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Effect of chicken manure amendment on lead burden in mice: exposure to lead-spiked soil

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Abstract

Purpose Adding organic soil amendments, like animal manure, can alleviate the mobility of lead (Pb). However, there is a scarcity of in vivo studies examining the impact of animal manure as a soil amendment on mammals.

Methods Six C57BL/6 mice per group were raised for 30 and 98 days on soil containing 3000 mg Pb/kg. On Pb-spiked soil, chicken manure (40 t/ha) was applied as an immobilizer. Pb concentrations in the tissues were analyzed using ICP-MS after microwave digestion. RT-PCR was used to measure the mRNA expression of antioxidant and cell apoptosis genes in the liver, kidneys, and brain.

Results When chicken manure was used as the immobilizer in Pb-spiked soil, Pb concentrations in the brain, kidneys, and lungs at day 30 were significantly different ($p < 0.05$) with reduction percentages of 28.58, 26.63, and 25.62, respectively. The brain, kidneys, liver, and bone, except for the lungs and trachea, also showed a similar reducing phenomenon on day 98, with percentages of 11.82, 4.63, 29.87, and 14.54, respectively. There was a significant difference in the levels of Nrf2, HMOX1, SOD1, CAT, BCL2, and Bax in the brain between Pb-exposed and remediated groups after 98 days of exposure.

Conclusions This study highlights that the utilization of chicken manure reduced Pb accumulation in tissues (6.25–28.58% at 30 days and 4.63–29.87% at 98 days). This reduction in Pb accumulation subsequently alleviated the body burden by activating antioxidant genes in the brain, notably HMOX1, SOD1, and CAT.

Keywords Remediation · Chicken manure · Lead-spiked soil · Oxidative stress · Apoptosis

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1 Introduction

Lead (Pb) is a ubiquitous toxicant that persists in the environment. Due to the weathering process and excessive anthropogenesis, Pb contamination in soil has increased during the last few decades. Improper discharge of industrial waste, extensive use of agricultural chemical products, mines, and smelting operations are the most common events leading to exacerbating soil pollution (Palansooriya et al. 2020). Humans and animals may experience exposure to Pb through accidental ingestion of contaminated soil or inhalation of contaminated dust. Once Pb enters the body, it is rapidly absorbed from the alimentary tract and distributed throughout the body by penetrating the biological barriers. Then, Pb may be detoxified or deposited to be excreted or stored in organs (Briffa et al. 2020).

In humans and animals, Pb has caused disturbances in physiological and biochemical systems (Yabe et al. 2015). Under normal conditions, the production of reactive oxygen species (ROS) is a common process at the cellular level, and several antioxidant enzymes, such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) balance redox homeostasis by regulating ROS production. Under stress conditions, an imbalance between ROS and antioxidant enzymes occurs and causes oxidative stress by stimulating the production of free radicals in many organs. Due to oxidative damage, cell death may follow in cellular macromolecules such as proteins or DNA by diminishing cellular events like cell division and immune response (Demirci-Çekiç et al. 2022). Apoptosis is a cellular self-destructive mechanism featuring distinct hallmarks such as chromatin condensation, cell and nuclear shrinkage, membrane blebbing, and DNA fragmentation (D'Arcy 2019). This phenomenon is controlled by a number of anti- and pro-apoptotic genes that encode homologous proteins. Among these genes, B-cell lymphoma 2 (BCL2) and BCL2-associated X (Bax) appear to play a major role in determining whether a cell proceeds down the pathway of apoptosis (Knudson and Korsmeyer 1997). Several studies showed that lead exposure promoted ROS production and imbalanced antioxidant activities by disturbing the antioxidant enzymes (Matović et al. 2015; Ye et al. 2015; Fan et al. 2020).

Application of soil amendments that are utilized for the recovery of Pb contamination in the soil has proven effective for immobilizing Pb in soils by changing the soil's physiochemical properties after application (Hashimoto et al. 2011; Lim et al. 2013; Vrînceanu et al. 2019). Organic soil amendments are rich in organic matter (OM) content and improve soil structure. Because of their abilities to restrain Pb through different mechanisms, organic soil amendments such as animal manure have been acknowledged as immobilizing agents (Hashimoto et al. 2009). On the other hand, further contamination, especially of essential elements, can occur by adding animal manure, but it depends on the quality and origin of the manure and physiochemical properties of the contaminated soil (Park et al. 2011). Studies on animals to investigate the bioavailability of Pb in soil remediated with amendments have been done using rats or mice but the route of exposure is only by ingestion or inhalation (Bradham et al. 2018; Kastury et al. 2019). One study used the soil from a closed mining site and exposed it to rats for 1 year under environmentally relevant conditions (Nakayama et al. 2019). To our knowledge, there are no reports of in vivo studies to evaluate the effect of soil amendments on mammals.

Therefore, this study aimed to evaluate the effect of chicken manure amended into the Pb-spiked soil on tissue distribution of Pb in mice under direct exposure and to investigate its effect on Pb-induced oxidative stress and cell apoptosis.

2 Materials and methods

2.1 Soil preparation

For the animal experiment, Arakida soil (Akagi Engei Co. Ltd., Isesaki, Japan), an alluvial clay soil with water-retentive and argilliferous features (Doi et al. 2011), was used as a control soil. The bulk soil samples were homogenized and sieved (< 4 mm). Ninety-nine percent pure lead chloride ($PbCl_2$) powder (Fujifilm Wako Pure Chemical Corporation, Osaka, Japan) was used as the Pb source. The Pb-contaminated soil had a condition of 3000 mg Pb/kg of $PbCl_2$. The dose used for this study fell within the Pb pollution level range (6.7 − 51,188 mg/kg dry weight) in the soil from Kabwe, the town with the most Pb-polluted soil (Ikenaka et al. 2010; Nakayama et al. 2011; Doya et al. 2020). For the remediation, a 1:5 ratio of 240 g of chicken manure (Akagi Engei Co. Ltd.) was applied to each cage (area = 600 cm^2) in the soil (1.15 kg), achieving 40 t/ha of chicken manure in the soil (Christian et al. 2021; Mwilola et al. 2020). Prior to adding chicken manure, $PbCl_2$ was mixed with the control soil shanking. Then, the treated soils were gently mixed and incubated at 25 °C for 2 weeks for equilibration. Then, soil spiked with $PbCl_2$, and Pb-spiked soil amended with chicken manure were kept at room temperature before the animal experiment.

2.2 Soil chemical properties

Soil chemical properties, encompassing total organic carbon (TOC), pH, and cation exchange capacity (CEC),

were measured from the bulk soils ($n = 3$). Briefly, TOC was determined by measuring the difference between total carbon content and inorganic carbon content using a total carbon analyzer with a solid sample combustion unit (TOC-VCSH-SSM-5000A, Shimadzu Corporation, Kyoto, Japan). Soil pH (soil (m): water (v) = 1:2.5) was measured by pH meter (HM-25R, TOA DKK, Tokyo, Japan). The measurement of CEC was done using the Schollenberger method (Schollenberger and Simon 1945) by Tokachi Federation of Agricultural Cooperatives (Obihiro, Japan). Standard reference material (2710a Montana I soil, Gaithersburg, USA) was digested in a microwave digestion system (Speed Wave MWS-2, Berghof, Eningen, Germany) after drying at 110 °C for 2 h. Digested samples were diluted with Milli-Q and total element concentrations were analyzed using an inductively coupled plasma–mass spectrometer, ICP-MS (7700 series, Agilent Technologies, Tokyo, Japan). Triplicate analyses of the 2710a showed good accuracy and recovery ranged from 93.6 to 136.2%. ICP-MS analysis was used to determine the elemental composition of the control soil and chicken manure samples before $PbCl_2$ was added, after microwave digestion.

2.3 Animals and experimental design

Forty-eight male C57BL/6 mice (7 weeks of age) were purchased from Sankyo Labo Service Corporation, Inc. (Tokyo, Japan). Mice were kept at 22 − 24 °C under a 12-h light/dark cycle. Food and water were provided ad libitum. Experiments were carried out after a 1-week acclimation period. All animal experiments were performed under supervision and with the approval of the Institutional Animal Care and Use Committee of Hokkaido University (approval number 20- 0143).

The mice (8 weeks of age) were divided into four groups ($n = 6$ for each group) as follows:

1. control group (control soil).
2. chicken manure group (chicken manure with control soil).
3. Pb-exposed group (soil spiked with $PbCl_2$).
4. remediated group (chicken manure added to Pb-spiked soil).

A total of 1.15 kg of soil was used for each group and spread thoroughly in the cage. Then, mice (three mice per cage, two cages per group) were exposed to untreated or treated soil for 30 and 98 days by keeping them on soil bedding (Supplemental Fig. S1). After 30 and 98 days of exposure, the mice were euthanized by carbon dioxide inhalation. The brain, trachea, lungs, liver, kidney, and bone (tibia) were collected for further analysis.

2.4 Lead quantitation

According to the method described previously by Nakayama et al. (2019), brain, trachea, lungs, liver, kidney, and bone samples were digested in a microwave for Pb detection. After sample digestion, the concentration of Pb was measured using ICP-MS. Detailed explanations for the sample digestion and measurement of Pb from samples are mentioned in the "Materials and methods" section of the Supplementary material.

2.5 RNA extraction and cDNA synthesis from liver, kidney, and brain tissues

Total RNA was extracted from the liver, kidney, and brain tissues using a modified combination of two protocols (Kataba et al. 2021). Detailed descriptions of RNA extraction can be found in the "Materials and methods" section within the Supplementary materials. The synthesis of cDNA was performed using the ReverTra Ace® qPCR RT Master Mix with gDNA Remover (TOYOBO Co., Ltd.) from 0.5 µg of extracted RNA.

2.6 Plasmid construction

Plasmids were generated to construct standard curves for quantifying cDNA copy numbers of target genes. The primer sets for antioxidant genes and apoptosis marker employed in this study were sourced from NCBI and previously published works, as detailed in Supplementary Table S1. B-actin served as the internal control. PCR protocol and primer efficiency are mentioned in the "Materials and methods" section of the Supplementary material.

2.7 Quantitative reverse transcription polymerase chain reaction (qRT- PCR)

qRT- PCR was conducted using the Quant Studio 12 K Flex Real-Time PCR system (Applied Biosystems). The total PCR reaction mixture (10 µl) was composed of 5 µl 2 × Fast SYBR Green Master Mix (Applied Biosystems), 0.4 µl 5 µM forward and reverse primers (Thermo Fisher Scientific), 2.2 µl RNase-free water, and 2 µl cDNA sample template. The following qPCR conditions were used: 95 °C for 20 s, followed by 40 cycles of 95 °C for 3 s, and 60 °C for 30 s. mRNA expressions of analyzed genes were performed using the standard curve technique, and copy numbers were normalized against β-actin.

2.8 Statistical analysis

Statistical analyses were conducted using JMP Pro 16.0.0 (SAS Institute, USA), with a significance level of $p < 0.05$. The concentration of Pb in tissues and expression levels of

Table 1 Chemical properties of the soil from different groups (mean ± SD)

Soil	pH	TOC (%)	CEC (meq/100 g)
Control	6.5 ± 0.4	0.7 ± 0.01	16.3 ± 1.1
Chicken manure	8.1 ± 0.5	3.0 ± 0.49	19.4 ± 0.9
$PbCl_2$	5.0 ± 0.1	0.7 ± 0.02	15.3 ± 0.1
$PbCl_2$ + chicken manure	8.2 ± 0.4	3.0 ± 0.18	19.0 ± 0.3

antioxidant and apoptosis-related genes among groups were analyzed by Tukey HSD test.

3 Results

3.1 Soil chemical properties

The pH, TOC, and CEC in bulk soils from groups are shown in Table 1. The pH of control soil was slightly acidic (6.5 ± 0.4) with low total organic carbon (0.7 ± 0.01%). The clean soil with chicken manure had a moderate base condition (pH: 8.1 ± 0.5) and a high percentage of TOC (3.0 ± 0.49). In the Pb-spike soil, soil pH was slightly acidic (5.0 ± 0.1) with low total organic carbon (0.7 ± 0.02%). The pH of Pb-spike soil becomes moderately base (8.2 ± 0.4) and has a high content of TOC (3.0 ± 0.18%) when chicken manure is added. The CECs of control soil and Pb-spiked soil were 16.3 ± 1.1 meq/100 g and 15.3 ± 0.1 meq/100 g, respectively. Soil with chicken manure and chicken manure amended Pb-spiked soil had increased CEC when compared to the control and Pb-spiked soil. The concentrations of Pb in control soil and soil with chicken manure were lower than Ecological Soil Screening Level (Eco-SSL) values for mammalian and avian species (Table 2). However, the concentration of Cd in chicken manure (0.41 mg/kg in dry weight) was slightly higher than Eco-SSL values for mammals (0.36 mg/kg in dry weight). Among the other trace elements of interest, copper (Cu) and zinc (Zn) levels in control soil and chicken manure were higher than Eco-SSL values for both mammalian and avian species.

3.2 Lead accumulation in the body

The concentrations of Pb in mouse tissues were determined (Table 3). Both Pb-exposed and remediated groups were significantly different from the control group and chicken manure group in all tissues after 30 days of exposure. In the brain, lungs, and kidneys, Pb accumulation was significantly reduced when chicken manure was added as an immobilizer. Pb accumulation was also significantly different between control groups (control and chicken manure groups) and Pb-exposed groups (Pb-exposed and remediated groups) in 98 days of exposure. However, no significant difference was observed between Pb-exposed group and remediated group. The reduction percentages in the brain, kidneys, lungs, trachea, bone, and liver were 28.58, 26.63, 25.62, 25.01, 19.23, and 6.25, respectively, at day 30 (Fig. 1). A similar reducing phenomenon was observed in most tissues on day 98 except the lungs and trachea, which showed a slightly higher level in the remediated group. The reduction percentages in liver, bone, brain, and kidneys were 29.87 and 14.54, 11.82, and 4.63, respectively, at day 98 (Fig. 1).

Table 2 Element concentration in control soil and chicken manure (mg/kg in dry weight), $n = 3$

Element	Control soil median (range)	Chicken manure median (range)	Eco-SSL (mammalian)[a]	Eco-SSL (avian)[a]
As	8.07 (7.58 – 8.58)	0.75 (0.74 – 0.78)	46	43
Cd	0.18 (0.16 – 0.22)	0.41 (0.40 – 0.41)	0.36	0.77
Pb	20.1 (18.0 – 26.6)	0.71 (0.70 – 0.77)	56	11
Co	25.4 (22.3 – 27.3)	1.32 (1.23 – 1.36)	230	120
Ni	85.4 (77.1 – 92.8)	8.76 (8.02 – 9.08)	130	210
Cu	61.1 (55.1 – 66.2)	52.8 (50.0 – 53.9)	49	28
Zn	124 (104 – 154)	596 (589 – 614)	79	46

[a]*Eco-SSL*, Ecological Soil Screening Level (US EPA 2007)

3.3 mRNA expression of oxidative stress and apoptosis genes in liver, kidney, and brain

mRNA expressions of essential antioxidants and apoptosis genes following exposure in liver, kidney, and brain are shown in Figs. 2, 3, 4, 5, 6, and 7.

3.3.1 Thirty-day exposure

BCL-2, SOD1, GPx, and glutathione S-transferase (GST) were significantly upregulated, and kelch-like ECH associated protein 1 (Keap1) was significantly downregulated in the liver of Pb-exposed group when compared with control group. Heme oxygenase 1 (HMOX1) was significantly upregulated in the remediated group compared to the control group. In kidney, no significant difference was observed among groups. Upregulations of SOD1, GPx, and GST were observed in Pb-exposed and remediated groups, but all genes examined showed no significant difference among the groups in the brain.

Table 3 Concentration of Pb in tissues of mice (mg/kg in dry weight)

	30 days (mean ± SD)				98 days (mean ± SD)			
	control	Chicken manure	$PbCl_2$	$PbCl_2$ + chicken manure	control	Chicken manure	$PbCl_2$	$PbCl_2$ + chicken manure
Brain	0.02 ± 0.01^a	0.03 ± 0.01^a	0.70 ± 0.11^b	0.50 ± 0.09^c	0.02 ± 0.01^a	0.03 ± 0.02^a	1.20 ± 0.42^b	1.06 ± 0.36^b
Trachea	0.12 ± 0.03^a	0.36 ± 0.20^a	14.38 ± 3.68^b	10.79 ± 4.31^b	0.40 ± 0.46^a	0.23 ± 0.15^a	15.59 ± 13.04^b	17.11 ± 3.17^b
Lungs	0.04 ± 0.01^a	0.04 ± 0.02^a	0.65 ± 0.16^b	0.49 ± 0.08^c	0.06 ± 0.03^a	0.05 ± 0.02^a	0.45 ± 0.11^b	0.45 ± 0.10^b
Liver	0.02 ± 0.01^a	0.10 ± 0.21^a	1.46 ± 0.34^b	1.37 ± 0.34^b	0.02 ± 0.003^a	0.04 ± 0.03^a	1.54 ± 0.52^b	1.08 ± 0.26^b
Kidneys	0.13 ± 0.03^a	0.14 ± 0.05^a	9.39 ± 1.07^b	6.89 ± 0.95^c	0.13 ± 0.02^a	0.20 ± 0.06^a	9.88 ± 1.20^b	9.42 ± 0.86^b
Bone	0.38 ± 0.17^a	0.32 ± 0.10^a	31.54 ± 8.43^b	25.48 ± 3.41^b	0.20 ± 0.03^a	0.28 ± 0.03^a	53.49 ± 9.45^b	45.71 ± 5.45^b

SD standard deviation, same letters denoted no significant and different letters denoted as significant differences between group

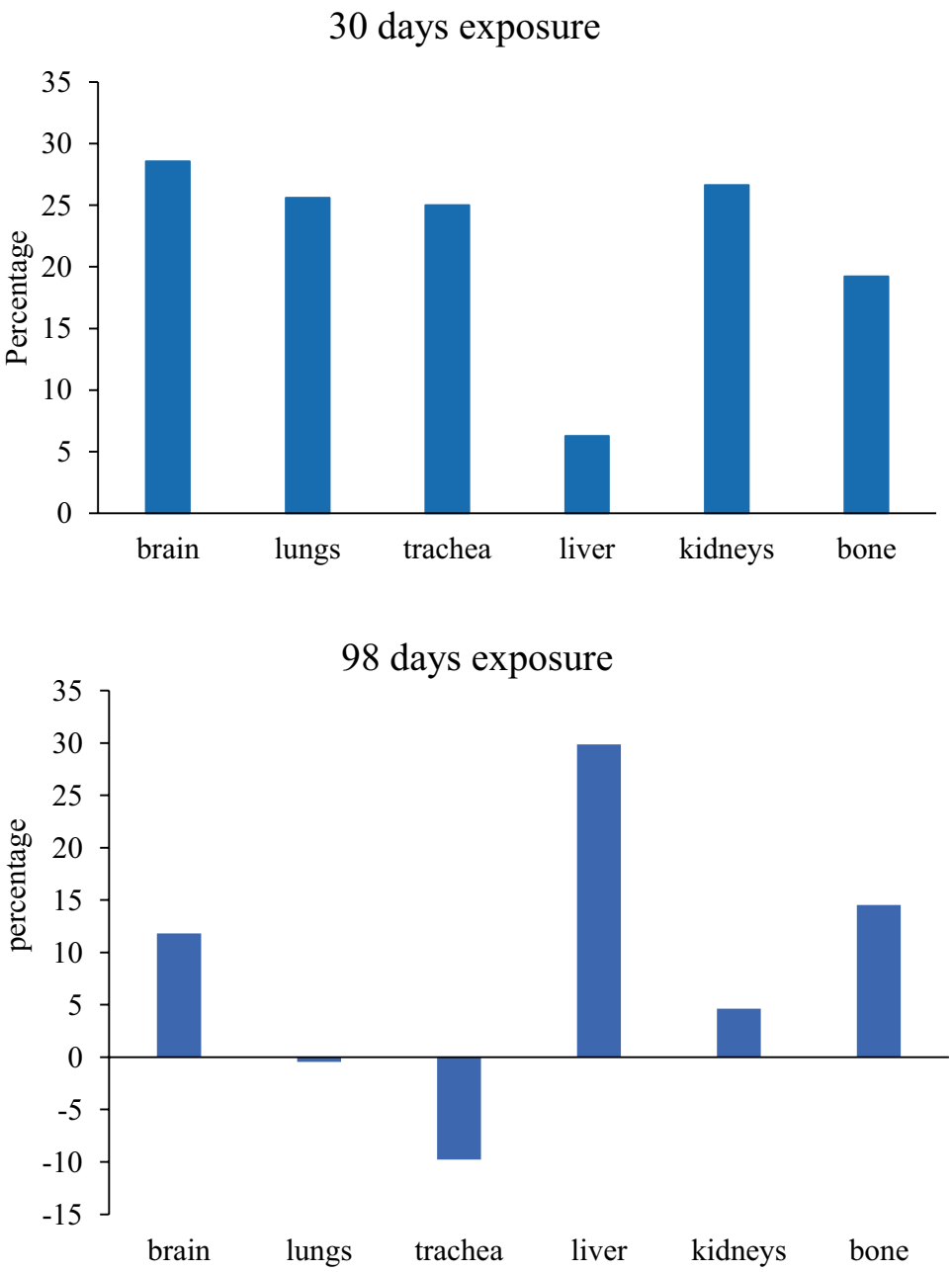

Fig. 1 Reduction percentage between Pb-exposed group and remediated group

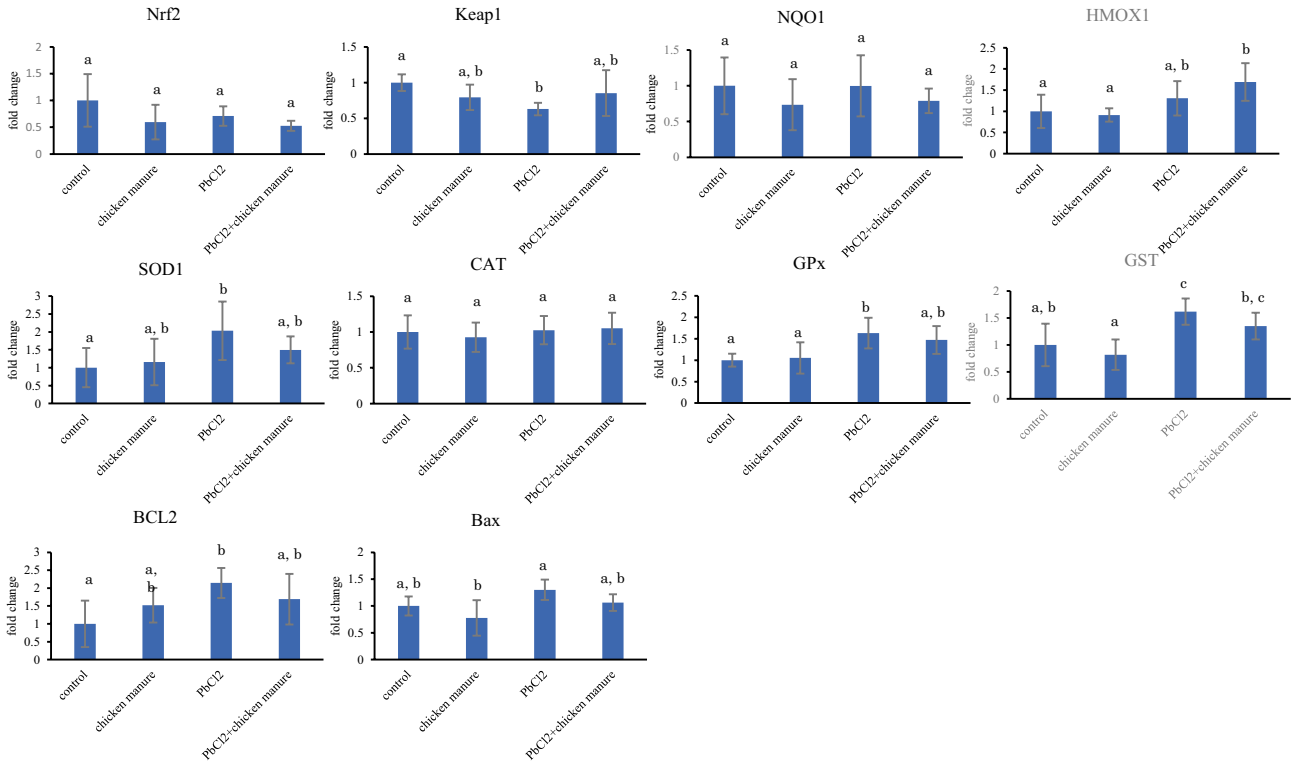

Fig. 2 Expression of antioxidant enzymes and apoptosis related genes in the liver at 30 days exposure ($n=6$) at $p<0.05$. Different letters symbols among groups denote significant difference

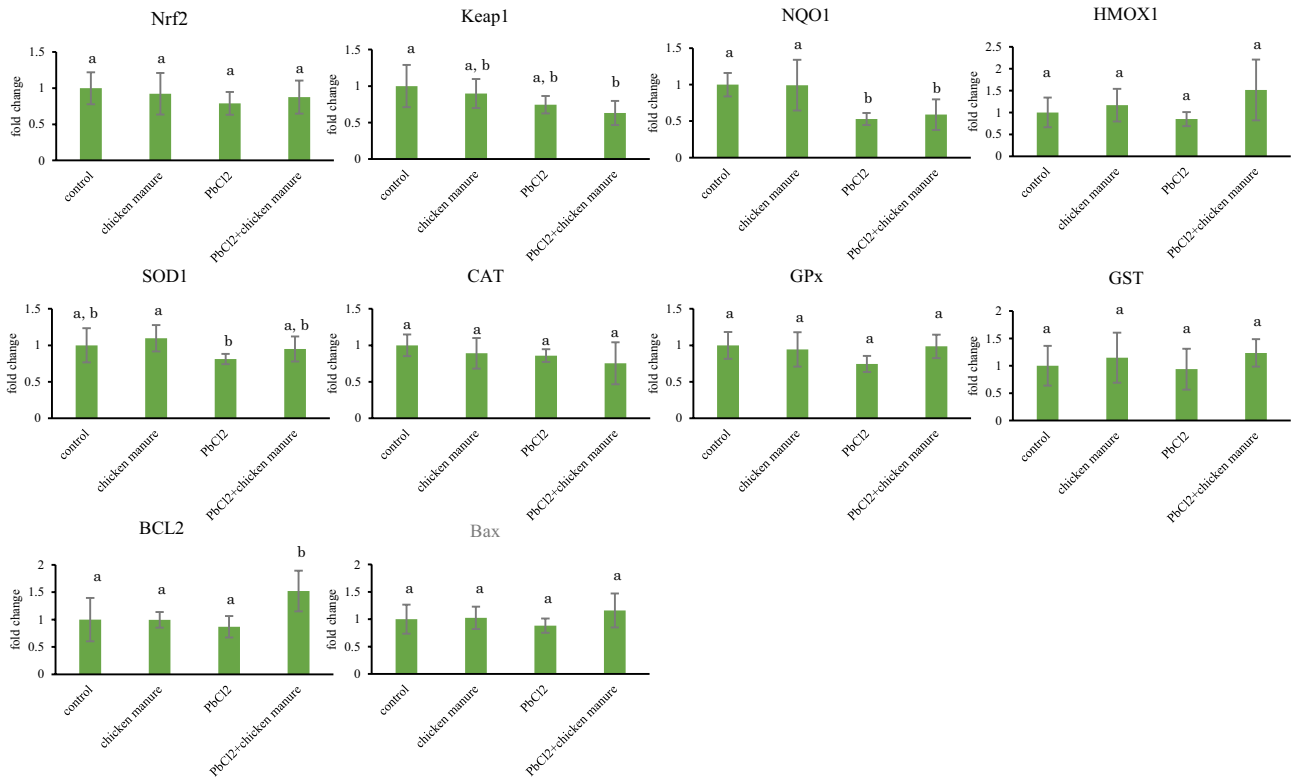

Fig. 3 Expression of antioxidant enzymes and apoptosis-related genes in the liver at 98-day exposure ($n=6$) at $p<0.05$. Different letter symbols among groups denote significant difference

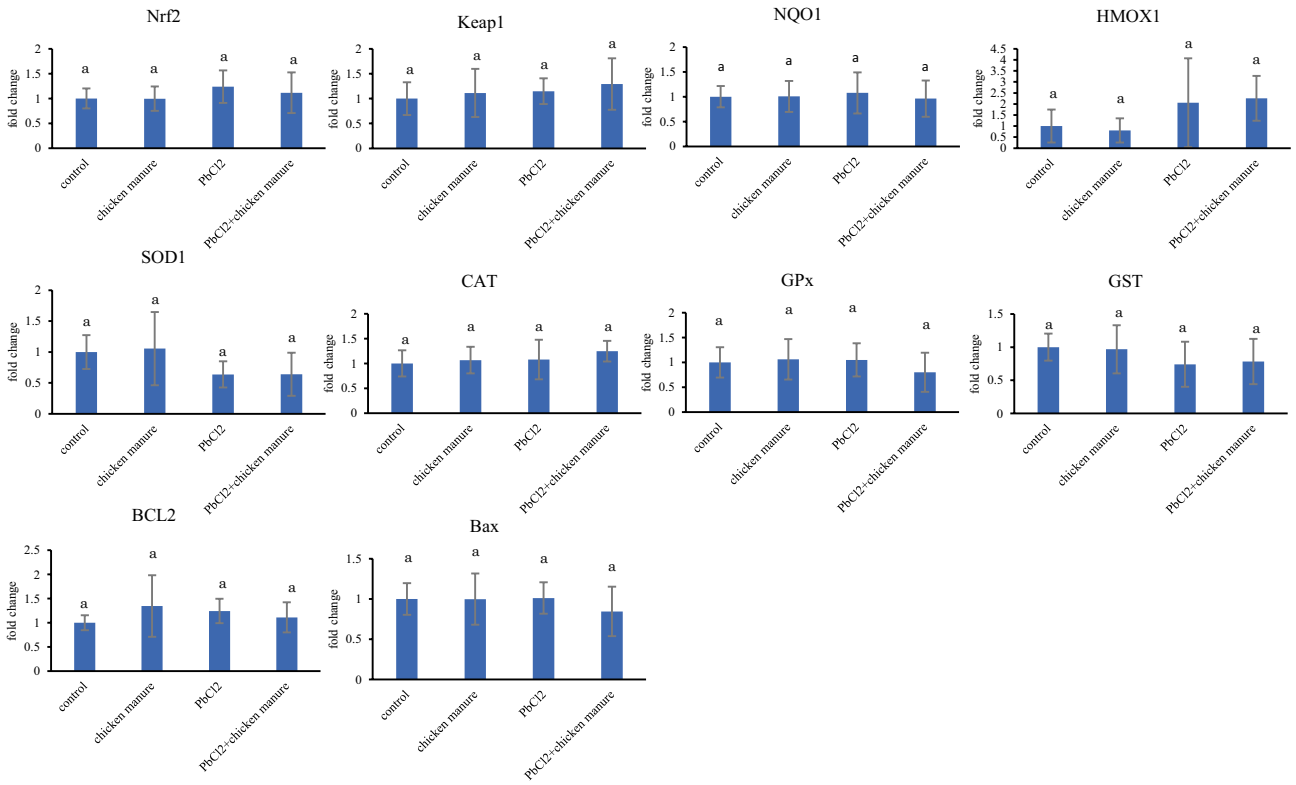

Fig. 4 Expression of antioxidant enzymes and apoptosis-related genes in the kidney at 30-day exposure ($n=6$) at $p<0.05$. Different letter symbols among groups denote significant difference

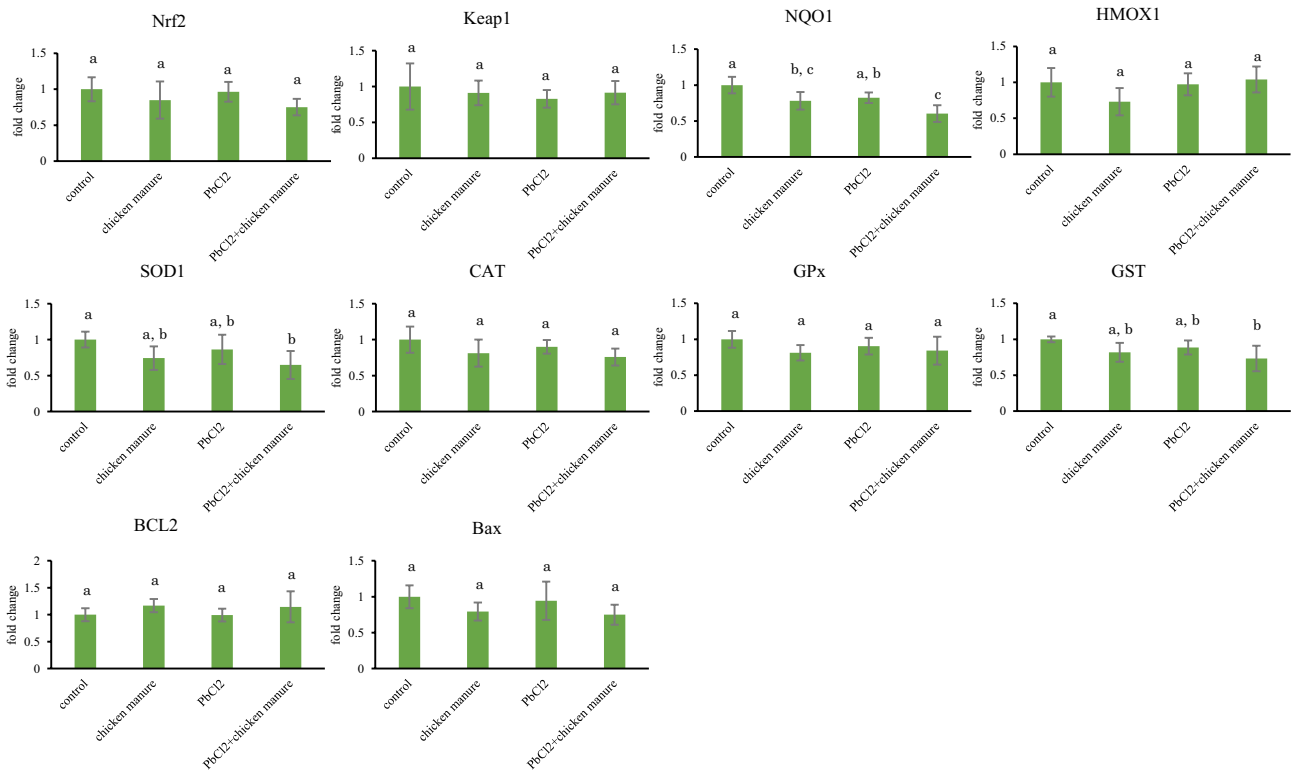

Fig. 5 Expression of antioxidant enzymes and apoptosis-related genes in the kidney at 98-day exposure ($n=6$) at $p<0.05$. Different letter symbols among groups denote significant difference

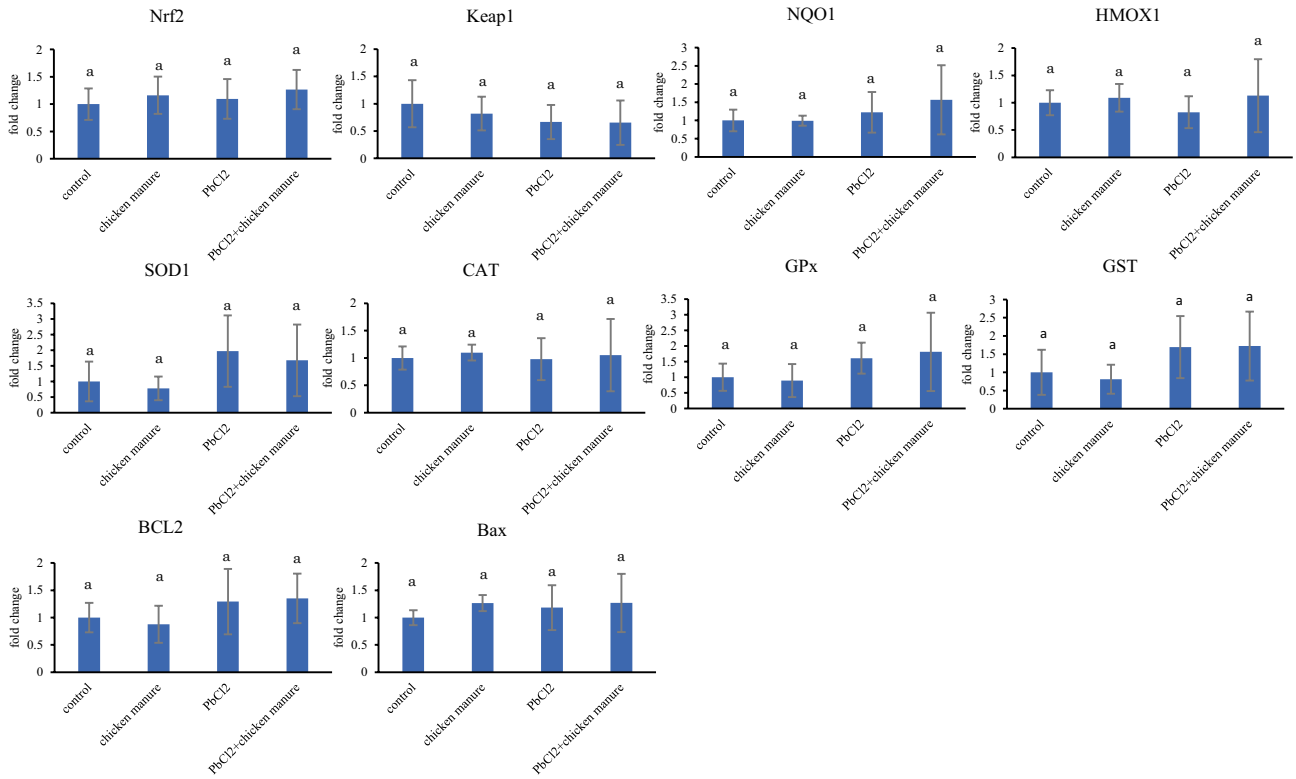

Fig. 6 Expression of antioxidant enzymes and apoptosis-related genes in the brain at 30-day exposure ($n=6$) at $p<0.05$. Different letter symbols among groups denote significant difference

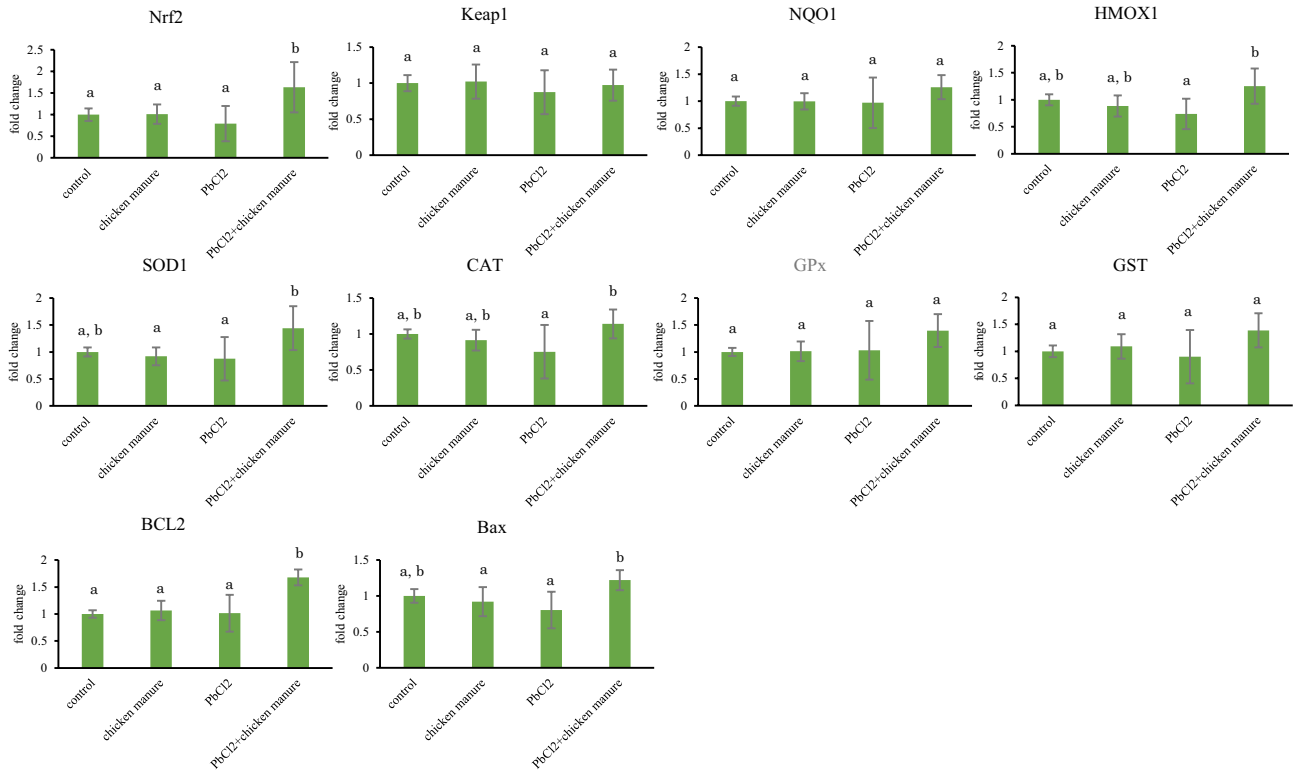

Fig. 7 Expression of antioxidant enzymes and apoptosis-related genes in the brain at 98-day exposure ($n=6$) at $p<0.05$. Different letter symbols among groups denote significant difference

3.3.2 Ninety-eight-day exposure

In the liver, BCL2 was significantly upregulated in the remediated group compared with other groups, while NAD(P)H quinone dehydrogenase 1 (NQO1) was significantly downregulated in the Pb-exposed and remediated groups when compared with other groups. mRNA expressions of NQO1, SOD1, and GST in the kidneys were significantly decreased in remediated group when compared with control group. In the brain, mRNA expressions of BCL2 and nuclear factor erythroid 2-related factor 2 (Nrf2) were significantly upregulated in remediated group compared among groups. Bax, HMOX1, SOD1, and CAT showed significant differences between Pb-exposed and remediated groups; however, no significance was found among the control, the chicken manure, and the remediated groups.

4 Discussion

The current study presents the exposure of mice to Pb-spiked soil by accidental ingestion and ingestion pathways. Accumulation of Pb was observed in mice's tissues after 98-day exposure. To our knowledge, this is the first study to evaluate the effect of chicken manure as an immobilizer in Pb-spiked soil on the cellular mechanisms of oxidative stress and apoptosis in mice.

Soil exposure causes uneven Pb accumulation in several tissues of mice among groups. As expected, moderately high accumulations of Pb in the kidneys and liver were observed. The levels of accumulated Pb in the lungs and brain were relatively smaller than those in the kidneys and liver. The bone showed the highest levels of Pb accumulation, followed by the trachea in the whole body. The liver is the main detoxification organ and kidneys are the main excretion organ in the body (Gundacker et al. 2010; Gu and Manautou 2012). Both organs are also rich in Pb-binding proteins like metallothionein (Yu et al. 2009; Blondet et al. 2018), which may lead to high accumulated Pb. Unlike the liver and kidneys, the lungs and brain have low expression of Pb-binding proteins. A study has proved that Pb exposure by soil/dust inhalation should be counted as exposure pathway in the environmentally relevant model. In that study, the lungs-to-liver ratio of Pb accumulation level was higher in rats exposed to Pb under environmentally relevant conditions for a year (0.432) (Nakayama et al. 2019) than in rats exposed to Pb by feed for 12 weeks (0.055) (Winiarska-Mieczan 2014). In the present study, the lungs-to-liver ratios of Pb in the Pb exposed group were 0.47 and 0.33 in 30 days and 98 days, respectively. Those for Pb in the remediated group were 0.33 and 0.44, in 30 days and 98 days, respectively. The higher ratios observed in this study suggest that respiratory exposure, rather than oral exposure, may contribute significantly to the accumulation of Pb in mice living in heavily contaminated soil environments. Significant differences in Pb accumulation in tissues were found in the brain ($p < 0.001$), lungs ($p < 0.05$), and kidneys ($p < 0.001$) between Pb-exposed and remediated groups after 30 days of exposure. The reduction percentages between these groups ranged from 19.3 to 30.4 in all tissues except the liver (6.3%). In the 98-day exposure group, the reduction percentages were lower in the brain, lungs, trachea, kidneys, and bone compared to the 30-day exposure group. It seems that continuous exposure over a longer period might increase accumulation in tissues. However, the reduction in the percentage of Pb in liver (29.7%) increased over time. It seems that younger mice tend to accumulate more Pb in the liver because of its higher absorption from the gastrointestinal tract than older mice (Guimarães et al. 2012).

Mobilization of Pb in the soil is controlled by soil properties such as pH, CEC, and OM content (Palansooriya et al. 2020). In this study, the pH of the control soil was neutral with low organic carbon content. Chicken manure was used as remediation for Pb spiked soil since it is high in OM content. Normally, OM immobilizes the Pb by various mechanisms such as ion exchange, complexation, and adsorption (Palansooriya et al. 2020). In this study, adding chicken manure to the Pb-spiked soil increased the soil pH and CEC. Generally, soils with high CEC have the ability to retain cationic metals (Weil and Brady 2017). A study proved that the formation of chloropyromorphite, the insoluble Pb form, was observed in the shooting range soils by adding chicken manure (Hashimoto et al. 2009). In the present study, the accumulation rate of Pb was decreased in some tissues by chicken manure. However, a higher rate of chicken manure application may be necessary in the environment because more complex interactions may occur in natural soil systems, such as increased amounts of soluble Pb forms in the soil, natural or fertilizer-induced acidification, incomplete formation or dissolution of insoluble Pb forms due to OM decomposition, and competition from other cation ions such as magnesium, aluminum, calcium, and iron (Schroth et al. 2008; Shaheen et al. 2013; Zhang et al. 2022).

The efficiency of chicken manure as a soil amendment on Pb-induced oxidative stress and cell apoptosis at molecular levels is unknown. In this study, the mRNA expression of antioxidant and cell apoptosis-related genes in response to Pb toxicity in the liver, kidneys, and brain of mice were examined. Upregulation of SOD1, GPx1, GST, and downregulation of Keap1 were observed in the liver of Pb exposed group at 30 days of exposure. Keap1 was downregulated in the Pb-exposed group compared with the control group. Keap1, a very cysteine-rich protein, interacts with Nrf2 to ameliorate reactive oxidative stress (Suzuki and Yamamoto 2015). Under oxidative stress, Nrf2 detaches from Keap1 and enters the nucleus, thereby regulating detoxifying

enzymes and antioxidants (Ye et al. 2015). Downregulation of Keap1 might protect tissue damage from oxidative stress through the Keap1-Nrf2 signaling pathway. Oxidative stress-induced cell damage is protected by antioxidant enzymes. Therefore, upregulation of SOD1, GPx1, and GST mRNA expressions suggests that Pb-induced oxidative stress was protected by antioxidant genes to prevent tissue damage in the mice (Luo et al. 2019).

Although regulation of NQO1, SOD1, and GST was observed in the liver and kidney, the expression of mRNA of genes examined between Pb exposed group and remediated group was not significantly different after 98 days of exposure. However, the amelioration effect of chicken manure in the soil somehow might have been effective on the Pb neurotoxicity by activating the Keap1-Nrf2 signaling pathway and producing HMOX1, SOD1, and CAT to defend against oxidative stress (Ye et al. 2015).

Cell apoptosis was also modulated by Pb toxicity. BCL2, the regulator of apoptotic cell death, prevents apoptosis by blocking cytochrome C release from mitochondria (Yang et al. 1997). Induction of BCL2 was observed in the liver and brain of the remediated group after 98 days of exposure, suggesting an antiapoptotic response.

5 Conclusion

Taken together, this study has demonstrated the toxic effects of Pb in mouse models exposed directly, as well as the immobilization effect of chicken manure on Pb-spiked soil through tissue accumulation. Results showed that Pb accumulation in the body was decreased by adding chicken manure, potentially through regulating soil properties such as pH, CEC, and OM in addition to forming insoluble Pb that reduced bioavailability. The application of chicken manure in Pb-spiked soil has been shown to mitigate Pb-induced oxidative stress and apoptosis. After 98 days of exposure, the brain of mice in the remediated group showed signs of HMOX1, SOD1, and CAT activation. There was, however, no clear evidence of oxidative stress and apoptosis in the liver or kidney following different exposure periods. Further studies are needed to investigate the potential effects of using chicken manure at different rates as an immobilizer with different doses of Pb in the soil to reduce Pb body burden.

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Data availability Available from the corresponding author on reasonable request.

Declarations

Competing interests The authors declare no competing interests.

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