Identification of root exudates from the Pb-accumulator Sedum alfredii under Pb stresses and assessment of their roles

Qing Luo, Shiyu Wang, Li-na Sun, Hui Wang, Tong Bao and Muhammad Adeel

Key Laboratory of Regional Environment and Eco-Remediation of Ministry of Education, College of Environment, Shenyang University, Shenyang, People’s Republic of China

ABSTRACT
The Pb-accumulator Sedum alfredii is a good phytoremediation material, and widely used in the phytoremediation research of soils contaminated with Pb. The root exudates from it may be playing a significant role in the process of phytoremediation. In this study, the metabolomics method which based on gas chromatography–mass spectrometry (GC–MS) and pattern recognition analysis was used to identify the remarkable root exudates from S. alfredii under different Pb stresses, including exposure concentrations (0, 10, 50, 200 and 1000 µmol/L) and times (4 and 8 days). And batch extraction experiments were used to verify the roles of these remarkable root exudates. According to the results, 11 metabolites were considered as the remarkable metabolites. Oxalic acid, galactonic acid and glyceric acid can remove Pb in soil, and the removal effect was: oxalic acid > galactonic acid > glyceric acid. Xylose, glucose and maltose have no removal effect for Pb in soil.

Introduction
Since the inception of global industrialization and urbanization, heavy metal-contaminated soils have become an emerging and serious concern due to deleterious effect on human and animal health (Rascio and Navari-Izzo 2011). In China, recently nationwide surveys reported that 16% of the soil samples, 19% for the agricultural soils, are polluted with heavy metals based on China’s soil environmental quality standards (Zhao et al. 2014). Most of the agriculture land across the world is highly polluted with heavy metals including Lead (Pb). Pb is considered the most harmful for human health and accumulated at highest rates in soils (Venkatachalam et al. 2017). Environment researchers have given high priority to reducing the bioavailability of heavy metals in agriculture sites in order to ensure food security and human health (Bian et al. 2014).

Phytoremediation is novel, cost-effective, efficient, environment friendly and solar-driven remediation technology, which can improve the soil quality (Pilon-Smits 2005; Ali et al. 2013). Sedum alfredii is a newly discovered Cd/Zn co-hyperaccumulator native to China (Lu et al. 2010) which growing in an old mining areas of southeast China (Yang et al. 2002a, 2004), and later it demonstrated as a Pb accumulator (He et al. 2002). Earlier studies has exposed the accumulation and transportation mechanisms of S. alfredii (Long et al. 2002; Yang et al. 2002a, 2002b, 2004, 2006a), but some mechanisms still have not been clearly understood, especially the role of root exudates.

Root exudates play crucial role in the process of phytoremediation as an emerging green and in situ remediation technology. Root exudates are plant metabolites which exuded from plant roots to improve plant nutrient uptake and alleviate the response to environment stresses (Luo et al. 2014, 2015). The chemical composition and quantity of root exudates depend on plant’s inherent biology, such as plant species and growth cycle and other is plant growth environment (Luo et al. 2017). Root exudates can alter the chemical form of heavy metals, and then add or subtract their bio-available content (Kuang et al. 2003). Low molecular weight organic acids (LMWOA) which released from plant roots were attested to enhance phytoextraction of heavy metals by stimulating the mobility of metals and changing the nutrient condition (Rajkumar et al. 2012). Dissolved organic matter (DOM) which released from the rhizosphere of S. alfredii could be markedly decrease Zn and Cd adsorption and enhance their mobility by form the soluble DOM–metal complexes (Jiang et al. 2013). Citric acid and oxalic acid can maximize the translocation of Cd, Cu and Pb from roots to shoots (Fan et al. 2001). However, these studies are the targeted researches which based on some specified compounds, mainly are organic acids. The not-targeted researches which based on the approximate global analysis of root exudates from accumulator were rarely reviewed.

Metabonomics method is a high flux and unbiased composite analysis method which can expose the different metabolic states of the creature and identify the remarkable metabolites among the different states of biological systems (Wang et al. 2014). Our group has been using this technique to evaluate the variation of root exudates and identify the potential biomarkers among the accumulating and non-accumulating ecotype of S. alfredii under Cd and Pb stresses (Luo et al. 2014, 2015, 2017). The present study focus on the identification of the remarkable root exudates from the Pb-accumulator S. alfredii under Pb stresses and the verification of role of these remarkable root exudates.
Materials and methods

Chemicals

Pb(NO₃)₂, oxalic acid, galactonic acid, glyceric acid, xylose, glucose and maltose (analytic grade) were purchased from Sinopharm (Shanghai, China). Methanol (HPLC grade) used for extracting the plant root exudates was obtained from Fisher Ltd., USA. Pyridine (HPLC grade), methoxamine hydrochloride and N-methyl-N-(trimethylsilyl) trifluoroacetamide (MSTFA) for GC–MS analysis were purchased from Sigma Ltd., USA.

Plant culture

Pb-accumulator S. alfredii was collected from an old Pb/Zn mined area in Quzhou, Southeast China. In order to minimize the internal heavy metal contents, plants were grown in a non-contaminated soil in Shenyang University for some generations before used. Then, uniform plants, measuring in height with shoots, were selected and cultivated in the mined area in Quzhou, Southeast China. In order to minimize the internal heavy metal contents, plants were grown in a non-contaminated soil in Shenyang University for some generations before used. Then, uniform plants, measuring in height with shoots, were selected and cultivated in the mined area in Quzhou, Southeast China.

Pb treatment

In order to prevent lead precipitation, the NH₄H₂PO₄ concentration in nutrition solution was adjusted to 5 µmol/L.

First, the experiment of different Pb exposure concentrations was conducted. Intact roots of two weeks old seedlings of S. alfredii were treated with five treatments: 0 (control), 10, 50, 200 and 1000 µmol/L Pb(NO₃)₂, each pot containing one plant and each treatment has 11 pots. After growing for 4 days, the root exudates were collected.

Then, the experiment of different Pb exposure times was carried out. According to the results of the experiment of different Pb exposure concentrations, 200 µmol/L is an important node. When the exposure concentration higher or lower than this node, the morphology, physiology and root exudates of plants are all changed significantly. So 0 (control) and 200 µmol/L Pb(NO₃)₂ were selected as the exposure concentrations for the experiment of exposure times. Intact roots of two weeks old seedlings of S. alfredii were treated with two Pb treatments: 0 (control) and 200 µmol/L Pb(NO₃)₂, each pot containing one plant and each treatment has 11 pots. After growing for 4 and 8 days, the root exudates were collected.

Collection and pretreatment of root exudates

The procedure for the collection of root exudates has been described by Hao et al. (2010). The plants were transplanted to the sterilized pots which contain 50 mL deionized water in every pot to collect the root exudates for 6 h (from nine in the morning to three in the afternoon).

Sample preparation, derivatization and detection procedure were modified on the basis of previous studies (Lisec et al. 2006; Katsumasa et al. 2009). The root exudates were frozen by liquid nitrogen and freeze-dried for 2 days. The dried residue was resuspended in 100 mL of deionized water and freeze-dried again, then redissolved in 10 mL of cold MeOH. The sample solution was gently blow dried by N₂. After that, the dried residue sample was suspended in 40 µL 20 mg/mL methoxamine hydrochloride pyridine solution and shaken for 2 h at 37°C. Then, 70 µL N-methyl-N-(trimethylsilyl) trifluoroacetamide (MSTFA) was added and shaken for 30 min at 37°C. The sample was waiting for the GC–MS detection.

GC–MS detection of root exudates

The Thermo fisher PolarisQ mass spectrometer coupled with Thermo Trace GC Ultra was used to detect root exudates. Chromatographic column was the TR-5MS capillary column (30 m × 0.25 µm × 0.25 mm). The initial GC oven temperature was set at 70°C and maintained for 1 min, then slowly raised by 1°C/min to 76°C, followed by 5°C/min to 330°C and maintained for 10 min. The sample was injected in the splitless mode and the inject volume was 1 µL. The carrier gas was helium and the flow rate was 1 mL/min. The temperatures of injection port, interface and ion source were set at 230°C, 250°C and 210°C, respectively. The mass selective detector was run in the electron impact (EI) mode and the electron energy was 70 eV. The mass analyzer was run in full scan mode (m/z 50–600), and the solvent delay time was set at 3 min.

Data processing and pattern recognition analysis

The automatic mass spectral deconvolution and identification system (AMDIS, version 2.71) was used to extract the GC–MS raw data, obtain the chromatographic retention time, peak area, mass spectrometry and other useful information. A plant metabolites database, containing mass spectrometric data of many plant metabolic compounds which collected from Fiehn and Golm Metabolome Database (GMD), was set as the AMDIS database to identify the detected chromatographic peak. And the qualitative standard was the similarity is greater than 70%. The output of AMDIS was direct import to the metabolomics ion-based data extraction algorithm (MET-IDEA, version 2.08) which used to preprocess the chromatographic data, including baseline calibration, peak alignment, and so on. The MET-IDEA parameters including: (i) chromatography was GC, average peak width was set at 3 min. maximum peak width was set as 6, peak start/stop slope was set as 1.5, adjusted retention time accuracy was set as 0.95 and peak overload factor was set as 0.3; (ii) mass spec was trap, mass accuracy was set as 0.1 and mass range was set as 0.5; (iii) the AMDIS exclude ion list was 73, 147, 281, 341 and 415, lower mass limit was set as 50 and ions per compound was set as 1; (iv) the retention time calibration was set as 9.30 min. Before pattern recognition analysis, the chromatographic peak area of every identified compound must be normalized. The normalization method was the peak area...
values were divided by the average of the compound which first appeared.

Pattern recognition analysis was accomplished by SIMCA-P 13.0 (Umetrics, Sweden). Principal component analysis (PCA) was firstly used to get an overview of the sample distribution and discover the possible outliers. Before the analyses, the normalized GC–MS data were unit-variance scaled. Then, the orthogonal partial least-squares discrimination analysis (OPLS-DA) was carried out to identify the remarkable metabolites which observably contribute to differentiation. The value of Variable Importance in the Projection (VIP) was utilized to assess the variable contribution and identify the potential biomarkers. The univariate statistical analysis was conducted by SPSS 19.0 for further recognition of the potential biomarkers, such as box figure analysis and analysis of variance (ANOVA), and the p-value was set as .05 for statistical significance.

**Batch extraction with the remarkable root exudates**

Soil samples (0–20 cm) were collected from the Shenyang Zhangshi Irrigation Area, western Shenyang, Northeast China. The soil was air-dried and sieved to <2 mm. The organic matter content of the soil sample was 19.8 g/kg, the pH_{H2O} was 6.4, the cation exchange capacity (CEC) was 16.8 cmol/kg and the soil was clay loam. The concentration of Pb is 72.4 mg/kg.

Two grams of soil sample were extracted with 20 mL 0.01 mol/L NaNO_3 aqueous solution in a 50 mL polypropylene centrifuge tube in a reciprocal shaker for 3 h at room temperature (~20°C). The NaNO_3 solution contained the every remarkable root exudates and the content was 0, 2, 4, 8 and 16 mmol/L, respectively. The soil suspension was centrifuged at 7000 rpm for 20 min and then filtered through Whatman No. 42 paper. The concentration of Pb in the extract was determined by AAS.

**Results**

**Metabonomic profiling by GC–MS**

Sixty-eight metabolites were detected and identified in root exudates of Pb-accumulator *S. alfredii* under 0, 10, 50, 200 and 1000 µmol/L Pb treatment for 4 days. The relative contents of these 68 metabolites were listed in the Supplemental file (Supplemental Table 1). The obvious differences of the composition or content of root exudates were discovered from the total ions chromatogram (Figure 1).

Seventy-two metabolites were detected and identified from Pb-accumulator *S. alfredii* under 0 and 200 µmol/L Pb treatment for 4 and 8 days. The relative contents of these 72 metabolites and the total ions chromatogram were listed in the Supplemental file (Supplemental Table 2 and Fig. 1).

**Pattern recognition analysis of identified root exudates**

The PCA and OPLS-DA were used to carry out the pattern recognition analysis of identified root exudates. The PCA scores plots of the identified metabolites from *S. alfredii* in different Pb treatment groups are shown in Supplemental file (Supplemental Figs. 2 and 3). In Supplemental Fig. 2, when we analyze all treatments together, samples from 0, 10 and 50 µmol/L treatments clustered together, not separated. But when we only analyze the samples from t0, 10 and 50 µmol/L treatments, an obviously separation was achieved. When we look at the samples from 0, 10 and 50 µmol/L Pb treatments as a whole, samples under low Pb concentration, a clearly separation was obtained when analyzed together with samples from 200 and 1000 µmol/L Pb treatment. In Supplemental Fig. 3, a clearly separation was observed between 0 and 200 µmol/L Pb treatment for 8 days, and this separation was superior to 4 days. Besides this, the separation condition between 4 and 8 days under 200 µmol/L Pb treatments was better than 0 µmol/L. These indicated that Pb exposure concentration

![Figure 1. The total ions chromatogram of root exudates from *S. alfredii* under different Pb exposure concentrations for 4 days. (A) 0 µmol/L Pb treatment; (B) 10 µmol/L Pb treatment; (C) 50 µmol/L Pb treatment; (D) 200 µmol/L Pb treatment and (E) 1000 µmol/L Pb treatment. Some identified compounds: (1) lactic acid-2TMS; (2) l-alanine-2TMS; (3) diethylene glycol-2TMS; (4) glycerol-3TMS; (5) succinic acid-1TMS; (6) nonanoic acid-1TMS; (7) decanoic acid-1TMS; (8) erythritol-4TMS; (9) fructose-1MEOX-5TMS; (10) glucose-1MEOX-5TMS; (11) 9-hexadecenoic acid-1TMS; (12) octadecanol-1TMS; (13) octadecanoic acid-1TMS and (14) beta-sitosterol-1TMS.](image-url)
and time all can change the composition and quantity of root exudates of *S. alfredii*, and the influence of high Pb concentration is greater than low Pb concentration, and the influence of long time is greater than short time.

For more significant classification, a supervised learning method, such as PLS-DA, OPLS-DA or two-way OPLS-DA (O2PLS-DA), was employed to remove nonessential factors and then improved the accuracy of classification. We used OPLS-DA to discriminate amongst different Pb treatment groups in this study (Figures 2 and 3). As shown in the scores plot, the result is similar to the result of PCA, just the aggregation and separation is more visible.

**Identification of remarkable metabolites**

Remarkable metabolites were selected by the VIP plots (Figures 4 and 5) and loading plot (Supplemental Figs. 4 and 5) from the pattern recognition model. The VIP value >1 and the points were relatively far away from the center in the loading plot indicate variables that have an above average influence on the classification (Lu et al. 2013). Furthermore, alternation of the remarkable metabolites was evaluated using ANOVA (*p* < .05). Consequently, 16 metabolites were considered as remarkable metabolites when *S. alfredii* under different Pb exposure concentrations for 4 days, and 18 metabolites gathered when *S. alfredii* under different Pb exposure times. The relative contents of these remarkable metabolites were listed in the Supplemental file (Supplemental Tables 3 and 4).

Under two experimental conditions, Pb exposure concentrations and times, 11 remarkable metabolites were the same. They were oxalic acid, hexanoic acid, glyceric acid, xylose, glucose, *n*-pentadecanoic acid, galactonic acid, *n*-docosane, hexadecanoic acid, 9-hexadecenoic acid, maltose,

![Figure 2. The OPLS-DA scores plot of *S. alfredii* under different Pb exposure concentrations for 4 days (●) 0 µmol/L Pb treatment, (●) 10 µmol/L Pb treatment, (▲) 50 µmol/L Pb treatment, (●) 200 µmol/L Pb treatment, (▲) 1000 µmol/L Pb treatment.](image2)

![Figure 3. The OPLS-DA scores plot of *S. alfredii* under different Pb exposure times (●) 0 µmol/L Pb treatment for 4 days, (▲) 200 µmol/L Pb treatment for 4 days, (●) 0 µmol/L Pb treatment for 8 days, (▲) 200 µmol/L Pb treatment for 8 days.](image3)
respectively. So we focused on the role of these 11 remarkable metabolites in the follow-up experiments.

**Verification of the role of remarkable metabolites**

For further verifying the role of remarkable metabolites, batch extraction experiments were performed. Due to poorly solubility or insolubility in water, some remarkable metabolites, such as hexanoic acid, \( n \)-pentadecanoic acid, \( n \)-docosane, hexadecanoic acid and 9-hexadecenoic acid were not considered in the batch extraction experiments. Because the content of root exudates around rhizosphere is low, series low concentrations of batch extraction experiments were conducted.

From Figure 6, you can see that the extraction amount of Pb increased with the increase of the concentration of galactonic acid and glyceric acid. When the concentration of oxalic acid was 2 mmol/L, the extraction amount of Pb was less than the control (0 mmol/L). However, when the concentration of oxalic acid was 4 mmol/L, the extraction amount of Pb was significantly higher, and increased with the increase of the concentration of oxalic acid. The extract effect was: oxalic acid > galactonic acid > glyceric acid. The extraction amount of Pb under different concentration of xylose, glucose and maltose was same.

Pb content is the amount of Pb in extracted solution divided by the soil weight.

**Discussions**

In this study, 68 and 72 metabolites were detected and identified when the Pb-accumulator *S. alfredii* were treated under 0, 10, 50, 200 and 1000 µmol/L Pb treatment for 4 days and 0, 200 µmol/L Pb treatment for 4, 8 days, respectively. And there have many chromatographic peaks or compounds were not identified. But, only based on these identified metabolites, we observed the obviously differences of quantity or composition of root exudates which released from the Pb-accumulator *S. alfredii* under different Pb exposure concentrations and times through PCA and OPLS-DA. These findings are in agreement with previous results, many factors can
change the quantity and composition of plant root exudates, such as plant species and their growth cycle and environmental stresses, etc. (Lilia et al. 2011; Selvakumar et al. 2012).

Based on the results of the loadings plots, the VIP values of OPLS-DA and ANOVA, 11 metabolites were considered as the remarkable metabolites when the Pb-accumulator S. alfredii treatment with different Pb exposure concentrations and times. We speculated that these 11 remarkable metabolites might play an important role in the process of S. alfredii response to Pb stress.

Batch extraction experiments showed that oxalic acid, galactonic acid and glyceric acid can removed the Pb in soil. The removal effect of xylose, glucose and maltose for Pb in soil were not obvious. Oxalic acid and glyceric acid also were selected as potential biomarkers when two ecotypes of S. alfredii treated with Pb and their roles were activated Pb in soil (Luo et al. 2017).

Several prior studies have reported that the LMWOA play an important role in the process of modification of bioavailability of heavy metal (Sun et al. 2006; Yang et al. 2006b; Montiel-Rozas et al. 2016). Organic acids can behave as natural chelating agent to activate heavy metal in soil (Kim et al. 2010; Agnello et al. 2014). Oxalic acid have a potentially ability to release Pb from pyromorphite in heavy metals contaminated soils (Debela et al. 2010). Oxalic acid and citric acid can inhibit the formation of pyromorphite and zinc phosphates (sparingly soluble minerals) in soils (Debela et al. 2013). Oxalic acid significantly increased Pb uptake when the seeds (Zinnia elegans Jacq.) was treated with Pb (Cui et al. 2007). Oxalic acid also can enhance As mobilization by dissolving As host minerals and competing for sorption sites (Sun et al. 2016). In this study, oxalic acid was considered as the remarkable metabolites and can efficiently remove Pb in soil. Under a high pH condition, oxalic acid could chelate react with Pb to form an insoluble Pb-oxalate complex on the soil surface, and then decrease the bioavailability of Pb (Wasay et al. 1998). So when the concentration of oxalic acid was 2 mmol/L, the removal was less than the control.

Organic acids could lead to the acidification of the rhizosphere by decrease the pH (Niu et al. 2013; Seshadri et al. 2015), and then mobilized the insoluble heavy metal chelates in soil, increased their bioavailability (Wang and Lin 1991). Some sugar acids, such as glucuronic acid and glucaric acid, have a good effect to remove heavy metals in soil (Burckhard et al. 1995; Wasay et al. 2001; Fischer and Bipp 2002). In this study, galactonic acid and glyceric acid were considered as the remarkable metabolites and they can remove Pb in soil. Due to glyceric acid is weak acid, the remove effect is general.

The main function of carbohydrate is to provide carbon source for microorganisms (Görke and Stülke 2008). In this study, carbohydrate, such as xylose, glucose and maltose, has no remove effect for Pb in soil. But carbohydrate plays an important role in the process of phytoremediation. It can change the number and activities of microorganisms, and then indirectly affect the bioavailability of heavy metals in soil.

Hexanoic acid, n-pentadecanoic acid, n-docosane, hexadecanoic acid and 9-hexadecenoic acid also were considered as remarkable metabolites, but they are poorly solubility or insolubility in water, they cannot be excessively used as removal product. In this study, we did not explore their roles. But these metabolites might be useful for phytoremediation, such as through microbes (Brace 2001).

**Conclusions**

In this study, we used the metabonomics method based on GC–MS technology and pattern recognition analysis method to identify the remarkable root exudates when the Pb-accumulator S. alfredii was exposed to different Pb concentrations and exposure times. The results indicated that Pb concentrations and exposure times can obviously change the quantity and composition of plant root exudates, and 11 metabolites were considered as remarkable metabolites. Batch extraction experiments showed that oxalic acid, galactonic acid and glyceric acid can remove Pb in soil, and the removal effect was: oxalic acid > galactonic acid > glyceric acid. Xylose, glucose and maltose have no removal effect for Pb in soil.

**Acknowledgements**

The funder had no role in study design, data collection and analysis, decision to publish or preparation of the manuscript.

**Disclosure statement**

No potential conflict of interest was reported by the authors.

**Funding**

We are grateful to the financial support from the National Program on Key Basic Research Project of China (973 Program) (No. 2014CB441100), National Natural Science Foundation of China (No. 41401352), the project of Shenyang Science and Technology plan (No. F14–133–9–00) and Competitive selection project of Shenyang scientific undertaking (Study on risk management and restoration technology of urban ecological environment).
Fan TWM, Lane AN, Shenker M, Bartley JP, Crowley D, Higashi RM, Debela F, Arocena JM, Thring RW, Whitcombe T. 2013. Organic acids as amendments to enhance the heavy metal uptake by the hyperaccumulator Sedum alfredii. J Hazard. Mater. 272:121–128.

Brace EP. 2001. Phytoremediation of contaminated soil and ground water at hazardous waste sites. EPA ORD Ground Water Issue (EPA/540/S-01/500).

Burckhardt SR, Schwab AP, Banks MK. 1995. The effects of organic acids on the leaching of heavy metals from mine tailings. J Hazard Mater. 41:135–145.

Cui S, Zhou QX, Wei SH, Zhang W, Cao L, Ren LP. 2007. Effects of root exudates on the dissolution of Pb from Pb-contaminated soils. Chemosphere. 80:450–456.

Debela F, Arocena JM, Thring RW, Whitcombe T. 2010. Organic acid-induced release of lead from pyromorphite and its relevance to reclamation of Pb-contaminated soils. Chemosphere. 80:505–509.

Hao WY, Ren LX, Ran W, Shen QR. 2010. Allelopathic effects of root exudates from the Cd hyperaccumulator Sedum alfredii on the growth of Euphorbia tirucalli. Acta Bot Sin. 52:614–619.

Fan TWM, Lane AN, Shenker M, Bartley JP, Crowley D, Higashi RM, Debela F, Arocena JM, Thring RW, Whitcombe T. 2010. Organic acid-induced release of lead from pyromorphite and its relevance to reclamation of Pb-contaminated soils. Chemosphere. 80:450–456.

Debela F, Arocena JM, Thring RW, Whitcombe T. 2010. Organic acid-induced release of lead from pyromorphite and its relevance to reclamation of Pb-contaminated soils. Chemosphere. 80:450–456.

Luo Q, Sun LN, Hu XM, Zhou RR. 2014. The variation of root exudates from the hyperaccumulator Sedum alfredii under cadmium stress: metabolomics analysis. PLoS ONE. 9(12):e115581.

Luo Q, Sun LN, Wang H, Hu XM. 2015. Metabolic profiling analysis of root exudates from the Cd hyperaccumulator Sedum alfredii under different Cd exposure concentrations and times. Anal Meth. 7:3793–3800.

Luo Q, Wang SY, Sun LN, Wang H. 2017. Metabolic profiling of root exudates from two ecotypes of Sedum alfredii treated with Pb based on GC-MS. Sci Rep. 7:39878.

Montiel-Rozas MM, Madejón E, Madejón P. 2016. Effect of heavy metals and organic matter on root exudates (low molecular weight organic acids) of herbaceous species: an assessment in sand and soil conditions under different levels of contamination. Environ Pollut. 216:273–281.

Niu ZX, Liao XD, Sun LN, Sun TH. 2013. Dynamics of three organic acids (malic, acetic and succinic acid) in sunflower exposed to cadmium and lead. Int J Phytoremediat. 15:690–702.

Pilon-Smits E. 2005. Phytoremediation. Annu Rev Plant Biol. 56:15–39.

Rajkumar M, Sandhya S, Prasad M, Freitas H. 2012. Perspectives of plant-associated microbes in heavy metal phytoremediation. Biotechnol Adv. 30:1562–1574.

Rascio N, Navari-Izzo F. 2011. Heavy metal hyperaccumulating plants: how and why do they do it? And what makes them so interesting? Plant Sci. 180:169–181.

Selvakumar G, Panneerselvam P, Ganeshamurthy AN, Maheshwari DK. 2012. Bacterial mediated alleviation of abiotic stress in crops. In: DK Maheshwar, editor. Bacteria in agrobiology: stress management. New York (NY): Springer; p. 205–224.

Seshadri B, Bolan N, Naidu R. 2015. Rhizosphere-induced heavy metal (lodd) transformation in relation to bioavailability and remediation. J Soil Sci Plant Nutr. 15:524–548.

Sun J, Bostick BC, Mailloux BJ, Ross JM, Chirrud SN. 2016. Effect of oxalic acid treatment on sediment arsenic concentrations and liability under reducing conditions. J Hazard Mater. 111:125–133.

Sun R, Zhou QX, Jin C. 2006. Cadmium accumulation in relation to organic acids in leaves of Solanum nigrum L. as a newly found cadmium hyperaccumulator. Plant Soil. 285:125–134.

Venkatachalam P, Jayalakshmi N, Geetha N, Sahi SV, Sharma NC, Rene ER, Sarkar SK, Favis PF. 2017. Accumulation efficiency, genotoxicity and antioxidant defense mechanisms in medicinal plant Acalypha indica L. under lead stress. Chemosphere. 171:544–543.

Wang JL, Lin ZY. 1991. Behavior of heavy metals in rhizosphere and accumulation in the hyperaccumulating and non-hyperaccumulating species of Zn. J Plant Nutr Soil Sci. 147:3–11.

Yang XE, Long XX, Ni WZ. 2010. Root exudation of sugars, amino acids, and organic acids by Zn hyperaccumulator. Plant Soil. 285:125–133.

Yang XE, Li TQ, Yang JC, He ZL, Lu LL, Meng FH. 2006b. Zinc components and soils by natural chelating agents. Part II. Soil extraction and accumulation. Acta Bot Sin. 44:152–157.

Yang XE, Long XX, Ye ZQ, Ni WZ. 2011. Root exudation of sugars, amino acids, and organic acids by Zn hyperaccumulator. Plant Soil. 285:125–133.

Yao H, Wang YN, Zhao M, Wang F, Zhao CJ. 2014. Metabolic profiling analysis of fatty acids from hyperlipidemic rats treated with Gynostemma pentphyllum and atorvastatin based on GC/MS. Anal Methods. 6:8660–8667.

Wasay SA, Barrington S, Tokunaga S. 2001. Organic acids for the in situ remediation of soils polluted by heavy metals: soil flushing in columns. Water Air Soil Pollut. 127:301–314.

Wasay SA, Barrington S, Tokunaga S. 1998. Remediation of soils polluted by heavy metals using salts of organic acids and chelating agents. Environ Technol. 19:369–379.

Yang YE, Li TQ, Long XX, Stoffella PJ. 2006a. Dynamics of zinc uptake and accumulation in the hyperaccumulating and non-hyperaccumulating ecotypes of Sedum alfredii Hance. Plant Soil. 284:109–119.

Yang YE, Li TQ, Yang JC, He ZL, Lu LL, Meng FH. 2006b. Zinc compartmentation in root, transport into xylem, and absorption into leaf cells in the hyperaccumulating species of Sedum alfredii Hance. Planta. 224:185–195.

Yang YE, Long XX, Ni WZ. 2002a. Physiological and molecular mechanism of heavy metal uptake by hyperaccumulating plants. Plant Nutr Fertil Sci. 8:8–15.

Yang YE, Long XX, Ni WZ. 2002b. Sedum alfredii Hance – a new ectype of Zn hyperaccumulator plant species native to China. Chin Sci Bull. 47:1003–1006.

Yang YE, Long XX, Ye HB, He ZL, Calvert DV, Stoffella PJ. 2004. Cadmium tolerance and hyperaccumulation in a new Zn-hyperaccumulating plant species (Sedum alfredii Hance). Plant Soil. 259:181–189.

Zhao FJ, Ma Y, Zhu YG, Tang Z, McGrath SP. 2014. Soil contamination in China: current status and mitigation strategies. Environ Sci Technol. 49:750–759.