

Effects of sepiolite on stabilization remediation of heavy metal-contaminated soil and its ecological evaluation

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Abstract Stabilization in the remediation of heavy metal contaminated soils has been gaining prominence because of its cost-effectiveness and rapid implementation. In this study, microbial properties such as microbial community and enzyme activities, chemical properties such as soil pH and metal fraction, and heavy metal accumulation in spinach (*Spinacia oleracea*) were considered in assessing stabilization remediation effectiveness using sepiolite. Results showed that soil pH values increased with rising sepiolite concentration. Sequential extraction results indicated that the addition of sepiolite converted significant amounts of exchangeable fraction of Cd and Pb into residual form. Treatments of sepiolite were observed to reduce Cd and Pb translocation from the soil to the roots and shoots of spinach. Concentrations of Cd and Pb exhibited 12.6%–51.0% and 11.5%–46.0% reduction for the roots, respectively, and 0.9%–46.2% and 43.0%–65.8% reduction for the shoots, respectively, compared with the control group. Increase in fungi and actinomycete counts, as well as in catalase activities, indicated that soil metabolic recovery occurred after sepiolite treatments.

Keywords stabilization remediation, heavy metals, sepiolite, soil quality, spinach (*Spinacia oleracea*)

1 Introduction

Sepiolite ($\text{Mg}_4\text{Si}_6\text{O}_{15}(\text{OH})_2 \cdot 6\text{H}_2\text{O}$) belongs to the natural clay family of phyllosilicates, with a formula of magnesium hydro-silicate that occurs as a fibrous chain structure [1]. The structure of sepiolite is nearly similar to those of other 2:1 trioctahedral silicates such as talc, although disconti-

nities and inversions of the silica sheet are observed, which give rise to the structural tunnels and blocks in sepiolite [2]. These tunnels contain H_2O molecules and exchangeable cations (K^+ , Na^+ , and Ca^{2+}). All corners of the silica tetrahedral are connected to adjacent blocks in the inner blocks, however, several corners are Si atoms bound to hydroxyls (Si-OH) in the outer blocks. These Si-OH groups are the major active centers for metal polycation and organic pollutant adsorption [3,4].

The unique fibrous structure with internal channels ($3.6 \times 10.6 \text{ \AA}$) enables incorporation of organic groups and inorganic ions into sepiolite structure [1,5–8]. Numerous studies have been conducted using sepiolite to reduce availability of heavy metals in soil and to inhibit trace element absorption in plants [9–11]. Treatment using 4% sepiolite dose reduced water soluble fraction of Cd and Zn by 57.3% and 41.4%, respectively, and exchangeable Cd and Zn fraction by 42.8% and 24.7%, respectively. Mobility of Cd and Zn in mining-polluted soil, as determined by leaching studies, substantially decreased by approximately 69% and 52%, respectively [9]. Cd and Pb contents of *Lactuca sativa* L. under the combined treatment of sepiolite and phosphate fertilizer likewise decreased by 51.9% and 55.3%, respectively, compared with the control group [10].

Stabilization of trace elements is a remediation technique used by adding soil to reduce heavy metal mobility, and which can be achieved by using immobilizing amendments [12]. Immobilization of polluted soil does not decrease total concentration of heavy metals. Thus, the effectiveness of remediation should be monitored using a chemical method, i.e., sequential extraction and leaching test, to measure the amounts of bioavailability fraction of heavy metals [11,13,14]. However, this method does not provide enough information about the environmental impact of stabilization remediation. Biochemical, microbiological, and plant-based tests need to be employed in

soil toxicity assessment. For example, soil microbial biomass is considered to be a transformation agent of soil organic materials and a labile pool for plant nutrients [15]. Soil enzyme activities are also useful for detecting changes in soil quality because they boost nutrient cycling, and also function as signals of altered microbial community structure caused by environmental impact [16]. The present research investigated the potential of sepiolite for stabilization remediation of Cd and Pb contaminated soils. Mobility and bioavailability of heavy metals were investigated using sequential chemical extraction. The influence of soil treatment on microbial toxicity, enzyme activities, and heavy metal concentrations in plants were evaluated to measure efficiency of the stabilization procedure.

2 Materials and methods

2.1 Soil characterization and plant culture

0–20 cm surface soil samples were collected from Hechi Country, Guangxi, China. The soil samples passed through a 0.25 mm sieve were used for physical and chemical fraction analysis, and passed through 0.15 mm sieve were determine the concentrations of heavy metals. The following soil properties were determined: pH 7.72, clay 10.26%, sand 49.30%, silt 40.44%, Organic matter (OM) 2.49%, Cation exchange capacity (CEC) 8.62 cmol·kg⁻¹, total N 0.96%, available P 36.76%, available K 29%, Cd 13.44 mg·kg⁻¹, and Pb 1993.9 mg·kg⁻¹. 1.0 kg of surface soil samples which were ground to pass through a 4 mm mesh were placed in each plastic pot. Then the sepiolite was blended into the contaminated soils at a proportion of CK (0%), 0.5%, 1%, 3% and 5%, respectively. Each treatment was performed in triplicate and the soil was incubated for 5 weeks. 6 seeds of (*Spinacia oleracea*) were planted into each pot. Loss of water was made up using tapwater (no Cd detected) to reach 75% of the field water-holding capacity and maintained this humidity by daily watering throughout the cultivation, and a petri dish was placed under each pot to collect potential leachate during the experiment. After 72 day growth, the plants were harvested and washed with tap water and then rinsed 3–

4 times deionised water. The plant samples were separated into roots and shoots, and oven-dried (75°C) to a constant weight. Samples of plant were ground with stainless steel mill and passed through a 0.25 mm sieve prior to analysis.

2.2 Analytical methods

2.2.1 Soil pH and heavy metal speciation

The pH was measured at a soil: water ratio of 1:2.5 (v/v) using a pH meter (PB-10, Sartorius, German). Cd and Pb fractionation in the soil was performed using sequential extraction by Tessier et al. [17]. The extraction was carried out progressively on an initial weight of 1.00 g test soil, which was contained in a centrifuge tube (polypropylene, 100 mL) and shaking with variable speed on a reciprocal shaker at 220 strokes·min⁻¹. The extractant and operationally defined chemical fractions were shown in Table 1. After each successive extraction, separation was done after centrifuging for 30 min. The supernatant was filtered and placed in a tube for measurement.

2.2.2 Microorganisms communities and soil enzyme activities

Number of soil microorganisms was estimated by the dilution plate technique (three replicates from each dilution and soil dish). The compositions and preparation of media were as follows [18].

Soil urease activity was determined by the method of Tabatabai [19]. Urease activity was determined with urea as substrate, incubating at pH 7.1 (0.2 mmol·L⁻¹ phosphate buffer) and 37°C for 24 h. The residual urea was determined by a colorimetric method. The enzymatic activity is expressed as mg NH₄⁺–N·g⁻¹·h⁻¹.

The catalase was analyzed by titration with KMnO₄. Soil samples were added to 40 mL distilled water with 5 mL of 0.3% H₂O₂. The mixture was shaken for 20 min and then 5 mL of 1.5 mol·L⁻¹ H₂SO₄ were added. Afterwards the solution was filtered and titrated using 0.1 mol·L⁻¹ KMnO₄. The reacted amount of 0.1 mol·L⁻¹ KMnO₄, calculated per gram of dry soil, was used to express the activity of catalase. The enzymatic activity is expressed as

Table 1 Sequence extraction processes of heavy metals in soil

sequence	speciation	extractant
fraction 1	water soluble plus exchangeable (SE)	8 mL of 1.0 mmol·L ⁻¹ MgCl ₂ at pH 7.0 for 1 h at 25°C
fraction 2	bound to carbonate or weakly specifically adsorbed (WSA)	8 mL of 1.0 mmol·L ⁻¹ NaAc adjusted to pH 5.0 with acetic acid for 5.0 h
fraction 3	bound to Fe-Mn oxides (OX)	20 mL of 0.04 mmol·L ⁻¹ NH ₂ ·HCl in 25% (v) acetic acid (pH 2.0) for 6.0 h at 96°C
fraction 4	bound to organic matter (OM)	3 mL of 30% H ₂ O ₂ and 0.02 mol·L ⁻¹ HNO ₃ (pH 2.0) for 2.0 h at 85°C, followed by 3 mL 30% (v) H ₂ O ₂ (pH 2.0) for 3.0 h at 85°C and then 5 mL of 3.2 mmol·L ⁻¹ NH ₄ Ac in 20% HNO ₃ diluted to 20 mL at room temperature for 0.5 h
fraction 5	residual (RES)	the above four fractions subtracted from the total metal content

$\text{mL} \cdot \text{g}^{-1} \cdot \text{h}^{-1}$ [20].

Invertase activity was determined using a sucrose solution as a substrate and incubation at 37°C for 24 h, before measuring the produced glucose with a colorimetric method. The enzymatic activity is expressed as $\text{mg} \cdot \text{g}^{-1} \cdot \text{h}^{-1}$ [21].

2.2.3 Cd and Pb determination

The plant and soil samples were digested with a solution of $\text{HNO}_3\text{-HClO}_4$ and $\text{HCl-HNO}_3\text{-HF-HClO}_4$, respectively. The concentrations of Cd were determined using atomic absorption spectrophotometry (Solaar M6, Thermo Fisher Scientific, USA). A certified reference material, bush leaf material (GBW07603, China), was used to verify the accuracy and precision of the digestion procedure and subsequent analysis.

2.3 Statistical analysis

All treatments were replicated three times in the experiments. The means and standard deviations (SD) were calculated by the Microsoft Office Excel 2003. One-way analysis of variance was carried out with SPSS10.0. When a significant ($P < 0.05$ or $P < 0.01$) difference was observed between treatments, multiple comparisons were made by the least significant difference (LSD) test.

3 Results and discussion

3.1 Effects of sepiolite on soil pH and heavy metal fractionation

The pH value is a key factor that governs solid-solution equilibrium of trace elements in the soil [22]. Competition between H^+ and dissolved metals for ligands (e.g., SO_4^{2-} , S^{2-} , CO_3^{2-} , Cl^- , OH^- , and phosphates) usually becomes increasingly significant when soil pH decreases. This competition subsequently reduces adsorption abilities, and then increases mobility and bioavailability of heavy metals. As listed in Table 2, unamended soil used for the

experiments had a relatively low pH. After the addition of sepiolite, soil pH was significantly enhanced compared with the control group, and it subsequently increased when the concentration of sepiolite was increased because of the significant percentage of CaCO_3 , which gives alkaline properties (pH 10.1) to sepiolite. This result coincided with those reported by Liang et al. [10] and Sun et al. [11,23].

Sequential extraction procedures are often applied in investigating relative bioavailability of soil-sorbed metal by revealing operationally defined speciation of a metal in the solid phase [17,24], and then evaluating the efficacy of remediation treatments. When sepiolite was not applied, the highest proportion of Cd were bound to residual fractions or RES (48.0%), followed by Fe-Mn oxides bound or OX (23.3%), soluble plus exchangeable or SE (15.2%), weakly specifically absorbed or WSA (9.0%), and organic matter complexed or OM (4.5%) (Fig. 1). However, after adding consecutive concentrations of sepiolite, the proportion of Cd in the RES fraction increased from 51.5% to 53.6%, and that in SE decreased from 14.5% to 11.8%. The highest proportion of Pb were predominantly bound to RES (41.6%), followed by OM (36.5%), WSA (10.8%), OX (6.4%), and SE (4.7%). The exchangeable fraction of Pb treated with sepiolite was remarkably reduced compared with CK. The proportion of SE decreased by 1.1%–2.9%, whereas RES increased by 2.5%–7.7%. Results indicated that the addition of sepiolite was efficient in transforming Cd and Pb from SE to RES forms. Similarly, a 4% increase in the amount of sepiolite in soil decreased Cd and Zn mobility by 57.3% and 41.4%, respectively [9].

3.2 Plant biomass and metal accumulation

As shown in Table 2, shoot biomass of spinach was inhibited upon the application of sepiolite, resulting in a 7.5%–29.8% reduction compared with the control treatment. The dry weight of shoots decreased significantly when sepiolite treatment was up to 1%. The presence of large amounts of sepiolite in soil may cause nutrient imbalances and relative nutrient deficiencies. Decrease in nutrient availability may have also been caused by high soil pH [25]. However, our previous study indicated that in

Table 2 Response of pH, Cd and Pb accumulation factor and microbial properties under different treatments

sepiolite/%	pH	shoot dry weight	Cd accumulation factor		Pb accumulation factor		bacteria / $(10^7 \cdot \text{g}^{-1}$ soil)	fungi / $(10^5 \cdot \text{g}^{-1}$ soil)	actinomycete / $(10^6 \cdot \text{g}^{-1}$ soil)
			shoot	root	shoot	root			
0	7.72±0.02d	2.69±0.05a	0.67	1.83	0.0076	0.0876	1.17a	2.25ab	1.05b
0.5	7.81±0.01c	2.49±0.08ab	0.66	1.60	0.0043	0.0775	0.76b	2.30ab	1.07b
1	7.85±0.00c	2.13±0.11cd	0.63	1.52	0.0035	0.0670	0.64bc	2.85a	1.44ab
3	7.92±0.02b	2.38±0.13bc	0.51	1.18	0.0031	0.0597	0.41c	2.05b	1.45ab
5	8.03±0.02a	1.89±0.10d	0.36	0.90	0.0026	0.0473	0.29cd	2.00b	1.75a

Notes: Data are means±S.D. ($n = 3$). Means with different letters in the same column are significantly different from each other ($P < 0.05$) according to the LSD test. Accumulator factor refers the ratio of metal concentration in plants to that in soil

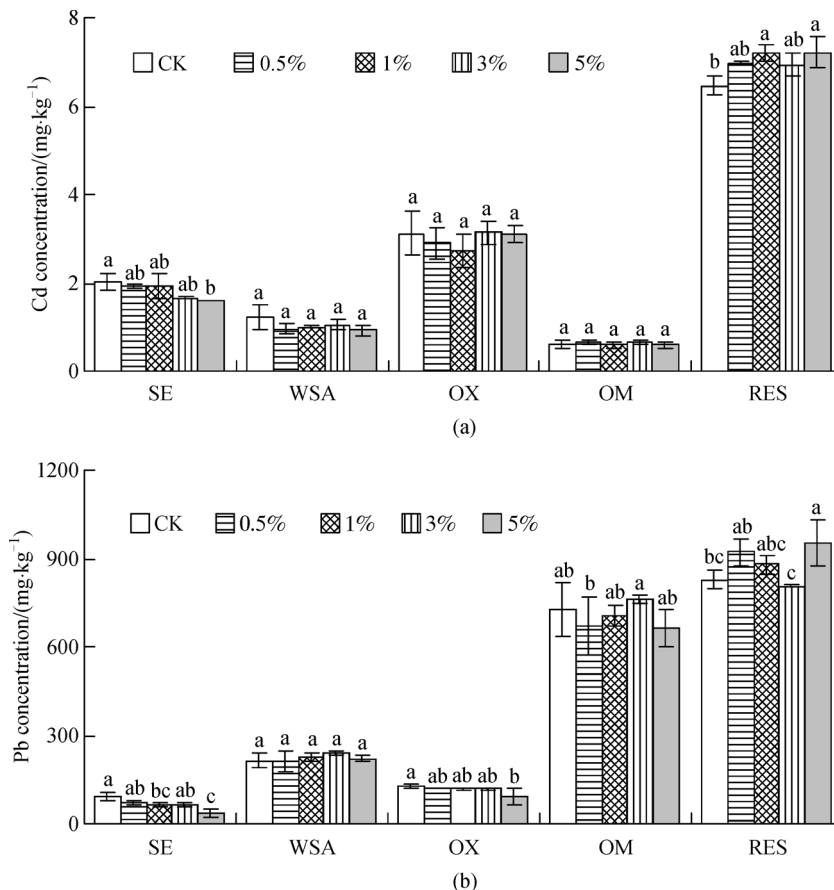


Fig. 1 Cd (a) and Pb (b) distribution into separate fractions in the studied soils

acidic soil, shoot biomass was significantly enhanced after sepiolite addition (1%–10%) under a Cd concentration of 1.25, 2.5, and 5 mg·kg⁻¹, thus resulting in an increase of 2.76 to 3.25, 0.17 to 1.40, and 1.48 to 7.12 times, respectively, when compared with unamended soil [11]. In alkaline soil, dry weight of plants increased by 58.5%–65.5% when the application of sepiolite was ≤ 10 g·kg⁻¹, however, zeolite had a prohibitive effect on plant growth when its concentration was up to 15 g·kg⁻¹ [25].

Cd and Pb contents in the roots and shoots of spinach grown in the soil sample are shown in Fig. 2. Cd and Pb uptake of spinach generally accumulate mainly in roots, and this phenomenon can be attributed to the important role of roots in restraining translocation of metals from roots to aerial parts. Accumulator factors of Cd and Pb in roots are higher than those in shoots, as shown in Table 2. In unamended soil, the concentrations of Cd and Pb were 24.6 and 9.0 mg·kg⁻¹ in roots, respectively, and 174.7 and 15.1 mg·kg⁻¹ in shoots, respectively. These results correspond with those found by other authors [26,27]. Maximum values of Cu, Mn, Zn, Pb, Ni, and Cd in spinach reached 16.1, 36.8, 161.3, 11.4, 5.6, and 8.3 mg·kg⁻¹, respectively, when spinach was irrigated with wastewater from Quetta City. Chunalall et al. [27] found

that Cd content in spinach plants grown on soil contaminated with 10 mg·kg⁻¹ Cd after a 35-day and 70-day exposure was 22.0 and 26.14 mg·kg⁻¹, respectively. High concentrations of heavy metal accumulation in spinach pose a potential hazard to human health through the food chain [13,28].

The addition of sepiolite efficiently decreased Cd and Pb uptake of spinach (Fig. 2). Cd and Pb concentrations in all parts of the plant were negatively proportional to the rate of sepiolite. The corresponding relationship can be expressed by using the following regression equations:

$$\text{Cd} : Y_{\text{roots}} = -2.349X_{\text{sepiolite}} + 23.346$$

$$(R^2 = 0.972; P < 0.05), \quad (1)$$

$$Y_{\text{shoots}} = -0.8564X_{\text{sepiolite}} + 9.2367$$

$$(R^2 = 0.9926; P < 0.01), \quad (2)$$

and

$$\text{Pb} : Y_{\text{roots}} = -114.159X_{\text{sepiolite}} + 162.35$$

$$(R^2 = 0.9037; P < 0.05), \quad (3)$$

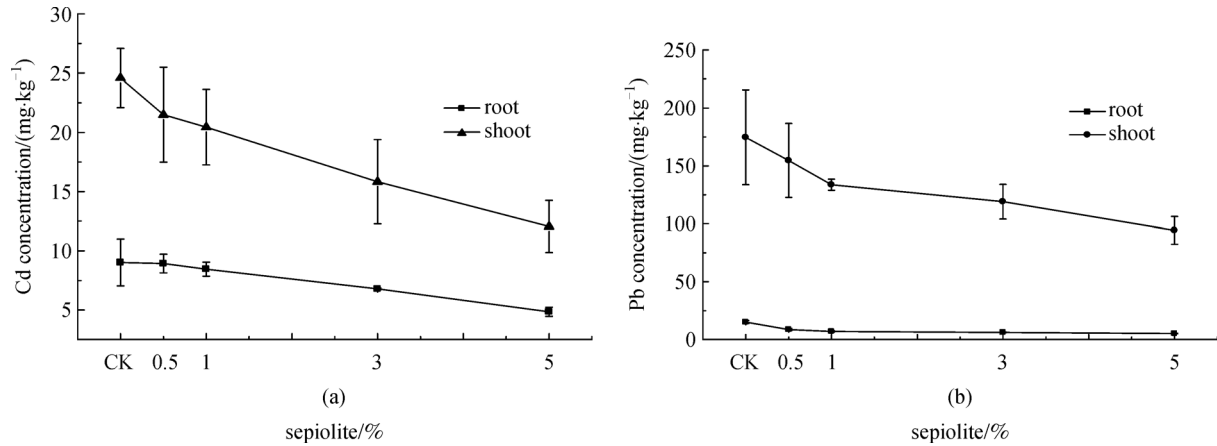


Fig. 2 Cd (a) and Pb (b) concentrations in edible part of spinach treated with sepiolite

$$Y_{\text{shoots}} = -1.4108X_{\text{sepiolite}} + 11.085 \quad (4)$$

$$(R^2 = 0.5457; P > 0.05),$$

where Y_{roots} and Y_{shoots} are Cd and Pb concentrations in roots and shoots, respectively. X is the concentration of sepiolite in soil.

When compared with CK, Cd concentrations in the shoots and roots of plants decreased by 0.9%–46.2% and 12.6%–51.0%, respectively, through the application of 0.5%–5% sepiolite. In the case of Pb concentrations in the shoots and roots after remediation, Pb contents under different treatments of sepiolite (0.5%–5%) were significantly reduced, with a decrease of 43.0%–65.8%, and 11.5%–46.0% of the control value, respectively. These results concurred with those found by Liang et al. [10]. Cd and Pb contents in *Lactuca sativa* L. under the combined treatment of sepiolite and phosphate fertilizer decreased by 51.9% and 55.3%, respectively, compared with the control group [8]. Immobilization materials such as alkaline compounds, clay minerals, and phosphorus-containing amendments have been proven to be very effective in reducing bioavailability of metals in soil, thus hampering plant absorption and translocation of these metals [11,29,30]. In the present study, values of Cd and Pb bioaccumulation factors in the shoots and roots of plants gradually decreased when sepiolite concentration was increased, with a decrease of 0.9%–46.2% and 12.6%–51.0%, and 43.4%–65.8% and 11.5%–46.0%, respectively, compared with CK.

3.3 Soil enzyme activities and microbial community analysis

Soil microbes are the driving force behind numerous soil processes, including the transformation of organic matter, nutrient release, and degradation of xenobiotics. Changes in soil microbial community structure can help detect

changes in the physical and chemical properties of soil, thus providing an early signal of soil improvement or soil degradation [31]. Evaluation of both biologic presence and activity should be considered with regard to soil amendments [11]. The number of bacteria decreased with the application of an increasing amount of sepiolite to soil, with a reduction of 35.0%–75.2% compared with the control group. By contrast, treatments of sepiolite increased the amount of actinomycetes by approximately 1.9%–66.7% more than that of CK. As for fungi, the addition of sepiolite first increased fungi count, and then the count was subsequently decreased when the amount of sepiolite was increased. Bacteria, fungi, and actinomycetes play important roles in the formation of soil aggregates in good soil structure because they promote detoxification of soil contaminants and production of plant growth promoters and organic chelating agents. The positive effect of sepiolite on fungi and actinomycete count may be attributed to the effects of amendments on alleviating metal toxicity [32], which demonstrates soil functional recovery after applying sepiolite [31].

Soil enzymes play an essential role in mediating biochemical transformations involving decomposition of organic residues and nutrient cycling in soil [33]. Soil enzyme activities are usually considered as sensitive and early indicators of both natural and anthropogenic disturbances [32]. As shown in Fig. 3, Catalase activities were observed to gradually increase with rising sepiolite concentration, resulting in a 3.4%–20.7% increase compared with CK (Fig. 3). Observed catalase activities had a significant ($P < 0.05$) increase when sepiolite concentration in soil was up to 1%. By contrast, urease activities were inhibited when soil sepiolite concentration was increased, and their values were reduced by 32.9%–76.8% compared with that of the control. However, no significant ($P > 0.05$) difference was found among sepiolite treatments of 0%–3%. In the case of invertase, activities declined when sepiolite treatments were 0.5%–

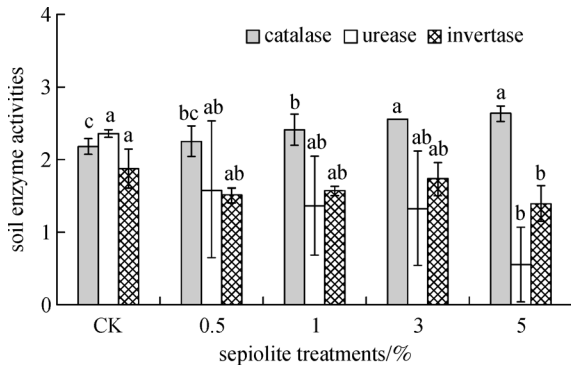


Fig. 3 Soil enzyme activities under different treatments of sepiolite

5%, with a decrease of 7.5%–25.4% compared with CK. Low catalase activities recorded in the unamended soil indicate the poor functional state of the soil, whereas increased values observed in the treated samples proved that a certain metabolic recovery had occurred [34]. The higher catalase activity in the sepiolite-soil, with respect to the control group, showed that catalase was possibly influenced by mobility and bioavailability of Cd and Pb, and by pH. However, inhibition of urease and invertase under different treatments of sepiolite revealed that they may not be useful in assessing soil recovery after heavy metal contamination. Similar to the case of catalase, pH change might possibly have been mainly responsible for such behavior [34,35].

3.4 Relationship between pH, shoot biomass, enzyme activities, and Cd and Pb in soil and plants

Table 3 shows Pearson's correlation coefficients between soil pH, shoot biomass, SE-Cd, SE-Pb, enzyme activities, as well as Cd and Pb concentrations in the edible parts of spinach. Generally, soil pH values were negatively

correlated with SE-Cd, SE-Pb, shoot biomass, urease and invertase activities, as well as Cd and Pb concentrations in spinach. Stabilization amendments reduce heavy metal leaching and their bioavailability by various chemical processes, such as adsorption to mineral surfaces, formation of stable complexes with organic ligands, surface precipitation, and ion exchange [12]. Outstanding sorptive and colloidal properties of sepiolite provide a specific remediation agent for widespread heavy metal contamination. Because pH is one of the most important parameters affecting trace element bioavailability [30], an increase in soil pH in sepiolite treatments was an additional mechanism of sepiolite-induced reduction in metal bioavailability in soil, thus prohibiting translocation and bioaccumulation in plants.

Soils can reduce mobility and bioavailability of heavy metals because these substances are retained in soil by sorption, precipitation, and complexation reactions. This natural attenuation process can be accelerated by the application of amendments [31]. Heavy metals in plants usually have highly positive correlations with their total and mobile properties in soil [11,13]. The proportion of SE-Cd and SE-Pb under sepiolite treatments decreased by 0.7%–3.4% and 2.5%–7.7%, respectively, compared with the control group (Fig. 1). Subsequently, the concentrations and accumulation factors of Cd and Pb in spinach exhibited decreases in varying degrees (Fig. 2 and Table 2). As shown in Table 3, significant and positive correlations were observed between SE-Cd in soil and Cd concentration in the shoots, and between SE-Pb in soil and Pb accumulation in the shoots, as reported by Sun et al. [28] and Liang et al. [10].

4 Conclusions

Assisted remediation using sepiolite improved chemical and biologic status of soil, which contributes to enhancing

Table 3 Correlation coefficients between pH, available metals, soil enzyme activities and biomass and metal concentration in spinach

	pH	SE-Cd	SE-Pb	shoot biomass	catalase	urease	invertase	shoot Cd concentration	shoot Pb concentration
pH	1	-0.79	-0.99**	-0.99**	0.86	-0.97**	-0.82	-0.83	-0.86
SE-Cd		1	0.85	0.72	-0.97**	0.87	0.48	0.96**	0.80
SE-Pb			1	0.96**	-0.89*	0.99**	0.83	0.87	0.88*
shoot biomass				1	-0.83	0.94*	0.80	0.78	0.82
catalase					1	-0.90*	-0.51	-0.92*	-0.86
urease						1	0.83	0.86	0.92*
invertase							1	0.51	0.74
shoot Cd concentration								1	0.68
shoot Pb concentration									1

Notes: * Correlation is significant at the 0.05 level; ** Correlation is significant at the 0.01 level

soil pH and decreasing heavy metal bioavailability in soil and absorption in spinach, and restoring soil functional properties. Amendments increased soil pH values and reduced Cd and Pb soluble concentrations compared with non-amended soil, thus reducing the risk of heavy metals entry into the food chain. Microbial parameters (fungi and actinomycete) and catalase activities seem to be the best indicators of soil function after immobilization remediation for contaminated soil. Although the application of sepiolite can provide a cost-effective and sustainable solution for the remediation of heavy metal-contaminated soil, further monitoring is necessary to prevent adverse effects to soil environment.

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