Original Research

Influence of Dissolved Organic Matter on Cd Speciation in Rhizosphere Soil Solution and Phytoextraction by *Sedum alfredii* and *Sedum sarmentosum*

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Abstract

Cd contamination in soil is a global environmental issue and the remediation of contaminated soil is necessary. Phytoextraction, as a promising friendly technique, has a main drawback: low mobility and bioavailability of Cd. In this study, the influences of dissolved organic matter derived from chicken manure (DOMc), humus soil (DOMs), and rice hull (DOMr) on Cd speciation in soil solution, and the uptake of Cd by a hyperaccumulating ecotype (HE) and a non-hyperaccumulating ecotype (NHE) of Crassulaceae were investigated. After the addition of exogenous DOM and subsequent plant growth, the dissolved organic carbon (DOC) concentration increase resulted in a higher proportion of Cd-DOM complexation. Moreover, DOM derived from the chicken manure had the highest degree of Cd complex formation in rhizosphere soil, followed by DOMs and DOMr. Furthermore, because of the increasing fraction of Cd-DOM, the solvation of Cd in the soil matrix was remarkably favored. However, growth of HE decreased the dissolved Cd in rhizosphere soil solution compared to bulk soil, and may be attributed to the phytoaccumulation of Cd by HE plants. In addition, the amount of Cd phytoextracted by plants significantly increased with DOM concentration. The Cd concentrations in the HE shoots were in the range of 754.3~1234.2 mg·kg^{-1} (DOMc), 714.9~1015.8 mg·kg^{-1} (DOMs), and 656.7~900.4 mg·kg^{-1} (DOMr), and were 15.4~17.0, 17.4~23.9, and 16.9~22.6 times higher than the NHE samples, respectively.

Keywords: dissolved organic matter, Cd, speciation, rhizosphere, phytoaccumulation

Introduction

Heavy metal contamination of soil is a salient global environmental issue. The remediation of contaminated soil is necessary not only to preserve soil resources, but also to safeguard human health [1]. Conventional remediation technologies such as soil washing or flushing, excavation, and dumping are generally expensive and can impair critical soil properties [2]. Phytoextraction is the use of green plants to extract pollutants from the contaminated soil to accumulate them in harvestable aboveground biomass.

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Table 1. Physical and chemical characteristics of the soil samples.

Soil pH	Total N	Total P	MFWC*	Organic C	CEC	Total soil Cd	Particle Size Distribution** (%)		
-	g·kg^{-1}	g·kg^{-1}	%	g·kg^{-1}	cmol·kg^{-1}	mg·kg^{-1}	Sand	Silt	Clay
6.82	2.31	0.64	21.7	30.70	17.08	0.11	26.4	44.5	29.1

*maximum field water capacity;

**Sand (2-0.02 mm), Silt (0.02-0.002 mm), and Clay (≤0.002 mm)

Phytoremediation has been advocated as a cost-effective and environmentally friendly alternative to conventional engineering technologies [3]. However, Krishnamurti and Naidu [4] reported that heavy metal speciation and solubility in soil solutions are two important factors that control the efficacy of phytoextraction and the metal-accumulating capacity of plants. A previous study demonstrated that different metal species in soil solutions, such as free hydrated metals and metal-ligand complexes, can be taken up by plant roots to varying extents [5].

Factors such as elemental concentrations, soil pH, redox potential, soil organic matter (SOM), and dissolved organic matter (DOM) can significantly influence trace element speciation and bioavailability in the rhizosphere [6, 7]. DOM is an important factor that affects the mobility of metals in a soil matrix [8]. Extensive research has already been conducted to characterize DOM from soil, natural water, forest litter, and sewage sludge. Most of these studies investigated the adsorption characteristics of DOM on soil constituents and its effects on metal adsorption and solubility in the soil matrix [9]. However, there is considerable controversy regarding the influence of DOM on metal solubility and uptake by plants. Some researchers have reported that DOM can form soluble metal-DOM complexes, and is preferentially adsorbed onto the soil surface in place of metals, which reduces the metal adsorption onto the soil, promoting metal phytoavailability when the soil pH is above a certain value [10, 11]. However, other researchers have reported that DOM contains mono- or multidentate functional groups that exhibit the capability to form strong heavy metal complexes. Depending on the nature of the soil solution, complex formation by DOM can decrease the soluble heavy metals through adsorption of these complexes to the soil surface [12].

Little has been done to investigate the role of DOM on Cd solubility and speciation in the rhizosphere. *Sedum alfredii* Hance (Crassulaceae) is a recently identified Zn/Cd co-hyperaccumulator native to the Pb/Zn rich regions of China [13]. 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. Many studies have been carried out to investigate the physiology and molecular mechanisms of heavy metal hyperaccumulation in *S. alfredii* [14, 15], but there is a lack of information concerning metal solubilization in the rhizosphere of this plant. The rhizosphere has distinct root-modified chemical, physical, and biological parameters from bulk soil. Therefore,

the dynamics, transformations, bioavailability, and toxicity of Cd are expected to differ markedly from those in bulk soil. In this study, we chose three exogenous natural DOM in lieu of high-cost, synthetic chelating agents, to investigate their influence on Cd solubility and speciation in contrasting ecotypes of Crassulaceae. The two ecotypes, in the same plant family, were categorized as Cd hyperaccumulating ecotype (*Sedum alfredii* Hance) and non-hyperaccumulating ecotype (*Sedum sarmentosum* Bunge). They grew in the same environment and under the same treatment. We believe that both species are great enough to use them in a comparative study and would be better known by the different mechanism in uptake and accumulating Cd between them.

The aims of this study were:
1) to investigate the effects of DOM on plant growth and Cd accumulation in both ecotypes,
2) to assess the concentration and interactions of DOM on Cd solubility and speciation in rhizosphere soil solutions, in comparison to bulk soil solutions.

Materials and Methods

Soil Sampling

For this study, soil samples were collected from a private woodland in Guchengzi Village, Dongling District of Shenyang, Liaoning Province, PR China (GPS coordinates: E123.589585, N41.747574). Select properties of the soil are given in Table 1. Soil pH was measured in a 1:2.5 soil:water (w/v) suspension using a pH meter (Sartorius PB-10) following 1 h equilibration period. Mechanical analysis of the soil was carried out by gravity sedimentation after dispersion of the soil with 5% sodium hexametaphosphate. The organic C content was determined by the Walkley-Black wet oxidation method. The Cd concentration was extracted by digesting with HNO_3-HF-$HClO_4$ and then measured by flame atomic absorption spectrometry.

Incubation of Soil with Addition of Cd

Soil samples were air-dried, mildly ground, passed through a 2 mm stainless steel sieve, and then homogenized. A Cd solution (as $3CdSO_4 \cdot 8H_2O$) was added in an open plastic container to achieve a soil Cd concentration of 20 mg Cd·kg^{-1} soil. Deionized distilled water was then added to bring the soil to saturation. The soil was allowed to dry under ambient conditions for 10 days before again being re-wetted to saturation with deionized distilled water.

Table 2. Properties of three dissolved organic matters.

DOM type	pH	Organic carbon	Electric conductivity	FA*	HA*	Cd
	—	mg·L^{-1}	ms·cm^{-1}	%	%	mg·L^{-1}
DOMc	7.52	1722	7.81	44.4	55.6	nd
DOMr	6.68	831	2.78	93.2	6.8	nd
DOMs	6.95	639	3.45	35.5	64.5	nd

*Proportion of fulvic acid (FA) or humic acid (HA) of active DOM, nd – not detected

The soil was subjected to seven such wetting and drying cycles; at the end of the last cycle, the soil was thoroughly mixed, homogenized, and stored for subsequent use.

DOM Extraction and Characterization

Exogenous DOM extracted from humus soil (obtained from a private woodland in Guchengzi Village, Dongling District of Shenyang), chicken manure (obtained from Xinmin of Shenyang), and rice hull (homegrown production) were used in this study. To designate the origins of the samples, the DOM samples are labeled as DOMs, DOMc, and DOMr. Before extraction, the chicken manure and rice hull were air-dried and ground to powder. All samples were extracted with deionized-distilled water with a ratio of 1:20 (w/v) and maintained at a constant temperature for fermentation for one month. Samples were then shaken at 200 r·min^{-1} at 25±1ºC for 24 h on a reciprocal shaker. After centrifugation at 10,000×g for 20 min, the supernatant was collected and filtered through a 0.45μm membrane filter. The filtrates were stored at 4ºC until use and properties of three dissolved organic matters are shown in Table 2.

Characterization of the three exogenous DOM was achieved by using attenuated total reflectance-Fourier transform infrared (ATR-FTIR). FTIR spectra were carried out in the 4000-400 cm^{-1} wavelength range using a Varian 3100 FT-IR spectrophotometer on KBr pellets. The pellets were generated by pressing uniformly prepared mixtures of 1.0 mg of freeze-drying sample and 200 mg of spectroscopic-grade KBr under reduced pressure; precautions were taken so as to avoid moisture uptake [16].

Experimental Design

Subsets of the 2.0 kg Cd-contaminated soils (as described above) were amended with four levels each of DOM at 25, 50, 100, and 200 mgC·kg^{-1} soil, respectively. Two plants were used in the experiment. The hyperaccumulating ecotype (HE) of *S. alfredii* was collected from the Institute of Soil Science, Chinese Academy of Sciences, Nanjing, while the non-hyperaccumulating ecotype (NHE) of *S. sarmentosum* was obtained from a garden in Anshan, Liaoning Province. A rhizobox was designed as described by Weng et al. [7]. The rhizobox was divided into three sections, a rhizosphere zone (20 mm in width), which was surrounded by nylon mesh (300 mesh), and left and right non-rhizosphere zones (60 mm in width). Root growth was lim-

ited to the central compartment. About 0.5 kg of soil was placed in the rhizosphere zone and 1.5 kg was placed in the non-rhizosphere zone. One week before the study, the soil equilibrated at field capacity. Then three *S. alfredii* and *S. sarmentosum* plants were transplanted in the rhizosphere zone and each treatment was replicated in quadruplicate. Control pots without plants were included. The plants were watered throughout the study to keep the soil at approximately 65% of its field capacity and allowed to grow for 90d in a greenhouse with controlled light and temperture conditions (26/20ºC day/night temperature, 60% relative humidity, 16 h light per day). At the end of the experiment, plants were harvested and each plant was separated into root and shoot. Both the shoots and roots were thoroughly washed with deionized water several times to avoid soil contamination. They were oven dried for 48h at 65ºC, weighed, and ground for chemical analysis.

Soil Solution Analysis

Prior to harvest, each pot was saturated with Milli-Q water and allowed to equilibrate for 18 h for further extraction and the soil solution was collected following centrifugation. The moist soil was packed into 25-mL filtration tubes, 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 12,000 g for 30 min and filtered through a 0.45μm membrane filter [17]. Dissolved organic carbon (DOC) in soil solution was determined immediately after the isolation of soil solution using a total organic carbon (TOC) analyzer (analytik jena multi N/C 3100). The pH was measured before filtration. Solutions were then passed through 0.22 μm cellulosic membranes, while the major cations (K$^+$, Na$^+$, Ca^{2+}, Mg^{2+}) and anions (F$^-$, Cl$^-$, NO$_3^-$, NO$_2^-$, SO$_4^{2-}$) were analyzed by ion chromatography (ICS 5000, DIONEX). The total dissolved Cd was quantified by graphite furnace atomic absoption spectrometry (GFAAS) with a Varian SpectrAA 220 instrument.

Speciation of Cd in Soil Solution

The speciation of Cd in soil solution was assessed using Visual MINTEQ version 3.0. The modeling was performed using soil solution pH, DOC, anions (F$^-$, Cl$^-$, NO$_3^-$, NO$_2^-$, SO$_4^{2-}$), and cations (K$^+$, Na$^+$, Ca^{2+}, Mg^{2+}) as input data. The NICA-Donnan model was selected to evaluate the

Table 3. General assignments of the FTIR spectra of DOM.

Wavenumber (cm⁻¹)	Assignment
3500-3300	Stretching vibration of -COOH or O-H in phenols or alcohols or H-bonded N-H
3000-2800	Aliphatic C-H, C-H$_2$, C-H$_3$ stretching vibration
1675-1635	C=C stretching vibration of alkenes
1630-1590	C=C stretching of aromatic rings and C=O stretching of conjugated carbonyl groups in ketones and amides
1465-1440	Aliphatic C-H deformation
1450-1400	O-H deformation vibration of carboxylic groups, C-H asymmetric vibrations of methyl and methylene groups of aliphatic chains
1350-1280	Stretching vibrations of C-O-C in ethers and esters and stretching vibrations of C-O in phenols
1240-1150	C-O asymmetric stretching vibration and O-H deformation vibration of -COOH
1080-1000	C-O asymmetric stretching vibration of alcohols or phenols

metal binding to DOM [18]. The active DOM was assumed to contain 50% carbon and was simulated with a mixture of fulvic acid (FA) and humic acid (HA). The proportion of FA or HA in active DOM is listed in Table 2.

Statistical Analysis

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

Results and Discussion

Characterization of ATR-FTIR Spectroscopy

Infrared spectroscopy provides valuable information about the structural and functional properties of DOM [19, 20]. The functional groups corresponding to the respective wavenumbers are shown in Table 3. As shown in Fig. 1, all three DOM spectra exhibited a prominent band at approximately 3450 cm⁻¹, which can be attributed to O-H stretching vibrations of hydroxyl groups involved in hydrogen links, carboxyl groups, and phenol groups [21], and the band of DOMc exhibited strongest intensity. There was also a band in the 2923 cm⁻¹ region for DOMc, which is attributed to C-H stretching of methyl and methylene groups of aliphatic chains. Some differences were observed between samples in intensity of the bands in 1646 cm⁻¹, indicating alkene (C=C) stretching vibration in aromatic structure, COO-, H-bonded C=O [20]. However, it has a considerably lower relative intensity in the spectrum of DOMr. Furthermore, only DOMc exhibit a stonger band at approximately 1324 cm⁻¹, which is usually assigned to vibrations of C-O-C in ethers and esters and stretching vibrations of C-O in phenols [21]. There was a band at 1080 cm⁻¹ for DOMr and DOMs, which can be attributed to C-O asymmetric stretching vibrations of alcohols or phenols. Nevertheless, DOMc failed to display this spectral. DOMs displayed

lower relative intensities approximately 1420 cm⁻¹, which is probably due to O-H deformation vibrations of carboxylic groups and, to a lesser extent, C-H asymmetric vibrations of methyl and methylene groups of aliphatic chains [21]. Compared to DOMs and DOMr, DOMc exhibited a stronger -COOH and O-H peak at 3445 cm⁻¹, C=C and C=O peak at 1646 cm⁻¹, and C-O peak at 1324 cm⁻¹. Prior studies have indicated that these bands can act as potential chelating centers of metal ions, thus enhancing the solubility of heavy metals [19].

Soil Solution Composition and Properties

Metal transport and mobility in a soil system typically occur through the soil solution phase, and considered relating to the soil solution composition and properties. Select soil solution properties of the soils planted with *S. alfredii* and *S. sarmentosum* are listed in Tables 4 and 5. Earlier experiments indicated that the addition of DOM to soils did not significantly affect major ion concentrations and soil solution pH, so only soil properties of relevance are shown in Table 4. At the end of the experiment, no significant differences in pH were observed between rhizosphere and bulk soil solution of NHE *S. sarmentosum*. However, the rhizosphere soil solution pH of HE *S. alfredii* was reduced by 0.3 units in comparison to the bulk soil solution. This finding is in congruency with previous studies [11, 22], which suggested that rhizosphere acidification is involved with metal mobilization in rhizosphere soil. Li et al. [22] demonstrated that the reduction in soil solution pH was probably due to increased release of root exudates, which is consistent with the enhanced DOC concentration in the rhizosphere soil solution of both ecotypes plants (Table 5). The final DOC concentrations in the rhizosphere of both ecotype plants were greater than those in the bulk soil. Li et al. [11] proposed that root exudates as well as rhizosphere microorganisms may have significantly contributed to DOC concentrations. In comparison to the other two DOM applications, DOMc soils had the highest DOC concentra-

Table 4. Concentrations of major ions in soil solutions.

Soil solution		pH	Na	K	Mg	Ca	Cl⁻	NO₂⁻	SO₄²⁻	NO₃⁻	F⁻
		-	mg·L⁻¹								
S. alfredii	Rz*	6.61	25.71	234.16	57.75	216.3	32.73	1.15	59.89	542.0	2.76
	Bk**	6.92	25.23	230.35	46.18	203.2	31.87	1.21	54.07	547.3	2.64
S. sarmentosum	Rz	6.82	23.45	220.28	59.27	208.8	30.55	1.08	56.57	557.1	2.03
	Bk	6.89	20.36	227.67	51.58	205.1	33.63	1.13	55.26	546.9	2.15

*rhizosphere soil solution, **bulk soil solution

tion, followed by DOMs and DOMr. Fitz and Wenzel [23] proposed that hyperaccumulators may enhance metal solubility in soil solutions via root exudation, consequently increasing the plant bioavailbility of the metals. As the HE plants exuded about 4.97~74.3% more DOC in the rhizosphere than NHE, our experiment appears to corroborate this hypothesis. Li et al. [24] demonstrated that DOC significantly affects metal mobility, microbial activity, and soil properties. Wenzel et al. [25] found a highly significant correlation between Ni and DOC in soil solutions (R^2=0.85), providing evidence for the propensity of formation of Ni-DOM complexes in the rhizosphere of *T. goesingense*.
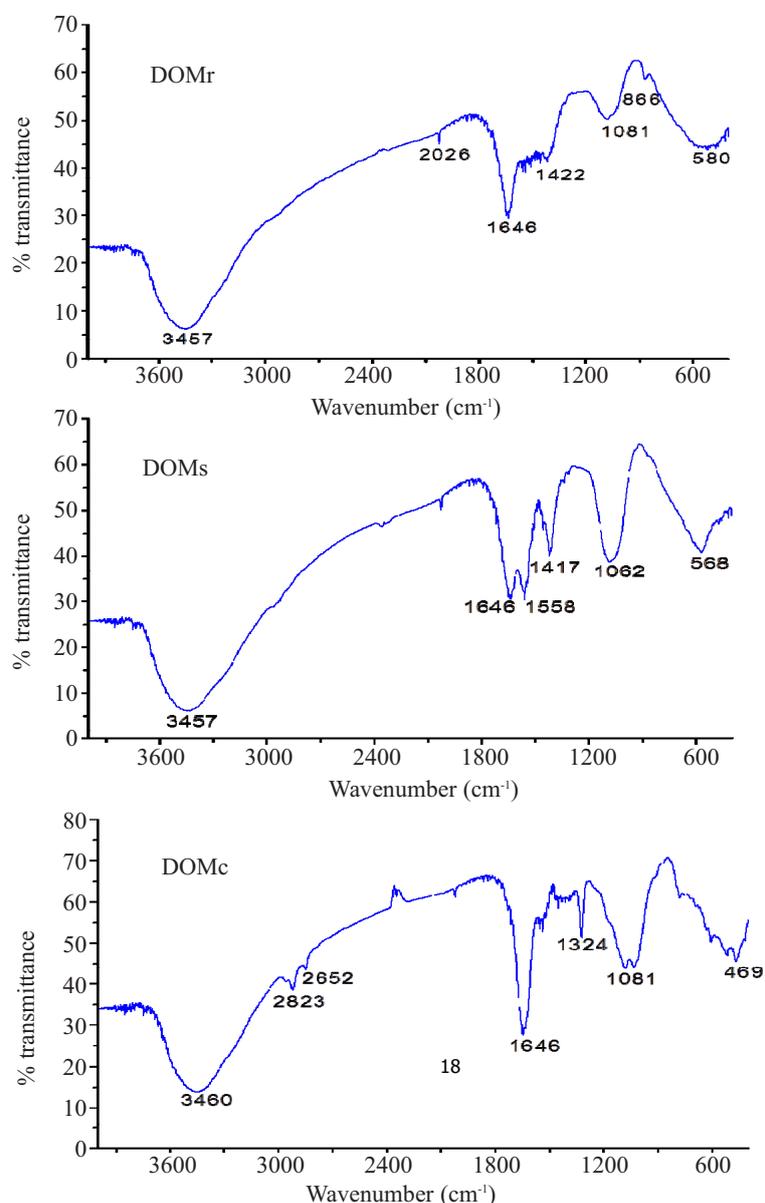
Fig. 1. ATR-FRIR spectra of the DOM derived from chicken manure (DOMc), humic soil (DOMs), and rice hull (DOMr).

Table 5. Concentrations of DOM and total dissolved Cd in soil solution.

Soil solution		T25		T50		T100		T200	
		Cd	DOC	Cd	DOC	Cd	DOC	Cd	DOC
		mg·L^{-1}							
S. alfredii									
DOMr	Rz	0.113	67.1	0.199	88.2	0.287	137.5	0.536	234.2
	Bk	0.121	41.4	0.204	64.5	0.356	118.4	0.633	216.4
DOMs	Rz	0.218	70.6	0.243	90.6	0.354	145.1	0.789	251.9
	Bk	0.229	45.3	0.274	72.5	0.398	121.2	0.846	223.1
DOMc	Rz	0.423	85.2	0.591	109.4	0.632	182.7	0.813	294.2
	Bk	0.456	64.5	0.618	75.1	0.649	148.9	0.867	254.4
S. sarmentosum									
DOMr	Rz	0.123	50.6	0.216	69.2	0.296	115.6	0.545	223.1
	Bk	0.141	38.2	0.249	57.3	0.332	103.2	0.581	213.5
DOMs	Rz	0.234	61.3	0.258	72.8	0.377	125.4	0.792	235.3
	Bk	0.255	40.5	0.294	55.7	0.409	107.5	0.834	223.9
DOMc	Rz	0.335	72.4	0.431	89.8	0.569	154.3	0.868	261.7
	Bk	0.422	57.8	0.474	68.9	0.645	128.6	0.917	231.6

The concentrations of Mg, Ca, Na, and other major ions in the soil solutions were determined for both plants growing in control soils (Table 4). However, the growth of HE and NHE plants did not trigger significant changes of these ion concentrations in both rhizosphere and bulk soil solutions. Results also showed that the total dissolved Cd in the soil solution increased with DOC concentration. Moreover, with growth of HE came decreased dissolved Cd in the rhizosphere soil solution in comparison to the bulk soil. This is consistent with previous research demonstrating that the decrease of Cd concentration in the rhizosphere may be attributed to the excessive metal uptake by HE plants [25]. Organic complexes have been recognized as having a strong impact on the migration of metals (Pb, Cu, Zn, and Cd) in soils [3]. For explanation of the effects of different DOM on complex formations, qualitative aspects of DOM must be considered. The variation in the composition of DOM is a putative etiological agent for the discrepancies in Cd complexation and mobilization. Compared to DOMs and DOMr, the addition of DOMc to soils resulted in the highest amount of dissolved Cd. Inaba and Takenaka [26] reported that the abundance of carboxylic acid contained in DOM demonstrated the highest capacity to mobilize heavy metals by forming metal complexes. This claim is supported by the results and analysis above.

Effects of DOM on Cd Speciation in Soil Solution

The bioavailability of Cd to the plants can be better illuminated in terms of its speciation. Cd speciation of soil solution using Visual MINTEQ 3.0 indicated that Cd-DOM complexes and free Cd^{2+} were the two dominant Cd species in soil solutions. The results of Cd-DOM complexes in soil solution are shown in Table 6. After subtracting the Cd-DOM complexes from the total Cd in soil solution, Cd left in solution is approximately regarded as the free Cd^{2+} fraction. After the addition of exogenous DOM and subsequent plant growth, the increasing DOC concentrations resulted in a higher fraction of Cd-DOM complexes than in control soil solution, while the proportion of Cd^{2+} decreased. The results confirmed that the DOMc exhibited the greatest capability to form complexes with Cd, followed by DOMs and DOMr. For the four concentrations of DOMc, about 54.4%, 58.7%, 73.3%, and 84.0% of Cd were complexed with DOM in rhizosphere soil solutions of HE and 47.6%, 51.7%, 69.9%, and 80.6% of Cd-DOM in bulk solutions. For NHE, however, only 51.8%, 56.1%, 68.7%, and 81.7% of the Cd formed complexes with DOM in rhizosphere soil solutions, and 43.8%, 50.6%, 66.6%, and 80.0% in bulk solutions. For the three DOM sources, the degree of complexation increased with DOM concentration. The source of the DOM determined its influence of Cd interactions and may be a result of parameters, such as differences in ionic strength, cation composition, and complexing ability, as well as the present functional groups of DOM [19]. Moreover, the fractions of Cd-DOM in the rhizosphere of HE was higher than in bulk soil solution, while the fraction of free Cd^{2+} in the rhizosphere soil solution was lower. For NHE, however, no significant differences were observed between bulk and rhizosphere soil solution.

Table 6. Calculated Cd-DOM concentration in soil solution after the addition of DOM using Visual MINTEQ model ($\mu g \cdot L^{-1}$). Values in parentheses are percentage (%) of Cd-DOM in soil solution.

DOM	T25		T50		T100		T200	
	Rz	Bk	Rz	Bk	Rz	Bk	Rz	Bk
S. alfredii								
DOMr	62.5 (55.3)	56.1 (46.3)	122.1 (61.4)	115.7 (56.7)	207.6 (72.3)	243.5 (68.4)	437.9 (81.7)	467.1 (73.8)
DOMs	130.0 (59.6)	105.5 (46.1)	153.6 (63.2)	150.5 (54.9)	256.4 (72.4)	270.4 (67.9)	636.9 (80.7)	607.5 (71.8)
DOMc	230.1 (54.4)	217.1 (47.6)	346.9 (58.7)	307.1 (51.7)	463.3 (73.3)	453.7 (69.9)	682.9 (84.0)	698.8 (80.6)
S. sarmentosum								
DOMr	63.9 (52.0)	67.1 (47.6)	120.1 (55.6)	129.0 (51.8)	203.1 (68.6)	216.0 (65.0)	432.2 (79.3)	453.3 (78.0)
DOMs	118.5 (50.6)	114.5 (44.9)	137.9 (53.5)	136.9 (46.6)	262.3 (69.6)	268.5 (65.6)	637.3 (80.5)	648.7 (77.8)
DOMc	173.5 (51.8)	184.8 (43.8)	241.8 (56.1)	239.8 (50.6)	390.9 (68.7)	429.6 (66.6)	709.2 (81.7)	733.6 (80.0)

The different amounts of Cd speciation in soil solutions and divergence between two ecotypes may be a result of root exudates. Li et al. [24] reported that LMWOA concentrations in root exudates of HE were higher than those of the root exudates of NHE. It was also demonstrated that the root exudates, especially LMWOA, have the ability to form complexes with heavy metals, and are likely to increase the solubility and phytoavailability of metals in soil [27].

Generally speaking, soil solution pH and DOC concentration are the two main factors governing metal speciation in soil solutions. Cornu et al. reported that Cd was dominated by free Cd^{2+} in soil solution. While other studies showed that the dominant Cd speciation in most soil solution was Cd-organic complex [4, 6]. Schmidt [28] demonstrated that the increase in DOC induced Cu mobility, which was more pronounced at high than low pH. Weng et al. [7] showed that
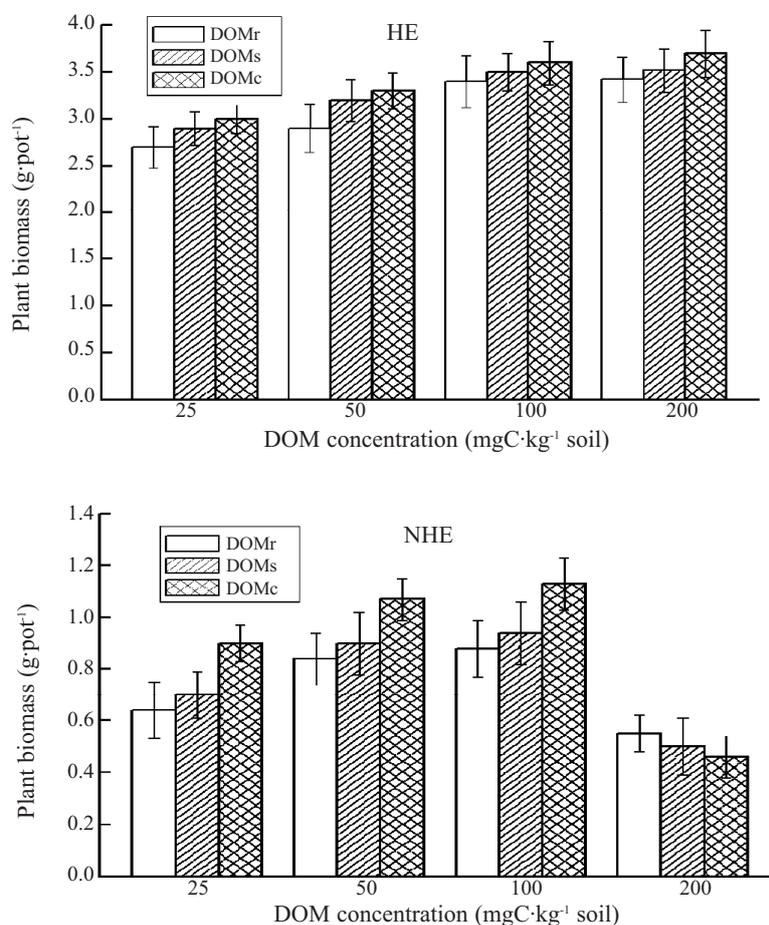
Fig. 2. Effect of concentration of DOM on plant biomass. Error bars are standard deviations of duplicates.

Table 7. Cd concentrations of two plants (mg·kg⁻¹) after the addition of different concentrations of DOM.

DOM	T25		T50		T100		T200	
	shoot	root	shoot	root	shoot	root	shoot	root
S. alfredii								
DOMc	754.3±66.3*	135.2±12.4	864.5±53.5*	200.8±19.7*	1013.2±85.6*	189.5±15.1*	1234.2±106.7*	135.4±11.4
DOMs	714.9±71.2*	168.8±17.6*	827.6±47.8*	143.1±12.3	955.3±78.8*	156.4±14.5*	1015.8±92.9*	129.7±15.3
DOMr	656.7±54.4*	104.2±13.5	703.4±35.9*	115.8±10.6	797.1±68.6*	143.2±9.7*	900.4±89.8*	124.5±10.2
S. sarmentosum								
DOMc	45.8±2.45	85.3±7.43	50.9±5.62	94.6±6.17	65.7±10.9	104.2±11.4	74.3±5.15	115.8±8.43
DOMs	41.2±3.41	76.5±6.84	43.4±5.07	89.4±7.26	43.7±3.98	82.8±6.02	42.4±3.48	110.5±7.67
DOMr	38.7±3.13	72.3±7.25	42.3±4.89	81.3±9.77	37.9±4.52	83.9±7.57	39.8±4.36	104.5±9.15

Data are means±SD (n=4).

*indicates significant differences (p＜0.05) between HE and NHE that underwent the same treatment.

metal binding to DOM does not contribute significantly to the control of metal activity in soil, and can affect the solubility of metals to a considerable extent. Effects of DOM on complexation with metals are more significant at high pH, and any factors that lead to an increase in the DOM concentration enhance the solubility and mobility of metals. This study confirms that Cd complexation with DOM can increase the concentration of dissolved Cd by more than 5 times. However, Sauve et al. [6] found DOM had little impact on the Cd speciation in soil solution, and the addition of DOM to their equation $pCd^{2+} = 5.14 + 0.61pH - 0.79 \log$ (total Cd) improved R^2 by less than 2%.

Influence of DOM on Plant Growth and Uptake of Cd

During the 90 d growth period, there were no explicit symptoms of Cd toxicity observed for HE *S. alfredii*. Growth of *S. alfredii* was promoted by the addition of all three exogenous DOM. With the increase of DOM concentration, there were diminishing returns for plant biomass production, especially for DOMc (Fig. 2). Under the four DOC concentration treatments (T25, T50, T100, T200), the HE *S. alfredii* biomass increased by 3.8%, 11.5%, 30.8%, and 31.5% for DOMc, 11.5%, 23.1%, 34.6%, and 35.3% for DOMs, and 15.3%, 26.9%, 38.5%, and 42.3% for DOMr, respectively. The NHE *S. sarmentosum* biomass increased for the three T25, T50, and T100 treatments. However, growth of *S. sarmentosum* was inhibited with the treatment of 200 mgC·kg⁻¹ soil for all three DOM sources and showed both chlorotic and necrotic symptoms. NHE *S. sarmentosum* biomass decreased by 8.3%, 16.7%, and 23.3% as compared to those grown in control soil for DOMc, DOMs, and DOMr, respectively. This may be attributed to the increased phytoavailbility of Cd. Cytosolic disorders, and because disturbances of metabolic processes are reported for concentrations of Cd in solution between 100 and 200 μg L [29].

As the concentrations of DOM increased in Cd-inoculated soil, Cd concentrations in both ecotype plants increased. However, the Cd concentrations in shoots of HE *S. alfredii* were significantly higher than those of NHE *S. sarmentosum* (P<0.05) (Table 7). Concentrations of Cd in the shoot of HE *S. alfredii* plants were in the range of 754.3~1234.2 mg·kg⁻¹, 714.9~1015.8 mg·kg⁻¹, and 656.7~900.4 mg·kg⁻¹ for DOMc, DOMs, and DOMr, which were 15.4~17.0, 17.4~23.9, and 16.9~22.6 times higher than NHE *S. sarmentosum*, respectively. The concentrations of Cd in the roots of two ecotype plants did not exhibit significant discrepancies, with the exception of T100 treatment. Results suggested that the NHE plant didn't exhibit a higher ability to transport and uptake Cd as HE, even if the total dissolved Cd was higher in soil solution for it. These are supported by previous findings [19, 30] and confirmed that, compared to NHE, HE exhibits promise as a phytoremediation agent for Cd-polluted soils.

Three factors affect metal concentrations in plants and soil:
1) the total amount of bioavailable metals,
2) activity of metals in the soil solution,
3) the rate of metals transferred from the solid to the liquid phase and to the plant roots [1, 28].

The augmented Cd mobility in soil as a result of DOM interactions may be attributed to influence on factors 1 and 3. The high concentration of DOM added to the soil may act as ligands for Cd, enhancing Cd solubility in soil solution and its phytoavailability. This proposal was supported by the fact that the higher DOC content in soil resulted in a greater proportion of dissolved Cd in the soil matrix. Antoniadis and Alloway [31] reported that an increase in the concentration of DOM increased Cd extractability from the soil through elevated metal uptake by plants. Metal-binding by humic acids in sediment has been shown to affect the mobility of metals in the environment and could promote the transformation of heavy metals from less bioavailable fractions to potential bioavailable fractions [32]. One mechanism described by

Dessureault-Rompre et al. showed the free metal ions transported to the root are taken up [33]. Following this, metal-complexes dissociate to replenish the pool of free metal ions. Nevertheless, some authors [4] observed that low molecular-weight organic acids, which comprise DOM, can be taken up by plant roots along with the metals that they have bound. Hernandez-Soriano and Jimenez-Lopez [34] described how, although the metal aqueous complexes might be less available species, they may constitute a pool of readily available metal for plant uptake, and a significant increase on Pb and Zn uptake occurred concomitantly with an enhancement of the percentage of metal-organic complex in solution.

Conclusions

The goal of this study was to investigate the complexation of Cd by DOM derived from different materials in the rhizosphere and their resulting phytoavailability. The following conclusions are drawn on the basis of the experimental results:

1) DOM derived from chicken manure exhibits the greatest ability to complex with Cd compared to those derived from humic soil and rice hull; the dissolved Cd concentration in the soil solution increased with DOM concentration.

2) For all treatments, Cd-DOM complexes were the dominant species in rhizosphere soil solution. The proportion of DOM-complexed species is generally more significant for HE than for NHE.

3) This study also demonstrated the positive effect of DOM on the Cd uptake by HE *Sedum alfredii*, and the amount of Cd uptake by plants increased with DOM concentration.

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