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Effect of soil washing with biodegradable chelators on the toxicity of residual metals and soil biological properties

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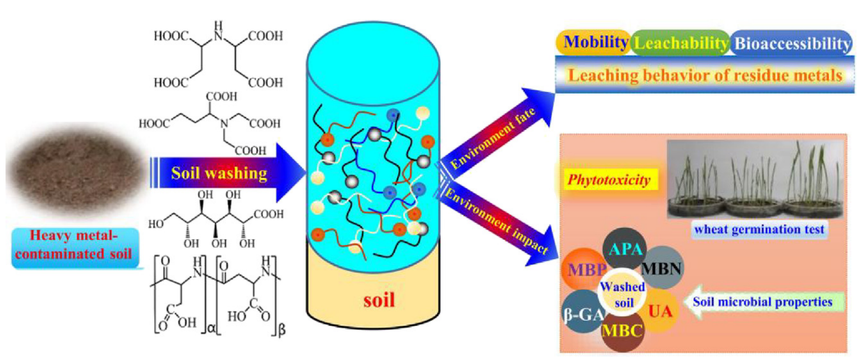
HIGHLIGHTS

- GLDA and ISA efficiently removed 25–85% of Cd, Pb, and Zn from polluted soils.
- Leachability and bioaccessibility of metals reduced by 24–92% in GLDA and ISA washing.
- Biodegradable chelates allow higher soil enzyme activity than that of EDTA treatment.
- Wheat seed germination bioassay was used to evaluate the phytotoxicity of washed soil.

GRAPHICAL ABSTRACT



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ABSTRACT

Soil washing with chelators is a promising and efficient method of remediating metals-contaminated soils. However, the toxicity of residual metals and the effects on soil microbial properties have remained largely unknown after washing. In this study, we employed four biodegradable chelators for removal of metals from contaminated soils: iminodisuccinic acid (ISA), glutamate-N,N-diacetic acid (GLDA), glucomonocarbonic acid (GCA), and polyaspartic acid (PASP). The maximum removal efficiencies for Cd, Pb, and Zn of 85, 55, and 64% and 45, 53, and 32% were achieved from farmland soil and mine soil using biodegradable chelators, respectively. It was found that the capacity of ISA and GLDA to reduce the labile fraction of Cd, Pb, and Zn was similar to that of the conventional non-biodegradable chelator ethylenediaminetetraacetic acid (EDTA). The leachability, mobility, and bioaccessibility of residual metals after washing decreased notably in comparison to the original soils, thus mitigating the estimated environmental and human health risks. Soil β-glucosidase activity, urease activity, acid phosphatase activity, microbial biomass nitrogen, and microbial biomass phosphorus decreased in the treated soils. However, compared with EDTA treatment, soil enzyme activities distinctly increased by 5–94% and overall microbial biomass slightly improved in the remediated soils, which would facilitate reuse of the washed soils. Based on soil toxicity tests that employed wheat seed germination as the endpoint of assessment, the washed soils exhibited only slight effects especially after ISA and GLDA treatments, following high-efficiency metal removal. Hence, ISA and GLDA appear to possess the greatest potential to rehabilitate polluted soils with limited toxicity remaining.

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1. Introduction

Heavy metals-polluted soil is one of the pervasive environmental issues worldwide, as caused by many anthropogenic activities including mining and smelting, waste disposition, and pesticide and fertilizer application (Wiggenhauser et al., 2016; Beiyuan et al., 2017a; Mu Azu et al., 2018). There is an imperative need to restore contaminated soils, among others due to the environmental and health implications induced by the presence of heavy metals. Soil washing with chelating agents for the treatment of metals-polluted soils is considered to be an emerging remedial method (Chae et al., 2017; Trellu et al., 2017) as it removes contaminants from soil rapidly and/or efficiently relative to other techniques (Im et al., 2015; Beiyuan et al., 2018). Chelating agents such as ethylenediaminetetraacetic acid (EDTA) and [S,S]-stereoisomer of ethylenediaminedisuccinic acid (EDDS) have been extensively proposed since they possess strong chelating ability for different metals and induce minimal effects on soil properties compared with inorganic acids (Deng et al., 2017; Qiao et al., 2017). However, EDTA has poor biodegradability and high persistence in the soil environment (Jez and Lestan, 2016), which usually results in deterioration of soil functions (Jelusic and Lestan, 2014; Guo et al., 2016). EDDS has recently been proposed as a substitute for EDTA in view of its biodegradability in soils (Beiyuan et al., 2017b). Unfortunately, it displays an insufficient extraction efficiency whereas its applicability is limited because of a relatively high price (Tsang et al., 2013; Wang G. et al., 2016). Searching for alternatives such as biodegradable washing reagents, is therefore highly recommended.

Biodegradable chelators, such as iminodisuccinic acid (ISA), glutamate-N,N-diacetic acid (GLDA), glucomonocarbonic acid (GCA), and polyaspartic acid (PASP), have been suggested as alternatives to EDTA and EDDS because they decreased environmental persistence and should therefore also have fewer of the above-mentioned negative effects (Pinto et al., 2014; Ferraro et al., 2016). When compared with conventional chelators that persist in the environment for years (Jez and Lestan, 2016), these chelators have excellent biodegradability characteristics and short half-lives (days). Studies conducted by Kołodyńska (2013) and van Ginkel and Geerts (2016) have demonstrated that >80% of ISA and 60% of GLDA were degraded within 28 days. Moreover, the alternative chelators are characterized by low potential toxicity (Pinto et al., 2014) and a powerful ability to develop soluble complexes with polyvalent ions over an extensive pH range (Lingua et al., 2014; Suanon et al., 2016; Fu et al., 2017); therefore they may comprise another environmentally-friendly alternative next to EDDS for substitution of persistent EDTA.

The metal removal efficiency is crucial for achieving efficient remediation in soil washing with chelators; however, much more attention should be paid to the toxicity of residual metals involved in the safe reuse of the washed soil. Metals associated with labile fractions, which are highly toxic, mobile, and more bioavailable to biota than non-labile fractions, can easily be removed by soil washing (Wang G. et al., 2016). Nevertheless, the metals remaining in washed soils may destabilize and transform from non-labile fractions to labile fractions (Udovic and Lestan, 2009), which will induce an undesirable increase in their mobility and bioaccessibility (Tsang et al., 2013; Im et al., 2015; Beiyuan et al., 2017b), Tsang and Hartley (2014) reported that a portion of residual metals were destabilized and shifted to the exchangeable fraction in soils after washing of the soil with EDDS and natural humic substances, which increased metals mobility. Although the removal efficiency and redistribution of metals have been extensively reported, the information concerning the toxicity of the residual fraction in soil is still limited (Fedje et al., 2013; Kulikowska et al., 2015; Suanon et al., 2016).

It is also important to consider that soil washing will change soil characteristics such as texture, pH, cation exchange capacity, organic matter, and nutrient concentrations (Jelusic and Lestan, 2014; Fedje and Strömvall, 2016; Wang G. et al., 2016) as well as lead to a possible change in bioavailability of residual metals in soils (Im et al., 2015). Soil enzyme activities and microbial biomass can serve as representative evaluation indices of soil functional restoration (Udovic and Lestan, 2012; Im et al., 2015) since washing solutions and conditions as well as residual metals may convert the substrate or enzyme-substrate complexes in soils. Specifically, β-glucosidase is the rate-limiting enzyme in the microbial degradation of cellulose to glucose, catalyzing the hydrolysis of cellulose and playing a vital role in C cycling (Tabatabai, 1994). Urease catalyzes the hydrolysis of urea into carbon dioxide and ammonia (Lloyd and Sheaffe, 1973), which represents microbial activity connected with N cycling. Acid phosphatase catalyzes the hydrolysis of diverse organic monoester compounds to inorganic P (Udovic and Lestan, 2012; Abad-Valle et al., 2016). They reflect the capacity of soil to perform specific reactions related to soil carbon (C), nitrogen (N), and phosphorus (P) cycling (Yoo et al., 2016; Chae et al., 2017). Nevertheless, the impact of washing on the above-mentioned soil enzyme activities and microbial biomass remains poorly understood (Jelusic and Lestan, 2014).

The objectives of this work were therefore to: (1) assess the environmental effects of residual metals in soils treated with biodegradable chelators by means of assessing leachability, mobility, and bioaccessibility of metals, and by performing sequential extraction as well as by evaluating the potential risks to human health associated with residual metals; (2) evaluate the influence of washing treatments on the soil enzyme activities and microbial biomass; and (3) examine the potential phytotoxicity of the washed soils by performing bioassays.

2. Materials and methods

2.1. Soil sampling and analysis

Field-contaminated soils were collected from the surface (0–20 cm) at a lead zinc contaminated mine wasteland (mine soil; 29°24′ N, 102°39′ E) and from farmland near a non-ferrous metal smelter (farmland soil; 30°59′N, 103°57′E) in Sichuan, China. Soil samples were air-dried and passed through a 2-mm sieve, and stored in airtight containers prior to analysis. The metal concentrations in the samples were measured using a flame atomic absorption spectrophotometer (FAAS, Thermo Solaar M6, Thermo Fisher Scientific Ltd., USA) after addition of a mixture of concentrated HNO_3-HCl-$HClO_4$ at a volume ratio of 1:2:2 in a microwave digestion system (GHZ-16, Beijing Guohuan Institute of High-tech Automation, China). The methods for determining the physicochemical characteristics of the contaminated soils are presented in the Supplementary Information (SI) and the results of the analyses are listed in Table 1.

Table 1
Selected physicochemical properties of the contaminated topsoils (0–20 cm).

Soil property	Mine soil	Farmland soil
Clay/silt/sand, %	52.2/7.6/40.2	65.2/16.7/18.1
Texture	Sandy clay	Clay
Soil pH	6.25–6.31	6.97–7.16
Electrical conductivity, dS cm^{-1}	1.78 ± 0.21	3.06 ± 0.43
Available nitrogen, mg kg^{-1}	32.1 ± 2.0	78.4 ± 6.9
Available phosphorus, mg kg^{-1}	27.6 ± 3.6	17.7 ± 0.6
Available potassium, mg kg^{-1}	106 ± 6	87 ± 7
Soil organic carbon, g kg^{-1}	19.2 ± 1.1	23.9 ± 2.4
Total nitrogen, g kg^{-1}	1.20 ± 0.17	1.51 ± 0.36
Total phosphorus, g kg^{-1}	1.02 ± 0.15	0.790 ± 0.160
Total potassium, g kg^{-1}	13.4 ± 1.1	17.7 ± 0.9
Cation exchange capacity, cmol kg^{-1}	13.0 ± 0.6	19.1 ± 1.4
Cd, mg kg^{-1}	15.4 ± 1.1	36.2 ± 3.6
Pb, mg kg^{-1}	1293 ± 102	268 ± 14
Zn, mg kg^{-1}	2278 ± 146	1082 ± 44

Experimental results are reported as mean ± standard deviation (n = 3).

2.2. Biodegradable chelators

The following biodegradable chelators were used in this study: a solution of GLDA with a solid content of 47% and a density of 1.40 g cm^{-3} (Akzo Nobel Chemicals (Ningbo), Co., Ltd., China), a solution of ISA with a solid content of 34% and a density of 1.68 g cm^{-3} (Lanxess Chemical (Shanghai) Co., Ltd., China), PASP with an average molecular mass of 4000 and a purity of ≥99.50% (Hebei Think-Do Environment Co., Ltd., China), and GCA with a purity of ≥99.90% (Hubei Giant Technology Co., Ltd., China). EDTA, the most widely used and effective non-biodegradable synthetic chelator (Jez and Lestan, 2016; Deng et al., 2017), was selected as the reference compound for comparison with the performance of biodegradable chelators. The molecular structures of the tested chelators are given in the Fig. S1.

2.3. Batch soil washing

The soil washing procedures using the biodegradable chelator solution are reported in detail in our previous study (Wang G. et al., 2016). Briefly, air-dried soils (100 g) were placed in a 2.0 L acid-rinsed plastic bottle, after which 50.0 mM solutions (pH 5.00) of one of the four reagents (GLDA, ISA, PASP, and GCA) were slowly added to achieve a soil-to-solution (*S/L*) ratio of 1:5. Subsequently, the mixed suspensions were shaken at 150 rpm and room temperature for 2 h. Following the reaction, the suspensions were centrifuged (4000 rpm for 10 min) and filtered through 0.45-μm filters, after which the metal concentrations in the supernatants were determined using FAAS. The washed soils were subsequently rinsed with deionized water by shaking for 10 min at a 1:5 g mL^{-1} ratio before subsequent experiments to eradicate the influence of entrapped and lightly bound metal-chelator complexes, then air-dried for further analysis after discarding the supernatant. The EDTA soil washing procedure was same as that of biodegradable chelators. All tests were conducted in triplicate.

2.4. Soil phytotoxicity

Wheat seed germination and growth test is a rapid and practical technique that is extensively used for monitoring the toxicity of environmental samples (Wang T. et al., 2016; Mohamed et al., 2017) especially in assessing the change of soil properties and the toxicity of residual metals after soil washing (Im et al., 2015; Sastre-Conde et al., 2015; Yoo et al., 2016). To evaluate the potential toxicity of remediated soils before and after washing with various biodegradable chelators, wheat seed germination and growth tests were carried out as described by Gil-Díaz et al. (2017) with some modifications. The seeds were sterilized with 3% H_2O_2 solution for 30 min to prevent fungal growth and to stimulate germination, then copious washing with deionized water. Next, seeds were soaked in ultrapure water for 4 h, after which 50 grains were placed on a sterilized petri dish (90 mm) containing washed soil equivalent to 100 g. Subsequently, the petri dishes were placed in a thermostatic incubator with a 12/12 h of light/dark and a corresponding temperature of 25/20 °C per day. All petri dishes were watered daily to 80% of the soil water holding capacity based on weight. After seven days of incubation, the seed germination rate was counted and 20 seedlings from each petri dish were randomly selected to determine the total root length using a caliper rule. Radicle protrusion was considered the criterion for germination. The germination rate, germination index, and vigor index were used to assess the phytotoxicity of the washed soils, as calculated by the following equations (Wang T. et al., 2016):

$$\text{Germination rate }(\%) = \frac{\text{number of seeds germinated in 7 d}}{\text{total number of seeds}} \times 100\% \quad (1)$$

$$\text{Germination index} = \sum(G_t/D_t) \quad (2)$$

$$\text{Vigor index} = \text{Root length (cm)} \times \text{Germination index} \quad (3)$$

where G_t is the number of germinated seeds on day t, and D_t represents the number of days for which seeds were allowed to germinate.

2.5. Analytical methods

2.5.1. Leachability, bioaccessibility, and sequential extraction of residual metals and health risk calculation

The untreated and washed soils were leached according to the TCLP (toxicity characteristic leaching procedure, EPA Method 1311) and SPLP (synthetic precipitation leaching procedure, EPA Method 1312) (Koralegedara et al., 2017). The bioaccessibility of residual metals in remediated soils if ingested into the human gastrointestinal system was evaluated by one-step simplified bioaccessibility extraction test (SBET, Beiyuan et al., 2017a, 2017b). The SBET procedure described by Rahman et al. (2017). In addition, the distribution of residual metals in soils was determined according to the modified Tessier's sequential extraction procedure (Suanon et al., 2016). The detailed steps of the method are given in Table S1. Moreover, the non-cancer and cancer risks for children and adults were determined based on the SPLP and SBET results. The details of the method are available in the SI.

2.5.2. Soil enzyme activities and microbial biomass

The washed soils were pre-incubated for 7 days at 80% of the soil water holding capacity and a temperature of 25 °C under dark conditions before analysis. The tested *exo*-enzymes were β-glucosidase (β-GA, EC 3.2.1.21), urease (UA, EC 3.5.1.5), and acid phosphatase (APA, EC 3.1.3.2). β-GA and APA were measured according to the method proposed by Tabatabai (1994), while soil UA was assayed by measuring the NH_4^+ produced by means of colorimetric methods after the addition of urea to soil subsamples (Lloyd and Sheaffe, 1973). In addition, soil microbial biomass carbon (MBC), microbial biomass nitrogen (MBN) and microbial biomass phosphorus (MBP) were analyzed by the chloroform-fumigation-extraction method as previously described (Brookes et al., 1982; Brookes et al., 1985; Vance et al., 1987). A brief description of the analysis of soil enzyme activities and microbial biomass is provided in the SI.

2.6. Quality control and statistical analysis

A reference material (GBW07405) was analyzed for QA/QC purposes during the digestion procedure. The recovery rates of metals from the reference soils were approximately 93–107%. Analytical duplicates and reagent blanks were also devoted where appropriate to ensure the accuracy and precision of the analysis. The data were subjected to analysis of variance (ANOVA, one way), and the mean differences were compared by Fisher's LSD test using SPSS version 20.0 (SPSS Inc., USA) from three replicates. The results were considered significant at $P < 0.05$.

3. Results and discussion

3.1. Effect of biodegradable chelator washing on speciation of residual metals in soils

As shown in Fig. 1, PASP and GCA were less effective in extracting Cd (7–38%), Pb (0–8%), and Zn (5–43%) from polluted soils. Conversely, 85% of Cd, 55% of Pb, and 53% of Zn were removed from the farmland soil and 45% of Cd, 53% of Pb, and 32% of Zn were removed from the mine soil by GLDA washing, while ISA removed 52, 45, and 64% and 25, 38, and 30% of Cd, Pb, and Zn from farmland soil and mine soil, respectively. GLDA and ISA achieved significantly higher metal removal efficiency than PASP and GCA ($P < 0.05$). This difference in efficiency might be related to the fact that GLDA and ISA contain more carboxylic

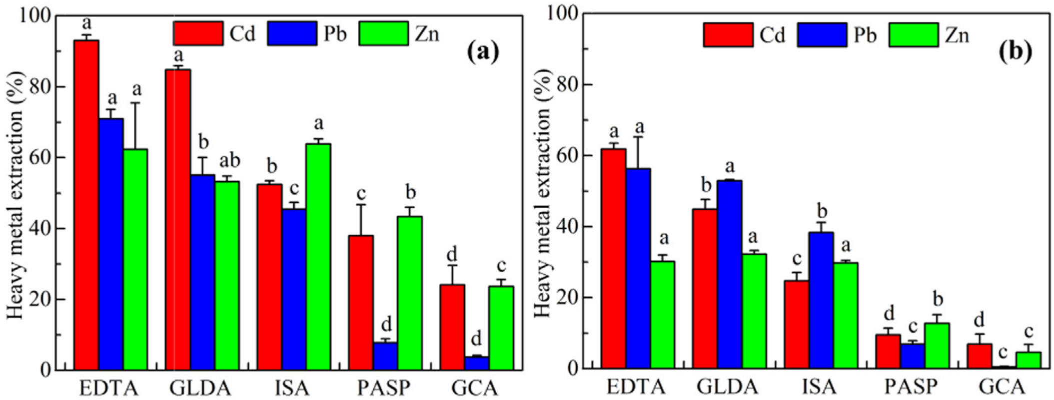

Fig. 1. Effect of addition of biodegradable chelators on heavy metals extraction from farmland soil (a) and mine soil (b). The error bars represent the standard deviation of the mean from triplicate samples and same letters above the bar indicate that the results are not significantly different according to the Fisher's LSD test at $P < 0.05$.

groups than PASP and GCA (Fig. S1), which facilitates more efficient ligand-metal ion complexation and formation of metal-chelate complexes (Yip et al., 2010). Previous works have confirmed that EDTA possess strong chelating ability for multi-heavy metals even over a wide pH ranges (Udovic and Lestan, 2012; Deng et al., 2017; Lestan, 2017). The Cd, Pb, and Zn removal efficiencies obtained in this work for farmland soil were 93, 71, and 62% after washing with EDTA, respectively, but washing the mine soil removed less metals (62% of Cd, 56% of Pb, and 32% of Zn).

The chelator-induced washing process altered the chemical forms of the residual metals in the soils (Fig. 2). Before washing, the dominant chemical forms of Cd in the farmland and mine soils were the exchangeable (46 and 29%), carbonate-bound (20 and 36%), and residual fractions (21 and 19%). In contrast, the dominant portions of Pb in soils were the carbonate-bound and residual fractions. Overall, these two fractions accounted for 73% of total Pb in the farmland soil and 84% in the mine soil. The distribution of Zn differed, with the organic matter-bound and residual fractions of Zn accounting for approximately 45% and 60% in the farmland and mine soils, respectively, while only 33% of the total Zn was found in the carbonate-bound fraction in both soils. The relatively high proportion of Zn in the organic matter-bound and residual fractions indicates that Zn is strongly incorporated within the crystalline lattice of the soils, and may be less easily extractable, even by chemically-enhanced washing (Tsang and Hartley, 2014; Wang G. et al., 2016). However, in comparison with the initial distribution, the EDTA-washing resulted in a marked reduction of Cd, Pb, and Zn concentrations, which was likely because it was associated with greater mineral dissolution (Udovic and Lestan, 2009; Jez and Lestan, 2016). Cd and Zn in the water-soluble, exchangeable, and carbonate-bound fractions were most efficiently extracted by GLDA and ISA, as shown by a corresponding significant reduction of 17–97% for farmland soil and 19–75% for mine soil. In the case of Pb, the water-soluble and carbonate-bound fractions were notably reduced (by 27–90%). Additionally, PASP and GCA were also able to partially remove Cd and Zn from exchangeable and carbonate-bound fractions from soils (by 13–79% removal), but extraction of Pb by these chelators was negligible

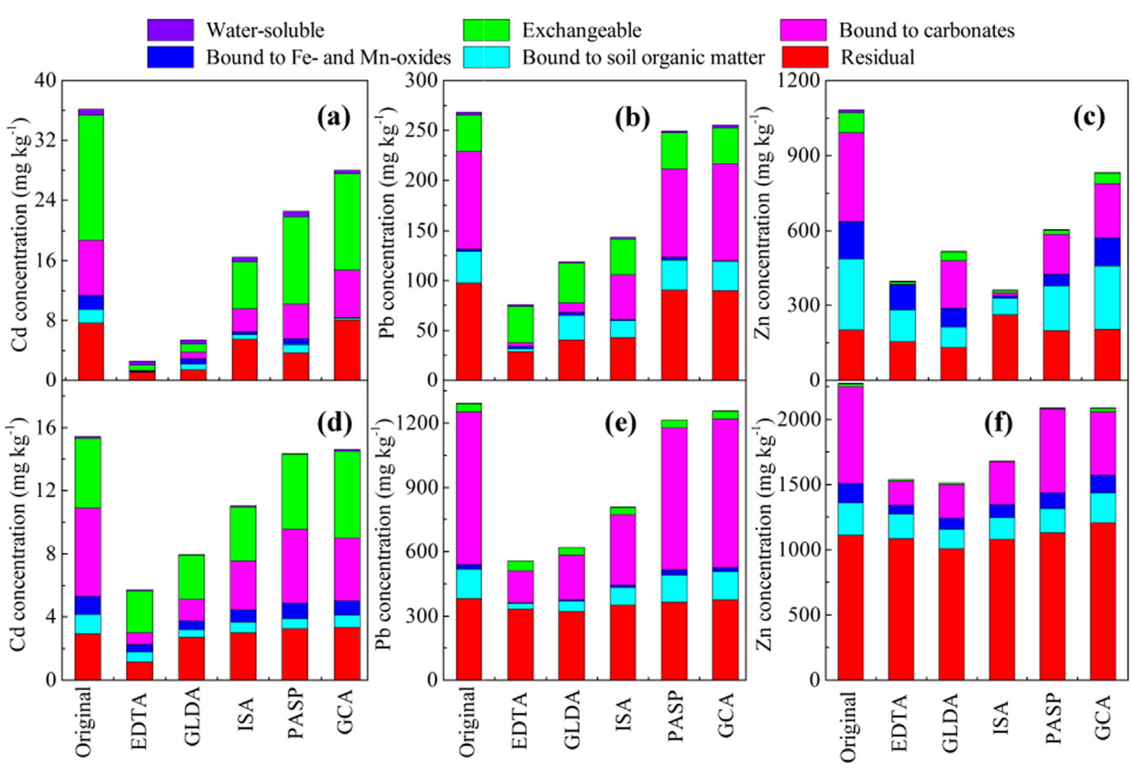

Fig. 2. Chemical forms of Cd, Pb and Zn in farmland soil (a–c) and mine soil (d–f) before and after soil washing.

as only 1–7% of Pb bound to Fe-Mn oxides and 4–9% of Pb bound to organic matter could be extracted. Begum et al. (2013) also stated that the Cd, Cu, Pb, and Zn contents in these fractions were much more arduous to remove during sequential soil washing with biodegradable aminopolycarboxylate chelators.

The chemical speciation of metals in soil could exert a great impact on their fate concerning the leaching and subsequent environmental risks (Gusiatin et al., 2017; Rahman et al., 2017). Previous works have demonstrated Fe-Mn oxides, organic matter-bound, and residual fractions are commonly at steady state and induce relatively minor adverse effects in the environment (Yang et al., 2017; Zhang et al., 2017). Conversely, the other fractions are unstable and could induce large environmental risks because of their high bioactivity and bioaccessibility (Wu et al., 2015). The biodegradable chelator enhanced soil washing could effectively remove much of the active fraction of Cd, Pb, and Zn from soils, which in turn reduces the environmental risk and bioaccessibility of metals, especially after GLDA and ISA treatments.

3.2. Leachability, mobility, and bioaccessibility of residual metals

The TCLP and SPLP procedures were selected to assess the leachability and mobility of residual metals in the washed soils. In comparison with the untreated soils, the leachability and mobility of residual Cd, Pb, and Zn after washing with biodegradable chelators were dramatically reduced in most cases ($P < 0.05$, Fig. 3). Specifically, GLDA and ISA washing reduced the TCLP leachability and SPLP mobility of Cd, Pb, and Zn by 27–80% and 17–100%, respectively. Similarly, the metals remaining in treated soils were leached and mobilized by >10% after PASP and GCA washing, particularly in the SPLP mobility test of the mine soil (>75%), despite the extraction capacity of PASP and GCA for Cd, Pb, and Zn being relatively limited (0–43%, Fig. 1). However, it should be noted that the non-biodegradable EDTA increased the leachability and mobility of Pb from the mine soil (Fig. 3b and d), which was validated by an increase in the exchangeable fraction of Pb after EDTA sequential washing (Fig. 2e), which is most likely due to a portion of these EDTA destabilized Pb via surface complexation are not yet detached (Zhang et al., 2010). Tsang et al. (2013) and Jelusic et al. (2013) also found that residual metal-EDTA complexes resulted in an increased leachability and exchangeable fraction.

The bioaccessibility of metals is of greater interest than their total concentration in soil (Udovic and Lestan, 2009; Mele et al., 2015; Rahman et al., 2017). The bioaccessible concentration is the concentration that can actually be absorbed by organisms for metabolism via the ingestion of soil. In the present study, the SBET-extractable concentrations of Cd, Pb, and Zn extracted from the washed soils were considerably reduced compared with the original soils, particularly in the EDTA, GLDA, and ISA treatments ($P < 0.05$, Fig. 4). The SBET-extractable concentrations decreased by 44–95% in the farmland soil and by 18–66% in the mine soil. However, the bioaccessible fraction (%, calculated as the ratio of SBET-extractable metal to the total concentration) of Zn increased from 76% in the initial mine soil to 89% in the remediated soil. This can probably be attributed to the high proportion of Zn in the organic and residual fractions (Fig. 2f), whereas the substantial amount of newly released Zn was prone to re-adsorption onto the soil surface (Beiyuan et al., 2017b). This turns the residual Zn into an extremely mobile and bioaccessible form. Moreover, PASP and GCA enhanced-washing had only weak effects on the bioaccessibility of remaining Cd, Pb, and Zn, which was in line with their limited removal effectiveness (Fig. 1).

The biodegradable chelators effectively extracted Cd, Pb, and Zn from contaminated soils, especially GLDA and ISA. These chelators can, however, also alter the leachability, mobility, and bioaccessibility of remaining Cd, Pb, and Zn in the washed soils, implying that reuse of the remediated soil may still pose potential risks to the surrounding environment (Jelusic et al., 2013; Jelusic and Lestan, 2014).

3.3. Enzyme activities and microbial biomass of the treated soils

The physiochemical characteristics of the restored soil inevitably changed upon removal of metals during the remediation process, thus

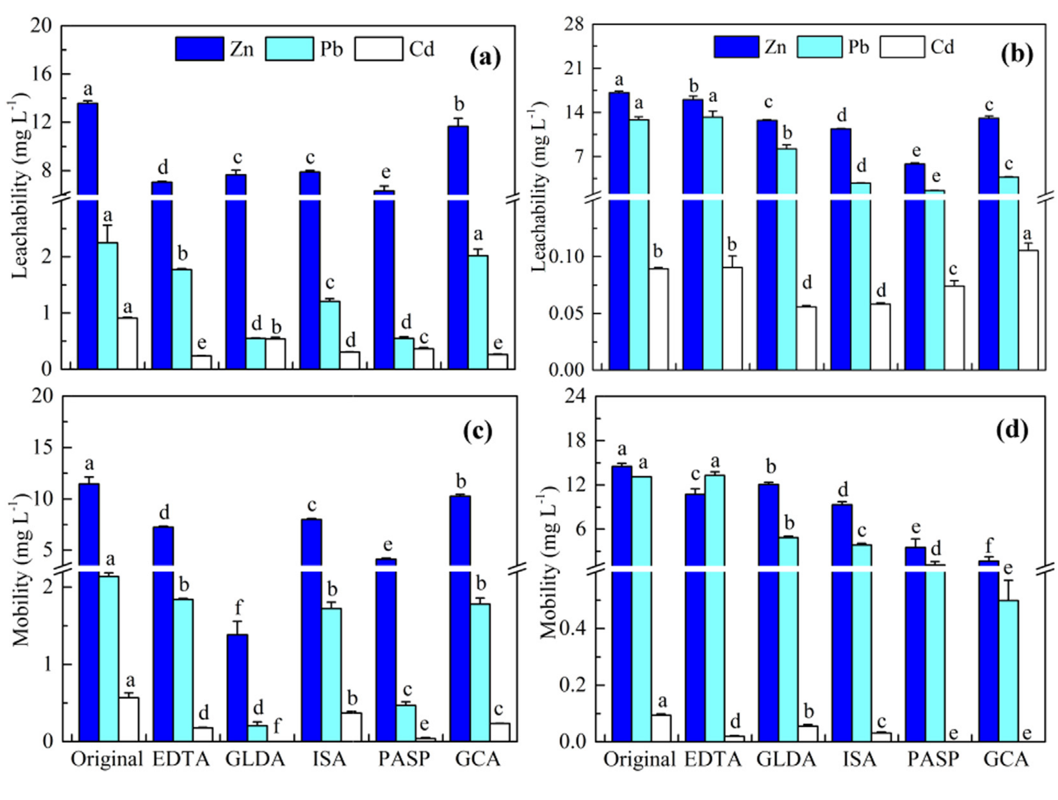

Fig. 3. TCLP leachability and SPLP mobility of Cd, Pb and Zn in farmland soil (a & c) and mine soil (b & d) after washing. The error bars represent the standard deviation of the mean from triplicate samples and same letters above the bar indicate that the results are not significantly different according to the Fisher's LSD test at $P < 0.05$.

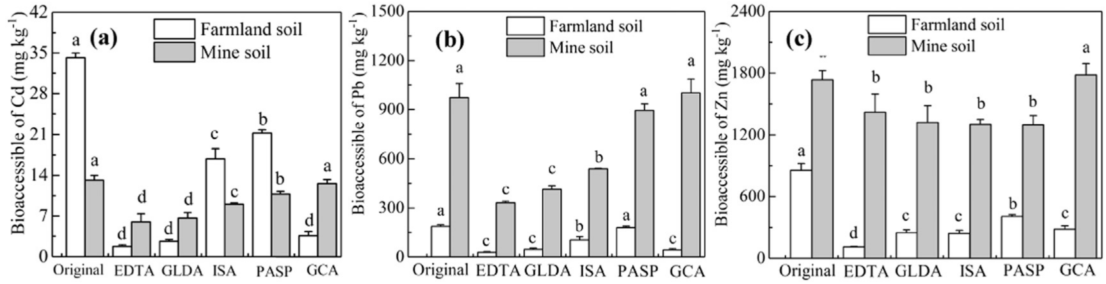

Fig. 4. SBET bioaccessible concentrations of Cd (a), Pb (b) and Zn (c) in the contaminated soils after washing. The error bars represent the standard deviation of the mean from triplicate samples and same letters above the bar indicate that the results are not significantly different according to the Fisher's LSD test at $P < 0.05$.

influencing the soil microbial activity (Udovic and Lestan, 2012; Chae et al., 2017). Soil enzyme activities and microbial biomass are considered to be bio-indicators because of their rapid response and sensitivity to early soil environmental changes caused by remediation with soil washing (Im et al., 2015; Yoo et al., 2016; Chae et al., 2017; Kaurin et al., 2018). In the present study, soil washing with biodegradable chelators induced significant adverse effects on soil microorganisms ($P < 0.05$), resulting in suppressed biological responses (Table 2). β-Glucosidase, urease, and acid phosphatase enzyme activities in both soils decreased remarkably by 4–50%, 1–38%, and 19–74% after soil washing ($P < 0.05$), respectively. Previous studies have also reported decreased enzyme activities in washed soils (Im et al., 2015; Yoo et al., 2016; Chae et al., 2017). However, the activities of the aforementioned enzymes slightly improved by 15–74%, 5–19%, and 8–94%, respectively, after washing with the biodegradable chelator solutions when compared with EDTA, which possible be associated with the concentrations of soil organic matter and nutrients in remediated soils (Im et al., 2015). The toxicity of EDTA could also facilitate inhibition the activity of the microbial communities present to a certain extent (Epelde et al., 2008). Increasing the activities of these enzymes after washing with the biodegradable chelators thus leads to enhanced microbial activity related to organic matter breakdown and N and P circulation when compared with EDTA washing, which might have positive effects on nutrient availability in washed soils.

The levels of MBN and MBP in the treated soils were also considerably decreased by approximately 3–27% and 12–31% respectively upon washing with biodegradable chelators ($P < 0.05$, Table 2). The employed chelating agents were not only effective in extracting soil Cd, Pb, and Zn, but also likely induced the release of nitrogen and phosphorus associated with soil colloids and organic matter leaching because the washing process simultaneously facilitated the solubility of soil nitrogen and Fe/Al-bound phosphate. In contrast, washing with these chelating agents increased the MBC concentrations by 14–40% in the treated soils, indicating the presence of residual chelators (Beiyuan et al., 2017b). Stringent conditions during soil washing induced the disaggregation of loosely bound soil structures, which presumably caused lysis of microbial cells and release of enzymes (Kaurin et al., 2018). Therefore, our results imply that special attention should be paid to replenishment of N and P to ensure the quality of remediated soil after washing with biodegradable chelators. Besides, there may be adverse effects on the restored soil structures and on microbial activities associated with the presence of biodegradable chelators as there might be loss of available nutrients, inorganic minerals, and soil organic matter during the washing process (Udovic and Lestan, 2012; Jelusic et al., 2014; Im et al., 2015; Fedje and Strömvall, 2016; Yoo et al., 2016).

3.4. Soil phytotoxicity analysis

The wheat germination rates increased dramatically by 13–40% after soil washing (Fig. 5, $P < 0.05$). The germination rates in the GLDA and ISA treatments were 80–88%, respectively, which in both cases were significantly higher than the EDTA treatment of 70% for the farmland soil and 60% for the mine soil ($P < 0.05$). Conversely, the germination indices of 20.4 and 15.2 of the initial farmland soil and mine soil increased considerably by 53–81% and 39–100% after remediation ($P < 0.05$), respectively. Thereupon, the differences in germination indices for PASP and GCA treatments did not differ notably ($P > 0.05$). Additionally, the root length and vigor index were remarkably enhanced in both soils after the biodegradable chelator washing when compared with the control ($P < 0.05$). This was especially true for the vigor index, which increased by about 2-fold, whereas the changes in these indicators were not significantly different among the GLDA, ISA, PASP and GCA treatments ($P > 0.05$).

Table 2
Soil enzyme activities and microbial biomass in the washed soils.

Soils	Treatments [a]	Soil enzyme activities			Soil microbial biomass [b]		
		β-Glucosidase (μg *p*-nitrophenol g^{-1} h^{-1} soil)	Urease (μg NH_3-N g^{-1} h^{-1} soil)	Acid phosphatase (μg *p*-nitrophenol g^{-1} h^{-1} soil)	MBC (mg kg^{-1})	MBN (mg kg^{-1})	MBP (mg kg^{-1})
Farmland soil	Original	14.6 ± 2.3a	7.45 ± 0.66a	52.2 ± 6.7a	170 ± 15b	89.6 ± 5.6a	13.0 ± 1.3a
	EDTA	10.3 ± 1.4b	4.62 ± 0.33b	28.8 ± 3.9d	206 ± 32a	65.1 ± 6.1c	9.15 ± 0.94c
	GLDA	12.8 ± 1.7ab	5.05 ± 0.39b	42.4 ± 2.9b	201 ± 16a	80.5 ± 3.7b	11.1 ± 1.2b
	ISA	13.8 ± 1.1a	4.89 ± 0.48b	31.3 ± 2.4 cd	206 ± 32a	79.6 ± 7.2bc	10.7 ± 0.9b
	PASP	13.7 ± 0.9a	4.86 ± 0.12b	38.2 ± 7.5bc	200 ± 26a	85.1 ± 3.2b	8.94 ± 0.79c
	GCA	14.0 ± 2.1a	5.08 ± 0.81b	35.6 ± 3.3bcd	193 ± 26a	85.8 ± 4.1b	9.77 ± 1.06bc
Mine soil	Original	51.1 ± 1.0a	1.92 ± 0.09a	89.4 ± 5.5a	90.3 ± 6.5c	42.1 ± 3.6a	26.4 ± 2.0a
	EDTA	25.6 ± 1.9c	1.60 ± 0.19a	23.3 ± 0.9c	126 ± 12a	34.2 ± 2.9b	18.7 ± 1.5c
	GLDA	30.8 ± 3.2c	1.81 ± 0.30a	25.2 ± 6.0bc	113 ± 11ab	42.8 ± 4.2a	23.1 ± 2.3b
	ISA	29.5 ± 2.2c	1.90 ± 0.05a	35.5 ± 12.7b	107 ± 10bc	35.1 ± 3.1b	22.1 ± 1.7b
	PASP	33.1 ± 2.3c	1.75 ± 0.65a	45.3 ± 1.6b	126 ± 10a	33.2 ± 2.2b	18.8 ± 1.6c
	GCA	44.7 ± 5.8b	1.70 ± 0.35a	35.3 ± 5.6b	125 ± 9a	40.8 ± 4.1a	19.0 ± 0.9bc

Original, contaminated soils that were not washed. Experimental results are reported as mean ± standard deviation (n = 3). The different letters designate significant differences according to the Fisher's LSD test at $P < 0.05$.

[a] For soil washing, solid-to-liquid ratio of 1:5, chelator concentration of 50 mM, pH of 5.0 and contact time of 120 min were used for all the treatments.

[b] MBC, microbial biomass carbon, MBN, microbial biomass nitrogen, and MBP, microbial biomass phosphorus.

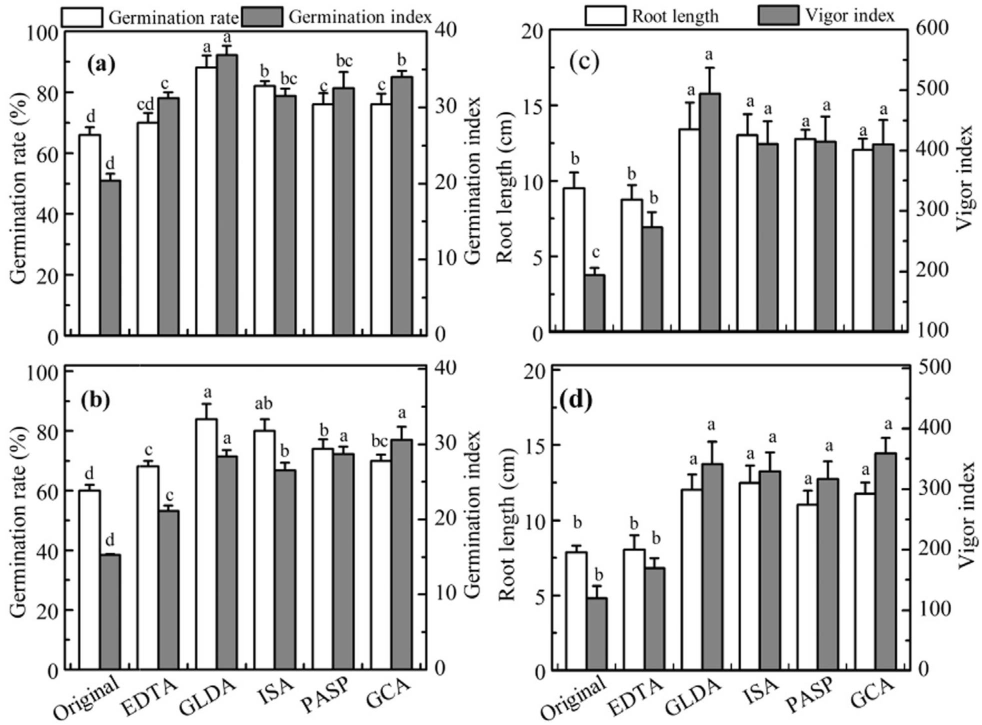

Fig. 5. Germination characteristics, root length and vigor index of wheat seeds under various treatments from the polluted farmland soil (a & c) and mine soil (b & d), respectively. The error bars represent the standard deviation of the mean from triplicate samples and same letters above the bar indicate that the results are not significantly different according to the Fisher's LSD test at $P < 0.05$.

The original soils presented an induced toxicity that hindered the germination and growth of wheat seed, and this toxicity persisted even after the soils had been washed using EDTA. High concentrations of bioaccessible Cd, Pb, and Zn in the initial soils (Fig. 4) were found to be extremely toxic to plants, restraining plant growth and causing low germination rates (Gil-Díaz et al., 2017). Although EDTA has a relatively strong chelating ability to extract various chemical forms of metals (Udovic and Lestan, 2012; Jelusic and Lestan, 2014; Deng et al., 2017), the residual EDTA inhibition of seed germination might be attributed to insufficient substances and energy needed for seed germination, as shown by the reduced breakdown of starch and proteins in seed storages as related to the limited biodegradability of EDTA (Shahid et al., 2012; Jez and Lestan, 2016) after washing. However, toxicity decreased substantially after remediation of the contaminated soils with GLDA, ISA, PASP, and GCA, with the lowest toxicity observed when the contaminated soil was washed with GLDA.

3.5. Mitigation of risks to human health

Human health risks were calculated by assessing the pathways of direct oral ingestion and dermal absorption based on the SPLP. The goal of the calculations was to indicate the extent of risk related to untreated versus washed soil under acid rain precipitation, whereas the SBET results were intended to mimic the risk associated with untreated/washed soil through accidental soil ingestion. In the present study, the non-cancer risk to children was acceptable (hazard index <1, Fig. 6a and d) because of the higher Cd, Pb, and Zn removal efficiencies by the biodegradable chelators after sequential washing (Fig. 1). Consequently, biodegradable chelator washing reduced the non-cancer risk posed by the contaminated soils. However, children might be exposed to a considerable non-cancer risk in the original soils via accidental soil ingestion (Fig. 6b and e). This is because Cd and Pb could trigger severe injuries to the brain, kidneys and nervous system (Beiyuan et al., 2017b), of especially in children. These risks were dramatically decreased after washing with biodegradable chelators by decreasing the bioaccessible concentrations of metals in the treated soils (Fig. 4). Even though only weakly bound metals were extracted by chelators, the potential risk of cancer in children posed by the washed soil was still at an unacceptable level (Fig. 6c and f): whereas the acceptable risk is in the range of 10^{-6}–10^{-4} (Ferreira-Baptista and De Miguel, 2005), the remaining risk after soil washing were over 2 orders of magnitude larger. Similar changes were observed for adults (Fig. S2), although they are less prone to exposure to metal pollution. However, it is important to note that these risk calculations were theoretical with limitations; therefore, the results of the present study should be elucidated cautiously for comparison purpose. The SPLP extraction is an operationally defined method (Koralegedara et al., 2017), while the SBET is an in vitro chemical extraction procedure (Rahman et al., 2017). Thus, a nondeterminacy assessment is highly recommended for extrapolating the in vitro results to in vivo bioaccessibility values (Scheckel et al., 2009). Moreover, a follow up study should be conducted to determine actual metal uptake by plants in the field as well as the metal contents of overland runoff and of infiltration in washed soil.

4. Conclusions

This work evaluated the toxicity of residual metals in soils as well as the change of soil enzyme activities and microbial biomass after washing with the biodegradable chelators GLDA, ISA, PASP, and GCA, as compared with the conventional agent of EDTA. A solid-to-liquid ratio of 1:5, chelator concentration of 50.0 mM, pH of 5.00 and contact time of 2 h for GLDA and ISA washing led to approximately 25–85%, 38–55%, and 30–64% of removals for Cd, Pb, and Zn, respectively. Most of the extracted metals originated from the easily-extractable fractions, namely the water-soluble, exchangeable, and carbonate fractions. After washing with biodegradable chelators, the leachability, mobility, and bioaccessibility of residual metals were considerably reduced due to the removal of the labile fractions. The human health risk via water consumption was diminished and the risk related to soil ingestion was decreased by more than half. Compared with the conventional agent EDTA washing

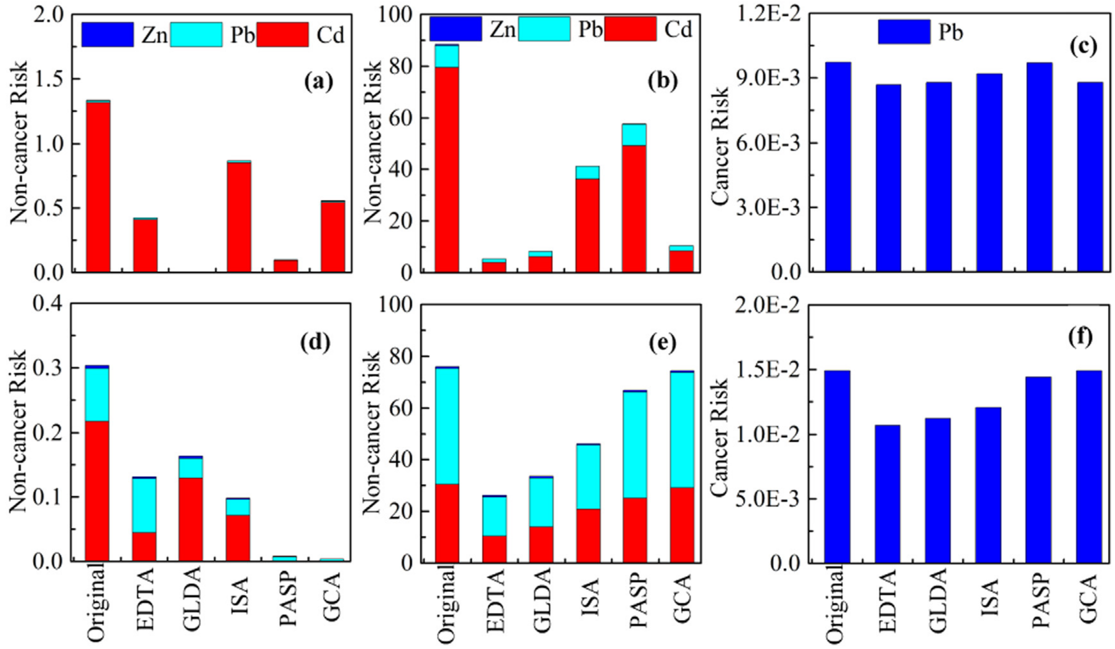

Fig. 6. Estimated health risks for children after the biodegradable chelators dissolution of the contaminated soils. Non-cancer risk of water consumption based on the SPLP results from the farmland soil (a) and mine soil (d); non-cancer risk of accidental soil ingestion based on the SBET results from the farmland soil (b) and mine soil (e); and cancer risk of accidental soil ingestion based on the SBET results from the farmland soil (c) and mine soil (f).

only, GLDA, ISA, PASP, and GCA washing were found to improve the soil enzyme activities and microbial biomass and the biodegradable chelators significantly decreased the phytotoxicity of the treated soils. It is recommended that, besides the removal efficiency, the toxicity of residual metals and the effect on the soil microbial characteristics should be taken into account when considering the reuse of washed soils. Nevertheless, future studies are recommended to explore the effects of this biodegradable chelator washing on change of the soil microbial community before reuse the washed soil.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2018.01.019.

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