Accumulation and subcellular distribution of cadmium in ramie (*Boehmeria nivea* L. Gaud.) planted on elevated soil cadmium contents

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ABSTRACT

The tolerance, accumulation and subcellular distribution characteristics of cadmium (Cd) in ramie (*Boehmeria nivea* L. Gaud.) were investigated using a 2-year field experiment. The results indicated that ramie has a certain extent of tolerance to soil Cd (≤ 20 mg/kg) contamination with no significant decrease in shoot biomass and fibre yield relative to control conditions. Although ramie did not hyperaccumulate Cd, it accumulated considerable amount of Cd in the aboveground parts (approximately 0.19 to 1.09 kg/ha annually). The Cd contents retained in ramie tissues were found in order of roots > stems > leaves. Further, regarding the subcellular distribution of Cd in ramie tissues, 80% of the total Cd was bound to the cell walls of the roots and stems, whereas in leaves the proportion of Cd stored in the cell wall fraction was around 60% and a lesser amount of Cd was stored in the soluble fraction (24.1–25.5%). Our collective results indicated that ramie adapts to Cd stress via the store of a large amount of Cd in cell walls, and suggested potential usefulness of ramie in the phytoremediation of Cd-contaminated farmlands.

Keywords: Cd; phytoremediation; cell wall; field experiment

Elevated cadmium (Cd) levels in agricultural soils, resulting from mining activities, industrial emissions and the application of sewage sludge or phosphorus fertiliser, are becoming a major environmental problem due to the high toxicity of Cd and its mobility from soil to plants and therefore into the food chain (Zhu et al. 2012). In China, approximately 2.8×10^5 ha of farmland is contaminated with Cd (State Environmental Protection Agency 2003). However, the majority of conventional remediation approaches do not provide acceptable solutions to soil Cd pollution. Phytoremediation is regarded as an alternative technology to remove Cd from contaminated soils, and many studies focus on hyperaccumulator plants (Baker and Brooks 1989, Kacálková et al. 2009). Unfortunately, many known hyperaccumulators exhibit slow growth rate and small biomass, leading to limited usefulness for remediation of Cd-contaminated soils (Wang et al. 2008). Therefore, high-biomass plants that are characterized by high content of Cd may be useful due to their high biomass.

Ramie (*Boehmeria nivea*) is a perennial herb plant with high shoot biomass (more than three crops per year) and strong root system (Yang et al. 2010). As an industrial crop, ramie has been widely cultivated in southern China for more than 5000 years, and ramie fibre is mainly used for textile materials due to its fine characteristics. Yang et al. (2010) studied the tolerance and accumulation of heavy metals in 8 populations and 2 germplasms of ramie using a field survey and hydroponic experiments. In addition, She et al. (2011) conducted a field micro-plot experiment and investigated the ac-

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cumulation of Cd in the aboveground parts of 9 ramie cultivars under Cd stress. Various cultivars of ramie, such as Zhongzhu-1, were found to possess a certain extent of Cd tolerance (Yang et al. 2010, Zhou et al. 2010, She et al. 2011). Liu et al. (2007) and Wang et al. (2008) investigated the effects of Cd stress on oxidative stress, the ascorbate-glutathione cycle, Cd accumulation, the chemical speciation of Cd and the subcellular distribution of Cd in ramie using short term hydroponic experiments. They found that the protective mechanisms acquired by ramie play an important role in Cd detoxification at relatively low Cd concentrations (≤ 3 mg/L Cd) but become restricted to maintaining internal homeostasis at higher levels of Cd stress (7 mg/L Cd). Previous studies indicated that ramie could be an economically ideal crop for use in the phytoremediation of Cd polluted areas. This present work describes a 2-year field micro-plot trail designed and conducted to explore the effects of different concentration of Cd on the growth, Cd accumulation and subcellular distribution of Cd in ramie under field conditions.

MATERIAL AND METHODS

Field experiment and sampling. The experiment was conducted in Zhuzhou city, Hunan province, China (27°49'N, 113°01'E). This area is located in a subtropical zone with a humid monsoon climate. The soil is Acrisol derived from Quaternary red clay and was contaminated by Cd due to waste water discharged from a nearby electroplating factory (ceased operation in April 2006). Basic characteristics of the tested soil (0–20 cm) were as follows: pH, 5.23; organic matter, 25.8 g/kg (humic acid, 2.7 g/kg and fulvic acid, 7.4 g/kg), total N, 2.46 g/kg; available P, 7.0 mg/kg; available K, 152.0 mg/kg; and total Cd, 1.91 mg/kg. Based on the Langmuir equation, the calculated maximum sorption capacity was 625.0 mg/kg for the tested soil ($R = 0.99$, $P < 0.01$).

The experimental area was divided into micro-plots using cement to a size of 100 × 100 × 100 cm. Cadmium treatments were started in February 2008. Briefly, a $CdCl_2 \cdot H_2O$ solution was first added to approximately 2 kg of soil and then mixed into the upper layer of soil (0–30 cm) in the micro-plot. Soil Cd was prepared in triplicate for 6 Cd pollution levels at 0 (T0); 10 (T1); 20 (T2); 35 (T3); 65 (T4), and 100 (T5) mg/kg. The actual Cd contents of the soil (0–20 cm) of T0, T1, T2, T3, T4 and T5 measured 1 week prior to cultivation were 1.91 ± 0.02, 11.44 ± 0.58, 19.57 ± 0.69, 33.28 ± 2.10, 60.81 ± 1.79 and 94.41 ± 3.52 mg/kg, respectively. Two months after the Cd addition, six ramie plants were planted in each micro-plot at a density of 30 × 55 cm.

A cultivar named Zhongzhu-1 originating from the Hunan province was tested in this study. Routine cultivation, fertilisation and winter care were performed, and the ramie plants were cut to stalks in July 2008 and harvested twice in October 2008 and May 2009, respectively. At the time of harvest, we recorded the fresh weights of the shoots and fibres (raw textile materials) in each plot and measured the water contents by oven-drying. The aboveground parts of ramie were sampled as shoots and fibres at the first harvest (stems were separated into fibres and residues using a raspador), and were sampled as leaves, bark, sticks and fibres at the second harvest (stems were firstly separated into bast and sticks by hand, and then the bast was separated into fibres and bark with a blade by hand). The samples were dried at 65°C to a constant weight and ground to pass a 0.3-mm sieve. During the second harvest, the roots, tender stems (10 cm top to bottom) and leaves (the first 5 leaves counted from top to bottom) from treatments T0, T4 and T5 were collected and frozen in liquid N_2 for the determination of Cd in the subcellular fractions.

Analysis. Frozen ramie tissues cells were separated into three fractions: cell wall fraction, soluble fraction and organelle containing fraction using a differential centrifugation technique according to Wang et al. (2008). To determine the Cd contents, three fractions of ramie tissues, frozen tissues and dried plant materials were digested (open system) with HNO_3-$HClO_4$ (5:1, v/v), and the soil samples (1.0 g dry weight (DW)) were digested (open system) with *aqua regia*-$HClO_4$ (20:3, v/v); and the concentrations of Cd in the digested solutions were determined by atomic absorption spectroscopy (AAS; GBC, Melbourne, Australia) (Bao 1999). Organic C and total N were measured using a CN auto-analyzer (Vario MAX C/N, Hanau, Germany), and Olsen-P and available K were extracted with 0.5 mol/L $NaHCO_3$ and 1 mol/L NH_4OAC, respectively (Bao 1999). The composition of organic matter (humic acid and fulvic acid) and the sorption capacity of Cd in tested soil were analysed according to the methods described by Kumada (1987) and Adhikari and Singh (2003).

The translocation factor (C_{shoot}/C_{root}, C_{shoot} means Cd concentration in shoot and C_{root} means Cd concentrations in root) and enrichment factor (C_{shoot}/C_{soil},

C_{soil} means Cd concentration in soil) were calculated to characterise the accumulation of Cd in ramie. All of the values were expressed as the mean ± SE (standard error) of the three replicates. Data were analysed using one-way ANOVA tests with the *LSD* tests to separate means. Differences were considered significant at $P < 0.05$. Data were processed using the SPSS 11.5 (SPSS, Chicago, USA) software.

RESULTS AND DISCUSSION

Cadmium inhibits the growth and fibre yield of ramie. The biomass and yield of fibre in the second season were significantly higher than the first season except for treatments T4 and T5 (Tables 1–2). This may be attributed to the number of ramie tillers and the plant height significantly (data not shown) increased after one year of growth, and the ramie biomass and fibre yield could reach normal levels by the second year (Wang et al. 2000). As compared with the T0 treatment, Cd treatments led to a significant decrease in the ramie biomass by 29.9–42.3% (Cd addition at 20 to 100 mg/kg) and 26.7–67.4% (Cd addition at 35 to 100 mg/kg) for the first and second seasons, respectively. Similarly, the ramie fibre yield was significantly decreased by 21.1–60.3% after Cd addition at 35 to 100 mg/kg in the second season. However, a significant decrease in the ramie fibre yield did not occur in the Cd treatments during the first season. The significant effect of Cd stress on the ramie biomass and fibre yield was also reported by She et al. (2011). These results indicated that ramie possesses a certain degree (≤ 20 mg/kg) of constitutional Cd tolerance.

Accumulation of Cd in ramie. The Cd contents of the ramie shoot system significantly increased in correlation with increases in the soil Cd concentration during both the first and second seasons ($y = 0.49x + 20.97$, $r = 0.85$, $P < 0.01$ and $y = 0.82x + 12.68$, $r = 0.98$, $P < 0.01$, respectively). The highest Cd contents in ramie shoots were 61.55 (T4) and 84.57 (T5) mg/kg, which were approximately 7.4-fold and 13.2-fold higher than in the T0 treatment during the first and second seasons, respectively (Tables 1–2). The C_{shoot}/C_{soil} values decreased with an increase in the soil Cd concentration and were greater than 1.0 in the majority of treatments (except for T5 treatment) during both seasons. A similar decrease in the C_{shoot}/C_{root} values (< 1.0 in all three tested treatments) was observed. According to the definition of Cd hyperaccumulator plants (Baker and Brooks 1989), ramie was not a Cd hyperaccumulator plant. However, ramie could be harvested three times in one year, and there was no significant difference in the shoot biomass among the three time points. Therefore, the annual Cd accumulation by ramie shoots was between 0.19 and 1.09 kg/ha in the present study, which was close to the reported phytoextraction potential of willow and lower than poplar under similar soil Cd concentrations (Kacálková et al. 2009).

Ramie stem consisted of bark, fibre and sticks. The Cd contents of these three parts of the stem were 1.0- to 9.4-fold higher than in the leaves in the Cd-amended treatments (Table 2). The bark Cd contents were between 22.9 to 379.4 mg/kg, which were 3.5- to 7.5-fold and 3.9- to 7.3-fold higher than the Cd contents of the stick and fibre samples from the 6 treatments (Table 2). The Cd contents of the ramie roots were significantly higher than in the shoots, especially for treatments T4 and T5. Similar distributions of Cd among plant tissues in ramie (Yang et al. 2010, She et al. 2011), grass-like species and wetlands plants were observed (Deng et al. 2004). These results indicated that the Cd tolerance strategy of ramie was its limited mobility once inside the plant when ramie grown in Cd contaminated soils.

Subcellular distribution of Cd in ramie tissues. We investigated the subcellular distribution of Cd

Table 1. The characteristics of Cd accumulation in ramie after the first season (DW)

Treatment		T0	T1	T2	T3	T4	T5
Cd content (mg/kg)	shoot	8.31 ± 0.41^{d}	25.01 ± 2.07^{c}	34.60 ± 2.60^{c}	49.39 ± 4.36^{b}	61.55 ± 5.11^{a}	56.54 ± 5.18^{ab}
	fibre	4.74 ± 0.27^{e}	24.52 ± 3.60^{d}	40.93 ± 5.80^{c}	45.99 ± 3.45^{bc}	61.56 ± 2.80^{a}	56.04 ± 5.35^{ab}
Shoot biomass	(g/m^2)	807.8 ± 127.0^{a}	585.1 ± 91.3^{ab}	566.2 ± 64.2^{b}	526.1 ± 44.4^{b}	559.5 ± 40.1^{b}	464.5 ± 36.2^{b}
Fibre yield		52.1 ± 7.7^{a}	50.4 ± 4.3^{a}	50.4 ± 7.7^{a}	51.5 ± 3.7^{a}	48.2 ± 4.9^{a}	43.3 ± 2.3^{a}
C_{shoot}/C_{soil}		4.35	2.19	1.77	1.48	1.01	0.6

DW – dry weight. Results are expressed as the mean (± SE) of three samples. Within each row, the data accompanied by the same letter are not significantly different ($P = 0.05$). T0 – 0 mg Cd/kg; T1 – 10 mg Cd/kg; T2 – 20 mg Cd/kg; T3 – 35 mg Cd/kg; T4 – 65 mg Cd/kg; T5 – 100 mg Cd/kg

Table 2. The accumulation and distribution of Cd in ramie tissues after the second season (DW)

Treatment		T0	T1	T2	T3	T4	T5
Cd content (mg/kg)	leaf	7.29 ± 0.46^{e}	12.69 ± 0.64^{de}	21.30 ± 1.66^{cd}	25.98 ± 3.04^{bc}	33.57 ± 2.01^{ab}	48.44 ± 6.16^{a}
	bark	22.93 ± 2.01^{d}	96.53 ± 3.64^{c}	177.66 ± 15.06^{b}	232.49 ± 18.12^{b}	314.53 ± 23.69^{a}	379.38 ± 45.89^{a}
	stick	3.56 ± 0.09^{d}	12.79 ± 0.66^{cd}	28.81 ± 3.19^{cd}	35.94 ± 6.23^{bc}	62.41 ± 12.72^{ab}	107.11 ± 19.27^{a}
	fibre	3.16 ± 0.21^{e}	13.58 ± 0.12^{de}	30.45 ± 1.60^{cd}	39.07 ± 3.56^{c}	67.20 ± 7.67^{b}	97.06 ± 17.01^{a}
	root	8.72 ± 2.54	–	–	–	123.68 ± 32.81	234.29 ± 31.39
	shoot	6.43 ± 0.19^{e}	18.37 ± 0.37^{de}	35.80 ± 3.97^{cd}	45.87 ± 5.55^{bc}	67.30 ± 9.86^{ab}	84.57 ± 16.81^{a}
Shoot biomass	(g/m^2)	985.7 ± 64.8^{a}	885.5 ± 13.0^{a}	866.3 ± 46.0^{a}	722.4 ± 7.0^{b}	540.0 ± 11.9^{c}	321.3 ± 42.6^{d}
Fibre yield		79.0 ± 6.7^{a}	71.6 ± 4.7^{a}	68.9 ± 8.9^{a}	62.3 ± 0.6^{ab}	50.1 ± 1.1^{b}	31.4 ± 3.4^{c}
C_{shoot}/C_{soil}		3.37	1.61	1.83	1.38	1.11	0.90
C_{shoot}/C_{root}		0.74	–	–	–	0.54	0.36

DW – dry weight; – not determined. Results are expressed as the mean (± SE) of three samples. Within each row, the data accompanied by the same letter are not significantly different (P = 0.05). T0 – 0 mg Cd/kg; T1 – 10 mg Cd/kg; T2 – 20 mg Cd/kg; T3 – 35 mg Cd/kg; T4 – 65 mg Cd/kg; T5 – 100 mg Cd/kg

in ramie leaves, stems and roots for treatments T0, T4 and T5 (Figure 1). The majority of the Cd was found to occur in the cell wall fraction (56.1–83.0%), whereas a minor fraction was associated with the soluble (8.0–25.5%) and organelle (3.9–18.4%) fractions. With Cd levels in the soil increasing, the relative accumulation of Cd in the cell wall fractions of the roots decreased, and the proportion of Cd in the soluble fractions of roots increased. And in leaves, a decrease in proportion of Cd in the cell wall fraction and increase in soluble and organelle fractions was observed with the increasing soil Cd concentration. However, in the stems, the relative accumulation of Cd in the cell wall and soluble fractions decreased and the proportion of Cd in the organelle fraction increased with an increasing supply of Cd in the soil.

A similar large proportion (48.2–61.9%) of Cd bound to the cell wall fraction in ramie tissues was observed in a 20-day hydroponic experiment (Wang et al. 2008). Previous studies have also demonstrated that cell walls store the large fraction of Cd in different tissues of cattail and lettuce (Ramos et al. 2002, Xu et al. 2011). Therefore, the large proportion of Cd in the cell wall fraction of ramie tissues suggested that the cell wall may function as the primary barrier protecting the protoplast from Cd toxicity. Plant cell walls, which are mainly composed of polyose (including cellulose, hemicellulose and pectin) and protein, display negatively charged sites on their surfaces and thus can bind and restrict the transportation of Cd ions across the cytomembrane (Fu et al. 2011).

The complexation of Cd with organo-ligands (mainly sulphur-rich peptides and organic acids) and Cd stor-

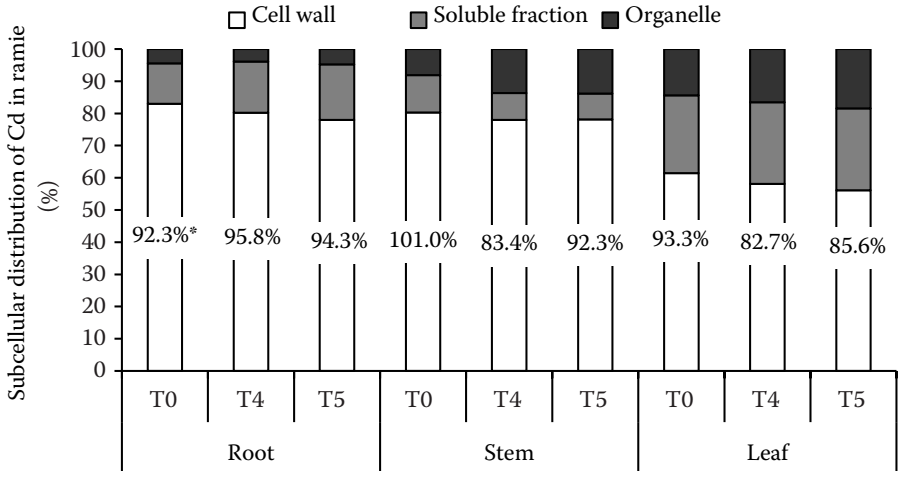

Figure 1. Subcellular distribution of Cd in ramie tissues after the second season. *recovery percentage. T0 – 0 mg Cd/kg; T4 – 65 mg Cd/kg; T5 – 100 mg Cd/kg

age in the soluble fraction (mainly in vacuoles) were reported to be essential detoxification responses to Cd stress for several plants (Fu et al. 2011). A considerable proportion of Cd stored in the soluble fraction (30.2–38.1%) of ramie tissues was observed in a short-term Cd exposure hydroponic experiment (Wang et al. 2008). Similarly, the proportions of Cd stored in the soluble fractions of leaves of ramie were 24.1–25.5% in the present study. However, in this present study, the proportions of Cd stored in the soluble fractions of the roots and stems of ramie were only 12.5–17.3% and 8.0–11.6%, respectively. These present results were, to some extent, in agreement with those of Xu et al. (2011), who used energy dispersive X-ray analysis of cattail plants to determine that the proportions of Cd stored in the vacuoles of roots and leaves were 12.0% and 6.0%, respectively. Moreover, the proportion of Cd bound to organelles was much higher in the leaves than in the roots and stems for the three treatments, which was consistent with the results of Wang et al. (2008). This distribution may be attributed to the preferential accumulation of Cd in chloroplasts (Ramos et al. 2002). However, the subcellular distributions of Cd in ramie tissues presented here were not entirely consistent with those reported in previous studies. As noted by Wang et al. (2008) and Fu et al. (2011), these differences may occur due to the varied experimental conditions that were used as well as the diverse plant species and tissues that were studied.

In summary, ramie was shown to possess a certain extent of tolerance to Cd and was capable of accumulating Cd present in the soil, which demonstrated that ramie is a potential candidate for the phytoremediation of Cd-contaminated soils. In addition, the Cd accumulated by ramie was primarily bound to the cell walls of the roots, stems and leaves.

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