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Mobilization of cadmium by dissolved organic matter in the rhizosphere of hyperaccumulator Sedum alfredii

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HIGHLIGHTS

- Cd speciation in soil solution of hyperaccumulator was simulated using Visual MINTEQ.
- Cd–DOM complex were the dominant Cd species in soil solutions followed by free Cd$^{2+}$ species.
- DOM from the rhizosphere of HE S. alfredii showed greater ability to form complexes with Cd.
- DOM from the rhizosphere of HE S. alfredii increased the solubility of Cd minerals significantly.

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ABSTRACT

Pot experiments were conducted to investigate the role of dissolved organic matter (DOM) in the Cd speciation in the rhizosphere of hyperaccumulating ecotype (HE) and non-hyperaccumulating ecotype (NHE) of Sedum alfredii and its effects on Cd mobility. After growing HE S. alfredii, the rhizosphere soil solution pH of heavily polluted soil (HPS) and slightly polluted soil (SPS) was reduced by 0.49 and 0.40 units, respectively, due to enhanced DOC derived from root exudation. The total Cd concentration in soil solution decreased significantly but the decrease accounted for less than 1% of the total Cd uptake in the shoots of HE S. alfredii. Visual MINTEQ speciation predicted that Cd–DOM complexes were the dominant Cd species in soil solutions after the growth of S. alfredii for both soils, followed by the free metal Cd$^{2+}$ species. However, Cd–DOM complexes fraction in the rhizosphere soil solution of HE S. alfredii (89.1% and 74.6% for HPS and SPS, respectively) was much greater than NHE S. alfredii (82.8% and 64.7% for HPS and SPS, respectively). Resin equilibration experiment results indicated that DOM from the rhizosphere (R-DOM) of both ecotypes of S. alfredii had the ability to form complexes with Cd, whereas the degree of complexation was significantly higher for HE-R-DOM (79–89%) than NHE-R-DOM (63–74%) in the undiluted sample. The addition of HE-R-DOM significantly ($P < 0.05$) increased the solubility of four Cd minerals while NHE-R-DOM was not as effective at the same concentration. It was concluded that DOM in the rhizosphere of hyperaccumulating ecotype of S. alfredii could significantly increase Cd mobility through the formation of soluble DOM–metal complexes.

1. Introduction

Cadmium (Cd) is toxic to organisms, even at low concentrations. Cadmium pollution of the soil has occurred through the application of Cd-containing phosphate fertilizers/manures, sewage sludge, and municipal sewage-wastes to agricultural soils (Das et al., 1997; Singh et al., 2006). The clean-up of soils contaminated with heavy metals (HMs) is one of the most difficult tasks for environmental engineering. Phytoextraction has attracted attention as an environmentally friendly low-input remediation technique. This technology makes use of metal-(hyper)accumulating plants that extract HMs from the soil and accumulate them in the harvestable above-ground biomass (Chaney et al., 1997; McGrath and Zhao, 2003). 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 (Pence et al., 2000; Ebbs et al., 2009; Barcelo and Pochsenrieder, 2011). However, the potential use of hyperaccumulating plants in phytoremediation is limited by a lack of knowledge of many basic plant processes. In particular, the mechanisms...
involved in the acquisition of metals from soils with low concentrations of soluble metals are not clear.

Hyperaccumulators are known for their exceptional capacity for metal uptake, but even for these plants, metal uptake is also dependent upon metal availability in the soil (McGrath et al., 1997; Wenzel et al., 2003). Much of the previous work has shown a great reduction in the water-soluble or NH₄NO₃-extractable concentration of metals in the rhizosphere of hyperaccumulators (Knight et al., 1997; McGrath et al., 1997; Whiting et al., 2001; Gonzaga et al., 2006; Li et al., 2011a). However, the reductions observed in water-soluble or labile pools of metals generally accounted for a low percentage of the total metal uptake by the plants, indicating that the metals are mainly acquired from less available pools (Fitz et al., 2003; Li et al., 2011a). Plants can modify their rhizosphere to enhance uptake of nutrients such as Fe, P, and Zn. Common mechanisms involved are acidification, decrease of redox potential, and release of root exudates (Zhao et al., 2001; Tu et al., 2004). Whereas, rhizosphere processes that regulate metal hyperaccumulation phenomenon have not yet been fully elucidated, the mechanisms of how hyperaccumulators mobilize and take up metal from contaminated soils are still unclear (Kidd et al., 2009; Wenzel, 2009).

There are many factors that influence trace element speciation and bioavailability in the rhizosphere, such as elemental concentrations, pH, pCO₂, pO₂, redox potential, organic ligand concentrations, and microorganisms (Puschenreiter et al., 2005). Dissolved organic matter (DOM) consists of both hydrophilic (organic acids, carbohydrates, amino acids, and amino sugars) and hydrophobic (aromatic phenols, hydrocarbons, fats, and nucleic acids) components, and is one of the important factors affecting metal mobility and phytotoxicity in the soils (Kaiser et al., 2002). These organic compounds can form soluble organometallic complexes, or is preferentially adsorbed onto soils surface in place of metals, which reduce the metal adsorption onto soil surfaces, promoting metal availability for plants (Antoniadis and Alloway, 2002; Weng et al., 2002; Cornu et al., 2011). Much of the previous works have been conducted to characterize DOM from soil, natural water, forest litter, and sewage sludge (Kalbitz et al., 1999; Leenheer and Croue, 2003; Bolan et al., 2011), and most of these studies have involved the adsorption characteristics of DOM on soil constituents and its effect on metal adsorption and solubility in the soil solution (Kaiser and Guggenberger, 2000; Weng et al., 2002; Yamashita and Jaffe, 2008). Little has been done to investigate the DOM in the rhizosphere of hyperaccumulator plants in relation to metal accumulation. Sedum alfredii (Crassulaceae) grow in old Pb/Zn mining areas of southeast China, and has been identified as a Zn/Cd-hyperaccumulator. To date, a lot of work has been conducted to investigate the mechanisms of uptake, transport, and detoxification of metal in S. alfredii (Yang et al., 2006; Tian et al., 2010; Li et al., 2011a,b). There is a lack of information concerning metal solubilization in the rhizosphere of this plant. Previous studies have shown that the DOM derived from the rhizosphere of the hyperaccumulating ecotype of S. alfredii can significantly reduce metal sorption on soils as compared to non-hyperaccumulating ecotype (Li et al., 2011b). We speculate that DOM derived from rhizosphere of hyperaccumulating ecotype of S. alfredii could significantly increase HMs mobility through the formation of soluble DOM–metal complexes, which need to be further confirmed. The objectives of this study were (1) to assess the role of DOM in the Cd speciation in soil solutions and (2) to investigate the effects of DOM from the rhizosphere of S. alfredii on Cd solubility. Results of the present study will help in the understanding of the mechanisms by which S. alfredii mobilizes and takes up Cd from soil and will optimize the management practices for maximum Cd phytoextraction.

2. Materials and methods

2.1. Experimental design

This was a continued study from earlier experiments. The experimental design has been described in detail previously (Li et al., 2011a). In brief, a rhizobox was used in this study and the dimensions of the rhizobox were 150 × 140 × 200 (length × width × height in mm). It was divided into three sections: a root compartment or rhizosphere zone (20 mm in width), which was surrounded by nylon mesh (<25 µm), and left and right soil compartment or non-rhizosphere zone (60 mm in width). Root growth was limited to the root compartment and no root hairs can enter the adjacent soil zones. Two soils were used in the pot experiment. The paddy soil (heavily polluted soil, HPS) used in the pot experiment was collected from the surface layer (0–20 cm) of an abandoned site in Fuyang county of Hangzhou, Zhejiang Province, PR China. The slightly polluted soil (SPS) was collected from the top soil (0–20 cm) in the farm of Zhejiang University, Hangzhou. 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.

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. After pre-culturing for 3 weeks in Hoaglands solution, three S. alfredii plants were transplanted in rhizosphere zone and each treatment was replicated four times. Control pots without plants were included. Soil moisture was adjusted to 65% of its field capacity by watering to weight weekly with deionized water. The plants were allowed to grow for 90 d in a greenhouse with a 12-h photoperiod (natural light) and an average night/day temperature of 26/20 °C, and day/night humidity of 70/85%.

2.2. Soil solution extraction and analysis

Prior to harvest, each pot was saturated with Milli-Q water and allowed to equilibrate for 24 h for further extraction of field moisture. After harvest of the plants, soil solution was collected following centrifugation. The moist soil was packed into 25-ML filtration tubes. Soils were then centrifuged at 8000 g for 30 min with the filtration tube inside a 50-ML centrifuge tube that contained a small spacer in the bottom. Extracted solutions were then centrifuged at 12,000g for 30 min and filtered through a 0.45 µm membrane filter. Dissolved organic carbon (DOC) in soil solution was determined immediately after the isolation of soil solution using a

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Physical and chemical characteristics of the soil used.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soil</td>
<td>HPS</td>
</tr>
<tr>
<td>pH</td>
<td>6.83</td>
</tr>
<tr>
<td>Organic matter (g kg⁻¹)</td>
<td>27.6</td>
</tr>
<tr>
<td>Total N (g kg⁻¹)</td>
<td>1.43</td>
</tr>
<tr>
<td>Total P (g kg⁻¹)</td>
<td>0.84</td>
</tr>
<tr>
<td>CEC (cmol kg⁻¹)</td>
<td>8.72</td>
</tr>
<tr>
<td>Total Cd (mg kg⁻¹)</td>
<td>21.05</td>
</tr>
<tr>
<td>NH₄NO₃-Cd (mg kg⁻¹)</td>
<td>0.21</td>
</tr>
<tr>
<td>Particle size distribution (g kg⁻¹)</td>
<td>Sand 265, Silt 336, Clay 399</td>
</tr>
</tbody>
</table>

a 1:2.5 Soil/water ratio.
b Cation exchange capacity.
c Particle size distribution (2–0.02 mm), Silt (0.02–0.002 mm), and Clay (<0.002 mm).
total organic carbon analyzer (TOC-5050A, Shimadzu, Japan). The solution pH was measured using a 1:2.5 soil:water ratio. The concentrations of total Ca, Mg, K, Na and Cd were determined by Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES) (ICPE-9000, Shimadzu, Japan), and the concentrations of anions (Cl\(^{-}\), SO\(_4^{2-}\), and NO\(_3^{-}\)) were analyzed by Ion Chromatography (LC-20A, Shimadzu, Japan). The speciation of Cd in soil solution was assessed by calculation using Visual MINTEQ version 2.61. The modeling was performed using soil solution pH, anions (Cl\(^{-}\), SO\(_4^{2-}\), and NO\(_3^{-}\)) and total dissolved Ca, Mg, K, Na and Cd as input data (Cornu et al., 2011). DOM was assumed to contain 50% carbon. The NICA-Donnan model was used to evaluate the metal binding to DOM (Milne et al., 2003). The DOM was simulated by 100% fulvic acid (FA) as almost no humic acid (HA) were detected in the soil solutions.

### 2.3. Complexation of Cd by dissolved organic matter

After harvest of the plants, the DOM was extracted according to the method of Jones and Willett (2006). Field moist soil was extracted with deionized-distilled water using a solid:water ratio of 1:2.5 (w/v) on a dry weight basis and shaken at 200 rpm for 2 h at 20 °C on a reciprocal shaker. The suspension was centrifuged at 10,000g for 25 min, and the supernatant was filtered through a 0.45 μm membrane filter. The samples were stored in the dark at 1 °C. DOC, anions, heavy metals and major cations were determined as described above. Complexation of Cd by DOM was measured using a resin equilibrium method described by (Christensen and Christensen, 2000). Cation exchange resin (polystyrene with sulfonic acid groups, 100–200 mesh, Na\(^+\) form) was converted from the Na\(^+\)-form by exposure to the synthetic reference solutions which were made up of Ca\(^{2+}\), Mg\(^{2+}\), Na\(^{+}\), K\(^{+}\) and NH\(_4^{+}\) salts of chloride, sulfate and nitrate to mimic the composition of the DOM samples from the rhizosphere of two ecotypes of S. alfredii with respect to cations, chloride, and ionic strength. The resin equilibration experiments were carried out in 50-ml polyethylene bottles containing 25 ml of solution (A sample solution that containing different concentrations of DOC in its original matrix, or a reference solution that is DOC-free but is otherwise identical to the DOC-containing sample) and 150 mg of the corresponding resin. Cadmium was added to each bottle at a predetermined concentration. The bottles were equilibrated for 3 d (based on preliminary test). During equilibration, pH was adjusted by addition of small amounts of HNO\(_3\) or NaOH to maintain constant and identical pH in the bottles (pH 6.0). After equilibration, solutions were separated from the resin by centrifugation. The distribution of heavy metal between the solution and the resin in the absence of DOC was determined in triplicate and the mean value used in the calculations. Cadmium in solution was determined by ICP-OES (ICPE-9000, Shimadzu, Japan). The effect of metal-DOM complexation can be determined by comparing the metal distribution in two solutions equilibrated with a resin. The degree of DOC–Cd complexation is defined as (1 - \(K_{D,DOC}/K_{D,RES}\)), where \(K_{D,DOC}\) is the distribution of Cd between the solution and the resin in the presence of DOC, and \(K_{D,RES}\) is the distribution of Cd between the solution and the resin in the absence of DOC.

### 2.4. Cd extracting experiment

An extracting experiment was conducted to evaluate the effect of DOM concentration on Cd mobility. The DOM in the rhizosphere of S. alfredii was extracted as described above, DOC was determined using a total organic carbon analyzer, and then defined DOM concentration (25, 50, 100 mg C L\(^{-1}\)) was achieved by diluting original DOM with deionized water. Four Cd minerals of phosphate, carbonate, hydroxide or oxalate with different solubility in water (0.06 mg L\(^{-1}\), 0.39 mg L\(^{-1}\), 2.70 mg L\(^{-1}\), 60.04 mg L\(^{-1}\) at 25 °C, respectively) were selected. These minerals were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). A sample of 2.0 g mineral was placed into 50-ml polyethylene centrifuge tubes and equilibrated with 20 ml rhizosphere DOM in different concentrations (25, 50, 100 mg C L\(^{-1}\)). Deionized water was added to each Cd mineral as a control. The suspensions were shaken on a reciprocal shaker at 200 rpm and 25 °C for 4 h and then centrifuged at 5000g for 30 min and filtered. Cadmium concentrations in the filtrates were determined by ICP-OES (ICPE-9000, Shimadzu, Japan).

### 2.5. Statistical analysis

Data were analyzed statistically using analysis of variance (ANOVA). Means of significant difference were separated using least significant difference (LSD, \(P < 0.05\)).

### 3. Results and discussion

#### 3.1. Physico-chemical properties of soil solutions

Solution pH is an important parameter known to impact on the speciation of metals in soil solution (Cornu et al., 2009). Table 2 shows that at the end of the experiment, no significant changes in soil solution pH were observed for both rhizosphere and bulk soil of NHE S. alfredii, as compared to the control soil solution, probably indicating the ability of the soil to buffer any plant induced changes in pH. However, the rhizosphere soil solution pH was reduced by 0.49 and 0.40 units for HPS and SPS, respectively, after the growth of HE S. alfredii, compared to the control soil solution. In general, pH changes in the rhizosphere are dominated by the inorganic cation–anion balance in the plant and the associated excretion of H\(^{+}\) or OH\(^{-}\) from the roots. In this study, the reduction in solution pH was probably due to increased release of root exudates, which was consistent with the enhanced DOC derived from root exudation in the rhizosphere of HE S. alfredii (Table 2). The decreased pH suggests that HE S. alfredii does exhibit a solution acidification mechanism in the rhizosphere and thus solubilise metals (Li et al., 2011a). The acidification of rhizosphere soil has also been found in other metal-(hyper)accumulating plants. Loosemore et al. (2004) found that a significant acidification occurred in the rhizosphere of Zn accumulator Nicotiana tabacum cv. The pH of soil solution of Cd hyperaccumulator Solanum nigrum was significantly lower than non-hyperaccumulator Solanum hycopersicum, because large amount of low molecular weight organic acids were excreted in soil by S. nigrum. However, Bernal et al. (1994) and McGrath et al. (1997) found that the Ni hyperaccumulator Alysum murale and the Zn hyperaccumulator Thlaspi caerulescens did not cause a significant decrease in the pH of the rhizosphere. Luo et al. (2000) found that T. caerulescens rhizosphere soil had even higher pH than non-rhizosphere soils. Kim et al. (2010) found that after cropping with Indian mustard, soil solution pH increased by 1.3 units. These conflicting results indicated that acidification of rhizosphere is not the only approach for mobilizing metals in the soil by hyperaccumulators.

The nature of DOM in soil pore water is poorly established, especially in the rhizosphere of hyperaccumulator. Growth of both ecotypes of S. alfredii significantly increased the DOC concentration in the rhizosphere soil solution for both heavily and slightly polluted soil (Table 2), as compared to control soil solution, indicating that the plant roots contributed to the increase of DOC by exudation. However, the magnitude of increase in DOC for HE S. alfredii (by 83.8% and 52.6% for HPS and SPS, respectively) was much greater than for NHE S. alfredii (by 34.2% and 25.7% for HPS and SPS, respectively) (Table 2). This result is consistent with previous
findings (Wenzel et al., 2003; Kim et al., 2010) in which different plant species (T. goesingense, Indian mustard) were used and similar changes in DOC were reported. Root exudates as well as rhizosphere microorganisms, may have significantly contributed to the DOM. Wenzel et al. (2003) found exudation of organic ligands may contribute to increased DOC in the of rhizosphere Thlaspi goesingense. Tu et al. (2004) also demonstrated that when exposed to higher arsenic in the solution, P. vittata released more root exudates.

The concentration of Cd, Mg, Ca, Na and K in soil solution was determined for both ecotypes of S. alfredii (Table 2). For both heavily and slightly polluted soil, no change in total Cd concentration in both rhizosphere and bulk soil solution was observed after the growth of NHE S. alfredii, as compared to control soil solution. However, growth of HE S. alfredii significantly decreased the total Cd concentration in the rhizosphere soil solution as compared to control soil solution. This consistent with previous observations that the labile Cd fraction (1 M NH4NO3-extractable) was also depleted (Li et al., 2011b) due to excessive Cd uptake. Water soluble Mg, Ca, Na and K tended to be generally larger in rhizosphere soil solution, as compared to bulk soil solution, but the difference was not significant for both ecotypes. It is proposed that hyperaccumulators may enhance metal solubility in the rhizosphere via root exudation, consequently increasing plant metal uptake (Fitzlators may enhance metal solubility in the rhizosphere via root

enhanced Ni concentrations in soil solutions from depleted rhizospheres of T. goesingense were associated with increased levels of DOC. Kim et al. (2010) found that increased Cu and Pb solubility in the rhizosphere of Indian mustard was mainly attributed to increases in rhizosphere DOC. However, other studies found that DOC does not contribute to Zn/Cd hyperaccumulation in T. caerulescens (Zhao et al., 2001). The present study shows a significant decrease of water soluble Cd after the growth of HE S. alfredii (Table 2), however, the decreases in this fraction in both rhizosphere and bulk soils for both soils accounted for less than 1% of the total uptake in the shoots of HE S. alfredii (3.2 mg pot⁻¹ and 2.8 mg pot⁻¹ for HPS and SPS, respectively). Therefore, more than 99% of the total Cd uptake must have come from the non-mobile fractions. This apparent contradiction can only be resolved if an additional mechanisms of Cd mobilization are assumed. Our results indicate that enhanced DOC derived from root exudation in the rhizosphere of HE S. alfredii appears to control this mobilization process.

3.2. Solution Cd speciation

The bioavailability of trace metals to the plant can be better understood in terms of their chemical speciation. Table 3 shows the speciation of Cd in soil solution calculated by Visual MINTEQ after the growth of S. alfredii. The data indicated that Cd–DOM complexes were the dominant (63.77–89.14%) Cd species in all soil
solutions, followed by the free metal Cd\(^{2+}\) species (9.90–29.56%), with low amounts of Cd-inorganic complexes (0.95–6.66%). These findings are consistent with previous studies that showed the presence of dominant amounts of Cd-organic complex in most of the soil solutions (Sauve et al., 2000; Krishnamurti et al., 2004). However, other studies have reported speciation data showing that Cd is dominated by free metals ions (Lorenz et al., 1997; Corru et al., 2009).

In control soil solution, the fraction of metal-DOM complex calculated by Visual MINTEQ using DOM parameters were 78.41% and 63.77% of total dissolved Cd for HPS and SPS, respectively. The Cd-DOM complexes were lower than the data commonly reported in the literature (Krishnamurti and Naidu, 2003; Nolan et al., 2003). After the growth of S. alfredii, the increased DOC concentration induced by root exudates resulted in a higher fraction of metal-DOM complex than in control soil solutions for both soils, however, the magnitude of increase for HE S. alfredii was much greater than for NHE S. alfredii. For instance, after the growth of HE S. alfredii, the increases in DOC concentrations resulted in 89.14% of Cd being complexed with DOM and only 9.90% Cd remaining free in the rhizosphere soil solutions of heavily polluted soil (Table 3). However, the fraction of metal-DOM complex as a percentage of soluble Cd in the rhizosphere of NHE S. alfredii was 82.85%. Generally, soil solution pH and the concentration of DOC are considered the two main factors governing metal speciation in soil solution. Numerous researchers have reported that metal speciation was governed by DOC rather than soil pH when the soil pH exceeded a certain level (pH > 6.5) (Christensen and Christensen, 2000). In this study, the rhizosphere soil solution pH of both soils was reduced after plant growth of HE S. alfredii (Table 2). The observed decrease in pH may suggest that the change in Cd speciation in soil solution is mainly governed by the enhanced DOC in the rhizosphere HE S. alfredii.

### 3.3. Cd complexation with DOM in the rhizosphere of S. alfredii

The results of Visual MINTEQ calculation suggest that DOM in the rhizosphere of HE S. alfredii may form stronger complexes with Cd than DOM derived from the rhizosphere of NHE S. alfredii, thus the complexation of Cd with DOM derived from the rhizosphere of S. alfredii as function of concentration were conducted. The formation of Cd complex with DOM from the rhizosphere of S. alfredii is shown in Fig. 1, expressed as the degree of complexation. The results of the resin equilibration experiment confirmed that the DOM derived from the rhizosphere of S. alfredii has a significant ability to form complexes with Cd. For both soils, the degree of complexation increased with DOM concentration, and DOM derived from the rhizosphere of HE S. alfredii (HE-R-DOM) showed greater complexation ability than DOM derived from the rhizosphere of NHE S. alfredii (NHE-R-DOM). Over the last several years, the complexation of trace metals with dissolved organic matter has been of particular interest and much work has been devoted to investigate the effect of DOM on the solubility of metals like Cu, Pb, Zn, Ni (Christensen and Christensen, 1999, 2000; Weng et al., 2002; Kim et al., 2010; Bolan et al., 2011). Previous studies have shown that DOM-complexed species are generally more significant for Cu and Pb than for Cd, Zn, and Ni (Weng et al., 2002; Kim et al., 2010) found that after the growth of Indian mustard, less than 60% Cd in soil solution was complexed with DOM, whereas more than 91% of Cu and Pb were predominantly complexed with DOM. In this study, more than 60% of the total Cd content in solution was bound in DOM complexes at the maximal DOC concentration, this result confirmed the visual MINTEQ results (Table 3) and consistent with previous studies (Sauve et al., 2000; Krishnamurti et al., 2004). However, great difference was observed between both ecotypes, DOM derived from the rhizosphere of hyperaccumulating ecotype of S. alfredii showed higher complexation ability than NHE-R-DOM, even at relatively low DOC concentrations. Therefore, the formation of soluble DOM–metal complexes could explain why DOM derived from the rhizosphere of the hyperaccumulating ecotype of S. alfredii can significantly reduce metal sorption on soils and thus increase metal mobility as compared to non-hyperaccumulating ecotype (Li et al., 2011b).

### 3.4. Cd extractability by DOM from the rhizosphere of S. alfredii

Different Cd minerals have different solubility in water (25 °C), and the addition of DOM from the rhizosphere of S. alfredii grow in both heavily and slightly polluted soil significantly (P < 0.05) increased the solubility of four Cd minerals as compared to water (Fig. 2). For instance, Cd concentrations extracted with 100 mg L\(^{-1}\)
The extracting ability of dissolved organic matter derived from the rhizosphere of hyperaccumulating ecotype (HE-R-DOM) and non-hyperaccumulating ecotype (NHE-R-DOM) of *S. alfredii* grown in polluted soil. Bars represent standard deviations of three replicates. Bars sharing the same letter code among different DOM for a given mineral are not significantly different at *P* < 0.05.

![Graph showing extraction ability of DOM](image)

**Fig. 2.** The extracting ability of dissolved organic matter derived from the rhizosphere of hyperaccumulating ecotype (HE-R-DOM) and non-hyperaccumulating ecotype (NHE-R-DOM) of *S. alfredii* grown in polluted soil. Bars represent standard deviations of three replicates. Bars sharing the same letter code among different DOM for a given mineral are not significantly different at *P* < 0.05.

DOC from the rhizosphere of hyperaccumulating ecotype of *S. alfredii* were 1.5–5.8 times higher than those extracted with water. Furthermore, the Cd extractability increased with the increase of DOM concentration (Fig. 2). Antoniadis and Alloway (2002) also reported that an increase in the concentration of DOM increases metal extractability from the soil. This might be due to the formation of soluble DOM–metal complexes, in this study, the degree of complexation also increased with an increase in DOM concentration (Fig. 1). On the other hand, DOM from the rhizosphere of HE *S. alfredii* showed much greater extracting ability for Cd than NHE-R-DOM (Fig. 1). On the other hand, DOM from the rhizosphere of HE *S. alfredii* showed much greater extracting ability for Cd than NHE-R-DOM. Von der Borch et al. (2000) observed that DOM or low molecular-weight organic acids, which comprise DOM, can be taken up by plant roots along with the metals that they have bound. However, the mechanism by which plants uptake heavy metals bound to DOM is not yet well understood, especially for hyperaccumulator.

### 4. Conclusions

The goal of this study was to assess the role of DOM in the Cd speciation in soil solutions and to investigate the effects of DOM from the rhizosphere of *S. alfredii* on Cd solubility. The following conclusions are drawn on the basis of the experimental results:

1. Significant decrease in rhizosphere soil solution pH was observed for both heavily and slightly polluted soil following HE *S. alfredii* cultivation due to enhanced DOC by exudation, this result suggests that HE *S. alfredii* exhibit a solution acidification mechanism in the rhizosphere.

2. Visual MINTEQ speciation predicted that Cd–DOM complexes were the dominant Cd species in the rhizosphere soil solutions after the growth of *S. alfredii*, followed by the free Cd$^{2+}$ species. However, Cd–DOM complexes fraction in the rhizosphere soil solution of HE *S. alfredii* were much greater than NHE *S. alfredii*.

3. Due to the greater ability to form complexes with Cd, HE-R-DOM derived from both soils showed much greater extracting ability for Cd than NHE-R-DOM at the same DOC concentration.

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