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Full Length Research Paper

Study of tin accumulation strategy by *Cyperus* species in pot experiments

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The present investigation reports the results of Sn (tin) accumulated by *Cyperus* sp. in a pot experiment on different levels of Sn supply (0, 0.5, 2, 6, 25, 60 mg/kg). All tested *Cyperus species* showed the different abilities to remove Sn which depend on species and concentrations level. Sn accumulated by the leaves, twigs and roots linearly increased with increasing Sn supply levels. The higher concentration Sn treatments significantly promoted the Sn accumulation. *Cyperus rotundus L* performed the stronger ability of Sn accumulation under different Sn supply treatments, while *Cyperus alternifolius* and *Cyperus fastigiatus Rottb.* had the poorest accumulation ability. Sn in soil was more intensively absorbed in the leaves and twigs for all three *C.* species, was not retained in roots and was transferred to plant tissues above the ground. The results indicated that *Cyperus* sp. have excellent potential for Sn phytoremediation because of their high accumulation ability.

Key words: *Cyperus* sp., tin, accumulation, phytoremediation.

INTRODUCTION

Soil is a complex mixture of mineral particles that can interact with organic matter, water, air, gas and pollutants. Each of these entities will interact with one another that could alter the intrinsic values. Industrial process, agricultural productions, mining and other human activities have results in considerable contamination of soils with heavy metals. Soils polluted with metals may threaten ecosystems and human health (Pulford and Watson, 2003). As indicated by (Shacklette and Boerngen, 1984), the average Sn content in the surface soil in the US ranged between 0.6 to 1.7 ppm. Other studies shows that standard soil samples contain about 4.5 ppm Sn (tin) (Ure and Bacon, 1978), while in some contaminated sites it has been reported to contain up to 800 ppm Sn (Kabata-Pendias and Pendias 1999). Metals such as Sn in its various forms stannous (Sn2+), stannic (Sn4+), complex anions of oxides and hydroxides, could then be made bio available to plants growing in the soil especially in tin tailings. The mobility of Sn depends greatly on pH, where Sn2+ serves as a strong reducing agent, present in acidic conditions (Pendias and Pendias, 2001).

The presence of heavy metal in soil could be leached out by mobilization due to precipitation, adsorption or complexation (Impens et al., 1991). Traditional remediation technologies of soils contaminated with toxic metals are generally too costly, and often result in detoriation of soil properties (Meers et al., 2004). The potential uses of plants as a suitable vegetation cover for heavy metal-contaminated land, with their lower cost and environmental friendly nature, have attracted increasing attentions. (Lee et al., 2007; Perez-Sirvent et al., 2008). This kind of technology, known as “phytoremediation”, represents a harmless and low cost technique, lacking of distinctive side effects (Cunningham and Owen, 1996). Most of the studies on phytoremediation have mainly focus on metal hyperaccumulating plants (Blaylock and Huang, 2000). 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).

Field observations at tin tailing areas indicated that *Cyperus rotundus* was one of the dominant plants in the secondary succession. It belong to the Cyperaceae

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family, and shows similar morphological feature as the grass. Its other features are the triangle shaped stem that grow to a height between 15 to 50 cm (Raby and Wong, 1972). Earlier studies (Griffitts and Milne, 1977) indicated that sedges and mosses were Sn accumulators. It was thought that *C. rotundus L* could be screened as a hyperaccumulator plant that could be used in future photoremediation studies. Different strategies have been proposed for the use of *Cyperus* for phytoremediation. One is *Cyperus* 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 *Cyperus* that accumulate relatively high amounts of metal are desirable if soil remediation is to be achieved by phytoextraction and plant harvesting.

The aim of the present study was to investigate species variation in Sn uptake and accumulation of three *Cyperus* species grown in soil containing a range of Sn concentrations. More specific, Sn concentration in different plant parts under a series of Sn contamination conditions was investigated.

MATERIAL AND METHODS

Cultivated experiments

In March 2010, the seedling of three *Cyperus* (*C. rotundus* L., *Cyperus alternifolius and Cyperus fastigiatus Rottb.)* were planted in 180 10-litre plastic pots with 10kg of sands. Six replications for each species were made in 6 Sn treatments with 0, 0.5, 2, 6, 25, 60 mg/kg. The pots contained 3 seedlings and were placed in a greenhouse. For 6 months of experiment, the night/daylight period of the greenhouse was 10/14h and the temperature varied between 15.2 and 32.5℃. 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 our 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 80ºC 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 500ºC 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 Sn 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, poplar species and parts of plants.

RESULTS

Accumulation of Sn in twig, leaf and root of *Cyperus* under different Sn supply levels

The Sn accumulation in twig, leaf and root in three *Cyperus* species treated with Sn (0.5 mg/kg to 60 mg/kg) is shown in Figures 1 and 2. A two-way ANOVA showed that the amounts of Sn accumulation in the twig leaf and root were significantly affected by different species ($P<0.01$), Sn treatment ($P<0.01$) and interaction ($P<0.01$) between species and Sn treatment (Table 1). The contents of the Sn in the twigs (one-way ANOVA: F_5 = 184.08, $p < 0.001$ for *C. rotundus L*; F_5 = 256.51, $p < 0.001$ for *C. alternifolius*; F_5 = 237.99, $p < 0.001$ for *C. fastigiatus Rottb*), leaves (F_5 = 485.77, $p < 0.001$ for *C. rotundus* L; F_5 = 345.68, $p < 0.001$ for *C. alternifolius*; F_5 = 1192.34, $p < 0.001$ for *C. fastigiatus Rottb*) and roots (F_5 = 940.26, $p < 0.001$ for C. *rotundus L*; F_5 = 213.19, $p < 0.001$ for *C. alternifolius*; F_5 = 72.83, $p < 0.001$ for *C. fastigiatus Rottb*) were significantly different among different Sn treatments in all three *Cyperus* species. For all *Cyperus* species, the lowest Sn accumulation in twig, leaf and root always occurred in lowest Sn concentration treatments. Compared with the control, higher concentration Sn treatments (6, 25, 60 mg/kg) significantly promoted Sn uptake of the twig, leaf and root for all species (Figure 1).

For *C. rotundus L,* the amounts of Sn accumulation in twig and leaf dramatically and significantly increased with the addition of Sn concentration, however, under the highest Sn concentration (60 mg/kg), the amount of Sn accumulation in root was significantly lower than the 25 mg/kg Sn treatment, and higher in other Sn treatments. The amounts of Sn accumulation in twig, leaf and root of *C. alternifolius* and in twig of *C. fastigiatus Rottb* under the highest Sn concentration (60 mg/kg) were significantly lower than the 25 mg/kg Sn treatment, and higher than other Sn treatments. The amount of Sn accumulation in all three parts of *C. alternifolius* under the 6 mg/kg Sn treatment was significantly higher than other lower Sn concentration treatments and the control.

The higher Sn concentration treatments (25, 60 mg/kg) significantly promoted the Sn accumulation in root and leaf of *C. fastigiatus Rottb*, there was no significant

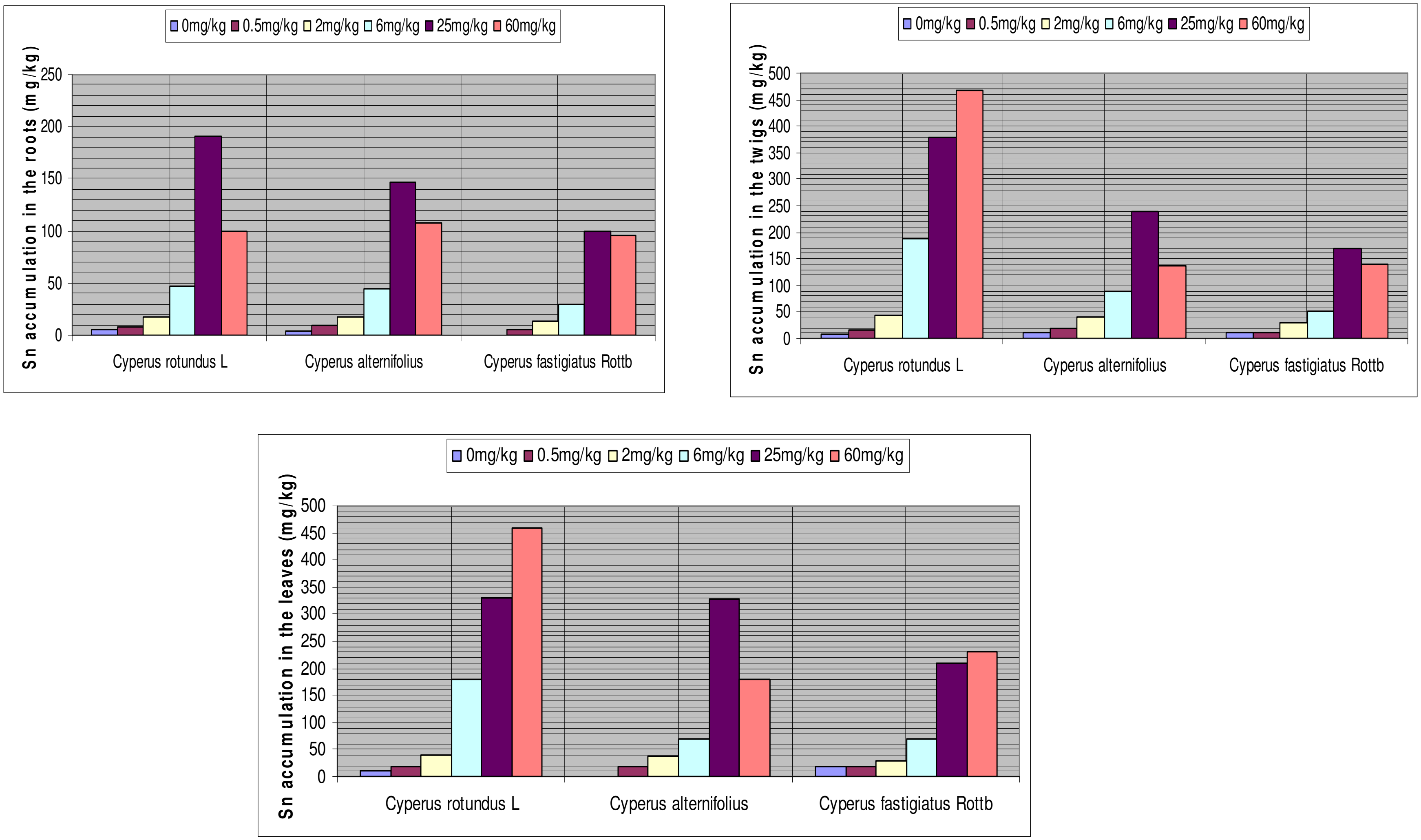


Figure 1. Accumulation difference of six different Sn treatments in twig, leaf and root among three *Cyperus* species. Values with the same letters are not significantly different among treatments at $p<0.05$ according to Duncan's multiple comparison tests.

difference for the amounts of Sn accumulation between the 25 mg/kg and 60 mg/kg Sn treatments, among the control and the lower concentration treatments (0.5, 2 mg/kg). The amounts of Sn accumulation in root and leaf of *C. fastigiatus Rottb* under the 6 mg/kg Sn treatment were significantly higher than other lower Sn concentration treatments and the control, and lower than the 25 and 60 mg/kg Sn treatments (Figure 1). There were the significant correlations between the amounts of Sn accumulation in twig, leaf and root and Sn concentration for all three

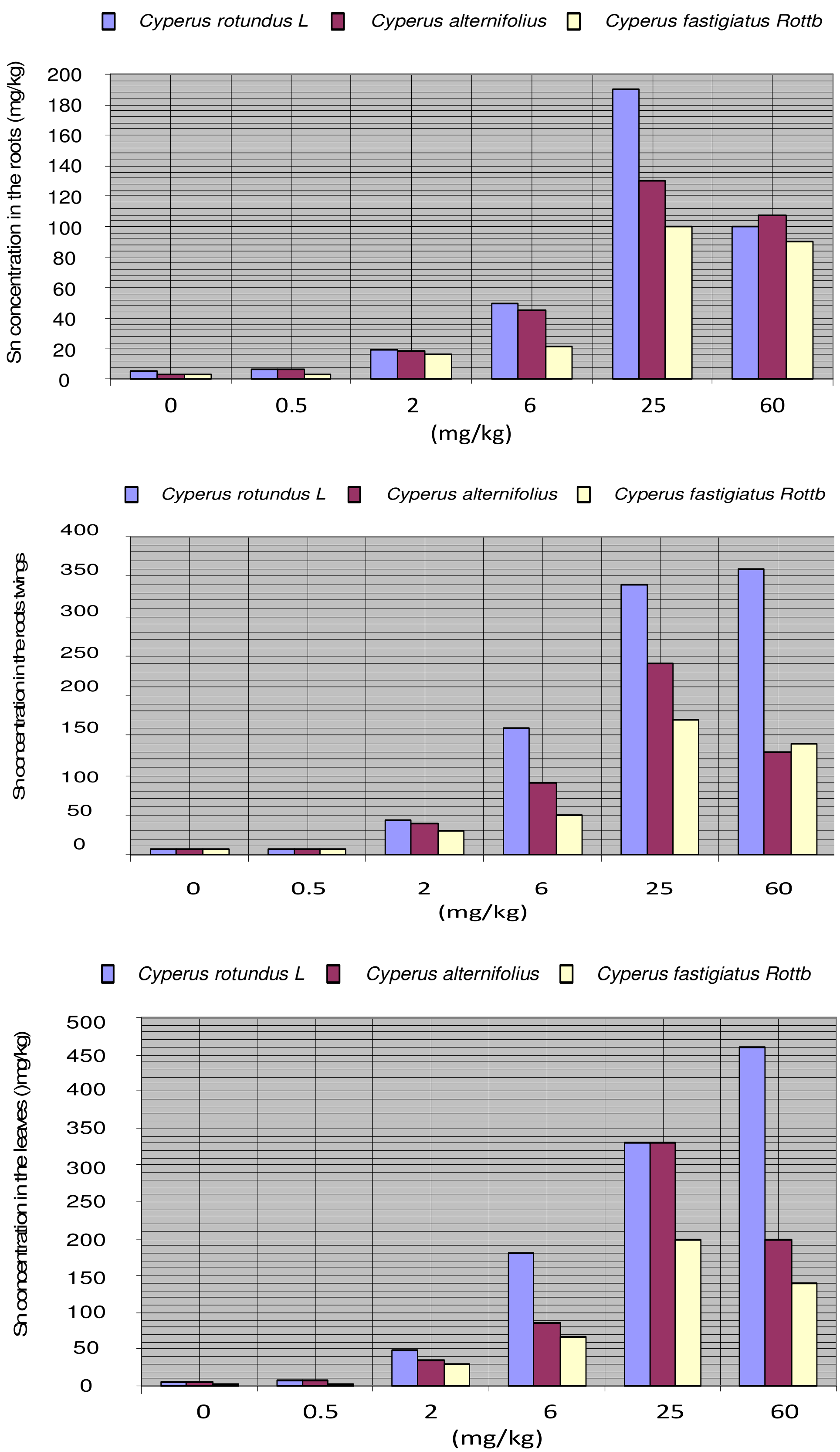


Figure 2. Accumulation difference of three *Cyperus* species in twig, leaf and root among six different Sn treatments. Values with the same letters are not significantly different among treatments at p<0.05 according to Duncan's multiple comparison tests.

Table 1. Analysis of variance for the effects of different species, Sn treatments, and their interaction on accumulation of the leaves, twigs and roots for three *Cyperus* species.

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

Table 2. Correlations of Sn concentration to the amount of Sn accumulation in leaves, twigs and roots.

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

Cyperus species (Table 2).

Difference of Sn accumulation in twig, leaf and root among *Cyperus* species

The contents of the Sn 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 Sn treatment; F_2 = 18.42, p<0.001 for the 2 mg/kg Sn treatment; F_2 = 39.37, p<0.001 for the 6 mg/kg Sn treatment; F_2 = 33.79, p<0.001 for the 25 mg/kg Sn treatment; F_2 = 163.79, p<0.001 for the 60 mg/kg Sn treatment) and leaves (F_2 = 9.86, p = 0.0019 for the control; F_2 = 13.66, p = 0.004 for the 0.5 mg/kg Sn treatment; F_2 = 11.81, p = 0.008 for the 2 mg/kg Sn treatment; F_2 = 86.32, p < 0.001 for the 6 mg/kg Sn treatment; F_2 = 51.97, p < 0.001 for the 25 mg/kg Sn treatment; F_2 = 183.29, p<0.001 for the 60 mg/kg Sn treatment) were significantly different among three *Cyperus* species under all Sn treatments. There were significantly difference for the amount of Sn accumulation in the roots among three *Cyperus* 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 no for the control and the 60 mg/kg Sn treatment.

C. rotundus L and *C. alternifolius* did not perform significant difference in the amounts of Sn accumulation in the twigs and leaves at 0.5 mg/kg treatment, in the roots 6 mg/kg treatment, in the leaves at 2 and 25 mg/kg treatment, were significantly higher than *C. fastigiatus Rottb*. *C. rotundus L* always performed the biggest amounts of Sn accumulation in twig at the 2, 6 and 25 mg/kg treatments, in root at 25 mg/kg treatment, and *C. fastigiatus Rottb* performed the poorest accumulation ability significantly. The leaves at the 6 and 60 mg/kg and the twigs at 60 mg/kg Sn treatments of *C. rotundus L* always accumulated the most amount of Sn than other two species which did not performed significant difference in the amounts of Sn accumulation (Figure 2).

Accumulation of Sn in different part of three *Cyperus* species

The amounts of Sn accumulation under the control (F_2 = 62.32, p < 0.001 for *C. rotundus L*; F_2 = 17.08, p < 0.001 for *C. alternifolius*; F_2 = 53.92, p < 0.001 for *C. fastigiatus*

Rottb), the 0.5 mg/kg (one-way ANOVA: F_2 = 8.48, p = 0.0034 for *C. rotundus L*; F_2 = 9.23, p = 0.0024 for *C. alternifolius*; F_2 = 19.17, p < 0.001 for *C. fastigiatus Rottb*), 2 mg/kg (F_2 = 28.52, p < 0.001 for *C. rotundus L*; F_2 = 68.17, p < 0.001 for *C. alternifolius*; F_2 = 28.47, p < 0.001 for *Cyperus fastigiatus Rottb*), 6 mg/kg (F_2 = 24.68, p < 0.001 for *C. rotundus* L; F_2 = 2.72, p = 0.0984 for *C. alternifolius*; F_2 = 81.67, p < 0.001 for *C. fastigiatus Rottb*), 25 mg/kg (F_2 = 28.42, p < 0.001 for *C. rotundus L*; F_2 = 74.55, p < 0.001 for *Cyperus alternifolius*; F_2 = 63.73, p < 0.001 for *C. fastigiatus Rottb*) and 60 mg/kg (F_2 = 261.38, p < 0.001 for *C. rotundus L*; F_2 = 37.08, p < 0.001 for *C. alternifolius*; F_2 = 59.99, p < 0.001 for *C. fastigiatus Rottb*) Sn treatment were significantly different among three parts of *C. species*, but no for *C. alternifolius* at the 60 mg/kg Sn treatment.

At the control, the amounts of Sn 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 Sn treatment, there is not significant difference in the amounts of Sn accumulation between the twigs and leaves for *C. rotundus L*, and *C. alternifolius*, and the amounts of Sn accumulation in aboveground parts were significantly higher than underground part for these two species, for *C. fastigiatus Rottb*, the amounts of Sn accumulation in the leaves were significantly higher than the twigs and roots which did not performed significant difference with each other. At the 2, 6 and 25 mg/kg Sn treatments, the amounts of Sn accumulation in the twigs and leaves for *C. rotundus L*, were not significantly different, but significantly higher than the root, for *C. fastigiatus Rottb*, the amounts of Sn accumulation in the twigs were significantly higher than the roots and lower than the leaves.

The amounts of Sn accumulation in the twigs under 2 and 25 mg/kg Sn treatments for *C. alternifolius*, and 60 mg/kg Sn treatment for C. *fastigiatus Rottb* and *C. rotundus L,* were significantly higher than the roots and lower than the leaves. The amount of Sn accumulation in the leaves under 60 mg/kg Sn treatment was significantly higher than the twigs and roots which did not perform significant difference with each other (Figure 3).

CONCLUSIONS AND DISCUSSION

We investigated the accumulation of Sn in three *Cyperus* species grown in a pot experiment on different levels of Sn contamination. Generally, the Sn accumulation in the leave, twigs and root of the tested *Cyperus* species always increased with the addition of Sn supply, only at the highest Sn supply conditions, the Sn accumulation in the twigs and root for *C. rotundus L, C. alternifolius* and *C. fastigiatus Rottb* in the leaves for *C. alternifolius* performed significant decrease. Plants showed the different abilities to remove Sn which depend on species and level of concentrations. For all tested *Cyperus* species, Sn accumulated by the leaves, twigs and roots linearly increased with increasing Sn supply levels, peaked at the Sn level of 60 mg/kg in twigs and leaves of *C. rotundus L*, and 25 mg/kg for *C. alternifolius* which then decreased with further increasing external Sn level. For *C. fastigiatus Rottb*, the maximum amount of Sn taken up by the twigs and roots were as high as 161.62 and 97.64 mg/kg, respectively, grown at the Sn level of 25 mg/kg, and the highest amount of Sn accumulation in the leaves performed at the treatment of 60 mg/kg.

Much more information is available on the possibilities to remediate soils contaminated with heavy metals through the use of *Cyperus* plants. Several studies indicated that there is a pronounced uptake of Sn into the aboveground parts of *Cyperus* plants (Musharifah et al., 2005). In this experiment, the tested *Cyperus* species confirmed high aboveground Sn accumulation potentials. By comparison of individual aboveground parts of plants, Sn was intensively accumulated in leaves than in twigs. Sn was not retained in roots and was transferred to aboveground plant tissues in all tested species. The result of this study indicated that *Cyperus* have excellent potential for Sn phytoremediation because of their high accumulation ability. If the plant-uptake in field conditions is similar to that which has been achieved in these experiments then *Cyperus* could be used to decontaminate soils highly contaminated with Sn. The use of *Cyperus* as heavy metal collectors seems thus most feasible if only Sn is targeted or when the metal input to the stands can be controlled. Moreover, metal removal by *Cyperus* would be of additional value if it depletes the fractions of bio-available or environmentally active metals in the substrate (Ledin, 1998; Wilkinson, 1999).

In addition to the possibilities of actively remediating contaminated substrates, the more extensive use of *Cyperus* stands in phytostabilisation and revegetation applications looks very promising. Metals can be stabilized and prevented from leaching as water infiltration is reduced through plant mediated hydraulic control. This is the result of the increased evapotranspiration and the interception of rainwater in the stands canopy. A root system allowed less percolation than a barren soil, a good grass cover and a clay cap. Through the development of a root system and theaccumulation of organic matter and litter on the stand surface, the dispersal of contaminants by wind or runoff can be prevented (Wilkinson, 1999). At the same time the site is landscaped and integrated into its surroundings. When the *Cperus* Sp. are harvested at regular intervals, these contaminated dredged sediment sites with little other beneficial use can be made useful through the production of biomass for energy purposes.

However, it is important to determine whether tree planting under any phytoremediation strategy will contaminate the wider environment through leaf fall. Translocation of large amounts of metals to the leaves

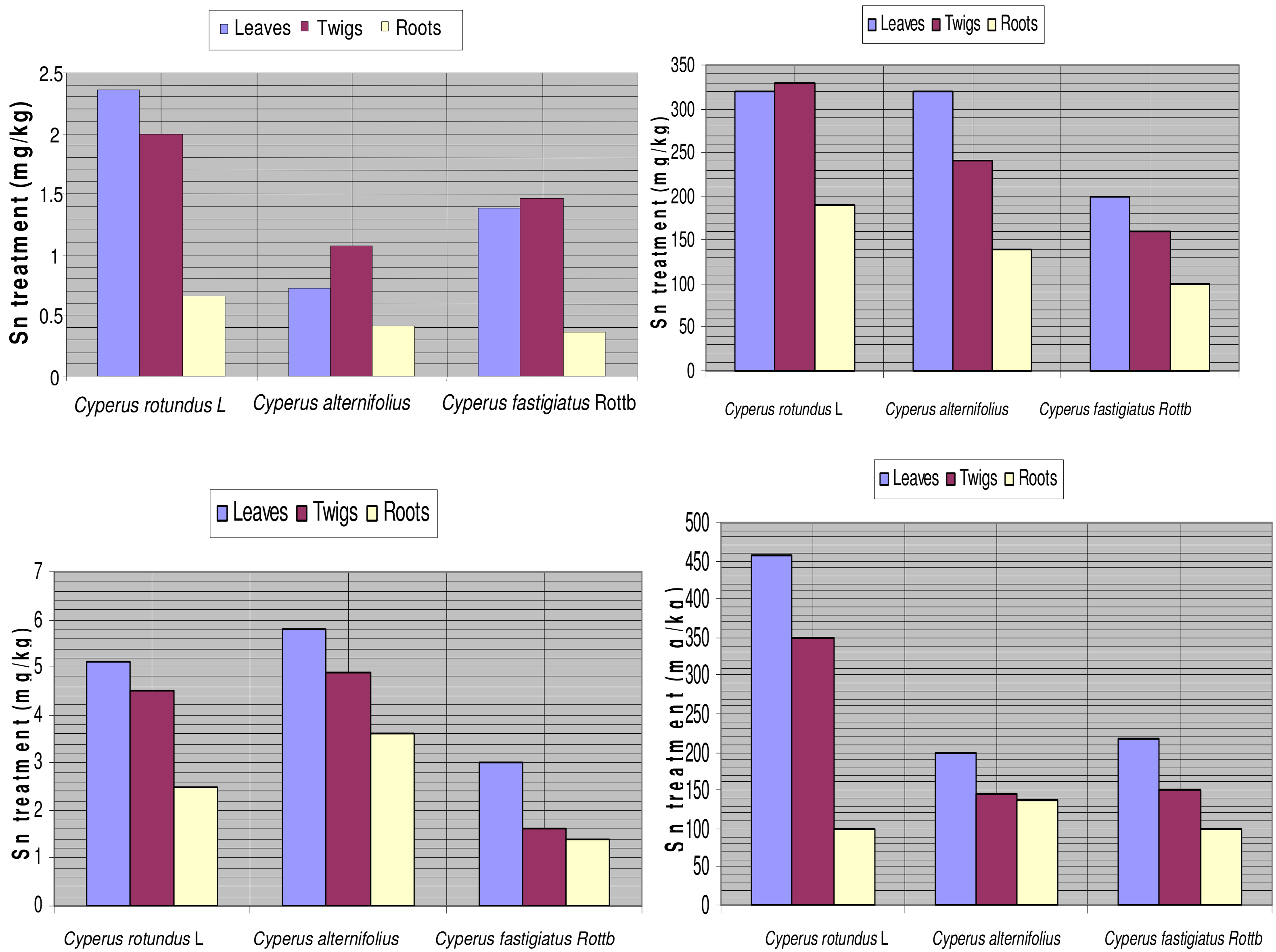
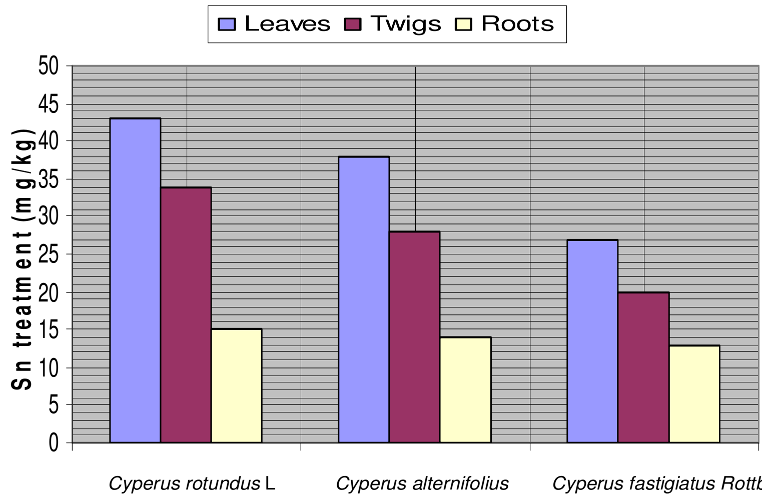


Figure 3. Accumulation difference of three parts for three *Cyperus* under six different Sn treatments. Values with the same letters are not significantly different among treatments at $p<0.05$ according to Duncan's multiple comparison tests.



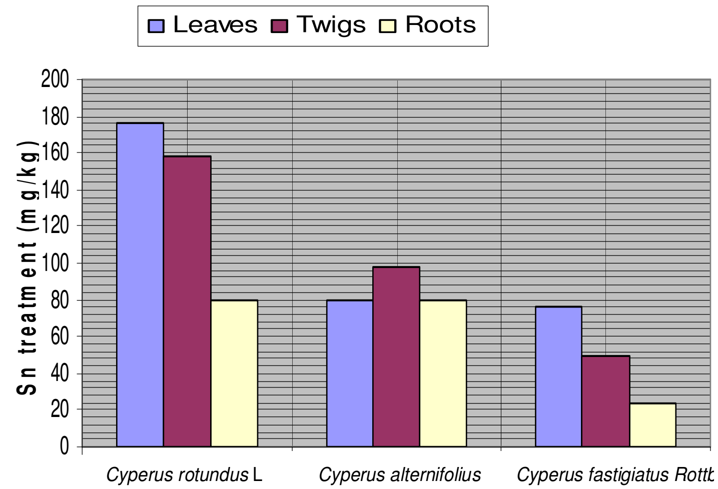


Figure 3. Accumulation difference of three parts for three *Cyperus* under six different Sn treatments. Values with the same letters are not significantly different among treatments at $p<0.05$ according to Duncan's multiple comparison tests (continued).

and twigs may be an undesirable source of food chain accumulation of metals. Therefore, the use of *Cyperus* for phytoremediation would require annual harvesting to avoid the recycling of leaf- and twig-bound Sn.

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