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Phytoremediation of Cadmium-Contaminated Farmland Soil by the Hyperaccumulator *Beta vulgaris* L. *var*. *cicla*

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Abstract A field study was conducted to evaluate the phytoremediation efficiency of cadmium(Cd) contaminated soil utilizing the Cd hyperaccumulator *Beta vulgaris* L. *var*. *cicla* during one growing season (about 2 months) on farmland in Zhangshi Irrigation Area, the representative wastewater irrigation area in China. Results showed that *B*. *vulgaris* L. *var*. *cicla* is a promising plant in the phytoremediation of Cd contaminated farmland soil. The maximum of Cd phytoremediation efficiency by *B*. *vulgaris* L. *var*. *cicla* reached 144.6 mg/ha during one growing season. Planting density had a significant effect on the plant biomass and the overall Cd phytoremediation efficiency ($p < 0.05$). The amendment of organic manure promoted the biomass increase of *B*. *vulgaris* L. *var*. *cicla* ($p < 0.05$) but inhibited the Cd phytoremediation efficiency.

Keywords Phytoremediation · Farmland soil · Cadmium · *Beta vulgaris* L. *var*. *cicla*

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Heavy metal-contaminated soil caused by various human activities is one of the widespread global problems. The clean up of heavy metal-contaminated soils is necessary but difficult for their potential toxicity and high persistence. Phytoremediation is an environment-friendly and cost-effective green remediation technology, using the hyperaccumulator or accumulator to remove toxic pollutants from soil, and presents a promising alternative to current environmental methodologies especially for the large field application at low to moderate concentration of contaminants (Kim and Lee 2010; Wei et al. 2010). The phytoremediation efficiency depends on the heavy metal contents in the plant and the biomass production of the plant (Blaylock et al. 1997). Therefore, large biomass and high accumulating capacity of plant work well in the phytoremediation practice. Until now, *Solanum nigrum* L., *Salix*‘*calodendron*’, *Populus* spp., and *Arabis paniculata* (Wei et al. 2005; French et al. 2006; Maxted et al. 2007) may be good candidates among the limited Cd hyperaccumulators for field conditions. In order to enhance the Cd removal efficiency of these hyperaccumulators, some natural or synthetic chelators such as EDTA, NTA, DTPA, and EDDHA have been studied and suggested for field application (Quartacci et al. 2007; Marques et al. 2008). However, the application of chelators in field may result in secondary pollution of soils and have potential risk on groundwater contamination. Therefore, suitable agronomic practices to maximize biomass of hyperaccumulators in field conditions may be a safe and ideal phytoextraction strengthening method (McGrath et al. 2006).

In this study the *Beta vulgaris* L. *var*. *cicla*, previously proved to be a Cd-hyperaccumulator by pot experiment (Li et al. 2007), was investigated to verify its viability as an alternative in the Cd phytoremediation practice in field scale. In addition, effects of the amendment of chicken manure, the most commonly used fertilizer in agricultural production in China, and planting density on the phytoremediation efficiency were also investigated.

Materials and Methods

The experimental site (123°26′90.5″E, 41°46′18.2″N) located in the Shenyang Zhangshi Irrigation Area (SZIA), which is in a temperate zone with a semi-moist continental climate, a mean annual temperature of 5–9°C, an average annual rainfall of 650–700 mm, and an annual frost-free season of 127–164 days. The SZIA is a famous Cd contamination region in China (Song et al. 2006), where paddy field was irrigated by industrial wastewater together with surface water since 1956. From 1983, the discharge of wastewater was gradually ceased and most contaminated region of SZIA was rezoned from paddy field to an industrial land in 1993. However, the remaining regions are still utilized as agricultural land but the farming style has been changed from paddy field to dry land in order to decrease the migration of Cd in soil.

The top 20 cm soil was ploughed to homogeneity by normal agronomic machinery. The field trial was designed with two factors – planting density and manure addition, and each factor consisted of three replications as described in Table 1. Fifteen plots with 9.0 m^2 (3.0 m × 3.0 m) each were arranged randomly. 50 cm thick and 30 cm high ridges were constructed between plots to minimize the near-neighbor effects. In the manure addition plots (Group 2) the mature chicken manure was applied and mixed thoroughly in the top 20 cm soil. The chicken manure was taken as organic manure which provided TOC content of 19.9%, Cd content of 0.28 mg/kg, and pH of 7.71.

Seeds of *B. vulgaris* L. *var. cicla* were sown in the 15 random plots according to the designed row space in late April. The seedlings were thinned out matching the planting densities given in Table 1 10 days after the emergence. During the whole growth stage the sample field was irrigated by well water and weeded by hand. The aboveground parts were harvested in early July and the plants in each plot were sampled for the laboratory analysis.

Table 1 Experimental design of the plots

Group1 (planting density, cm × cm, without manure amendment)		Group 2 (manure amendment, kg/m^2, planting density, 15 cm × 20 cm)	
D1	10 × 15	M1 (D2)	0
D2	15 × 20	M2	2.5
D3	20 × 20	M3	5.0

Basic physico-chemical characteristics of test soil were analyzed. The pH value was determined using demonized water (w/v = 1/2.5). Cation exchange capacity (CEC) was calculated by the sum. Particle size distribution was determined by the hydrometer method. Total organic carbon (TOC) was determined by ferrisulfas titration after the oxidation of organic matter by $K_2Cr_2O_7$ (Lu 2000).

Soil samples were air-dried, grinded and passed through a 0.149 mm sieve. Then the soil samples were digested by concentrated HNO_3 and $HClO_4$ (Li et al. 2007). The filtrate was analyzed for Cd content by atomic absorption spectrophotometer (AAS, Varian Spectr AA 220). The measured values of Cd content in soil samples were checked using certified standard reference material (GBW07401, dark brown soil) for quality assurance. The recovery rate and detection limit of Cd were 92.9% and 0.010 mg/L, respectively. The harvested plants were washed thoroughly first with running tap water followed by demonized water. The roots and leaves were separated, and dried in an oven at 105°C for 10 min, and then at 70°C for 8 h until the plant samples reached constant weight. 0.5 g of dried plant samples were weighed into porcelain crucibles, decomposed on an electric hot plate, then incinerated in a muffle furnace at 550°C for 6 h. The ash was dissolved in aqua regia. The filtrate was determined for Cd content by AAS. The measured values of Cd content in plant samples were checked using certified standard reference material (CTA–OTL–1, oriental tobacco leaves) for quality assurance. The recovery rate and detection limit of Cd were 93.6% and 0.010 mg/L, respectively.

The phytoremediation efficiency (PE) was calculated, which represented the total amount of heavy metals extracted per ha of soil in one single phytoremediation cycle. It was calculated as follows: $PE = Cd_{plant} \times B_{plant}$, where Cd_{plant} is the content of Cd in the dry aboveground part (mg/kg) and B_{plant} is the dry biomass yield (kg/ha).

Statistical analyses were performed using software SPSS 17.0. All experimental results were expressed as means ± standard deviation. Analysis of variance (ANOVA) with subsequent Duncan test was performed to evaluate the statistical significance of the differences between groups at the significance level of $p < 0.05$.

Results and Discussion

The field soil was classified as meadow burozem and the soil physico-chemical characteristics were summarized in Table 2. The Cd content in the top 20 cm soil ranged from 2.82 to 3.17 mg/kg, which was 9.4–10.6 times of the Grade II (for agricultural purpose) environmental quality standard for soils of China (National Environmental Protection

Table 2 Basic physico-chemical characteristics of the farmland soil (top 20 cm)

Parameters	Content
pH (H_2O)	5.92 ± 0.14
CEC (cmol/kg)	14.9 ± 0.2
TOC (%)	2.8 ± 0.3
Particle size distribution (%)	
Sand (%)	66.2
Silt (%)	31.9
Clay (%)	1.9
Soil texture	Loam
Total heavy metal contents (mg/kg) (n = 3)	
Cd	3.02 ± 0.14
Pb	41.3 ± 4.7
Cu	46.1 ± 7.3
Zn	169.8 ± 12.4

Agency of China. 1995). The contents of the Pb, Cu, and Zn were below the Grade II standard. The field soil was moderately acid with pH value of 5.92. Heavy metal content analysis showed that the field was under the moderate Cd contamination, which might deteriorate the quality of farm products so as to threaten people's health. Therefore, it was necessary to remediate the farmland soil.

Results of plant biomass and Cd extraction by *B. vulgaris* L. *var. cicla* in the three different planting densities were listed in Table 3. The average plant height aboveground ranged from 106.0 to 116.1 cm, and the root length was from 13.6 to 14.3 cm. The average plant height and average root length increased with the density decreased, but no significant differences of plant aboveground height and root length were detected in different density treatments ($p > 0.05$).

Planting density had a significant influence on aboveground biomass yield per unit area. For example, the dry aboveground biomass at the low planting density (D3) was only 5,490 ± 330 while 7520 ± 130 kg/ha at the high planting density (D1) (as shown in Table 3). Statistical analysis also showed a significant positive correlation between the aboveground biomass per unit area and the planting density (r = 0.975, $p < 0.05$).

Cd concentrations in the aboveground biomass by dry weight in the planting density group (D1, D2, and D3) were 19.23 ± 0.54, 20.10 ± 1.26, and 19.37 ± 1.65 mg/kg, respectively. No significant differences were detected (shown in Table 3), which indicated that planting density had no significant effect on the Cd uptake and accumulation in the plant tissue of *B. vulgaris* L. *var. cicla*. As discussed that the concentration of heavy metals in plant tissue depends on the genetic predisposition of the individual plant and soil contamination level (Ma et al. 2001).

Since planting density had no significant effect on the Cd concentrations in the aboveground parts, the phytoextraction efficiency should depend on the plant aboveground biomass. Statistical analysis showed that there was a significantly positive correlation between the total Cd phytoremediation efficiency (PE) and plant aboveground biomass (r = 0.988, $p < 0.05$). The PE value in the high density treatment D1 was 144.6 mg/ha, 1.36 times of that in the low density treatment D3, in which the Cd extraction amount was 106.3 mg/ha (Table 3).

Beta vulgaris L. *var. cicla* is a good candidate as Cd hyperaccumulator in field condition for its relatively large biomass and high Cd extraction capacity. Compared with *S. nigrum*, a famous Cd hyperaccumulator in field condition (Sun et al. 2008; Wei et al. 2010; Ji et al. 2011), the growth period of *B. vulgaris* L. *var. cicla* is shorter about two-thirds of that of *S. nigrum* despite its PE value of Cd in unit area in one batch is relatively lower. *B. vulgaris* L. *var. cicla* as a herbaceous plant can grow in either spring, summer or autumn and it can be cultivated for two to three batches annually, thus the Cd extraction efficiency can be expected to a certain high level as *S. nigrum* (0.233 kg/ha, Ji et al. 2011).

The biomass and Cd concentrations in *B. vulgaris* L. *var. cicla* in the three manure amendment treatments (M1, M2, and M3) were measured and shown in Fig. 1. Manure amendment elevated the biomass of *B. vulgaris* L. *var. cicla*. For instance, in M1 treatment (no manure amendment), the aboveground biomass was the lowest 6,410 kg/ha, while in the chicken manure amendment treatments M2 and M3, the aboveground biomass were 6,560 and 6,980 kg/ha, respectively. As known that organic manures

Table 3 Effect of planting density on biomass and Cd extraction by *Beta vulgaris* L. *var. cicla*

Treatment	D1	D2	D3
Height aboveground (cm)	106.0 ± 9.5 a	112.3 ± 4.0 a	116.1 ± 10.8 a
Root length (cm)	13.6 ± 0.7 a	14.0 ± 0.6 a	14.3 ± 0.5 a
Dry biomass yield (kg/ha)	7,520 ± 130 a	6,410 ± 30 b	5,490 ± 330 c
Cd in aboveground (mg/kg)	19.23 ± 0.54 a	20.10 ± 1.26 a	19.37 ± 1.65 a
PE value (mg/ha)	144.6	128.8	106.3

Experimental data were expressed as mean ± SD. Figures followed by different letters in a same line were significantly different at $p < 0.05$, n = 3

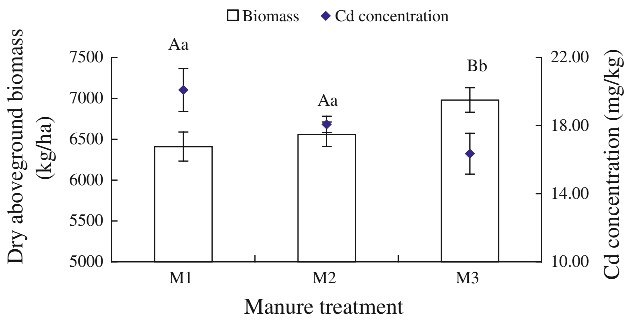

Fig. 1 Plant dry aboveground biomass of *Beta vulgaris* L. *var. cicla* in the different manure amendment treatments. Data shown are means ± SD (n = 3). *Capital letters* and *lower-case letters* represent the dry aboveground biomass and the Cd concentration, respectively. Data with the *same letter* represent statistically identical values ($p < 0.05$)

have abundant nutrition needed by plant such as N and P, therefore promote the increase of plant biomass (Ma et al. 2011). However, the Cd concentration in the aboveground decreased with the manure amendment, as shown in Fig. 1. In M1 treatment (no manure amendment), the Cd concentration was 20.10 mg/kg (dry weight), while it was only 18.07 and 16.35 mg/kg (dry weight) in M2 and M3. Statistical analysis indicated that Cd concentrations in manure amendment treatments were significantly lower than that of plants unfertilized ($p < 0.05$).

In total, the PE values of Cd in different manure amendment treatments were not significantly different ($p > 0.05$). The PE values of Cd in M1, M2, and M3 were 128.8, 118.5 and 114.1 mg/ha, respectively. This indicated that chicken manure amendment, the most commonly used method for increasing crop production (Yang 2002), played no role in increasing the Cd accumulation in plants. This is consistent with previous reports, where organic manure decreased the Cd uptake in plants because the solubility and mobility of the heavy metal in soil were reduced and thus inhibiting the accumulation (Berti and Cunningham 1997). Therefore, manure amendment may not be an appropriate way to enhance phytoremediation efficiency of Cd in farmland soil.

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