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Uptake of Cadmium by Different Cultivars of *Brassica pekinens* (Lour.) Rupr. and *Brassica chinensis* L. and Their Potential for Phytoremediation

C. P. Liu,[1,2] Z. G. Shen,[1] X. D. Li[3]

[1] College of Life Sciences, Nanjing Agricultural University, Nanjing 210095, People's Republic of China
[2] Guangdong Key Laboratory of Integrated Control of Agro-Environment, Guangdong Institute of Eco-Environment and Soil Sciences, Guangzhou 510650, People's Republic of China
[3] Department of Civil and Structural Engineering, The Hong Kong Polytechnic University, Hung Hom, Kowloon, Hong Kong

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Heavy metal contamination of soils due to intensive industrial activities and agricultural development can usually cause environmental problems. Elevated levels of heavy metals not only decrease soil microbial activity and crop production, but also threaten human health through the food chain (Wagner, 1993; McLaughlin et al. 1999). Uptake of heavy metals by plants has been well studied in the last few decades. It has been reported that different plant species and even cultivars vary in their uptake of heavy metals from soils or hydroponic solutions. Some plants can efficiently translocate heavy metals from roots and accumulate them in shoots. Baker and Brooks (1989) defined these plants accumulating > 10 000 μg g^{-1} of Zn or Mn, or > 1000 μg g^{-1} of Cu, Pb, Co, Cr or Ni, or > 100 μg g^{-1} of Cd in the shoots as hyperaccumulator species. Metal accumulation by plants, particularly hyperaccumulation, have attracted considerable interests in recent years due to their potential use for remediation of contaminated sites.

Cadmium has no essential biological function, and is highly toxic to plants and animals. It is of particular concern to human health as Cd can be readily absorbed by plant roots and be concentrated by many cereals, potatoes, vegetables and fruits (Wagner, 1993). In most plant species, Cd is mainly accumulated in roots, although the translocation to shoots may occur (Sanità di Toppi and Gabbrielli, 1999). A few plant species have been found to accumulate Cd above 100 μg g^{-1} in shoot DM (Reeves and Baker, 2000). It has been found that some members of the *Brassica* genus are capable of accumulating high levels of Cd as well as other toxic metals in shoots (Kumar, et al.1995; Ebbs et al. 1997; Shen et al. 2002; Ru et al. 2004). This ability may be inherited from some wild species in the Brassicaceae family (Kumar, et al. 1995). Some species or cultivars of plants with high biomass and high capacity of metal accumulation in shoots can be used for phytoremediation of contaminated soils. In this study, the uptake of Cd by twenty cultivars of *Brassica pekinensis* (Lour.) Rupr. and *Brassica chinensis* L. was studied, and their potential use in phytoremediation process was evaluated based on the results of the pot experiment in the present study.

Correspondence to: Z. Shen

MATERIALS AND METHODS

Soil samples were collected from the 0-30 cm layer of an agricultural field at Nanjing Agriculture University. Soils were air-dried, crushed to pass through a 4 mm diameter sieve, and then artificially amended to 100 mg kg^{-1} dry soil Cd in the form of $CdCl_2$. The Cd-amended soils were allowed to undergo water saturation and air-drying cycles for two weeks. The air-dried soils (1 000 g) were placed in plastic pots. Basal fertilizers applied to the soils were 100 mg N kg^{-1} dry soil as urea, 80 mg P kg^{-1} and 100 mg K kg^{-1}as KH_2PO_4 (Shen et al. 2002). About 15 seeds of *B. pekinensis* (10 cultivars) and *B. chinensis* (10 cultivars) were sown in each pot. After germination, the seedlings were thinned to five plants per pot. The pots were watered daily to 65% of the field water capacity. Each cultivar was replicated three times. After 36 days growth under natural light, the shoots were cut, washed with tap water thoroughly, and then rinsed with de-ionized water. The samples of plants were dried at 80°C for 72 h. The dry weights of the plants were measured. The plant materials were ground in a carnelian mortar and were acid-digested with a mixture of HNO_3-$HClO_4$ (87:13 v/v). The concentrations of Cd in the solutions were determined by a flame atomic absorption spectrometer model TAS-986 (Purkinje General Co. Ltd., Beijing). A certified standard reference material (tomato leaves) of the National Institute of Standards and Technology, U.S.A., was used to verify the accuracy of metal analysis. Reagent blank and analytical duplicates were also used where appropriate to ensure accuracy and precision in the analysis.

Seeds of *B. pekinensis* (cv. Jiaobai-6, Qingyan 87-114, Xiakang-50, Xiawang) were surface-sterilized in 0.5% NaClO and rinsed thoroughly with de-ionized water, then germinated for 3 d. Afterwards, 20 seedlings were transferred to 2 dm^3 pots containing Hoagland nutrient solutions. The seedlings were grown in a green house under natural light for 30 days. The pH of nutrient solutions was adjusted to 6.0 with 1 mM HCl or 1 mM NaOH. Nutrient solutions were renewed every three days. The $CdCl_2$ was added into solution at the concentration of 0 and 50 μM. Each treatment was replicated three times. The shoots were harvested 7 d after the Cd treatment. The plant materials were washed, dried at 80 °C for 72 h and weighted, and then were ground in a carnelian mortar. The Cd concentrations in plants were determined with a flame atomic absorption spectrometer as previously described.

Data reported in this paper were the mean values based on the three replicate results. The one-way analyses of variance were calculated by the standard procedure using the statistical software SPSS 11.0 (SPSS Inc., California). The means were separated by Least Significant Difference (LSD) test, when the F-test was significant at $p \leq 0.05$.

RESULTS AND DISCULTION

Compared with *B. chinensis*, all cultivars of *B. pekinensis* had higher shoot dry matter yields during the 36 days of plant growth period (Figure 1). Among the 10 cultivars of *B. pekinensis*, the Xiawang had the highest shoot biomass, followed by the Qingyan, Xiakang-50 and Jiaobai-6. No significant differences were found among the 10 cultivars of *B. chinensis* in the shoot dry weight ($P > 0.05$).

There were significant differences in the concentration of Cd in the shoots among the plant species and cultivars (Table 1). The average concentrations of Cd in *B. pekinensis* and *B. chinensis* were 198 and 227 $\mu g\ g^{-1}$ DM, respectively. The maximum Cd concentration and total amount of Cd accumulation in shoots were obtained in cultivar Xiawang of *B. pekinensis*, which reached 255 $\mu g\ g^{-1}$ DM and 458 $\mu g\ pot^{-1}$ (1 kg soil), respectively. Noticeably, the average amount of Cd accumulation in the shoots of 10 *B. pekinensis* cultivars (265 $\mu g\ pot^{-1}$) was significantly higher than that in the 10 cultivars of *B. chinensis* (160 $\mu g\ pot^{-1}$).

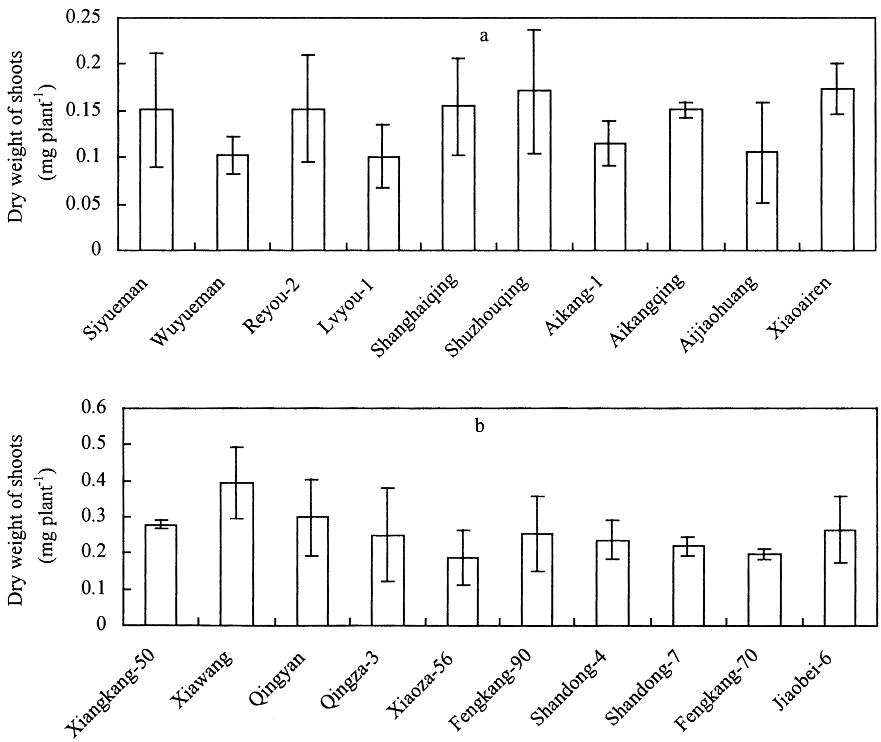

Figure 1. Shoot dry matter yields of different cultivars of *B. chinensis* (a) and *B. pekinensis* (b) grown in Cd-amended soil. Bars represent SEs of three replicates.

The treatment with 50 μmol L^{-1} Cd affected the growth of the plants and the shoot biomass. The Qingyan and Xiakang-50 cultivars showed relatively less response to the 50 μmol L^{-1} Cd treatment than the Jiaobai-6 and Xiawang cultivars (Figure 2). However, there were no significant differences of the shoot dry yields among the different cultivars ($P > 0.05$). The concentrations of Cd in the shoots of Xiakang-50, Xiawang and Qingyan reached 239, 216 and 219 μg g^{-1} (Figure 3),

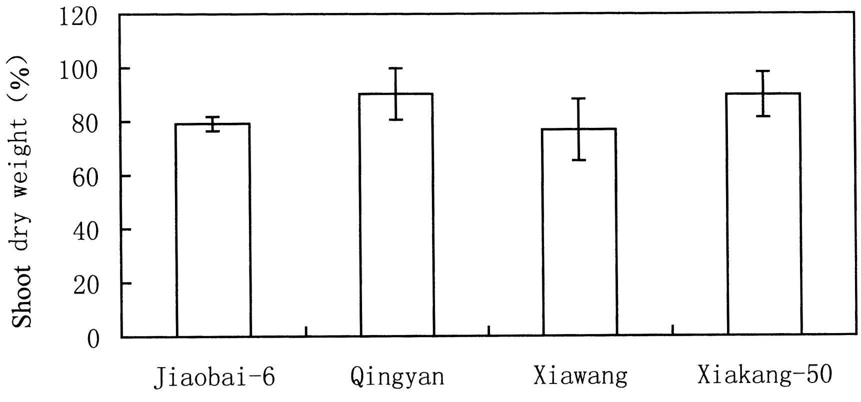

Figure 2. Effect of 50 μmol L^{-1} Cd on the shoot dry matter yields of four *B. pekinensis* cultivars in the hydroponic culture experiment. Bars represent SEs of three replicates. (The relative dry weight of the shoots = the dry weight of the shoots treated with 50 μmol L^{-1} Cd / the dry weight of the shoots in the control group × 100%)

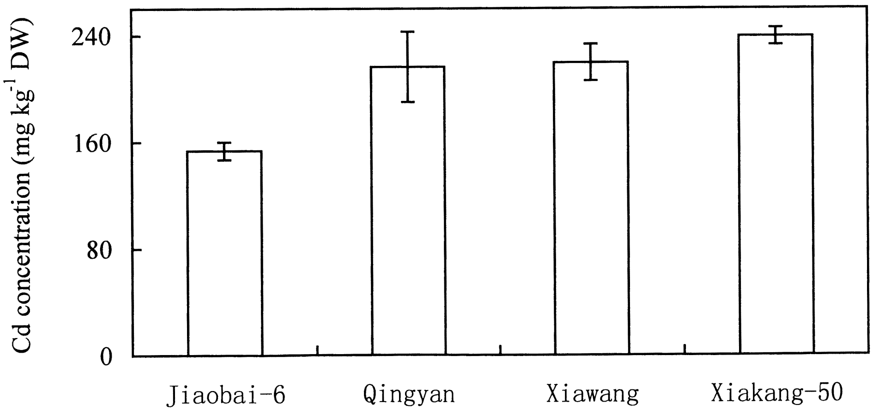

Figure 3. The concentrations of Cd in the shoots of four *B. pekinensis* cultivars treated with 50 μmol L^{-1} Cd. Bars represent SEs of three replicates.

respectively. The shoots of the Jiaobai-6 cultivar had significant lower Cd concentration than other three cultivars ($P < 0.05$), but was still able to accumulate more than 150 μg g^{-1} Cd in the shoots.

Leafy vegetables play an important role in well-balanced diets for human. The normal concentration of Cd in vegetable leaves ranged from 0.09 to 0.88 μg g^{-1} with a mean of 0.56 μg g^{-1} dry weight (McLaughlin et al. 1999). According to the recommendation of the National Research Council of the United Sates (1980), the concentration of Cd in forage crops should not be more than 0.5 μg g^{-1}. In previous studies, a few plant species have been found to accumulate Cd above 100 μg g^{-1} in shoot DM (Reeves and Baker, 2000; Liu et al. 2003; McGrath and Zhao, 2003; de la Rosa et al. 2004). Some members of the *Brassica* genus, such as *Brassica juncea*, *Brassica napus*, *Brassica oleracea*, are capable of accumulating high levels of Cd as well as other toxic metals in shoots (Kumar et al. 1995; Ebbs *et al.* 1997; Shen et al. 2002; Ru et al. 2004). In the present study, all cultivars of *B. chinensis* and *B. pekinensis* were found to accumulate more than 150 μg g^{-1} DM of Cd in their shoots (Table 1), indicating that these plants were able to take up Cd from contaminated soils and transport this metal to the shoots. This may pose potential health risks for people consuming these vegetables grown on metal contaminated soils. Furthermore, high levels of citric and malic acids were found in Brassicae vegetables, which would facilitate the absorption of minerals by human (Lucarini et al. 1999). Thus, stringent guidelines should be set up to reduce

Table 1. The uptake of Cd in the shoots of *B. chinensis* and *B. pekinensis* grown in Cd-amended soil.

B. chinensis			*B. pekinensis*		
Cultivar	Cd concentration	Cd uptake	Cultivar	Cd concentration	Cd uptake
Siyueman	216 bc	168 cd	Xiangkang-50	243 a	336 b
Wuyueman	196 bc	99.0 g	Xiawang	255 a	458 a
Reyou-2	244 ab	205 b	Qingyan	214 ab	355 b
Lvyou-1	273 a	126 ef	Qingza-3	245 a	306 c
Shanghaiqing	233 ab	184 bc	Xiaoza-56	209 ab	194 f
Shuzhouqing	225 bc	167 cd	Fengkang-90	157 c	193 e
Aikang-1	225 bc	117 fg	Shandong-4	220 ab	260 d
Aikangqing	181 c	156 de	Shandong-7	171 bc	187 f
Aijiaohuang	200 bc	143 de	Fengkang-70	154 c	155 g
Xiaoairen	278 a	242 a	Jiaobei-6	155 c	204 f

Means sharing the same letter within one column are not significantly different from each other (Duncan's multiple range test; $p < 0.01$), the unit of Cd concentration is (μg g^{-1}) and the unit of Cd uptake is (μg pot^{-1}).

metal concentration and the consumption of *Brassica* vegetables in metal contaminated areas. Plant breeding and agronomic management can minimize the accumulation of Cd in vegetables, but it remains important that inputs of metals to soil should be minimized, and the contaminated soil should be well controlled. On the other hand, the accumulation of metals by plants can be used to remove toxic heavy metals from contaminated soils. In the Brassicaceae family, several hyperaccumulators have been identified. Cd hyperaccumulator is defined as being able to accumulate more than 100 μg g^{-1} Cd in shoots (Baker and Brooks, 1989). Instead of this rather arbitrary value, the following common traits are now known to be shared by all hyperaccumulators: the bioconcentration factor is usually greater than 1; the shoot to root ratio of metal concentration is greater than 1; and the plants possess much enhanced tolerance to metals in the exposure medium and inside plant cells (McGrath and Zhao, 2003). Up to 10, 000 μg g^{-1} DM of Cd and a bioconcentration factor of 60 were reported in the shoots of *Thlaspi caerulescens*, a population from Southern France, without suffering significant phytotoxicity under hydroponic conditions (Lombi et al. 2000). In the present study, the maximum Cd concentrations in the shoots of *B. chinensis* (278 μg g^{-1}) and *B. pekinensis* (255 μg g^{-1}) (Table 1) were much lower than those in *T. caerulescens*, *A. halleri* (Küpper et al. 2000; Lombi et al. 2000), *Viola baoshanensis* (Liu et al. 2003), and *Salsola kali* (de la Rosa et al. 2004). However, the concentrations of Cd in the shoots of all cultivars investigated in both soil and solution culture experiments were still much higher than the criterion set by Baker and Brooks (1989). In addition, *B. chinensis* and *B. pekinensis* accumulated Cd in the shoots by a factor > 1.0 in comparison with the concentrations of Cd in the soil, although the shoot/root ratios of the concentration of Cd in *B. chinensis* and *B. pekinensis* were generally less than 0.1 in all the solution experiments (Liu et al. 2005). No visual symptoms of metal toxicity was found on the plants grown in Cd-contaminated soil and 50 μmol L^{-1} Cd solutions, indicating that these plants could be tolerant to high levels of Cd. More importantly, *B. chinensis* and *B. pekinensis* plants grow much more rapidly and are able to yield higher biomass in comparison with *T. caerulescens*. The current results showed that the amount of Cd phytoextracted in one crop cycle was 99-242 μg kg^{-1} soil (with an average of 160 μg kg^{-1} soil) by *B. chinensis* cultivars, and 155-458 μg kg^{-1} soil (with an average of 265 μg kg^{-1} soil) by *B. pekinensis* cultivars during the 36-day plant growth period. These values accounted for 0.099-0.242 % and 0.155-0.458 % of the total Cd in soil (100 mg kg^{-1}), respectively. The extraction efficiencies were comparable with the data reported by Kayser et al. (2000) for the Cd phytoextraction assisted with the application of nitrilotrotriacetate, and higher than the results reported by Ebbs et al. (1997) in six plant species including *T. caerulescens*, *B. juncea*, *B. rapa* and *B. napus* after six weeks of growth, and by Lehmann and Rebele (2004) in *Calamagrostis epigejos*. After three growing seasons for 28 months, only 0.11 to 0.25% of the total soil Cd was removed by *C.*

epigejos from soil and concentrated in above-ground living and dead biomass (Lehmann and Rebele, 2004). Ru et al. (2004) reported a maximum Cd uptake of 577 μg pot^{-1} (3 kg soil) by oilseed rape (*B. juncea*) shoots grown in a soil amended with 84 mg kg^{-1} Cd after 42 days of growth. In our previous experiments, 1.9 μg $g^{-1}$of Cd was measured in the shoots of *B. chinensis* (cv. Xinza No 1) grown in the soil containing 15 μg g^{-1} Cd, which was probably due to the high concentrations of Zn, Cu and Pb in the soil (Shen et al. 2002). It has been reported that high concentration of Zn can significantly reduce the Cd uptake by plants (Küpper et al. 2000). The uptake of Cd seems to be in competition for the same transmembrane carrier with other metal ions, such as Ca, Mg, Fe, Mn, Cu and Zn (Sanità di Toppi and Gabbrielli, 1999).

In conclusion, between the two species tested, *B. pekinensis* plants grew much more rapidly and were able to yield higher biomass in comparison with *B. chinensis*. The species *B. pekinensis* was also more tolerant to Cd than *B. chinensis*. Compared with the control, 10 to 50 μM Cd in solutions had no significant effect on the shoot dry matter (DM) yields of *B. pekinensis*, whereas the negative effect on *B. chinensis* was observed at 20 μM Cd and above (Liu et al. 2005). These results suggested that *B. pekinensis* might have higher potential for use in the remediation of metal-contaminated soils.

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