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EFFECT OF NITROGEN FERTILIZER ON GROWTH AND CADMIUM ACCUMULATION IN *SEDUM ALFREDII* HANCE

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□ *We characterized the effect of nitrogen (N) fertilizer on root morphology, photosynthetic pigments and cadmium (Cd) accumulation in a series of hydroponic experiments by using hyper-accumulating ecotype of* Sedum alfredii *H. (HE) and non-hyperaccumulating ecotype (NHE) plants. Cd accumulation in both ecotypes increased with increase of N levels until it reached the peak at 16 mM and then decreased suddenly. Among all the three N forms tested, ammonium sulfate [$(NH_4)_2SO_4$] was found to be an optimum choice. Based on these findings, a pot experiment was also conducted to evaluate the effect of three N fertilizers on phytoextraction from a slightly Cd-contaminated soil by the hyper-accumulating plant* Sedum alfredii *H.*

Keywords: cadmium, nitrogen, phytoextraction, *Sedum alfredii* H.

INTRODUCTION

With the development of industry and modernization of agriculture, soil contamination has increasingly become a public concern (Min et al., 2007), as it impacts human life and health through the food chain (Baker and Brooks, 1989). Phytoextraction of heavy metals from contaminated soil is an emerging technology that aims to remove metals from soils (Brooks et al., 1998; Salt et al., 1998). It has grabbed attention in recent years for the

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low cost of implementation and environmental benefits (Quartacci et al., 2006). However, for the slow growth speed, small biomass and low phytoremediation efficiency of most hyper-accumulators, the technique of phytoremediation faced some problems (Glass, 2000). As a result, many measures were taken to enhance the biomass and uptake ability of hyper-accumulators (Chen et al., 2006; Luo et al., 2006; Nouairi et al., 2006; Santos et al., 2006). Among them, some agricultural measures that work through amending soil environment becomes a big attraction to the public for its low potential environmental risk and high increasing effects.

There are many measures to amend soil environment and enhance soil fertility, involving chemical fertilizer, organic material and green manure (Gray et al., 2002). Nitrogenous fertilizers are a major accelerating factor among all of them, which could increase plant biomass significantly when applied properly. Li et al. (2000) reported that nitrogenous fertilizer had significant effects on Cd absorption, de-sorption, chemical transformation and transportation. Many studies have reported about different nitrogen (N) forms [ammonium (NH_4) and nitrate (NO_3)] on plant growth; however, because of different materials and treatment dosage, the results were not similar. At the same time, more and more studies indicated that combination of more than one nitrogenous fertilizer will accelerate plant growth significantly than others (Drinkwater et al., 2007). Some field experiment shows that with increase of N dosage, the concentration of available cadmium (Cd) in soil increased accordingly (Mitchell et al., 2000).

Sedum alfredii H. is a new zinc (Zn)/Cd-hyperaccumulator found in an old lead (Pb) / Zn mining area in China (Yang et al., 2002, 2004a). Zinc (Zn) concentration in its shoots can reach over 20 g kg^{-1} when grown under 80 mg Zn L^{-1} in nutrient solution without showing any toxic symptoms (Yang et al., 2004b). Amount of Cd accumulation in the shoots can reach 2.9 and 3.2 mg $plant^{-1}$ at external Cd levels of 0.2 and 0.4 mM, respectively. In addition, it has characteristics of fast growth, large biomass, asexual reproduction, and perennial habitat, which can grow up to 40 cm height, and propagate 3–4 times in a year if the environmental conditions are favorable (Long et al., 2002).

However, most of the studies before on *Sedum alfredii* H. mainly focused on the physiological mechanism and transportation (Yang et al., 2002, 2004a, 2004b, 2006a, 2006b; Xiong et al., 2005) while studies on phytoextraction through application of some agricultural measures were ignored. The present objective of this study are as follows: i) The effect of nitrogen dose and organic chemicals on plant growth and Cd accumulation in *S. alfredii* in hydroponics ii) The effect of N fertilizers on phytoextraction from slightly Cd-contaminated soils, and to provide references for Cd-hyperaccumulating plant in the application of agricultural measures.

MATERIALS AND METHODS

Plant Materials and Soil Characterizations

Two different ecotypes of *Sedum alfredii* H., i.e. the hyperaccumulating ecotype (HE) and non-hyperaccumulating ecotype (NHE), were collected from an old Pb/Zn mine area and a tea garden of Hangzhou, Zhejiang Province of China respectively. Healthy and equal-sized shoots of the two ecotypes of *Sedum alfredii* H. were chosen and grown for two weeks in the basic nutrient solution containing (in mM) calcium nitrate [$Ca(NO_3)_2 \cdot 4H_2O$] 2.00, monopotassium phosphate (KH_2PO_4) 0.10, magnesium sulfate ($MgSO_4 \cdot 7H_2O$) 0.50, potassium chloride (KCl) 0.10, potassium sulfate (K_2SO_4) 0.70; (in μM) boric acid (H_3BO_3) 10.00, manganese sulfate ($MnSO_4 \cdot H_2O$) 0.50, zinc sulfate ($ZnSO_4 \cdot 7H_2O$) 1.0, copper sulfate ($CuSO_4 \cdot 5H_2O$) 0.20, ammonium molybdate [$(NH_4)_6 Mo_7O_{24}$] 0.01, iron (Fe)-ethylenediaminetetraacetic acid (EDTA) 100 (Long et al., 2002).

Agricultural soil was taken from Huajiachi campus of Zhejiang University, Hangzhou, Zhejiang Province, China. The samples were sieved through a 2 mm sieve and air-dried for 3 d. The soils were contaminated artificially with Cd as cadmium chloride ($CdCl_2$) at the content of 1 mg kg^{-1} soil. After adding Cd, the soil were kept for acclimatizing for 15 d, undergoing five cycles of saturation with distilled water and air-drying. After acclimatizing the soil for 15 days, following parameters were determined: pH value (solid distilled water 1:2.5, w/v); total organic matter (450 and 600°C, after heating for 6 h in a muffle furnace); total nitrogen content; total phosphorus (P) and water-soluble P; water-soluble nitrogen (N); water-soluble potassium (K); total Pb, Zn, copper (Cu) and Cd contents [mixed acid digestion with concentrated nitric acid (HNO_3), hydrochloric acid (HCl), and hydrofluoric acid (HF) = 3:1:1, v/v]; and water-soluble metal contents (solid:distilled water 1:2.5, w/v) (Bao, 2000). The agrochemical properties of the tested soil were shown in Table 1.

Hydroponics Experiments

Experiment 1

Nitrogen concentration dependent experiment was conducted after preculturing for 15 d, the plants were transferred to a 2.5 L pots. Fourteen different treatments were used (in mM), i.e., (1) 0.01 Cd (CK1); (2) 4 N/0.01 Cd; (3) 8 N/0.01 Cd; (4) 16 N/0.01 Cd; (5) 32 N/0.01 Cd; (6) 64 N/0.01 Cd; (7) 0.1 Cd (CK2); (8) 4 N/0.1 Cd; (9) 8 N/0.1 Cd; (10) 16 N/0.1 Cd; (11) 32 N/0.1 Cd; (12) 64 N/0.1 Cd. N and Cd were used as urea [$CO(NH_2)_2$] and $CdCl_2 \cdot 2.5H_2O$, respectively. The experiment was randomly arranged with three replicates for each treatment. Plants were grown in glasshouse with natural light, day/night temperature of 25–30°C

TABLE 1 Physico-chemical properties of the soil used in the study

Property	Value
pH	7.12
Organic matter (g kg^{-1})	22.55
Total N (g kg^{-1})	1.05
Water soluble N (mg kg^{-1})	62.8
Total P (g kg^{-1})	0.52
Water-soluble P (mg kg^{-1})	6.5
Total K (g kg^{-1})	14.6
Water-soluble K (mg kg^{-1})	66.3
Total metal content (mg kg^{-1})	
Pb	37.84
Zn	105.33
Cu	15.68
Cd	0.53
Water-soluble metal content (mg kg^{-1})	
Pb	0.082
Zn	0.151
Cu	0.231
Cd	0.002

and humidity of 70–90%. Nutrient solution pH value was adjusted daily to 5.5 with 0.1 M sodium hydroxide (NaOH) or 0.1 M hydrochloric acid (HCl) and was continuously aerated and renewed with treatments after every three days. The experiment was terminated after 15 d of treatment. Root and shoot were separated, washed thoroughly with distilled water and fresh weight was taken. These parts were then oven-dried at 70°C after which dry weight were recorded.

Experiment 2

Nitrogen source dependent experiment was carried out when cultured plants were transferred to a 2.5 L pots. Eight different treatments were used (in mM), i.e., (1) 0.01 Cd (CK1); (2) 16 ammonium sulfate [$(NH_4)_2SO_4$]/ 0.01 Cd; (3) 16 $Ca(NO_3)_2{\cdot}4H_2O$ /0.01 Cd; (4) 16 NH_4NO_3/0.01 Cd; (5) 0.1 Cd (CK2); (6) 16 $(NH_4)_2SO_4$/0.1 Cd; (7) 16 $Ca(NO_3)_2{\cdot}4H_2O$/0.1 Cd (CK2); (8) 16 ammonium nitrate (NH_4NO_3)/ 0.1 Cd. The level of N was the optimum concentration obtained from Experiment 1, and Cd was used as $CdCl_2{\cdot}2.5H_2O$. The experimental conditions were the same as Experiment 1.

Pot Experiment

Cultured HE plants were transferred to pots containing 1 kg soil. Seven different treatments were used (in mM), *i.e.* (1) control (CK); (2) 0.25 g kg^{-1} $CO(NH_2)_2$; (3) 1 g kg^{-1} $CO(NH_2)_2$; (4) 0.25 g kg^{-1} $Ca(NO_3)_2{\cdot}4H_2O$; (5) 1 g kg^{-1} $Ca(NO_3)_2{\cdot}4H_2O$; (6) 0.25 g kg^{-1} $(NH_4)_2SO_4$; (7) 1 g kg^{-1} $(NH_4)_2SO_4$. The soil moisture content was maintained at 60% of the water-holding capacity (by weight) adding distilled water after every 2 d. Plants were grown in greenhouse at 30°C and 24°C during the day and night, respectively. After three months, plants were harvested.

Measurement of Root Morphological Parameters

After harvesting the plant root morphological parameters such as root length, surface area, diameter, and volume were determined by root automatism scan apparatus (Min Mac, STD1600^{+}; Régent Instruments Inc., Québec, Canada), equipped with WinRHIZO software manufactured by Régent Instruments Inc., Québec, Canada.

Chlorophyll Content Determination

Fresh leaves of both ecotypes were collected for the determination of chlorophyll contents. Leaves were crushed in 5 mL of (80%, v/v) chilled acetone and the extract was centrifuged at 10,000 g for 10 min. Chlorophyll content was estimated by the method of Arnon (1949) and Maclachlan and Zalik (1963).

Elemental Analysis

After harvesting, plant parts were washed with tap water and de-ionized water to remove adhered soil and then oven-dried at 70°C for 72 h, weighed, and ground to 0.5 mm for metal analysis. 0.1 g of the plant sample was digested using $HNO_3/HClO_4$ (10:1) digestion method. Diluted digest were quantified for Pb, Zn, Cu and Cd concentration using inductively coupled plasma atomic emission spectrometry (ICP-AES).

Statistical Analysis

Statistical analysis was performed using the SPSS statistical package (version 11.0; SPSS, Chicago, IL, USA). All values reported in this work are means of three independent replicates. Data were tested at significant levels of $P < 0.05$ through one-way ANOVA.

RESULTS AND DISCUSSION

Effects of N Interaction on Root Morphology at Low and High Cd

Heavy metal uptake can be directly affected by root morphology (Marschner, 1995). In the present hydroponics experiment, root morphology of both ecotypes of *S. alfredii* H. treated with different N level and form under two concentrations of Cd were presented in Tables 2 and 3. The root morphology responses under Cd stress had been studied before (Li et al.,

TABLE 2 Effects of different N levels on root morphology of two ecotypes of *Sedum alfredii* H. under two concentrations of Cd

Treatment		Root length ($cm\ plant^{-1}$)		Root surface ($cm^2\ plant^{-1}$)		Root diameter ($mm\ root^{-1}$)		Root volume ($cm^3\ plant^{-1}$)	
Cd (mM)	N (mM)	HE	NHE	HE	NHE	HE	NHE	HE	NHE
0.01	CK	208bc	109b	43b	22b	0.61bc	0.32b	0.62bc	0.33bc
	4	233b	118ab	48ab	23ab	0.68b	0.35ab	0.69b	0.36b
	8	251ab	166a	52ab	33a	0.73ab	0.48a	0.74ab	0.50a
	16	273a	123ab	56a	25ab	0.79a	0.37ab	0.81a	0.38b
	32	245ab	101b	51ab	20b	0.68b	0.28b	0.69b	0.39b
	64	186c	96b	37b	19b	0.55c	0.26b	0.56c	0.27c
0.1	CK	296bc	92b	60bc	18b	0.82bc	0.25c	0.85bc	0.26c
	4	335b	101b	68b	20b	0.91b	0.28bc	0.96b	0.29bc
	8	498ab	108ab	81ab	21b	1.09ab	0.31b	1.13ab	0.33b
	16	546a	136a	89a	28a	1.28a	0.42a	1.32a	0.43a
	32	289bc	109ab	58bc	22b	0.81bc	0.32b	0.82bc	0.33b
	64	253c	88b	52c	17b	0.72c	0.23c	0.73c	0.25c

Values are means ± SD (n = 3) from three individual replicates. Different letters indicate significant differences ($P < 0.05$) among the treatments.

2005a, 2005b), but the effects of N on the root morphology of *S. alfredii* H. were ignored. In the present study, with the increase of N level, root morphology parameters involving root length, surface, diameter and volume of both ecotypes of *S. alfredii* H. increase firstly and then decreased suddenly, which was occurred in both Cd concentrations (Table 2). For example, under low Cd concentration (0.01 mM), root length of HE enhanced significantly with the increase of N application, reached the peak (273 cm $plant^{-1}$) at 16 mM N concentration.

After treating with different N form, all the root morphology parameters varied significantly (Table 3). It could be seen that under low Cd

TABLE 3 Effects of different N forms on root morphology of the two ecotypes of *Sedum alfredii* H. under two concentrations of Cd

Treatment		Root length ($cm\ plant^{-1}$)		Root surface ($cm^2\ plant^{-1}$)		Root diameter ($mm\ root^{-1}$)		Root volume ($cm^3\ plant^{-1}$)	
Cd (mM)	N (16 mM)	HE	NHE	HE	NHE	HE	NHE	Cd (mM)	N (mM)
0.01	CK	231b	115b	45b	21b	0.65b	0.33b	0.68b	0.35b
	$(NH_4)_2SO_4$	295a	132ab	63a	26a	0.89a	0.38ab	0.92a	0.39ab
	$Ca(NO_3)_2{\cdot}4H_2O$	263a	146a	55a	29a	0.76ab	0.42a	0.79ab	0.43a
	NH_4NO_3	271a	131ab	58a	25ab	0.81a	0.37ab	0.83ab	0.38ab
0.1	CK	333b	102c	66b	18b	0.89b	0.26b	0.96b	0.28b
	$(NH_4)_2SO_4$	486a	128b	83a	25a	1.11a	0.37a	1.15a	0.32ab
	$Ca(NO_3)_2{\cdot}4H_2O$	465a	132ab	81a	26a	1.09a	0.39a	1.12a	0.33ab
	NH_4NO_3	423a	151a	73ab	29a	1.01ab	0.43a	1.02ab	0.36a

Values are means ± SD (n = 3) from three individual replicates. Different letters indicate significant differences ($P < 0.05$) among the treatments.

concentration (0.01 mM), after treating with 16 mM $(NH_4)_2SO_4$, the root length, surface, diameter and volume of HE plants increased by 27.7, 40, 36.9 and 35.3%, respectively, as compared with CK. By contrast, the application of $Ca(NO_3)_2{\cdot}4H_2O$ was more effective for NHE plants. The similar results were also found in high Cd (0.1 mM) concentration.

Effects of N on the Chlorophyll Contents at Low and High Cd

Heavy metal toxicity adversely interrupted photosynthetic activity by causing distortion of chloroplast ultra-structure, inhibition of the synthesis of photosynthetic pigments and enzymes of Calvin cycle (Mishra et al., 2006). Reduction in chlorophyll content might be attributed to inhibition of δ-aminolevulinic acid dehydratase (ALAD) caused by heavy metal uptake (Prasad and Prasad, 1987), impaired uptake of essential elements such as Mn and Fe or damaged photosynthetic apparatus or due to chlorophyll degradation by increased chlorophyll activity (Sharma et al., 2005).

The chlorophyll a, b and total chlorophyll of the HE plants were significantly ($P < 0.05$) higher than that of the NHE plants in all treatment durations (Table 4). Under the stress of 0.01 mM Cd, the chlorophyll a content of HE plants increased by 4.7, 13.3, 15.7, and 17.2% respectively along with the increase of N level range from 4 to 32 mM; chlorophyll b content increased by 12.5, 24.9, 29.6, and 32.7% respectively as compared with CK. The same trends were recorded under 0.1 mM Cd concentration. For NHE plants, the chlorophyll content reached the peak at 8 mM of N at low Cd while 16 mM of N for high Cd concentration.

TABLE 4 Effects of different N levels on chlorophyll contents in leaves of two ecotypes of *Sedum alfredii* H. under two concentrations of Cd

Treatment		Chlorophyll a ($mg\ g^{-1}$)		Chlorophyll b ($mg\ g^{-1}$)		Total chlorophyll ($mg\ g^{-1}$)	
Cd (mM)	N (mM)	HE	NHE	HE	NHE	HE	NHE
0.01	CK1	0.431c	0.401bc	0.191c	0.163b	0.622c	0.563bc
	4	0.451bc	0.419ab	0.215bc	0.181a	0.666b	0.601ab
	8	0.488ab	0.438a	0.238ab	0.193a	0.726ab	0.632a
	16	0.498a	0.385c	0.248a	0.145c	0.746a	0.529cd
	32	0.505a	0.354d	0.253a	0.137c	0.758a	0.491d
	64	0.482ab	0.326e	0.240ab	0.092d	0.722ab	0.418e
0.1	CK2	0.418d	0.352e	0.179e	0.162e	0.598e	0.513d
	4	0.503c	0.405c	0.251d	0.196d	0.754d	0.601c
	8	0.529b	0.431b	0.268c	0.216c	0.798c	0.647b
	16	0.537b	0.511a	0.292b	0.280a	0.829b	0.791a
	32	0.563a	0.416bc	0.329a	0.239b	0.892a	0.656b
	64	0.399e	0.379d	0.164f	0.234b	0.563f	0.613c

Values are means ± SD (n = 3) from three individual replicates. Different letters indicate significant differences ($P < 0.05$) among the treatments.

TABLE 5 Effects of different N forms on chlorophyll contents in leaves of two ecotypes of *Sedum alfredii* H. under two concentrations of Cd

Treatment		Chlorophyll a ($mg\ g^{-1}$)		Chlorophyll b ($mg\ g^{-1}$)		Total chlorophyll ($mg\ g^{-1}$)	
Cd (mM)	N (16 mM)	HE	NHE	HE	NHE	HE	NHE
0.01	CK	0.398bc	0.323a	0.162c	0.141a	0.549bc	0.463a
	$(NH_4)_2SO_4$	0.506a	0.355a	0.208a	0.165a	0.715a	0.521a
	$Ca(NO_3)_2{\cdot}4H_2O$	0.471ab	0.336a	0.199a	0.148a	0.671ab	0.485a
	NH_4NO_3	0.465ab	0.325a	0.182ab	0.152a	0.648ab	0.478a
0.1	CK	0.433b	0.251b	0.181b	0.116b	0.615b	0.367b
	$(NH_4)_2SO_4$	0.512a	0.296a	0.218a	0.137a	0.729a	0.433a
	$Ca(NO_3)_2{\cdot}4H_2O$	0.479ab	0.278ab	0.216a	0.128ab	0.695ab	0.406ab
	NH_4NO_3	0.486ab	0.281ab	0.203ab	0.125ab	0.689ab	0.405ab

Values are means ± SD (n = 3) from three individual replicates. Different letters indicate significant differences ($P < 0.05$) among the treatments.

Under both concentrations of Cd, with the application of $(NH_4)_2SO_4$, $Ca(NO_3)_2{\cdot}4H_2O$, or NH_4NO_3 increasing trend of chlorophyll content was recorded in HE plants respectively. Among all the three N fertilizers, $(NH_4)_2SO_4$ was the most effective one (Table 5). In case of NHE plants, there were no significant increase after all treatment except for the application of $(NH_4)_2SO_4$ under high Cd concentration which increased chlorophyll content significantly.

Effects of N on Cd Accumulation at Low and High Cd

Various studies on fertilizer application have been conducted to enhance the heavy metal accumulation in shoots of plants (Klang-Westin and Perttu, 2002; Leggo et al., 2006). In the present study, after application of N fertilizer lower than 0.16 mM, Cd accumulation in shoots of HE plants exposed to both concentrations of Cd increased accordingly ($P < 0.05$) under solution culture conditions (Table 6); and the similar trends were also found in roots. But it was noticed that Cd accumulation in shoot of HE was remarkably higher than in root. However, quite contradictory to HE, though Cd content in shoot and root both increased with the application of N fertilizer and Cd content in roots was still significantly lower than that in shoots, it especially increased Cd accumulation in roots significantly for NHE.

After application of $(NH_4)_2SO_4$, $Ca(NO_3)_2{\cdot}4H_2O$, or NH_4NO_3 Cd accumulation in shoots and roots of both ecotypes of *S. alfredii* H. increased as compared with CK (Table 7). Under both low and high Cd concentrations, $(NH_4)_2SO_4$ was the most effective one among all the three fertilizers tested on HE plants. The effects of three N fertilizers on Cd concentrations in NHE were similar with those of HE plants, while Cd concentrations in roots of NHE were significant higher than those of HE.

TABLE 6 Effects of different N levels on Cd accumulation in shoots and root of two ecotypes of *Sedum alfredii* H. under two concentrations of Cd

Treatment		Cd accumulation in shoots (mg plant^{-1})		Cd accumulation in roots (mg plant^{-1})	
Cd (mM)	N (mM)	HE	NHE	HE	NHE
0.01	CK	0.192f	0.026d	0.023c	0.026c
	4	0.326e	0.036c	0.036c	0.032b
	8	0.482c	0.058b	0.051ab	0.049a
	16	0.804a	0.062b	0.071a	0.050a
	32	0.714b	0.073a	0.082a	0.052a
	64	0.414d	0.036c	0.052ab	0.030bc
0.1	CK	1.306d	0.048f	0.128cd	0.073c
	4	1.667c	0.076e	0.147c	0.077c
	8	2.105b	0.134c	0.180ab	0.100b
	16	2.971a	0.164b	0.209a	0.154a
	32	1.884bc	0.187a	0.186ab	0.157a
	64	1.261d	0.110d	0.134bc	0.115b

Values are means ± SD (n = 3) from three individual replicates. Different letters indicate significant differences ($P < 0.05$) among the treatments.

Effects of N on the Biomass in Slightly Cd Contaminated Soil

Klang-Westin and Perttu (2002) reported that stem biomass production of willow significantly ($P < 0.05$) increased with a higher N supply, independent of harvest occasion. In the present study, HE plants had no evident toxic symptom after being planted in slightly Cd-contaminated soil, and grew better after the application of N fertilizers. Both shoots and roots dry weight increased significantly as compared to CK ($P < 0.05$) with all treatments (Table 8). Among all the three fertilizers, $(NH_4)_2SO_4$ was the most effective

TABLE 7 Effects of different N forms on Cd accumulation in shoots and root of two ecotypes of *Sedum alfredii* H. under two concentrations of Cd in solution culture

Treatment		Cd accumulation in shoot (mg plant^{-1})		Cd accumulation in root (mg plant^{-1})	
Cd (mM)	N (16 mM)	HE	NHE	HE	NHE
0.01	CK	0.499c	0.014c	0.036c	0.011b
	$(NH_4)_2SO_4$	0.685a	0.021a	0.066a	0.016a
	$Ca(NO_3)_2{\cdot}4H_2O$	0.543b	0.018b	0.053b	0.018a
	NH_4NO_3	0.612ab	0.019b	0.055b	0.016a
0.1	CK	1.809c	0.068c	0.069c	0.017b
	$(NH_4)_2SO_4$	2.347a	0.085a	0.115a	0.038a
	$Ca(NO_3)_2{\cdot}4H_2O$	2.118ab	0.083a	0.093b	0.039a
	NH_4NO_3	2.031b	0.077b	0.113a	0.045a

Values are means ± SD (n = 3) from three individual replicates. Different letters indicate significant differences ($P < 0.05$) among the treatments.

TABLE 8 Effects of different N dosage and fertilizers on dry weight of two ecotypes of *Sedum alfredii* H. grown in the slightly Cd-contaminated soil

Nitrogen forms	N dosage ($g\ kg^{-1}$ soil)	Dry weight ($g\ plant^{-1}$)	
		Shoot	Root
$CO(NH_2)_2$	CK	0.431b	0.131c
	0.25	0.471ab	0.149b
	1	0.585a	0.183a
$Ca(NO_3)_2{\cdot}4H_2O$	CK	0.431c	0.131c
	0.25	0.596b	0.158b
	1	0.699a	0.182a
$(NH_4)_2SO_4$	CK	0.431c	0.131c
	0.25	0.606b	0.162b
	1	0.738a	0.188a

Values are means ± SD (n = 3) from three individual replicates. Different letters indicate significant differences ($P < 0.05$) among the treatments

one. With 0.25 and 1 $g\ kg^{-1}$ N, shoot dry weight of HE plant increased by 40.6 and 71.2%, respectively, as compared to CK.

Effects of N on the Cd Accumulation in Slightly Cd Contaminated Soil

In the pot experiment, Cd accumulation in shoots and roots of HE plants increased significantly after application of three N fertilizers as compared with CK ($P < 0.05$) (Table 9). Ammonium sulfate was the most effective N fertilizer, which could increase Cd accumulation in shoots of HE by 16.1 and 32.3% respectively as compared with CK with the application of 0.25 and 1 $g\ kg^{-1}$ soil N.

TABLE 9 Effects of different N dosage and fertilizers on Cd accumulation in shoots and root of two ecotypes of *Sedum alfredii* H. grown in the slight Cd-contaminated soil

Nitrogen forms	N dosage ($g\ kg^{-1}$ soil)	Cd accumulation ($mg\ plant^{-1}$)	
		Shoot	Root
$CO(NH_2)_2$	CK	0.031b	0.0033b
	0.25	0.033ab	0.0035ab
	1	0.037a	0.0039a
$Ca(NO_3)_2{\cdot}4H_2O$	CK	0.031b	0.0033a
	0.25	0.035ab	0.0035a
	1	0.039a	0.0037a
$(NH_4)_2SO_4$	CK	0.031b	0.0033b
	0.25	0.036ab	0.0036ab
	1	0.041a	0.0039a

Values are means ± SD (n = 3) from three individual replicates. Different letters indicate significant differences ($P < 0.05$) among the treatments.

CONCLUSIONS

From our present study, it could be concluded that N fertilizer was effective on root morphology, chlorophyll content and Cd accumulation. In hydroponics experiments, 16 mM $(NH_4)_2SO_4$ was the optimum application level. In pot experiment, $(NH_4)_2SO_4$ was also the most effective among all the three N fertilizers. In a word, it is effective to enhance the biomass of *Sedum alfredii* Hance and increase its heavy metal accumulation in shoots when N fertilizer is properly applied. To determine if it is also a good agricultural method for other hyper-accumulators, a further study is needed.

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