Rhizosphere characters of zinc and cadmium contaminated soil after continuous phytoextraction by *Sedum plumbizincicola*

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Abstract: Growth chamber pot experiment and field plot trial were conducted, and rhizobags were installed, to study the effects of continuous phytoextraction by Cd and Zn hyperaccumulator *Sedum plumbzincicola* on the bioavailability of Cd and Zn in rhizosphere and bulk soils. Results showed that for heavy metal contaminated soil with continuous phytoextraction, the Cd and Zn concentrations in both rhizosphere and bulk soil solutions were significantly lower than that without phytoextraction ($p<0.01$). After continuous phytoextraction by *S. plumbizincicola* (S1-SP5, S4-SP5), total heavy metal concentrations in heavily contaminated soil were decreased; and the soil NH_4OAc extractable Cd and Zn were also decreased markedly. With the increase of total metal concentrations in contaminated soil, Cd and Zn uptake by plants has an increasing trend, from 23.8 to 258 μg per pot, from 2.78 to 11.8 mg per pot, respectively. The NH_4OAc extraction procedure was appropriate to predict the bioavailability of Cd and Zn in the rhizosphere soil to plant shoots with positive correlation coefficients of 0.9013 ($p < 0.01$), 0.7382 ($p < 0.01$), respectively. The plot trial results showed that slight decrease was found in waster soluble and NH_4OAc extractable metals, showed a similar trend to that of pot experiment.

Key words: Zn, Cd, Continuous phytoextraction, *Sedum plumbizincicola,* rhizosphere characters

1. Introduction

Heavy metals are well known to be highly toxic in soils when present in excessive concentrations [4]. Therefore, phytoextraction strategy using hyperaccumulator plants has been recognised as a potential technique for the decontamination of metal-polluted soils [2-3, 24].

Metal hyperaccumulator plants comprise species that accumulate (in mg kg^{-1}) > 10000 (Zn or Mn), or > 100 (Cd) in their shoots [1, 27-28]. These plants have attracted the interests of plant and soil scientists because of their role in the development of phytoremediation technologies for the treatment of metal-polluted soils [15, 27].

Sedum plumbizincicola has been described as a hyperaccumulator by Wu *et al.* [30-31] and confirmed in hydroponic experiments and in a field study. Much of previous work was focused on improving the extraction rate and shoots biomass of the plants; little was known about the changes of bioavailable heavy metals after several crops of plants for phytoremediation.

The rhizosphere is a small but important session of pedosphere, commonly defined as the zone where root activity significantly influences soil properties. The properties of rhizosphere are different from those of bulk soils in a range of biochemical, chemical and physical processes that occur as a consequence of root growth, water and nutrient uptake, respiration and rhizodeposition [12, 26]. Studies showed that characteristics of trace elements in the rhizosphere are supposed to be different from that in the bulk soil and may have effect on their availability [4].

The bioavailability is frequently predicted by correlating data with organism accumulation with amounts in soil determined by single or sequential extraction [25]. The heavy metal concentrations in the soil were considered to reflect the plant available metal in the soil [18-19]. Despite current progress in understanding availability of metals in the soil, rhizosphere characters of zinc and cadmium contaminated soil after continuous phytoextraction by *S. plumbizincicola* were still poorly characterised.

In this paper, a pot experiment and a field trial were conducted to: (1) compare the changes in bioavailability of heavy metals in the rhizosphere soil and in the bulk soil with continuous phytoextraction; (2) study the relationship between bioavailable metals and uptake by the hyperaccumulator *S. plumbizincicola*. The paper focuses on soil solution and NH_4OAc extractable heavy metal concentrations after continuous phytoextraction for the examination of changes in the bioavailable Cd and Zn in contaminated soil. The results in this study were supposed to better understand rhizosphere characters of zinc and cadmium contaminated soil after continuous phytoextraction by hyperaccumulator.

2. Materials and methods

2.1 Characterization of soils and pot experiment design

The soil for pot experiment was based on the former phytoextraction experiment, which was conducted from April 2006 to November 2007 in the Institute of Soil Science, Chinese Academy of Sciences, Nanjing. The 19-month pot experiment was conducted with soil collected from the top layer (0-15 cm) of a farmland nearby a copper smelter near Hangzhou City [13], Zhejiang Province, east of China. The soil was a typic agri-udic ferrosols [9].

In December 2007, four treatments of the former phytoextraction experiment were selected for the rhizosphere pot experiment: Two treatments of low heavy metal contamination level (S1-SP5: planted with five crops of *S. plumbizincicola*; S1-CK5: unplanted) and two treatments of high heavy metal contamination level (S4-SP5: planted with five crops of *S. plumbizincicola*; S4-CK5: unplanted). These selected soils physical-chemical properties were listed in Table 1. Total Zn and Cd concentrations were listed in Table 2.

Table 1. Physical and chemical characteristics of soil used in the pot experiment

Items	S1-SP5	S1-CK5	S4-SP5	S4-CK5
pH (H_2O)	6.02±0.21	6.38±0.24	6.91±0.12	7.15±0.13
TC (g kg^{-1})	32.3±0.48	32.2±0.47	24.8±0.76	21.9±0.28
Total N (g kg^{-1})	3.45±0.06	4.06±0.05	2.27±0.02	2.61±0.06
Total K (g kg^{-1})	18.3±0.3	21.4±0.5	19.6±1.8	24.5±0.3
Total P (g kg^{-1})	0.73±0.03	0.53±0.04	0.73±0.04	0.51±0.02
Avai.-N (mg kg^{-1})	153±15	143±7	98.4±9.7	123±8
Olsen-P (mg kg^{-1})	42.3±3.5	8.2±0.4	42.3±5.5	13.1±1.3
NH_4OAc-K (mg kg^{-1})	32.8±4.4	33.0±2.4	29.5±3.6	128±13

Values are means±SE.

Table 2. Zn and Ca concentrations in soil used in the pot experiment (mg kg^{-1})

Treatment	Total Zn	Total Cd	Note
S1-SP5	224±11	0.22±0.03	planted with *S. plumbizincicola*
S1-CK5	321±8	1.11±0.14	unplanted
S4-SP5	5485±445	5.33±0.34	planted with *S. plumbizincicola*
S4-CK5	6499±324	15.3±0.6	unplanted

These four soils were air-dried and sieved (40 mesh), and then put into a plastic cubic rhizo-pot (90 mm in length, 70 mm in width and 70 mm in height), with 40 g in the rhizosphere and 320 g as bulk soil. Plastic frames (10 mm in length, 70 mm in width and 70 mm in height) covered with 500-mesh nylon mesh cloth were used to separate the rhizosphere soil from the bulk soil in the rhizo-pot. Two porous soil moisture samplers (Rhizon SMS, Rhizosphere

Research Products, Wageningen, The Netherlands) were installed both in the rhizobag and in the bulk soil 5 cm away from the rhizobag to allow the samples of the soil solution to be extracted. There were four replicates of each treatment in randomised design. Four healthy seedlings of *S. plumbizincicola* were transplanted into the rhizo-bag.

The plants were allowed to grow 60 days in a greenhouse with an average night/day temperature of 20/25±2 °C and an average photosynthetically active radiation flux of 60 w m^{-2}. The plants were watered with deionised water during plant growth to keep the soil at approximately 70% soil water-holding capacity. Soil solution was collected on 42^{nd} day and 56^{th} day after transplanting, respectively.

2.2 Characterization of agricultural soils and the design of field trial

Agricultural soil pH (H_2O) was 7.24, organic carbon was 29.1 g kg^{-1}, and cation exchange capacity (CEC) was 11.8 cmol (+) kg^{-1}. Total N, P, and K were 2.21, 0.22 and 22.9 g kg^{-1}, respectively. Available N was 105 mg kg^{-1}, Olsen-P was 6.70 mg kg^{-1}, and NH_4OAc extractable K was 160 mg kg^{-1}.

There were four plant densities for field trial, plant density of 110 (D11), 250 (D25), 440 (D44), and 1000 (D100) thousand seedlings per hectare, respectively. Each treatment was replicated three times. Each plot was 6 m in length and 2 m in width. Seedlings of *S. plumbizincicola* were transplanted on May 14^{th}, 2007. Afterwards, soil was fertilized with 150 kg hm^{-2} carbamide, 225 kg hm^{-2} compound fertilizers (N: 15%; P_2O_5: 15%; K_2O: 15%). On April 2008, field soil was fertilized with 75 kg carbamide per hectare, and 75 kg compound fertilizers per hectare. Plants were harvested on June 28^{th}. Then, these soils were used as soil with *S. plimbizincicola* phytoextraction, and kept for field rhizosphere study.

The field rhizosphere experiment was started from September 14^{th}, 2008 to May 21^{st}, 2009, using three treatments (D11, D25 and D44).

For field experiment, the rhizosphere soil was separated from bulk soil by a 500-mesh nylon mesh cloth (10 cm height and 5 cm in diameter). Ten rhizobags were installed in each plot, according to grid method. Every rhizobag contained a seedling of *S. plumbizincicola*.

2.3 Chemical analysis

(1) Determination of heavy metals in soils and plants

For pot experiment, the soil in the rhizobag was sampled, as rhizosphere soil; the soil 5 cm away from the rhizobag was collected as bulk soil. For field trial, soil in the rhizobag was considered as rhizosphere soil, while soil sampled at the centre site between two plants was collected as bulk soil.

Then all samples were air dried, homogenised in agate mortar, passed through a 100-mesh nylon sieve. Soil total heavy metal concentrations were determined using atomic absorption spectrophotometer (Varian SpectrAA 220FS, 220Z, Varian, Palo Alto, CA, USA) after digestion of ~0.25 g samples with 14 ml of HCl-HNO_3-$HClO_4$ (4: 2: 1, v/v). Quality control of heavy metal analysis was included using standard reference material GBW07401.

At the end of the experiment, plants were harvested. The plants were washed thoroughly with tap water and then rinsed with deionised water. The shoots were oven dried at 105 °C, weighed and ground. Plant samples (~0.5 g) were digested with 10 ml of HNO_3-$HClO_4$ (3: 2, v/v) mixture, for determination using AAS. For quality control, certified reference material, GBW07603, was used.

The data determined by the method above were within the certified range of Zn and Cd (data not shown), indicating that the metal concentrations determined in this work were reliable.

(2) Extraction procedure

Rhizosphere and bulk soil in the pot experiment were evaluated for soil solution Cd and Zn, obtained directly from the SMS. In field conditions, rhizosphere and bulk soils were evaluated for water-soluble Cd and Zn in water content 40%, obtained after adding distilled water, incubating for 16 h, centrifuging (15 min at 4500 rpm) and filtering (0.45 μm syringe filter).

Soil was shaken at 25 °C for 16 h with 1.0 M NH_4OAc (soil to extractant ratio was 1: 5), then centrifuged (15 min 4500 rpm) and filtered. Cd and Zn concentrations were then determined by AAS [17].

2.4 Statistical analysis

Differences between heavy metal concentrations were analysed (i.e., one-way ANOVAs) using the SPSS for Windows, to least significant difference at 5% level, when significance is observed at the p < 0.05 level, Turkey's post hoc test was used to carry out multiple comparisons.

3. Results

3.1 Soil solution and NH_4OAc extractable Zn and Cd in pot experiment

Soil solution was sampled on day 42 and day 56, respectively, for determination of heavy metal concentration. The continuous phytoextraction by *Sedum plumbizincicola* significantly affected zinc and cadmium concentration (Fig. 1). The cadmium and zinc concentration in both the rhizosphere and bulk soil solution of the treatment S1-CK5 was higher than that of the treatment S1-SP5 (p<0.01). The same trend was also observed between the treatment S4-CK5 and S4-SP5.

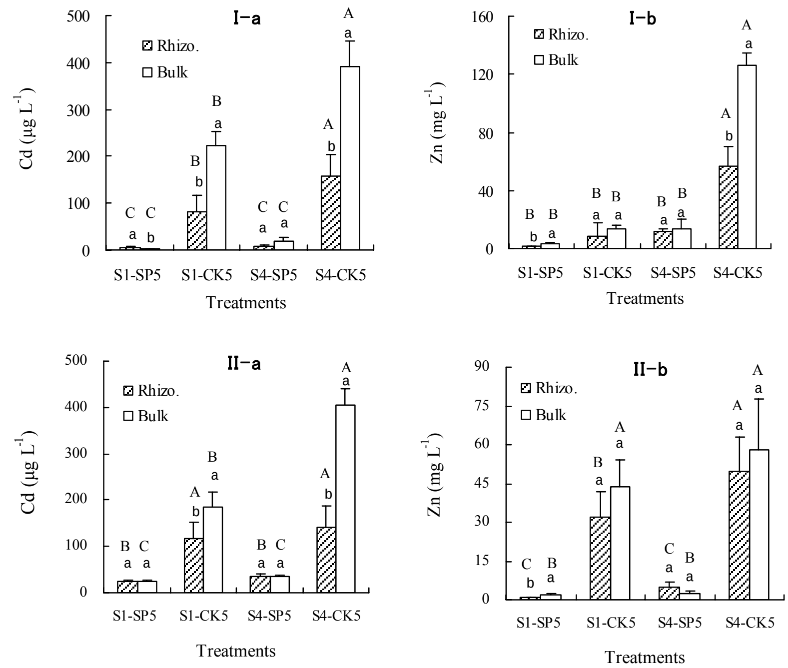

Fig. 1 Cadmium and zinc concentration in soil solution on day 42(I) and on day 56(II)

Values followed with different capital letters are significantly different (at least p < 0.01) between four treatments, and lowercase letters are significantly different (at least p < 0.05) between rhizosphere and bulk soil for every treatment according to Ducan's post hoc test.

Total Cd and Zn concentrations of the treatment S1-CK5 were 1.11 and 321 mg kg^{-1}, respectively, lower than that of the treatment S1-SP5 (Cd: 5.33 mg kg^{-1}; Zn: 5485 mg kg^{-1}). However, after 56 days' culture with *S. plumbizincicola*, the treatment S1-CK5 had about three times Cd concentration in soil solution and about six times of Zn concentration in soil solution of the treatment S4-SP5 (Fig. 1 II-a & II-b). These results confirmed that while continuous phytoextracting with *S. plumbizincicola*, soil total heavy metal concentrations decreased distinctively, and the rapid decrease of Cd and Zn bioavailability was also obtained at the mean time.

For the treatment with *S. plumbizincicola* continuous phytoextraction (S1-SP5, S4-SP5), the NH_4OAc extractable Cd and Zn concentrations in the rhizosphere and bulk soils were low compared to the corresponding treatment without any phytoextraction (S1-CK5, S4-CK5) (Table 3). The depletion rates of NH_4OAc extractable Zn in the rhizosphere soil in each treatment (S1-SP5, S1-CK5, S4-SP5, S4-CK5) were 59.7%, 18.0%, 16.3%, and 18.6%, respectively. The depletion rates of NH_4OAc extractable Cd in the rhizosphere soil in each treatment (S1-SP5, S1-CK5, S4-SP5, S4-CK5) were 6.67%, 29.4%, 40.3%, and 41.4%, respectively. The Zn and Cd concentrations in the rhizosphere soils were significantly (p<0.05) lower than that in bulk soils. With the increase of NH_4OAc extractable heavy metal concentrations, the Cd depletion rate was increased. *S. plumbizincicola* continuous phytoextracting has no distinct effect on depletion of NH_4OAc extractable Zn in the rhizosphere soil (Table 3).

Table 3. NH_4OAc extractable Cd and Zn in the rhizospheric and bulk soils (mg kg^{-1})

Treatment	Cd		Zn	
	Rhizo.	Bulk	Rhizo.	Bulk
S1-SP5	0.28±0.10 C	0.30±0.08 C	2.90±0.90 C	7.22±1.69 C
S1-CK5	0.36±0.02 C	0.51±0.04 C	27.8±0.5 C	33.9±1.4 C
S4-SP5	1.35±0.22 B	2.26±0.06 B	273±29 B	326±15 B
S4-CK5	4.83±0.45 A	8.24±0.78 A	464±103 A	570±42 A

Values are means±SE. Mean values followed with different capital letters are significantly different (at least p < 0.01) between four treatments according to Ducan's post hoc test.

3.2 Biomass and heavy metal uptake by *S. plumbizincicola* in pot experiment

Shoot biomass of *S. plumbizincicola* was listed in Fig. 2. Mean shoot biomass of the plants decreased in the order S4-SP5 > S4-CK5 > S1-SP5 > S1-CK5, suggesting that soil contamination levels play an important role in the growth of the hyperaccumulator. Shoot biomass of *S. plumbizincicola* planted in the soil without any phytoextraction was below that with continuous phytoextraction. That may result from the improvement of soil physical and chemical conditions after phytoextraction by the hyperaccumulator.

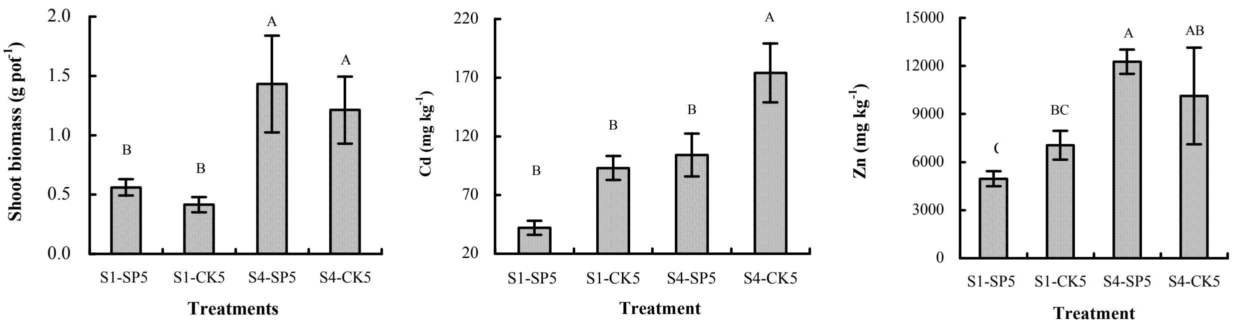

Fig. 2 Shoot biomass and Cd and Zn concentrations of *Sedum plumbizincicola*

Fig. 2 also showed the heavy metal concentrations of *S. plumbizincicola*. Plant Cd concentration tended to be much high in the heavily contaminated soil. Further, the treatment S1-SP5 gave the lowest Cd concentration of *S. plumbizincicola*. However, although total Cd concentration in heavily contaminated soil with continuous phytoextraction (S4-SP5) decreased from 15.3 to 5.33 mg kg^{-1} and total Cd of the treatment S1-CK5 was only 1.11 mg kg^{-1}, showing that the former treatment has about five times soil Cd concentration of the latter treatment, but no distinct difference was found for Cd concentration of the hyperaccumulator between these two treatments. This could be attributed to the decreased of Cd bioavailability after continuous phyoextracting as mentioned above.

The *S. plumbizinciloca* plants of the treatment S1-SP5 harvested 60 days after transplanting had average biomass of 0.56 g per pot, and Zn concentration of 4961 mg kg^{-1}, and therefore a calculated uptake of 2.78 mg Zn per pot. With the increase of total metal concentrations in contaminated soil, Cd uptake by the hyperaccumulator has an increasing trend. For heavy metal contaminated soils after continuous phytoextraction (S1-SP5, S4-SP5), Cd uptake by *S. plumbizinciloca* was lower compared to the corresponding soils without any phytoextraction (S1-CK5, S4-CK5). For heavily polluted soils (S4-SP5, S4-CK5), Zn uptake by the hyperaccumulator plants was higher compared to the treatments S4-SP5 and S4-CK5. In the pot experiment, the difference of heavy metal uptake by *S. plumbizinciloca* shoot between each treatment might be accounted for plant shoot biomass (p<0.01), since shoot biomass of the plants increased significantly with the increase of soil total metal concentrations (Fig. 3).

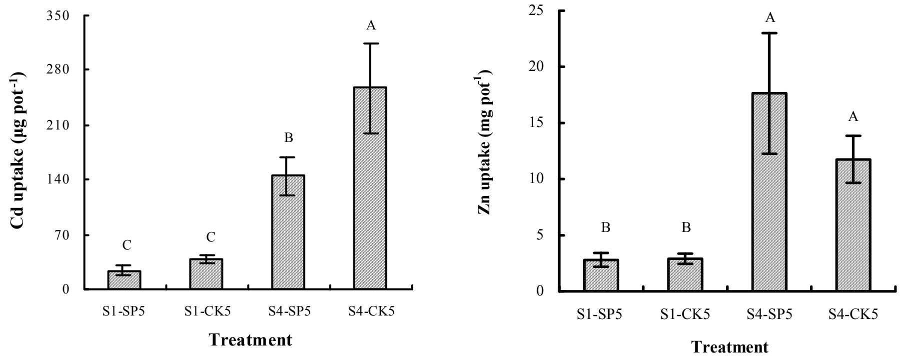

Fig. 3 Heavy metal uptake by aboveground *Sedum plumbizincicola*

Correlation analysis was performed to investigate the relationship between NH_4OAc extractable Cd and Zn in rhizosphere and bulk soils and their plant (shoot) concentrations. The correlation coefficients of NH_4OAc-Cd in the rhizosphere and bulk soils to plant concentrations were found to be good (0.9013 and 0.9401, respectively). Zinc accumulated in shoot correlated well to the concentration of NH_4OAc extractable Zn in rhizosphere and bulk soils (0.7382 and 0.8111, respectively). The results above suggested that the moist rhizosphere soils could be used in predicting the bioavailability of heavy metals to the hyperaccumulator *S. plumbizincicola*.

3.3 Bioavailability of Zn and Cd in field trial

The data of the field plot trial showed a decrease of NH_4OAc extractable Cd and Zn in the rhizosphere of hyperaccumulator *S. plumbizincicola* compared to bulk soil, (Table 4). With increasing planting density, the concentration of NH_4OAc-Cd tended to be generally decreased in rhizosphere soil, though the difference was not significant. Similar results were found for Zn.

Table 4. Cd and Zn concentrations extracted by NH_4OAc in field conditions

Treatment	Cd		Zn	
	Rhizo.	Bulk	Rhizo.	Bulk
D11	0.70±0.14	0.75±0.11	52.3±15.4	59.2±15.1
D25	0.68±0.11	0.75±0.10	50.0±14.2	55.0±13.1
D44	0.66±0.15	0.75±0.14	52.1±14.8	58.7±16.6

Values are means±SE in means.

The average water soluble Cd and Zn were lower in rhizosphere soil compared to concentration in bulk soil. For the treatment D44 with continuous phytoextraction, there was no difference between rhizosphere and bulk soils in water soluble Cd concentration. This could be attributed to the large planting density (440000 plants per hm^2). As a result, water soluble Cd was higher in the treatment D44 than that in treatment D11 (Table 5).

Table 5. Cd and Zn concentrations in soil solution in field conditions

Treatment	Cd		Zn	
	Rhizo.	Bulk	Rhizo.	Bulk
D11	0.31±0.06	0.75±0.41	33.9±2.0	35.8±2.8
D25	0.31±0.02	0.70±0.38	26.1±0.6	38.0±7.3
D44	0.39±0.02	0.37±0.05	46.9±1.0	34.7±1.3

Values are means±SE in means.

3.4 Biomass and heavy metal accumulation by *S. plumbizincicola* in field trial

The treatment with plant density of 110 thousand plants per hectare were used to provide soil of low phytoextraction rate, while the treatments with planting density of 440 thousand plants per hectare were included to give the soil with high rate continuous phytoextraction. There were significant differences between the treatments in the shoot biomass of *S. plumbizincicola* (Fig. 4). The shoot biomass in the treatment D44 were higher than in treatment D11 (p<0.05); the shoots of the plants in the former treatment were between three and four times the mass of plants in the latter treatment.

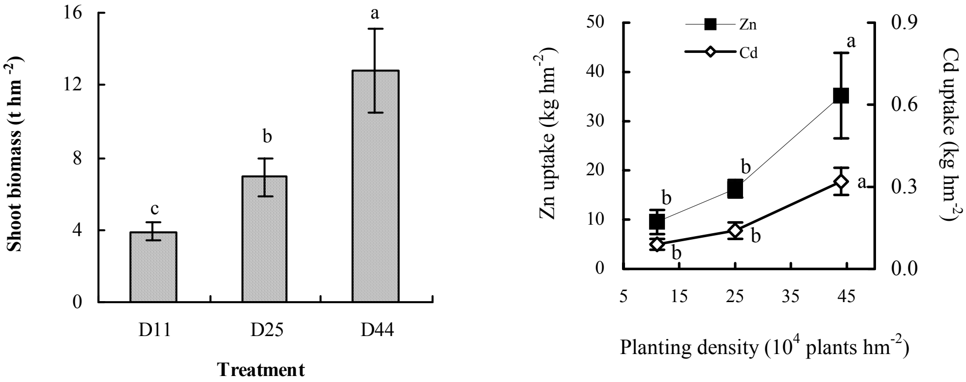

Fig. 4 Shoot biomass and heavy metal uptake of *Sedum plumbizincicola* in the field experiment

When comparing the heavy metal concentrations in each treatment, there was no significant differences between treatments for concentrations of Zn and Cd in the shoot of *S. plumbizincicola*, whereas Zn and Cd concentrations of the plants increased gradually with increasing plant density (Fig. 5). The results of heavy metal uptake showed a similar trend to the shoot biomass (Fig. 4).

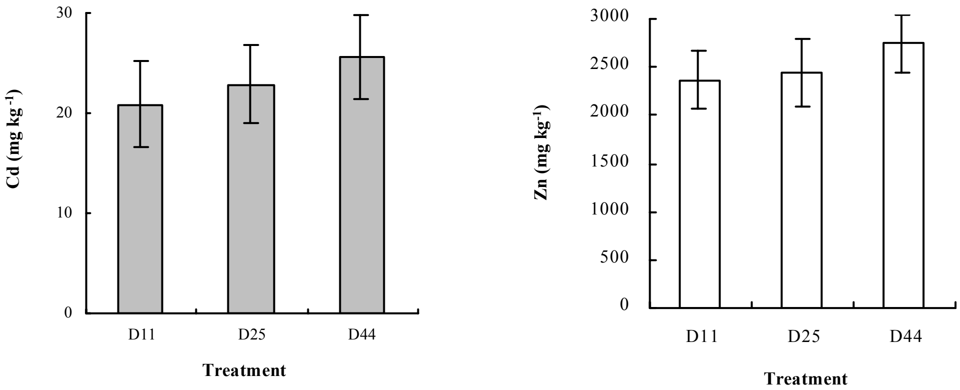

Fig. 5 Cadmium and zinc concentrations in aboveground *Sedum plumbizincicola* in the field conditions

4. Discussion

The rhizosphere is a dynamic region where multiple interactive processes take place [5]. Root growth, water and nutrient uptake, respiration and rhizodeposition are all components of the dynamic system [8, 12]. Contaminants exhibit special behaviours in rhizosphere soil that occur as a consequence of heavy metal speciation, distribution, mobilization and bioavailability affected by a range of biochemical, chemical and physical processes in rhizosphere soil.

Single extraction procedures were based on the assumption that there is a relationship between the extractable fraction of heavy metals and the phytoavailability of heavy metals to plants; that is to say, a good correlation indicated that a certain fraction of the total metal and the phytoavailability was bioavailable to plants [6]. Our results demonstrated that NH_4OAc extractable method in the moist rhizosphere soil could provide a way to estimate the phytoavailability of Zn and Cd to *S. plumbizincicola*, based on the correlation coefficients.

When released into soil, heavy metal bioavailability to plants was decreased [16, 21]. Knight *et al.* (1997) compared changes in the soil solution Zn and Cd concentrations after growth of *T. caerulescens* in seven contaminated soils. Both soluble Zn and Cd decreased considerably at the end of experiment [14]. Gonzaga (2009) also observed a depletion of solution As in the rhizosphere of the hyperaccumulator *P. Biaurita* [10]. It is suggested that the driving force for the observed decrease in available Zn and Cd was mainly the heavy metal uptake by the plant [11]. From previous literatures, little was known about rhizosphere characters of Zn and Cd contaminated soil after continuous phytoextraction.

NH_4OAc extraction method and soil solution were adapted to study the rhizosphere characters of zinc and cadmium contaminated soil after *Sedum plumbizincicola* continuous phytoextraction. In the pot experiment, five consecutive crops of plants resulted in a significant reduction of total and bioavailable Cd and Zn in the rhizosphere soils. In the field plot experiment, *S. plumbizincicola* was planted from May 2007 to June 2008 and the rhizosphere experiment lasted from September 2008 to May 2009. Soil water soluble and NH_4OAc extractable metal concentrations showed a similar trends to the data in the pot experiment.

Fitz and Wenzel (2002) proposed that hyperaccumulators might enhance heavy metal solubility in the rhizosphere soils, consequently increasing plant metal uptake [7]. Our results seemed to support this hypothesis since the decrease of Zn and Cd concentrations in rhizosphere soils during the pot experiment phytoextracting did not account for the mass of Zn and Cd accumulated by *S. plumbizincicola*. The NH_4OAc extractable Cd concentration was only 0.30 μg kg^{-1} in bulk soil, and 0.28 μg kg^{-1} in rhizosphere soil. Since the mass of soil in each "rhizobag" was 40 g, we calculated that the NH_4OAc extractable Cd had been depleted by 0.8 μg Cd pot^{-1}. However, this 0.8 μg decrease just represents 3.36 % of the Cd accumulated in the shoots of the plants (Table 6). Furthermore, the most pronounced percentage was found in the treatment S4-CK5; the 136 mg decrease only represents 52.7% of the Cd accumulated in plant shoots. But the depletion of metals in rhizosphere could not be attributed to the uptake of metals by the hyperaccumulator plants, since the difference of extractable metal in 1 mol L^{-1} NH_4OAc between rhizosphere and bulk soils was less than the uptake of metals by *S. plumbizincicola*. Hence, a certain amount of heavy metal accumulation by plants was from the previous labile fractions in soil [29]. Similarly, McGrath *et al.* (1997) reported that the decrease in the ammonium nitrate extractable fractions of Zn accounted for only ten percent of the Zn accumulated by *T. caerulescens* [19]. It was reported that despite the hperaccumulator species' influence on water soluble As mobilization in the rhizosphere, this labile As accounted for < 1.2% of the total As accumulated by plants [10]. Authors suggested that this might be a result of either the buffering capacity of the soil re-supplying Zn^{2+} to the soil solution as plants remove it [14, 19]. In our pot experiment, decrease of NH_4OAc extractable fractions explained < 53% and < 36% of the total Cd and Zn uptake, respectively, by the plants. These results suggested that a large proportion of heavy metal uptake by hyperaccumulator plants must have been driven from other fractions, which is in correspondence with McGrath's results (2001) [20].

Table 6. The depletion of NH_4OAc extractable Cd and Zn in the rhizospheric soil

Treatment	NH_4OAc extractable metal		Heavy metals in shoots	
	Cd (μg pot^{-1})	Zn (mg pot^{-1})	Cd (μg pot^{-1})	Zn (mg pot^{-1})
S1-SP5	0.80	0.17	23.8±6.6	2.78±0.62
S1-CK5	6.00	0.24	38.3±4.5	2.91±0.45
S4-SP5	28.4	2.12	145±24	17.6±5.4
S4-CK5	136	4.24	258±57	11.8±2.1

The total amount of Cd and Zn accumulated in the shoots of *S. plumbizincicola* were also listed.

S. plumbizincicola is a Zn and Cd hyperaccumulator [30-31]. There are fluctuations in shoot biomass of plants among planting seasons, without any obvious increase or decrease. Some researchers reported that total metal uptake could reach the same extraction rate in every single growing season [19, 22-23]. In contrast to these literatures, our study confirmed that metal extraction efficiency of plants decreased with the extension of phytoextraction seasons, which could be attributed to bioavailability of metals in the phytoremediation process. Hence, metal phytoavailability, as affected by continuous phytoextraction and total metal concentrations in soil, *et al.*, is considered to be an important factor. Further research is needed to investigate whether the bioavailability of metals is a limiting factor for metal uptake by hyperaccumulator, and provide promising techniques for phytoextraction [20].

Acknowledgments

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