Effects of dissolved organic matter from the rhizosphere of the hyperaccumulator *Sedum alfredii* on sorption of zinc and cadmium by different soils

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ABSTRACT

Pot experiments were conducted to investigate the changes of the dissolved organic matter (DOM) in the rhizosphere of hyperaccumulating ecotype (HE) and non-hyperaccumulating ecotype (NHE) of *Sedum alfredii* and its effects on Zn and Cd sorption by soils. After planted with HE, soil pH in the rhizosphere reduced by 0.5–0.6 units which is consistent with the increase of DOM. The hydrophilic fractions (51%) in DOM from the rhizosphere of HE (HE-DOM) was much greater than NHE-DOM (35%). In the presence of HE-DOM, Zn and Cd sorption capacity decreased markedly in the following order: calcareous clay loam > neutral clay loam > acidic silty clay. The sorption isotherms could be well described by the Freundlich equation ($R^2 > 0.95$), and the partition coefficient (K) in the presence of HE-DOM was decreased by 30.7–68.8% for Zn and 20.3–59.2% for Cd, as compared to NHE-DOM. An increase in HE-DOM concentration significantly reduced the sorption and increased the desorption of Zn and Cd by three soils. DOM derived from the rhizosphere of the hyperaccumulating ecotype of *S. alfredii* could significantly reduce metal sorption and increase its mobility through the formation of soluble DOM–metal complexes.

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1. Introduction

Phytoextraction, the use of green plants to clean up contaminated soil, has attracted attention as an environmentally friendly low-input remediation technique. This technology makes use of pollutant-accumulating (usually hyperaccumulator) that extract pollutants from the soil and accumulate them in the harvestable above-ground biomass [1,2]. Hundreds of hyperaccumulators have been identified in the past three decades, and many studies have been carried out to investigate the mechanisms of uptake, transport, and detoxification of pollutants [3–5]. However, potential use of hyperaccumulators in phytoremediation is limited by a lack of knowledge of many basic plant processes [6]. Most heavy metals have low mobility in soils, and are not easily absorbed by plant roots. However, hyperaccumulating plants show an extraordinary ability to absorb metals from the soil and accumulate them in the shoots under both low and high metal levels. For example, the decrease in the concentration of Zn in the soil solution during the growth of *Thlaspi caerulescens* accounted for only 1% of the Zn accumulated by the plant [7]. Similarly, decreases in the NH_4NO_3-extractable concentration of Zn in the soil after the growth of *T. caerulescens* has been found to account for only 10% [8] and 25% [9] of the Zn accumulated in the shoots. These results suggest that *T. caerulescens* was effective in mobilizing Zn from less soluble fractions of the soils. Many studies have investigated factors regulating the growth of hyperaccumulator plants for maximum metal removal from contaminated sites [10,11]. Whereas, rhizosphere processes that regulate hyperaccumulation have not yet been fully elucidated, the mechanisms of how hyperaccumulator plants solubilize and take up heavy metals from contaminated soils are still unclear [12].

Dissolved organic matter (DOM) consists of several types of low molecular weight organic compounds, such as polyphenols, simple aliphatic acids, amino acids and sugar acids. It is one of the most important factors affecting metal mobility and phytotoxicity in soils [13]. For example, plants release organic acids to take up Fe from the soil, especially under conditions of Fe deficiency [14]. DOM present in root exudates, such as carboxylic acids, is involved in mobilization of inorganic phosphorus (P) in the rhizosphere by changing soil pH, displacing P from sorption sites, chelating P-immobilizing metal cations and forming soluble metal chelate complexes with P [15]. Fitz et al. [16] found that dissolved organic carbon (DOC) concentrations in the rhizosphere soil solution of *Pteris vittata* were increased by 86% and appeared to enhance total Fe solubility due to complexation reactions. To date, although

many workers have dealt with the adsorption characteristics of DOM on soil constituents and its effect on metal adsorption and solubility in the soil solution [17–19], there is a lack of information concerning the influence of DOM on the availability of heavy metals in the rhizosphere of hyperaccumulator [20,21].

Sedum alfredii grows in old Pb/Zn mining areas of southeast China, and has been identified as a Zn/Cd-hyperaccumulator [22]. This plant has exceptional abilities to tolerate and accumulate high concentrations of Zn/Cd, and the characteristics of large biomass, rapid growth, asexual propagation, and perennial growth make it an ideal plant for studying mechanisms responsible for hyperaccumulation as well as for enhancing phytoremediation practices. To date, studies on the mechanism of metal tolerance and hyperaccumulation of *S. alfredii* were mainly focused on the uptake [23,24] and subcellular distribution characteristics [25,26], while no information is available about DOM in the rhizosphere of this plant. The aims of this study were (1) to investigate the changes of DOM in the rhizosphere of *S. alfredii* grown in contaminated soils; and (2) to assess the effects of DOM from the rhizosphere of *S. alfredii* on Zn and Cd sorption via batch studies by three different soils with different physicochemical properties.

2. Materials and methods

2.1. Plant propagation and soil characterization

The hyperaccumulating ecotype (HE) of *S. alfredii* was collected from an old Pb/Zn mine area in Zhejiang Province, PR China, and the non-hyperaccumulating ecotype (NHE) of *S. alfredii* was obtained from a tea garden in Hangzhou, Zhejiang Province, PR China. Plants were grown in non-contaminated soil for several generations to minimize internal metal concentrations. The healthy and equal-sized plant shoots were selected and grown for three weeks in the greenhouse using a basic nutrient solution [22].

The paddy soil used in the pot experiment was collected from an abandoned site in Fuyang county of Hangzhou, Zhejiang Province, PR China. The site was heavily contaminated due to mining activities and not suitable for crop growth. Soil samples were air-dried, ground to pass through a 2-mm sieve, and stored in plastic bottles until use. Selected physical and chemical characteristics of this soil are shown in Table 1.

2.2. Experimental design

The experimental design has been described in detail previously by Li et al. [27]. In brief, the dimensions of the rhizobox were $150 \times 140 \times 200$ (length × width × height in mm). It was divided into three sections, a central zone or rhizosphere zone (20 mm in width), which was surrounded by nylon cloth (300 mesh), and left and right non-rhizosphere zones (60 mm in width). Root growth was limited to the central compartment and within the nylon cloth. 0.5 kg of soil was placed in the rhizosphere zone and 3.0 kg was placed in the non-rhizosphere zones. One week before the study,

the soil equilibrated at field capacity. After pre-culturing for three weeks in hydroponic solution, three plants were transplanted in rhizosphere zone and each treatment was replicated six times. The plants were watered throughout the study to keep the soil at approximately 65% of its field capacity. The plants were allowed to grow for 90 days in a greenhouse with natural light and an average night/day temperature of 30/24 °C, and day/night humidity of 70/85%. At the end of the experiment, the plants were harvested and each plant was separated into root and shoot. The plant were washed thoroughly with tap water and then rinsed with distilled water. Then were oven dried for three days at 65 °C, weighed, and ground to a 60-mesh fineness for chemical analysis. Rhizosphere soil (soil in rhizosphere zone) and bulk soil (soil in non-rhizosphere zones) were separated.

2.3. Extraction and fractionation of soil dissolved organic matter

The dissolved organic matter (DOM) was extracted according to the method of [28] with some modification. Both rhizosphere and bulk soils were extracted with deionized–distilled water using a solid: water ratio of 1:5 (w/v) on a dry weight basis and shake at 200 rpm for 16 h at 25 °C on a reciprocal shaker. The suspension was centrifuged at $10,000 \times g$ for 25 min, and the supernatant was filtered through a 0.45 μm membrane filter and subsequently through a sodium cation exchanger to remove cations (e.g., Zn^{2+}, Cd^{2+}, Mg^{2+}). The filtrates were stored at 4 °C until use. The compositions of the DOM were fractionated into hydrophilic acid (HiA), hydrophilic base (HiB), hydrophilic neutral (HiN), hydrophobic acid (HOA), hydrophobic base (HoB) and hydrophobic neutral (HON) using the method developed by Leenheer [29].

2.4. Zinc and cadmium sorption experiments

Three different soils were used in the Zn and Cd sorption experiments (Table 1), a acidic silty loam (Typic Acrorthox, developed on quaternary red earths), a neutral clay loam (Inceptisol, derived from alluvial sediments) and a calcareous clay loam (yellow podzol). The soils were collected from the surface layer (0–20 cm) from Longyou, Jiaxing and Deqing County of Zhejiang province, PR China, respectively. A 1.0 g sample of each soil type was weighed into 50-ml polyethylene centrifuge tubes and equilibrated with 20 ml of mixed solution containing 10 concentration levels (0, 10, 25, 50, 100, 200, 300, 400, 500, 600 mg L⁻¹) of nitrate salts of Zn or Cd in a 0.01 M NaNO₃ background in the absence or presence of rhizosphere DOM. All the suspensions were adjusted to the pH of it original soils. Two drops of 1 M NaN₃ were added to each tube to inhibit DOM decomposition during the sorption experiments. The soil suspensions were shaken on a reciprocal shaker at 200 rpm and 25 °C for 4 h and then equilibrated in the dark for 2 h. The tubes were then centrifuged at $5000 \times g$ and filtered. Zinc or cadmium concentrations in the filtrates were determined by flame atomic absorption spectrophotometry (FA-AAS, AA-6800, Shimadzu). The quantity of Zn or Cd adsorbed was calculated by subtracting the

Table 1
Selected physical and chemical properties of the soils.

Soils	pH[a]	OM[b]	Total N	Total P	CEC[c]	Sand	Silt	Clay	Total Zn	Total Cd
		g kg⁻¹			cmol kg⁻¹	%			mg kg⁻¹	
Paddy soil	6.83	27.6	1.43	0.84	8.72	26.5	33.6	39.9	2001.6	21.05
Acidic silty clay	4.75	25.9	0.93	0.61	10.86	11.9	46.3	41.8	81.6	0.26
Neutral clay loam	6.90	20.8	1.48	0.89	13.45	32.2	42.4	25.4	118.5	0.35
Calcareous clay loam	8.08	21.8	0.98	0.79	15.59	35.5	40.3	24.2	96.9	0.40

[a] 1:2.5 soil/water ratio.
[b] Organic matter.
[c] Cation exchange capacity.

Zn or Cd concentration in the equilibrium solution from the total initial concentration.

Another series of experiments were performed to evaluate the effect of DOM concentration on Zn and Cd sorption and desorption. To compare the rhizosphere DOM from the two ecotypes of *S. alfredii,* the DOM from the rhizosphere of the NHE was concentrated to $200\,mg\,C\,L^{-1}$ by rotary evaporation under $40\,^{\circ}C$. A soil sample of 1.0 g was shaken with 20 mL of solution containing $200\,mg\,Zn\,L^{-1}$(or $200\,mg\,Cd\,L^{-1}$) and different DOM concentrations of 0, 25, 50, 100, 200 $mg\,C\,L^{-1}$ at a background ionic strength of 0.01 M $NaNO_3$. Two drops of 1 M NaN_3 were added to each tube. The rest of the procedure including shaking and centrifugation was the same as described above. The residues obtained after sorption were washed using double-distilled water, then 20 mL of 0.01 M $NaNO_3$ (pH 7) was added into the tube and shaken at 200 rpm for 2 h at $25\,^{\circ}C$, and then centrifuged at $5000 \times g$ and filtered. Zinc or cadmium concentrations in the filtrates were determined to obtain desorption of Zn and Cd previously sorbed by soils.

2.5. Chemical analysis

Plants were digested using EPA Method 3050A for the Hot Block Digestion System (Environmental Express, Mt. Pleasant, SC) and then determined using FAAS (AA6800, Shimadzu, Japan). Soil pH was measured using a 1:2.5 soil to water ratio (Orion 902A ion analyzer). NH_4NO_3-extractable Zn and Cd in the rhizosphere and bulk soils were determined using a 1:2.5 soil to NH_4NO_3 solution ratio, shaking (1 h), centrifuging (15 min at $4000 \times g$) and filtering (0.45 μm syringe filter). Zinc and Cd concentration were determined using ICP-AES (ICPE-9000, Shimadzu, Japan). The concentrations of DOC were measured using a total organic carbon (TOC) analyzer (TOC-5050A, Shimadzu, Japan).

All statistical analyses were conducted with SPSS 11.0. Means were compared by *t* test or least significant difference (LSD) test at $P < 0.05$.

3. Results and discussion

3.1. Plant growth and uptake of Zn and Cd

During the 14-week growth, no visual symptoms of Zn and Cd toxicity were observed for the HE, whereas the growth of the NHE was inhibited significantly. The shoot and root biomass of the HE were 2.1–3.6 times higher than the NHE (Table 2). After growing in contaminated soil, Zn and Cd concentrations in the shoots of HE were 8.7 and 9.9 times higher than in the roots whereas in the NHE the shoot/root ratios of the Zn and Cd concentrations were 0.31 and 0.45, respectively. These data indicate that the HE was much more efficient in translocating Zn and Cd from the roots to the shoots than the NHE. Concentrations of Zn and Cd in the shoots of the HE were 26.4 and 20.3 times higher than in those NHE. HE accumulated significantly more Zn and Cd in the shoots and roots than did NHE. These results agree with previous papers [22,23] and suggest that

Table 2
Plant biomass, Zn and Cd concentration and accumulation of *S. alfredii* after growing for 14 weeks in Zn/Cd-contaminated soils.

Plant parameters	HE *S. alfredii*	NHE *S. alfredii*
Shoot biomass (g pot^{-1})	4.18 ± 0.42[*]	2.03 ± 0.20
Root biomass (g pot^{-1})	0.51 ± 0.04[*]	0.14 ± 0.01
Shoot Zn (mg kg^{-1})	8982.5 ± 451.4[*]	340.5 ± 21.4
Root Zn (mg kg^{-1})	1032.0 ± 89.2[*]	1109.8 ± 98.8
Shoot Cd (mg kg^{-1})	810.4 ± 72.4[*]	39.8 ± 3.4
Root Cd (mg kg^{-1})	82.0 ± 9.4	85.5 ± 7.2
Shoot Zn (mg pot^{-1})	37.55 ± 3.42[*]	0.69 ± 0.04
Root Zn (mg pot^{-1})	0.67 ± 0.04[*]	0.16 ± 0.02
Shoot Cd (mg pot^{-1})	3.39 ± 0.22[*]	0.08 ± 0.01
Root Cd (mg pot^{-1})	0.04 ± 0.00[*]	0.01 ± 0.00

Data are means ± SD ($n = 6$).
[*] Significant differences ($P < 0.05$) between HE *S. alfredii,* and NHE *S. alfredii.*

the hyperaccumulating ecotype of *S. alfredii* has a great potential for removal of Zn and Cd from contaminated soil.

3.2. Changes in mobile soil Zn and Cd, pH and dissolved organic carbon before and after growth of S. alfredii

The concentration of NH_4NO_3-extractable Zn and Cd in the soil was determined before and after growth of *S. alfredii* (Table 3). No significant change was found for the NHE. However, the concentrations of mobile Zn and Cd in both rhizosphere and bulk soils decreased compared to the initial values before the HE was planted (Table 3), and the decreases in the rhizosphere were much greater than in the bulk soils. The results agree with the study on *T. caerulescens* [7,8] and *P. vittata* [12]. Although the mobile Zn and Cd fraction in soils decreased considerably after planting HE, the decreases in this fraction in both rhizosphere and bulk soils represented only 7.1% and 9.3% of the total uptake in the shoots. Therefore, more than 90% of the total Zn and Cd uptake must have come from the non-mobile fractions. The results suggest that the HE is highly efficient at mobilizing Zn and Cd which is not soluble initially.

Soil pH is considered to be one of the most important chemical factors controlling the availability of heavy metals [16]. Table 3 shows that at the end of the experiment, no change in pH was observed in both rhizosphere and bulk soil of the NHE. However, the rhizosphere soil of the HE was reduced 0.5–0.6 pH units compared to the bulk soil and initial values. This result agree with Gonzaga et al. [12] who found that arsenic-induced root exudates reduced soil pH by 0.74–0.92 units in the rhizosphere of the As hyperaccumulator *P. vittata*. Reduction in rhizosphere pH increases the chemical activity of most metals in solution, thereby increasing Zn and Cd uptake by the HE. This suggests that rhizosphere acidification maybe an important mechanism for mobilizing metals in soil by the HE. However, Bernal and McGrath [30] and McGrath et al. [8] found that the Ni hyperaccumulator *Alysum murale* and the Zn hyperaccumulator *T. caerulescens* did not cause a significant decrease in the pH of the rhizosphere, suggesting that the increased metal uptake is not simply a function of low rhizosphere pH. Luo

Table 3
pH, mobile Zn and Cd and dissolved organic carbon in the bulk and rhizosphere soil of *S. alfredii*.

Soil	HE *S. alfredii*				NHE *S. alfredii*			
	pH	NH_4NO_3-Zn (mg kg^{-1})	NH_4NO_3-Cd (mg kg^{-1})	DOC (mg L^{-1})	pH	NH_4NO_3-Zn (mg kg^{-1})	NH_4NO_3-Cd (mg kg^{-1})	DOC (mg L^{-1})
Rhizosphere	6.26 ± 0.16b[*]	2.88 ± 0.12b[*]	0.14 ± 0.02b[*]	210.0 ± 18.1a[*]	6.88 ± 0.09a	3.48 ± 0.15a	0.20 ± 0.02a	156.6 ± 12.7a
Bulk soil	6.74 ± 0.14a	3.34 ± 0.13a	0.19 ± 0.04a	156.1 ± 12.5b[*]	6.79 ± 0.10a	3.56 ± 0.33a	0.21 ± 0.01a	134.8 ± 10.2b
Before planting	6.83 ± 0.19a	3.61 ± 0.10a	0.22 ± 0.02a	122.6 ± 9.6c	6.80 ± 0.15a	3.58 ± 0.24a	0.21 ± 0.03a	121.5 ± 10.1b

Data are means ± SD ($n = 6$). Values followed by different letters for different soils are significantly different at $P < 0.05$.
[*] Significant differences ($P < 0.05$) between HE *S. alfredii* and NHE *S. alfredii*.
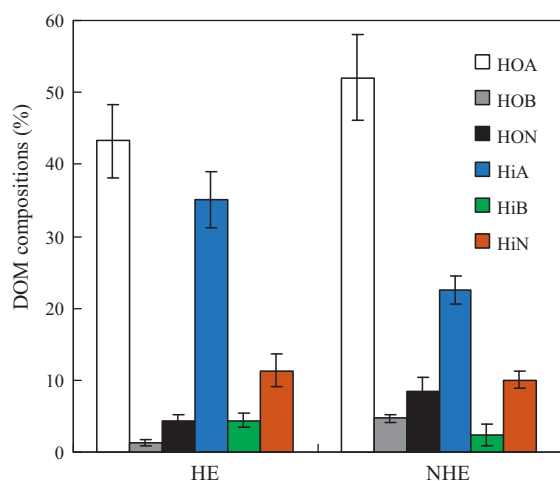
Fig. 1. The distribution of hydrophobic and hydrophilic fractions of DOM derived from the rhizosphere of the hyperaccumulating ecotype (HE) and non-hyperaccumulating ecotype (NHE) of *S. alfredii* (percent of total DOM).

et al. [31] found that *T. caerulescens* rhizosphere soil had a higher pH than non-rhizosphere soils. These conflicting results indicated that different hyperaccumulators have different mechanisms for mobilizing metals in soil. The reduction in pH was probably due to increased release of root exudates, which was consistent with the greater DOC concentrations in the rhizosphere of the HE.

The DOC concentrations in the rhizosphere of both ecotypes of *S. alfredii* were greater than those in the bulk soil (Table 3). The DOC concentration in the rhizosphere of the HE was almost double compared to initial values. Fitz and Wenzel [32] proposed that hyperaccumulators may enhance metal solubility in the rhizosphere via root exudation, consequently increasing plant metal uptake. Our experiment seemed to support this hypothesis since the HE exuded about 34% more DOC in the rhizosphere than the NHE (Table 3). Despite the fact that DOC accounts for a small portion of total organic carbon, it significantly affects nutrient and contaminant mobility, microbial activity and soil properties [13]. Wenzel et al. [20] found a highly significant correlation between Ni and DOC in soil solution ($R^2 = 0.85$), providing evidence for enhanced formation of Ni–DOM complexes in the rhizosphere of *T. goesingense*.

Further analysis found that the major composition of DOM in the rhizosphere of both ecotypes of *S. alfredii* were acid fraction (HoA + HiA) (Fig. 1), which accounted for 76% and 72% of total DOM in the rhizosphere of HE and NHE, respectively. However, the distribution of chemical fractions differed markedly according the source of DOM. Dissolved organic matter from the rhizosphere of HE constituted of 51% of hydrophilic fractions and 49% of hydrophobic fractions. In contrast, the hydrophobic fraction (65%) of the DOM from the rhizosphere of NHE was much greater than the hydrophilic fractions (35%). Meanwhile, the content of DOM HiA, HiB, and HiN from the rhizosphere of HE was 1.6, 1.9, and 1.1 times higher than corresponding content of DOM from the rhizosphere of NHE. The hydrophilic acids are considered to be the most chemically active class of DOM because they contain functional components that can chelate and dissolve metal from soil [33,34]. Thus, the reaction of DOM in the rhizosphere of the HE with Zn and Cd is highly expected and has the potential to influence Zn and Cd sorption and mobility.

3.3. Sorption of Zn and Cd by different soils in the presence of DOM

The equilibrium isotherms of Zn and Cd sorption for the three soils with or without the addition of DOM derived from the rhizosphere of *S. alfredii* are given in Fig. 2. The sorption amount
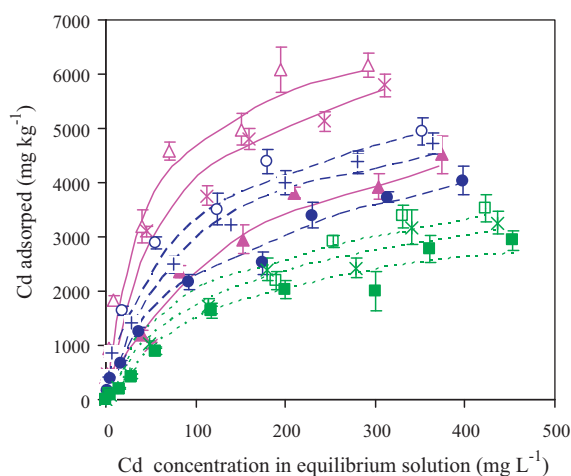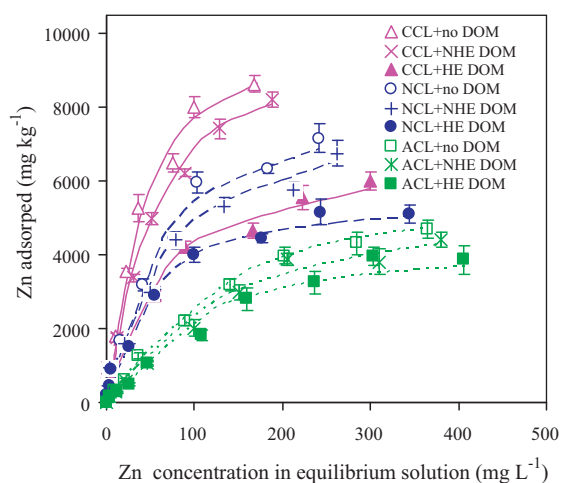

Fig. 2. Sorption isotherms of Zn and Cd for the acidic silty loam (ASL), neutral clay loam (NCL), and calcareous clay loam (CCL) in the absence or presence of DOM derived from the rhizosphere of the hyperaccumulating ecotype (HE-DOM) and non-hyperaccumulating ecotype (NHE-DOM) of *S. alfredii*.

increased with an increase in equilibrium Zn or Cd concentrations and eventually attained a plateau value at higher equilibrium concentrations for the soils. The Zn and Cd sorption capacities for the three soils followed the order: calcareous clay loam > neutral clay loam > acidic silty clay at the same equilibrium concentration of Zn or Cd in the absence or presence of different kind of DOM. The acidic silty clay showed much less ability to adsorb Zn and Cd due to its lower pH even though its clay content was more than the neutral and calcareous clay loam. These results agree with a previous study showing that increasing pH generally caused an increase in metal sorption [35,36]. It also implies that pH play a more important role in metal sorption. There is no general agreement on a single mechanism responsible for this behavior, but the changes in surface variable charge density and the formation of metal hydroxide precipitates might be responsible for the increased metal sorption at higher pH [37].

The Zn or Cd sorption was analyzed using the Freundlich equation:

$$\lg Y = \lg K + \frac{1}{n}\lg C \tag{1}$$

where Y is the amount of metal adsorbed ($\mathrm{mg\,kg^{-1}}$), K is the equilibrium partition coefficient, $1/n$ is the sorption intensity, and C is the equilibrium metal concentration ($\mathrm{mg\,L^{-1}}$).

The Zn and Cd sorption isotherms are well described by the Freundlich equation (Table 4). Generally, the higher the sorption

Table 4
Freundlich parameters for Zn and Cd sorption by three soils in the presence or absence of DOM derived from the rhizosphere of the hyperaccumulating ecotype (HE DOM) and the non-hyperaccumulating ecotype (NHE DOM) of *S. alfredii*.

Soils	DOM type	k		$1/n$		R^2	
		Zn	Cd	Zn	Cd	Zn	Cd
Acidic silty clay	No DOM	75.6	33.1	0.732	0.794	0.988	0.971
	HE DOM	52.4	26.4	0.750	0.818	0.984	0.967
	NHE DOM	62.6	30.5	0.737	0.810	0.989	0.969
Neutral clay loam	No DOM	501.0	230.1	0.490	0.562	0.989	0.900
	HE DOM	291.8	143.3	0.533	0.605	0.987	0.971
	NHE DOM	405.1	172.2	0.509	0.591	0.991	0.967
Calcareous clay loam	No DOM	1107.4	509.3	0.385	0.476	0.965	0.986
	HE DOM	347.8	208.0	0.522	0.527	0.965	0.952
	NHE DOM	996.8	390.4	0.407	0.494	0.974	0.985

intensity parameter ($1/n$), the lower is the binding affinity of the metal to the soil. The equilibrium partition coefficient (K) is positively related to the metal sorption capacity of the soils. From the calculated parameters of the Freundlich sorption isotherms, we can found that the addition of DOM from the rhizosphere of the HE significantly reduced the Zn and Cd sorption capacity of the three soils as compared to DOM from the rhizosphere of the NHE and control without DOM, and this decrease in turn depended on the soil types. The sorption intensity parameter ($1/n$) of the three soils followed the order: acidic silty clay > neutral clay loam > calcareous clay loam and increased significantly with the addition of DOM from the rhizosphere of the HE. This result indicated that DOM from the rhizosphere of the HE could decrease the affinity of the three soils for Zn and Cd, especially for the calcareous clay loam. The DOM from bulk soil of both ecotypes had no effect on Zn and Cd sorption on the three soils as compared to DOM from the rhizosphere of the NHE (data are not shown in Fig. 1). Thus, we only compared the DOM from the rhizosphere of two ecotypes of *S. alfredii*.

The distribution coefficient (K) is a useful index for comparing the sorption capacities of different soils for a particular ion under the same experimental conditions [38,39]. The addition of DOM from the rhizosphere of the HE significantly decreased the Freundlich K values by 30.7–68.8% for Zn and 20.3–59.2% for Cd, respectively, with the decreases being in the following order: calcareous clay loam > neutral clay loam > acidic silty clay, these results suggesting that DOM from the rhizosphere of the HE led to a greater reduction in Zn sorption than Cd sorption. These results are consistent with Wong et al. [40] who found that DOM from digested dewatered sludge significantly reduced the Cd and Zn sorption capacity by a factor of 2.1–5.7 for Cd and 2.3–13.7 for Zn in different soils. The DOM could reduce Zn and Cd sorption, especially in higher pH soil due to the formation of soluble metal–organic complexes or by competing with the metal for the sorption sites on the soil surface, since DOM contains many different metal chelating groups [17]. Zinc forms slightly more stable complexes with organic molecules than Cd which may explain the higher reduction in Zn sorption after the addition of DOM from the rhizosphere of the HE than for Cd [41].

3.4. DOM concentration effect on Zn and Cd sorption and desorption

Increasing the DOM concentration caused a significant reduction on the sorption of Zn and Cd by three soils (Fig. 3), especially in the calcareous clay loam. However the reduction was much greater for DOM from the rhizosphere of the HE compared to DOM from the rhizosphere of the NHE, e.g., the sorption amount decreased 18.6–24.8% for Zn and 17.8–22.8% for Cd for the three soils with the addition of 200 mg C L^{-1} HE-DOM, whereas its only decreased 2.5–9.8% for Zn and 8.9–12.2% for Cd for NHE-DOM. A significant

negative linear correlation between DOM concentration and Zn or Cd sorption was observed for all three soil types (Table 5), indicating that metal mobility increased with an increase in DOM concentration. Likewise, we found that the DOM from the rhizosphere of the HE had a greater inhibition on metal sorption than the DOM from the rhizosphere of the NHE, based on the slope of the linear equation. The effect of DOM concentration on metal sorption was dependent on soil type. At a given DOM concentration, the inhibition on metal sorption increased in the following order: calcareous clay loam > neutral clay loam > acidic silty clay.
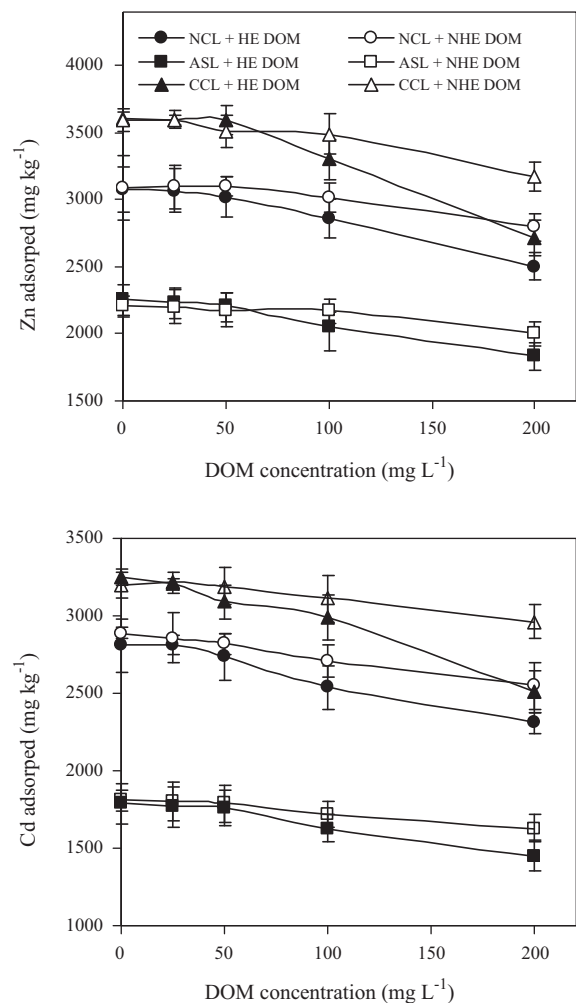

Fig. 3. Effect of rhizosphere DOM concentration on Zn and Cd sorption on acidic silty loam (ASL), neutral clay loam (NCL), and calcareous clay loam (CCL) at an initial Zn/Cd concentration of 200 mg L^{-1}.

Table 5
Linear equations for DOM concentration and metal adsorbed at an initial Zn/Cd concentration of 200 mg L^{-1}.

Soils	DOM	n	Equation	R^2
Acidic silty clay	HE DOM	5	$[Zn]_{adsorbed} = -1.648[DOC] + 2282.9$	0.961
		5	$[Cd]_{adsorbed} = -1.870[DOC] + 1815.5$	0.924
	NHE DOM	5	$[Zn]_{adsorbed} = -0.270[DOC] + 2201.0$	0.886
		5	$[Cd]_{adsorbed} = -0.862[DOC] + 1810.3$	0.897
Neutral clay loam	HE DOM	5	$[Zn]_{adsorbed} = -2.468[DOC] + 3231.3$	0.928
		5	$[Cd]_{adsorbed} = -2.825[DOC] + 2819.0$	0.994
	NHE DOM	5	$[Zn]_{adsorbed} = -1.069[DOC] + 3220.3$	0.928
		5	$[Cd]_{adsorbed} = -1.513[DOC] + 2829.6$	0.861
Calcareous clay loam	HE DOM	5	$[Zn]_{adsorbed} = -4.234[DOC] + 3698.5$	0.956
		5	$[Cd]_{adsorbed} = -3.703[DOC] + 3316.6$	0.959
	NHE DOM	5	$[Zn]_{adsorbed} = -1.798[DOC] + 3620.3$	0.943
		5	$[Cd]_{adsorbed} = -2.045[DOC] + 3225.1$	0.914

The origin and concentration of DOM affected not only the Zn or Cd sorption of the three soils, but the desorption of Zn or Cd sorbed previously in the sorption study (Fig. 4). A relatively higher desorption was found for Zn and Cd sorbed in the presence of a higher concentration of DOM, especially for HE-DOM treatment. The desorption rate of the three soils were 5.6–7.8% for Zn and 6.8–10.9% for Cd in the presence of 200 mg L^{-1} HE-DOM, which were 1.2–2.7 times and 1.1–1.9 times of NHE-DOM, respectively.
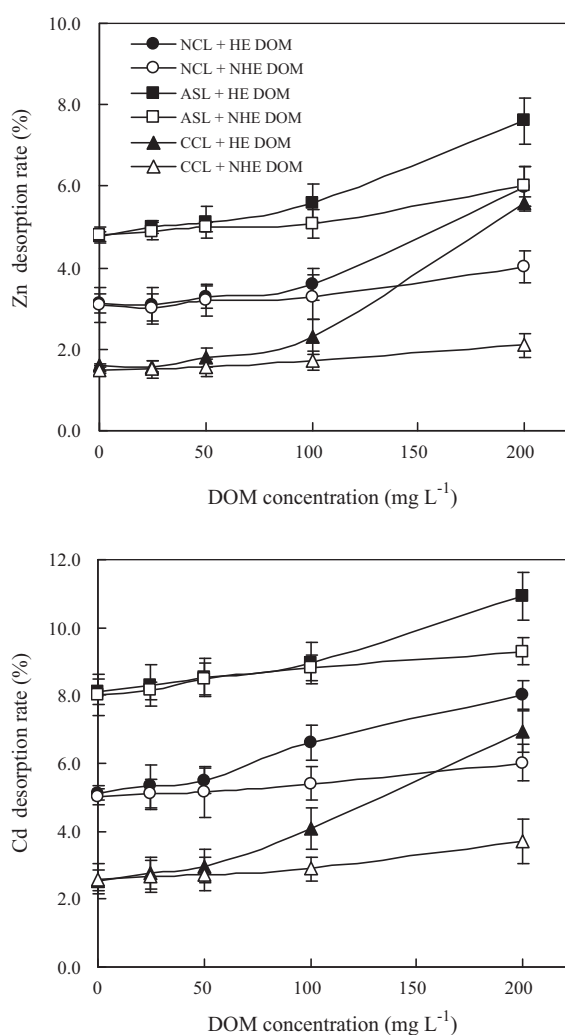

Fig. 4. Desorption of Zn or Cd sorbed by the different soils under the addition of various concentrations of DOM derived from the rhizosphere *S. alfredii* with an initial Zn or Cd concentration of 200 mg L^{-1}.

This might result from a stronger binding affinity of Zn and Cd with HE-DOM, and as a result it hinders partly the precipitation of Zn or Cd, as compared with NHE-DOM. This implied that Zn or Cd mobility might be facilitated greatly in the soil receiving DOM, especially for HE-DOM, because of the reduction of Zn or Cd sorption onto soil and the relatively ready desorption of Zn or Cd sorbed previously in the presence of DOM. The differences in Zn or Cd sorption and desorption behavior caused by DOM from the rhizosphere of two ecotype of *S. alfredii* appeared to be closely related to the chemical components of DOM. HE-DOM contained a relatively greater amount of hydrophilic fractions, especially hydrophilic acid (HiA) and hydrophilic base (HiB), but fewer hydrophobic fractions, than that of NHE-DOM (Fig. 1). Many researchers revealed that hydrophobic fractions of DOM bind more strongly on soils and minerals than the hydrophilic fractions [42], while the hydrophilic fractions (HiA and HiB) which was richer in carboxyl and polyhydroxyl groups and was not readily sorbed by soils but could strongly associate with Zn and Cd [33,34]. Thus, HE-DOM had a stronger capability to reduce Zn and Cd sorption by soils than did NHE-DOM.

The main sources of DOM in soils are plant litter, soil humus, and root exudates [43]. It has been reported that the As hyperaccumulator *P. vittata* releases various low molecular weight organic acids via root exudation [21]. In this study, the effect of DOM from the rhizosphere of two ecotype of *S. alfredii* on Zn and Cd sorption and desorption are totally different, this result imply that the DOM component is the probable factor affecting the environmental chemical behavior of Zn and Cd. Thus further studies should be conducted to elucidate the characterisation of the organic component compounds (e.g., organic acids, amino acids and sugar acids) in the DOM from the rhizosphere of *S. alfredii*.

4. Conclusions

The present study confirmed that the HE *S. alfredii* was more efficient in Zn and Cd accumulation than the NHE *S. alfredii*. Root-induced pH reduction and DOM increase in the rhizosphere of the HE helped to solubilize Zn and Cd in the rhizosphere. The hydrophilic fractions in DOM from the rhizosphere of HE (HE-DOM) was much greater than NHE-DOM. In the presence of HE-DOM, Zn and Cd sorption capacity decreased markedly in the following order: calcareous clay loam > neutral clay loam > acidic silty clay, as compared to NHE-DOM. Increasing DOM concentration reduced Zn and Cd sorption, especially in the calcareous clay loam. A relatively higher desorption was found for Zn and Cd sorbed in the presence of a higher concentration of DOM, especially for HE-DOM treatment. DOM derived from the rhizosphere of the HE could significantly reduce metal sorption and increase its mobility through the formation of soluble DOM–metal complexes. This may be one of the mechanisms by which the hyperaccumulating ecotype of *S. alfredii* activates metals in the rhizosphere.

Acknowledgments

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