Effects of DTPA on Cadmium Accumulation of Galinsoga parviflora

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Abstract: The effects of diethylene triamine pentaacetic acid (DTPA) on cadmium (Cd) accumulation of hyperaccumulator *Galinsoga parviflora* were investigated through pot experiment. The results showed that the biomass, chlorophyll content of *G. parviflora* decreased with the increase of DTPA concentrations in the soil. Applying DTPA increased Cd content in *G. parviflora*, and the maxima of Cd content in shoots of *G. parviflora* were the dose of 4 mmol/kg DTPA, which increased by 347.39% compared with the control. Applying DTPA also increased Cd extraction by *G. parviflora*, and the dose of 4 mmol/kg DTPA was the best concentration in this experiment to improve Cd extraction by *G. parviflora*. Therefore, DTPA enhanced the Cd extraction ability of *G. parviflora* from Cd-contaminated soil, and 4 mmol/kg DTPA demonstrated to be the best dose that could be used to enhance the phytoremediation ability of *G. parviflora* efficiently.

Introduction

Hyperaccumulator is the main material for heavy metal phytoremediation [1]. However, the screened hyperaccumulators grow slowly and have small biomass, which are lead to low efficiency of phytoremediation [2]. The chelator is the newly auxiliary phytoremediation material that can improve the bioavailability of heavy metal from soil [3]. When the chelator applying in the soil, the form of heavy metal changes, and increases the bioavailability of heavy metal concentration in the soil, which contributes to heavy metal absorption of hyperaccumulator [4]. When ethylene diamine tetraacetic acid (EDTA) applying in the soil, the available concentration of Pb, Cd, Cu improve, but the proportion of chelation nickel is only 23% of the full amount nickel [5]. For hyperaccumulator, all of EDTA, diethylene triamine pentaacetic (DTPA) and citric acid promote heavy metals absorption of hyperaccumulator A. corsicum in the low concentrations of heavy metals, and the same chelator is quite different with the effect of different heavy metals [6]. Other studies also have confirmed the chelator can improve the phytoremediation ability of hyperaccumulator [7].

Galinsoga parviflora is a Cd-hyperaccumulator for remedy Cd contaminated soil [8]. However, compared with other high biomass hyperaccumulator [9], the remedy ability of *G. parviflora* is low, and remains to be improved. Therefore, to enhance the remedy ability to Cd-contaminated soil, the different concentrations of DTPA were applied to treat *G. parviflora* in this study. The aim of the study was to screen DTPA concentration which could enhance the phytoremediation ability of *G. parviflora*, and provide a reference for improving phytoremediation ability of other hyperaccumulators.

Materials and Method

Materials. The inceptisol soil samples were collected from Ya'an campus farm of the Sichuan Agricultural University (29°59′N, 102°59′E), China, in March 2014. The basic properties of the soil were the same as reference [8], and the total Cd content was 0.101 mg/kg. *Galinsoga parviflora* seedlings with two pairs of euphyllas were collected from the Ya'an campus farm (from uncontaminated soil) in April 2014.

Experimental Design. The experiment was conducted at the Ya'an campus farm from March to Jun in 2014. The soil samples were air-dried and passed through a 5-mm sieve. Three kilograms of the air-dried soil was weighed into each polyethylene pot (15 cm high, 18 cm in diameter). Cd was added to soils as $CdCl_2 \cdot 2.5H_2O$ at 10 mg kg^{-1}. The pots were soaked in the Cd solutions for 4 weeks, and then the soil in each pot was mixed with DTPA powder, and the soil moisture was maintained at 80% of field capacity for 1 week. The DTPA treatments in the experiment were 0, 0.5, 1, 2, 4 mmol/kg [7], and each treatment was replicated three times using a completely randomized design with 10-cm spacing between pots. Four uniform seedlings of *G. parviflora* were transplanted into each pot and the soil moisture content was maintained at 80% of field capacity from the time the plants were transplanted into the pots until the time the plants were harvested.

Sample Analysis. After *G. parviflora* had matured (after 50 d), the upper mature leaves of *G. parviflora* were collected to determine the photosynthetic pigment (chlorophyll a, chlorophyll b, total chlorophyll and carotenoid) contents [10]. The plants were then gently removed from the soil. The roots and shoots of *G. parviflora* were harvested and washed with tap water. The roots were immersed in 10 mM/L HCl for 10 min to remove Cd adhering to the root surface. Then, the treatments and analyses of plants were described as in reference [8].

Statistical Analyses. Statistical analyses were performed using SPSS 13.0 statistical software (IBM, Chicago, IL, USA). Data were analyzed by one-way analysis of variance with least significant difference at a 5% confidence level.

Results and Discussion

Biomass of *G. parviflora*. With the increase of DTPA concentrations in the soil, the root, stem, leaf and shoot biomass of *G. parviflora* decreased (Table 1). The root biomass decreased by 0.58% (p > 0.05), 2.31% (p > 0.05), 3.85% (p < 0.05) and 5.39% (p < 0.05) at 0.5, 1, 2, 20 and 4 mmol/kg DTPA, respectively, compared with the control, and the shoot biomass decreased by 5.06% (p < 0.05), 8.82% (p < 0.05), 12.55% (p < 0.05) and 18.49% (p < 0.05) at 0.5, 1, 2, 20 and 4 mmol/kg DTPA, respectively, compared with the control. These results indicate that DTPA could inhibit the growth of *G. parviflora*, which because of DTPA improves Cd effectiveness in the soil and increases Cd toxicity to *G. parviflora* [7]. The root/ shoot ratio of *G. parviflora* had increasing trend with the increase of DTPA concentration, but there was no obvious in low concentrations (Table 1), indicating that *G. parviflora* could increase root proportion to enhance the resistance to Cd stress.

Table 1 Effects of DTPA on biomass of *Galinsoga parviflora*

DTPA concentrations (mmol/kg)	Roots (g/plant)	Stems (g/plant)	Leaves (g/plant)	Shoots (g/plant)	Root/ shoot ratio
0	0.519±0.006a	1.425±0.035a	1.284±0.030a	2.709±0.065a	0.192
0.5	0.516±0.004a	1.364±0.020b	1.208±0.017b	2.572±0.037b	0.201
1	0.507±0.010ab	1.334±0.018b	1.136±0.013c	2.470±0.031bc	0.205
2	0.499±0.006b	1.258±0.024c	1.111±0.014c	2.369±0.038c	0.211
4	0.491±0.003b	1.203±0.014c	1.005±0.007d	2.208±0.021d	0.222

Photosynthetic Pigment Contents of *G. parviflora*. When applying DTPA in the soil, the photosynthetic pigment contents of *G. parviflora* decreased (Table 2), which were consistent with the biomass. With the increase of DTPA concentrations in the soil, the chlorophyll a, chlorophyll b, total chlorophyll and carotenoid contents of *G. parviflora* decreased. The total chlorophyll content decreased by 17.79% (p < 0.05), 18.01% (p < 0.05), 26.63% (p < 0.05) and 32.43% (p < 0.05) at 0.5, 1, 2, 20 and 4 mmol/kg DTPA, respectively, compared with the control, and the carotenoid content decreased by 19.80% (p < 0.05), 21.45% (p < 0.05), 30.86% (p < 0.05) and 35.31% (p < 0.05) at 0.5, 1, 2, 20 and 4 mmol/kg DTPA, respectively, compared with the control. The carotenoid can receive the surplus energy of excited chlorophyll molecule, and avoids formatting the singlet oxygen, which plays the protective effect of light [11]. DTPA decreased carotenoid contents of *G. parviflora* suggesting that the damage of *G. parviflora* by light enhanced with the increase of DTPA concentrations in the soil. The chlorophyll a/b of *G. parviflora* decreased when the dose of DTPA was not more than 2 mmol/kg, and increased from the dose of 2 mmol/kg to 4 mmol/kg (Table 2). So, under the low concentrations of DTPA, the photosynthesis of *G. parviflora* could weaken, and with strong photosynthesis under the high concentrations of DTPA.

Table 2 Effects of DTPA on photosynthetic pigment contents of *Galinsoga parviflora*

DTPA concentrations (mmol/kg)	Chlorophyll a (mg/g)	Chlorophyll b (mg/g)	Total chlorophyll (mg/g)	Chlorophyll a/b	Carotenoid (mg/g)
0	1.790±0.003a	0.486±0.003a	2.276±0.006a	3.683	0.606±0.006a
0.5	1.471±0.040b	0.400±0.011b	1.871±0.051b	3.678	0.486±0.017b
1	1.460±0.044b	0.406±0.007b	1.866±0.051b	3.596	0.476±0.012b
2	1.313±0.068c	0.357±0.015c	1.670±0.083c	3.675	0.419±0.002c
4	1.214±0.060c	0.324±0.004d	1.538±0.064c	3.747	0.392±0.009c

Cd Content in *G. parviflora*. With the increase of DTPA concentrations in the soil, the Cd content in *G. parviflora* increased compared with the control (Table 3), which was related to DTPA improving available Cd concentration in the soil [7]. The Cd contents in roots, stems, leaves and shoots of *G. parviflora* increased with the increase of DTPA concentrations in the soil. The Cd content in roots increased by 12.31% ($p < 0.05$), 62.11% ($p < 0.05$), 111.01% ($p < 0.05$) and 218.22% ($p < 0.05$) at 0.5, 1, 2, 20 and 4 mmol/kg DTPA, respectively, compared with the control, and the Cd content in shoots increased by 14.79% ($p < 0.05$), 101.68% ($p < 0.05$), 176.22% ($p < 0.05$) and 347.39% ($p < 0.05$) at 0.5, 1, 2, 20 and 4 mmol/kg DTPA, respectively, compared with the control. These results indicate that the Cd content increase rate of shoots was higher than that in roots. The translocation factor (TF) of *G. parviflora* also increased with the increase of DTPA concentrations in the soil, indicating that DTPA promoted Cd transporting from roots to shoots of *G. parviflora*, which could be benefit to improve the phytoremediation ability of *G. parviflora*.

Table 3 Effects of DTPA on Cd content in *Galinsoga parviflora*

DTPA concentrations (mmol/kg)	Roots (mg/kg)	Stems (mg/kg)	Leaves (mg/kg)	Shoots (mg/kg)	TF
0	12.35±0.92d	10.39±0.86d	13.57±0.61d	11.90±0.74d	0.96
0.5	13.87±0.33d	12.04±0.74d	15.48±0.74d	13.66±0.75d	0.98
1	20.02±1.39c	20.80±0.99c	27.75±1.71c	24.00±1.32c	1.20
2	26.06±1.05b	26.64±1.22b	39.92±1.53b	32.87±1.39b	1.26
4	39.30±0.99a	42.33±2.35a	66.30±2.40a	53.24±2.40a	1.35

The translocation factor (TF) is defined as Cd content in shoot/ Cd content in root.

Cd Extraction by *G. parviflora*. The Cd extraction by *G. parviflora* increased when applied DTPA in the soil compared with the control (Table 4). With the increase of DTPA concentrations in the soil, the Cd extractions by roots, stems, leaves, shoots and whole plant increased. The Cd extraction by roots increased by 11.70% ($p > 0.05$), 58.35% ($p < 0.05$), 102.81% ($p < 0.05$) and 201.09% ($p < 0.05$) at 0.5, 1, 2, 20 and 4 mmol/kg DTPA, respectively, compared with the control; the Cd extraction by shoots increased by 8.97% ($p > 0.05$), 83.90% ($p < 0.05$), 141.72% ($p < 0.05$) and 264.72% ($p < 0.05$) at 0.5, 1, 2, 20 and 4 mmol/kg DTPA, respectively, compared with the control; the Cd extraction by whole plant increased by 9.42% ($p > 0.05$), 79.66% ($p < 0.05$), 135.14% ($p < 0.05$) and 254.17% ($p < 0.05$) at 0.5, 1, 2, 20 and 4 mmol/kg DTPA, respectively, compared with the control. Therefore, DTPA can effectively improve phytoremediation ability of *G. parviflora*, which was consistent with the other studies [7].

Table 4 Effects of DTPA on Cd extraction by *Galinsoga parviflora*

DTPA concentrations (mmol/kg)	Roots (μg/plant)	Stems (μg/plant)	Leaves (μg/plant)	Shoots (μg/plant)	Whole plant (μg/plant)
0	6.41±0.41d	14.81±0.86d	17.42±0.38d	32.23±1.24d	38.64±1.65d
0.5	7.16±0.11d	16.42±0.77d	18.70±0.63d	35.12±1.40d	42.28±1.51d
1	10.15±0.50c	27.75±0.94c	31.52±1.59c	59.27±2.53c	69.42±3.03c
2	13.00±0.38b	33.51±0.89b	44.35±1.13b	77.86±2.02b	90.86±2.40b
4	19.30±0.39a	50.92±2.23a	66.63±1.95a	117.55±4.17a	136.85±4.55a

Conclusions

Applied DTPA in the soil decreased the biomass of *G. parviflora* with the increase of DTPA concentrations. The chlorophyll a, chlorophyll b, total chlorophyll and carotenoid contents also decreased with the increase of DTPA concentrations in the soil. DTPA promoted the Cd absorption of *G. parviflora* from soil, and increased Cd content and Cd extraction of *G. parviflora*. The maximum of Cd extraction by *G. parviflora* was at 4 mmol/kg DTPA. Therefore, DTPA can effectively improve phytoremediation ability of *G. parviflora*, and could be applied in the field.

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