

## Evaluation of Cadmium Phytoremediation Potential in Chinese Cabbage Cultivars

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**ABSTRACT:** Selecting a phytoextraction plant with moderate to high Cd-accumulating ability and high biomass based on the plant's compatibility with mechanized cultivation techniques may yield more immediately practical results. In the present study, six Chinese cabbage cultivars were grown in three soils, ranging from 0.15 to 2.25 mg Cd kg<sup>-1</sup> soil, to examine uptake and translocation of Cd in their tissues. The results indicated that the order of the shoot Cd concentration values in the cultivars was as follows: Beijingxiaoza 56 > Suancaiwan > Quansheng and Qiubo 60 > Xianfengkuaicai and Chunkang. Similar order was also found in the bioaccumulation factor (BAF), translocation factor (TF), and metal extraction ratio (MER). Several soil Cd fractions after Beijingxiaoza 56 harvesting decreased most. Beijingxiaoza 56 is thus promising for phytoextraction of Cd from soils with low to moderate (<2.25 mg kg<sup>-1</sup>) Cd contamination.

**KEYWORDS:** Chinese cabbage (*Brassica pekinensis* L.), cadmium pollution, phytoextraction, irrigation

### INTRODUCTION

Cadmium (Cd), one of the most toxic heavy metals, has been released into natural and agricultural environments principally through anthropogenic activities.<sup>1,2</sup> In China, Cd has been a public concern due to its high toxicity to plants and human beings.<sup>3</sup> Previous studies have highlighted that, even at low level, soil Cd contamination could pose a significant risk to human health through the soil–crop–human exposure pathway.<sup>4</sup> Therefore, it is urgent to establish remediation programs not only for heavily Cd-contaminated sites but also for slightly or moderately Cd-contaminated sites.

In recent years, phytoremediation, defined as the use of plants to remove pollutants from the environment or to render them harmless, is attractive as it can provide a cost-effective, long-lasting, and aesthetic solution for remediation of contaminated sites.<sup>5</sup> One of the most effective strategies of phytoremediation is phytoextraction; that is, plants remove metals from the soil and concentrate them in the harvestable parts of the plants.<sup>6</sup> In the past decades, much interest has been focused on studies of hyperaccumulator plants capable of accumulating potentially phytotoxic elements to concentrations >100 times those found in nonaccumulators.<sup>7–9</sup> So far, more than 400 species of hyperaccumulators belonging to 45 families have been documented in the world, but others are still being sought worldwide.<sup>10</sup>

Although great progress has been made in the studies of phytoremediation, its application is still very limited due to low biomass of hyperaccumulators, unavailability of suitable plant species, and long growing seasons required.<sup>11</sup> The culturing of these hyperaccumulator species could be hampered by their susceptibility to certain diseases. Therefore, to maximize phytoextraction efficiency, it is important to select a fast-growing and high-biomass plant with high Cd-accumulating ability, which is also compatible with mechanized cultivation techniques and local weather conditions.<sup>12,13</sup> For example, it has been

documented that some crops such as maize (*Zea mays* L.), oat (*Avena sativa*), Indian mustard (*Brassica juncea*), and barley (*Hordeum vulgare*) can tolerate and accumulate relatively high concentrations of metals in soils.<sup>12,14,15</sup>

Interestingly, genotype and/or cultivar differences in the accumulation of metals has been confirmed in many plant species.<sup>16</sup> Cultivar differences in terms of metal uptake were observed in diploid and tetraploid chamomile (*Matricaria chamomilla* L.) cultivars; diploid plants accumulated higher amounts of Cd in both shoots and roots compared to tetraploid plants.<sup>17</sup> Our previous studies highlight that a significant cultivar difference ( $p < 0.05$ ) occurs in shoot Cd concentrations in Chinese cabbage (*Brassica pekinensis* L.), a leafy vegetable with a 1500-year history that is widely cultivated in China and other Asia countries.<sup>18,19</sup> However, no study on phytoextraction using Chinese cabbage has yet been reported. Chinese cabbage tends to be a Cd accumulator even under low-Cd treatment (1.0 mg kg<sup>-1</sup>), and the cultivation system for Chinese cabbage has been well established and highly mechanized in China.<sup>18,19</sup> Therefore, it is possible to screen out high Cd accumulating Chinese cabbage cultivars suitable for phytoextraction.

The purpose of this study is to select a promising cultivar for the phytoextraction of soils contaminated with relatively low concentrations of Cd based on a comparison of Cd uptake, bioaccumulation factor (BAF), and translocation factor (TF) in shoots of six Chinese cabbage cultivars. Simultaneously, changes of soil Cd fractions before sowing and after harvesting were also examined to explore the effect of plant growth on available Cd in soils.

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**Table 1.** Basic Physicochemical Properties in the Soils of Shenyang Station of Experimental Ecology (SSEE), Shenfu Irrigation Area (SIA), and Zhangshi Irrigation Area (ZIA)

	soil		
	SSEE	SIA	ZIA
location	41° 31' N, 123° 41' E	42° 35' N, 123° 55' E	41° 45' N, 122° 48' E
type	meadow brown	meadow brown	meadow brown
main source of contamination	none	wastewater from lead/zinc smelter	wastewater from petrochemical plant
pH	6.5 ± 0.7 <sup>a</sup>	5.8 ± 0.5	5.9 ± 0.6
CEC (cmol kg <sup>-1</sup> )	17.2 ± 3.1	16.3 ± 2.7	18.6 ± 1.4
total N (%)	0.84 ± 0.12	1.13 ± 0.16	1.05 ± 0.17
available P (mg kg <sup>-1</sup> )	0.32 ± 0.08	0.43 ± 0.06	0.45 ± 0.07
available K (mg kg <sup>-1</sup> )	11.74 ± 1.36	13.55 ± 1.24	12.68 ± 1.49
TOC (%)	1.45 ± 0.21	1.88 ± 0.26	1.79 ± 0.32
total Cu (mg kg <sup>-1</sup> )	69.4 ± 8.1	72.6 ± 9.7	116.8 ± 16.2
total Pb (mg kg <sup>-1</sup> )	36.4 ± 5.8	48.5 ± 6.6	79.7 ± 26.4
total Zn (mg kg <sup>-1</sup> )	74.1 ± 8.5	69.2 ± 9.7	185.4 ± 23.5
total Cd (mg kg <sup>-1</sup> )	0.15 ± 0.06	1.15 ± 0.12	2.25 ± 0.31

<sup>a</sup> Mean ± SD (*n* = 4).

## MATERIALS AND METHODS

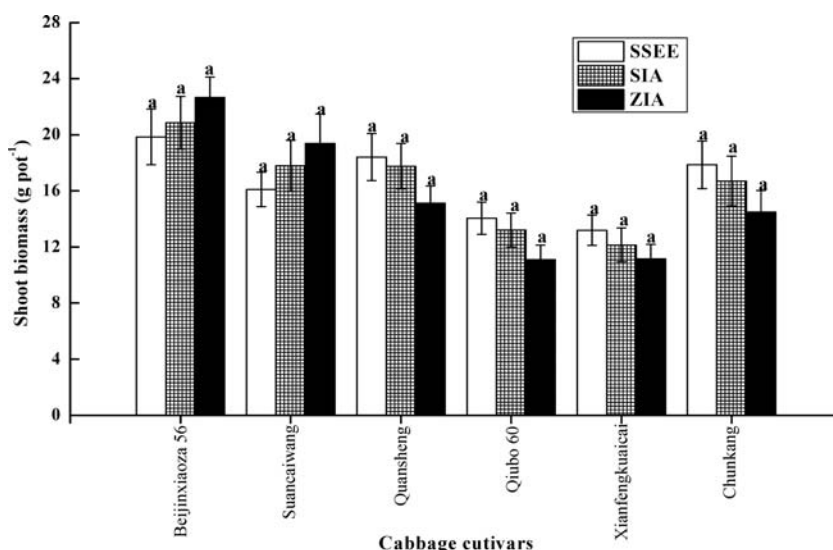
**Experimental Setup.** Soil samples were collected from sites chosen for their industrial activities and/or historical backgrounds in Shenyang, Liaoning province, China. The soils used in this pot experiment were collected from the surface (0–20 cm topsoil) of three fields without or with low to moderate Cd concentration in Shenyang District: Shenyang Station of Experimental Ecology (SSEE, without Cd pollution in soils) of Chinese Academy of Sciences (CAS), Shenfu Irrigation Area (SIA, with low Cd contamination), and Zhangshi Irrigation Area (ZIA, with moderate Cd contamination). The soils collected from the three sites are classified as meadow burozem. Each collected soil was air-dried at room temperature, crushed, and passed through a 2 mm sieve to remove rocks and undecomposed organic materials. Then 2.5 kg soil samples were placed in each plastic pot (20 cm in diameter and 15 cm in height). Basic physical and chemical properties of the collected soils were analyzed according to the routine analytical methods of agricultural chemistry in soil.<sup>20</sup> The details of the basic properties in soils are shown in Table 1. Moreover, Cd is known to interact with several elements in metal uptake.<sup>21</sup> It has been reported that nitrate deficiency reduces Cd accumulation in chamomile plants and that mineral nutrients are affected by a given metal rather than by N deficiency.<sup>22</sup> Thus, the copper (Cu), lead (Pb), and zinc (Zn) concentrations in the collected soils were also measured. Total metals in soils were determined by digestion with a 12 mL solution containing 87% of concentrated HNO<sub>3</sub> and 13% of concentrated HClO<sub>4</sub> (v/v).<sup>19</sup>

Six Chinese cabbage cultivars, that is, Beijingxiaozha 56, Suancai wang, Quansheng, Qjubo 60, Xianfengkuaicai, and Chunkang, were tested in the pot experiment due to their relatively high Cd accumulation in shoot according to our previous studies.<sup>18,19</sup> Seeds of these cultivars were collected from a seed company from the Shenyang Agricultural University, Shenyang City, Liaoning Province, China. First, seeds were sterilized in 2% (v/v) hydrogen peroxide for 10 min, washed several times with distilled water, and then soaked in water overnight. Subsequently, they were sowed directly into plastic pots. The pot experiment followed a randomized block design, with four replicates per soil–cultivar treatment. The experiment was carried out in a greenhouse illuminated with natural light, and no fertilizers were applied. The experiment was performed under the following conditions: 14 h (28 °C)/10 h (15 °C) day/night cycle; and relative humidity, ~50%. The moisture content in the soil was maintained at 75% of the field

water-holding capacity using tap water (no Cd detected), and a Petri dish was placed under each pot to collect potential leachate during the experiment. Two weeks after sowing, the seedlings were thinned to two plants per pot. The plants were harvested for analysis after 9 weeks of the growth.

**Soil and Plant Analysis.** To identify which soil Cd fractions were decreased by plant growth, a Cd analysis of the soils was conducted using a single-extraction method with 0.01 and 0.1 mol L<sup>-1</sup> HCl (1:5 w/v, 1 h shaking side-by-side) and a sequential extraction method.<sup>12,23</sup> The soil Cd fractions determined by this sequential extraction method were as follows: exchangeable Cd fraction, extracted with 0.05 mol L<sup>-1</sup> Ca(NO<sub>3</sub>)<sub>2</sub> (1:10 w/v, 24 h shaking); inorganically bound Cd fraction, extracted with 2.5% acetic acid (1:10 w/v, 24 h shaking) from the residue of the exchangeable Cd fraction; organically bound Cd fraction, extracted with 2.5% acetic acid (1:10 w/v, 24 h shaking) after decomposition of organic matter with 6% H<sub>2</sub>O<sub>2</sub> from the residue of the inorganically bound Cd fraction; and oxide-occluded Cd fraction, extracted with a mixture of 0.1 mol L<sup>-1</sup> H<sub>2</sub>C<sub>2</sub>O<sub>4</sub> and 0.175 mol L<sup>-1</sup> (NH<sub>4</sub>)<sub>2</sub>C<sub>2</sub>O<sub>4</sub> (1:30 w/v) in a boiling water bath for 1 h, with occasional stirring, from the residue of the organically bound Cd fraction.<sup>12</sup>

At 63 days after sowing, the shoots of all plants were harvested by cutting the stems approximately 1 cm above the soil. After the shoots had been harvested, the roots were carefully removed from the soil, and then the soil from each pot was separately air-dried and passed through a 2 mm sieve. Roots and shoots of harvested cabbage samples were rinsed with tap water to remove soil and then carefully washed with deionized water for approximately 3 min.<sup>24</sup> The samples were then dried at 105 °C for 5 min and then at 70 °C in an oven until completely dry. Then the shoot biomass was measured according to the shoot dry weight by use of an analytical balance. The plant samples (0.50 g) and soil samples (0.50 g) were digested with a 12 mL solution containing 87% of concentrated HNO<sub>3</sub> and 13% of concentrated HClO<sub>4</sub> (v/v).<sup>19</sup> The concentrations of heavy metals in digested solutions were determined using an atomic absorption spectrophotometer (AAS, Hitachi 180-80). The detection limit for the heavy metal analysis is 0.01 mg kg<sup>-1</sup>. A certified reference material, bush leaf material (GBW07603, Qinghai Province, China), was used to monitor the recovery of metals from the plant samples. The geochemistry standard samples were used in this study to validate the soil analyses. The recovery rates for the certified references, material bush leaf material and geochemistry standard samples, were 90–105 and 95–104%, respectively.



**Figure 1.** Shoot biomass of six cultivars in the soils of Shenyang Station of Experimental Ecology (SSEE), Shenfu Irrigation Area (SIA), and Zhangshi Irrigation Area (ZIA). Means in the same group with the same letter are not significantly different at  $p < 0.05$  by Tukey's multiple-range test. Error bars show standard error ( $n = 4$ ).

**Data Processing and Statistical Analysis.** All treatments were replicated four times in the experiment. Data were analyzed with statistical package SPSS 13.0, Origin 8.5, and Excel 2003 for Windows. ANOVA was conducted for all data, and Duncan's multiple comparison was used to test the difference between genotypes or Cd levels to evaluate whether the means were significantly different, taking  $p < 0.05$  and 0.01 as significant. All results were expressed on a dry weight basis.

## RESULTS

**Cd Tolerance of the Six Chinese Cabbage Cultivars.** The Cd tolerance of plants can be measured by their variation in shoot biomass response to Cd toxicity (compared with control).<sup>18,19</sup> In this study, the shoot biomass of six cultivars in the soils of SIA and ZIA did not decrease significantly ( $p > 0.05$ ) compared with those in SSEE (control) (Figure 1). Moreover, the shoot biomass of Beijingxiaoza 56 and Suancaiwang even increased in soils of SIA and ZIA. Similarly, no visual phytotoxicity symptoms or yield reduction were found in the tested cultivars. Therefore, the six Chinese cabbage cultivars had considerable tolerance to Cd toxicity.

**Cd Concentration of the Six Chinese Cabbage Cultivars.** Significant differences ( $p < 0.05$ ) in shoot Cd concentrations were observed among the six Chinese cabbage cultivars, which were tested in soils collected from SSEE, SIA, and ZIA (Figure 2). The Cd concentrations in cultivar shoots ranged from 0.16 to 0.46 mg kg<sup>-1</sup>, from 1.12 to 5.15 mg kg<sup>-1</sup>, and from 2.86 to 11.78 mg kg<sup>-1</sup>, with average values 0.28, 2.90, and 6.27 mg kg<sup>-1</sup>, respectively. Similarly, there were significant differences ( $p < 0.05$ ) in root Cd concentrations among the six Chinese cabbage cultivars in the three soils (Figure 2). For each soil, the order of the shoot Cd concentration values in the cultivars was as follows: Beijingxiaoza 56 > Suancaiwang > Quansheng and Qiubo 60 > Xianfengkuaicai and Chunkang. The shoot Cd concentrations of Beijingxiaoza 56 and Suancaiwang were significantly higher than those of the other cultivars in each soil ( $p < 0.05$ ). In contrast, the root Cd concentrations of Beijingxiaoza 56 and Suancaiwang were lowest in each soil.

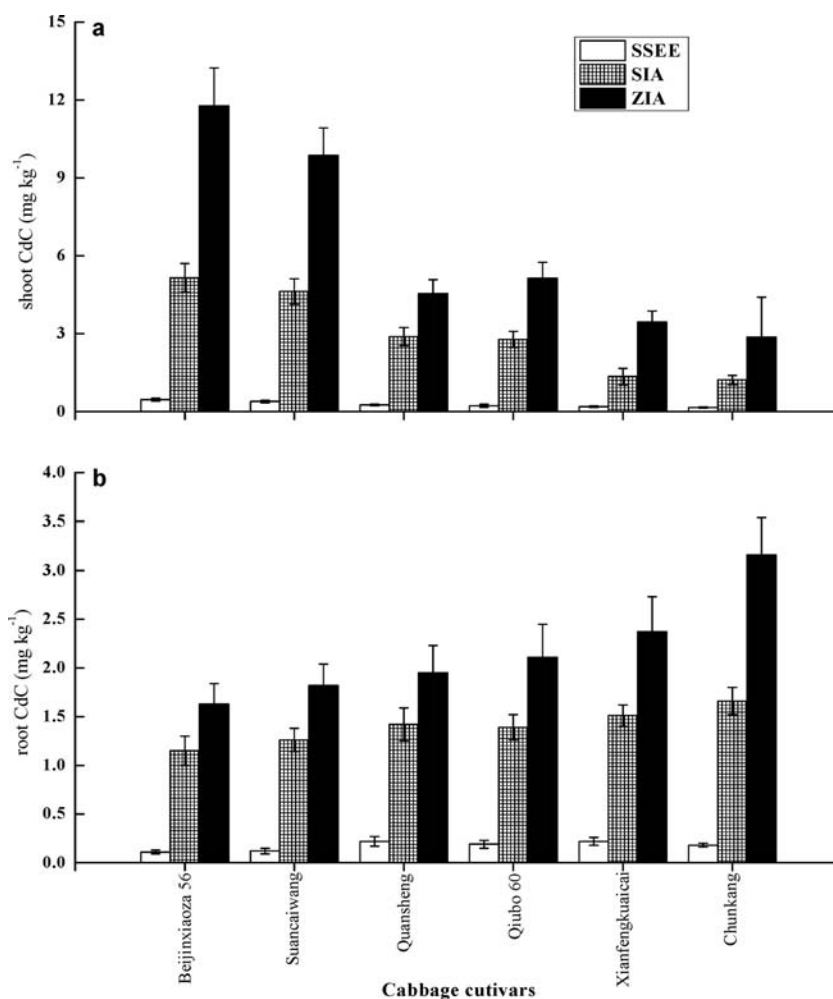
**Bioaccumulation Factor and Translocation Factor of the Six Chinese Cabbage Cultivars.** The BAF can be used to

evaluate the ability of plants to accumulate heavy metals. It is calculated as the ratio of the Cd concentration in shoots to the Cd concentration in soils.<sup>25</sup> BAF > 1.0 and TF > 1.0 were considered as two critical standards for the selection of hyperaccumulators. In the present experiment, the average values of BAF in the six cabbage cultivars all exceeded 1.0, with significant difference among different cultivars (Table 2). For each soil, Beijingxiaoza 56 had the highest BAF values, showing high efficient accumulation of Cd from soil by this plant.

The TF of heavy metals can be used to evaluate the capacity of plants to translocate heavy metals from roots to shoots. It is calculated as the ratio of the Cd concentration in plant shoots to the Cd concentration in plant roots.<sup>26</sup> Table 2 shows the average values of TF of the six Chinese cabbage cultivars in soils of SSEE, SIA, and ZIA, ranging from 0.89 to 4.18, from 0.82 to 4.28, and from 0.91 to 7.23, respectively. Similar to the BAF, the highest TF values in each soil were found for Beijingxiaoza 56.

**Cd Concentrations in Soil Fractions.** The soil pH and Cd concentration values of the seven Cd fractions (exchangeable, inorganically bound, organically bound, oxide occluded, 0.01 mol L<sup>-1</sup> HCl extractable, 0.1 mol L<sup>-1</sup> HCl extractable, and total) in SIA and ZIA before sowing and after harvesting are shown in Tables 3 and 4, respectively. In the soils of SIA, the soil pH after harvesting did not vary significantly ( $p > 0.05$ ) compared with that before sowing (Table 3). For soil Cd concentrations determined by means of sequential extraction, we found significant ( $p < 0.05$ ) decreases in each cultivar's soil after harvesting compared with the control (before sowing) in every fraction. The most decreased Cd concentration fraction in the soils of each cultivar was as follows: the exchangeable fraction, the exchangeable and inorganically bound fractions for Beijingxiaoza 56; and the oxide occluded fraction for Beijingxiaoza 56 and Suancaiwang. The postharvesting soil Cd concentrations by single extraction (0.01 mol L<sup>-1</sup> HCl and 0.1 mol L<sup>-1</sup> HCl) and the total Cd decreased in the following order: Beijingxiaoza 56 > Suancaiwang > Quansheng and Qiubo 60 > Xianfengkuaicai and Chunkang (Table 3).

In the soils of ZIA, we did not find significant changes in soil pH between before sowing and after harvesting (Table 4). For



**Figure 2.** Cd concentration (CdC) in shoot (a) and root (b) of six cultivars grown in soils of Shenyang Station of Experimental Ecology (SSEE), Shenfu Irrigation Area (SIA), and Zhangshi Irrigation Area (ZIA).

**Table 2. Bioaccumulation Factor (BAF) and Translocation Factor (TF) in Six Cultivars Grown in Soils of Shenyang Station of Experimental Ecology (SSEE), Shenfu Irrigation Area (SIA), and Zhangshi Irrigation Area (ZIA)**

cultivar	BAF			TF		
	SSEE	SIA	ZIA	SSEE	SIA	ZIA
Beijingxiaoza 56	3.07 ± 0.32 a <sup>a</sup>	4.48 ± 0.52 a	5.24 ± 0.61 a	4.18 ± 0.42 a	4.48 ± 0.45 a	7.23 ± 0.71 a
Suancai wang	2.60 ± 0.25 a	4.03 ± 0.43 a	4.39 ± 0.42 a	3.25 ± 0.31 a	3.67 ± 0.32 a	5.42 ± 0.53 a
Quansheng	1.73 ± 0.16 b	2.51 ± 0.26 b	2.02 ± 0.23 b	1.18 ± 0.12 b	2.04 ± 0.23 b	2.33 ± 0.22 b
Qjubo 60	1.53 ± 0.14 b	1.98 ± 0.21 b	2.28 ± 0.24 b	1.21 ± 0.13 b	1.64 ± 0.15 c	2.43 ± 0.26 b
Xianfengkuaicai	1.27 ± 0.13 bc	1.17 ± 0.12 c	1.53 ± 0.15 c	0.86 ± 0.11 c	1.22 ± 0.12 c	1.46 ± 0.13 c
Chunkang	1.07 ± 0.12 c	1.06 ± 0.11 c	1.27 ± 0.12 c	0.89 ± 0.12 c	0.82 ± 0.09 d	0.91 ± 0.11 c

<sup>a</sup> Mean ± SD ( $n = 4$ ). Means in the same column followed by the same letter are not significantly different at  $p < 0.05$  based on Duncan's multiple-comparison test.

soil Cd in the exchangeable fraction, significant ( $p < 0.05$ ) difference occurred among cultivars. For the other three fractions, no significant differences were found among cultivars. Moreover, significant ( $p < 0.05$ ) decreases were found in each cultivar's soil after harvesting compared with the control (before sowing) in every fraction. Similarly, the postharvesting soil Cd concentrations by single extraction (0.01 mol L<sup>-1</sup> HCl and 0.1 mol L<sup>-1</sup> HCl) and the total Cd also decreased in the following order:

Beijingxiaoza 56 > Suancai wang > Quansheng and Qjubo 60 > Xianfengkuaicai and Chunkang (Table 4).

## DISCUSSION

For many years, the capacity of plants to accumulate metals in their tissues was considered to be a detrimental trait. Indeed, being at the bottom of many food chains, metal-accumulating plants are



**Table 3. pH and Cd Concentration (CdC) in Seven Fractions in Soil of Shenfu Irrigation Area (SIA) before Sowing and after Harvesting**

treatment	pH	CdC by sequential extraction (mg kg <sup>-1</sup> )				CdC by single extraction (mg kg <sup>-1</sup> )		total CdC (mg kg <sup>-1</sup> )
		exchangeable	inorganically bound	organically bound	oxide occluded	0.01 mol/L HCl	0.1 mol/L HCl	
before sowing	5.8 ± 0.5 a <sup>a</sup>	0.25 ± 0.03 a	0.16 ± 0.05 a	0.18 ± 0.05 a	0.12 ± 0.02 a	0.23 ± 0.05 a	0.53 ± 0.05 a	1.15 ± 0.05 a
after harvesting								
Beijingxiaoza 56	5.5 ± 0.4 a	0.06 ± 0.00 c	0.08 ± 0.00 bc	0.09 ± 0.01 b	0.05 ± 0.01 b	0.09 ± 0.01 c	0.20 ± 0.05 c	0.95 ± 0.05 b
Suancaiwan	5.5 ± 0.5 a	0.08 ± 0.01 c	0.09 ± 0.01 bc	0.11 ± 0.01 b	0.05 ± 0.01 b	0.11 ± 0.02 bc	0.23 ± 0.05 c	0.99 ± 0.05 b
Quansheng	5.7 ± 0.6 a	0.12 ± 0.02 b	0.11 ± 0.00 b	0.13 ± 0.02 ab	0.07 ± 0.02 ab	0.13 ± 0.02 b	0.28 ± 0.05 bc	1.06 ± 0.05 ab
Qjubo 60	5.8 ± 0.6 a	0.14 ± 0.01 b	0.11 ± 0.02 b	0.14 ± 0.03 ab	0.07 ± 0.03 ab	0.14 ± 0.01 b	0.27 ± 0.05 bc	1.06 ± 0.05 ab
Xianfengkuaicai	5.7 ± 0.7 a	0.15 ± 0.02 b	0.12 ± 0.01 b	0.15 ± 0.02 ab	0.09 ± 0.02 ab	0.16 ± 0.02 b	0.34 ± 0.05 b	1.12 ± 0.05 a
Chunkang	5.8 ± 0.3 a	0.17 ± 0.03 b	0.12 ± 0.02 b	0.15 ± 0.03 ab	0.09 ± 0.01 ab	0.17 ± 0.04 b	0.33 ± 0.05 b	1.12 ± 0.05 a

<sup>a</sup> Mean ± SD ( $n = 4$ ). Means in the same column followed by the same letter are not significantly different at  $p < 0.05$  based on Duncan's multiple-comparison test.

**Table 4. pH and Cd Concentration (CdC) in Seven Fractions in Soil of Zhangshi Irrigation Area (ZIA) before Sowing and after Harvesting**

treatment	pH	CdC by sequential extraction (mg kg <sup>-1</sup> )				CdC by single extraction (mg kg <sup>-1</sup> )		total CdC (mg kg <sup>-1</sup> )
		exchangeable	inorganically bound	organically bound	oxide occluded	0.01 mol/L HCl	0.1 mol/L HCl	
before sowing	5.9 ± 0.5 a <sup>a</sup>	0.57 ± 0.05 a	0.50 ± 0.05 a	0.45 ± 0.04 a	0.22 ± 0.02 a	0.35 ± 0.03 a	1.34 ± 0.16 a	2.25 ± 0.25 a
after harvesting								
Beijingxiaoza 56	5.6 ± 0.4 a	0.18 ± 0.03 c	0.33 ± 0.04 b	0.27 ± 0.03 c	0.18 ± 0.01 a	0.11 ± 0.00 a	0.88 ± 0.08 c	1.96 ± 0.19 b
Suancaiwan	5.6 ± 0.5 a	0.23 ± 0.04 c	0.36 ± 0.03 b	0.33 ± 0.03 bc	0.22 ± 0.02 a	0.13 ± 0.01 a	0.93 ± 0.09 c	1.99 ± 0.22 b
Quansheng	5.9 ± 0.6 a	0.33 ± 0.03 b	0.41 ± 0.05 ab	0.29 ± 0.02 c	0.20 ± 0.01 a	0.18 ± 0.01 a	0.96 ± 0.08 c	2.17 ± 0.24 a
Qjubo 60	5.9 ± 0.6 a	0.37 ± 0.02 b	0.43 ± 0.04 ab	0.34 ± 0.03 b	0.22 ± 0.03 a	0.20 ± 0.02 a	0.95 ± 0.07 c	2.18 ± 0.22 a
Xianfengkuaicai	5.8 ± 0.7 a	0.39 ± 0.04 b	0.49 ± 0.05 a	0.46 ± 0.05 a	0.23 ± 0.02 a	0.25 ± 0.03 a	1.07 ± 0.12 b	2.21 ± 0.23 a
Chunkang	5.8 ± 0.3 a	0.40 ± 0.03 b	0.47 ± 0.05 a	0.45 ± 0.04 a	0.22 ± 0.02 a	0.23 ± 0.02 a	1.09 ± 0.15 b	2.22 ± 0.21 a

<sup>a</sup> Mean ± SD ( $n = 4$ ). Means in the same column followed by the same letter are not significantly different at  $p < 0.05$  based on Duncan's multiple-comparison test.

directly or indirectly responsible for a large proportion of the dietary uptake of toxic metals by animals and human beings.<sup>13</sup> Only in recent years have fast-growing, high-biomass crop plant species that accumulate moderate levels of metals in their shoots been actively tested for their metal phytoremediation potential. Interestingly, some of these fast-growing, high-biomass crop plant species are known to display a significant heavy metal tolerance, particularly those from the genus *Brassica*.<sup>6,13</sup> In this study, the six Chinese cabbage cultivars did not show significant decreases in shoot biomass when exposed to Cd stress. The shoot biomass of Beijingxiaoza 56 and Suancaiwan even increased when they were cultured in SIA and ZIA (compared with in SSEE), showing high tolerance to Cd toxicity, which is similar to our previous results.<sup>18</sup> Similar positive and neutral responses of biomass to Cd stress have also been found in a wide range of species.<sup>19</sup> Although the durations of the experiments were identical, the values of shoot biomass in this experiment are nearly 2 times higher than those found in our earlier study,<sup>19</sup> which may be caused by variation of the experiment condition and the soil source. The experiment was carried out in greenhouse condition, whereas the previous experiment was performed under open field condition.

Moreover, the phytoextraction performance of cultivars varies significantly depending on the screening method used.<sup>13</sup> Data

from hydroponic experiment and pot experiments using spiked toxic elements cannot be directly extrapolated to real field conditions, as some studies revealed that Cd is more available in spiked soils than in natural aging soils. Therefore, soils used in this study were collected from natural contaminated sites to imitate real field conditions.

All plants take up metals to a certain extent.<sup>26</sup> It was reported that plant uptake of heavy metals from soil occurs either passively with the mass flow of water into the roots or through active transport across the plasma membrane of root epidermal cells.<sup>24</sup> Metal concentrations in plants vary with plant species and genotypes.<sup>16</sup> In this study, significant difference ( $p < 0.05$ ) occurred in shoot Cd concentration among the six Chinese cabbage cultivars. Beijingxiaoza 56 showed the highest Cd uptake potential, and the shoot Cd concentration in Beijingxiaoza 56 was up to 2.88–4.60-fold higher than that in Chunkang in each soil (Figure 2). BAF and TF are two basic standards to evaluate Cd accumulation and translocation efficiency in plants. Meanwhile, Beijingxiaoza 56 had the highest TF and BAF values among the six Chinese cabbage cultivars in each soil, ranging from 3.07 to 5.24 and from 4.18 to 7.23, respectively (Table 2).

The plant effective number (PEN) has been applied to evaluate the ability of remedying contaminated soils by plants.

**Table 5. Metal Extraction Ratio (MER) and Plant Effective Number (PEN) of Six Chinese Cabbage Cultivars in the Soils of Shenfu Irrigation Area (SIA) and Zhangshi Irrigation Area (ZIA)**

cultivar	MER (%)		PEN (shoots)	
	SIA	ZIA	SIA	ZIA
Beijingxiaoza 56	0.037	0.048	9308.47	3744.58
Suancaiawang	0.029	0.034	12133.86	5225.23
Quansheng	0.018	0.012	19472.19	14558.12
Qjubo 60	0.011	0.010	33201.85	17569.34
Xianfengkuaicai	0.006	0.007	60966.32	26019.31
Chunkang	0.007	0.007	53432.50	24130.46

PEN is defined as the number of plants needed to extract 1.0 g of metal when the biomass of shoots and of total plants is considered.<sup>27</sup> To remove 1.0 g of Cd from soils of SIA and ZIA, more than 9308.47 and 3744.58 shoots of Beijingxiaoza 56 would be needed, respectively (Table 5). An evaluation of the extraction capacity should take into account the depth of the rooting zone, the density of the soil, and the biomass production and mortality of the aboveground biomass components harvested. Expressing the extraction capacity as a ratio that takes into account the produced biomass and the soil volume to be cleaned would be more informative.<sup>28</sup> Therefore, the metal extraction ratio (MER) is proposed and defined as the ratio of metal accumulation in the shoots to that in soil, which is calculated as

$$MER = (C_{\text{plant}} \times M_{\text{plant}} / C_{\text{soil}} \times M_{\text{rootedzone}}) \times 100\%$$

where  $C_{\text{plant}}$  is the metal concentration in the harvested component of the plant biomass,  $M_{\text{plant}}$  is the mass of the harvestable aboveground biomass produced in one harvest,  $C_{\text{soil}}$  is the metal concentration in the soil volume, and  $M_{\text{rootedzone}}$  is the mass of the soil volume rooted by the species under study.<sup>28</sup> The MER decreased in the following order: Beijingxiaoza 56 > Suancaiawang > Quansheng > Qjubo 60 > Chunkang > Xianfengkuaicai. The MER of Cd for Beijingxiaoza 56 in soils of SIA and ZIA were 0.037 and 0.048%, respectively (Table 5), which is close to a reported MER of Cd for a Cd hyperaccumulator, *Rorippa globosa*.<sup>29</sup> These results indicate that Beijingxiaoza 56 is a promising cultivar for phytoextraction of Cd from contaminated soils.

The results of metal fractions in soils are useful for obtaining information about the origin, mode of occurrence, and bioavailability of these elements.<sup>30</sup> Several metal pools may be selectively affected by plant uptake, and changes in their relative proportions may provide insights into the mechanisms responsible for uptake of metals.<sup>31</sup> In the selection of a suitable plant for phytoextraction, it is thus necessary to assess the effects of potential species on several metal fractions and the potential of these plants to mobilize and deplete metals from these fractions.<sup>12</sup> In the present study, the soil Cd fractions decreased by plant cultivation were the exchangeable, inorganically bound, organically bound, and oxide occluded fractions in soil of SIA and the exchangeable, inorganically bound, and organically bound fractions in soil of ZIA (Tables 3 and 4), which are similar to a former study by Murakami et al.<sup>12</sup> The soil Cd concentrations in the 0.1 mol L<sup>-1</sup> HCl extractable fraction equaled 90% (in soil of SIA) and 88% (in soil of ZIA) of the sum of the exchangeable, inorganically bound, and organically bound fractions. The result suggests that the 0.1 mol L<sup>-1</sup> HCl extraction is a useful diagnostic

measure for evaluating the decrease in soil Cd concentration caused by crop cultivation. The 0.1 mol L<sup>-1</sup> HCl extraction also decreased in the following order: Beijingxiaoza 56 > Suancaiawang > Quansheng and Qjubo 60 > Xianfengkuaicai and Chunkang (Tables 3 and 4), which indirectly indicated that Beijingxiaoza 56 is a suitable cultivar for phytoextraction. Several studies show that plant-root exudates can lower soil pH in the rhizosphere and thus can affect metal bioavailability.<sup>13</sup> In this study, the cultivation of Chinese cabbage did not affect pH significantly ( $p > 0.05$ ) in each soil, but the cultivation of Beijingxiaoza 56 and Suancaiawang does lower soil pH to some extent.

The results mentioned above show that the cultivar Beijingxiaoza 56 is suitable to phytoextract Cd in slightly to moderately contaminated soils. Moreover, the cultivation systems for Chinese cabbage are well established and highly mechanized in China, which will promote the use of Beijingxiaoza 56 for phytoextraction. Soil amendments have been reported in the literature that could render soil trace metals more phytoavailable, among which ethylenediaminetetraacetate (EDTA) and ethylenediaminedisuccinate (EDDS) have been proved to be highly efficient.<sup>32,33</sup> Thus, the combination of Beijingxiaoza 56 with use of soil amendments may be more efficient in phytoremediation, which will be studied in our subsequent research. However, a field trial is needed to test the phytoremediation efficiency in future studies. Recently, it was reported for the first time that soluble phenols may be involved in Cd shoot-to-root translocation and that 2-aminoindane-2-phosphonic acid (AIP), a potent phenylalanine ammonia-lyase (PAL) inhibitor, plays an important role in the accumulation of Cd in chamomile (*M. chamomilla*).<sup>34,35</sup> The effects of endogenous metabolites on the uptake of Cd in Beijingxiaoza 56 also need to be explored in future work.

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