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Feasibility of Chinese cabbage (*Brassica bara*) and lettuce (*Lactuca sativa*) cultivation in heavily metals–contaminated soil after washing with biodegradable chelators

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ABSTRACT

Soil washing with biodegradable chelator is a promising technique for treating metal-contaminated soil. However, limited information is available on the effects of such treatments on plant growth and the accumulation of residual metals from remediated soil. Four biodegradable chelators including glutamate–N,N–diacetic acid (GLDA), iminodisuccinic acid (ISA), polyaspartic acid (PASP), and glucomonocarbonic acid (GCA) were employed to remove Cd, Pb, and Zn from polluted soils, and two common vegetables (*Brassica bara* and *Lactuca sativa*) were used to verify the fitness of plants grown on the washed soil. The ISA and GLDA demonstrated excellent Cd, Pb, and Zn removal efficiencies (25–85%) compared with GCA and PASP. Moreover, the phytoavailability of soil Cd, Pb, and Zn decreased after washing, and this effect was more pronounced for GLDA than ISA. *B. bara* and *L. sativa* grew well in the washed soils but considerably less biomass was produced than by plants grown in unwashed soil. Their photosynthetic capacities, oxidation defense abilities, and nutritional qualities were improved, compared with plants cultivated in the original soils. The ISA and GLDA treatments decreased the Cd, Pb, and Zn concentrations in *B. bara* and *L. sativa* shoots but the GCA and PASP treatments had no effect or increased the metal concentrations. *B. bara* and *L. sativa* grown in remediated soils were unacceptable for human consumption because the Cd and Pb concentrations in the edible parts were higher than the European legal limits. Soil washing with biodegradable chelators especially ISA and GLDA successfully removed toxic metals and allowed the plants to grow well while decreasing the uptake of metals remaining in the remediated soil.

1. Introduction

Soil contamination with potentially toxic elements (PTEs) through mining and smelting activities, wastewater irrigation, and improper chemical fertilizer application poses potential risks to ecosystems and food security (Boente et al., 2017). Cadmium (Cd), lead (Pb), and zinc (Zn) are non–biodegradable PTEs that are extremely persistent in soil (Alqadami et al., 2018). Much attention is being paid to soil Cd, Pb, and Zn because they are toxic, carcinogenic, and teratogenic (Zhou et al., 2018; Mu'azu et al., 2018). The development of cost–effective and environmentally benign techniques is regarded as a viable and ecologically sustainable approach for remediation PTEs–contaminated soil.

Various treatments such as solidification/stabilization (Beiyuan et al., 2018; Yoo et al., 2018; Tang et al., 2018), phytoremediation (Luo et al., 2018; Komínková et al., 2018), and electroremediation (Tang et al., 2017; Fu et al., 2017) have been proposed and implemented to mitigate and/or minimize the risks posed by PTEs to human health and the environment. Soil washing with chelators (Beiyuan et al., 2018; Lestan, 2017), among the remediation techniques, is one of the few available treatments for permanently removing metals from polluted soil that has recently been highly recommended as a feasible strategy. The simplicity of this technology, its short operation time and high efficiency, make it a

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Abbreviations

AAS	Atomic absorption spectrophotometer
CAT	Catalase
Cd	Cadmium
Chl a	Chlorophyll a
Chl b	Chlorophyll b
DTPA	Diethylenetriaminepentaacetic acid
EDDS	[S,S]–ethylenediaminedisuccinic acid
EDTA	Ethylenediaminetetraacetic acid
EU	European Union
GCA	Glucomonocarbonic acid
GLDA	Glutamate–N,N–diacetic acid
GSH	Glutathione
ISA	Iminodisuccinic acid
MDA	Malondialdehyde
NBT	Nitro–blue tetrazolium
PASP	Polyaspartic acid
Pb	Lead
POD	Peroxidase
PTEs	Potentially toxic elements
ROS	Reactive oxygen species
SOD	Superoxide dismutase
Zn	Zinc

practical option for PTEs removal (Asadzadeh et al., 2018; Tang et al., 2017). Selecting a suitable chelator is critical to the efficiency of the washing process (Asadzadeh et al., 2018). One of the most prevalent chelators is ethylenediaminetetraacetic acid (EDTA). It is frequently used for removing PTEs with soil washing (Golmaei et al., 2018; Sawai et al., 2017) but has been criticized because of its poor biodegradability and its persistence in soil (Mu'azu et al., 2018; Wang et al., 2016). [S,S]–ethylenediaminedisuccinic acid (EDDS) has been proposed as an environmentally–friendly substitute for EDTA (Beiyuan et al., 2017, 2018; Yoo et al., 2018) because it is very biodegradable and gives comparable soil washing efficiencies to EDTA (Wang et al., 2018). However, more eco–friendly and effective biodegradable chelators for soil washing are still desired (Mu'azu et al., 2018).

Biodegradable chelators such as polyaspartic acid (PASP) (Mu'azu et al., 2018; Fu et al., 2017), glutamate–N,N–diacetic acid (GLDA) (Tang et al., 2017; Suanon et al., 2016; Wang et al., 2016), iminodisuccinic acid (ISA) (Wu et al., 2015), and glucomonocarbonic acid (GCA) (Wang et al., 2018) have been described in many publications because they are biodegradable and have low toxicities (Pinto et al., 2014; Sawai et al., 2017). PASP and GCA are much more biodegradable than other common chelators such as EDTA (Wang et al., 2018; Mu'azu et al., 2018), and more that 60% of GLDA (Suanon et al., 2016) and ISA (Wu et al., 2015) in soils has been found to degrade within 28 d. Moreover, like other biodegradable chelators, and in contrast to strong acid or basic solutions, these chelators generally affect the soil composition, aggregate structure, and the fertility level little (Komínková et al., 2018; Wang et al., 2018). In addition, because they contain different types and amounts of groups (Fig. S1), their abilities for complexation polyvalent ions over an extensive pH range are different. Recent biodegradable chelator washing studies have predominantly been focused on identifying optimum operational conditions (Ferraro et al., 2016; Mu'azu et al., 2018; Wang et al., 2016) and determining metal removal efficiencies (Beiyuan et al., 2017; Suanon et al., 2016). We have previously investigated the fate and toxicity of residual metals (Wang et al., 2018) and changes in soil properties (Wang et al., 2016) in soil remediated by biodegradable chelator washing. However, the effects of washing with these biodegradable chelators on the overall health (Hu et al., 2014), ecological function, and reuse of remediated soil (Jelusic et al., 2013, 2014a) have not been studied sufficiently.

Decreasing pollutant concentrations is a crucial remediation objective, but attention should also be paid to reestablishing a healthy ecosystem capable of supporting fauna, flora, and microbes to ensure the treated soil is safe and can be sustainably reused (Guo et al., 2016; Jelusic and Lestan, 2015). Soil washing processes may adversely affect the activities of soil enzymes such as dehydrogenase (Im et al., 2015), acid phosphatase (Im et al., 2015; Wang et al., 2018), arylsulfatase (Yi and Sung, 2015), β–glucosidase (Chae et al., 2017; Kaurin et al., 2018), and urease (Chae et al., 2017). These enzymes play critical roles in organic matter turnover and nutrient cycling in soil (Im et al., 2015; Kaurin et al., 2018). Soil washing can also simultaneously solubilize mineral elements (i.e., Ca, Mg, Al, and Fe) with the removal of metals (Wang et al., 2017; Yip et al., 2010). These changes in soil properties caused by soil washing can affect the long–term function and health of the soil and may make remediated soil less suitable as a substrate for plants than untreated soil (Fedje and Strömvall, 2016). It has been found that residual metal–chelant complexes in treated soils can transfer metals from strongly to weakly bound fractions and prevent co–precipitation (Beiyuan et al., 2017, 2018), thus arousing concerns about on–site reuse of washed soil for cultivating plants. Consequently, there is an urgent need to verify whether the remediated soils using biodegradable chelator washing are suitable for plant cultivation or agricultural production.

Concern about crops cultivated in remediated soil has recently increased, but the available information is contradictory. Hu et al. (2014), Jelusic et al. (2014a), and Zupanc et al. (2014) reported that growth and biomass production were suppressed but Cd, Pb, and Zn uptake decreased when several common vegetables and grasses were grown in EDTA–washed compared with when the plants were grown in unwashed soil. Jelusic and Lestan (2015), however, showed that ornamental plants and grasses grew better in EDTA–remediated soil than in original soil, and this was corroborated in other studies (Ye et al., 2016; Kim et al., 2017). Larger amounts of Cd, Pb, and Zn have been found in the aboveground parts of *Brassica rapa* (Jelusic et al., 2013) and *Zea mays* (Guo et al., 2016) planted in washed soil than in initial soil.

Little information is available on the feasibility of plants cultivation in soils remediated with biodegradable chelators such as PASP, GLDA, and ISA. Chinese cabbage (*Brassica bara*) and lettuce (*Lactuca sativa*) are commonly grown around the world (Jelusic et al., 2013; Komínková et al., 2018), so we performed experiments in which these plants were grown in washed soil. This work aimed at examining the fitness of *B. bara* and *L. sativa* cultivated in heavily metals–contaminated soil before and after washing with PASP, GLDA, GCA, and ISA. The novelty of this investigation was to quantify the influence of these treatments on (i) the Cd, Pb, and Zn removal efficiencies and bioavailabilities and (ii) the growth response and physiological changes such as photosynthetic capacity, oxidation defense ability, and nutritional quality, and accumulation of residual metals in *B. bara* and *L. sativa*. The results are expected to support the sustainable management and agricultural use of remediated soil.

2. Materials and methods

2.1. Soil sample collection and characterization

Two field–contaminated soils were collected from Sichuan, China. One (later called 'mine soil') was obtained from an abandoned lead–zinc mine (29°24′ N, 102°39′ E) and the other (later called 'farmland soil') from a paddy field near a non–ferrous metal smelter (30°59′N, 103°57′E). The soils were chosen on the basis of the results of previous studies (Wang et al., 2016, 2018); moreover, they represented different sources and degree of pollution, making the experiments representative of more situations than would have been possible using only one soil. The total Cd, Pb, and Zn concentrations in each soil were determined by digesting a 0.5 g aliquot of the soil in a mixture of 5.00 mL of HNO_3 and 1.00 mL of HF in a GHZ–16 microwave digestion system (Beijing Guohuan Institute of High-tech Automation, Beijing, China) and analyzing the digest using a Thermo Solaar M6 atomic absorption spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). The potentially plant–available metal contents in soils were assessed using diethylenetriaminepentaacetic acid (DTPA) solution as reported by Beiyuan et al. (2018) and Kulkarni et al. (2018). Soil (5.0 g) was extracted with 50 mL of solutions containing 0.005 mol L^{-1} DTPA, 0.01 mol L^{-1} $CaCl_2$, and 0.1 mol L^{-1} triethanolamine buffer at pH 7.3 in an end–over–end rotator (30 rpm) for 2 h at room temperature. Their physicochemical characteristics are presented in Table S1. A detailed description for determining these properties is available from previous work (Wang et al., 2016).

2.2. Soil washing experiments

Four biodegradable chelators (ISA, GLDA, PASP, and GCA) were selected as washing agents. The structures of the chelators are shown in Fig. S1. Each chelator solution was freshly prepared by dissolving or diluting an appropriate amount of the chelator in deionized water and adjusted pH to 5.00 using dilute HNO_3 and NaOH solutions. Biodegradation was prevented by added 200 mg L^{-1} NaN_3 (0.05–0.10 mL) and photodegradation was prevented by covering each container with aluminum foil (Beiyuan et al., 2018; Gan et al., 2017). The solutions were stored at 4 °C before use. Polluted soil (1 kg) was mixed with a washing liquid (50 mmol L^{-1}, pH 5.00) in an acid–rinsed polyethylene bottle fixing the liquid–to–soil ratio to 5:1 as suggested in previous publications (Wang et al., 2017, 2018; Ye et al., 2016). The mixture was stirred in an end–over–end rotator at 150 rpm for 2 h, then centrifuged at 4000 rpm for 10 min and passed through a 0.45–μm filters. The Cd, Pb, and Zn concentrations in the supernatants were determined using atomic absorption spectrophotometer (AAS). Additional 5–min washing with deionized water was carried out to displace entrapped and loosely bound metal–chelator complexes and residual reagents (Yip et al., 2010), then the remediated soil was centrifuged and air-dried before being analyzed and used in the plant culture experiments. EDTA, being the most frequently cited non–biodegradable synthetic chelator in chelator–assisted soil washing remediation procedure (Mu'azu et al., 2018; Sawai et al., 2017), was selected as the reference to which the performance of the biodegradable chelators was compared. Each experiment was executed with four replicates to ensure the results were reproducible and reliable. The Cd, Pb, and Zn removal efficiencies were calculated using Equation (1), from the ratio of the extracted metals through soil washing to the total content of metals in the soil as obtained by microwave digestion.

$$\text{Removal } efficiency(\%) = \frac{C_{solution} \times V}{M \times C_{total}} \tag{1}$$

where $C_{solution}$ is the metal concentrations in the leachate (mg L^{-1}); V is the washing solution volume (L); M is amount of soil washed (kg); C_{total} is the total metal concentrations (mg kg^{-1}).

2.3. Plant culture

The effects of remediated soils on *B. bara* and *L. sativa* were investigated by growing plants in a greenhouse at the ambient temperatures and light conditions. A series of 15 cm–diameter and 20 cm–height pots were prepared, each containing a 2.0 kg aliquot of unwashed or washed soil. The soil in each pot was allowed to equilibrate for one month. Nitrogen ($CO(NH_2)_2$), phosphate ($Ca(H_2PO_4)_2\ H_2O$), and potassium (KCl) fertilizers were applied at rates of 0.65, 2.00, and 0.50 g pot^{-1}, respectively. Seeds were sterilized in H_2O_2 solution (2%, v/v) for 15 min, rinsed 4–5 times with tap water, and soaked in deionized water overnight. Ten seeds were sown in each pot, and after two weeks seedlings were removed to leave one *B. bara* seedling or two *L. sativa* seedlings. The plants were subsequently grown for eight weeks, irrigated with tap water that did not contain detectable PTE concentrations.

2.4. Plant sampling and biochemical analysis

After eight weeks, the aboveground (shoots) and underground (roots) of the plants were harvested separately, rinsed thoroughly with tap water followed by deionized water, and the fresh weight recorded. Each sample was then dried at 70 °C for 72 h in an oven up to constant weight, ground to a fine powder, and stored in a polyethylene bag.

2.4.1. Photosynthetic pigment

The chlorophyll a (Chl a), chlorophyll b (Chl b), and carotenoid contents of the plant leaves were quantified using a method described by Li et al. (2018). Fresh leaves (0.2 g) were ground with 5 mL of 80% (v/v) ice–cold acetone, and the mixture was centrifuged at 4000 rpm for 15 min in a TGL–20 M refrigerated centrifuge (Shanghai Lu Xiangyi centrifuge instrument co., Ltd., Shanghai, China). The supernatant was transferred to a fresh tube and the residue re–extracted twice with 2.5 mL of 80% (v/v) ice–cold acetone. The Chl a, Chl b, and carotenoid contents were determined using a 7600CRT UV–vis spectrophotometer (Shanghai Jinghua Instruments, Shanghai, China) at wavelengths of 663, 645, and 470 nm, respectively and expressed as mg g^{-l} fresh weight.

2.4.2. Oxidative damage and oxidative defense system

Fresh shoots were stained with 3,3–diaminobenzidine (Thordal-Christensen et al., 1997) and nitro–blue tetrazolium (NBT) (Romero-Puertas et al., 2004) as presented in literature (see details in Supporting Information (SI), Section S1). The hydrogen peroxide (H_2O_2) and superoxide radical ($O_2^{\bullet -}$) concentrations were determined by the method suggested by Romero-Puertas et al. (2004). The malondialdehyde (MDA) was characterized with mixed 2.00 mL of enzyme extract and 2.00 mL of 0.6% (w/v) thiobarbituric acid, placing the mixture in a boiling water bath for 15 min, and then recorded absorption at 450, 532, and 600 nm as described by Liang et al. (2008).

Fresh shoots (0.5 g) were ground to a fine powder in liquid nitrogen using a mortar and pestle and subsequently transferred to another pre-cooled mortar and pestle (4 °C) with 5 mL of 50 mmol L^{-1} potassium phosphate buffer at pH 7.8 which containing 0.1 mmol L^{-1} EDTA and polyvinylpolypyrrolidone. The homogenates were centrifuged at 10,000 rpm for 20 min at 4 °C, and the enzyme activities in the supernatant were determined (Farooq et al., 2015). The superoxide dismutase (SOD) activity was estimated by monitoring the inhibition of the photochemical reduction of NBT (Velikova et al., 2000). Absorbance was recorded at 560 nm and one SOD activity unit (U) was defined as 50% inhibition of NBT reduction. The catalase (CAT) activity was measured using a method described by Cao et al. (2004) by analyzing H_2O_2 consumption. One CAT activity unit defined (U) was defined as a 0.01 unit decrease in absorbance at 240 nm within 60 s at 25 °C. The peroxidase (POD) activity was assayed by guaiacol substrates following the method described by Wu and von Tiedemann (2002). Absorbance was recorded at 470 nm within 60 s after H_2O_2 addition and one enzyme activity unit (U) was expressed as a 0.01 unit increase in absorbance at 470 nm in 60 s. The glutathione (GSH) content was performed according to Nagalakshmi and Prasad (2001). All enzyme activities were expressed in U g^{-1} fresh weight. The procedures are detailed in SI, Section S2.

2.4.3. Nutritional quality

The soluble protein contents of the edible parts of *B. bara* and *L. sativa* were estimated according to Bradford (1976). Soluble sugar was extracted with distilled water in a water bath at 85 °C and analyzed using the anthrone–sulfuric acid method (Fairbairn, 1953). Vitamin C was analyzed using the method illustrated by Arakawa et al. (1981). The detailed protocols are displayed in SI, Section S3.

2.4.4. Cd, Pb, and Zn concentrations in plant tissue

The Cd, Pb, and Zn contents of the dried shoots and roots were measured. A powdered sample (1.0 g) was digested in 5.00 mL of concentrated HNO_3 in the microwave digestion system, then the digest was filtered through 0.45–μm filters and diluted with deionized water. The Cd, Pb, and Zn concentrations in each samples were analyzed by AAS.

2.5. Quality assurance, quality control, and statistical analysis

A standard soil reference material (GBW07405) provided by the China National Center for Standard Materials (Beijing, China), was digested and analyzed, and the metal recoveries were 96–105%. Analytical duplicate and reagent blanks were run to determine the accuracy and precision of the method. All the data presented are means ± standard deviations of a specified number of replicates in which the error limits of the analysis within 5%. The significant differences between metal removal efficiencies, biomass, stress response, nutritional quality, and metal content in plants were evaluated using Tukey's multiple range tests, performed using the Statistical Packages for the Social Sciences software version 22.0 (SPSS Inc., Chicago, USA). $P < 0.05$ was considered significantly.

3. Results and discussion

3.1. Removal efficiencies and plant-availability of metals

The mine soil and farmland soil were heavily polluted with PTEs: 15.42, 1292, and 2278 mg kg^{-1} of Cd, Pb, and Zn and 36.18, 268.2, and 1082 mg kg^{-1} of Cd, Pb, and Zn, respectively. The effects of different biodegradable chelators on soil Cd, Pb, and Zn removal are shown in Fig. 1. The soil Cd, Pb, and Zn removal efficiencies by individual biodegradable chelator washing had significant difference. They ranged in between 7 and 93%, 0–71%, and 5–64%, respectively, depending on the washing reagents, the metal considered, and the soil used. The ISA and GLDA exhibited excellent removal capabilities compared with EDTA, and were considerably more effective than GCA and PASP ($P < 0.05$, Fig. 1). The potential mechanisms responsible for cleaning metals from contaminated soil by washing with biodegradable chelators are mainly through chelation, ion exchange, and solubilization (Ferraro et al., 2016). The ISA and GLDA are exceptionally effective at removing Cd, Pb, and Zn (Fig. 1), as was previously observed by Suanon et al. (2016), Wang et al. (2016), and Wu et al. (2015). They have more carboxylic groups (Fig. S1), which could explain Cd, Pb, and Zn being more mobilized by them than by the other two biodegradable chelators (Fig. 1). These carboxylic groups will facilitate ligand–metal ion complexation and the formation of metal–chelator complexes (Sawai et al., 2017; Wang et al., 2018; Yip et al., 2010).

Metal bioavailability needs to be taken into account in addition to the metal removal efficiency. DTPA–extraction has widely been used to evaluate the bioavailability of residual PTEs in soil (Beiyuan et al., 2018; Yoo et al., 2018). The bioavailability of Cd, Pb, and Zn in the washed soils decreased remarkably in comparison with the original soils ($P < 0.05$, Fig. 1) except for Pb in the soils washed with GCA and PASP ($P > 0.05$). No significant changes in plant–availability of Cd, Pb, and Zn in the remediated soils between the EDTA and GLDA treatments were observed ($P > 0.05$) except for Zn in the farmland soil (Fig. 1F). The accumulation of PTEs from remediated soil to the edible part of plant was positively correlated to their bioavailable concentrations (Zhou et al., 2018). Reduction of bioavailability of PTEs could therefore alleviate the impacts of PTEs on plants and allow the remediated soil to be used for agriculture. In the present study, the DTPA–extractable Cd, Pb, and Zn concentrations were lower in the washed soil compared with the non–treated soils (Fig. 1). This indicated that biodegradable chelator washing diminished metal mobility to different extents and removed the most labile metals (Jelusic et al., 2013; Suanon et al., 2016; Yoo et al., 2018). A similar decrease in metal plant–availability was observed through soil washing with EDTA (Hu et al., 2014; Voglar and Lestan, 2012).

3.2. B. bara and L. sativa cultivated in washed soil

3.2.1. Plant growth, morphology, and pigment content

Both *B. bara* and *L. sativa* could grow well in washed soils throughout the experiment, with no visible symptoms of toxicity such as yellow leaves or dysplasia. However, *B. bara* and *L. sativa* had significantly lower biomasses when grown in the washed soils than when grown in the untreated soils ($P < 0.05$; Fig. 2). For the mine soil, the highest decrease of the biomass of shoots and roots in *B. bara* was obtained by a factor of 11.7 and 4.9 for EDTA, followed by GCA and PASP treatments, respectively. While these factors were 3.1 and 2.9 in case of PASP treatment, followed by EDTA and ISA treatments for *L. sativa*. Similarly, the plant biomass in the treated farmland soil was also significantly decreased to approximately 1.3–5.9 times of the control ($P < 0.05$), except for *B. bara* grown in the ISA–washed soil (Fig. 2). Whereas the maximum decrease values for shoots and roots of *B. bara* and *L. sativa* were observed by 1.9 and 2.5–fold and 5.9 and 5.4–fold of the control by the EDTA treatment, respectively, followed by PASP treatment.

It has previously been found that plants produced more biomass when grown in washed soil than unwashed soil. Jelusic and Lestan (2015) reported for example that the fresh weight of ornamental and grass plants on remediated soil was remarkably higher (up to 3.5 times) than those of them grown on the original soil. This was also in line with Ye et al. (2016), who observed that the root and leaf fresh mass of *L. sativa* cultivated in the washed soil increased by 24 and 6%, respectively, compared with plants grown in the non–treated polluted soil. However, in the present study, a decrease of plant biomass (4–91%) for all treatments was observed except for ISA–washed farmland soil as compared to the control (Fig. 2). Zupanc et al. (2014) found that *Trifolium repens* dry mass yield was up to 40% higher on original contaminated soil than on the EDTA–remediated soil. These results also were corroborated with Hu et al. (2014) in which the fresh weight of pak choi grown in

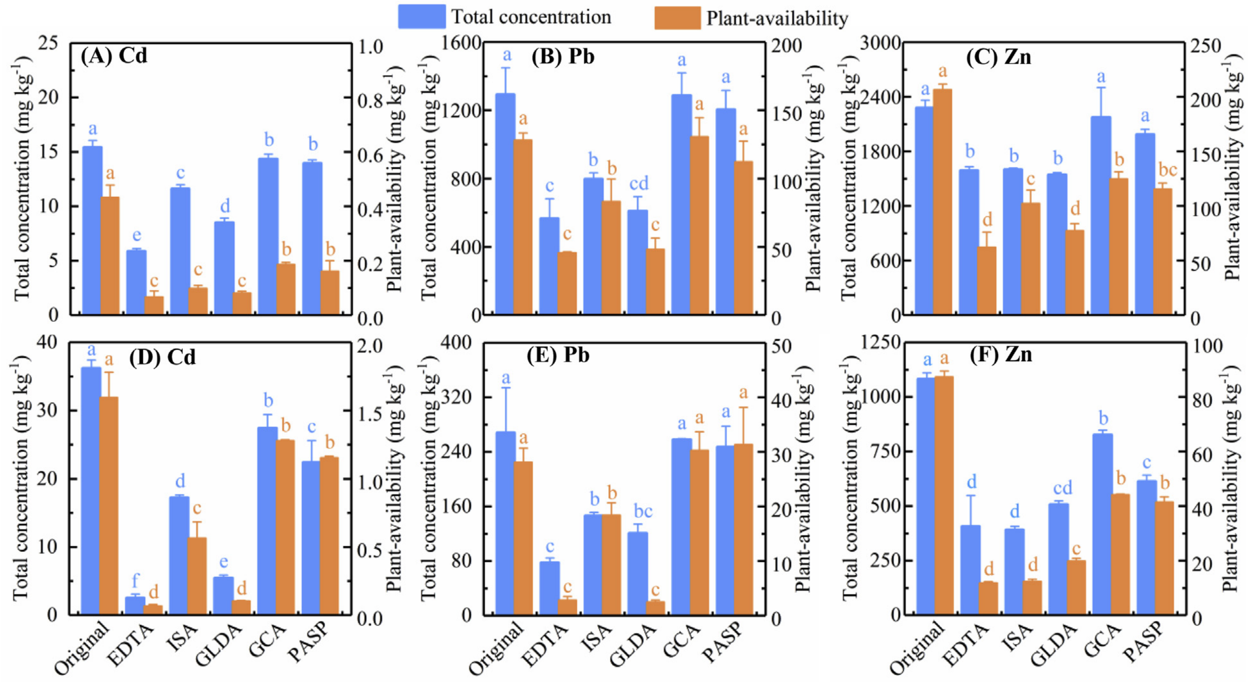

Fig. 1. Total and DTPA–extractable concentrations of Cd, Pb, and Zn before and after washing with biodegradable chelator with a contact time of 2 h, L/S ratio of 5:1, reagent dose of 50 mM, and pH of 5.00 in mine soil (A, B & C) and in farmland soil (D, E & F). Means ($n = 4$) followed by different lowercase letters indicate significant differences at the $P < 0.05$ level between treatments according to the Tukey's test. Error bars indicate the standard deviation of the mean.

Fig. 2. The morphology and biomass of *B. bara* (A, B, E & F) and *L. sativa* (C, D, G & H) for different treatments in mine soil (A, C, E & G) and in farmland soil (B, D, F & H). Error bars represent the standard deviation of four replicates. Values followed by different lowercase letters within the same group differ significantly the $P < 0.05$ level (Tukey's test). FW: fresh weight.

EDTA–washed soil was 90% less in the first harvest and 12% less in the second harvest compared with the initial soil. Adverse effects of the chelator application to soil on plant yield were also reported by Jelusic et al. (2014a, 2014b).

Plant growth in washed soils could be affected by several factors, including the fertility level and the existence of toxic metal–chelate complexes (Cheng et al., 2018; Jelusic et al., 2013; Komínková et al., 2018). The loss of soil macro– and micronutrients such as K, Fe, Ca, Mn, and Mg during washing process could be a potential growth–inhibiting factor (Fedje and Strömvall, 2016; Im et al., 2015; Jelusic et al., 2014b; Wang et al., 2016; Yi and Sung, 2015). The reduction of biomass of *B. bara* and *L. sativa* grown on the remediated soils in this work may be due to soil available nitrogen and potassium being removed by washing with the biodegradable chelators (Table S1). In addition, the metal–chelator complexes formed during soil washing probably remained bound to the solid phases through adsorption with the mineral surface such as crystalline iron oxides, particularly goethite (Guo et al., 2016; Jelusic et al., 2013; Voglar and Lestan, 2012; Yip et al., 2010) and this could affect plants' health. Phytoremediation experiments have also demonstrated that the application of EDTA could severely hinder the plant growth, because of the increased essential metal dissolution or, through its interference with cellular structures and/or functions (Luo et al., 2018). More effective revitalization approaches, such as amendment with biochar, are therefore clearly required to re–establish and restore the nutrient pool to washed soil to allow the soils to be used as fertile plant substrates (Beiyuan et al., 2018; Kaurin et al., 2018; Yoo et al., 2018).

The *B. bara* and *L. sativa* photosynthetic pigment contents, as an indicator of photosynthetic capacity and biomarker of contaminant stress and contributor to the capture of light energy (Li et al., 2018), were generally higher in washed soil as compared to the untreated soil (Fig. S2). No significant changes were found in Chl a, Chl b, and carotenoid contents for *L. sativa* in both soils ($P > 0.05$) except for carotenoid contents in the PASP treatment of farmland soil. However, the impact of remediated soil on the photosynthetic pigment contents for *B. bara* planting was more obvious. The highest Chl a and Chl b contents (1.1 and 1.4–fold higher, respectively, than in the controls) were found in ISA–washed mine soil. Correspondingly, these contents were enhanced by 1.2 and 3.5 times in the farmland soil as compared to the control. The enhancement of chlorophyll and carotenoid contents could be attributed to the lowered loss of essential macro– and microelements supplied with the pigment biosynthesis as well as immobilization of availability of PTEs (Chu et al., 2018) after biodegradable chelator washing. Ye et al. (2016) showed that the chlorophyll contents of *L. sativa* increased by 47% after cultivation in a soil that was washed twice, in comparison with plants cultivated in the original contaminated soil. In a recent study, photochemical energy conversion of *B. bara* cultivated in 10–60 mmol kg^{-1} EDTA washed garden soil was slightly higher than unwashed soil (Jelusic et al., 2013).

3.2.2. *Reactive oxygen species (ROS) level*

The MDA is a widely accepted physiological indicator of lipid peroxidation in plants (Liang et al., 2008). H_2O_2 and $O_2^{\bullet-}$ are representative ROS, the overproduction of which could pose oxidative damage to proteins, DNA, and lipids and may increase the MDA content of a plant (Etesami, 2018). Plants balance ROS production and MDA content under natural conditions, but this delicate balance is destroyed under stressful conditions (Farooq et al., 2015; Romero-Puertas et al., 2004) such as exposure to PTEs. In this work, the MDA contents were 11–69% lower for the washed soils than the controls (Fig. S3). Specifically, the MDA contents displayed no significant decrease in the GCA– and PASP–washed mine soils ($P > 0.05$), while they decreased substantially by 26–64% in farmland soil ($P < 0.05$) compared with the control, respectively. A plant subjected to external stress could generate ROS such as $O_2^{\bullet-}$ and H_2O_2 as aerobic metabolism byproducts. These were identified using a histological staining method. Relatively deep blue staining was observed in the treatments of EDTA, GCA, and PASP and the control (Fig. S4). The quantified formazan spots indicated that less formazan precipitate was produced by plants grown in ISA– and GLDA–washed soils than in control, EDTA, GCA, and PASP treatments (Fig. 3E–H). These results demonstrated that ISA and GLDA treatments induced oxidative damage slightly. 3,3' –diaminobenzidine staining, in which red–brown staining indicates the presence of H_2O_2, provided further evidence for this (Fig. 3A–D). The plants in the soil treated with ISA and GLDA were almost transparent (except *L. sativa* grown in ISA–washed farmland soil), while the plants in the other treatments went deep brown (Fig. S5).

3.2.3. *Antioxidant defense system*

To control ROS levels, plants are well–equipped with a detoxification system. This system involves synthesizing antioxidant enzymes (Liang et al., 2008) such as SOD, POD, and CAT and antioxidants (Yadav et al., 2018) such as GSH to prevent or alleviate oxidative stress. SOD, the first defence against oxidative damage, catalyzes the transformation of $O_2^{\bullet-}$ into H_2O_2 and the excess H_2O_2 is converted into H_2O by CAT and POD (Abdel Latef et al., 2017). Antioxidant enzyme activities were significantly different in plants grown in remediated and unwashed soils (Table 1). The SOD activities in shoots of plants grown in the remediated soils reduced as compared to the control. The decrease in the activity was more remarkable for ISA and GLDA treatments. The *L. sativa* POD activities were not significantly change among treatments ($P > 0.05$); but POD activities in *B. bara* were1.3 and 1.7 times higher for the EDTA– and GCA–washed mine soils, respectively, and 1.3 and 1.6 times higher for the EDTA– and ISA–washed farmland soils, respectively, than for the controls. The CAT activity in plants, another antioxidant enzyme, showed differences in both mine and farmland soils. Compared with the control, CAT activity dramatically increased by 44–243% for plants grown in mine soil ($P < 0.05$) except for *B. bara* in the GCA treatment, while no obvious changes were recorded for the farmland soil. Non–enzymatic antioxidative systems involving antioxidants such as GSH also perform crucial roles in the scavenging of overproduced ROS. The GSH contents changed in similar ways to the SOD activities. The GSH contents were slightly lower in the GCA and PASP–treated group relative to the control ($P > 0.05$), but were remarkably reduced to 24–58% of the GSH contents in the control for the EDTA, ISA, and GLDA treatments.

The activities of all the examined antioxidant enzymes and antioxidant generally decreased (Table 1), and the MDA and ROS levels were clearly lower in *B. bara* and *L. sativa* grown in washed soil than unwashed soil (Fig. 3 and Figs. S3–S5). There could have been two reasons for this (Abdel Latef et al., 2017): either (i) the time to activate the antioxidant enzymes did not coincide with the increase of ROS production or (ii) the decrease in enzymatic antioxidant activities was not abundant to dominate ROS generation. Washing with ISA and GLDA boosted enzymatic antioxidant defenses more than did washing with EDTA, and the plants more effectively managed the ROS generated through exposure to contaminated soil. Application of biodegradable chelator for washing the polluted soil mitigated the oxidative stress in *B. bara* and *L. sativa* (Fig. 3 and Table 1), possibly by decreasing the total metal concentrations and plant–available Cd, Pb, and Zn concentrations (Bashir et al., 2017; Duan et al., 2018).

3.2.4. *Vitamin C, soluble protein and sugar*

The vitamin C, soluble protein and sugar contents (John et al., 2008) in plants were severely affected when plants were cultivated in heavily metals–contaminated soils. The application of soil washing with biodegradable chelators obviously increased their contents in *B. bara* and *L. sativa* when compared with the control (Fig. 4). These responses were more pronounced in the ISA and GLDA treatments than in the case of GCA and PASP treatments. Washing with ISA and GLDA increased the vitamin C contents by 90–132% for *B. bara* and 59–107% for *L. sativa* and the soluble sugar contents by 51–106% for *B. bara* and 11–51% for *L. sativa* relative to the controls. These results agreed with the results of Ye et al. (2016), who found soluble protein contents of *L. sativa* planted in soils

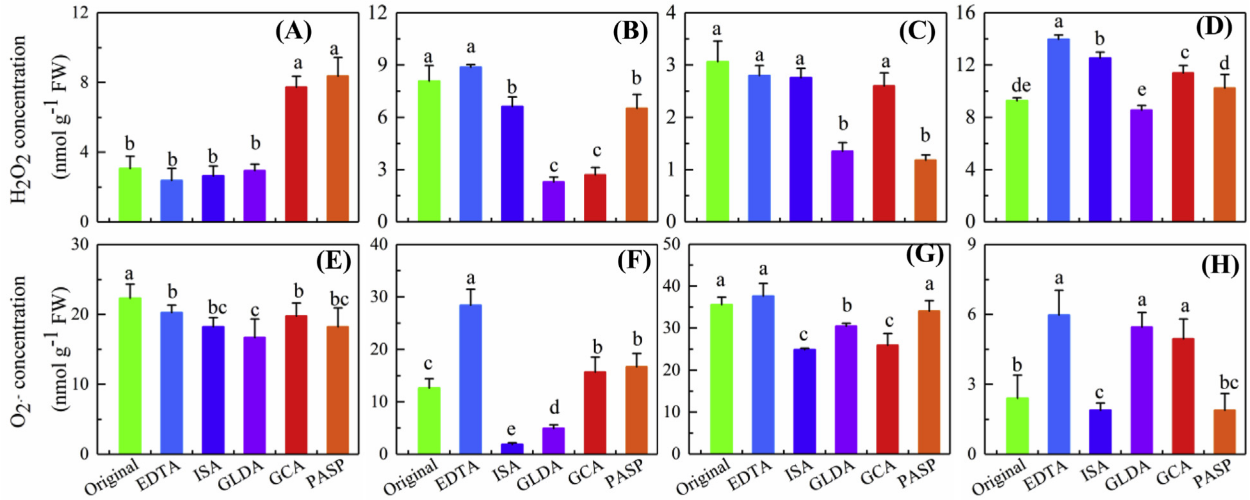

Fig. 3. The concentrations of H_2O_2 and $O_2^{\cdot-}$ accumulation in the shoots of *B. bara* (A, C, E & G) and *L. sativa* (B, D, F & H) for different treatments of mine soil (A, B, E & F) and of farmland soil (C, D, G&H). Error bars represent the standard deviation of four replicates. Values followed by different lowercase letters within the same group differ significantly the $P < 0.05$ level (Tukey's test). FW: fresh weight.

Table 1
The antioxidant system of *B. bara* and *L. sativa* grown on the remediated soils. Error bars represent the standard deviation of four replicates. Values followed by different lowercase letters within the same group differ significantly at the $P < 0.05$ level (Tukey's test). FW: fresh weight.

Soils	Plants	Washing treatments	Antioxidant capacity			
			GSH (μmol g^{-1} FW)	SOD (U g^{-1} FW)	POD (U min^{-1} g^{-1} FW)	CAT (U min^{-1} g^{-1} FW)
Mine soil	*B. bara*	Original soil	96.84 ± 10.12a	150.37 ± 25.61a	16.00 ± 1.42c	13.00 ± 1.03d
		EDTA	73.61 ± 6.51b	121.95 ± 15.62b	20.22 ± 2.69b	21.83 ± 2.04bc
		ISA	62.00 ± 10.74b	126.43 ± 17.46b	6.11 ± 2.36d	26.00 ± 2.16a
		GLDA	71.03 ± 9.65b	73.07 ± 7.62c	13.78 ± 0.25c	22.83 ± 1.95b
		GCA	88.45 ± 6.74a	66.33 ± 8.76c	27.50 ± 1.92a	4.67 ± 0.14e
		PASP	83.29 ± 10.24a	80.80 ± 9.15c	15.06 ± 0.59c	18.67 ± 1.95c
	L. sativa	Original soil	49.74 ± 5.63a	156.11 ± 26.51a	1.11 ± 0.19a	5.00 ± 0.36c
		EDTA	20.71 ± 2.51b	75.06 ± 4.75c	1.06 ± 0.26a	9.67 ± 1.07b
		ISA	23.94 ± 3.14b	46.38 ± 9.61d	1.89 ± 0.12a	10.17 ± 1.09b
		GLDA	22.00 ± 4.01b	72.07 ± 8.49c	1.11 ± 0.49a	17.17 ± 2.08a
		GCA	46.52 ± 6.37a	97.51 ± 10.34b	0.61 ± 0.26a	11.83 ± 0.61b
		PASP	41.35 ± 5.47a	134.41 ± 18.46a	2.16 ± 0.14a	13.33 ± 0.74b
Farmland soil	*B. bara*	Original soil	173.61 ± 25.64a	132.17 ± 12.36a	11.44 ± 1.67c	13.67 ± 2.18b
		EDTA	109.74 ± 14.15b	139.40 ± 20.42a	15.11 ± 1.27b	6.17 ± 0.94c
		ISA	109.10 ± 12.85b	115.46 ± 10.61a	18.72 ± 1.21a	13.33 ± 2.61b
		GLDA	118.77 ± 10.87b	138.90 ± 19.74a	4.94 ± 0.82d	7.33 ± 0.68c
		GCA	178.77 ± 30.26a	129.93 ± 15.36a	12.94 ± 0.51c	14.00 ± 1.61b
		PASP	163.94 ± 29.85a	124.94 ± 17.48a	4.39 ± 1.34d	21.50 ± 2.34a
	L. sativa	Original soil	47.16 ± 2.61b	108.23 ± 12.06a	2.11 ± 0.94a	21.50 ± 2.75a
		EDTA	28.45 ± 5.14c	83.04 ± 9.51b	1.50 ± 0.74a	16.00 ± 1.48b
		ISA	25.87 ± 3.94c	83.29 ± 7.86b	1.95 ± 0.25a	6.00 ± 0.85c
		GLDA	29.74 ± 4.18c	79.55 ± 8.91b	1.89 ± 0.99a	17.33 ± 1.29b
		GCA	65.87 ± 6.14a	106.23 ± 16.24a	2.94 ± 0.11a	24.86 ± 3.06a
		PASP	52.97 ± 4.19b	87.03 ± 8.59b	2.95 ± 0.36a	1.83 ± 0.09d

contaminated with Cd and antibiotics increased by 40% after sophoroliplid-enhanced soil washing. Similarly, the amendments with GCA and PASP increased vitamin C contents by 16–42% in *B. bara* and 15–40% in *L. sativa*; soluble sugar by 3–75% in *B. bara* and 3–25% in *L. sativa*, respectively. However, the soluble protein contents of *L. sativa* were not significantly different for any of the farmland soil ($P > 0.05$). Overall, soil remediated with biodegradable chelators substantially ameliorated the plant nutritional quality.

3.3. Uptake and translocation of metals from remediated soil

The Cd, Pb, and Zn concentrations in *B. bara* and *L. sativa* grown in the untreated contaminated soils were in most cases substantially higher than in the remediated soils (Fig. 5). The Cd, Pb, and Zn concentrations in the untreated mine soil in the edible parts of *B. bara* and *L. sativa* were 1.12, 20.14, and 350.1 mg kg^{-1} and 0.75, 16.25, and 296.5 mg kg^{-1}, respectively, while they reached 2.13, 5.26, and 195.1 mg kg^{-1} and 1.59, 3.26, and 150.6 mg kg^{-1} in the initial farmland soil, respectively. The European Union (EU) legal limits for Cd and Pb concentrations in foodstuffs should be less than 0.2 and 0.3 mg kg^{-1}, respectively (EC, 2006). The Cd and Pb concentrations in the edible parts of the plants grown in the original soils were 3.8–10.7 and 10.9–67.1 times higher, respectively, than these limits. However, in the remediated soils, the concentrations of toxic metals in the edible parts decreased considerably compared with the untreated soils but were still higher than the EU limits. Generally, the largest decline in the concentrations of toxic metals

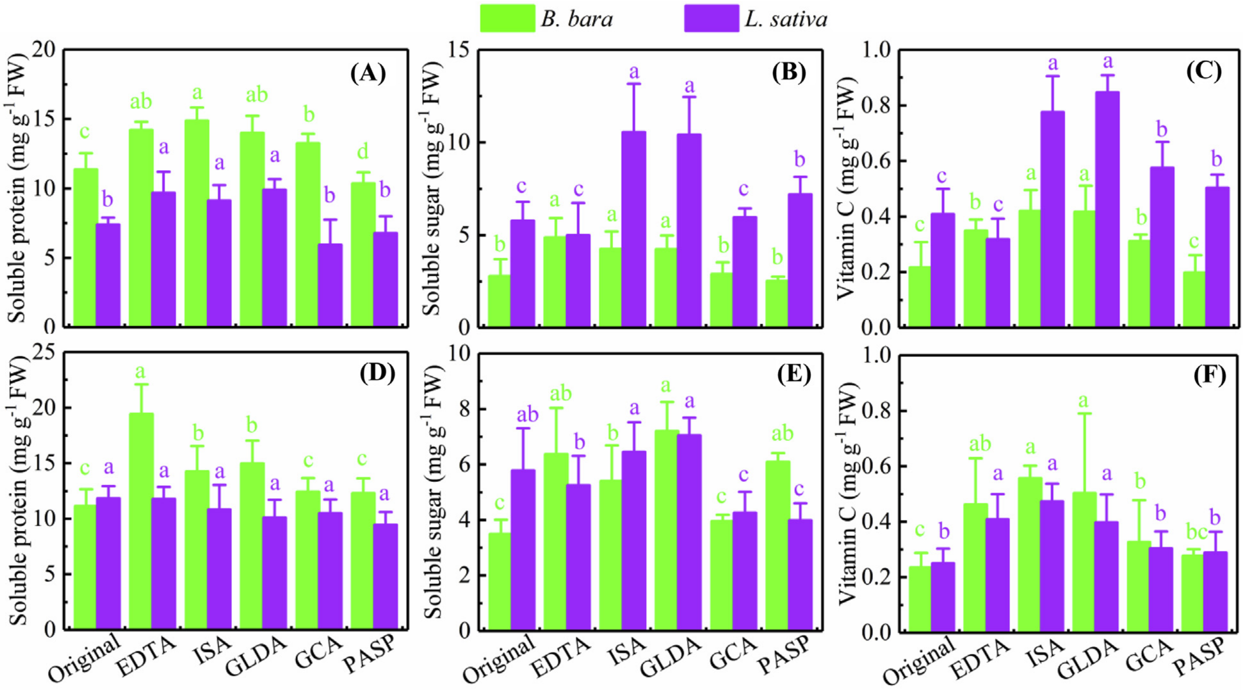

Fig. 4. Vitamin C, soluble protein and sugar contents for shoots of *B. bara* and *L. sativa* grown in the mine soil (A, B & C) and farmland soil (D, E & F) washed with different biodegradable chelators. Error bars represent the standard deviation of four replicates. Values followed by different lowercase letters within the same group differ significantly the $P < 0.05$ level (Tukey's test). FW: fresh weight.

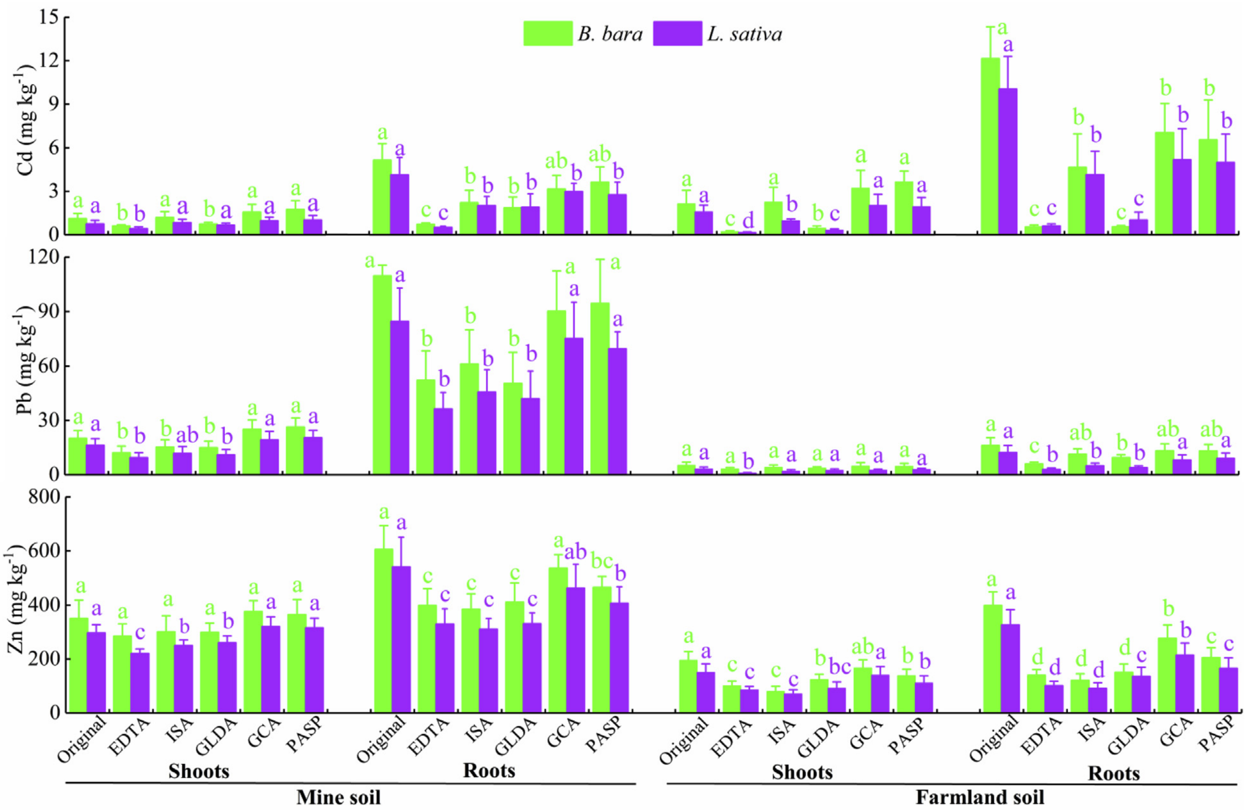

Fig. 5. Concentrations of Cd, Pb, and Zn in *B. bara* and *L. sativa* for different treatments. Error bars represent the standard deviation of four replicates. Values followed by different lowercase letters within the same group differ significantly the $P < 0.05$ level (Tukey's test).

in plants was observed for the treatments of EDTA, ISA, and GLDA with higher metal removal rates (Fig. 1). Moreover, the concentrations of Cd, Pb, and Zn were generally higher in roots than in shoots, with some exceptions. *B. bara* and *L. sativa* grown in original and remediated mine soils for example demonstrated similar values for Cd in shoots and roots in the treatment of EDTA. Meanwhile, *B. bara* accumulated more toxic metals than *L. sativa* in both soils of all treatments even when the total metal concentrations in soils were low.

Increased accumulation of PTEs in the root is a ubiquitous tactic of plants to reduce metal toxicity to the aboveground parts (Yadav et al., 2018; Etesami, 2018). This phenomenon is attributed to the metals binding with ligands present in the root. The Cd, Pb, and Zn concentrations were enriched more in the roots than shoots (Fig. 5), agreeing with the results of previous reports (Komínková et al., 2018; Ye et al., 2016; Jelusic et al., 2014a). Meanwhile, they in the roots and shoots of *B. bara* and *L. sativa* strongly correlated ($R^2 > 0.64$) with the DTPA–extractable metal concentrations in soils (Fig. 6). These results are also in accordance with the work conducted by Shahbaz et al. (2018) in which Ni concentrations in red clover significantly correlated with the DTPA–extractable Ni from polluted soil. The considerable decrease in DTPA–extractable Cd, Pb, and Zn concentrations and their correlation in plant tissues could be attributed to PTE mobilization by the biodegradable chelators. In addition, soil washing with biodegradable chelator reduced soil–root transfers of Cd, Pb, and Zn by up to 85% (Table S2) but did not prevent Cd and Pb accumulation in aboveground parts of *B. bara* and *L. sativa* (Fig. 5). This agreed with the results of a study by Jelusic et al. (2013) in which *B. bara* cultivated in EDTA–washed soils accumulated more Pb and Cd in its upper parts. Accumulation of PTEs in the leaves of *B. bara* and *L. sativa* could be explained by a higher internal translocation of Cd and Pb from roots to shoots for the plants cultivated in the washed soils compared with the untreated soils (Lestan, 2015). It has been indicative of that enhanced plant uptake of metal–chelator complexes could transpire in points at which suberization of the root cell walls has not yet developed and at breaks in the Casparian strip and the root endodermis (Nowack et al., 2006; Jelusic et al., 2013). This could indicate that *B. bara* and *L. sativa* will accumulate Cd and Pb from washed soils even when the total Cd and Pb concentrations in the remediated soil are low. In the present work, Pb and Cd concentrations in the green parts of *B. bara* and *L. sativa* dropped considerably (Fig. 5). However, the metal concentrations in the *B. bara* and *L. sativa* grown in all the treatments were still above the EU limits for foodstuffs (EC, 2006). A similar situation of high accumulation of metals in some vegetables after EDTA washing was reported (Jelusic et al., 2013; Jelusic et al., 2014a; Hu et al., 2014; Jelusic and Lestan, 2015).

The chelators ISA, GLDA, PASP, and GCA are biodegradable and are not toxic or are poorly toxic (Pinto et al., 2014; Jani and Hogland, 2018), but this is not the case for EDTA (Asadzadeh et al., 2018; Golmaei et al., 2018). Consequently, the formation of metal–chelator complex residues in washed soils and the transformation of metals into more unstable forms could cause plants to take up metals more easily. Several previous studies reported that most plants absorbed Pb on their roots and only a small part is transferred to the aboveground parts (Jelusic and Lestan, 2015). However, Pb could be transported to shoots as Pb–chelator complexes when EDTA is present in soil (Luo et al., 2018), increasing the Pb concentration in the aboveground parts and decreasing it in the roots. Cd is more accessible than Pb to plants and is more mobile than Pb and less mobile than Zn (Jelusic et al., 2014a; Lestan, 2015; Komínková et al., 2018), which is an essential element for plants. Cd shares transport pathways with Zn in plants (Pence et al., 2000). Because of lowered Zn availability, as confirmed by previous work (Wang et al., 2018) and also due to the presence of residual chelator in the remediated soil, as discussed above, Cd could also have been more readily transferred into the plants grown in washed soils (Jelusic et al., 2013; Hu et al., 2014; Jelusic and Lestan, 2015). In addition, Zn was still absorbed by *B. bara* and *L. sativa* cultivated in the washed soil since residual metal–chelate complexes were left in the soil after washing, in concentrations below to those found in

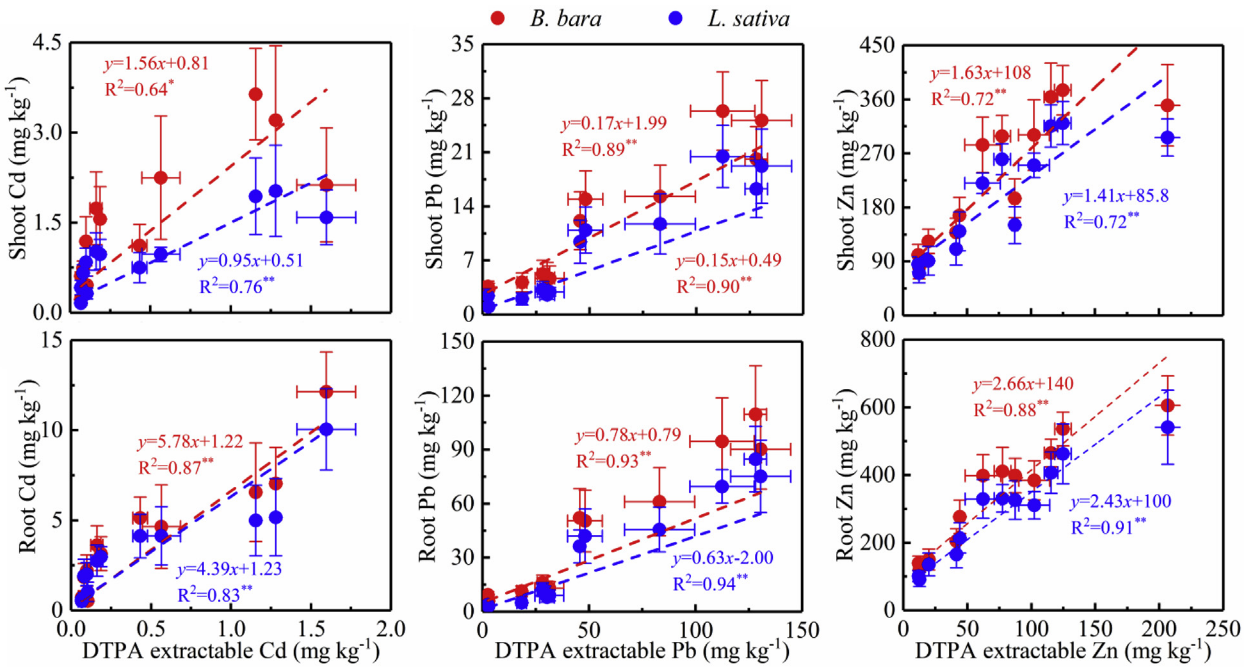

Fig. 6. Correlations between Cd, Pb, and Zn concentrations in the shoots and roots of *B. bara* and *L. sativa* with DTPA–extractable Cd, Pb, and Zn from soils. Error bars represent the standard deviation of four replicates. $^{*}P < 0.05$, $^{**}P < 0.01$.

the original soils. Komínková et al. (2018) also presented that *L. sativa* accumulated more Zn in leaves after the contaminated soil washing with EDDS.

3.4. Environmental implications and economic costs

The final target of successful remediation of polluted soil is to effectively remove PTEs and decrease their availabilities without major modification of the properties and qualities of the remediated soils (Guo et al., 2016; Jelusic and Lestan, 2015). Wang et al. (2017) found that >30% of soil organic matter was lost after the soils were washed with NaOH or EDTA. However, we found only 1–24% of soil organic matter was lost when the soils were washed with biodegradable chelators and that washing did not significantly change the available nitrogen and potassium concentrations ($P > 0.05$, Table S1). The available phosphorous increased in the washed soils (Table S1), probably by enhancing the solubility of Fe–/Al–bound phosphate in the soil, as was found in previous studies (Beiyuan et al., 2017; Hu et al., 2014; Kaurin et al., 2018). These results confirmed that the washed soil was adapted for agricultural use (Komínková et al., 2018).

The metal removal efficiency is crucial for achieving efficient remediation in soil washing with chelators; however, much more attention should be paid to the cost for the technology. Based on the laboratory–scale experiment, such energy as apparatus and water consumption expenses can't be estimated easily. Therefore, the preliminary cost analysis was conducted for the four biodegradable chelators selected in this study. The average price of PASP, GLDA, GCA, and ISA is 1.5, 1.7, 5.1, and 1.5 times that of EDTA, respectively, which is the most common chelator applied in soil washing (internet source http://www.alibaba.com, average price of four sellers). Considering the environment impact and heavy metal removal efficiencies, GLDA and ISA could be the better eco–suitable alternative for soil washing treatment among the investigated options.

However, as an environmentally sustainable technology, the possibilities of industrialization and commercialization of sequential application of soil washing and phytoremediation should be discussed. Phytoremediation exploits the abilities of green plants to take up, stabilize, and/or metabolize the pollutants. Moreover, it is a cost–effective and environmentally safe approach as compared to conventional methods to solve the problems of soil pollution. Phytoremediation has successfully been applied to treat various contaminated sites and pollutants in actual fields (Luo et al., 2018; Dhanwal et al., 2017). Therefore, there is a huge potential for industrialization and commercialization. In recent years, several plant species, predominantly commercially available agricultural grasses have been successfully used in phytoremediation (Dickinson et al., 2009). Also, cost is an important factor that we need to consider when using phytoremediation for polluted soil. A two–year phytoremediation project of soil contaminated with arsenic, Cd, and Pb was implemented to determine the essential parameters for soil remediation (Wan et al., 2016). Results showed highly efficient PTE removals. The total cost of phytoremediation was US$ 75,375.20/m^2h or US$ 37.70/m^3, which was lower than the reported values of other remediation technologies. One of the most economical and protected approaches of using the contaminated biomass is the combination of phytoextraction with the production and commercial application of the biomass as a source of energy for production of biofuel (Mahar et al., 2016). The sale of carbon credits is another possible profit source from phytomining.

4. Conclusions

Reusing polluted soil washed by biodegradable chelators could allow sustainable and environmentally benign remediation, which is a relatively new concept that has focused attention on the environmental remediation. In the present work four biodegradable chelators, GLDA, ISA, PAS, and GCA as well as the commonly used non–biodegradable chelator EDTA were employed to wash the PTEs polluted soils. The characteristics of *B. bara* and *L. sativa* cultivated in the washed soils were then evaluated. Soil washing with biodegradable chelator had positive effects on removal of Cd, Pb, and Zn, especially for ISA and GLDA where cleanup 25–85% of metals, and reduced the bioavailability of all three metallic contaminants. Residual metals and metal–chelator complexes adsorbed by the soil were phytotoxic, and decreased the biomasses of *B. bara* and *L. sativa* grown in the washed soils by 4–91% relative to plants grown in the unwashed soils. In the remediated soils, the photosynthetic capacities and nutritional qualities of *B. bara* and *L. sativa* were increased but oxidative stress was found, indicating that the plants suffered external stress. The metals accumulated in the edible parts of *B. bara* and *L. sativa* less from the washed soils than from the unwashed soils, but the metal concentrations in the plants grown in the washed soils would still pose risks to health.

The results indicate that soil washing with biodegradable chelators and growing plants on the washed soil is a sustainable and cost–effective approach to managing PTEs–contaminated soil. The approach should be performed with considerable care, and all residual metal–chelator complexes should be removed before the washed soil is used for agriculture to prevent risks and phytotoxicity associated with these compounds. The safe cultivation of plants in washed soils will require additional measures such as soil amendments with phosphates, compost, and biochar to immobilize residual metals to protect the soil and human health.

Competing financial interests

The authors declare no competing financial interests.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.jclepro.2018.06.225.

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