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Effects of Phosphate, Red Mud, and Biochar on As, Cd, and Cu Immobilization and Enzymatic Activity in a Co-Contaminated Soil

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Abstract: Arsenic (As), cadmium (Cd), and copper (Cu) are the primary inorganic pollutants commonly found in contaminated soils. The simultaneous stabilization of the three elements is a preferred approach for mixture-contaminated soils which has received extensive research attention. However, few studies have focused on the immobilization efficiency of a single amendment on the three elements. In this study, phosphate, red mud, and biochar were used to remediate As (237.8 mg kg⁻¹), Cd (28.72 mg kg⁻¹), and Cu (366.5 mg kg⁻¹) co-contaminated soil using a 180-day incubation study. The BCR (European Community Bureau of Reference) extraction method, NH₄H₂PO₄-extractable As, and diethylenetriamine penta-acetic acid (DTPA)-extractable Cd and Cu were analyzed at different time intervals. The results indicated that the application of red mud and biochar significantly reduced soil DTPA-Cd and Cu concentrations during the incubation, while the decrease in soil NH₄H₂PO₄-As was much less than that of soil DTPA-Cd and Cu. After 180 days of incubation, the concentrations of NH₄H₂PO₄-As in red mud and biochar treatments decreased by 2.15~7.89% and 3.01~9.63%, respectively. Unlike red mud and biochar, phosphate significantly reduced the concentration of soil DTPA-Cd and Cu, but failed to lower that of As. The BCR extraction method confirmed that red mud and biochar addition increased the reducible fraction of As due to the surface complexes of As with Fe oxide. Canonical correspondence analysis (CCA) demonstrated that soil pH in addition to available As, Cd, and Cu concentrations were the primary factors in driving the changes in soil enzymatic activity. Soil pH showed positive correlation with soil urease and catalase activities, while negative correlation was observed between soil-available As, Cd, and Cu, and soil enzyme activities. This study revealed that it is difficult to simultaneously and significantly reduce the bioavailabilities of soil As, Cd, and Cu using one amendment. Further research on modifying these amendments or applying combined amendments will be conducted, in order to develop an efficient method for simultaneously immobilizing As, Cd, and Cu.

Keywords: amendments; heavy metals; immobilization; bioavailability; enzyme activity



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1. Introduction

Soil heavy metal pollution has long been a severe environmental problem of widespread concern because of these metals' high toxicity and non-biodegradable properties [1]. Once heavy metals contaminate the soil, they will exist in the soil for a long time unless treated or transported. Long-term exposure to heavy metals in soil may result in severe negative effects to kidneys, skin, bones, and affect cardiovascular function [2,3]. According to the Chinese Ministry of Environmental Protection and Ministry of Land and Resources, soil

cadmium (Cd), nickel (Ni), arsenic (As), and copper (Cu) rank in the top four inorganic pollutants, with 7.0%, 4.8%, 2.7%, and 2.1% soils identified as contaminated, respectively. High contents of As, Cd, and Cu in agricultural soils have been identified in many countries [4]. Therefore, there is an urgent demand and a great challenge to develop effective treatments and remediation methods for soil heavy metal pollution.

There are currently two main strategies to remediate heavy metal polluted soils: to eliminate heavy metals from the contaminated soil, e.g., phytoremediation and chemical washing [1]; and to reduce mobility and availability of heavy metals by altering the chemical states of soil heavy metals, e.g., chemical stabilization technology [5,6]. In situ immobilization is generally considered a feasible technology since it is a sustainable, highly efficient, and cost-effective method to manage soil heavy metal contamination [1,7]. However, selecting suitable amendments has been challenging, as they may alter soil properties and soil quality [8]. Tang et al. [9] reported that biochar, compost, and their combinations all significantly improve soil pH and organic carbon in heavy metal-polluted soil. Jiang et al. [10] also demonstrated that the application of calcium and iron-combined amendment increased soil urease and catalase activities respectively by 15.8% and 25.9% in Cd and As co-contaminated soils. Thus far, numerous chemical and biological materials have been successfully applied in order to decrease soil heavy metals contamination, including phosphatic compounds, clay minerals, red mud, biochar, and so on.

Phosphate is a cost-effective amendment that can stabilize soil heavy metals by transforming bioavailable heavy metal fractions into more stable forms [11]. The possible reaction mechanisms include precipitation and ion exchange processes [12]. Red mud is a highly alkaline, ultrafine-grained waste material generated from the aluminum industry in large quantities, which has been intensively investigated for its stabilization of heavy metals in the soil [13,14]. It is highly alkaline with various iron-aluminum oxides, silicates, and active functional groups, resulting in beneficial immobilization capabilities [15]. Numerous studies have indicated that red mud as an ameliorator has a strong adsorption capacity to immobilize heavy metals. For example, Zhou et al. [1] found that red mud addition increased soil pH and decreased the availability of soil Cu and Cd. Hua et al. [16] and Pavel et al. [17] confirmed that red mud application could reduce the Cd bioavailability by ions adsorption, and facilitate the transformation of the soluble fraction to Fe-Mn oxide-associated fractions. Biochar, as a carbon-rich solid product derived from agroforestry biomass waste, is produced by thermal decomposition of organic materials under conditions of anoxia or limited oxygen supply [18]. Biochar as a stabilization material to control heavy metal contamination, has been researched for a long time. Luo et al. [3] indicated that the concentration of leachable As in biochar-treated soil was reduced by 14.77% compared to the control treatment. Wu et al. [19] observed that the calcium-based biochar reduced the unstable fraction of As, resulting in a decrease in soil bioavailability of As. Biochar mainly reduces the bioavailability of soil heavy metals through adsorption, since it has a large specific surface area, high porosity, and active surface functional groups [20].

Previous studies have evaluated the effectiveness of phosphate, red mud, and biochar to immobilize soil heavy metals [1,13,15]. However, the majority of these studies have focused on soils contaminated by a single heavy metal. Anthropogenic activities such as smelting and mining are the main sources of heavy metal pollution [19]. As, Cd, and Cu can co-exist in soil and lead to co-contaminated pollution. It is crucial to study whether and how the three amendments perform for the simultaneous immobilization of As, Cd, and Cu co-contamination in soil. Moreover, there is also limited knowledge of the dynamic changes in soil physicochemical properties and enzymatic activity during the remediation of the three amendments on As, Cd, and Cu co-contaminated soil.

In this study, a 180-day incubation experiment was conducted to elucidate the hypothesis that phosphate, red mud, and biochar at different rates can effectively immobilize soil As, Cd, and Cu, and will reduce the phytoavailability of heavy metals in the co-contaminated soil. The dynamic impacts on soil pH, dissolved organic carbon (DOC), soil As, Cd, and Cu bioavailability, in addition to the dynamic changes of soil enzymatic activity, were

examined over the incubation time. The key soil environmental factors in determining soil enzymatic activities were explored. The results of this study provide valuable insights to evaluate the effect of phosphate, red mud, and biochar on the immobilization of As, Cd, and Cu co-contamination in soil.

2. Materials and Methods

2.1. Soil and Amendments

The soil samples were collected from the surface layer (0~20 cm) of farmland near a smelter in Dazu District, Chongqing, southwest China. The soil was air-dried and ground to sieve through a 2-mm mesh, in order to analyze its basic physicochemical properties. The soil pH, cation exchange capacity (CEC), and DOC were 9.52, 15.09 $\text{cmol}_{(+)}\text{kg}^{-1}$, and 25.63 mg L^{-1} , respectively. The content of sand, silt, and clay particles was 69.25%, 18.72%, and 12.03%, respectively. The soil was co-contaminated by As, Cd, and Cu, with total concentrations of 237.8, 28.72, and 366.5 mg kg^{-1} , respectively. The concentrations of As and Cd exceeded the risk intervention values of agricultural land according to the Environmental Quality Standard for Soils of China (GB15618-2018, 100 mg kg^{-1} for As and 4.0 mg kg^{-1} for Cd).

Three amendments (phosphate, red mud, and biochar) were used in this study. Phosphate (KH_2PO_4) was purchased from Sigma-Aldrich Chemical Reagent Co., Ltd. (Burlington, MA, USA). Red mud was collected from Rio Tinto Alcan Yarwun refinery, Australia. The detailed mineralogical composition of red mud was given in the previous study [21]. Biochar was made from wood shavings under oxygen-limited conditions at 650 °C for 4 h. Red mud and biochar were sieved through a 2-mm mesh for use. The detailed physical and chemical properties of the three amendments were presented in Table 1.

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

	pH (1:5)	Surface Area ($\text{m}^2 \text{g}^{-1}$)	Organic Carbon (g kg^{-1})	Total As (mg kg^{-1})	Total Cd (mg kg^{-1})	Total Cu (mg kg^{-1})
Phosphate	4.62	–	–	ND	ND	ND
Red mud	10.48	24.3	5.17	1.66	0.05	2.13
Biochar	8.81	1.58	335.5	0.07	ND	0.12

ND: Not detected.

2.2. Experimental Design

2.2.1. Soil Incubation Experiment

The incubation experiment was carried out in a constant temperature chamber at 25 °C for 180 days. Soil samples (100 g) were placed in a plastic box, and then the phosphate, red mud, and biochar were added into the soil at ratios of 2%, 5%, and 10% (w/w), namely, 2P, 2R, 2B, 5P, 5R, 5B, 10P, 10R, 10B, respectively. The treatment without the addition of an amendment was named CK. Each treatment was performed in triplicate. During the incubation time, deionized water ($18.2 \text{ M}\Omega \text{ cm}^{-1}$, Milli-Q, Merck Millipore, Burlington, MA, USA) was added to the soil every three days, in order to maintain the moisture content at 40% of field capacity. The soil sample was collected on days 1, 5, 10, 14, 28, 56, 90, and 180, in order to analyze the dynamic changes in soil physicochemical characteristics, heavy metal contents, and enzymatic activity.

2.2.2. Soil Chemical Analysis

Soil pH was measured by pH meter (Orion Star™ A211, Thermo Fisher Scientific, Waltham, MA, USA) using a 1:2.5 (w/v) soil to water ratio. DOC was measured by TOC analyzer (Multi N/C 3100, Analytik Jena, Jena, Germany).

Soil-available As was extracted using 0.01 mol L^{-1} CaCl_2 and 0.05 mol L^{-1} $\text{NH}_4\text{H}_2\text{PO}_4$ [3]. Briefly, 1.0 g soil and 40 mL of 0.01 mol L^{-1} CaCl_2 solution were shaken for 2 h and

centrifuged at 4000 rpm for 20 min. Then, the residual was extracted with 25 mL of $0.05 \text{ mol L}^{-1} \text{ NH}_4\text{H}_2\text{PO}_4$, shaken for 16 h, centrifuged, and filtered for As analysis.

The availability of soil Cd and Pb was extracted using diethylenetriamine penta-acetic acid (DTPA) extractant solution [22]. Then, 5 g of soil was added into the extractant solution of 25 mL, which was mixed with 0.005 mol L^{-1} DTPA, 0.1 mol L^{-1} triethanolamine, and $0.1 \text{ mol L}^{-1} \text{ CaCl}_2$, and adjusted to pH 7.3 using HCl. The suspensions were shaken on an end-over-end oscillator at 180 rpm at 25°C for 2 h, then centrifuged and filtered for Cd and Cu analysis.

Soil As, Cd, and Cu were extracted with BCR (European Community Bureau of Reference) extraction method after 180 days of incubation [23]. Four different fractions, including acid-soluble, reducible, oxidizable, and residual fractions, were extracted using the following procedure. Firstly, 1.0 g soil was extracted with 40 mL of 0.11 mol L^{-1} acetic acid (HOAc) for 16 h, and centrifuged at 4000 rpm for 20 min. Secondly, 40 mL of 0.1 mol L^{-1} hydroxylamine hydrochloride ($\text{NH}_2\text{OH}\cdot\text{HCl}$, acidified at pH 2) was added to the residual soil of step 1, shaken for 16 h, and then centrifuged. Thirdly, a water bath (85°C) was conducted after adding 30% (*m/v*) H_2O_2 (10 mL) to the residual soil of step 2 until the volume of H_2O_2 was less than 3 mL. A similar procedure was repeated. Then, 50 mL of 1 mol L^{-1} of ammonium acetate (NH_4OAc) at pH 2 was added to the residual, which was shaken for 16 h and centrifuged. Lastly, air-dried residual soil from step 3 was digested using aqua regia in a microwave digester (CEM, MARS6, Matthews, NC, USA).

The As, Cd, and Cu concentrations in the filtrates of the previous extractions mentioned above and their sequential extractions were measured by inductively coupled plasma mass spectrometry (ICP-MS) analysis (Agilent 7500, Agilent, Santa Clara, CA, USA). The recovery rates of soil As, Cd, and Cu ranged from 92% to 107%.

2.2.3. Soil Enzymatic Activity Analysis

Soil urease activity was conducted using the sodium phenate-sodium hypochlorite colorimetry assay, and was expressed as $\text{mg NH}_4^+-\text{N g}^{-1} \text{ soil } 24 \text{ h}^{-1}$ [24]. Soil catalase activity was determined using the permanganometric assay with hydrogen peroxide, and was described as $\text{mL } (0.1 \text{ mol L}^{-1} \text{ KMnO}_4) \text{ h}^{-1} \text{ g}^{-1}$. Blank matrix control was set for each treatment, and blank samples and matrix control were established throughout the experiment [25].

2.3. Statistical Analysis

All the data, including soil pH, DOC, soil available, and bioaccessible As, Cd, and Cu, were recorded as the means with standard deviations (SD). Statistical analysis was conducted using SPSS 20.0 software (IBM, New York, NY, USA). The effects of three amendments on soil enzymatic activity and available heavy metals were analyzed using a one-way ANOVA at a significance level of 0.05. The possible correlations among the soil enzyme activity and soil physicochemical properties were conducted using canonical correspondence analysis (CCA) (CANOCO 4.5).

3. Results and Discussion

3.1. Effects of Three Amendments on Soil pH and DOC during the Incubation

The soil pH in all treatments increased initially, reached a peak on day 14 or 28, and then stabilized with incubation time (Figure 1a). Moreover, soil pH increased with the increasing application rate of amendments for red mud and biochar. Compared to the control treatment (CK), the application of red mud increased soil pH by 0.17–1.12 units during the incubation, especially, with the addition amounts of 5% (5R) and 10% (10R). The increase of soil pH may be ascribed to the high alkalinity of red mud (pH 10.48) and a large number of OH^- ions released from the surface of red mud particles [26]. Besides, red mud contains abundant SiO_2 (13.88%), TiO_2 (6.84%), and Na_2O (9.41%) [21], which play an essential part in increasing soil pH. Phosphate addition decreased soil pH by 0.01–0.89 units compared to the control. This may be related to the exchange of K^+

produced by the ionization of phosphate (KH_2PO_4) and H^+ on the surface of soil particles, which resulted in H^+ entering the soil solution and reducing soil pH.

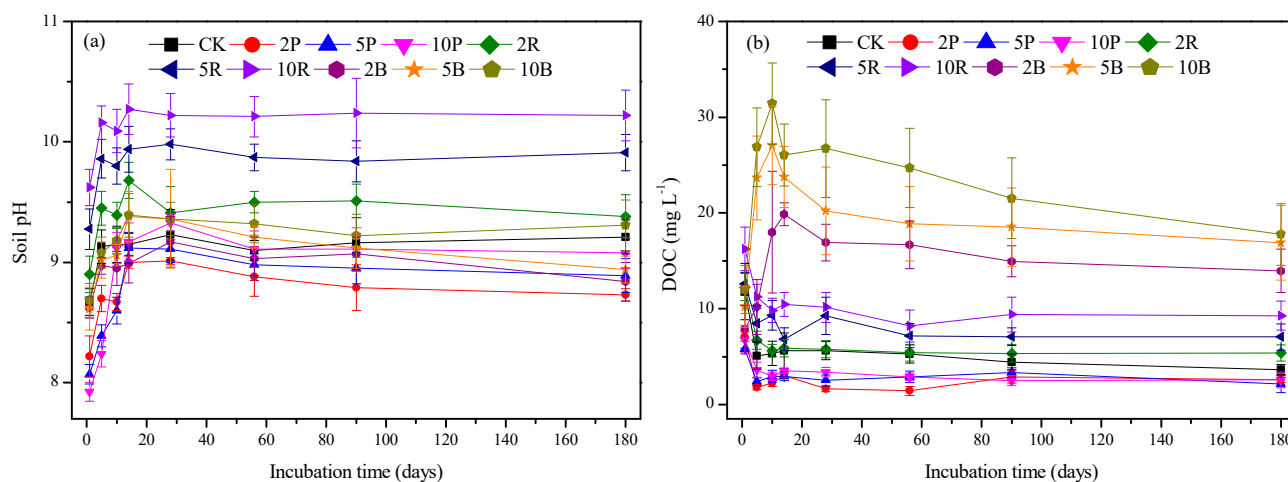


Figure 1. Changes in soil pH (a) and DOC concentration (b) over 180 days incubation with phosphate, red mud, and biochar (CK: control; 2P: 2% phosphate; 5P: 5% phosphate; 10P: 10% phosphate; 2R: 2% red mud; 5R: 5% red mud; 10R: 10% red mud; 2B: 2% biochar; 5B: 5% biochar; 10B: 10% biochar). Error bars represent \pm standard error.

The DOC concentrations initially increased and peaked on day 10 or 14, then showed a stable trend in biochar treatments (Figure 1b). Soil DOC concentrations maintained the highest level of all the treatments during the incubation. Compared to the control, the application of biochar significantly increased the DOC concentrations, and the increment was 0.52–7.41 times. This may be caused by the high input of organic C by biochar. For both phosphate and red mud treatments, soil DOC concentrations dramatically declined on day 5 and stabilized after 14 days. The decrement may be attributed to the mineralization of soil organic carbon by microorganisms [27].

3.2. Effects of Three Amendments on Chemical Fractions of Soil As, Cd, and Cu

The speciation of soil As, Cd, and Cu was dramatically affected by applying the three amendments (Figure 2). In the original soil, the As concentrations of the four fractions were in the order of reducible fraction > acid soluble fraction > residual fraction > oxidizable fraction (Figure 2a). After 180 days of incubation, the proportion of residual fraction of As in phosphate, red mud, and biochar treatments decreased by varying degrees, indicating that the residual fraction of As transformed into other fractions. The proportion of acid-soluble fraction in phosphate, red mud, and biochar treatments decreased by 18.46–24.48%, 11.84–18.22%, and 2.62–13.28%, respectively, compared to the control treatment.

Unlike As, the primary Cd fraction in the initial soil was acid-soluble fraction (60.50%), followed by the reducible fraction (19.83%). The oxidizable and residual fraction of Cd was 13.41% and 6.26%, respectively (Figure 2b). The application of amendments promoted the transformation of acid-soluble fraction of Cd to other fractions. Compared to the control treatment, the acid-soluble fraction of Cd in 5P, 5R, and 5B treatments decreased by 22.96%, 45.91%, and 40.33%, respectively. The residual fraction of Cd increased by 1.32, 2.94, and 2.59 times, respectively. No significant difference was observed for the proportions of reducible and oxidizable Cd with increasing amendment quantity.

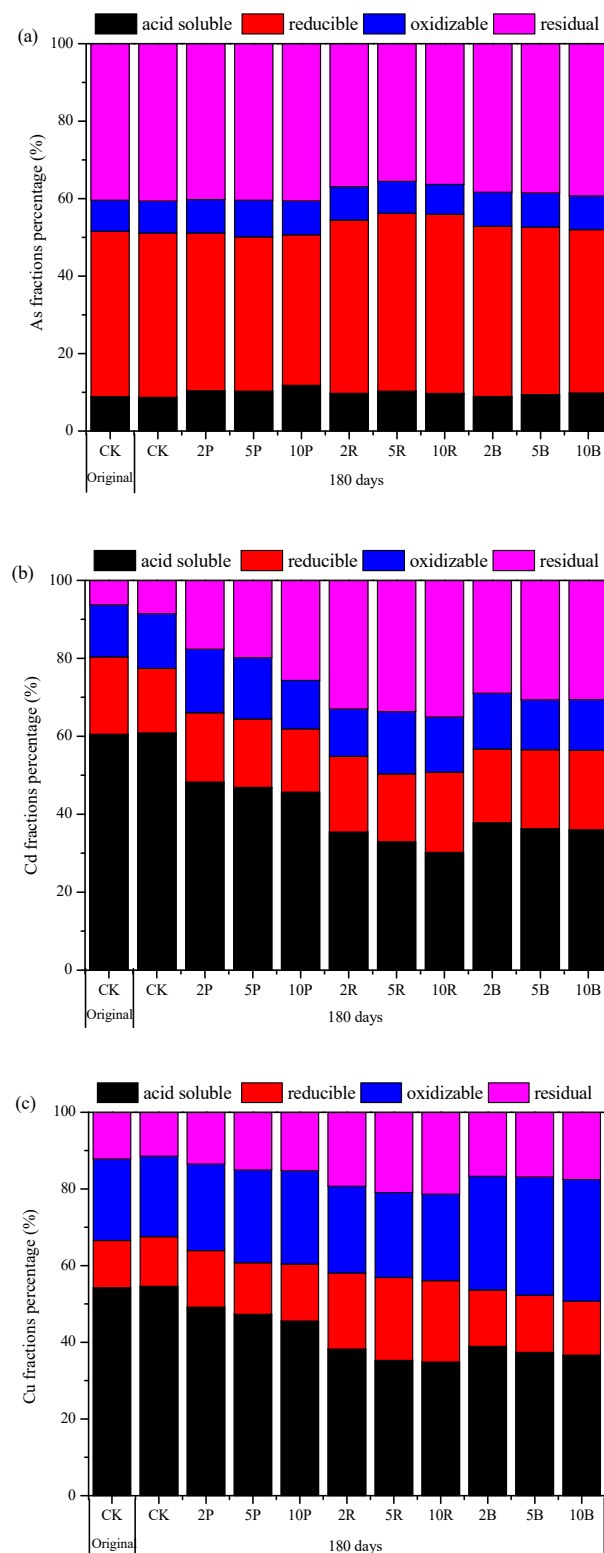


Figure 2. Proportions of different As (a), Cd (b), and Cu (c) fractions after 180 days of incubation (CK: control; 2P: 2% phosphate; 5P: 5% phosphate; 10P: 10% phosphate; 2R: 2% red mud; 5R: 5% red mud; 10R: 10% red mud; 2B: 2% biochar; 5B: 5% biochar; 10B: 10% biochar).

As shown in Figure 2c, the addition of amendments dramatically decreased the acid-soluble Cu fraction, and increased the residual Cu fraction. Compared to the control treatment, the application rate of phosphate at 2% (2P), 5% (5P), and 10% (10P) decreased

the acid-soluble fraction of Cu by 9.95%, 13.44%, and 16.62%, respectively. Similar to the phosphate, the increasing application rate significantly reduced the acid-soluble fraction of Cu from 30.04% to 36.15% in red mud treatments, and from 28.70% to 32.87% in biochar treatments. For the residual fraction, a significant increase was found in phosphate, red mud, and biochar treatments, with a rise of 17.91~33.43%, 68.59~86.49%, and 45.89~53.26%, respectively.

The acid-soluble fraction is usually regarded as the labile fraction with high mobility and bioavailability. The evaluation of the effectiveness in situ immobilization is often based on the concentrations of the labile fraction [2]. Recently, studies demonstrated that phosphorus (P) was the strongest competitor for As, since P and As have similar physicochemical properties [28,29]. The application of soluble phosphate amendment may lead to the release of oxyanions of As as a result of the competitive substitution of phosphate in soil exchange sites [12]. This may be the reason for the increase in acid-soluble As fraction in the soil after applying phosphate. In this study, phosphate addition was beneficial to the Cd and Cu transformation from acid-soluble fraction to the residual fraction. The possible mechanisms may be the formation of Cd- and Cu-containing phosphates, such as $\text{Cd}(\text{PO}_3)_2$, $\text{Cd}_{1.5}\text{Ca}_{1.5}(\text{PO}_4)_2$, and $\text{Cu}_{3.04}(\text{PO}_4)_2\text{OH}_{0.08}\cdot 2\text{H}_2\text{O}$ [30].

Red mud application observably transformed the residual fraction of As into acid-soluble and reducible fractions in the present study (Figure 2). The main reason was that red mud application significantly increased soil pH and promoted the release of HAsO_4^{2-} and AsO_4^{3-} . Moreover, red mud is rich in Fe_2O_3 (34.48%) and Al_2O_3 (22.53%) [21], and has the potential to raise the number of soil oxides, leading to an increase in Fe–As complexation. A previous study also proved that As was mainly chelated and distributed on Fe oxide by μ -XRF [19]. In addition, red mud application promoted both Cd and Cu hydroxides and carbonates at high pH and increased heavy metals adsorption capacity. A similar result was found by Xu et al. [31]. Pavel et al. [17] reported that red mud addition significantly decreased the proportion of mobile fraction Cd from 76.6% to 12.6%, and the fraction bound to crystalline Fe oxides increased from 3.8% to 7.1%.

In this study, biochar induced the transformation of Cd and Cu from acid-soluble fractions to residual fractions. Nie et al. [32] and Tu et al. [24] indicated that biochar addition facilitated the labile fractions of Cd and Cu to recalcitrant fractions, such as carbonate and organically bound species. Unlike As and Cd, biochar addition significantly increased the proportion of oxidizable Cu. This may be ascribed to the notable increase in soil DOC concentrations (Figure 1). The result was in good agreement with previous studies [33,34]. Some researchers demonstrated that soil organic matter plays an essential part in soil Cu speciation, resulting in the combination of Cu and organic matter [35].

3.3. Effects of Three Amendments on Phytoavailability of Soil As, Cd, and Cu

The concentration of $\text{NH}_4\text{H}_2\text{PO}_4$ -extractable As ($\text{NH}_4\text{H}_2\text{PO}_4$ -As) increased initially, reached a peak on day 10, then decreased after that. Compared to the control, adding phosphate, red mud, and biochar dramatically increased the concentration of $\text{NH}_4\text{H}_2\text{PO}_4$ -As ($p < 0.05$) on day 10. The increasing application rate of amendments had little effect on $\text{NH}_4\text{H}_2\text{PO}_4$ -As during the incubation (Figure 3a). After 180 days of incubation, the concentration of $\text{NH}_4\text{H}_2\text{PO}_4$ -As in red mud and biochar treatments decreased by 2.15~7.89% and 3.01~9.63%, while applying phosphate increased soil $\text{NH}_4\text{H}_2\text{PO}_4$ -As concentration by 1.01~5.82%.

During the incubation, DTPA-extractable Cd (DTPA-Cd) declined gradually with increasing incubation time (Figure 3b). Phosphate additions dramatically reduced the concentration of DTPA-Cd by 41.83~53.34% compared to the control. However, the increasing amounts of phosphate hardly affected the concentration of DTPA-Cd. Unlike phosphate, the application rate of red mud showed a marked impact on the concentration of DTPA-Cd from day 28 to day 180. DTPA-extractable Cd concentration was decreased by 71.09% for 2R, 73.69% for 5R, and 82.71% for 10R compared to the control on day 180. Biochar addition slightly reduced the concentration of DTPA-Cd with the incubation time, and the addition

ratio had no significant effect on the concentration of DTPA–Cd. Compared to the control, biochar decreased the concentration of DTPA–Cd by 71.60~78.85% during the incubation.

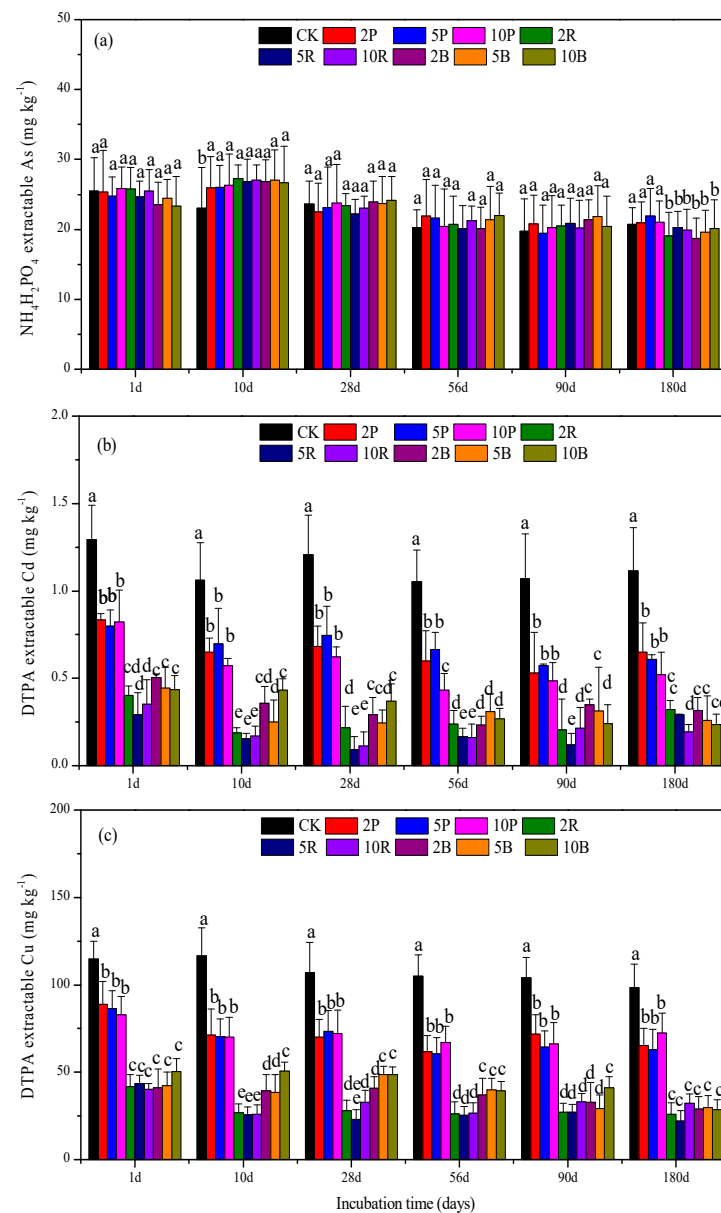


Figure 3. Changes in the concentrations of $\text{NH}_4\text{H}_2\text{PO}_4$ -extractable As (a), DTPA-extractable Cd (b), and DTPA-extractable Cu (c) over 180 days with phosphate, red mud, and biochar (CK: control; 2P: 2% phosphate; 5P: 5% phosphate; 10P: 10% phosphate; 2R: 2% red mud; 5R: 5% red mud; 10R: 10% red mud; 2B: 2% biochar; 5B: 5% biochar; 10B: 10% biochar). Error bars represent \pm standard error. Lowercase letters above the error bars indicate significant differences among different treatments at $p < 0.05$ level.

As shown in Figure 3c, compared to the control, adding phosphate, red mud, and biochar dramatically decreased the concentration of DTPA-extractable Cu (DTPA–Cu) by 22.43~41.19%, 62.16~78.48%, and 54.22~71.89%, respectively (Figure 3c). For both phosphate and red mud, the increasing application rate hardly affected the concentration of DTPA–Cu. However, the rising application rate of biochar significantly increased the concentration of DTPA–Cu on day 10 and day 28. This phenomenon may be attributed to the increasing concentration in DOC due to biochar application. High concentrations of DOC may chelate Cu and increase mobility and phytoavailability [24].

The application of phosphate enhanced the concentration of soil-bioavailable As, especially on days 10 and 56. This may be attributed to the competition of P and As for soil non-specific sorption sites due to their similar adsorption mechanisms, resulting in the release of soil-absorbed As [36]. Deng et al. [37] reported that phosphate strongly inhibited both As (III) and As (V) adsorption in goethite, and Pincus et al. [38] also demonstrated that phosphate (H_2PO_4^-) was the strongest adsorptive competitor with As, resulting in the reduction of As removal efficiency. In addition, high DOC concentrations can result in higher bioavailability of As by inhibiting secondary Fe (III) hydroxide precipitation and As adsorption onto it [39].

Soil pH plays a crucial role in controlling the mobility and phytoavailability of heavy metals. An increase in soil pH is beneficial to the immobilization of Cd and Cu, due to the formation of more stable and nonbioavailable forms (i.e., hydroxide, carbonate, or phosphate precipitates), especially $\text{pH} > 8$ [8,19]. Besides, more negatively-charged adsorption sites increased attraction for Cd and Cu. In this study, the soil amended with red mud had the highest pH to cause precipitation and to co-precipitate with Fe, Al, and Ca compounds in red mud [31]. The addition of biochar exhibited significant effects on Cd and Cu stabilization, mainly caused by the complexation with hydroxyl, carboxyl, and phenolic groups [24]. Moreover, surface complexation and cation exchange with mineral components in biochar can be conducive to the adsorption of Cd and Cu [35].

Unlike Cd and Cu, As becomes more available as soil pH increases. The increase in the negative charge resulted in a release of AsO_4^{3-} from their exchange positions, and enhanced repulsive forces against soil-absorbed AsO_4^{3-} [36]. Moreover, the competition for the surface sites between As ions and OH^- may also contribute to the reduced sorption with increasing pH [40]. Therefore, an increase of soil-bioavailable As was observed in red mud treatments at the outset of the incubation. With increased incubation time, red mud can fix As in lattices of iron-aluminum minerals to form stable compounds, resulting in a decrease in bioavailability of As [41]. Besides, As can be adsorbed and oxidized on the surfaces of hematite and goethite in red mud. Using transmission electron microscopy and elemental mapping, Wang et al. [30] also demonstrated the formation of precipitation on crystalline Ca-As and amorphous Fe-As compounds. DOC can compete with As and inhibit the adsorption of As on soil particles. Therefore, the increase in DOC concentrations results in an increase in soil-bioavailable As. This may be the reason for the increment in phytoavailability of soil As with biochar application on day 10. Moreover, soil-bioavailable As in biochar treatments decreased gradually with increased incubation time, indicating that other factors may affect the immobilization process. Previous studies demonstrated that high pH, in the presence of sulfate and carbonates, can produce co-precipitation of As in the subsequent formation of oxyhydroxides and sulfates [42].

The key problem in in situ chemical immobilization is whether the immobilization efficiency is stable for a long time. In this study, the remediation efficiency of the three amendments after 180 days of incubation was calculated as below:

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

The results from our study confirmed that the highest remediation efficiencies of As, Cd, and Cu treated by 2% addition of red mud and biochar were 7.89~9.63%, 71.09~71.60%, and 70.72~73.54%, respectively. This result showed that red mud and biochar could be efficient amendments for the immobilization of As, Cd, and Cu in the tested co-contaminated soil, even if the reduction of soil-phytoavailable As was much less than that of soil-phytoavailable Cd and Cu. Due to the different properties of As, Cd, and Cu, the availability of soil heavy metals cannot be controlled only by changing the physico-chemical properties of the soil. It is necessary to modify the amendment materials, for example, combining Fe oxide with biochar, in order to develop novel biochar to enhance the adsorption capacity for As.

3.4. Effects of Three Amendments on Soil Enzymatic Activity

Soil enzymatic activities have been suggested as sensitive indicators of soil quality, and have been widely used to evaluate the effects of soil amendments on biological functions [24]. The dynamic changes of soil urease and catalase activities with phosphate, red mud, and biochar during the incubation of 180 days are shown in Figure 4. Soil urease activity gradually decreased following incubation for 28 days, and then elevated with the increased incubation time (Figure 4a). The reduction in enzymatic activity during the initial period may be caused by the direct disturbance of amendments addition, resulting in harm to microorganism and enzyme production [9]. With increasing incubation time, the application of amendments can decrease soil-available heavy metal concentrations, which provides more substrates for soil enzymes and promotes increases in soil enzyme activity [43]. At the end of the incubation, urease activity in biochar treatments was higher than those in phosphate and red mud treatments. A possible mechanism for this may be that biochar contains an excellent pore structure that efficiently stores and supplies good moisture, dissolved substances, and hydrothermal conditions for microorganisms [44]. Soil catalase activity with phosphate addition first sharply decreased and lowered on day 28, then gradually elevated until the end of the incubation. However, no significant change was observed in red mud and biochar treatments over time (Figure 4b). Compared to the red mud and biochar treatments, the application of phosphate significantly decreased ($p < 0.05$) soil catalase activity during the incubation. The results may be ascribed to the higher heavy metal concentrations in phosphate treatments. Heavy metals can coordinate with the active site of the enzyme, compete with the substrate, inhibit the growth of soil microorganisms, and reduce the activity of soil enzymes. Besides, the decrease in soil pH for phosphate addition treatments may also contribute to the reduction in catalase activity.

Soil enzymatic activity can be restricted by many environmental factors. In this study, CCA was performed to determine the effects of soil pH, DOC, $\text{NH}_4\text{H}_2\text{PO}_4\text{-As}$, DTPA-Cd , DTPA-Cu , the application rate of amendment, and incubation time, on the activities of urease and catalase (Figure 5). CCA axis 1 explained 43.9% of the activity of soil urease and 51.2% of the activity of soil catalase. CCA results further demonstrated that the effects of these environmental factors on soil enzyme activities follows the order of $\text{pH} > \text{DTPA-Cd} > \text{DTPA-Cu} > \text{NH}_4\text{H}_2\text{PO}_4\text{-As} > \text{application rate of amendments} > \text{DOC}$. Soil pH and available heavy metal concentrations were the primary factors in driving the changes of soil enzymatic activity. Soil pH and DOC had positive correlations with urease and catalase activities, while soil-available heavy metals had a negative correlation with soil enzyme activities. This result further proved that the decrease in soil enzyme activities caused by phosphate addition may be due to the decrease in soil pH. Similar results were also found by Jiang et al. [10] and Tang et al. [45], who reported that the reduction in soil enzyme activities was related to the decrease in soil pH and the increase in available heavy metals.

3.5. Issues with Real-Scale Application

In situ chemical immobilization technology is usually used to manage soil heavy metal contamination by reducing mobility and availability of heavy metals, in order to alleviate the accumulation of heavy metals in plants. However, stable heavy metals may return to a mobilized phase as a result of uncontrolled climate or weather changes [46]. Although the utilized sophisticated laboratory technology successfully separated clean soil medium with heavy metal from the stable heavy metal itself, it is hard to separate the immobilized metal from the medium in real-scale applications. Low efficiency is found under real-scale conditions due to the limitation of equipment and energy consumption [47]. Moreover, this study was conducted on a laboratory scale using an incubation experiment. The experimental conditions were artificially controlled, and the results cannot be directly applied to real-scale environments. Long-term field experiments are needed in order to confirm the results of this study.

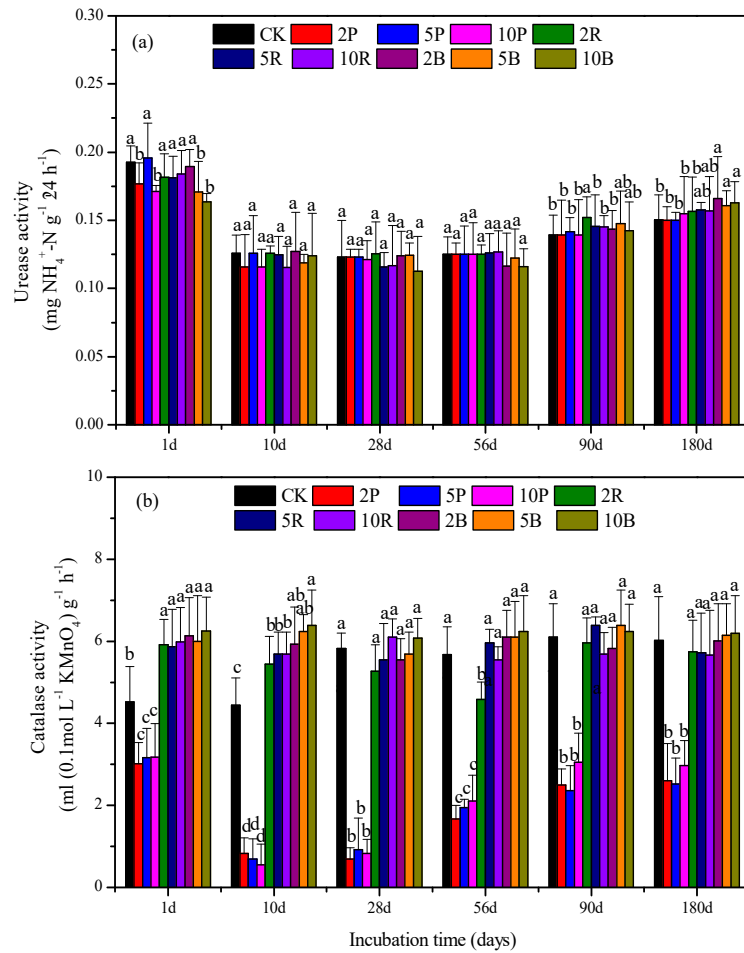


Figure 4. Changes in the activities of urease (a) and catalase (b) over 180 days with phosphate, red mud, and biochar (CK: control; 2P: 2% phosphate; 5P: 5% phosphate; 10P: 10% phosphate; 2R: 2% red mud; 5R: 5% red mud; 10R: 10% red mud; 2B: 2% biochar; 5B: 5% biochar; 10B: 10% biochar). Error bars represent \pm standard error. Lowercase letters above the error bars indicate significant differences among different treatments at the $p < 0.05$ level.

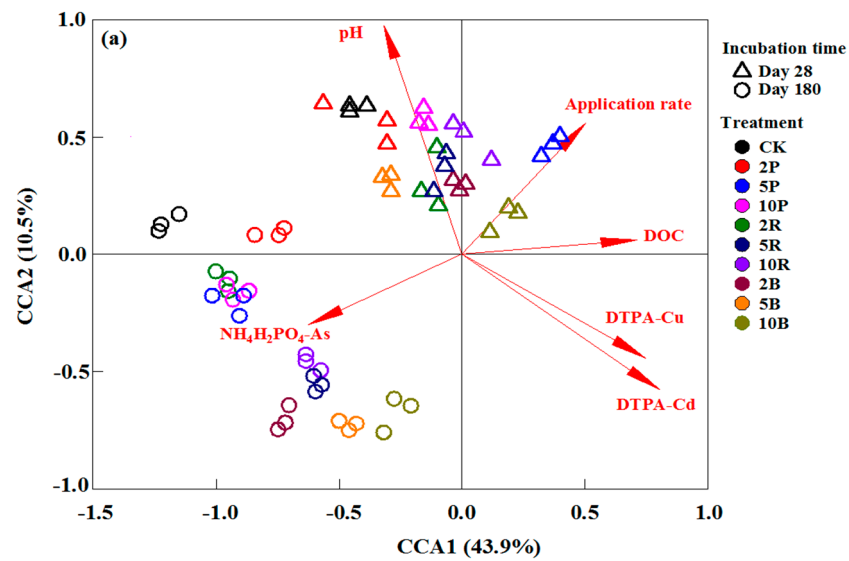


Figure 5. Cont.

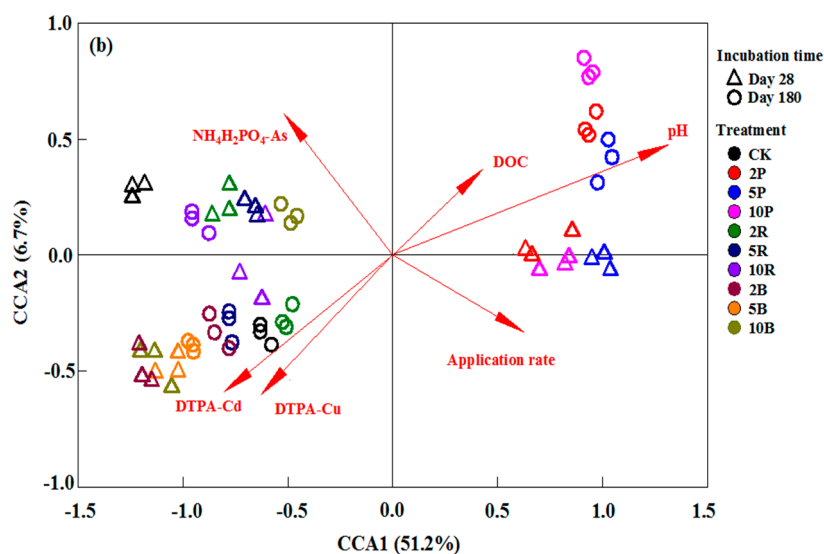


Figure 5. Canonical correspondence analysis (CCA) of soil urease activity (a) and catalase activity (b) with phosphate, red mud, and biochar after 180 days of incubation (CK: control; 2P: 2% phosphate; 5P: 5% phosphate; 10P: 10% phosphate; 2R: 2% red mud; 5R: 5% red mud; 10R: 10% red mud; 2B: 2% biochar; 5B: 5% biochar; 10B: 10% biochar).

4. Conclusions

The red mud and biochar addition effectively reduced the phytoavailabilities of soil Cd and Cu after 180 days of incubation, while the decrease in soil-phytoavailable As was much less than that of soil-phytoavailable Cd and Cu. Phosphate significantly decreased the concentrations of soil-available Cd and Cu but failed to lower that of As. The BCR extraction method demonstrated that red mud and biochar addition increased soil-reducible fraction of As as a result of the surface complexes of As with Fe oxide. The results of CCA showed that soil pH and available As, Cd, and Cu concentrations were the primary factors in driving changes in soil enzymatic activity. Soil pH had positive correlations with soil urease and catalase activities, while negative correlations were observed between soil-available heavy metals and soil enzyme activities. This study indicated that it is difficult to simultaneously and significantly lower the phytoavailabilities of As, Cd, and Cu using one amendment addition. Therefore, further research on modifying these amendments, or applying combined amendments, is imperative in order to develop an efficient method to immobilize co-contamination of As, Cd, and Cu.

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