BC, in reducing heavy metal transfer from contaminated soils to poultry. This approach has the potential to enhanced food safety and protect public health in regions burdened by metal contamination. However, further long-term and field-scale studies are needed to establish the effectiveness and sustainability of soil amendment use.

1. Introduction

Heavy metal contamination is a major global environmental challenge, with elements such as lead (Pb), cadmium (Cd), and arsenic (As)

posing serious threats to ecosystem stability, animal health, and human well-being (Futsaeter and Wilson, 2013; Luo, 2024; Phaenark et al., 2024; WHO, 2010). Previous studies have indicated heavy metal accumulation across diverse environmental matrices and biological taxa,

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including aquatic ecosystems (Ikenaka et al., 2010), wild rats (*rattus*) (Nakayama et al., 2011), avian species (Ishii et al., 2017; Maruyama et al., 2024), canines (Soe et al., 2024), livestock (Bataa et al., 2022; Yabe et al., 2013), and humans (Kataba et al., 2023; Toyomaki et al., 2020; Yabe et al., 2020). These findings demonstrate the transfer of heavy metals from the environment to animals and, ultimately, to humans via the food chain.

Pb exposure has been linked to neurotoxicity, cognitive impairment, developmental delays, and cardiovascular diseases (WHO, 2010), while Cd exposure is associated with kidney dysfunction, bone demineralization, and increased risk of cancer (EFSA, 2011). These health threats are particularly critical in regions affected by mining, smelting, and industrial activities, where environmental contamination remains persistent.

Kabwe, the provincial capital of Zambia's central province (14.45° S, 28.45° E), is recognized as one of the most Pb-contaminated towns worldwide, a legacy of prolonged Pb-Zn (Zn) mining and smelting operations (Mufalo et al., 2023; Nakata et al., 2022; Yamada et al., 2020). High soil concentrations of Pb and Cd, often exceeding international safety thresholds (US EPA, 2012), have been documented in Kabwe (Ikenaka et al., 2010), and residues have been detected in local wildlife, livestock, and the human population (Křibek et al., 2019; Nakayama et al., 2011; Yabe et al., 2015). Local communities rely on subsistence agriculture and free-range livestock production, making dietary exposure to heavy metals a pressing concern (Bataa et al., 2022). In particular, free-range chickens that scavenge on contaminated soil, insects, and vegetation are highly vulnerable to heavy metal exposure (Yabe et al., 2013), and their edible tissues, including muscle, liver, kidney, heart, and gizzard, represent a direct pathway to human exposure (Hossain et al., 2023; Zhuang Ping, 2014).

The bioavailability of Pb and Cd in soil largely determines their transfer into the food chain (Munir et al., 2021; Nakata et al., 2016). A wide range of soil amendments have been studied for heavy metal immobilization, including biochar (BC), calcined dolomite (CD), lime, phosphoric acid (PA), triple superphosphate (TSP), red mud, coal fly ash-derived zeolitic materials, alkaline compounds, and phosphate rock (Gray et al., 2006; Lee et al., 2013; Querol et al., 2006; Xu et al., 2013; Soe et al., 2025; Mocek-Płóciniaak et al., 2023; Mufalo et al., 2023). Among these, BC, TSP, PA, and CD were selected for the current study due to their demonstrated capacity to immobilize Pb and Cd through adsorption, precipitation, and complexation (Abbas et al., 2018; Qiu et al., 2022; Sun et al., 2015). Adsorption refers to the binding of Pb and Cd ions onto the surface of the amendment materials, while complexation involves the formation of stable chemical bonds between the metals and functional groups such as carboxyl, hydroxyl, or phosphate (Bolan et al., 2014). These processes reduce the solubility and mobility of the metals, thereby lowering their bioavailability (Zia et al., 2011). In addition to proven efficacy, these amendments were chosen for their ease of application, cost-effectiveness, and availability in Kabwe and similar contaminated regions.

Despite extensive studies on soil amendments in plant systems (Mench et al., 1994; Mwilola et al., 2020) and experimental rodent models (Soe et al., 2025). Their application in livestock, particularly poultry, remains underexplored. The bioaccumulation of Pb and Cd in chickens raised on contaminated soils is a critical food safety and public health issue that has received limited attention, especially in African mining towns such as Kabwe, where poultry is a primary protein source (Nakata et al., 2016). Addressing this knowledge gap is essential to protecting both animal and human health.

The current study directly investigates the efficacy of soil amendments (BC, TSP, PA, and CD) in reducing Pb and Cd bioavailability in contaminated Kabwe soils and limiting their accumulation in poultry organs. Using an integrated *ex-situ* soil amendment experiment combined with *in-vivo* poultry exposure, this research offers a poultry-specific perspective on heavy metal transfer in contaminated environments. This approach provides new insights into food safety risks and evaluates practical, cost-effective remediation strategies relevant to

communities living in mining-affected regions.

2. Materials and methods

2.1. Soil sample collection and preparation

Heavy metal-contaminated soil was collected from Kabwe, a town located at approximately 14.46374° S, 28.42703° E, shown in Fig. 1. Despite the closure of mining and smelting activities in Kabwe, the pollution of heavy metals, particularly Pb and Cd, has continued affecting the environment (Ikenaka et al., 2010; Nakata et al., 2022).

The soil was collected approximately 52 m away from the mine tailings dump, the primary source of Pb exposure. About 500 kg of topsoil was collected to a depth of approximately 10 cm, was homogenized, sieved using 4 mm mesh, and subsequently packed into polyethylene bags for transportation. The heavy metal-contaminated soil samples were transported to the University of Zambia research site in Lusaka using a closed container truck, under approval of the Zambia Environmental Management Agency (ZEMA), license number ZEMA/INS/101/4/1.

The physicochemical properties of the bulk soil were measured following the protocols outlined by Soe et al. (2025). Before any amendments were applied, the concentrations of Pb and other metals in the soil were determined using an inductively coupled plasma-mass spectrometer (ICP-MS; 7850 series, Agilent Technologies, Tokyo, Japan) after microwave digestion. Appropriate quality assurance procedures, including reagent blanks and certified reference materials (CRM), were employed to ensure accuracy and reliability of the measurements. Details of the control measurements are provided in the materials and methods section of the Supplementary Materials. Following the method described by Soe et al. (2025), with minimal modifications, briefly, 10 % of the following amendments were added to the contaminated soil: wood-based BC, TSP, PA, and CD, while unamended soil served as the positive control (PC). The 10 % dosage was selected based on the method of Nkoh et al. (2022), who demonstrated that amendment rates in this range effectively reduce Pb and Cd bioavailability while maintaining soil nutrient balance and ensuring practical applicability under field conditions.

The amended soils were spread in the concrete-covered chicken cages, encompassing an area of approximately 55 sq. ft to a depth of about 1 cm, and were left to cure for a minimum of 10 days. During the curing process, approximately 5 liters of distilled water was sprinkled and mixed into the soil to facilitate equilibrium. After curing, samples of all amended soils were collected to measure their physico-chemical properties and concentrations of Pb, Cd, Zn, and Cu (Soe et al., 2025).

2.2. Animal and experimental design

Day-old broilers ($n = 50$) and free-range chickens ($n = 60$) were purchased from Livestock Services Cooperative Society in Lusaka, Zambia. Broilers are genetically and physiologically different from free-range chickens, as they are bred for rapid growth and high feed efficiency in controlled environments, while free-range chickens exhibit slower growth rates, increased activity, and enhanced scavenging adaptations. The inclusion of both types in the study facilitated a comparative analysis of the responses of different poultry systems to Pb and Cd exposure, as well as an evaluation of the effectiveness of soil amendments across different management and physiological profiles.

Upon arrival, the chicks were housed separately in brooding cages that had been cleaned and disinfected with Virukill (ICA International Chemicals (Pty) Ltd, 28 Planken Street, Plankenbrug Ind., Stellenbosch, South Africa) for three weeks. Each cage was prepared with approximately 10 cm of wood shavings as bedding, and all feeders and drinkers were cleaned and disinfected before use. The brooding area was pre-heated for 24 h before the chicks' placement to ensure appropriate thermal conditions, as shown in Fig. S4.



Fig. 1. Sampling area (14.46374° S, 28.42703° E), near the Broken Hill Mine dumping site in Kabwe (outlined in red).

The chicks were fed commercial feed (Novatek Animal Feed, Lusaka, Zambia) and provided with clean, fresh distilled water (Table S4.a). The temperature was maintained as 30–32 °C for the first 7 days, 28–30 °C from day 8 to 14, for day 15 to 21 temperature 25–27 °C, and 23–25 °C thereafter. Both free-range and broiler chickens remained in the brooding facility for three weeks before exposure to the contaminated soil. Additionally, all bedding materials, feed, and water were screened for metal contamination to prevent any pre-exposure to heavy metals before the experimental phase.

2.3. Exposure

At week 3, broiler chickens were placed in cages containing different soil amendments, with 8 chickens per group. The groups included:

1. Contaminated soil only (positive control, PC),
2. Contaminated soil + biochar (BC),
3. Contaminated soil + triple super phosphate (TSP),
4. Contaminated soil + phosphoric acid (PA),
5. Contaminated soil + calcined dolomite (CD),
6. A non-exposed group (negative control, NC) with a floor covered in wood shavings.

Similarly, free-range chickens were placed in separate cages with the same group designations, except that each group contained 10 chickens.

Body weight measurements of each chicken were recorded biweekly, and monthly blood collections were done to monitor the lead (Pb) trends, following the protocol described by Soe et al. (2025). Broiler chicken tissues were collected at week 6 (market-ready age), while free-range chicken tissues were collected at the 5th month (market-ready age).

2.4. Sample collection

All laboratory materials and instruments used for metal extraction were pre-washed with 2 % nitric acid (HNO₃) and rinsed with double-deionized water, then oven-dried at 50 °C. Chickens were taken to the dissection room, where they were euthanized by exsanguination before dissection. The pectoral muscle, liver, kidneys, spleen, heart, gizzard muscle, trachea, blood, lungs, bone, large intestine content, and gizzard content were collected. All samples were stored at a –20 °C freezer until analysis.

The frozen chicken organ and blood samples were transported to the Toxicology Laboratory, Department of Environmental Veterinary Toxicology, Faculty of Veterinary Medicine, Hokkaido University, Sapporo, Japan, for analysis.

2.5. Measurement of heavy metal concentration

To measure heavy metal concentrations, the method described by Maruyama et al. (2024), with minor modifications, where 150–300 mg of organ or 200 µL of blood were used. Organ samples were dried at 50 °C for 48 h. After drying, the samples were digested using 5 mL of 30 % nitric acid (HNO₃) (atomic absorption spectrometry grade, Kanto Chemical Corp., Tokyo, Japan) and 1 mL of 30 % hydrogen peroxide (Cica reagent, Kanto Chemical Corp., Tokyo, Japan) in a speed wave MWS-2 microwave digestion system (Berghof, Eningen, Germany). The specific parameters for the digestion procedure are shown in Table S1.

After cooling, extracted solutions were transferred into 15 mL Falcon tubes (Fisher Scientific, Waltham, MA, USA) and diluted to a final volume of 10 mL with ultrapure water produced with a Milli-Q water system (Merck, Darmstadt, Germany). To prevent contamination, the digestion vessels were thoroughly cleaned after each digestion cycle using 60 % HNO₃.

All the chemicals, reagents, and standard solutions used were of analytical grade, and ultrapure water was used throughout the sample processing and analysis. The various concentrations of elements (Pb, Cd, Zn, and Cu) were measured using ICP-MS. The detailed operating set conditions are outlined in Table S2. Analytical quality control was performed using the DOLT-5 (fish liver, National Research Council Canada, Ottawa, Canada) and 2710a (Montana I soil; National 93 Institute of Standards and Technology, Gaithersburg, U.S.) certified reference material. Replicated analysis of the reference materials showed a good recovery rate, ranging from 97.5 to 108.3 % (Table S3.a).

The instrument method detection limit (MDL) for Pb was 0.0017 µg/g, and the method quantification limit (MQL) was 0.0045 µg/g. Detection and quantification limits for other metals are provided in Table S3.b. All the element concentrations are reported in milligrams per kilogram on a wet-weight basis (mg/kg, w.w.).

2.6. Statistical analysis

The relationship between blood Pb concentration and chicken body weight was assessed using Kendall's tau correlation coefficient (τ), chosen for its robustness to non-normal data distributions. This part of the statistical analyses was performed in JMP Pro 17.0.0 (SAS Institute, Cary, NC, USA).

For comparative analysis of metal accumulation across organs, we conducted a non-parametric approach using a Kruskal-Wallis test ($p < 0.05$) to evaluate overall differences in metal accumulation across the amendment and control groups. Significant Kruskal-Wallis tests were followed by a Dunn's test for pairwise comparisons with Bonferroni

adjustment. Specifically, we examined amendment efficacy for each amendment group vs. the positive (PC) and negative control (NC), respectively. For effect size quantification, we calculated Cliff's delta (δ) to assess the magnitude of observed differences, interpreting effects as: Negligible: $|\delta| < 0.147$, Small: $0.147 \leq |\delta| < 0.33$, Medium: $0.33 \leq |\delta| < 0.474$ and Large: $|\delta| \geq 0.474$ (Meissel and Yao, 2024).

To objectively quantify remediation performance, we developed the Median Remediation Index (MRI), using the formula below:

$$MRI = \left(\frac{PC_m - A_m}{PC_m - NC_m} \right) \times 100,$$

where PC_m , NC_m , and A_m represent median metal concentrations in positive control, negative control, and amendment groups, respectively. The MRI provides an intuitive metric where 100 indicates complete remediation (equivalent to NC levels), 0 shows no remediation effect (equivalent to PC levels), and values < 0 suggest metal accumulation/enhancement.

All analyses were conducted in R v4.4.2 (R Core Team, 2024) using the following packages: *tidyverse* for data wrangling, *dunn.test* for post-hoc comparisons, and *effsize* for effect size calculations.

3. Results

3.1. Physical and chemical properties of soil

A mean comparative analysis of the physicochemical properties of Kabwe soil used during the experiment for both free-range and broiler chickens is shown in Table 1. Notably, BC and CD treatments significantly increased pH to 6.32 and 7.02, respectively, compared to the untreated soil (PC), which recorded a pH of 5.83. In contrast, PA significantly reduced the pH to 2.87. Soils treated with PA and TSP exhibited the highest phosphate levels, at 127.47 mg/kg and 116.50 mg/kg, respectively, whereas PC showed minimal phosphate content. All treatments enhanced CEC, with the most significant increase recorded in the PA-amended soil (23.23 meq/100 g).

The impact of different soil amendments on the concentrations of metals, including Pb, Cd, Cu, and Zn, in contaminated Kabwe soil is shown in Table 2. High concentrations of Pb (15,006.93 mg/kg), Cd (112.90 mg/kg), Cu, and Zn were recorded in PC. All the amendments significantly reduced the concentrations of the analyzed metals compared to the PC. The most significant reduction occurred in the soil treated with PA, which resulted in Pb at 7280.30 mg/kg and Cd at 51.14 mg/kg. The concentrations of Pd, Cd, Cu, and Zn in both untreated and treated soils exceeded the Ecological Soil Screening Level (Eco-SSL) threshold set by the US EPA (2012).

3.2. Correlation of lead (Pb) concentration versus free-range chicken's body weight

The results in Fig. S1 show the correlations between body weight

Table 1

The mean \pm standard deviation (SD) for each soil treatment's pH, CEC (cation exchange capacity), and phosphate (PO_4^{3-}) levels.

Amendment – Groups	pH	CEC (meq/100 g)	Phosphate (PO_4^{3-} , mg/kg)
Contaminated Soil (PC)	5.83 \pm 0.15	2.49 \pm 0.05	1.18 \pm 0.03
Contaminated Soil + BC	6.32 \pm 0.08	9.15 \pm 0.04	7.30 \pm 0.10
Contaminated Soil + TSP	4.44 \pm 0.12	19.63 \pm 0.55	116.50 \pm 1.90
Contaminated Soil + PA	2.87 \pm 0.03	23.23 \pm 0.15	127.47 \pm 1.01
Contaminated Soil + CD	7.02 \pm 0.16	15.20 \pm 0.08	42.82 \pm 0.07

Table 2

Mean \pm standard deviation (SD) concentrations (mg/kg. dw) of heavy metals (Pb, Cd, Cu, and Zn) in various Kabwe soil treatments and untreated control.

Amendment	Pb (mg/kg. dw)	Cd (mg/kg. dw)	Cu (mg/kg. dw)	Zn (mg/kg. dw)
Group	Mean \pm SD	Mean \pm SD	Mean \pm SD	Mean \pm SD
Kabwe Soil	15,006.93 \pm 9.56	112.90 \pm 6.63	695.80 \pm 6.72	9363.15 \pm 1.95
PC	9.56	6.63	6.72	1.95
Soil + TSP	9616.36 \pm 7.57	67.98 \pm 4.88	543.78 \pm 9.10	4919.36 \pm 4.13
Soil + BC	8757.94 \pm 9.58	49.79 \pm 3.68	448.70 \pm 4.06	3393.51 \pm 9.83
Soil + PA	7280.30 \pm 2.91	51.14 \pm 13.42	318.18 \pm 1.41	3054.93 \pm 6.60
Soil + CD	11,106.68 \pm 7.39	56.14 \pm 6.70	559.60 \pm 9.22	6156.89 \pm 1.46

and blood lead levels (BLL) at weeks 2 and 5. At week 2, Kendall's tau (τ) values were -0.5 for PA and -0.222 for PC, while BC, TSP, and CD showed weak to moderate negative correlations. By week 5, τ values for TSP, PA, and CD shifted to positive, whereas the PC group remained negative (-0.222 at week 2, and -0.182 at week 5). These correlations did not yield new insights and are not discussed further. Cd showed lower overall accumulation and was therefore excluded from the correlation analysis.

3.3. Lead (Pb) concentrations in organs of free-range and broiler chickens

The Pb accumulation in free-range and broiler chicken organs across amendment groups (BC, TSP, PA, and CD), alongside PC and NC controls, are shown in Figs. 2 and 4. In both chicken types, the PC group displayed the highest Pb concentrations, often exceeding the reference threshold (indicated by the red dotted line), while the NC group exhibited the lowest accumulation, with levels remaining below established reference limits (Regulation Council, 2001). Amendment-treated groups consistently displayed significantly lower Pb levels than the PC, with concentrations close to those of the NC. Most differences were statistically significant ($p < 0.05$; Tables S6-S7).

Median remediation index (MRI) values (Figs. 3 and 5) confirmed amendment efficacy. BC was consistently the most effective, with average remediation of $\sim 81.5\%$ in free-range chickens and $\sim 72.4\%$ in broilers, followed by TSP, PA, and CD. All amendments yielded positive MRI values, indicating no enhancement effect.

The data presented in Tables S4-S9 further highlight organ-specific reductions. In free-range chickens, BC reduced Pb in pectoral muscle from 0.382 to 0.069 mg/kg and in lungs from 20.226 to 7.547 mg/kg, with notable decreases also in trachea, spleen, heart, bone, and gizzard content. In broilers, BC reduced Pb in kidneys (7.934 to 3.086 mg/kg), lungs (8.511 to 3.086 mg/kg), and blood (612.3 μ g/dL to markedly lower levels) (Regulation Council, 2001). CD consistently showed lower efficacy across both chicken types.

Together, these findings confirm that soil amendments, particularly BC, substantially limit Pb accumulation in edible and non-edible organs, enhancing food safety potential.

3.4. Cadmium (Cd) concentrations in various free-range and broiler chicken organs

Median remediation index (MRI) values for Cd in free-range chickens across different amendment groups are shown in Fig. 6. The remediation effects of four amendments, BC, TSP, PA, and CD, were evaluated, revealing variations in reduction rates based on organ and treatment type. The TSP treatment demonstrated the most effective remediation in the liver (93.1%), gizzard muscle (89.7%), and kidney (85.6%). Similarly, BC treatment achieved the effective remediation rates in the liver (87.1%), kidney (84.0%), and gizzard muscle (80.0%).

The results in broiler chickens (Fig. 7) indicated a wide range of

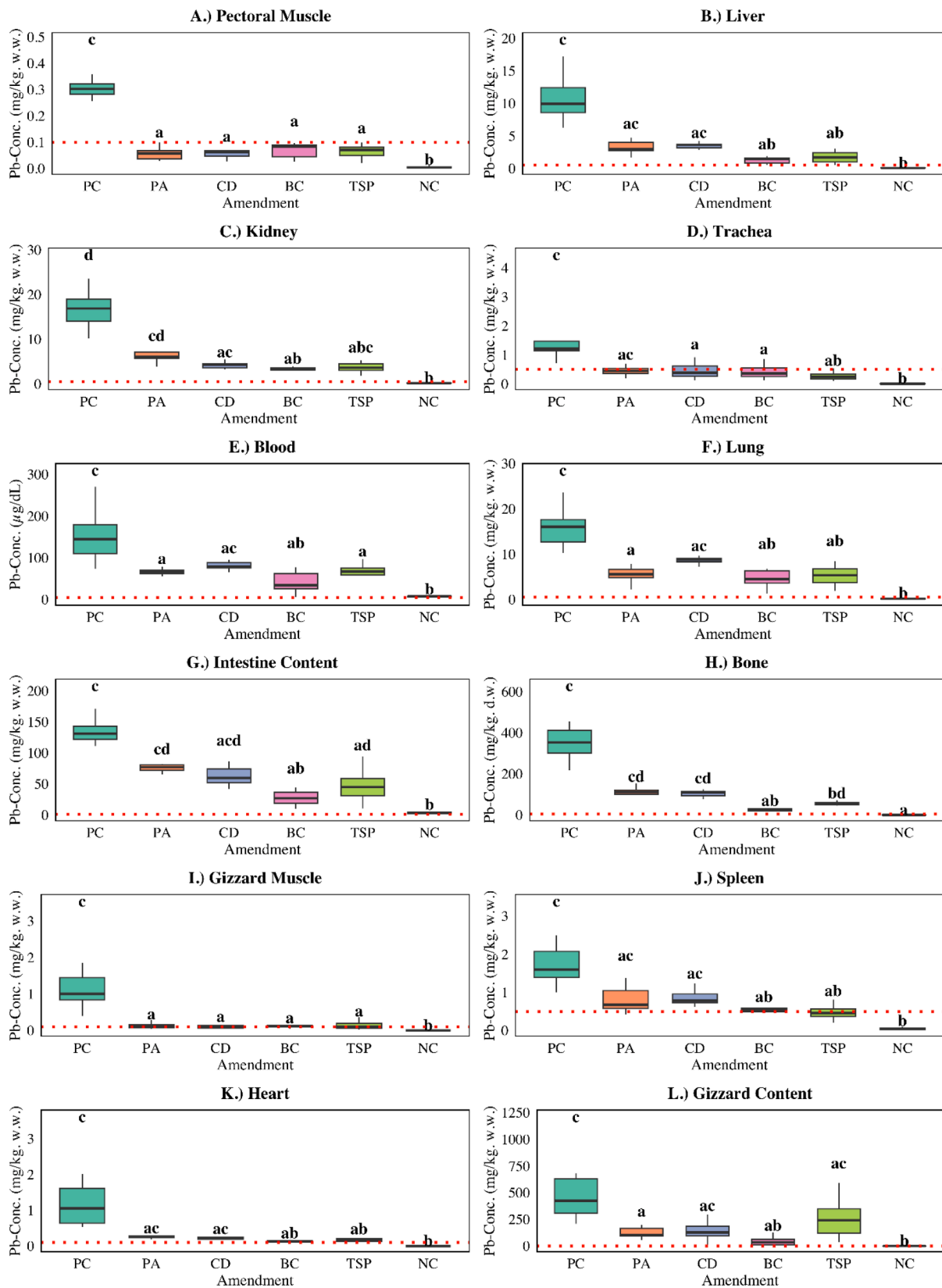


Fig. 2. Boxplots of Lead (Pb) concentration in (mg/kg) in various organs from A.) to L.) of free-range chickens across amendment groups and non-amended controls. Different letters above boxplots indicate significant statistical differences at $p < 0.05$. The red dotted line is the reference limit for lead metal in each organ.

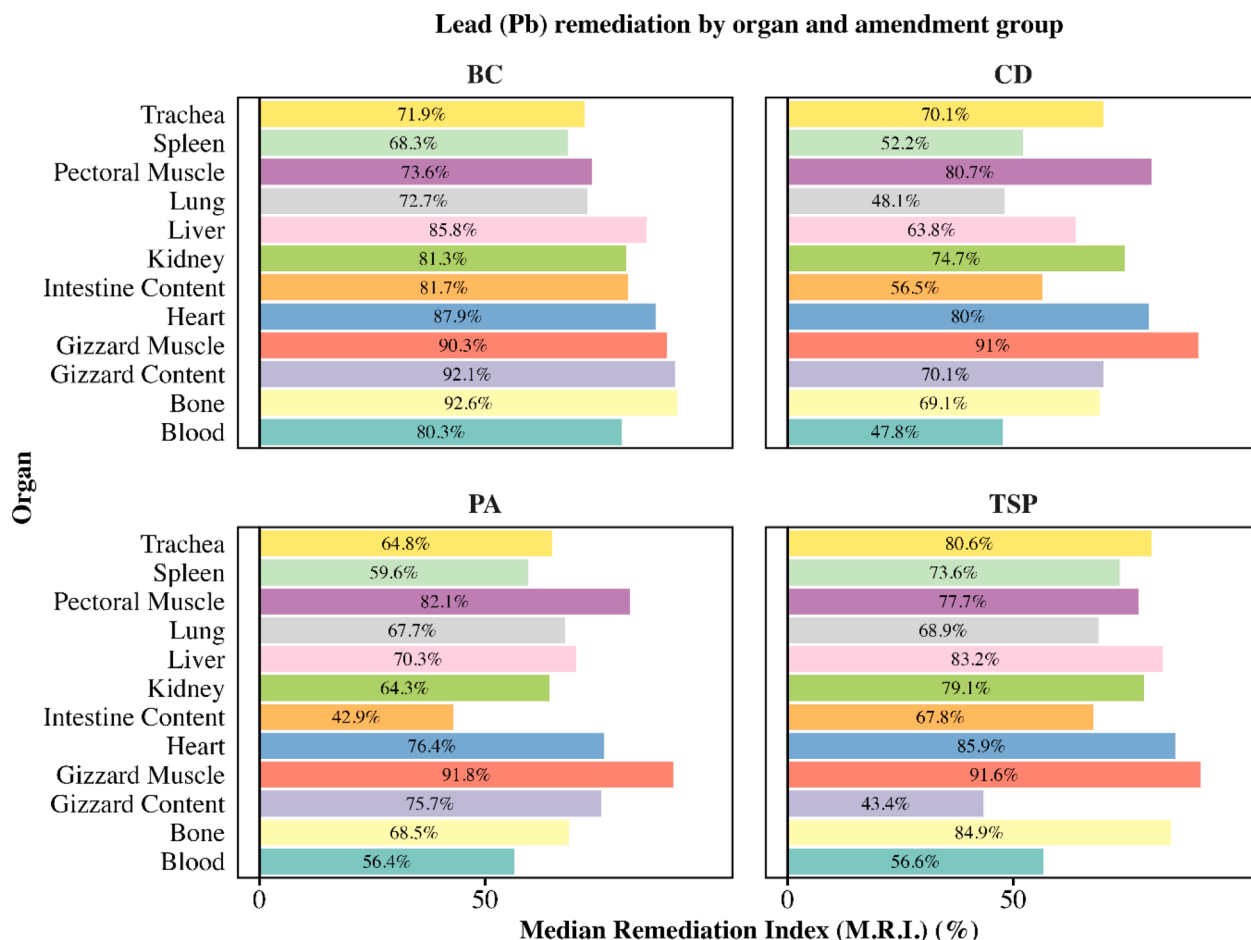


Fig. 3. Median remediation index (%) of lead (Pb) in free-range chickens across organs and amendment groups. x-axis shows Median remediation index (%) and the y-axis shows organ names.

efficacy across organs. The BC treatment resulted in substantial reductions of 87.7 % in blood, 77.5 % in gizzard muscle, and 80.6 % in gizzard content, demonstrating notable efficacy in these organs. Additional organs, such as the liver (74.6 %), kidney (71.2 %), and heart (69.5 %), exhibited moderate to high rates of remediation. CD treatment showed similar trends, with the highest reduction observed in blood (87.7 %), followed by gizzard muscle (78.8 %) and gizzard content (88.8 %). A similar pattern was noted with TSP treatment, which resulted in substantial reductions in blood (87.6 %) and gizzard muscle (57.5 %).

In most organs of both free-range and broiler chickens, Cd concentrations in the amendment groups were statistically significantly different ($p < 0.05$) from those in the PC group, as indicated by different letters above the boxplots in **Fig. S2 and S3**.

3.5. Copper (Cu) and zinc (Zn) concentrations in various free-range and broiler chicken organs

Data on Cu and Zn concentrations in various organs of free-range and broiler chickens are presented in **Tables S10 to S12**. The analysis indicated multiple instances where statistically significant differences were not observed ($p > 0.05$), despite varying effect sizes. Organs, including the pectoral muscle, liver, kidney, blood, lungs, gizzard muscle, and spleen, showed no significant differences. The effect sizes observed in these comparisons varied from negligible to large.

4. Discussion

The current study investigated the effectiveness of biochar (BC),

triple superphosphate (TSP), phosphoric acid (PA), and calcined dolomite (CD) amendments to immobilize Pb and other metals, such as Cd, Zn, and Cu, through adsorption, precipitation, and complexation mechanisms (Luo et al., 2023). The effects of these amendments on reducing toxic metal accumulation in chicken tissues reared on Pb and Cd-contaminated soils and the food safety implications are discussed.

4.1. Physicochemical properties of soil and immobilization of heavy metals

The experimental data reveal the varying impacts of soil amendments, BC, TSP, PA, and CD on the pH, CEC, and phosphate (PO_4^{3-}) levels in Pb-contaminated soil from Kabwe (**Table 1**). Among these, CD resulted in the most significant pH increase, due to its alkaline calcium and magnesium carbonate composition, whereas BC exhibited a balanced enhancement in CEC and phosphorus availability. These findings align with previous studies (Glaser et al., 2002; Lehmann et al., 2011), confirming BC's dual role in improving nutrient retention and stabilizing soil pH, especially in acidic, degraded soils. Conversely, PA significantly lowered soil pH, aligning with findings that mineral acids can enhance metal mobility while solubilizing phosphorous (Nziguheba et al., 2002). Acidification presents risks according to US EPA guidelines, as pH below 5.50 increases the bioavailability of heavy metals (US EPA, 2012). Notably, untreated contaminated soil from Kabwe (PC) contained Pb, Cd, Cu, and Zn concentrations exceeding Eco-SSL and EU thresholds (**Table 2**), highlighting significant ecological and health risks (Doya et al., 2020; Ikenaka et al., 2010; Nakayama et al., 2011). Amendments, particularly BC, markedly decreased extractable metals,

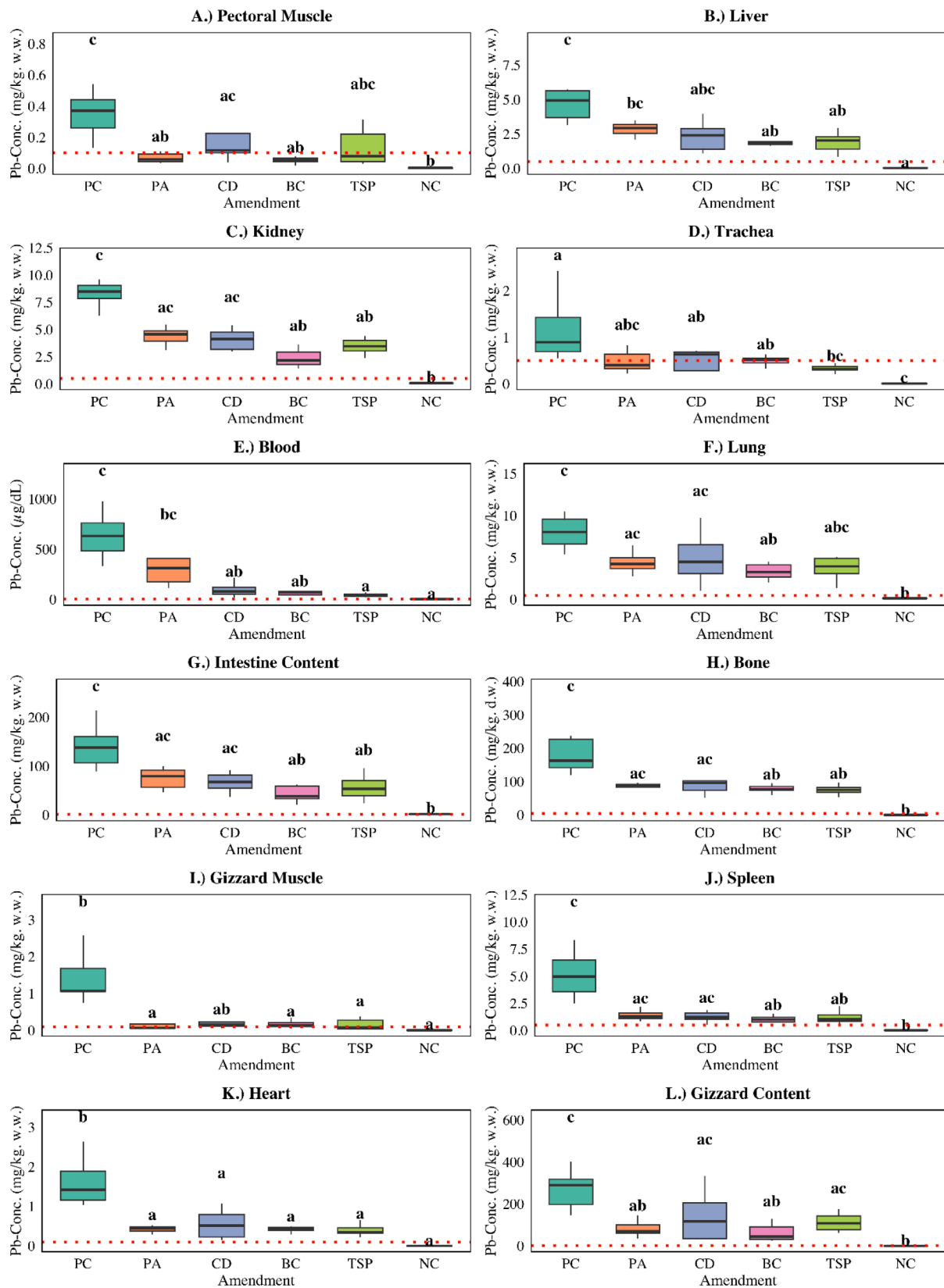


Fig. 4. Boxplots of Lead (Pb) concentration in (mg/kg) in various organs from A.) to L.) of broiler chickens across amendment groups and non-amended controls. Different letters above boxplots indicate significant statistical differences at $p < 0.05$. The red dotted line is the reference limit for lead metal in each organ.

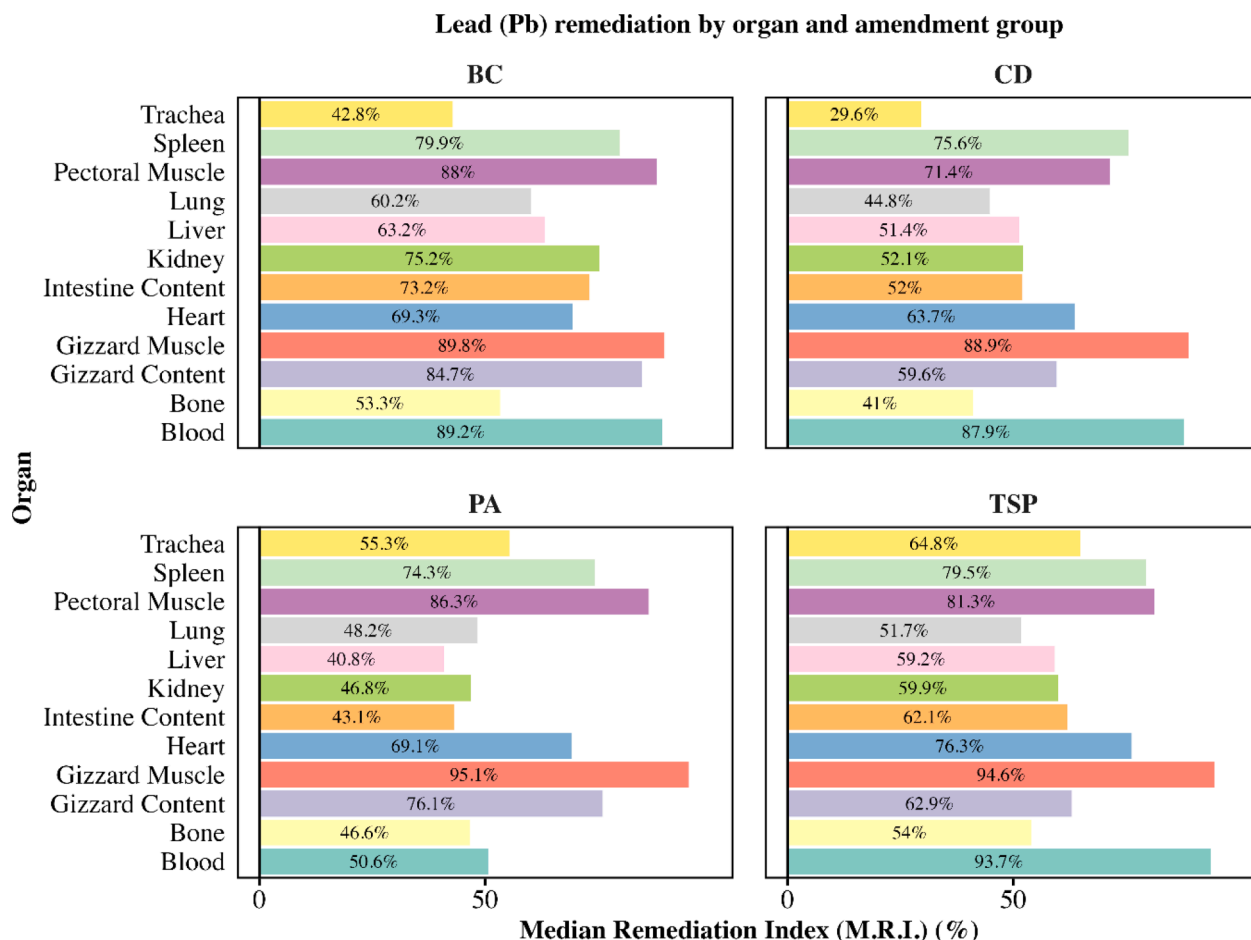


Fig. 5. Median remediation index (%) of lead (Pb) in broiler chickens across organs and amendment groups. x-axis shows Median remediation index (%) and the y-axis shows organ names.

likely through adsorption and complexation mechanisms (Beesley et al., 2011).

4.2. Lead (Pb) accumulation in free-range and broiler chickens

Findings in the current study revealed alarming levels of organ-specific Pb bioaccumulation in chickens exposed to untreated soil (PC), with concentrations in the liver, kidney, and gizzard content exceeding Codex Alimentarius limits (0.1–0.5 mg/kg). Poultry farming is an important source of protein, which is widely consumed globally (Mottet and Tempio, 2017; Hoque et al., 2023), owing to its low production cost and universal acceptance. However, consuming chickens that have been exposed to heavy metals such as Pb raises major concerns regarding food safety and public health (Yabe et al., 2013).

These elevated levels suggest possible health risks to the chickens, such as organ damage and impaired physiological functions (Kamaly and Sharkawy, 2023). The presence of Pb in edible organs presents a significant food safety concern for consumers, as chronic ingestion may result in Pb poisoning, particularly impacting vulnerable groups such as children and pregnant women (WHO, 2010).

However, the application of various amendments showed variations in their efficacy. For example, BC consistently outperformed other treatments, lowering Pb in pectoral muscle by >90% ($p < 0.001$; Cliff's $\delta = -1.000$), which can be attributed to its porous structure and high surface area (Ahmad et al., 2014). The efficacy of TSP was demonstrated through Pb-phosphate precipitation, whereby soluble phosphate ions react with Pb^{2+} in the soil to form highly insoluble minerals such as pyromorphites $[Pb_5(PO_4)_3Cl]$, which are stable and resistant to

dissolution under a wide range of environmental conditions (Cao et al., 2005), although its effects varied by organ (Fig. 3 and Table S6).

Broilers exposed for 31 days accumulated significant levels of Pb, emphasizing rapid uptake kinetics. BC again proved most effective, lowering pectoral muscle Pb to near-safe levels, while TSP exhibited variability, potentially attributable to soil-Pb speciation effects (Soo et al., 2025). The minimal Pb levels in NC confirmed amendment-driven reductions.

Importantly, the reduction of Pb in edible organs, especially muscle, is of primary focus, as it directly affects human health. This aligns with studies by Zhou et al. (2018) and Suanon et al. (2020), who reported biochar-based soil amendments are effective interventions for lowering heavy metal uptake in poultry and livestock.

4.3. Cadmium (Cd) accumulation in free-range and broiler chicken organs

The findings of the current study revealed that chickens in the PC group displayed Cd levels surpassing internationally accepted thresholds (0.05 mg/kg in muscle, 0.5 mg/kg in offal) as defined by EFSA (2011), highlighting the need for prompt implementation of effective remediation strategies in chickens reared on contaminated soils. Elevated Cd levels present considerable food safety risk and heighten the likelihood of chronic health problems in poultry consumers (Järup and Åkesson, 2009), such as renal impairment and carcinogenic consequences (WHO, 2010), hence emphasizing the public health significance of mitigating heavy metal pollution in chicken production systems.

Among the four soil amendments tested, BC, TSP, PA, and, CD; BC

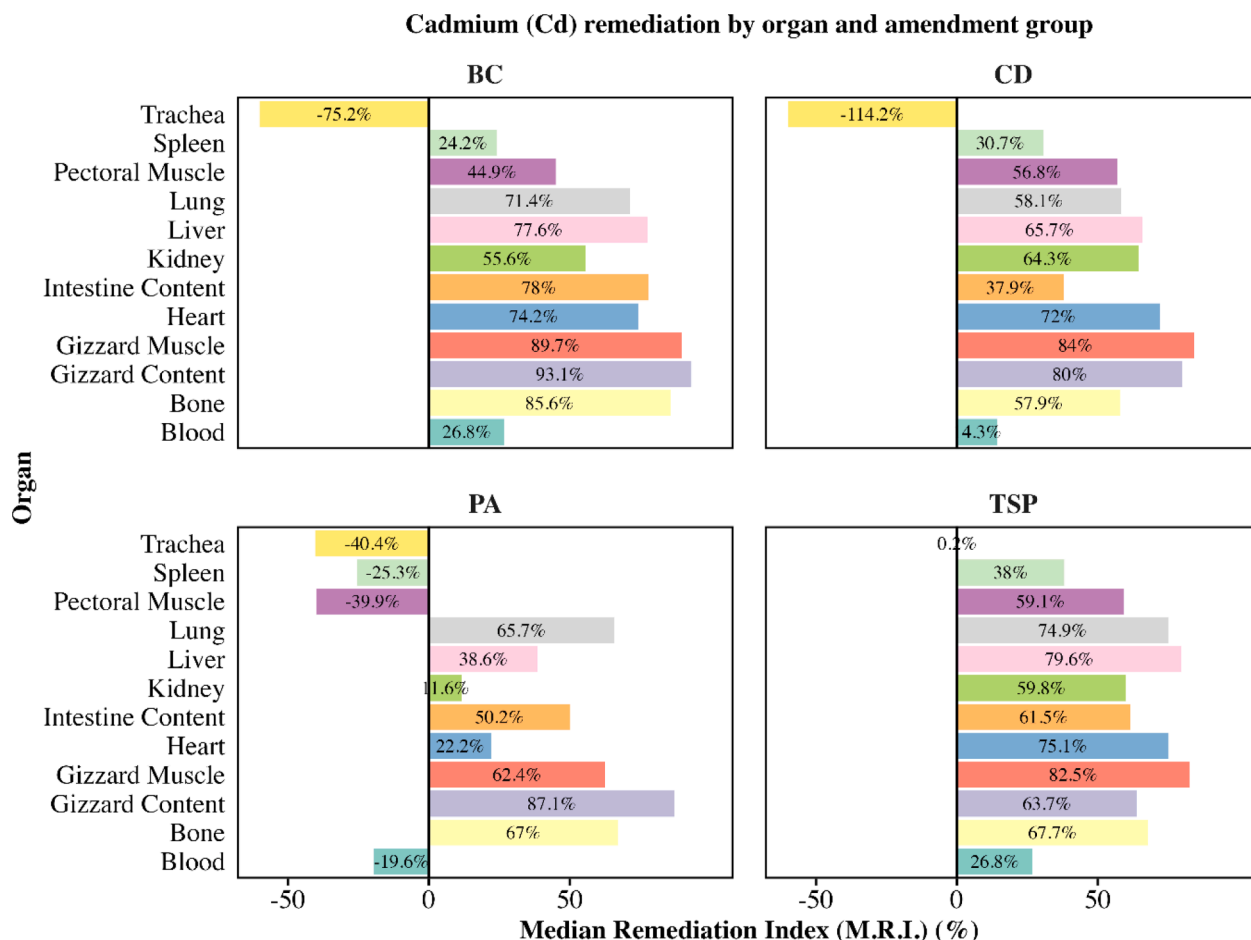


Fig. 6. Median remediation index (%) of cadmium (Cd) in free-range chickens across organs and amendment groups. x-axis shows Median remediation index (%) and the y-axis shows organ names.

and TSP exhibited the most effective remediation performance in both free-range and broiler chickens. Notably, TSP and BC showed similar reduction efficacy ranging from 84% to 93% in the liver and kidneys in free-range chickens. In broilers, BC and CD lowered Cd levels in blood up to 87.7%, suggesting reduced systemic exposure. This result underscores the ability of these amendments to impede Cd absorption during initial exposure, as blood Cd levels reflect recent and active exposure (Tchounwou et al., 2012). The observed reductions correspond with established mechanisms of Cd immobilization through phosphate precipitation and the high adsorption capacity of BC (Cao et al., 2005; Park et al., 2011; Beesley et al., 2011).

The digestive organs, especially the gizzard muscle and its content, exhibited remediation rates over 80%, indicating that the gastrointestinal tract could serve as a principal locus for amendment-mediated Cd mitigation. This could be due to the physiological conditions in the gastrointestinal tract, such as low pH, digestive enzymes, and the presence of organic ligands that enhance the interaction between ingested amendments and Cd (Bolan et al., 2014). Amendments such as BC, TSP, PA, and CD can bind, precipitate, or complex Cd within the gut lumen, thereby reducing its solubility and limiting intestinal absorption (Bolan et al., 2014; Zia et al., 2011). The results of the present study align with findings from both field and controlled research in livestock and crops (Heidari et al., 2020; Luo et al., 2023) and, significantly, extend this evidence to poultry under field-realistic exposure conditions.

4.4. Effects on copper and zinc homeostasis

Soil amendment application did not significantly impact essential

trace elements like Cu and Zn. Across organs, including the liver, kidneys, and pectoral muscle, Cu and Zn concentrations remained within expected physiological ranges (Tables S10 to S12), with no statistically significant differences found among treatment groups ($p > 0.05$). These findings are consistent with studies suggesting that amendments like BC and TSP selectively bind toxic metals while not disrupting the uptake of essential micronutrients (Beesley et al., 2011; Park et al., 2011).

The stability observed is essential, given that Cu and Zn play a vital role in enzymatic, immunological, and metabolic functions in poultry (Underwood and Suttle, 1999). Additionally, no adverse effects indicative of micronutrient deficiencies was observed, suggesting that the amendment dosages employed were within safe agronomic limits. However, effect size variability, particularly with CD, warrants further investigation into potential long-term changes in mineral bioavailability, especially in relation to varying soil chemistries or amendment dosages (Rengel, 2011).

4.5. Implications for public health

The presence of Pb and Cd in edible poultry organs provides a direct pathway for human exposure, especially in mining-affected areas such as Kabwe. Chronic exposure to Pb is linked to neurological, renal, cardiovascular, and developmental disorders, especially in children (Al-Saleh and Abduljabbar, 2017; WHO, 2010). Similarly, Cd has been classified as a carcinogen and is linked to renal dysfunction, bone demineralization, and reproductive toxicity (Feki-Tounsi and Hamza-Chaffai, 2014).

Our findings confirm that chickens raised on contaminated soil

Cadmium (Cd) remediation by organ and amendment group

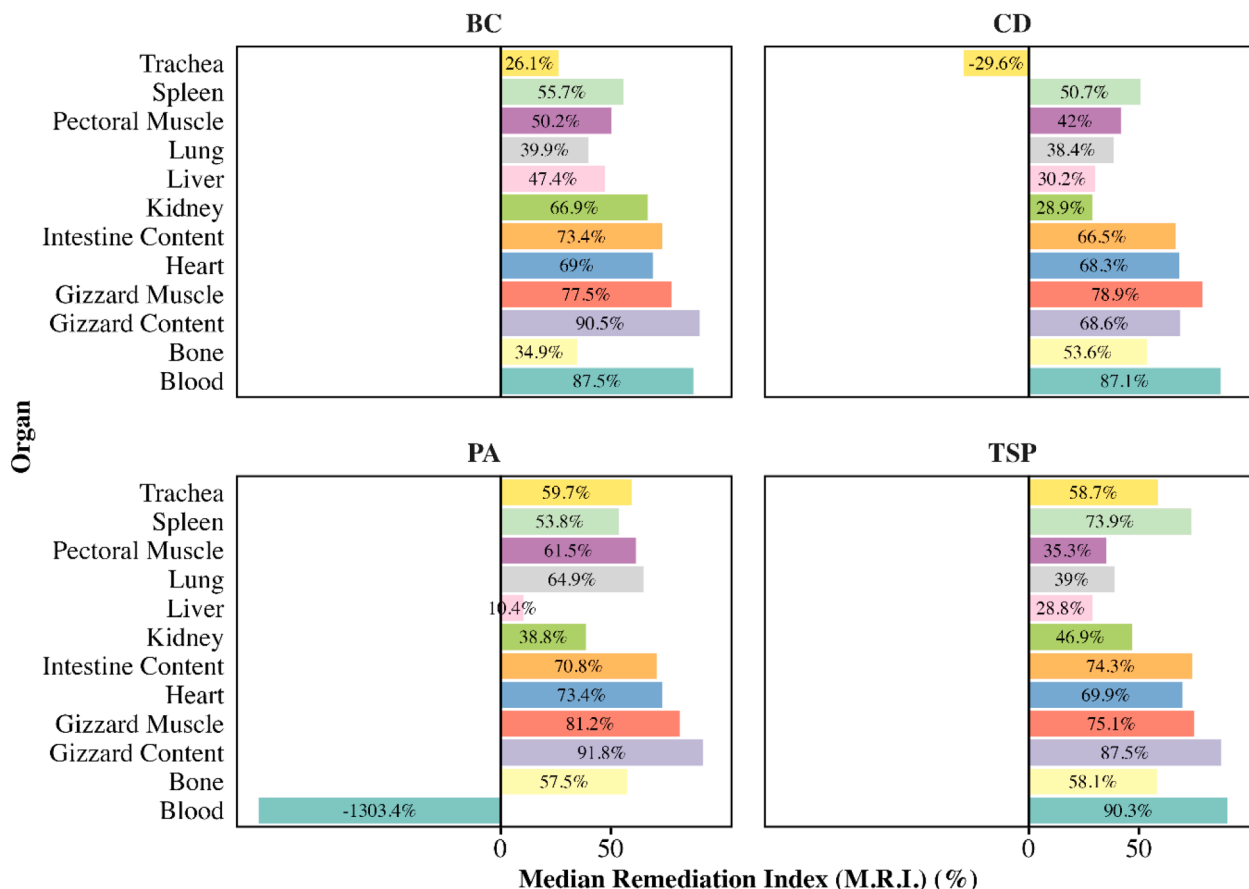


Fig. 7. Median remediation index (%) of cadmium (Cd) in broiler chickens across organs and amendment groups. x-axis shows Median remediation index (%) and the y-axis shows organ names.

without amendments accumulate heavy metals at levels that exceed safe consumption limits. This presents a serious risk to communities dependent on poultry as a dietary staple. The efficacy of BC and TSP in lowering metal burdens in various organs highlights their potential to reduce human exposure and mitigate health risks in contaminated regions. The findings endorse the integration of these elements into public health strategies designed to reduce dietary metal consumption from animal products.

4.6. Limitations of the study

This study examined fixed amendment concentrations (10 % w/w), without exploring optimal dosages or possible synergistic effects among amendments. Future research should investigate dose-response relationships and combinations of amendments to improve remediation results. The study did not account for the potential influence of chicken gender on metal bioaccumulation. Furthermore, although the ex-situ design allowed for controlled exposure, field-scale validation is necessary to confirm long-term effectiveness and stability under variable environmental conditions. Linking metal residues in poultry organs to human biomarker data would enhance the translational relevance of the findings and support more robust food safety risk assessments.

4.7. Conclusion

The study presents evidence that soil amendments, specifically BC and TSP, effectively lowered Pb and Cd accumulation in poultry raised on contaminated soils. BC demonstrated the most consistency, attaining

a decrease of up to 92.6 % in important organs such as pectoral muscle, liver, kidney, gizzard, and heart, whereas TSP showed similar efficacy. PA and CD had inconsistent outcomes; however, they remain viable owing to their cost-effectiveness and local accessibility. Crucially, the amendments did not disrupt essential mineral contents (Cu and Zn), hence maintaining the nutritious quality of poultry meat. These findings present a feasible, scalable solution for food safety in areas affected by heavy metals and establish a basis for legislation, agricultural risk management, and community-level implementation.

Future studies must concentrate on refining amendment strategies, understanding long-term ecological impacts, and developing field-based protocols to enhance agricultural resilience and protect public health in contaminated environments.

5. Environmental implication

This study shows that soil amendments, especially biochar, can effectively lower the risks of food chain contamination by reducing the transfer of Pb and Cd from contaminated soils into poultry. For areas affected by mining, such as Kabwe, Zambia, these amendments provide an affordable and easily accessible remediation method by maintaining essential trace elements while reducing toxic metal accumulation. The findings highlight their potential role in safeguarding food safety, protecting public health, and advance sustainable livestock production in environments contaminated by heavy metal.

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CRediT authorship contribution statement

Madalitso Tembo: Writing – original draft, Visualization, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Andrew Kataba:** Project administration, Methodology, Investigation, Data curation. **Nyein Chan Soe:** Methodology, Investigation. **Rio Doya:** Methodology, Investigation. **Richard Musonda:** Visualization, Software, Methodology, Formal analysis. **Golden Zyambo:** Methodology, Investigation. **Yoshinori Ikenaka:** Methodology, Investigation. **John Yabe:** Supervision, Investigation. **Kaampwe Muzandu:** Supervision, Investigation. **Mayumi Ishizuka:** Writing – review & editing, Supervision, Project administration, Funding acquisition. **Shouta M.M. Nakayama:** Writing – review & editing, Supervision, Project administration, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at [doi:10.1016/j.hazadv.2025.100907](https://doi.org/10.1016/j.hazadv.2025.100907).

Data availability

Data will be made available on request.

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