

Original Research

# Effects of Soil Amendments Applied on Cd and Pb Immobilization and Environmental Risks under Simulated Acid Rain

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## Abstract

A column leaching experiment was conducted to study the long-term remediation stability of hydroxyapatite, sepiolite, and biochar on soil Cd and Pb under simulated acid rain. The results indicated that simulated acid rain reduced soil pH and facilitated the conversion of residual fractions of Cd and Pb to acid-soluble fractions, thus increasing solubility, mobility, and availability of soil Cd and Pb. Lower leachate Cd and Pb contents were found in hydroxyapatite-treated soil than were found in sepiolite and biochar-treated soils. Meanwhile, Cd and Pb concentrations in hydroxyapatite met grade IV ( $Cd \leq 0.01 \text{ mg L}^{-1}$ ,  $Pb \leq 0.1 \text{ mg L}^{-1}$ ) at the end of leaching (48 weeks), according to the Environmental quality standards for groundwater in China (GB14848-93). The application of hydroxyapatite, sepiolite, and biochar effectively immobilized Cd and Pb in both the water and simulated acid rain treatments. The immobilization efficiencies of Cd treated by hydroxyapatite, sepiolite, and biochar were 37.97~43.55%, 21.52~25.81%, and 26.58~33.87%, respectively, after the long-term leaching experiment. For Pb, these values were 36.35~48.65%, 24.90~29.26%, and 20.91~22.41%, respectively. Hydroxyapatite has better immobilization efficiency and maintains stronger and more durable stability than sepiolite and biochar. Therefore, hydroxyapatite is recommended to use in the remediation of Cd and Pb-contaminated agricultural soils under the condition of soil acidification.

**Keywords:** hydroxyapatite, sepiolite, biochar, immobilization stability, simulated acid rain

## Introduction

Heavy metal contamination causes serious threats to the global environment because of its high toxicity and nonbiodegradability [1]. According to previous studies, about 19.4% of China's agricultural soil is contaminated, and Cd and Pb are considered the most

toxic in the list of major contaminants [2-4]. They are easy to accumulate in agricultural soils and can be transferred into the food chain, resulting in a great risk to human beings [5]. Therefore, the remediation of Cd and Pb co-contaminated agricultural fields has captured great concern in recent years. Several remediation techniques have been performed, such as soil dressing, vitrification, electrokinetic methods [3, 6], soil washing, chemical immobilization [7,8] and phytostabilization [9]. Among those techniques, agriculture-based *in situ*

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chemical immobilization attracts extensive attention due to the cost-effectiveness, rapid implementation, and environmental sustainability for the heavy metal contaminated soils [10, 11]. Chemical immobilization alleviates the solubility, mobility, and bioavailability of Cd and Pb by applying various amendments to increase soil pH, and by adsorbing, exchanging, complexing, or precipitating with heavy metals in soil [12, 13].

A feasible soil amendment is essential for chemical remediation technology and has attracted increasingly widespread attention. Various amendments, including in-organic materials (lime, phosphate compounds, and clay minerals) and organic materials (biochar, manure, organic fertilizer), have been successfully used to decrease the bioavailability of Cd and Pb during *in situ* chemical immobilization [4, 15]. For example, hydroxyapatite is considered an effective soil amendment for the remediation of Cd and Pb contaminated soils, due to its small size and high specific surface area [16, 12]. The application of clay minerals including sepiolite, bentonite, and zeolite can significantly increase adsorptive ability and ion exchange capacity and reduce the toxicity and extraction of Cd and Pb [5, 17]. Biochar is also recommended as a sustainable means of alleviating the Cd and Pb contamination because of its highly porous structure and various functional groups to increase adsorption capacity [18, 19].

It is worth paying attention to whether the immobilization ability by amendments is stable in a long-term process. Heavy metal re-mobilization is probably affected by environmental factors, local climate, amendment properties, and other uncertain factors [20]. Therefore, the immobilized heavy metals have the potential to release and become phytoavailable fractions with the change of environmental conditions [21]. Soil pH is a key factor governing heavy metals mobility and bioavailability, and it is significantly affected by acid rain. Acid rain is considered one of the three most severe environmental disasters, and China has the third-largest acid rain region, with about 40% of the land area currently suffering from acid rain [22]. Moreover, heavy metal contamination areas mainly occur in southern China and overlap with the areas affected by acid rain, aggravating soil acidification and the risk of remobilization of immobilized metals [3]. Even when applying alkaline amendments to heavy metal contaminated soils, the immobilization effect may decrease over time. Therefore, it is crucial to predict heavy metal stability for a long period under acid rain conditions, particularly when using alkaline amendments. At present, there is also a research gap in the long-term impacts of alkaline materials on the remediation stability of Cd and Pb under acid rain conditions. Further studies are needed to conduct and fill in the knowledge gaps on this subject.

In this research, a column leaching experiment was carried out to determine the effects of hydroxyapatite, sepiolite, and biochar on the long-term immobilization

of soil Cd and Pb under simulated acid rain. The overall objective is to provide valuable insights into (1) the dynamic changes of leachate properties, e.g., pH, electrical conductivity (EC), dissolved organic carbon (DOC), Cd, and Pb; (2) post-leaching changes in the mobility and bioavailability of soil Cd and Pb; and (3) the changes in the immobilization efficiency of the three amendments during the whole remediation period.

## Experimental

### Soil and Materials

Surface soils (0-20 cm) were sampled from a vegetable field contaminated by Cd and Pb in Nanjing, Jiangsu Province, China. It is classified as yellow-brown soil based on Chinese Soil Taxonomy while it would be Alfisol according to the US Soil Taxonomy. The soils were air-dried and impurities removed, then passed through a 2-mm sieve for use. Hydroxyapatite, sepiolite, and biochar were chosen as soil amendments in the present study. Hydroxyapatite and sepiolite were purchased from a local Chemical Reagent Co., Ltd. Biochar was prepared in laboratory according to the detailed process of Zhang and Ding [23]. The physico-chemical properties of the tested soil and applied materials are described in Table 1.

### Leaching Experimental Design

A laboratory column leaching experiment was carried out to determine the immobilization efficiency of applied materials on Cd and Pb contaminated soil under simulated acid rain. Four treatments were carried out in the experiment, i.e., control (without amendment, designated CK), H (hydroxyapatite), S (sepiolite), and B (biochar). Deionized water and simulated acid rain were used as leaching solutions for the column leaching experiment, which lasted for 48 weeks. Deionized water was set as a control with a pH value of 6.5. According to the characteristics of acid rain in Nanjing, the mother liquor of simulated acid rain was prepared with a mixture of  $H_2SO_4$  and  $HNO_3$  in the ratio of 4:1, and then diluted with deionized water to prepare a leaching solution (pH 4.0). Each amendment was evenly blended and applied with 2.5% (w/w) of soil, then placed into a uniform plexiglas column with each mixture replicated in triplicate. The plexiglas column was a diameter of 4.4 cm and a loading height of 20 cm. The loading sequence from bottom to top was porous plate, nylon net, 10-mm quartz sand, 2-mm soil, and 10-mm quartz sand. Deionized water was added to all the columns to stabilize for 48 h before the leaching experiment. After that, simulated acid rain leaching was conducted using a peristaltic pump with a flow rate of 10 mL  $h^{-1}$ . For each column leaching, 250 mL of simulated acid rain was used every two weeks. Twenty-four applications were conducted, and the cumulative

Table 1. The physical and chemical properties of the tested soil and three amendments.

	pH (1:2.5)	BET-surface area (m <sup>2</sup> g <sup>-1</sup> )	Soil texture	Organic carbon (g kg <sup>-1</sup> )	CEC (cmol <sub>(+)</sub> kg <sup>-1</sup> )	Total Cd (mg kg <sup>-1</sup> )	Total Pb (mg kg <sup>-1</sup> )
Soil	6.34	-	Silty clay	26.69	12.56	1.37	707.8
Hydroxyapatite	8.79	46.58	-	0	64.28	0.03	6.31
Sepiolite	8.42	41.23	-	0	136.6	0.006	1.27
Biochar	7.87	59.65	-	47.83	23.74	0.076	1.51

volume was equivalent to the annual rainfall (1000 mm) in this region. The same volume of deionized water was conducted in the control treatment. Leachates were collected every two weeks. The pH, EC, DOC, and total Cd and Pb concentrations of leachates were analyzed. About 20 g of soil was collected from each column and then naturally air-dried to determine the soil pH, the soil organic carbon (SOC), the soil available Cd and Pb contents, the toxicity characteristic leaching procedure (TCLP) extractable Cd and Pb, and the redistribution of soil Cd and Pb.

#### Chemical Analysis

The pH and EC of the leachate were determined using a pH meter (Orion Star™ A211, USA) and an electrical conductivity meter (DDSJ-318), respectively. The DOC was measured using a total organic carbon analyzer (Multi N/C 3100, Germany). The total Cd and Pb concentrations were analyzed by inductively coupled plasma mass spectrometry (ICP-MS, Agilent 7500, USA). The availability of soil Cd and Pb was extracted as described by Zhang et al. with 0.01 mol L<sup>-1</sup> CaCl<sub>2</sub> [23]. TCLP extractable method was conducted to assess the mobility of soil heavy metals, and the detailed experimental process is shown in the Supplementary Material [24]. The redistribution of Cd and Pb in soil was determined by the European Community Bureau of Reference (BCR) sequential extraction method [25]. Four different fractions designated by this method included: acid soluble, reducible, oxidizable, and residual fraction, of which the first three were extracted by 0.11 mol L<sup>-1</sup> HAc, 0.5 mol L<sup>-1</sup> NH<sub>2</sub>OH·HCl and 1.0 mol L<sup>-1</sup> NH<sub>4</sub>OAc, respectively, while the last was obtained by digesting the residual with HNO<sub>3</sub>-HF-HClO<sub>4</sub> mixture. The detailed process is contained in the Supporting Material. The soil Cd and Pb concentrations of the above-mentioned extraction were determined by ICP-MS. A certification reference material (GBW07412, National Research Center for Certified Reference Materials, China) was used to ensure the precision of the analytical procedure. The recovery rates of soil Cd and Pb ranged from 95% to 106%.

#### Data Analysis

All results were tabulated and presented as the means (n = 3) and standard deviations (S.D.). A two-way

ANOVA was conducted to test the differences of means using SPSS 19.0 statistical software. The least significant difference (LSD) test at a 5% probability level was employed.

## Results and Discussion

### Effects of Simulated Acid Rain and Amendments on Soil Leachate Chemistry

The dynamics of leaching water chemistry as affected by acid rain are shown in Fig. 1. The leachate pH of all treatments gradually increased with time and then tended to be stable after ten weeks. This may be caused by the exchange of H<sup>+</sup> with base ions (Na<sup>+</sup>, K<sup>+</sup>, Ca<sup>2+</sup>, Mg<sup>2+</sup>, and NH<sub>4</sub><sup>+</sup>) in the soil at the initial stage of leaching, thus increasing leachate pH [26]. With a large number of base ions consumed, the secondary buffering stage mainly depends on the weathering of minerals to decrease the effects of simulated acid rain [26]. This indicates that adequate time is needed for leachate pH to become stable due to the strong buffering capacity of the soil. The pH of leachate ranged from 5.39 to 6.93 in the acid rain treatment, which was much lower than that in the water treatment (6.33~8.49). The leachate pH in the control treatment was significantly higher than that of acid rain (pH 4.0). This was related to the specific adsorption of SO<sub>4</sub><sup>2-</sup>. After being adsorbed by soil, SO<sub>4</sub><sup>2-</sup> can exchange with hydroxyl groups on the soil oxide surface, and then the hydroxyl groups enter the solution from the soil surface to increase leachate pH [27]. Compared to the control treatment, amendments caused a significant increase in leachate pH in both the water and simulated acid rain groups. Of these, the pH in the hydroxyapatite (H) treatment increased the most and the magnitudes of the increase were 28.44 and 25.93%, respectively, in the water and simulated acid rain treatments. The increase of leachate pH may be attributable to the dissolution of hydroxyapatite, and the finding is consistent with that of Cui et al. [28].

As shown in Fig. 1, the leachate EC sharply decreased in the first ten weeks, and then it tended to be at a stable level. The EC decreased from 1.67 to 0.34 mS cm<sup>-1</sup> in the acid rain group and from 1.23 to 0.25 mS cm<sup>-1</sup> in the water group during the leaching period. This result may be the cause of the increased base ions following simulated acid rain and was

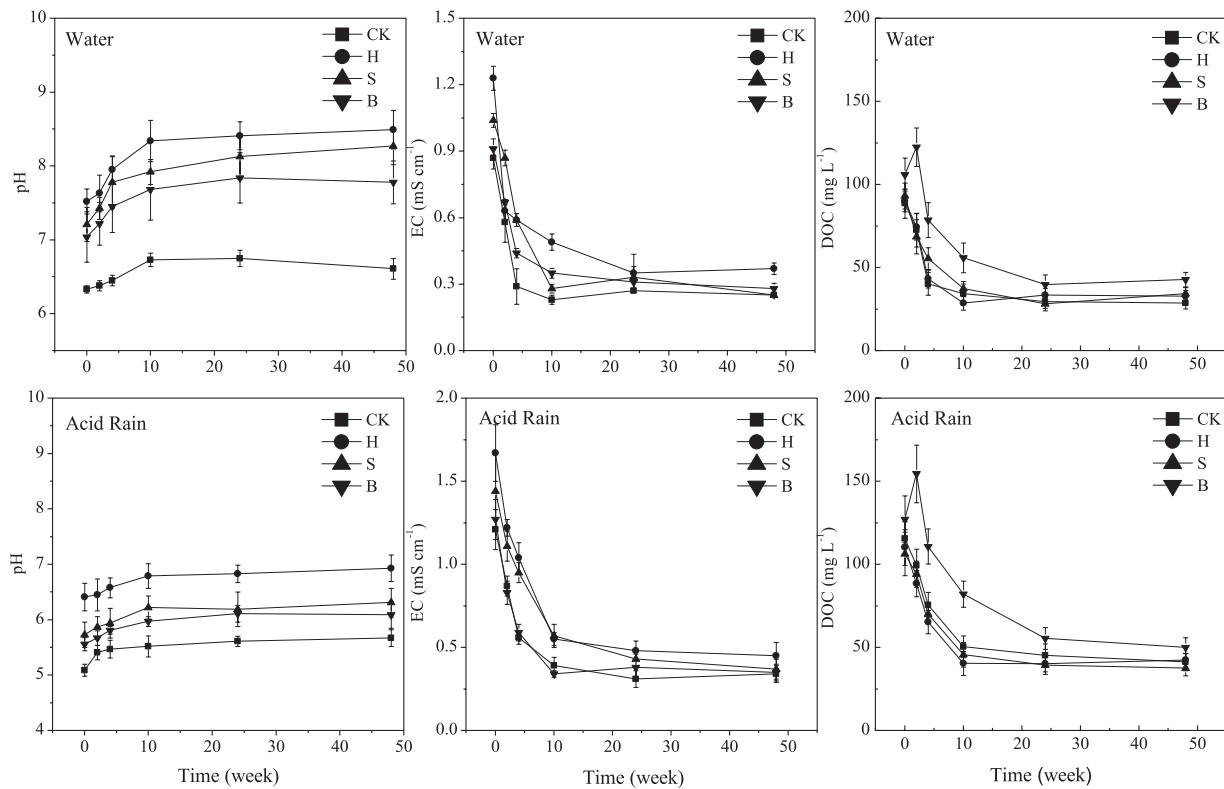


Fig. 1. Dynamics of pH, EC, DOC in leachate.

consistent with the previous study [28]. Compared with the control treatment, hydroxyapatite and sepiolite (S) caused a dramatic increase of leachate EC in the water and acid rain groups at the beginning of leaching (week 0). It is common to observe elevated EC due to the higher mineral contents after the dissolution of the amendments.

The DOC concentrations significantly decreased with time in the control, hydroxyapatite, and sepiolite treatments during the first ten weeks (Fig. 1). However, there was a fluctuation between collection periods in biochar (B). The DOC concentration dramatically increased by 10.02~74.89% and 17.33~78.74% in the first two weeks in the water and acid rain groups, respectively, compared with the other three treatments. Correspondingly, there was no significant difference among the control, hydroxyapatite, and sepiolite treatments. This result may be related to the increase of soil organic matter after applying biochar, leading to the increase of leachate DOC at the beginning of leaching. This study also indicated that the concentration of leachate DOC in the acid rain treatment was higher than that in the water treatment, which means simulated acid rain promoted the dissolution of organic carbon and accelerated the loss of DOC.

#### Effects of Simulated Acid Rain and Amendments on the Solubility of Cd and Pb

The Cd and Pb concentrations in leachate dramatically decreased with time in the first four weeks,

and then stabilized (Fig. 2). The concentrations of Cd and Pb were 9.45~120.6  $\mu\text{g L}^{-1}$  and 0.08~1.56  $\text{mg L}^{-1}$  in the water group during the leaching period, respectively. Correspondingly, Cd and Pb concentrations were 10.43~274.5  $\mu\text{g L}^{-1}$  and 0.11~2.75  $\text{mg L}^{-1}$ , respectively, in the simulated acid rain group. Simulated acid rain increased the solubility of  $\text{Cd}^{2+}$  and  $\text{Pb}^{2+}$ ; one reason for this is that the increasing concentration of  $\text{H}^+$  promotes Cd and Pb desorption by displacing metal cations from their binding sites [29]. On the other hand, simulated acid rain increased the concentrations of active  $\text{Al}^{3+}$  and  $\text{Fe}^{3+}$ , and increased the desorption of Cd and Pb due to the competitive adsorption and surface protonation [27, 28]. In addition, simulated acid rain can increase the solubility of heavy metal oxides and hydroxides in soil, reduce the adsorption of soil colloid to  $\text{Cd}^{2+}$  and  $\text{Pb}^{2+}$ , and then lead to the increase of Cd and Pb in leachate [27].

The application of hydroxyapatite, sepiolite, and biochar caused significant decreases in leachate Cd and Pb concentrations compared to the control treatment in the water and acid rain groups. The results indicate that the three amendments applied can decrease the mobility of Cd and Pb and reduce the risk of contaminating environment. According to the Environmental quality standards for groundwater in China (GB14848-93), the concentrations of leachate Cd and Pb in hydroxyapatite were below grade IV ( $\text{Cd} \leq 0.01 \text{ mg L}^{-1}$ ,  $\text{Pb} \leq 0.1 \text{ mg L}^{-1}$ ) at the end of leaching (48 weeks) both in the water and acid rain groups, and the Cd and Pb concentrations in other three treatments met grade V quality standard.

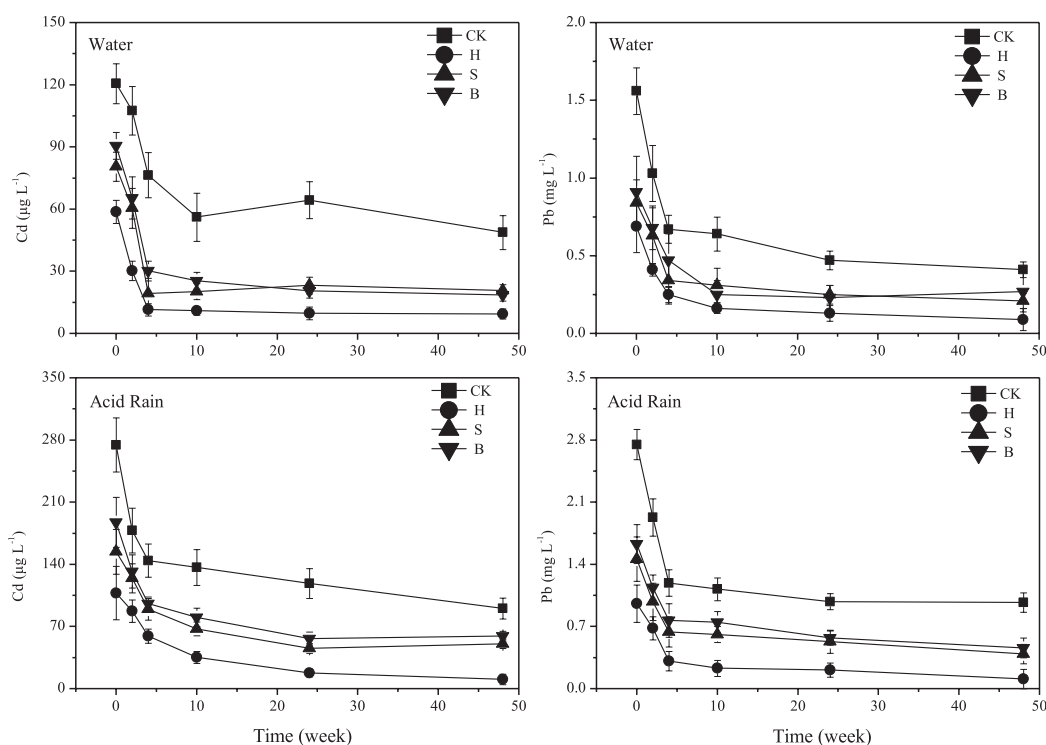


Fig. 2. Dynamics of total Cd and Pb concentrations in leachate.

Although sepiolite and biochar addition could alleviate the leaching risk of Cd and Pb into the environment, there is still a risk of water pollution during the first year, the hydroxyapatite appears to be the most optimal amendment to alleviate Cd and Pb leaching in a short time.

To evaluate the long-term environmental effects of Cd and Pb in the column leachate, the double constant model, parabolic model, exponential model and first-order kinetic model were analyzed [30]. The four simulated models and correlation coefficient ( $R^2$ ) are shown in Table 2. Of which, the parabolic model fit best to the cumulative leaching rate of Cd and Pb, the correlation coefficients reached a maximum of 0.998 and 0.995 in the water group, respectively, while the correlation coefficients were 0.996 and 0.993 for Cd and Pb in the acid rain group, respectively. Therefore, the parabolic model was used to predict the long-term effect of Cd and Pb leaching on water environment.

The model indicated that the concentrations of leachate Cd in hydroxyapatite, sepiolite and biochar may reach  $4 \mu\text{g L}^{-1}$ ,  $7 \mu\text{g L}^{-1}$  and  $6 \mu\text{g L}^{-1}$  over the next 5 years in the water group, respectively, while the concentrations of leachate Pb may reach  $0.03 \text{ mg L}^{-1}$ ,  $0.05 \text{ mg L}^{-1}$  and  $0.05 \text{ mg L}^{-1}$ , respectively. The parabolic model predicted that the concentrations of leachate Cd in hydroxyapatite, sepiolite and biochar may reach  $6 \mu\text{g L}^{-1}$ ,  $9 \mu\text{g L}^{-1}$  and  $10 \mu\text{g L}^{-1}$  over the next 5 years in the acid rain group, respectively, while the concentrations of leachate Pb may reach  $0.05 \text{ mg L}^{-1}$ ,  $0.08 \text{ mg L}^{-1}$  and  $0.10 \text{ mg L}^{-1}$ , respectively. The concentrations of leachate Cd and Pb with the three amendments were lower than the level III and level IV quality standard for groundwater in the water and acid rain group, respectively. This means that the Cd and Pb contaminated soil with hydroxyapatite, sepiolite and biochar remediation may be harmless to the water environment after adequate time even under acid rain conditions.

Table 2. Leaching models of Cd and Pb accumulated.

Leaching model	Equation	Water		Acid rain	
		$R^2$ values of Cd	$R^2$ values of Pb	$R^2$ values of Cd	$R^2$ values of Pb
Double constant model	$\ln \eta = a + b \ln t$	0.991	0.993	0.986	0.992
Parabolic model	$H = a t^2 + b t + c$	0.998	0.995	0.996	0.993
Exponential model	$1/\eta = a t^b$	0.994	0.990	0.989	0.990
First-order kinetic model	$\ln \eta = a + b t$	0.945	0.939	0.885	0.912

Notes: a, b and c are constants;  $\eta$  is the cumulative leaching rate, t is leaching time.



Table 3. Soil pH and SOC contents after water and acid rain leaching.

Treatment	pH (1:2.5)		SOC (g kg <sup>-1</sup> )	
	Water	Acid rain	Water	Acid rain
CK	6.31±0.08c	5.73±0.07b	26.14±2.43b	23.44±1.75b
H	7.14±0.12a	6.19±0.12a	27.39±1.95b	25.37±1.54b
S	7.09±0.07a	6.15±0.09a	26.65±3.04b	23.59±1.66b
B	6.62±0.06b	6.04±0.08a	35.03±4.11a	33.08±2.84a

Notes: values are presented as mean±standard deviation. Significant differences ( $p<0.05$ ) among treatments are indicated by different lowercase letters ( $n = 3$ , LSD test).

### Effects of Simulated Acid Rain and Amendments on Soil pH and Organic Carbon

The effects of water and acid rain on soil pH and SOC content are shown in Table 3. The increases of soil pH were 0.80, 0.75, 0.28 in hydroxyapatite, sepiolite, and biochar treatments, respectively, compared to the background soil in the water group. Meanwhile, the pH values in amendment treatments were about 0.15~0.61 lower than that of the background soil in the simulated acid rain group. This result indicated simulated acid rain caused a loss of soil base cations and reduced soil pH. Compared to the control, soil pH had a notable increase ( $p<0.05$ ) in the hydroxyapatite, sepiolite, and biochar treatments in the water and acid rain groups. The three amendments have high alkalinity and acid-neutralizing capacity, which can increase the soil pH to different degrees [31-33]. Notably, hydroxyapatite contains a large number of hydroxyl groups, which will consume H<sup>+</sup> through dissolution and increase the soil pH after being applied to the soil [16]. This result agrees with that of Zhao et al. [30] and Cui et al. [27], who showed that hydroxyapatite has a good liming effect for acidic soil and can effectively increase soil pH. The application of amendments caused different effects

on SOC contents. There was no marked difference between the hydroxyapatite, sepiolite, and control treatments, but a remarkable rise of SOC in biochar compared to other three treatments was observed ( $p<0.05$ ). Compared to the background soil, the increases in the SOC content were 8.34 and 6.39 g kg<sup>-1</sup> in the biochar treatment in the water and acid rain groups, respectively. Biochar is rich in organic carbon and can directly lead to an increase of soil stable organic carbon [34].

### Effects of Simulated Acid Rain and Amendments on the Distributions of Soil Cd and Pb

BCR sequential extraction techniques are suitable to indicate the effects of simulated acid rain on soil Cd and Pb fractionation, and the distributions of Cd and Pb in each solid phase of soil are shown in Fig. 3. The results indicated that amendments and simulated acid rain significantly changed the proportion of acid-soluble and residual fractions of Cd and Pb, but there were no significant changes in the oxidizable and reducible fractions. Compared to the water treatment, the percentages of acid-soluble fractions of Cd and Pb were increased, and the residual fraction of Cd and Pb

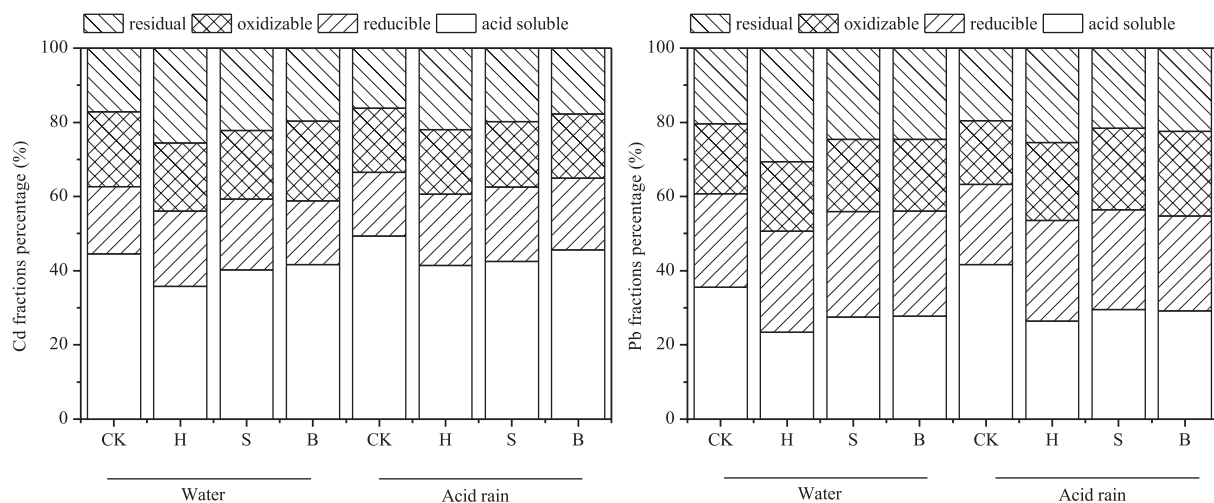


Fig. 3. Changes in Cd and Pb fractionation after water and acid rain leaching.

correspondingly decreased in the acid rain treatment. The percentages of the acid-soluble fraction of Cd and Pb increased by 5.64~15.65% and 5.53~17.12%, respectively, while the percentage of the residual fraction decreased by 5.42~14.16% and 4.41~16.25%, respectively. The acid-soluble fraction represents the easily labile fractions and can be used to evaluate the risks associated with heavy metal bioavailability and immobilization effectiveness [35]. Our study indicated that simulated acid rain transformed the less bioavailable metal fractions of soils into more bioavailable forms and decreased the metal immobilization. For the amended soils, compared to the control treatment, the greatest increase in residual fractions of Cd and Pb was found in the hydroxyapatite treatment. The proportions of residual Cd increased to 25.55 and 21.93% in the water and simulated acid rain groups, respectively, while the proportions of residual Pb increased to 30.63 and 25.41%, respectively. The immobilization of Cd and Pb by hydroxyapatite can be attributed to the formation of a large amount of metal precipitation. The increased soil pH caused by hydroxyapatite may lead to the formation of  $\text{Cd}(\text{OH})_2$  and  $\text{Pb}(\text{OH})_2$  [33]. Moreover, the dissolved hydroxyapatite will increase soil labile P and promote the formation of more stable phosphate such as  $\text{Cd}_x\text{Ca}_{10-x}(\text{PO}_4)_6(\text{OH})_2$  and  $\text{Pb}_x\text{Ca}_{10-x}(\text{PO}_4)_6(\text{OH})_2$  to increase the residual fraction [36]. The application of sepiolite and biochar also can decrease the proportions of acid-soluble fractions of Cd and Pb due to the effects of pH regulating, complexation or adsorption. Sepiolite has high pH and strong cation exchange capacity (Table 1) to increase immobilization of Cd and Pb. Biochar can increase surface area and binding sites to adsorb heavy metals. In addition, the surface complexation of oxygen-containing functional groups on biochar promotes the adsorption of soil Cd and Pb [37].

#### Effects of Simulated Acid Rain and Amendments on the Mobility and Bioavailability of Soil Cd and Pb

TCLP was used to evaluate the mobility of Cd and Pb under acidic conditions. Compared to the beginning of leaching (week 0), leaching at 48 weeks decreased TCLP-Cd by 8.33~41.56% and 7.96~35.16% in the water and acid rain groups, respectively (Fig. 4). For TCLP-Pb, the magnitudes of the decrease were 9.96~29.09% and 5.05~22.01% in the water and acid rain groups, respectively. At the end of leaching, compared to the water treatment, the increases of TCLP-Cd in the hydroxyapatite, sepiolite, and biochar treatments were 31.11, 47.06, and 28.07% in the acid rain treatment, respectively. Similarly, the TCLP-Pb increased by 39.11, 35.84, and 32.90%, in the acid rain group treated by hydroxyapatite, sepiolite, and biochar, respectively. Moreover, the three amendments decreased TCLP-Cd and TCLP-Pb remarkably ( $p < 0.05$ ) compared to the control, but no significant difference between amendments treatments was observed at the end of leaching (48 weeks). Similar to TCLP-Cd and TCLP-Pb, amendments and simulated acid rain caused marked changes in  $\text{CaCl}_2$ -Cd and  $\text{CaCl}_2$ -Pb (Fig. 5). At the end of leaching (48 weeks), compared to the water treatment, the increases of  $\text{CaCl}_2$ -Cd and  $\text{CaCl}_2$ -Pb were 0.11~0.17  $\text{mg kg}^{-1}$  and 0.82~1.53  $\text{mg kg}^{-1}$  in the acid rain group, respectively. Moreover, hydroxyapatite, sepiolite, and biochar showed a significant effect on soil  $\text{CaCl}_2$ -Cd and  $\text{CaCl}_2$ -Pb ( $p < 0.05$ ). Compared to the control treatment, the application of hydroxyapatite, sepiolite, and biochar decreased  $\text{CaCl}_2$ -Cd and  $\text{CaCl}_2$ -Pb by 22.41~43.55% and 25.81~45.65% in the water group, respectively. While the decrease was 21.52~37.97% and 20.91~36.35% in the acid rain group, respectively.

Simulated acid rain caused a significant effect on the mobility and bioavailability of soil Cd and Pb. The possible mechanism was the change of soil

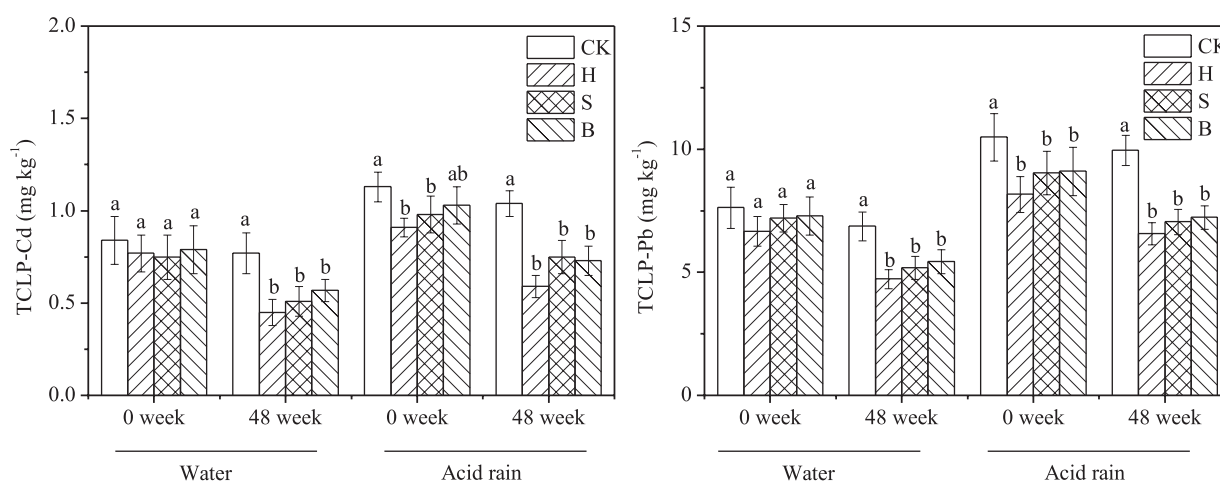


Fig. 4. Mobility of Cd and Pb as evaluated by TCLP. Values are the mean  $\pm$  SD, and different lowercase letters between bars indicate significant differences at  $p < 0.05$  level ( $n = 3$ , LSD test).

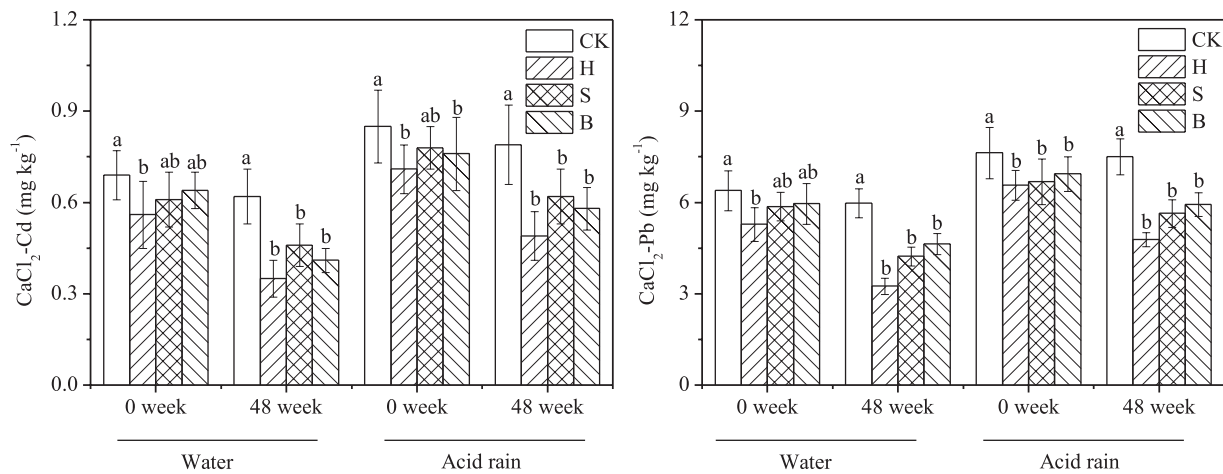


Fig. 5. Bioavailability of Cd and Pb as extracted by  $\text{CaCl}_2$ . Values are the mean $\pm$ SD and different lowercase letters between bars indicate significant differences at  $p < 0.05$  level ( $n = 3$ , LSD test).

physical-chemical properties affected by simulated acid rain. In particular, soil pH is a key factor governing Cd and Pb solubility, mobility, and bioavailability [38]. The decrease of pH increased the solubility of heavy metal hydroxides and oxides and reduced the adsorption of  $\text{Cd}^{2+}$  and  $\text{Pb}^{2+}$  by soil colloids [37]. Linear analysis confirmed that a significant and negative correlation ( $n = 24$ ,  $p < 0.01$ ) was observed between soil pH and TCLP-,  $\text{CaCl}_2$  extractable Cd and Pb. The determination coefficients ( $R^2$ ) were 0.76 and 0.73 in TCLP and  $\text{CaCl}_2$  extractable Cd, respectively, and 0.83 and 0.82 in TCLP and  $\text{CaCl}_2$  extractable Pb, respectively (Table 4). Previous studies also indicated that there was a negative correlation between pH and available Cd and Pb [32, 36, 39]. Furthermore, simulated acid rain promoted the release of  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  in soil and competed with  $\text{Cd}^{2+}$  and  $\text{Pb}^{2+}$  for soil adsorption, resulting in the decrease of the adsorption capacity of soil Cd and Pb [26].

In this study, the application of amendments decreased soil mobility and bioavailability compared to the control treatment. The main reason for this may be attributable to the increase of soil pH, which can promote the formation of hydroxide or carbonate precipitation and increase the negative charges of variably charged colloids, resulting in enhanced adsorption of  $\text{Cd}^{2+}$  and  $\text{Pb}^{2+}$  in soils [40-42]. In addition, hydroxyapatite can immobilize heavy metals by ion

exchange and substitution of Ca by other metals during recrystallization (coprecipitation) [16, 43]. Sepiolite belongs to the aluminosilicate family, and it has a large specific surface area and special pore structure, which can immobilize  $\text{Cd}^{2+}$  and  $\text{Pb}^{2+}$  through mineral surface adsorption, isomorphic substitution, and reaction coordination [44, 45]. Biochar also has a large surface area, microporous structure, abundant functional groups, and some soluble salts, and can reduce the solubility of Cd and Pb through surface adsorption, complexation, and precipitation [42, 46, 47]. Moreover, the application of biochar can increase the SOC content and form organo-metal complexes [48].

The key problem in *in situ* immobilization is whether the immobilization efficiency is stable for a long time [3]. In our study, the remediation efficiency of the three amendments was calculated as below:

$$\text{Immobilized Cd (Pb)(\%)} = \left(1 - \frac{\text{Bioavailable Cd(Pb) in amended soil}}{\text{Bioavailable Cd(Pb) in control soil}}\right) \times 100$$

After a long-term column leaching experiment, the remediation efficiencies of Cd treated by hydroxyapatite, sepiolite, and biochar were 43.55, 25.81, and 33.87%, respectively, in the water treatment. Meanwhile, the remediation efficiencies dropped to 37.97, 21.52, and 26.58%, respectively, in the simulated acid rain treatment. For Pb, the remediation efficiencies of hydroxyapatite, sepiolite, and biochar were 48.65, 29.26, and 22.41%, respectively, in the water treatment, and the values were 36.35, 24.90, and 20.91%, respectively, in the acid rain treatment. The results indicated that the immobilization efficiencies of the three amendments were different, following the order hydroxyapatite>sepiolite>biochar>control. Therefore, the immobilization effects of hydroxyapatite can be expected to be more stable than sepiolite and biochar in a long-term process.

Table 4. Linear analysis between soil pH and available metals.

Heavy metal	Linear regression models	$R^2$ values	$p$ values
Cd	$\text{TCLP}_{-\text{Cd}} = -0.33 \text{ pH} + 2.77$	0.76	<0.001
	$\text{CaCl}_{2-\text{Cd}} = -0.24 \text{ pH} + 2.08$	0.73	<0.001
Pb	$\text{TCLP}_{-\text{Pb}} = -2.96 \text{ pH} + 25.61$	0.83	<0.001
	$\text{CaCl}_{2-\text{Pb}} = -2.35 \text{ pH} + 20.29$	0.82	<0.001



## Conclusions

Simulated acid rain facilitated the conversion of a residual fraction of Cd and Pb to an acid-soluble fraction. Compared to the water group, the percentage of the residual fraction decreased by 5.42~14.16% and 4.41~16.25%, respectively, thus increasing the solubility, mobility and bioavailability of soil Cd and Pb. The application of hydroxyapatite, sepiolite, and biochar effectively reduced leaching and availability of Cd and Pb in both the water and simulated acid rain groups. Hydroxyapatite can transform a greater percentage of the acid-soluble fraction of metals to other more stable fractions, which may improve the immobilization efficiency and maintain stronger and more durable stability than sepiolite and biochar. In particular, hydroxyapatite appears to be the most appropriate amendment to mitigate Cd and Pb leaching. The leachate Cd and Pb concentrations in the hydroxyapatite treatment met the environmental quality standards for groundwater in China ( $Cd \leq 0.01 \text{ mg L}^{-1}$ ,  $Pb \leq 0.1 \text{ mg L}^{-1}$ ) and can eliminate the risk of Cd and Pb leaching into the environment in a short time. Therefore, this study recommends that hydroxyapatite can be used to remediate Cd and Pb-contaminated agricultural soils in the condition of soil acidification.

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## Conflict of Interest

The authors declare no conflict of interest.

## References

- ZHENG X.J., CHEN M., WANG J.F., LIU Y., LIAO Y.Q., LIU Y.C. Assessment of zeolite, biochar, and their combination for stabilization of multi metal-contaminated soil. *ACS Omega*, **5**, 27374, **2020**.
- BIAN R., JOSEPH S., CUI L., PAN G., LI L., LIU X., ZHANG A., RUTLIDGE H. WONG S., CHIA C., MARJO C., GONG B., MUNROE P., DONNE S. A three-year experiment confirms continuous immobilization of cadmium and lead in contaminated paddy field with biochar amendment. *J. Hazard. Mater.*, **272**, 121, **2014**.
- GUO F.Y., DING C.F., ZHOU Z.G., HUANG, G.X., WANG, X.X. Stability of immobilization remediation of several amendments on cadmium contaminated soils as affected by simulated soil acidification. *Ecotoxicol. Environ. Saf.*, **161**, 164, **2018**.
- LIU B., MO C.H., ZHANG Y. Using cadmium bioavailability to simultaneously predict its accumulation in crop grains and the bioaccessibility in soils. *Sci. Total Environ.*, **665**, 246, **2019**.
- BASHIR S., SHAABAN M., HUSSAIN Q., MEHMOOD S., ZHU J., FU Q., AZIZ O., HU H. Influence of organic and inorganic passivators on Cd and Pb stabilization and microbial biomass in a contaminated paddy soil. *J. Soil Sediment.*, **18**, 2948, **2018**.
- HE D., CUI J., GAO M., WANG W., ZHOU J., YANG J., WANG J., LI Y., JIANG C., PENG Y. Effects of soil amendments applied on cadmium availability, soil enzyme activity, and plant uptake in contaminated purple soil. *Sci. Total Environ.*, **654**, 1364, **2019**.
- SELVI A., ARULIAH R. A statistical approach of zinc remediation using acidophilic bacterium via an integrated approach of bioleaching enhanced electrokinetic remediation (BEER) technology. *Chemosphere*, **207**, 753, **2018**.
- MAKINO T., MAEJIMA Y., AKAHANE I., KAMIYA T., TAKANO H., FUJITOMI S., IBARAKI T., KUNHIKRISHNAN A., BOLAN N. A practical soil washing method for use in a Cd-contaminated paddy field, with simple on-site wastewater treatment. *Geoderma*, **270**, 3, **2016**.
- KHALID S., SHAHID M., NIAZI N.K., MURTAZA B., BIBI I., DUMAT C. A comparison of technologies for remediation of heavy metal contaminated soils. *J. Geochem. Explor.*, **182**, 247, **2017**.
- SUN Y., XU Y., XU Y., WANG L., LIANG X., LI Y. Reliability and stability of immobilization remediation of Cd polluted soils using sepiolite under pot and field trials. *Environ. Pollut.*, **208** (Pt B), 739, **2016**.
- RAHMAN Z., SINGH V.P. Bioremediation of toxic heavy metals (THMs) contaminated sites: concepts, applications and challenges. *Environ. Sci. Pollut. Res. Int.*, **27**, 27563, **2020**.
- WEI L., WANG S., ZUO Q., LIANG S., SHEN S., ZHAO C. Nano-hydroxyapatite alleviates the detrimental effects of heavy metals on plant growth and soil microbes in e-waste-contaminated soil. *Environ. Sci. Process Impacts*, **18**, 760, **2016**.
- NONG X., ZHANG C., CHEN H., RONG Q., GAO H., JIN X. Remediation of Cd, Pb and as Co-contaminated Paddy Soil by Applying Different Amendments. *Bull. Environ. Contam. Toxicol.*, **105**, 283, **2020**.
- HUANG G., GAO R., YOU J., ZHU J., FU Q., HU H. Oxalic acid activated phosphate rock and bone meal to immobilize Cu and Pb in mine soils. *Ecotoxicol. Environ. Saf.*, **174**, 401, **2019**.
- ZHANG D., LI T., DING A.F., WU X.X. Effects of an additive (hydroxyapatite-sepiolite-biochar) on Cd and Pb stabilization and microbial community composition in contaminated vegetable soil. *RSC Advances*, **11**, 12200, **2021**.
- YANG Z., LIANG L., YANG W., SHI W., TONG Y., CHAI L., GAO S., LIAO Q.S. Simultaneous immobilization of cadmium and lead in contaminated soils by hybrid bio-nanocomposites of fungal hyphae and nano-hydroxyapatites. *Environ. Sci. Pollut. Res. Int.*, **25**, 11970, **2018**.
- GU J.F., ZHOU H., YANG W.T., PENG P.Q., ZHANG P., ZENG M., LIAO B.H. Effects of an additive (hydroxyapatite-biochar-zeolite) on the chemical speciation of Cd and As in paddy soils and their accumulation and translocation in rice plants. *Environ. Sci. Pollut. Res. Int.*, **25**, 8608, **2018**.
- LIU Y., WANG Y., LU H., LONAPPAN L., BRAR S.K., HE L., CHEN J., YANG S. Biochar application as a soil amendment for decreasing cadmium availability in soil

- and accumulation in *Brassica chinensis*. *J. Soil Sediment.*, **18**, 2511, **2018**.
19. CHENG J., LI Y., GAO W., CHEN Y., PAN W., LEE X., TANG Y. Effects of biochar on Cd and Pb mobility and microbial community composition in a calcareous soil planted with tobacco. *Biol. Fert. Soils*, **54**, 373, **2018**.
  20. LIANG X., XU Y., XU Y., WANG P., WANG L., SUN Y., HUANG Q., HUANG R. Two-year stability of immobilization effect of sepiolite on Cd contaminants in paddy soil. *Environ. Sci. Pollut. Res. Int.*, **23**, 12922, **2016**.
  21. LIANG J., YANG Z.X., TANG L., ZENG G.M., YU M., LI X.D., WU H.P., QIAN Y.Y., LI X.M., LUO Y.A. Changes in heavy metal mobility and availability from contaminated wetland soil remediated with combined biochar-compost. *Chemosphere*, **181**, 281, **2017**.
  22. WEI H., LIU W., ZHANG J., QIN Z. Effects of simulated acid rain on soil fauna community composition and their ecological niches. *Environ. Pollut.*, **220**, 460, **2017**.
  23. ZHANG D., DING A.F. Effects of passivating agents on the availability of Cd and Pb and microbial community function in a contaminated acidic soil. *Bull. Environ. Contam. Toxicol.*, **103**, 98, **2019**.
  24. USEPA. Method 1311: toxicity characteristic leaching procedure. Test methods for evaluating solid waste, physical/chemical methods. Washington, DC: U.S. Environmental Protection Agency, Office of Solid Waste. U.S. Government Printing Office, **1992**.
  25. RIZWAN M.S., IMTIAZ M., CHHAJRO M.A., HUANG G., FU Q., ZHU J., AZIZ O., HU H. Influence of pyrolytic and non-pyrolytic rice and castor straws on the immobilization of Pb and Cu in contaminated soil. *Environ. Technol.*, **37**, 2679, **2016**.
  26. LING D.J., ZHANG J.E., OUYANG Y., HUANG Q.C. Role of simulated acid rain on cations, phosphorus, and organic matter dynamics in Latosol. *Arch. Environ. Con. Tox.*, **52**, 16, **2006**.
  27. CUI H.B., YI Q.T., YANG X., WANG X.M., WU H.J., ZHOU J. Effects of hydroxyapatite on leaching of cadmium and phosphorus and their availability under simulated acid rain. *J. Environ. Chem. Eng.*, **5**, 3773, **2017**.
  28. CUI H.B., ZHANG S., LI R., YI Q., ZHENG X., HU Y., ZHOU J. Leaching of Cu, Cd, Pb, and phosphorus and their availability in the phosphate-amended contaminated soils under simulated acid rain. *Environ. Sci. Pollut. Res.*, **24**, 21128, **2017**.
  29. LI F., LI Z., MAO P., LI Y., LI Y., MCBRIDE M.B., WU J., ZHUANG P. Heavy metal availability, bioaccessibility, and leachability in contaminated soil: effects of pig manure and earthworms. *Environ. Sci. Pollut. Res. Int.*, **26**, 20030, **2019**.
  30. ZHAO C., REN S., ZUO Q., WANG S., ZHOU Y., LIU W., LIANG S. Effect of nanohydroxyapatite on cadmium leaching and environmental risks under simulated acid rain. *Sci. Total. Environ.*, **627**, 553, **2018**.
  31. WU Y.J., ZHOU H., ZOU Z.J., ZHU W., YANG W.T., PENG P.Q., ZENG M., LIAO B.H. A three-year in-situ study on the persistence of a combined amendment (limestone+sepiolite) for remedying paddy soil polluted with heavy metals. *Ecotoxicol. Environ. Saf.*, **130**, 163, **2016**.
  32. YANG X., LIU J., MCGROUTHER K., HUANG H., LU K., GUO X., HE L., LIN X., CHE L., YE Z., WANG H. Effect of biochar on the extractability of heavy metals (Cd, Cu, Pb, and Zn) and enzyme activity in soil. *Environ. Sci. Pollut. Res. Int.*, **23**, 974, **2016**.
  33. HAMID Y., TANG L., HUSSAIN B., USMAN M., GURAJALA H.K., RASHID M.S., HE Z., YANG X. Efficiency of lime, biochar, Fe containing biochar and composite amendments for Cd and Pb immobilization in a co-contaminated alluvial soil. *Environ. Pollut.*, **257**, 113609, **2020**.
  34. ZHANG R.H., LI Z.G., LIU X.D., WANG B.C., ZHOU G.L., HUANG X.X., LIN C.F., WANG A.H., BROOKS M. Immobilization and bioavailability of heavy metals in greenhouse soils amended with rice straw-derived biochar. *Ecol. Eng.*, **98**, 183, **2017**.
  35. TU C., WEI J., GUAN F., LIU Y., SUN Y., LUO Y. Biochar and bacteria inoculated biochar enhanced Cd and Cu immobilization and enzymatic activity in a polluted soil. *Environ. Int.*, **137**, 105576, **2020**.
  36. ZHANG D., DING A.F., LI T., WU X.X., LIU Y., NAIDU R. Immobilization of Cd and Pb in a contaminated acidic soil amended with hydroxyapatite, sepiolite, and biochar. *J. Soil Sediment.*, **21**, 2262, **2021**.
  37. XU P., SUN C.X., YE X.Z., XIAO W.D., ZHANG Q., WANG Q. The effect of biochar and crop straws on heavy metal bioavailability and plant accumulation in a Cd and Pb polluted soil. *Ecotoxicol. Environ. Saf.*, **132**, 94, **2016**.
  38. ASHRAFI M., MOHAMAD S., YUSOFF I., SHAHUL HAMID F. Immobilization of Pb, Cd, and Zn in a contaminated soil using eggshell and banana stem amendments: metal leachability and a sequential extraction study. *Environ. Sci. Pollut. Res.*, **22**, 223, **2014**.
  39. WANG Y., XU Y., LI D., TANG B., MAN S., JIA Y., XU H. Vermicompost and biochar as bio-conditioners to immobilize heavy metal and improve soil fertility on cadmium contaminated soil under acid rain stress. *Sci. Total. Environ.*, **621**, 1057, **2018**.
  40. LI S., SUN X., LI S., LIU Y., MA Q., ZHOU W. Effects of amendments on the bioavailability, transformation and accumulation of heavy metals by pakchoi cabbage in a multi-element contaminated soil. *RSC Advances*, **11**, 4395, **2021**.
  41. TIAN H., WANG Y., XIE J., LI H., ZHU Y. Effects of Soil Properties and Land Use Types on the Bioaccessibility of Cd, Pb, Cr, and Cu in Dongguan City, China. *Bull. Environ. Contam. Toxicol.*, **104**, 64, **2020**.
  42. QUAN G., FAN Q., SUN J., CUI L., WANG H., GAO B., YAN J. Characteristics of organo-mineral complexes in contaminated soils with long-term biochar application. *J. Hazard. Mater.*, **384**, 121265, **2020**.
  43. WANG Y., LI R., LIU W., CHENG L., JIANG Q., ZHANG Y. Exploratory of immobilization remediation of hydroxyapatite (HAP) on lead-contaminated soils. *Environ. Sci. Pollut. Res. Int.*, **26**, 26674, **2019**.
  44. HAMIDPOUR M., AFYUNI M., KALBASI M., KHOSHGOFTARMANES A.H., INGLEZAKIS V.J. Mobility and plant-availability of Cd(II) and Pb(II) adsorbed on zeolite and sepiolite. *Appl. Clay Sci.*, **48**, 342, **2010**.
  45. SUN Y., SUN G., XU Y., LIU W., LIANG X., WANG L. Evaluation of the effectiveness of sepiolite, sepiolite, and phosphate amendments on the stabilization remediation of cadmium-contaminated soils. *J. Environ. Manage.*, **166**, 204, **2016**.
  46. TAN Z., YUAN S., HONG M., ZHANG L., HUANG Q. Mechanism of negative surface charge formation on

- biochar and its effect on the fixation of soil Cd. *J. Hazard. Mater.*, **384**, 121370, **2020**.
47. JING F., CHEN X., WEN X., LIU W., HU S., YANG Z., GUO B., LUO Y., YU Q., XU Y. Biochar effects on soil chemical properties and mobilization of cadmium (Cd) and lead (Pb) in paddy soil. *Soil Use Manage.*, **36**, 320, **2020**.
48. WU J., LI Z., HUANG D., LIU X., TANG C., PARIKH S. J., XU J. A novel calcium-based magnetic biochar is effective in stabilization of arsenic and cadmium co-contamination in aerobic soils. *J. Hazard. Mater.*, **387**, 122010, **2020**.

