Determining soil enzyme activities for the assessment of fungi and citric acid-assisted phytoextraction under cadmium and lead contamination

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Abstract Microorganism or chelate-assisted phytoextraction is an effective remediation tool for heavy metal polluted soil, but investigations into its impact on soil microbial activity are rarely reported. Consequently, cadmium (Cd)- and lead (Pb)-resistant fungi and citric acid (CA) were introduced to enhance phytoextraction by *Solanum nigrum* L. under varied Cd and Pb pollution levels in a greenhouse pot experiment. We then determined accumulation of Cd and Pb in *S. nigrum* and the soil enzyme activities of dehydrogenase, phosphatase, urease, catalase, sucrase, and amylase. Detrended canonical correspondence analysis (DCCA) was applied to assess the interactions between remediation strategies and soil enzyme activities. Results indicated that the addition of fungi, CA, or their combination enhanced the root biomass of *S. nigrum*, especially at the high-pollution level. The combined treatment of CA and fungi enhanced accumulation of Cd about 22–47 % and of Pb about 13–105 % in *S. nigrum* compared with the

phytoextraction alone. However, *S. nigrum* was not shown to be a hyperaccumulator for Pb. Most enzyme activities were enhanced after remediation. The DCCA ordination graph showed increasing enzyme activity improvement by remediation in the order of phosphatase, amylase, catalase, dehydrogenase, and urease. Responses of soil enzyme activities were similar for both the addition of fungi and that of CA. In summary, results suggest that fungi and CA-assisted phytoextraction is a promising approach to restoring heavy metal polluted soil.

Keywords Cadmium · Lead · Soil enzymes · *Solanum nigrum* L · Fungi · Citric acid

Introduction

Cadmium (Cd) and lead (Pb) are two trace elements that exert biological toxicity on many animals and plants (Xi et al. 2012). As by-products of industrialization and urbanization, heavy metal pollution severely threatens environmental safety and human health. Affordable technologies are therefore needed to deal with these challenges (Padmavathiamma and Li 2007). Remediation of heavy metal pollution has made great progress in recent years. Phytoextraction has attracted a great deal of research attention in recent decades because of its low cost and eco-friendly process (Kumar et al. 2014; Yu et al. 2015). However, the limited biomass of the hyperaccumulator and the low bioavailability of metals in soil restrict the wide application of phytoextraction (Gerhardt et al. 2009). Therefore, auxiliary materials such as chelate agents and microorganisms have been applied to phytoextraction. Chelates desorb metals from the soil matrix into the soil solution, thus, promoting metal transport into the xylem

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and increasing the translocation from the roots to the shoots of the hyperaccumulator (Gao et al. 2012). Microbial metabolite, such as low-molecular-weight acid, can also change the chemical availability of heavy metals in soil to assist the phytoextraction (Braud et al. 2009). Furthermore, application of beneficial microbial inoculants to soil promotes the uptake of nutrients by plants and then enhances phytoextraction effectiveness by promoting plant growth (Schoebitz et al. 2013). Nevertheless, the potential biotoxicity of chelate agents and microorganisms to soil and plant health limited the applications in soil (Leštan et al. 2008). Because of these potential risks, desired chelates such as nitrilotriacetate and citric acid (CA) of high biodegradability, low phytotoxicity, and strong chelating ability were suggested for the chemically assisted phytoextraction of some metals (Wenger et al. 2003). Research into the hyperaccumulator key characteristic of extracting heavy metals under microorganism or chelate-induced remediation has been carried out (Fine et al. 2014; Vaxevanidou et al. 2008). However, research into the response of soil biochemical characteristics and microbial activities after assisted phytoextraction is rarely reported.

Until now, the emphasis has mainly been on metal removal when assessing the success of a phytoextraction process. However, ideal soil remediation technologies are not only effective for removing contaminants but also helpful for soil function and health (Epelde et al. 2009). Soil enzymes are the primary mediators of soil biological processes, including organic matter degradation, mineralization, and nutrient recycling. They play an important role in maintaining soil ecosystem quality and functional diversity (Kandeler et al. 1996). Soil enzyme activity is sensitive to many types of heavy metal pollution (Hinojosa et al. 2004). Research has shown that heavy metal pollution can result in reduced enzyme activity in the contaminated soil (Chaperon and Sauvé 2008). However, soil enzyme activity could also be recovered through remediation (Jusselme et al. 2013). Hence, soil enzyme activity is widely used for monitoring soil functional restoration after various remediation processes (Ciarkowska et al. 2014).

Based on the information given above, the successful bioremediation of contaminated soils depends on a thorough understanding of the correlative microbial and enzymatic processes. Therefore, Cd and Pb accumulation in *Solanum nigrum* L. and soil enzyme activities such as dehydrogenase, phosphatase, urease, catalase, sucrase, and amylase involved in nutrient cycling and oxidative stress defending were determined in Cd- and Pb-polluted soil. This study aims to illustrate the effects of microorganism and citric acid-assisted phytoextraction on soil enzymes in Cd- and Pb-contaminated soil and to provide further data for remediation and soil health.

Materials and methods

Separation, screening, and biological identification of the microorganisms

Two fungal isolates were isolated, selected for their ability to tolerate Pb or Cd from industrial soil. Through gradient concentration inducing, culturing, and domesticating, the two strains could tolerate Cd and Pb at 1000 mg L⁻¹ and 2000 mg L⁻¹, respectively.

Genomic DNA was extracted using an EZ-10 Spin Column Genomic DNA Minipreps Kit (Bio Basic Inc.). The 18S *rRNA* gene was amplified by PCR. The primers ITS1 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS4 (5'-GCATATCAATAAGCGGAGGA-3') were used (Zhang et al. 2005). The PCR product was sequenced by Sangon Biotech Co. Ltd. (Shanghai, China), and the sequence was compared with the sequences in the GenBank database using the Blastn Program. The two fungal isolates were then identified as *Hypocrea virens* and *Paecilomyces lilacinus*.

Characterization, sampling, and treatment of soils and plants

The pot experiment was conducted in a greenhouse in Shanghai Jiaotong University, at an average temperature of 22–25 °C. The tested soil was a paddy soil which extracted from the Yangtse River Basin, Shanghai, China (N31°02', E121°5'), and the basic soil parameters were described as follows: organic matter, 16.17 g kg⁻¹; soil pH, 8.18; total P and N, 1.36 and 1.14 g kg⁻¹, respectively; cation exchange capacity, 15.60 cmol kg⁻¹; and Cu, Zn, Cd, As, and Pb concentrations were 22.9, 38.1, 0.18, 7.4, and 15.1 mg kg⁻¹, respectively. Fresh soil was sieved through a 4-mm sieve and thoroughly mixed with Cd, Pb, and basal fertilizers and then placed into plastic pots of 40 cm in height and 20 cm in diameter. Pb was applied as PbCl₂, and Cd was applied as CdCl₂·2.5H₂O. In total, five levels for combined Cd and Pb were built (Level 0 0+0, Level 1 5+50, Level 2 10+200, Level 3 20+400, and Level 4 50+600 mg kg⁻¹ of dry soil). The two isolated fungi and the citric acid were dissolved in liquid and added into the treatments of Cd and Pb combined polluted soil. The addition of the fungi was 4×10⁶ CFU kg⁻¹ of dry soil and that of citric acid was 20 mmol kg⁻¹ of dry soil. All treatments were performed in triplicate, and details are shown in Table 1.

The experimental plant was *S. nigrum* L. Five seedlings, each about 2 weeks old and 3–4 cm in height with 2–3 leaves were transplanted into each pot. Soil and plants were sampled at 40 days of growth after plants were transplanted into pots. For each pot, five soil samples (1.5 cm diameter) were taken at the depth of 10 cm and were then mixed into one sample (about 100 g).

Table 1 Various remediation treatments under different Cd and Pb pollution levels (unit mg kg⁻¹)

Treatments	None remediation	Planting	Planting + citric acid	Planting + fungi	Planting + citric acid + fungi
L 0 (Cd + Pb)	0+0	0+0	0+0	0+0	0+0
L1 (Cd + Pb)	5+20	5+20	5+20	5+20	5+20
L 2 (Cd + Pb)	10+200	10+200	10+200	10+200	10+200
L 3 (Cd + Pb)	20+400	20+400	20+400	20+400	20+400
L 4 (Cd + Pb)	50+600	50+600	50+600	50+600	50+600

Plants were harvested by cutting the shoots at the soil surface, and the roots were carefully separated from the soils. Plants were washed thoroughly with running water, followed by distilled water, and dried at 100 °C for 10 min then at 70 °C in an oven until completely dry. Plant samples were digested using a solution containing 87 % concentrated HNO₃ and 13 % concentrated HClO₄ (v/v) (Wei and Chen 2006). The concentration of Cd and Pb in digested solutions was determined using an AFS820 atomic absorption spectrophotometer (Beijing Titan Instruments, Beijing, China).

Determination of enzymatic activity

All soil enzyme activities were analyzed with three replicates. Soil urease activity was determined using the method of Tabatabai and Bremner (1972) and is expressed as NH₄-N mg kg⁻¹ h⁻¹. Dehydrogenase activity was tested using reduction of 2, 3, 5-triphenyltetrazolium chloride. After 24 h at 37 °C, the triphenyl formazan released was extracted with methanol and assayed at 485 nm by an ultraviolet spectrophotometer (CASIDA et al. 1964). The unit of dehydrogenase activity was triphenyl formazan (mg kg⁻¹ h⁻¹). Acid phosphatase activity was determined using the method of Tabatabai and Bremner (1969). The *p*-nitrophenol in the filtrate was determined colorimetrically at 410 nm after 1 h incubation with *p*-nitrophenyl phosphate, expressed as *p*-nitrophenol (mg kg⁻¹ h⁻¹). Catalase activity was determined using the method of Johnson and Temple (1964); the residual of the H₂O₂ in the filtrate was titrated with 0.2 mol L⁻¹ KMnO₄ solutions, expressed as 0.2 mol L⁻¹ KMnO₄ ml g⁻¹ h⁻¹. Sucrase and amylase activities were determined using the 3, 5-dinitrosalicylic acid colorimetric method (Guan 1986). Sucrase and amylase activities were expressed in glucose and maltose in mg kg⁻¹ h⁻¹, respectively.

Modified ecological model for soil enzyme activity

The toxicity coefficient was used to standardize the concentration of heavy metals for multivariable statistics. Using the toxicity coefficient, we can evaluate the inhibition effect for

enzyme activity subjected to multiple heavy metal synergic effects (Gao et al. 2010):

$$E_i = T_i \times C_i, \quad (1)$$

$$R_i = \sum_{i=1}^m E_i \quad (2)$$

where C_i is the concentration of heavy metal in soil (mg kg⁻¹ soil); T_i is the “toxic-response” factor for a given heavy metal, i.e., Pb=5, Cd=30 (He et al. 1998); E_i is the potential ecological toxicity coefficient for a given heavy metal; and R_i is total ecological toxicity coefficient under multiple heavy metal pollution. Using this method, we compared the soil enzyme activities under different pollution treatments after transforming Cd and Pb concentration to the total ecological toxicity coefficient. We then produced a detrended canonical correspondence analysis (DCCA) between soil enzymes activities and the heavy metal toxicity coefficient.

Statistical analysis

A DCCA was carried out to examine the relationship between remediation methods and soil enzyme activities under different heavy metal concentrations in soil. The CANOCO software for Windows (version 4.5, Microcomputer Power, Ithaca, NY, USA) was used for this work. The environmental variables were heavy metal concentration (Cd + Pb), single phytoextraction (P), fungus-assisted phytoextraction (P + F), citric acid-assisted phytoextraction (P + CA), and combined fungus and citric acid-assisted phytoextraction (P + F + CA). In the environmental variables, Cd and Pb concentrations were set as numerical variables, and the remediation method as a Boolean variable (False=0 and True=1). Other parameters used system defaults.

One-way analysis of variance and least significant difference at the 5 % confidence level were performed on each dependent variable using SPSS 19.0 software. Graphs were produced using Origin 8.0 and all quantitative data were expressed as means±SD.

Results

Plant growth and Cd and Pb accumulation

In the present study, *S. nigrum* showed no visual toxicity symptoms, such as necrosis or whitish-brown chlorosis, which suggested that *S. nigrum* could tolerate the Cd50 and Pb600 treatment. The changes in plant growth and heavy metal accumulation under the different remediation strategies are shown in Figs. 1 and 2. Generally, the biomass of the root, stem, or leaf of *S. nigrum* gradually decreased with the increase of Cd and Pb stress, especially in the sole plant treatment; however, the addition of citric acid or fungi alleviated heavy metal stress to plant organs. The difference in biomass was not significant between each treatment at the low heavy metal level, while clear difference was observed with the increasing concentration of Cd and Pb, and at Level 4, total plant dry weight was 43 % higher in the Plant + CA + Fungi treatment than in the Single Plant treatment. Moreover, the main differences in plant biomass between the remediation strategies were observed in the root or stem of *S. nigrum*.

The accumulation of Cd and Pb in *S. nigrum* showed a proportional increase in shoots and root growth with elevated Cd and Pb concentrations (Fig. 2). Moreover, the accumulation of Cd principally occurred in shoots, while Pb accumulation occurred mainly in the root. In general, the combined CA and fungi treatment enhanced Cd accumulation by about 22–47 % of the sole phytoextraction accumulation, in different organs of *S. nigrum*. CA or fungi-assisted phytoextraction also enhanced Cd accumulation by about 7–33 % relative to sole phytoextraction. Compared with sole planting in Level 4, Pb accumulation in the root (shoot) increased 105 (100), 32 (33), and 145 % (200 %) by P + CA + F, P + F, and P + CA, respectively.

Fig. 1 Change in *S. nigrum* dry biomass. Different letters indicate significant differences between remediation treatments at $P < 0.05$. Each value represents a mean $\pm$ SD of three independent experiments of each treatment in triplicate. L0 (lowest)–L4 (highest) represent different pollution levels of Cd and Pb

Changes of soil enzymes activities under remediations

The response of soil enzyme activities in the Cd and Pb combined polluted soil under different remediation strategies is shown in Fig. 3. Without any remediation, urease, amylase, and phosphatase activities decreased linearly with increased Cd and Pb stress, while dehydrogenase, catalase, and sucrase presented fluctuating changes. However, these phenomena disappeared after remediation. In general, most soil enzyme activities were enhanced by all remediation strategies to varying degrees, especially at high Cd and Pb concentrations, and dehydrogenase and urease were enhanced most of all. The combined CA and fungi-assisted phytoextraction (P + CA + F) exhibited promotion of dehydrogenase activity and that had increased around 35–65 % and 200–300 % compared with sole phytoextraction (P) and no remediation, respectively. Urease or catalase activity was also enhanced by the various remediation strategies compared with the no remediation treatment. However, phosphatase and amylase activities under various remediation strategies were decreased at low heavy metal levels but increased when levels were high. Furthermore, sucrase activity was slightly decreased after remediation.

Detrended canonical correspondence analysis

It is difficult to produce a visualized conclusion between soil enzyme activities and remediation strategies using a histogram because of the varying variables and heavy metal levels; however, multivariate statistical methods could be introduced to respond to this problem. To understand the correlation between different remediation methods and the soil enzyme activities intuitively, DCCA was introduced in this research. The Monte Carlo test result indicated that the first canonical axis reached a significant level ($P = 0.002$), and soil enzyme activity variables in the first ordination axis had a high correlation

Fig. 2 Change in Cd (a) and Pb (b) accumulation in the roots and shoots of *S. nigrum*. Different letters indicate significant differences between remediation treatments at $P < 0.05$. Each value represents a mean $\pm$ SD of three independent experiments of each treatment in triplicate. L0 (lowest)–L4 (highest) represent different pollution levels of Cd and Pb

with environmental variables ($R=0.93$); therefore, this may be considered an appropriate method to express the variables. Samples in the ordination diagrams (Fig. 4) were mostly distributed in three areas (I, II, III) depending on different remediation strategies. As shown in Fig. 4, most soil enzyme and environmental variables exhibited considerable changes when they were projected on the horizontal axis, which could reflect the soil enzyme response to environmental variables on this axis. The projective points of enzymes ranging from right to left on the horizontal axis implies an increasing tendency of enzymes activities improved by remediation in the following order: phosphatase, amylase, catalase, dehydrogenase, and urease. Compared with the histogram (Fig. 3), this result is not only similar but also visually clear.

The ordination diagrams also can express the relationship between remediation and soil enzyme activity by their site and distance. In Fig. 4, the distance between P + CA (planting + citric acid) and P + F (planting + fungus) was fairly close, which implies that the two remediation strategies had similar

effects on soil enzyme activities. However, the distance between P + CA + F (planting + citric acid + fungi) and P (planting) was a little larger. Urease, dehydrogenase, and catalase variables located in the second quadrant presented clear positive correlation with all remediation variables located in the same quadrant; nevertheless, sucrase located in the first quadrant was distant from them, which reveals a negative correlation. These results illustrate that specific soil enzyme activity had a characteristic response to different remediation types, and it can be considered as an indicator for soil ecological restoration.

Discussion

The large biomass of the hyperaccumulator and high availability of heavy metals are two key factors for phytoextraction (Padmavathiamma and Li 2007); therefore, chelating agents and microorganisms are usually used to ensure the

Fig. 3 Change in soil enzyme activities under different remediation strategies: **a** dehydrogenase, **b** phosphatase, **c** urease, **d** catalase, **e** amylase, and **f** sucrase. Where P represents single planting, P + CA represents planting + citric acid, P + F represents planting + fungi, and

P + CA + F represents planting + citric acid + fungi. Each value represents a mean±SD of three independent experiments of each treatment in triplicate. L0–L4 represents different pollution levels of Cd and Pb

requirements are met for successful remediation (Gaur et al. 2014). In the present study, no visual toxicity symptoms such as necrosis and whitish-brown chlorosis were observed in

plant growth, which indicates that *S. nigrum* could tolerate the high Cd and Pb stress. Moreover, adding fungi, CA, or a combination thereof enhanced the root biomass of *S. nigrum*,

Fig. 4 DCCA ordination graph of samples, enzyme activities, and remediation treatments. Where P represents single planting, P + CA represents planting + citric acid, P + F represents planting + fungi, and P + CA + F represents planting + citric acid + fungi. Asterisks represent the soil enzyme activities; arrows stood for the Cd and Pb stress; black triangles represent the various remediation treatments; and other patterns represent samples. Location of the symbol in DCCA ordination diagram can express the relationship between remediations and soil enzymes activities

especially at the high pollution level. These findings are similar to other studies in which increased root or shoot biomass was observed after CA or microorganism-assisted phytoextraction (Najeeb et al. 2009; Khan et al. 2015). The enhanced biomass may be related to the enhanced antioxidant enzyme activity of plants, which decreases the production of H_2O_2 and electrolyte leakage (Ehsan et al. 2014). However, Pb concentration in *S. nigrum* was considerably lower than that in soil. The enrichment factor (EF) and translocation factor (TF) are more efficient indices to evaluate hyperaccumulators than accumulation itself. The EF is defined as the ratio of the metal concentration in the plant to the metal concentration in the soil. The TF is defined as the ratio of the metal concentration in the shoots to that in the roots, and it can be used to measure a plant's effectiveness in translocating heavy metal from the roots to the shoots (Goswami and Das 2015). It was noted that adding fungi or CA also led to a higher transfer rate of Cd and Pb from soil into different part of *S. nigrum* compared with the treatments without CA and fungi addition. *Solanum nigrum* showed a clear hyperaccumulator characteristic for Cd with EF (3.72–8.34) and TF (1.43–2.13); however, EF (0.04–0.82) and TF (0.46–0.95) for Pb were both lower than ideal. For an ideal hyperaccumulator, TF values should be higher than 1 (Garbisu and Alkorta 2001). Hence, *S. nigrum* was not shown to be a hyperaccumulator for Pb, but it did have a high tolerance, or accumulation capacity, to Pb.

Soil enzyme activity showed either a linear decrease or fluctuation with the increase of Cd and Pb concentration in the treatments without remediation (Fig. 3). Enzyme activities have been reported to be valid biochemical indicators of the negative impact of heavy metals on the soil ecosystem (Hinojosa et al. 2004), as well as the effectiveness of the remediation processes (Gómez-Sagasti et al. 2012). However, the exact mechanisms of heavy metals interaction with diversiform enzymes in a complex environment remain unclear. The response of different enzymes to the same pollutant may vary greatly, and the same enzyme may respond differently to different pollutants (He et al. 2003). Hormesis is a dose–response concept that is characterized by low-dose stimulation and high-dose inhibition (Calabrese 2008). Most enzyme activities showed hormesis effects when treated with different concentrations of Cd and Pb (Fig. 3). Microorganisms require certain metals at low concentrations as essential micronutrients for vital cofactors for metalloproteinase and certain enzymes, but heavy metals become toxic to most microorganisms at high concentrations (Nies 1999). However, urease activity showed a linear decrease with increasing Cd and Pb concentration in the present study, and this result is consistent with other research (Jin et al. 2014). Soil urease, as an exocellular enzyme, mainly originates from soil microbial metabolites and root exudates, and heavy metal would inhibit microorganism or root growth and then reduce the urease source. Amylase can degrade polysaccharides (starch) to glucose, and invertase (sucrase) can hydrolyze sucrose, a common plant disaccharide, to two reducing hexoses (glucose and fructose) through the breakdown of the glycoside bond (Li et al. 2009). Our study revealed that amylase activity was inhibited, while sucrase activity was slightly stimulated. High heavy metal concentration could inhibit the amylase activity by destroying cells and restraining catabolic or anabolic reaction, while soil microbes could produce more invertin enzyme because microbes would require more C for maintenance (Mora et al. 2005). Catalase, as an important indicator of soil oxidation–reduction potential, can catalyze the decomposition of hydrogen peroxide to water and oxygen (Chelikani et al. 2004). Heavy metal stress could induce oxidative stress in organisms, and increased catalase activity is usually observed in organisms to resist oxidative stress (Patel and Patra 2015). This may explain why catalase activity was not seriously inhibited under the stress of combined Cd and Pb pollution.

Soil enzyme activities showed considerable changes after different phytoremediation strategies (Jusselme et al. 2013; Lu and Zhang 2014; Yang et al. 2015). Remediation could improve or inhibit soil enzyme activities by alleviating heavy metal stress or causing new environmental stress (Kelly and Tate 1998; Mora et al. 2005; Udovic and Lestan 2012). In the present study, amendments had positive effects on dehydrogenase, urease, and catalase activities but negative effects

on sucrose (Fig. 3). This response was also observed from the DCCA ordination graph (Fig. 4) in which sucrose was located in the right side of the ordinate far from remediations and other enzymes. The decreased sucrose activity after phytoextraction may be a response for alleviating heavy metal stress. Liu et al. (2007) reported that cadmium added to soil in doses from 5 to 100 mg kg⁻¹ stimulated the activity of invertase (sucrose), which was very similar to our results. Furthermore, soil enzyme activity usually changes with the varying demand for the metabolism of plants or microorganisms (Burns et al. 2013). Hence, decreased sucrose was also probably related to hydrolysed amylose providing more maltose by improved amylase activity under high metal stresses (Fig. 3), which alleviated the demand for glucose from hydrolyzing sucrose. Contrary to sucrose, oxidoreductase (dehydrogenase and catalase) was stimulated in the alleviation of Cd and Pb stress by remediation, especially in the combined CA and fungi treatment. These findings are similar to other studies in which dehydrogenase and catalase activities were improved in heavy metal polluted soil after remediation (Mandal et al. 2014; Yang et al. 2015).

Soil enzyme activities can be inhibited or stimulated under various environmental conditions, and their performance is mainly dominated by interactions within the plant/microorganism/soil system (Lyubun et al. 2013). In this investigation, adding microorganisms enhanced most soil enzyme activities because of the decreased heavy metal stress and increased plant biomass in the fungus-assisted phytoextraction. Furthermore, at high metal concentrations, the fungi may provide a protective function (filter function) to alleviate pollution stress (Sell et al. 2005). Microorganisms can produce organic acids such as fumaric acid, succinic acid, lactic acid, malic acid, and citric acid in their metabolic processes (Tsao et al. 1999). These microbial metabolites have the potential to alleviate the heavy metal stress on many soil enzyme activities by mobilizing heavy metals that can then be easily removed by plants (Becerra-Castro et al. 2013; Braud et al. 2009). Addition of citric acid can also enhance heavy metal availability by chelation, and high heavy metal availability is one of the key factors to increase the uptake of metals in the hyperaccumulator. Deng et al. (2013) also indicated that bioleaching (fungus) and chemical leaching (citric acid) have similar heavy metal removal efficiencies. This could partly explain why the two remediation strategies had similar effects on soil enzyme activities in assisting phytoextraction. Moreover, the potential toxicity of chelates could be reduced by controlling their type and dosage. In general, the responses of soil enzyme activities in microorganisms, plants, and chemistry combined remediation are very complex, which mainly results from the combined influence of pollution stress, exogenous microorganisms, and chelating agents in the original soil environment. However, in this study, a rough rule was observed that assisted phytoextraction with fungus, citric acid,

or their combination promoted hydrolase and oxidoreductases to different degrees. Hence, the assisted methods not only increased the uptake of heavy metals in phytoextraction but also improved many enzyme activities in soil, especially under high heavy metal stress.

Conclusions

Addition of citric acid or fungi alleviated heavy metal stress to plant growth and increased the accumulation of Cd and Pb in *S. nigrum*. However, *S. nigrum* was a hyperaccumulator for Cd, but not for Pb. Remediation efforts revealed positive effects on the activities of most soil enzymes under high Cd and Pb stress. The responses of soil enzyme activities with fungi addition were similar to those of citric acid addition. All remediation strategies promoted urease, dehydrogenase, and catalase activities. In conclusion, fungi and citric acid-assisted phytoextraction not only increased the accumulation of Cd and Pb in *S. nigrum*, but also produced a satisfactory result under high Cd and Pb stress through soil enzyme activity assessment.

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