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Effect of cadmium supply levels to cadmium accumulation by *Salix*

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ABSTRACT: The present investigation reports the results of the cadmium accumulated by *Salix matsudana*, *S. alba* var. *Tristis* and *S. babylonica* in a pot experiment at six different levels of cadmium supply (0, 0.5, 2, 6, 25, 60 mg/kg). All tested *Salix* species showed the different abilities to remove cadmium, which depend on species and concentrations level. Cadmium accumulated by the leaves, twigs and roots linearly increased with increasing cadmium supply levels. The higher concentration cadmium treatments significantly promoted the cadmium accumulation. *S. matsudana* always performed the stronger ability of cadmium accumulation under different cadmium supply treatments, while *S. alba* var. *Tristis* and *S. babylonica* had the poorer accumulation ability. Cadmium in soil was more intensively absorbed in the leaves and twigs for all three *Salix* species, was not retained in roots and was transferred to aboveground plant tissues. The results indicated that *Salix* has an excellent potential for cadmium phytoremediation because of its high accumulation ability.

Keywords: *Heavy metal; Phytoremediation; Plant; Uptake*

INTRODUCTION

Industrial process, agricultural productions, mining and other human activities have resulted in considerable contamination of soils with heavy metals (Goyal *et al.*, 2008; Atafar *et al.*, 2010). Soils polluted with metals may threaten ecosystems and human health (Pulford and Watson, 2003; Dobaradaran *et al.*, 2008a; b). Traditional remediation technologies of soils contaminated with toxic metals are generally too costly, and often result in decoration of soil properties (Meers *et al*., 2004). The potential use of trees as a suitable vegetation cover for heavy metal-contaminated land, with their lower cost and environmental friendly nature, has attracted increasing attentions (Lee *et al*., 2007; Perez-Sirvent *et al.*, 2008). Most of studies on phytoremediation have mainly focused on metal hyperaccumulating plants (Blaylock and Huang, 2000; Nouri *et al.*, 2009). Hyperaccumulators can accumulate several hundreds fold certain metals comparing normal plant species, with no adverse effects on their growth (Reeves and Baker, 2000; Lasat, 2002). However, hyperaccumulators are usually small biomasses with low growing rates and no economic value. Trees have been suggested as a low-cost, sustainable and ecologically sound solution to the remediation of heavy metal-contaminated land (Dickinson, 2000), especially when it is uneconomic to use other treatment or there is no time pressure on the reuse of the land. Benefits can arise mainly from stabilization of the soil or waste, although in some cases phytoextraction may be sufficient to provide cleanup of the soil. Therefore, some *Salix* species might be an interesting alternative with their high biomass production and fast-growing advantage (Greger and Landdberg, 1999; Pulford and Watson, 2003; Maria *et al*., 2007; Zacchini *et al*., 2009). *Salix* plant species that produce high biomass can be proposed for use in phytoremediation technology.

Among the heavy metals, Cd is of special concern due to its relatively high mobility in soils and potential toxicity to biota at low concentrations. Although Cd is naturally present in soil at trace amounts, high levels of Cd have been reported in some environment (Das *et al*., 1997). *Zea mays* L. seedlings accumulated more copper in roots but greater contents of zinc in their shoots (Mahmood *et al.*, 2005). Different species show different levels of tolerance to Cr^{2+} pollution

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(Shrestha *et al*., 2007; Nabulo *et al*., 2008; Jun *et al*., 2009; Ayari *et al*., 2010).

Some heavy-metal contaminated soils may be cleaned up by growing plants which accumulate the pollutants, then harvesting the plants and disposing of them in a 'safe area'. This kind of technology, known as "phytoremediation", represents a harmless and low cost technique, lacking of distinctive side effects (Cunningham and Owen, 1996; Ledin, 1998). *Triticum aestivum*, *Eruca sativa* and *Dumasia villosa* seedling length decreased in the high concentration of chromium treatment (Jamal *et al*., 2006; Perez-Sirvent *et al*., 2008). The ideal plants species to remediate a heavy metal-contaminated soil would be a high biomass producing species that can both tolerate and accumulate the contaminants of interest. For the purpose of phytoremediation, Punshon and Dickinson (1996) suggested that the following characteristics were beneficial for woody plants:

1) ability to grow on nutrient-poor soil;
2) deep root system;
3) fast rate of grow;
4) metal-resistance trait

Greger and Landberg (1999) discussed the potential of *Salix* for the decontamination of Cd-contaminated soils, reporting that 12 years of growth would be required to remove the Cd accumulated in Swedish soils during the last century.

Two possible strategies have been proposed for the use of *Salix* for phytoremediation. One is *Salix* that survive in contaminated soil with minimal uptake of metals into the aerial tissues would be most appropriate for use where distribution of heavy metals to the wider environment or transfer of metals into the food chain is to be avoided (Eriksson and Ledin, 1999; Vyslouzilova *et al*., 2003). Another one is *Salix* that accumulate relatively high amounts of metal are desirable if soil remediation is to be achieved by phytoextraction and tree harvesting.

The aim of the present study was to investigate species variation in Cd uptake and accumulation of three *Salix* species grown in soil containing a range of Cd concentrations. More specific, Cd concentration in different plant parts under a series of Cd contamination conditions was investigated. The experiment was carried in Institute of Environmental Ecology, Lanzhou Jiaotong University in April 2009.

MATERIALS AND METHODS

Cultivated experiments

In April 2009, the seedlings of three *Salix* (*S. matsudana*, *S. alba* var. *Tristis*, *S. babylonica*) were planted in 180 10-litre plastic pots with 10 kg of sands. Six replications for each species were made in 6 Cd treatments with 0, 0.5, 2, 6, 25, 60 mg/kg. The pots containing 3 seedlings were placed in a greenhouse. For 6 months of experiment, the night / daylight period of the greenhouse was 10/14 h and the temperature varied between 15.2 and 32.5 °C. The 180 pots were placed randomly in the greenhouse and watered three times a week with demineralized water in order to keep the sand's moisture content constant and care was taken to avoid leaching of water from the pots, a plastic tub was placed below each pot to collect the leachate. The collected leachate was again returned to the experiment pot. During the 6 months of the study, each pot was given about 600 mm of water.

Measurements of metal concentrations in plant samples

Aboveground biomass and root biomass were harvested after 6 months. Aboveground biomass was separated into leaves and twigs, roots were thoroughly washed by deionized water. Leaves, twigs and roots of each sampling were placed in a drying cabinet at 80 °C for 48 h until a constant weight was reached. Samples were weighed, and ground then passed 200-mesh screen. Approximately 0.5 (±0.0001) g of material from each sample was accurately weighed into 50 mL ceramic crucible and carbonized on the heating furnace until the smoke dissipated. Then the ceramic crucible were transferred to the muffle furnace at 500 °C for 8 h. The resulting ash was dissolved in 10 mL HNO_3 (1:1) and diluted to a final volume of 50 mL with deionized water and stored in polythene containers. Depending on detection limits, the Cd contents of the solutions were measured using the inductively coupled plasma optical emission spectrometry (ICP-AES).

Statistical analysis

Statistical analysis was performed based on STATISTICA (Statsoft, 1993). The data were analyzed through two-way and one-way analysis of variance (ANOVA) to determine the effect of treatments, species and parts of plants, and Duncan's multiple comparison tests were performed to determine the statistical significance of the differences among means of different Cd treatments,willow species and parts of plants.

RESULTS AND DISCUSSION

Accumulation of Cd in twig, leaf and root of Salix under different Cd supply levels

The Cd accumulation in twig, leaf and root in three *Salix* species treated with Cd (0.5 mg/kg to 60 mg/kg) is shown in Fig. 1 and 2. A two-way ANOVA showed that the amounts of Cd accumulation in the twig leaf and root were significantly affected by different species ($P<0.01$), Cd treatment ($P<0.01$) and interaction ($P<0.01$) between species and Cd treatment (Table 1).

The contents of the Cd in the twigs (one-way ANOVA: $F_5 = 184.08$, $p < 0.001$ for *S. matsudana*; $F_5 = 256.51$, $p < 0.001$ for *S. alba* var. *Tristis*; $F_5 = 237.99$, $p < 0.001$ for *S. babylonica*), leaves ($F_5 = 485.77$, $p < 0.001$ for *S. matsudana*; $F_5 = 345.68$, $p < 0.001$ for *S. alba* var. *Tristis*; $F_5 = 1192.34$, $p < 0.001$ for *S. babylonica*) and roots ($F_5 = 940.26$, $p < 0.001$ for *S. matsudana*; $F_5 = 213.19$, $p < 0.001$ for *S. alba* var. *Tristis*; $F_5 = 72.83$, $p < 0.001$ for *S. babylonica*) were significantly different among different Cd treatments in all three *Salix* species. For all *Salix* species, the lowest Cd accumulation in twig, leaf and root always occurred in lowest Cd concentration treatments. Compared with the control, higher concentration Cd treatments (6, 25, 60 mg/kg) significantly promoted Cd uptake of the twig, leaf and root for all species (Fig. 1).

For *S. matsudana*, the amounts of Cd accumulation in twig and leaf dramatically and significantly increased with the addition of Cd concentration, however, under the highest Cd concentration (60 mg/kg), the amount of Cd accumulation in root was significantly lower than that of 25 mg/kg Cd treatment, and higher than other Cd treatments. The amounts of Cd accumulation in twig, leaf and root of *S. alba* var. *Tristis* and in twig of *S. babylonica* under the highest Cd concentration (60 mg/kg) were significantly lower than that of 25 mg / kg Cd treatment and higher than that of the other Cd treatments. The amount of Cd accumulation in all three parts of *S. alba* var. *Tristis* under the 6 mg/kg Cd treatment was significantly higher than that of other lower Cd concentration treatments and the control. The higher Cd concentration treatments (25, 60 mg/ kg) significantly promoted the Cd accumulation in root and leaf of *babylonica*, there was no significant difference for the amounts of Cd accumulation between the 25 mg/kg and 60 mg/kg Cd treatments, among the control and the lower concentration treatments (0.5, 2 mg/kg). The amounts of Cd accumulation in root and leaf of *S. babylonica* under the 6 mg/kg Cd treatment were significantly higher than that of other lower Cd concentration treatments and the control and lower than that of the 25 and 60 mg/kg Cd treatments (Fig. 1). There were the significant correlations between the amounts of Cd accumulation in twig, leaf and root and Cd concentration for all three *Salix* species (Table 2).

Difference of Cd accumulation in twig, leaf and root among Salix species

The contents of the Cd in the twigs (one-way ANOVA: $F_2 = 4.09$, p=0.0383 for the control; $F_2 = 18.63$, p<0.001 for the 0.5 mg/kg Cd treatment; $F_2 = 18.42$, p<0.001 for the 2 mg/kg Cd treatment; $F_2 = 39.37$, p<0.001 for the 6 mg/kg Cd treatment; $F_2 = 33.79$, p<0.001 for the 25 mg/kg Cd treatment; $F_2 = 163.79$, p<0.001 for the 60 mg/kg Cd treatment) and leaves ($F_2 = 9.86$, p=0.0019 for the control; $F_2 = 13.66$,

Table 1: Analysis of variance for the effects of different species, Cd treatments, and their interaction on accumulation of the leaves, twigs and roots for three *Salix* species

Part of plant	Source of variation	df	*F*-value	*P*
Leaves	Cd treatment	5	1600.74	<0.001
	species	2	556.60	0.0018
	Cd treatment × species	10	539.81	<0.001
Twigs	Cd treatment	5	1423.23	<0.001
	species	2	317.30	0.0031
	Cd treatment × species	10	300.98	<0.001
Roots	Cd treatment	5	1266.91	<0.001
	species	2	440.35	0.0023
	Cd treatment × species	10	604.39	<0.001

p=0.004 for the 0.5mg/kg Cd treatment; F_2 = 11.81, *p*=0.008 for the 2 mg/kg Cd treatment; F_2 = 86.32, *p*<0.001 for the 6 mg/kg Cd treatment; F_2 = 51.97, *p*<0.001 for the 25 mg/kg Cd treatment; F_2 = 183.29, *p*<0.001 for the 60 mg/kg Cd treatment) were significantly different among three *Salix* species under all Cd treatments. There were significantly difference for the amount of Cd accumulation in the roots among three *Salix* species under 0.5 mg/kg (F_2 = 12.73, *p*<0.001), 2 mg/kg (F_2 = 4.48, *p*=0.0238), 6 mg/kg (F_2 = 229.37, *p*<0.001) and 25 mg/kg (F_2 = 47.38, *p*<0.001), but not for the control and the 60 mg/kg Cd treatment.

S. matsudana and *S. alba* var. *Tristis* did not perform significant difference in the amounts of Cd accumulation in the twigs and leaves at 0.5 mg/kg treatment, in the roots at 6 mg/kg treatment, in the leaves at 2 and 25 mg/kg treatment, were significantly higher than *S. babylonica*. *S. matsudana* always performed the biggest amounts of Cd accumulation in twig the 2, 6 and 25 mg/kg treatments, in root at 25 mg/kg treatment, and *S. babylonica* performed the poorest accumulation ability significantly. The leaves at the 6 and 60 mg/kg and the twigs at 60 mg/kg Cd treatments of *S. matsudana* always accumulated the most amount of Cd than the other two species which did not performed significant difference in the amounts of Cd accumulation (Fig. 2).

Accumulation of Cd in different part of three Salix species

The amounts of Cd accumulation under the control (F_2 = 62.32, p < 0.001 for *S. matsudana*; F_2 = 17.08, p < 0.001 for *S. alba* var. *Tristis*; F_2 = 53.92, p < 0.001 for *S. babylonicas*), the 0.5 mg/kg (one-way ANOVA: F_2 = 8.48, p = 0.0034 for *S. matsudana*; F_2 = 9.23, p = 0.0024 for *S. alba* var. *Tristis*; F_2 = 19.17, p < 0.001 for *S. babylonicas*), 2 mg/kg (F_2 = 28.52, p < 0.001 for *S. matsudana*; F_2 = 68.17, p < 0.001 for *S. alba* var. *Tristis*; F_2 = 28.47, p < 0.001 for *S. babylonicas*), 6 mg/kg (F_2 = 24.68, p < 0.001 for *S. matsudana*; F_2 = 2.72, p = 0.0984 for *S. alba* var. *Tristis*; F_2 = 81.67, p < 0.001 for *S. babylonicas*), 25 mg/kg (F_2 = 28.42, p < 0.001 for *S. matsudana*; F_2 = 74.55, p < 0.001 for *S. alba* var. *Tristis*; F_2 = 63.73, p < 0.001 for *S. babylonicas*) and 60 mg/kg (F_2 = 261.38, p < 0.001 for *S. matsudana*; F_2 = 37.08, p < 0.001 for *S. alba* var. *Tristis*; F_2 = 59.99, p < 0.001 for *S. babylonicas*) Cd treatment were significantly different among three parts of *Salix* species, but no for *S. alba* var. *Tristis* at the 60 mg/kg Cd treatment.

At the control, the amounts of Cd accumulation in the twigs and leaves for all three species were not significantly different, but significantly higher than the root. At the 0.5 mg/kg Cd treatment, there are not significantly difference in the amounts of Cd accumulation between the twigs and leaves for *S. matsudana* and *S. alba* var. *Tristis*, and the amounts of Cd accumulation in aboveround parts were significantly higher than underground part for these two species, for *S. babylonicas*, the amounts of Cd accumulation in the leaves were significantly higher than the twigs and roots which did not performed significant difference each other. At the 2, 6 and 25 mg/kg Cd treatments, the amounts of Cd accumulation in the twigs and leaves for *S. matsudana* were not significantly different, but significantly higher than that of the root, for *S. babylonicas*, the amounts of Cd accumulation in the twigs were significantly higher than that of the roots and lower than that of the leaves. The amounts of Cd accumulation in the twigs under 2 and 25 mg/kg Cd treatments for *S. alba* var. *Tristis*, and 60 mg/kg Cd treatment for *S. babylonicas*

Table 2: Correlations of Cd concentration to the amount of Cd accumulation in leaves, twigs and roots

Part of plant	Species	Linear model	R^2	*F*-value	*P*
Leaves	*S. matsudana*	y=-9813.07+96.44x	0.8965	304.1300	<0.001
	S. alba var. *Tristis*	y=-5775.93+56.84x	0.6693	71.8325	<0.001
	S. babylonica	y=-4934.21+48.49x	0.8341	177.0025	<0.001
Twigs	*S. matsudana*	y=-8257.89+81.20x	0.8643	223.9807	<0.001
	S. alba var. *Tristis*	y=-4085.65+40.27x	0.6197	58.0378	<0.001
	S. babylonica	y=-3434.73+33.77x	0.7709	118.7699	<0.001
Roots	*S. matsudana*	y=-3347.59+32.98x	0.6299	60.5756	<0.001
	S. alba var. *Tristis*	y=-2918.02+28.75x	0.7765	122.5741	<0.001
	S. babylonica	y=-2263.78+22.25x	0.7627	113.5081	<0.001

and *S. matsudana*, were significantly higher than that of the roots and lower than that of the leaves. The amount of Cd accumulation in the leaves under 60 mg/kg Cd treatment was significantly higher than that of the twigs and roots which did not performed significant difference each other (Fig. 3).

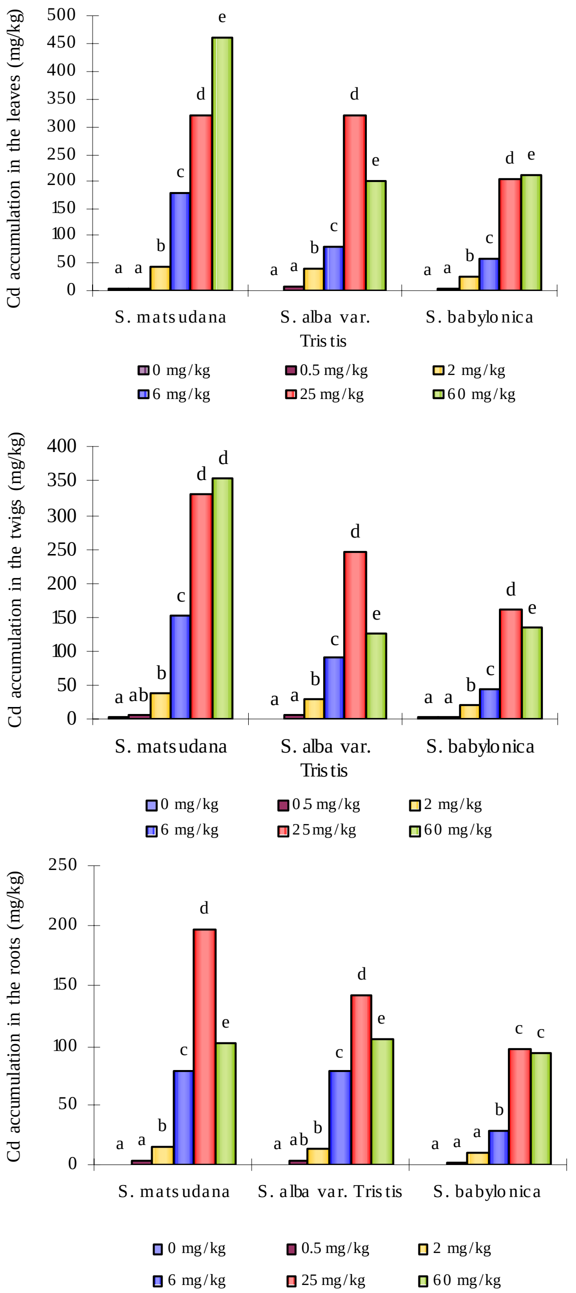

Fig. 1: Accumulation difference of six different Cd treatments in twig, leaf and root among three *Salix* species. Values with the same letters are not significantly different among treatments

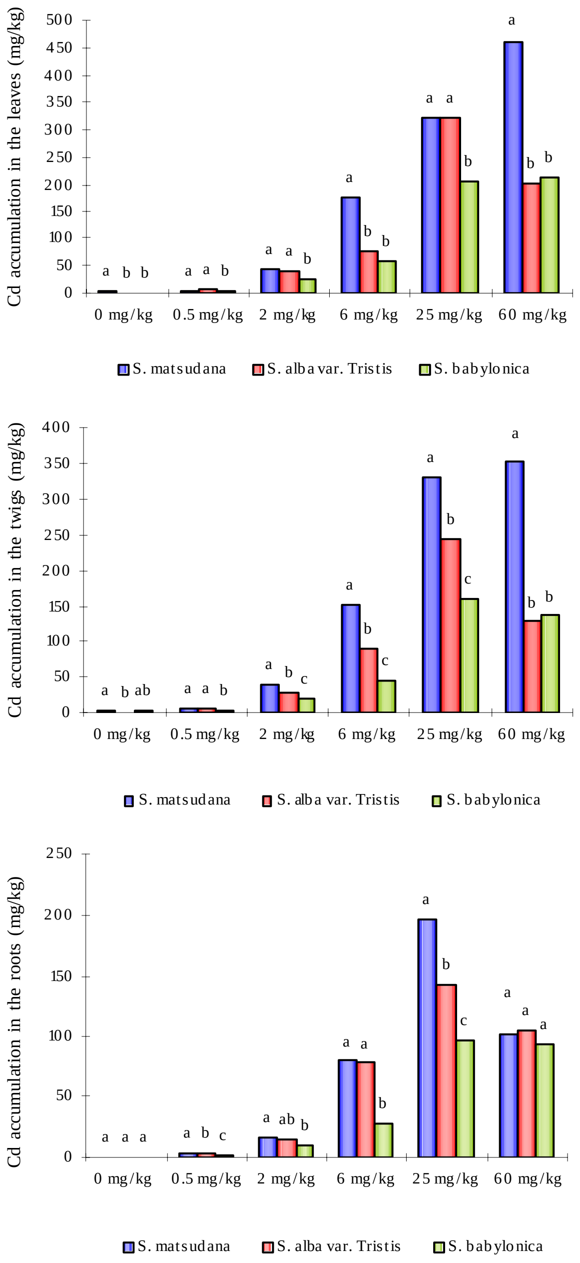

Fig. 2: Accumulation difference of three *Salix* species in twig, leaf and root among six different Cd treatments. Values with the same letters are not significantly different among treatments

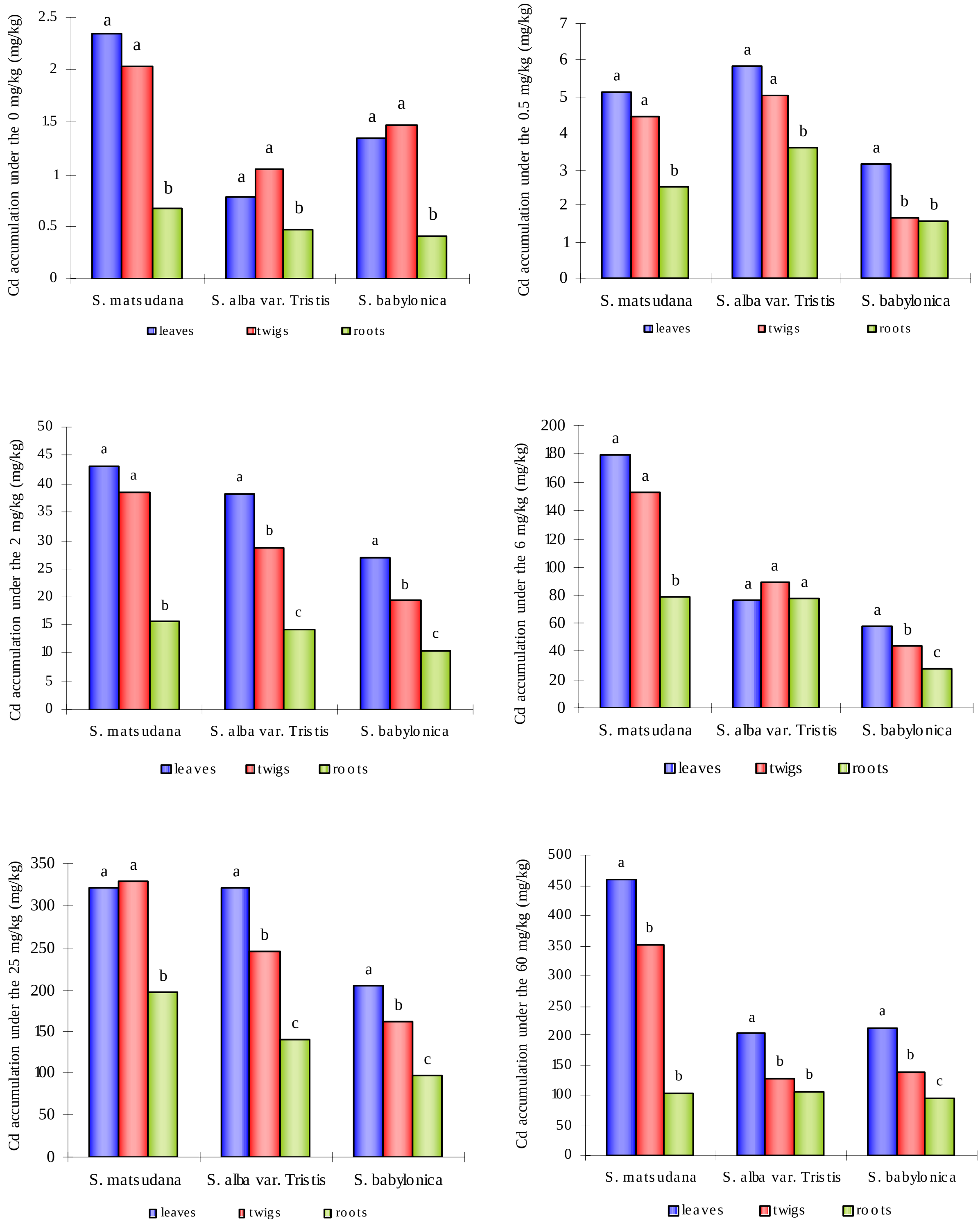

Fig. 3: Accumulation difference of three parts for three *Salix* under six different Cd treatments. Values with the same letters are not significantly different among treatments

CONCLUSION

The Cd accumulation in the leave, twigs and root of the tested *Salix* species always increased with the addition of Cd supply, only at the highest Cd supply conditions, the Cd accumulation in the twigs and root for *S. alba* var. *Tristis* and *S. babylonicas*, in the leaves for *S. alba* var. *Tristis* performed significant decrease. For all tested *Salix* species, Cd accumulated by the leaves, twigs and roots linearly increased with increasing Cd supply levels, peaked at the Cd level of 60 mg/kg in twigs and leaves of *S. matsudana* and 25 mg/kg for *S. alba* var. *Tristis* which then decreased with further increasing external Cd level. For *S. babylonica*, the maximum amount of Cd taken up by the twigs and roots were as high as 161.62 and 97.64 mg/kg, respectively, grown at the Cd level of 25 mg/kg, and the highest amount of Cd accumulation in the leaves performed at the treatment of 60 mg/kg.

The result of this study indicated that *Salix* has an excellent potential for Cd phytoremediation because of its high accumulation ability. By comparison of individual aboveground parts of plants, Cd was intensively accumulated in leaves than in twigs. Cd was not retained in roots and was transferred to aboveground plant tissues in all tested species.

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