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Repeated phytoextraction of metal contaminated calcareous soil by hyperaccumulator *Sedum plumbizincicola*

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ABSTRACT

Most studies on the phytoextraction of cadmium (Cd) and zinc (Zn) by the hyperaccumulator *Sedum plumbizincicola* (*S. plumbizincicola*) have been conducted in metal contaminated acid and neutral soils. However, little information is available on phytoremediation of calcareous soils. Two experiments were conducted to investigate the phytoextraction efficiency of *S. plumbizincicola* in a contaminated calcareous soil in He’nan province, north China. In a field experiment there was no significant decrease in shoot biomass production or metal (Cd and Zn) concentration in the shoots after three successive repeated phytoextractions. Repeated phytoextraction had no significant effect on the percentage distribution of Cd or Zn fractions in the soil even though the soil total Cd and Zn concentrations decreased by 32.8 and 19.7%, respectively. In a pot experiment the shoot biomass production and Zn and Cd uptake by *S. plumbizincicola* increased significantly with growth in metal contaminated calcareous soil amended with organic fertilizer, perlite and vermiculite. The results indicate that *S. plumbizincicola* can maintain sustainable uptake of Cd and Zn from the calcareous soil and enhancement of soil fertility and structure will significantly increase the phytoextraction efficiency.

Key words

Cd, hyperaccumulator, repeated phytoextraction, soil amendment, Zn

1 INTRODUCTION

Accumulation of heavy metals in agricultural soils has attracted considerable public concern in China due to the potential risks to human health (Liu *et al.* 2013; Zhao *et al.* 2015). The Ministry of Environmental Protection (MEP) and the Ministry of Land and Resources (MLR) of the People's Republic of China issued a joint report stating that 16% of soil samples (19% of agricultural soils) are contaminated with heavy metals and metalloids (MEP and MLR 2014). Cleanup of heavy metal contaminated soils is therefore a strategic aim for safe and sustainable agriculture in China. Phytoremediation is considered to be an alternative 'green' solution for the removal of heavy metals from agricultural soils and the option of repeated phytoextraction by hyperaccumulators is the main and most useful phytoremediation technique developed in recent years (Rascio and Navari-Izzo 2011; Ali, Khan, and Sajad 2013). Phytoextraction efficiency is determined mainly by the shoot metal concentration and shoot biomass and is also influenced by numerous factors including soil properties, heavy metal speciation and plant species (Audet and Charest 2007; Ali *et al*. 2013). The addition of organic amendments to a contaminated soil can increase soil fertility and enhance soil chemical properties to increase the plant biomass and uptake of heavy metals (Chirakkara and Reddy 2015; Rees, Sterckeman, and Morel 2016). The application of soil conditioners can stimulate root system development and biomass of *Thlaspi caerulescens* by decreasing soil bulk density, thus enhancing the phytoextraction efficiency of Cd in contaminated soil (Yang *et al*. 2010).

S. plumbizincicola has larger shoot biomass production and higher shoot Cd and Zn concentrations and is therefore considered to have a great capacity to extract Cd and Zn from contaminated soils. It has shown great potential in repeated phytoextraction of metal contaminated soils from both south and east China (Li *et al.* 2014; Deng *et al*. 2016). In a similar fashion to other hyperaccumulators, the phytoextraction efficiency of Cd and Zn by *S. plumbizincicola* is determined by both soil properties and environmental factors. Amendment of contaminated soil with rice straw or clover results in higher shoot concentrations of Cd and shoot biomass of *S. plumbizincicola* (Wu *et al*. 2012). The coarse soil particle fraction makes a major contribution to the decline in soil Cd and Zn concentrations rather than the fine soil fraction in whole soil during repeated phytoextraction (Li *et al*. 2014).

He'nan province is a major crop production area in north China and the typical cropping system is an annual rotation of winter wheat and summer maize. There is widespread agricultural soil contamination with heavy metals such as Cd, Pb and Zn, which are often derived from metal smelting industries (Zhang *et al*. 2012), and remediation of metal contaminated soil is critical for the safe production of food in He'nan province. The results of numerous successful phytoextraction experiments using *S. plumbizincicola* in south and east China have been reported, but no study has been conducted on repeated phytoextraction of calcareous soil in north China. In north China many agricultural soils experience different temperatures and precipitation, soil pH, structure and minerals, and tillage, irrigation and fertilizer practices compared with soils in south and east China. A pot experiment study found that the lower biomass of *Thlaspi caerulescens* obtained is a major factor constraining high phytoextraction efficiency in metal contaminated soil (Koopmans *et al*. 2008). However, long-term application of large amounts of chemical fertilizers resulted in a decline in soil organic carbon content and an increase in soil bulk density which were the two important factors adversely affecting the sustainability of crop production in parts of north China (Xin *et al*. 2016). Due to these differences between north and south in soil and environmental conditions it is not possible to transfer the findings of phytoextraction experiments from east and south China to the north of the country.

In the present study a field experiment was conducted to test the phytoextraction efficiency of the Cd/Zn hyperaccumulator *S. plumbizincicola* growing in a metal contaminated calcareous soil. In view of the soil factors limiting plant growth, a pot experiment was also conducted to investigate the plant growth conditions and phytoextraction efficiency using different soil amendments. The aim of this study was to test the phytoextraction efficiency of *S. plumbizincicola* in a calcareous soil to provide information that would help to develop management practices for the safe utilization of metal contaminated soils in north China.

2 MATERIALS AND METHODS

2.1 Site description

The study area is located in northwest He’nan province in north China (35°08'01.72“ N, 112°33'15.44” E) and is typical of the North China Plain. The region is characterized by a warm temperate monsoon climate with mean annual temperature and precipitation of 12.2°C and 617 mm, respectively. The soil is derived from alluvial sediments of the Yellow River and is classified as a Haplic Luvisols according to the World Reference Base for Soil Resources (IUSS Working Group WRB 2015). There is a large lead (Pb) smelting plant in the northwest of the study area (1.5 km) which has been in operation for more than 50 years. The smelter maintains an annual production capacity of 0.16 million tonnes of electrolytic Pb and 0.10 million tonnes of secondary Pb. It also generates considerable atmospheric emission of dust leading to high Cd and Pb contamination of agricultural fields in the vicinity of the smelting plant.

2.2 Field experiment

An upland field was selected to the southeast of the smelter where the Cd/Zn hyperaccumulator *S. plumbizincicola* was transplanted. There were five plots and each plot was 3 m × 20 m in area. Soil properties and total heavy metal concentrations before transplantation were determined and are shown in Table 1. The field experiment was conducted from October 2012 to July 2015 and was composed of three successive croppings of *S. plumbizincicola*. The first, second and third crops of the hyperaccumulator were transplanted in October 2012, 2013 and 2014 and harvested in July 2013, 2014 and 2015. Similar agricultural practices were performed each year. *S. plumbizincicola* shoots (10 cm height) were collected from a seedbed in a greenhouse near the study field and transplanted into each plot at a density of 4.44×10^5 plants ha^{-1} (15 × 15 cm). A compound fertilizer (N:P:K = 12:26:12, 675 kg ha^{-1}) was applied to the soil as a basal fertilizer before transplanting and urea (225 kg ha^{-1}) was applied at later growth stages. Throughout the experiment the plants were watered periodically depending on soil moisture conditions.

The shoots of *S. plumbizincicola* were harvested at flowering stage because of the high plant productivity and also because of the occurrence of uncontrolled death after this growth stage in the hot summer. Three plants were collected from each plot to obtain a mixed sample, washed with deionized water, oven dried at 80°C and then ground prior to metal analysis. At the same time 1 m^2 of shoots were harvested completely, washed with tap water and dried to calculate the aboveground yield. The same method was used to sample the plant shoots of all three crops. After the harvest of the third crop (2014–2015) a mixed topsoil (0–20 cm depth) sample was collected from each plot. The soil samples were air-dried and ground to pass a 0.15 mm nylon sieve for determination of total and fractions of heavy metals.

2.3 Pot experiment

A pot experiment was conducted in a glasshouse from 12 February to 30 July 2015 with one growth period at the Institute of Soil Science, Chinese Academy of Sciences, Nanjing. During the experiment the maximum, minimum and average temperatures were 35, 15 and 19–31°C in the glasshouse. Four treatments were included in the pot experiment, namely soil collected from the same region but with lower heavy metal concentrations than that of the field experiment in He’nan province (HN), a fertile soil collected from Zhejiang province (ZJ, soil properties are shown in Table 1), a mixture of the soils from HN and ZJ (1:1 by weight) to give the third treatment (HN+ZJ), and soil from HN amended with 2% organic fertilizer, 1% vermiculite and 1% perlite (HN+amendment). The ZJ soil used had received sewage sludge for many years. The concentrations of total N, K, P, Cd and Zn in the organic fertilizer were 30.7, 5.8 and 4.8 g kg^{-1}, and 0.05 and 66.2 mg kg^{-1}, respectively. Treatments ZJ and HN+ZJ were set up as a comparison in order to know whether amendment with organic fertilizer, vermiculite and perlite could lead to as high biomass production in HN soil as that in fertile soil. There were four replicates of each treatment.

Air-dried soil was ground to pass a 2-mm sieve and mixed thoroughly and 1.50 kg soil (oven dry basis) was placed in each 15-cm-diameter plastic pot. Eight seedlings of *S. plumbizincicola* (about 1 cm in height) were transplanted into each pot and thinned to four seedlings after three weeks. No basal fertilizers were applied in the pot experiment and 0.64 g kg^{-1} urea was added four weeks after transplantation. Deionized water was added to each pot to maintain the soil water content at about 70% of soil water holding capacity (WHC) during plant growth. At the end of the experiment all the plant shoots in each pot were harvested just above the soil surface, washed with deionized water, oven dried at 80°C, weighed and ground.

2.4 Chemical analysis

In the field experiment, subsamples of soil collected before and after repeated phytoextraction by *S. plumbizincicola* were used for Cd and Zn extraction. A modified fractionation procedure recommended by BCR was used to separate the soil heavy metals into four operationally defined fractions: acid-soluble, reducible, oxidisable and residual fractions. Full details of the method have been described previously by Li *et al*. (2014).

Soil organic carbon content (SOC) was determined by wet digestion with potassium dichromate. Soil pH was measured in a 1/2.5 (w/v) soil suspension with a glass electrode. Soil cation exchange capacity (CEC) was determined by exchange with ammonium acetate (1.0 mol L^{-1}, pH 7.0) and titration with HCl. Available phosphorus (P) was extracted with 0.5 mol L^{-1}

$NaHCO_3$ by the Olsen method. Available potassium (K) was determined by flame photometry after extraction with 1 mol L^{-1} NH_4OAc. All the determinations of soil properties were performed according to the protocols described by Lu (2000).

Total heavy metal concentrations in the bulk soils from both the field and pot experiments and in the soil residual fraction from BCR extraction were digested with 10 ml HCl-HNO_3 (1:1, v/v). Shoots of *S. plumbizincicola* from both the field and pot experiments were digested using a mixture of 6 ml HNO_3 and 2 ml H_2O_2. Heavy metal concentrations in the extract and digest solutions were determined by atomic absorption spectrometry (AAS) using a Varian SpectrAA 220FS or 220Z (Varian, Palo Alto, CA).

2.5 Statistical analysis

All data are expressed as mean ± standard deviation (SD). The significance of differences among treatments were analysed by one-way analysis of variance and mean values compared with Student's t-test and at $p < 0.05$ using the SPSS version 20 software package released in 2011 by IBM Corporation.

3 RESULTS

3.1 Shoot biomass and metal uptake in the field experiment

As shown in Fig.1a the shoot biomass of *S. plumbizincicola* showed significant differences among the three successive crops. The shoot biomass was 2.50 t ha^{-1} in the first crop (2012--2013) and increased significantly by 54.0 and 61.5%, respectively, in the second

(2013--2014) and third (2014--2015) crops. The shoot concentrations of Cd and Zn in each crop are shown in Fig. 1b. The lowest and highest shoot Cd concentrations were observed in the first and second crops, respectively. No significant differences in shoot Zn concentration were observed among the three successive crops. Total Cd and Zn uptake in each crop were calculated by multiplying shoot biomass by metal concentration (Fig. 1c). The highest Cd uptake was observed in the second crop followed by the third and first crops. In comparison to the value of 2523 g ha^{-1} in the first crop, the uptake of Zn increased significantly by 56.2% in the third crop but not significantly in the second crop.

3.2 Changes in soil total Cd and Zn and chemical speciation in the field experiment

Soil total Cd and Zn and chemical speciation before phytoextraction (unplanted) and in soil after three successive phytoextraction (planted) steps are shown in Table 2. After repeated phytoextraction the soil total Cd and Zn concentrations decreased significantly by 32.8 and 19.7%, respectively. In addition, the concentrations of soil acid-soluble, reducible, oxidisable and residual Cd decreased significantly by 41.8, 32.5, 26.6 and 37.9%, respectively, after repeated phytoextraction. Phytoextraction significantly decreased soil acid-soluble and residual fractions of Zn by 28.3 and 27.0%, respectively. As shown in Table 2 the acid-soluble and reducible fractions accounted for over 90% of soil total Cd. In contrast, soil total Zn comprised mainly the reducible, oxidisable and residual fractions (> 90%). In comparison to unplanted soil, repeated phytoextraction had no significant effects on the percentage distribution of Cd or Zn in planted soil. The recoveries of the sequential extraction in unplanted and planted soils were 104 and 106% for Cd and 94.2 and 88.0% for Zn, respectively. The results indicate that the sums of the four fractions were in good agreement with the total digestion results.

3.3 Shoot biomass and metal uptake in the pot experiment

The shoot biomass of *S. plumbizincicola*, metal concentrations and metal uptake are shown in Table 3. The shoot biomass in HN soil was lower than that in ZJ soil. In comparison to HN soil the shoot biomass in the mixed soil (HN+ZJ) increased significantly by 110%. Amendment of HN soil with organic fertilizer, vermiculite or perlite also significantly increased shoot biomass by 70.3% and the shoot biomass was similar to that in ZJ soil.

The mean shoot Cd concentration in ZJ soil was 2.85 mg kg^{-1}, a lower value than that of 19.2 mg kg^{-1} in HN soil. However, the shoot Cd concentration in mixed soil (HN+ZJ) was not significantly different from that in HN soil. Similarly, amendment of HN soil with organic fertilizer, vermiculite or perlite did not influence the shoot Cd concentration. Due to the high concentration of soil total Zn, the shoot Zn concentration in ZJ soil was the highest among the different treatments. However, there were no significant differences in shoot Zn concentration among treatments HN, HN+ZJ and HN+amendment. The use of fertile soil or application of organic or inorganic amendments (organic fertilizer, vermiculite or perlite) would therefore not affect the shoot Cd and Zn concentrations. The larger amounts of Cd and Zn taken up by the shoots in treatments HN+ZJ and HN+amendment compared with HN are attributable mainly to the significant increases in shoot biomass.

4 DISCUSSION

4.1 Field experiment

Li *et al*. (2013) reported that Cd concentrations in shoots of *S. plumbizincicola* decreased with harvest time in both acidic and neutral soils from Zhejiang province with repeated phytoextraction in a pot experiment. In contrast, in our study the shoot Cd concentration showed an increasing trend with time in a calcareous soil from He'nan province. This difference might be attributed to a higher desorption capacity of Cd from stable to labile fractions in the process of phytoextraction of the calcareous soil compared with acid and neutral soils (Li *et al.* 2016). Some studies report that the roots of hyperaccumulators can secrete H^+ ions and other exudates to acidify the rhizosphere soil, and the low rhizosphere pH will then promote the desorption of metal ions from the solid fraction to increase the heavy metal concentrations in the soil solution (McGrath, Zhao, and Lombi 2001; Ali *et al.* 2013; Jiang *et al.* 2013). In the present study the percentage (53%) of acid-soluble Cd in the calcareous soil is lower than that (64 ± 11%) reported in the acid and neutral soils (Li *et al*. 2014). The effect of acid conditions on Cd desorption may therefore be enhanced in calcareous soil. Although a significant decrease in the soil acid-soluble Cd fraction was found, the percentage distribution of the acid-soluble Cd fraction in the calcareous soil remained unaffected by repeated phytoextraction (Table. 2). In contrast, a reduction in the percentage distribution of acid-soluble Cd was found in acid and neutral soils (Li *et al*. 2014). However, the shoot Cd concentration (101--153 mg kg^{-1}) in *S. plumbizincicola* in the calcareous soil was not lower than that (25--162 mg kg^{-1}) in a neutral soil with a similar soil total Cd concentration (3.75 mg kg^{-1}, Deng *et al.* 2016). The results suggest that the supply of the most bioavailable Cd from the large pool of the reducible fraction may be adequate for plant uptake in calcareous soil. Due to the differences in soil pH and the percentage distribution of Cd fractions, the planting of *S. plumbizincicola* can maintain a stable phytoextraction efficiency of Cd for at least three successive crops in calcareous soil in comparison to acidic and neutral soils.

In contrast to the increasing trend of Cd, no significant difference in shoot Zn concentration was observed among the three successive crops in our calcareous soil. This might be due largely to the low soil total Zn concentration of 108 mg kg^{-1} which has reached the background value of natural soil according to the National Soil Environmental Quality Standard (First level: Zn 100 mg kg^{-1}). This indicates that total Zn in soil originates primarily from the weathering of soil parent materials and the residual fraction is the dominant chemical form of Zn in the soil. According to the results from the BCR extraction procedure, we also found that the acid-soluble Zn fraction accounted for only 8.6%, and large percentages of the Zn were present in the residual (36%), oxidisable (27%) and reducible (27%) fractions. The supply capacity of the most bioavailable Zn from the solid phase would therefore be lower than that of Cd. This might explain the absence of change in the shoot Zn concentration of *S. plumbizincicola* over three successive crops in the calcareous soil.

The results of our field experiment indicate that *S. plumbizincicola* can grow well and maintain relatively high shoot yields in the second and third crops compared with the first crop in the calcareous soil. This trend is similar to the results of a field experiment conducted in Zhejiang province, east China, in which there is no discernible reduction in the shoot biomass of *S. plumbizincicola* with increasing remediation time (Deng *et al*. 2016). In comparison to the first crop, the high uptake of shoot Cd and Zn in the subsequent crops might be attributed mainly to the large biomass yield in the calcareous soil. However, the yields (2.50--4.03 t ha^{-1}) obtained in our study are lower than the field results of 7.4--12.7 t ha^{-1} found in Zhejiang province at the same planting density (Deng *et al*. 2016). The lower SOC content (9.06 g kg^{-1}) of the calcareous soil used in this study than that of the soil (SOC > 29.1 g kg^{-1}) in Zhejiang province (Li *et al*. 2014; Deng *et al*. 2016) might be an important limiting factor. A large SOC content helps to enhance crop productivity and yield stabilization (Pan, Smith, and Pan 2009). In addition, weather conditions affect plant productivity. According to a meta-analysis study, experimental warming and increased precipitation generally stimulate plant biomass, productivity and ecosystem photosynthesis (Wu *et al*. 2011). *S. plumbizincicola* was first discovered in west Zhejiang province in east China (Wu *et al*. 2013), a region in which the mean annual temperature (15.9°C) and precipitation (1566 mm) are both higher than the values of 12.2°C and 617 mm found in the current study area in He’nan province. Low SOC content, temperature and precipitation may therefore be factors limiting the productivity of *S. plumbizincicola* in calcareous soil in He’nan province.

The total shoot uptake of Cd and Zn by *S. plumbizincicola* and the decrease in soil Cd and Zn after repeated phytoextraction are shown in Fig. 2. The decreases in soil Cd and Zn were 3.03 and 55.3 kg ha^{-1}, respectively, which were higher values than the total amounts of Cd (1.33 kg ha^{-1}) and Zn (10.4 kg ha^{-1}) in plant shoots. These trends are similar to the results of the field experiment conducted in Zhejiang province (Deng *et al*. 2016). The lower shoot uptakes than the decreases in soil Cd and Zn can be explained mainly by the substantial export of metals from surface runoff or leaching down the soil profile (El Khalil *et al*. 2008).

4.2 Pot experiment

Favourable soil structure and fertility are important for maintaining and increasing crop production but the Luvisols usually present problems due to their low SOC content and unfavourable physical properties in the study area (Xia *et al*. 2015; Xin *et al*. 2016). By comparing the shoot biomass of *S. plumbizincicola* in field experiments located in He’nan (this study) and Zhejiang provinces (Deng *et al*. 2016), we hypothesized that lower SOC content, temperature and precipitation are the important factors limiting the productivity of *S. plumbizincicola* in He’nan province. Enhancement of soil chemical and physical properties might therefore be a reasonable approach to enhance the yields of the hyperaccumulator, leading to a higher phytoextraction efficiency in metal contaminated soil under the same weather conditions.

The results of the pot experiment indicate that applications of fertile soil or organic or inorganic amendments can significantly increase the shoot biomass of *S. plumbizincicola* in calcareous soil in He’nan province (HN). There are several factors to be considered in relation to shoot biomass. The fertile soil collected from Zhejiang province (ZJ, Table 1) and the organic fertilizer applied both have large OC and nutrient contents which might increase soil fertility. In addition, organic matter plays a dominant role in the maintenance of soil bulk density because of its lower density than that of mineral particles and its aggregation effect on soil structure (Bronick and Lal 2005; Keller and Håkansson 2010). The expanded vermiculite and perlite also have low bulk density and high water retention (Doğan and Alkan 2004; Lanzón and García-Ruiz 2007; Jamei *et al*. 2011). In the present study we observed decreases in soil bulk density after amendment with fertile soil, organic fertilizer, vermiculite and perlite (Fig. 3). Increasing soil fertility and bulk density will therefore promote plant root growth and the ability of plants to take up water and nutrients (Bronick and Lal 2005; Keller and Håkansson 2010) and this may lead to higher plant productivity in metal contaminated soils. The perlite and vermiculite are also considered to be potential adsorbents for metals from aqueous solutions (Mathialagan and Viraraghavan 2002, 2003), thereby decreasing their mobility. However, amendment with perlite or vermiculite had no significant effect on Cd or Zn concentrations in the shoots of *S. plumbizincicola* after phytoextraction (Table 3). A previous study also reports that the major constraint for the successful phytoextraction of Cd contaminated soil by *T. caerulescens* is the requirement for increased biomass production (Koopmans *et al*. 2008). Therefore, the changes in chemical and physical properties of calcareous soil by organic and inorganic amendments will be management options that can be applied to enhance the phytoextraction efficiency of Cd and Zn in contaminated fields in He’nan province.

5 CONCLUSIONS

A field plot experiment indicates that *S. plumbizincicola* can maintain a stable level of Cd and Zn uptake in the calcareous soil after repeated phytoextraction. Due to the low SOC content, temperature and precipitation in the study area, low shoot biomass yield will be a major factor limiting phytoextraction efficiency in the fields of He’nan province. Enhancement of soil chemical and physical properties by the application of different amendments can significantly enhance shoot biomass and Cd and Zn uptake rates in calcareous soil. The planting of *S. plumbizincicola* might therefore represent a practical measure to achieve high phytoextraction efficiency for Cd and Zn with appropriate agricultural management practices in calcareous soils.

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Table 1 Properties of the soils used in the field and pot experiments

Site	Ph (H_2O)	OC (g kg^{-1})	CEC (cmol (+) kg^{-1})	Available P (mg kg^{-1})	Available K (mg kg^{-1})	Sand*(%)	Silt(%)	Clay(%)	Cd(mg kg^{-1})	Zn(mg kg^{-1})
HN-Field	7.91 ± 0.08	9.06 ± 0.54	16.8 ± 0.3	11.6 ± 0.8	76.7 ± 1.2	28.8	47.2	24.0	3.55 ± 0.34	108 ± 5
HN-Pot	8.08 ± 0.06	11.3 ± 0.8	16.1 ± 0.4	11.2 ± 0.8	97.0 ± 1.1	34.6	48.4	17.0	2.00 ± 0.01	87.7 ± 3.4
ZJ-Pot	6.37 ± 0.05	39.3 ± 0.9	23.7 ± 0.9	71.3 ± 0.8	82.7 ± 1.2	30.3	55.9	13.8	1.02 ± 0.02	677 ± 13

*determined by the ISSS (International Soil Science Society) soil texture classification, sand (20--2000 µm), silt (2--20 µm) and clay (0--2 µm). HN: He'nan province, ZJ: Zhejiang province. The sample sizes (n) are 5 in field experiment in HN and 3 in pot experiment in HN and ZJ. The mixed soil samples are used for measuring sand, silt and clay contents, respectively.

Table 2 Concentration of Cd and Zn in different fractions (mg kg^{-1}) and their percentage distribution (%) in soils without (Unplanted) and with (Planted) phytoextraction

Metal	Variable	Treatment	Acid-soluble	Reducible	Oxidisable	Residual	Sum	Total	Recovery (%)
Cd	Concentration	Unplanted	1.82±0.43 a	1.26±0.09 a	0.16±0.04 a	0.12±0.01 a	3.36±0.56 a	3.55±0.34 a	104±5
		Planted	1.06±0.15 b	0.85±0.14 b	0.12±0.01 b	0.08±0.01 b	2.10±0.27 b	2.39±0.27 b	106±2
	Percentage	Unplanted	53.8±3.7 a	37.8±3.7 a	4.74±0.64 a	3.73±0.34 a	100		
		Planted	50.4±3.3 a	40.3±3.0 a	5.69±1.07 a	3.68±0.59 a	100		
Zn	Concentration	Unplanted	9.61±0.47 a	30.6±1.6 a	30.7±3.5 a	40.8±4.1 a	112±5 a	108±5 a	94.2±8.0
		Planted	6.89±1.28 b	27.2±1.7 a	27.8±2.8 a	29.8±2.9 b	91.6±0.9 b	86.5±2.2 b	88.0±1.5
	Percentage	Unplanted	8.62±0.74 a	27.4±2.7 a	27.5±1.9 a	36.5±2.4 a	100		

		Planted	7.52±1.32 a	29.6±1.6 a	30.3±3.2 a	32.5±3.3 a	100		

Total metal concentration is obtained by digestion. The sum is obtained by four independent fractions. Recovery (%) = Sum/Total × 100. Different lowercase characters in the same column for each variable between unplanted and planted soils indicate significant differences by t-test at $p < 0.05$. Data are mean ± SD (n = 5).

Table 3 Shoot biomass, shoot Cd and Zn concentrations, and shoot Cd and Zn uptake of *S. plumbizincicola* under the different treatments

Treatment	Biomass(g pot^{-1})	Concentration (mg kg^{-1})		Total uptake (mg pot^{-1})	
		Cd	Zn	Cd	Zn
HN	3.98 ± 1.03 b	19.2 ± 2.6 a	88.1 ± 16.5 b	0.07 ± 0.01 b	0.35 ± 0.09 c
ZJ	8.96 ± 0.57 a	2.85 ± 0.98 b	2002 ± 182 a	0.02 ± 0.01 c	17.9 ± 1.6 a
HN+ZJ	8.38 ± 0.57 a	22.3 ± 4.2 a	159 ± 72 b	0.19 ± 0.02 a	1.31 ± 0.53 b
HN+amendment	6.78 ± 1.60 a	24.5 ± 5.7 a	131 ± 21 b	0.16 ± 0.02 a	0.87 ± 0.12 b

Different lowercase characters in the same column indicate significant difference by one-way analysis of variance at $p < 0.05$. Data are mean ± SD (n = 4).

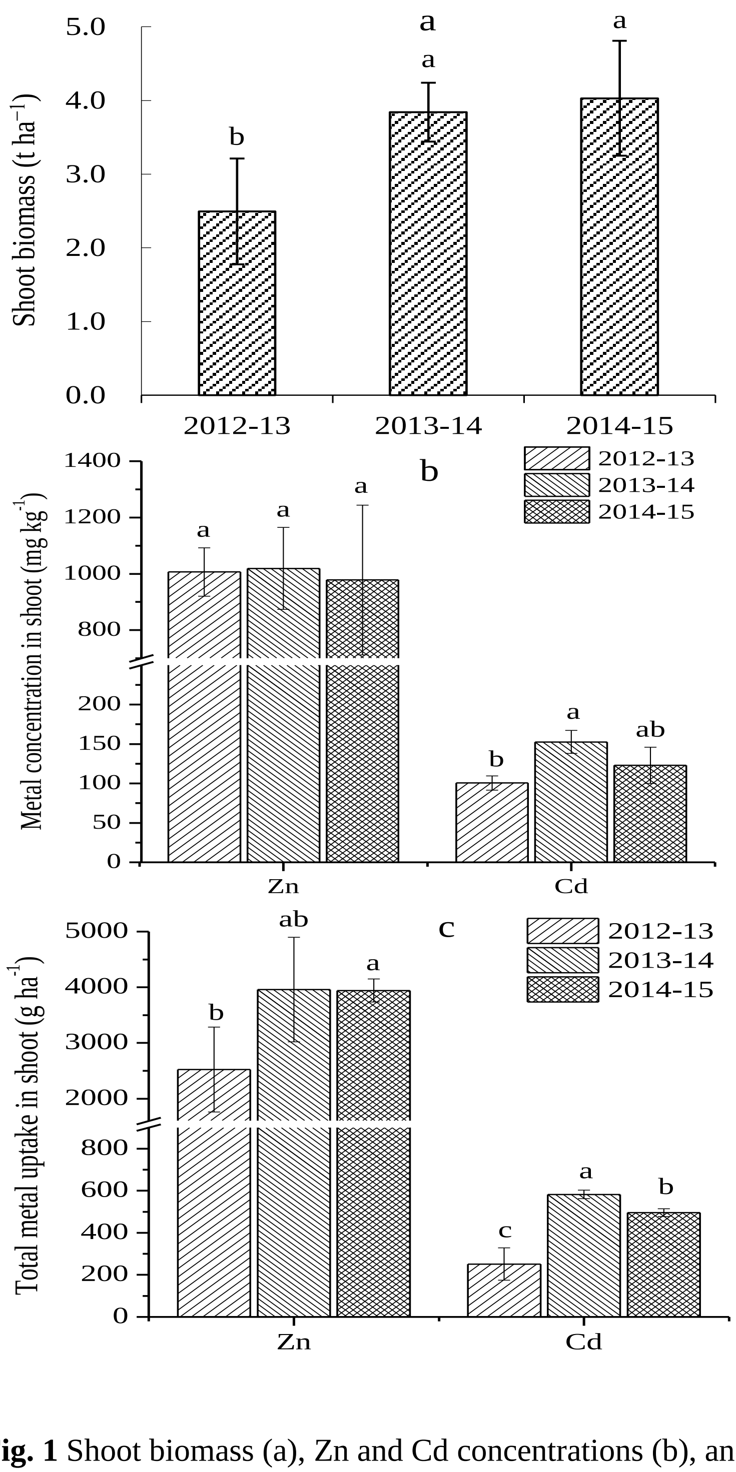

Fig. 1 Shoot biomass (a), Zn and Cd concentrations (b), and Zn and Cd uptake (c) of *S. plumbizincicola* over three successive crops in the field experiment. Different lowercase

characters for one variable indicate significant differences among three successive crops by one-way analysis at $p < 0.05$. The error bars are standard deviations (SD), n = 5.

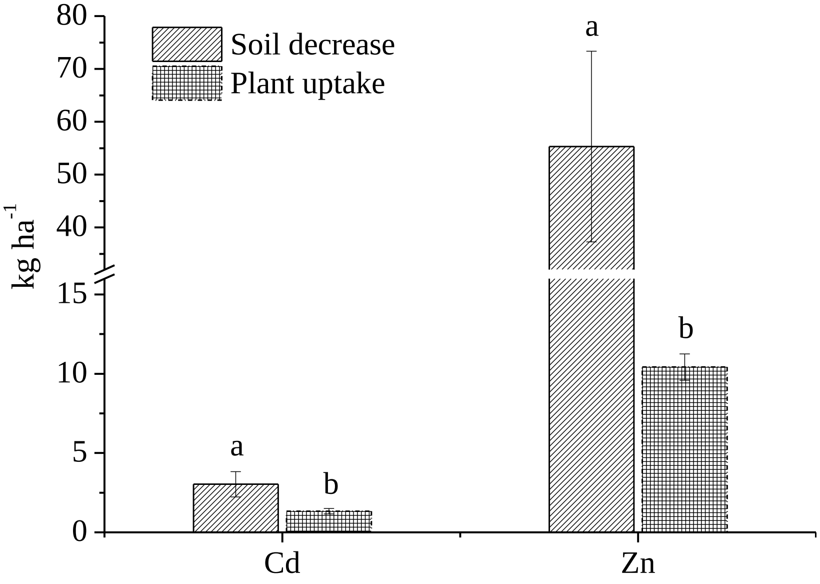

Fig. 2 Total metal uptake by plant shoots (sum of three crops, kg ha^{-1}) and total metal decrease in soil (total metal in top soils without phytoextraction minus that with phytoextraction, kg ha^{-1}) in the field experiment. Different lowercase characters for one variable (Cd or Zn) indicate significant differences between soil decrease and plant uptake by t-test at $p < 0.05$. The error bars are SD, n = 5.

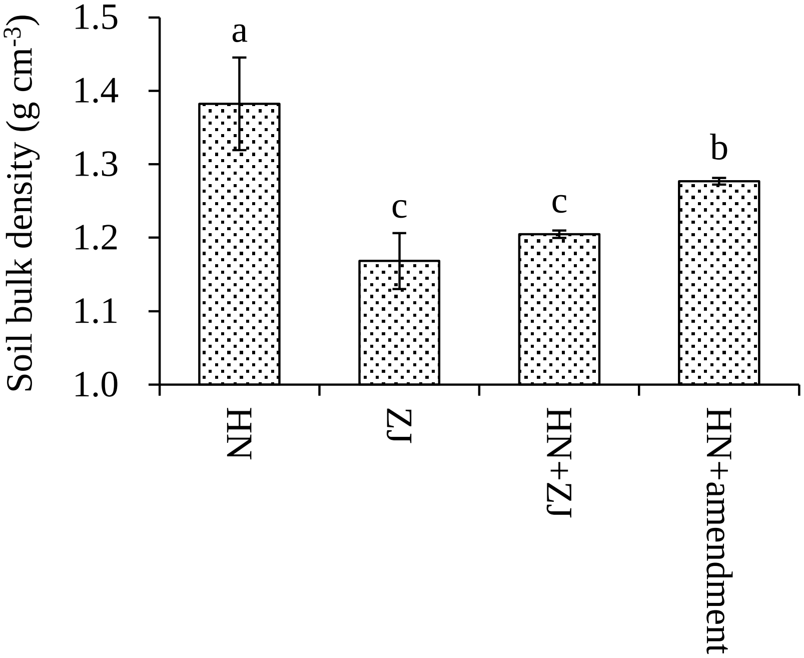

Fig. 3 Soil bulk density after the harvest of *S. plumbizincicola* shoots under the different treatments in the pot experiment. Different lowercase characters indicate significant differences by one-way analysis of variance at $p < 0.05$. The error bars are SD, n = 4.