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Full Length Research Paper

Uptake of Pb, Zn and Cu by roots and shoots of fast growing plants grown in contaminated soil in Vietnam

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The phytoremediation of soil contaminated with lead (Pb), zinc (Zn) and copper (Cu) from industrial wastewater discharge in a farming village near Hanoi City was investigated. The first growing plant species tested were the Spanish needle, water primrose, common willow herb, and water spinach. Uptake by roots and shoots per unit land area for each species was calculated and compared. The uptake of the heavy metals per unit area was higher in shoots than in roots for each plant species and was affected by the higher biomass production of the shoots. For the roots, the uptake did not differ by species, but did differ by heavy metal. For the shoots, the uptake differed by both plant species and heavy metal with the common willow herb having higher Pb and Zn uptake compared to those of the other three species. For the common willow herb, the uptake of heavy metal was in order of Pb > Zn > Cu. The results indicate that common willow herb has a superior phytoremediation capacity among the plants tested, particularly for the uptake of Pb.

Key words: Plant biomass, farming village, industrial wastewater, phytoremediation.

INTRODUCTION

In the large cities of Vietnam, surface water pollution due to the lack of wastewater treatment facilities for industrial waste is a serious problem. Some data shows high levels of heavy metals in surface water in some localities (DWRM/ESCAP/KICT (ed.), 2010). In the area around Hanoi, untreated industrial waste was discharged not only from large factories but also from small workshops including handicraft workshop. Surface water polluted by the waste was used to irrigate agricultural land, leading to heavy metal contamination of agricultural soils. Heavy metals in the soils are absorbed by crops and may adversely affect crop growth. Therefore, remediation measures to reduce soil contamination are necessary. In the area, phytoremediation, a technology of using plant to clean up pollutants in the environment is a promising method. This is due to the fact that it is an economical, energy efficient and environmental friendly method, and can be applied to large areas (Roongtanakiat, 2009).

According to Tong et al. (2004), an ideal plant for phytoremediation should be fast growing, develop a large biomass, be tolerant to and accumulate high concentrations of toxic metals in the shoot, and be easily cultivated and harvested. Much research on phytoremediation has been done using native plant species (Lorestani et al., 2011; Aziz et al., 2011; Barbafieri et al., 2011), hyperaccumulating plants (Revathi et al., 2011; Ji et al., 2011; Nazir et al., 2011), and agricultural crops (Mojiri, 2011; Poniedzialek et al., 2010; Garg and Kataria, 2009). In these studies, phytoremediation capacity was evaluated as heavy metal

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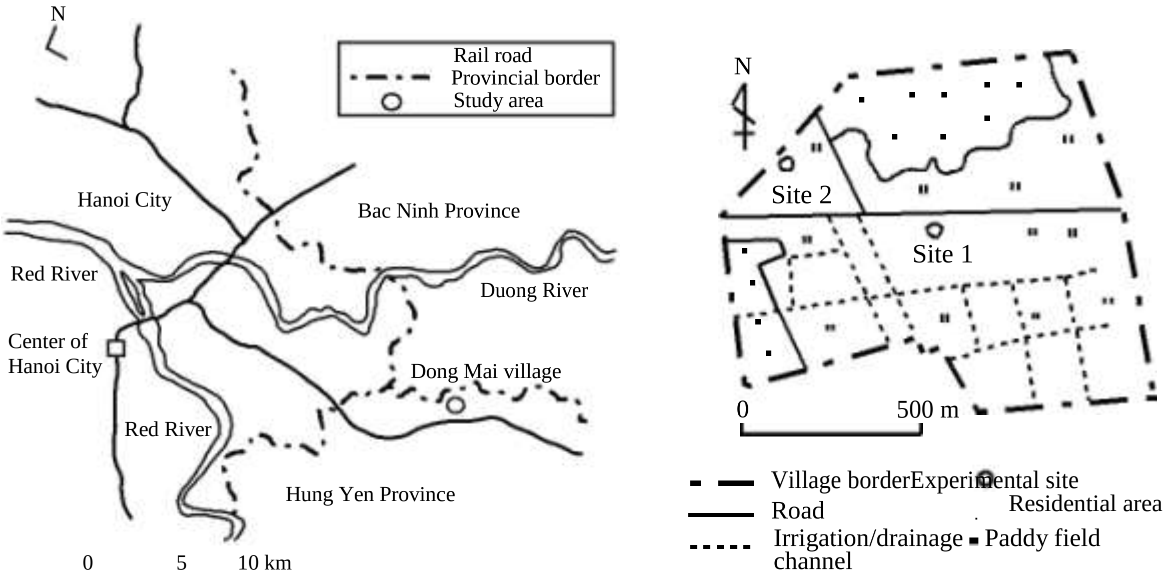

(A) (B)

Figure 1. Study site.

uptake per unit weight of plant roots and/or shoots. However, as demonstrated by Tlustos et al. (2006) and Dai et al. (2007), phytoremediation is a function of the plant assemblage of an area, and thus the remediation capacity should be evaluated as the uptake by all plants of a single species growing per unit area of land.

Since the phytoremediation capacity of plants has not been well investigated for contaminated soils in the Hanoi area, we tested the capacity of plants of different types that are known to have fast growth in the region. By using the uptake of heavy metals by roots and shoots per unit land area, plant with high phytoremediation capacity were identified.

MATERIALS AND METHODS

Study site characteristics

Agricultural and industrial activities

The study was conducted in Dong Mai Village, Chi Dao Commune, in the Van Lam District of Hung Yen Province, Vietnam. The village is located 20 km east of the center of Hanoi City (Figure 1A). The village has a total area of 170 ha and a population of 2,300. An agricultural irrigation system has been developed, and crops are planted in the irrigated area, which occupies 98 ha of the village. Rice is planted twice a year in most of the area. Vegetables are also planted in a portion of the area every year. In addition to agricultural activity, some villagers are engaged in producing handicrafts. From 1967 to 1999, many furnaces were used to smelt Pb for handicraft production, and these were consolidated to one location in 2000. There were no systems for treating the wastes from the smelting furnaces, and waste water containing heavy metals was discharged directly to ditches. The heavy metals were transferred to agricultural land through irrigation. Although, smelting furnaces were stopped in 2006, batteries containing Pb that were used in the old furnaces were abandoned along the ditches, resulting in the continued discharge of Pb into the ditches. These batteries are still contaminating the agricultural land.

Agro-ecological setting

According to meteorological data recorded from 2001 to 2009 in Hanoi City (General Statistics Office, 2002 to 2010), the mean annual air temperature was 24.5°C with a range of monthly mean temperatures of 17.0°C in January and 29.8°C in June. Relative humidity was very high throughout the year with an annual mean of 78.6%. In the rainy season from May to October, mean rainfall was 1,470 mm and mean daily sunshine was 4.8 h, while in the dry season from November to April, mean rainfall was 244 mm and mean daily sunshine was 2.8 h. The soil types in the village area were classified as Eutric Fluvisols according to the FAO/UNESCO soil classification system (VSSS-NIAP, 1996). The village is located in a low-lying area with an elevation of 5 to 6 m above sea level that is frequently flooded in the rainy season.

Field experiments on phytoremediation of soils

Plants tested and heavy metals targeted

Four plant species were tested in the experiments: Spanish needle (*Bidens pilosa* L.), water primrose (*Ludwigia adscendens* L.), common willow herb (*Ludwigia octovalvis* L.), and water spinach (*Ipomoea aquatica* L.). All of these plants are native to the Hanoi area and share the characteristics showing fast growth, producing large amounts of biomass and reproducing prolifically. The plant names are hereafter abbreviated as follows: SN for Spanish needle, WP for water primrose, CWH for common willow herb, and WS for water spinach. Pb, Zn and Cu were targeted because high concentrations of these heavy metals were previously reported in the agricultural soils of the Van Lam District, including in the study

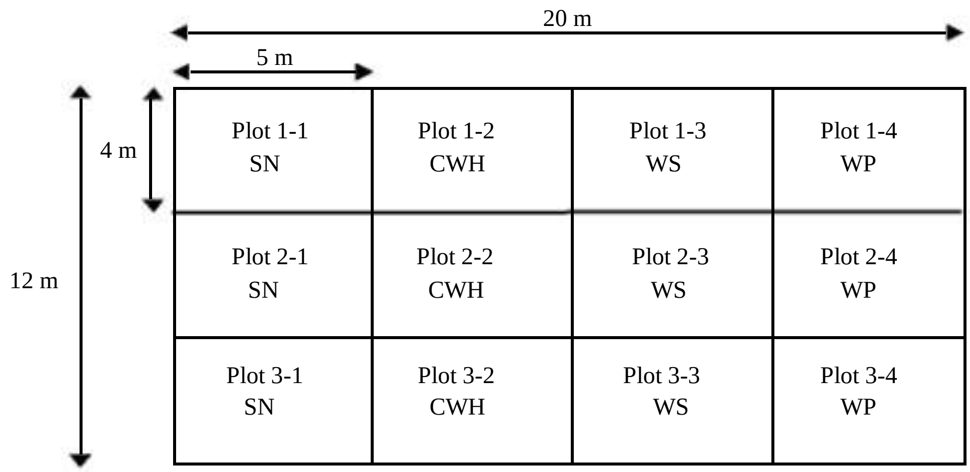

Figure 2. Arrangement of plots for testing plant species at the experimental sites

area of Dong Mai Village (Ho and Nguyen, 2003).

Experimental sites

Two experimental sites (240 m^2 divided into 12 plots) on agricultural land were designated in Dong Mai Village (Figure 1B) at 500 m (site 1) and 10 m (site 2) from the irrigation channel. At each site, each plant was planted in 3 of the 20 m^3 plots (Figure 2).

Transplantation

Plants were started in soil with low concentrations of Pb (11.5 mg/kg), Zn (38.0 mg/kg) and Cu (13.5 mg/kg) in the nursery and were transplanted to the designated plots at each experimental site on March 8, 2007. The planting density was 67 plants/m^2 for SN, WP, and WS, and 33 plants/m^2 for CWH. A total of 0.6 kg of urea fertilizer was applied to each plot 5 days after planting. The SN plots were kept moist without irrigation. Excess rain drained naturally from the plots. For WP, WS and CWH plots, the soil was kept in a wet or water-logged condition, matched to the optimal soil moisture for each plant. Except for sampling, plants were allowed to grow for 90 days after planting without disturbance, including weeding.

Plant sampling

All the plant shoots from an area of 1 m^2 at the four corners and in the center of each plot were collected by cutting all of the plants in all five sampling points of the three plots of the same species close to the ground at 30-day intervals (April 7th, May 7th, and June 6th, 2007), and biomass and heavy metals concentrations were determined. After the first and second samplings, shoots in the entire plots at each site were cut to promote regrowth of plants during the next month. The roots were sampled once on June 6th. At that time, the whole plants were taken from all five sampling points in all three plots for every plant type at both experimental sites. The reasoning for sampling over 90 days (3 months) was that shoots expand and come into contact with those of neighboring plants at 1 month. To get an average monthly growth of shoot under a constant environment, sampling was repeated three times during the 3 months. The root growth is not as fast as that of shoots in a 1 month, but it reached a high level of growth in 3 months.

Soil sampling

Soil was sampled at the two experimental sites on March 4th, 2007, immediately before the plants were transplanted. At each site, 0.5 kg of soil was taken from each of the five sampling points in each plot at a depth from 0 to 0.2 m. The samples for each plot were then combined, and a composite 0.5 kg soil sample was taken for analysis.

Analytical procedures

pH and organic matter content of soil

Soil pH was measured on a 1: 5 suspension of soil in 1 M KCl using a glass electrode. The organic matter (OM) content of soil was determined by the Walkley-Black method (Nelson and Sommers, 1996).

Concentrations of Pb, Zn and Cu in plants and soil

Plant samples (roots and shoots) were cleaned by washing with water, and fresh weight (biomass) was measured after surface drying. The samples were then oven-dried at 105°C for 10 min to neutralize enzymes, then at 70 to 80°C until the samples were completely dry. Soil samples were air-dried at room temperature for 3 to 4 days. After drying, all samples were preprocessed for heavy metal determination by finely grinding and passing the samples through a 1 mm sieve. Extraction of Pb, Zn and Cu from plant samples was done by combustion in a furnace maintained at 550°C, followed by dissolution of the ash with 3 M HCl (National Institute for Soils and Fertilizers, 1998). Soil was digested by hydrogen peroxide, hydrofluoric acid, nitric acid, and perchloric acid for Pb, Zn and Cu analyses (Amacher, 1996). Concentration of total Pb, Zn and Cu in the extracted or digested solutions was determined by atomic absorption spectrophotometry.

Data analysis

The mean difference test (t-test) was performed to assess differences between the two sites in soil heavy metal concentrations, and between the roots and shoots in biomasses productions and heavy metal uptakes. One-way analysis of

Table 1. pH, OM, and total heavy metal concentrations of agricultural soils at sites 1 and 2.

Site	pH	OM (%)	Heavy metal concentration (mg/kg)		
			Pb	Zn	Cu
1	4.67	2.84	864	204	177
2	4.32	3.52	3,352	281	180
Average	4.50	3.18	2,108	243	179
Permissible level (TCVN 7209-2002)			70	200	50

Table 2. Biomass growth of roots and shoots of the four plant species during the 3-month experimental period at sites 1 and 2 (g/m^2).

Roots/shoots	Site	SN	WP	CWH	WS
Roots	1	65	39	90	51
	2	72	30	104	47
	Average	69	35	97	49
Shoots	1	898	1,234	2,850	979
	2	930	1,181	3,338	1,017
	Average	914	1,207	3,094	998

Each value is the mean of three plots of the same species at each site.

variance (ANOVA) was used to identify significant differences in concentrations of heavy metals in soil. Two-way ANOVA was used to identify differences in heavy metal uptake by plant species and heavy metal type. Intergroup means shown to be significantly different were further analyzed by multiple comparison tests performed to assess the relative differences within plant species and/or within heavy metal types. For cases in which the interaction effect was significant between plant species and heavy metal type, the simple main-effect test was performed to determine effect of species and heavy metal type on these differences in detail. The Bonferroni method was applied for the multiple comparison and the simple main-effect tests. Microsoft Excel software was used for data analyses.

RESULTS AND DISCUSSION

Chemical properties and heavy metal concentrations of soil samples

The pH, OM, and Pb, Zn and Cu concentrations of agricultural soils at the two experimental sites are shown in Table 1. The values are the means of values from each of the 12 plots at each site. The soils at both experimental sites were strongly acidic (Table 1). The OM content indicates a medium level of soil fertility. The pH was slightly lower and the OM content somewhat higher for site 2 than for site 1. The soil Pb, Zn and Cu concentrations were all greater than the maximum permissible levels of heavy metals set by Vietnamese standards (TCVN 7209 - 2002), as shown in Table 1. According to one-way ANOVA performed on the differences between the Pb, Zn and Cu concentrations, there were no significant differences among the Pb, Zn and Cu concentrations ($F_{2,3}$=2.33, MSe=2,402,386, P>0.05). Further, t-test of soil heavy metal concentrations between sites 1 and 2 shows that there were no significant differences between the two sites (t(4)=0.80, p>0.05).

Biomass production of roots and shoots by plant species

Biomass production (dry matter quantity and means for three plots of the same species) of roots and shoots by plant species during the 3-month period at sites 1 and 2 are shown in Table 2. The harvested shoot mass was summed over 3 months as shoot growth for comparison with root growth, which was recorded only at the end of the 3-month period. In this table, CWH had the greatest shoot biomass per unit land area (2,850 to 3,338 g/m^2), followed by WP (1,181 to 1,234 g/m^2), and SN and WS had the least biomass (898 to 1,017 g/m^2). CWH also had the greatest root biomass per unit land area (90 to 104 g/m^2), followed by SN (65 to 72 g/m^2). WP and WS had the lowest root biomass (30 to 51 g/m^2). Shoot biomass was 31 to 39 times larger than root biomass for WP and CWH, 19 to 22 times larger for WS, and 13 to 14 times larger for SN. The paired t-test performed with data from the four species showed that shoots had significantly higher biomass than roots (t (7) =4 .46, p<0.05).

Uptake of Pb, Zn and Cu by roots and shoots per unit weight of plant material

Table 3 shows the Pb, Zn and Cu uptake per unit weight

Table 3. Pb, Zn and Cu uptake per unit weight of roots and shoots (mg/kg) for the four plant species during the 3-month period.

Roots/shoots	Heavy metal	Site	SN	WP	CWH	WS
Roots	Pb	1	387	576	661	243
		2	951	1,061	1,839	1,663
	Zn	1	97	60	83	67
		2	119	88	134	70
	Cu	1	35	42	83	25
		2	38	67	93	42
Shoots[1)]	Pb	1	125	48	357	179
		2	244	68	419	232
	Zn	1	149	141	129	149
		2	163	145	216	154
	Cu	1	35	62	80	64
		2	51	72	74	74

SN: Spanish needle, WP: water primrose, CWH: common willow herb, WS: water spinach. Each value is the mean of three plots of the same species at each site; [1)]The mean for the sampling with 1-month interval.

of roots and shoots (mg/kg) for the four species during the 3-month period. Each value is the mean of three plots of the same species at each site. Pb, Zn and Cu uptake for roots (mg/kg) ranged from 243 to 1,839, 60 to 134 and 35 to 93, respectively, for all species. The Pb, Zn and Cu uptake for shoots (mg/kg) ranged from 48 to 419, 129 to 216 and 35 to 80, respectively for all species. The uptake of each heavy metal for each plant was generally greater at site 2 than at site 1 for both roots and shoots. According to two-way ANOVA of uptake by the roots, significant differences by species were not observed ($F_{3,\ 12}$=0.30, MSe=49,515, P>0.05), but the differences by heavy metal were observed ($F_{2,\ 12}$=11.7, MSe=1,934,736, P<0.01), with the greatest uptake for Pb. There were no significant interactions between species and heavy metal type ($F_{6,\ 12}$=0.23, MSe=37,419, P>0.05).

However, for the uptake by shoots, significant differences were seen for both species and heavy metal type ($F_{3,\ 12}$=12.9, MSe=15,883, P<0.01, and $F_{2,\ 12}$=35.0, MSe=43,038, P<0.01, respectively), but since the interaction was also significant ($F_{6,\ 12}$=8.9, MSe=10,912, P<0.01), these results should be interpreted carefully.

Paired t-test of uptake by roots and shoots of all heavy metals showed no differences in uptake by shoots and roots of each plant species (t(5)=0.85-1.26, p>0.05). Yoon et al. (2006) measured the uptake of Pb, Zn and Cu for many native plant species grown in contaminated soils. For soil Pb, Zn and Cu concentrations similar to those in our study, the maximum uptake of Zn and Cu by the roots and shoots of the plants (524 and 250 mg/kg, respectively, for Zn; 375 and 210 mg/kg, respectively, for Cu) were larger than uptake measured in our study, but the maximum Pb uptake by the roots and shoots (881 and 491 mg/kg, respectively) was either smaller or only slightly larger than the uptake we measured for CWH (Table 3).

Uptake of Pb, Zn and Cu by roots and shoots per unit land area

Table 4 shows the Pb, Zn and Cu uptakes per unit land area of roots and shoots for the four species over the 3-month period. The uptake per unit land area was calculated by the uptake per unit weight and multiplied by plant weight for the unit land area. Each value is the mean of three plots of the same species at each site. Pb, Zn and Cu uptake by the roots (mg/m2) showed ranges of 12-199, 2-15 and, 1-10, respectively, for all plant species, and uptake was the greatest in CWH among all plant species tested at each site.

Pb uptake for the shoots (mg/m^2) ranged from 61 to 1,396 and was the smallest in WP (≤83), and greatest in CWH (≥1,056). Pb uptake in the other plant species was ≤241. Zn and Cu uptake for the shoots (mg/m^2) showed ranges of 137 to 718 and 32 to 247, respectively for all plant species. Uptake was the greatest for CWH.

Paired t-tests performed on the uptake (mg/m^2) of Pb, Zn and Cu by roots and shoots showed that the uptake by the shoots was significantly higher than by roots for each plant species (t(5)=3.72 to 6.77, p<0.05), which is different from the pattern shown for uptake per unit weight of shoots/roots discussed previously. This difference is likely attributable to the larger biomass production in shoots than in roots (Table 2).

Difference in uptake per unit land area according to the plant species and heavy metal type

Two-way ANOVA of uptake by the roots showed significant differences among heavy metal types ($F_{2,12}$=7.81, MSe=8,636, P<0.01), where the uptake of Pb was higher than those of Cu and Zn and those of Cu and

Table 4. Pb, Zn and Cu uptake of roots and shoots per unit land area (mg/m^2) for the four plant species during the 3-month period.

Roots/shoots	Heavy metal	Site	SN	WP	CWH	WS
Roots	Pb	1	24	23	57	12
		2	67	33	199	78
	Zn	1	6	2	7	3
		2	8	3	15	3
	Cu	1	2	2	7	1
		2	3	2	10	2
Shoots[1]	Pb	1	115	61	1,056	180
		2	232	83	1,396	241
	Zn	1	137	175	378	148
		2	152	174	718	158
	Cu	1	32	77	238	64
		2	48	86	247	78

SN: Spanish needle, WP: water primrose, CWH: common willow herb, WS: water spinach. Each value is the mean of three plots of the same species at each site; 1The sum of values for each month.

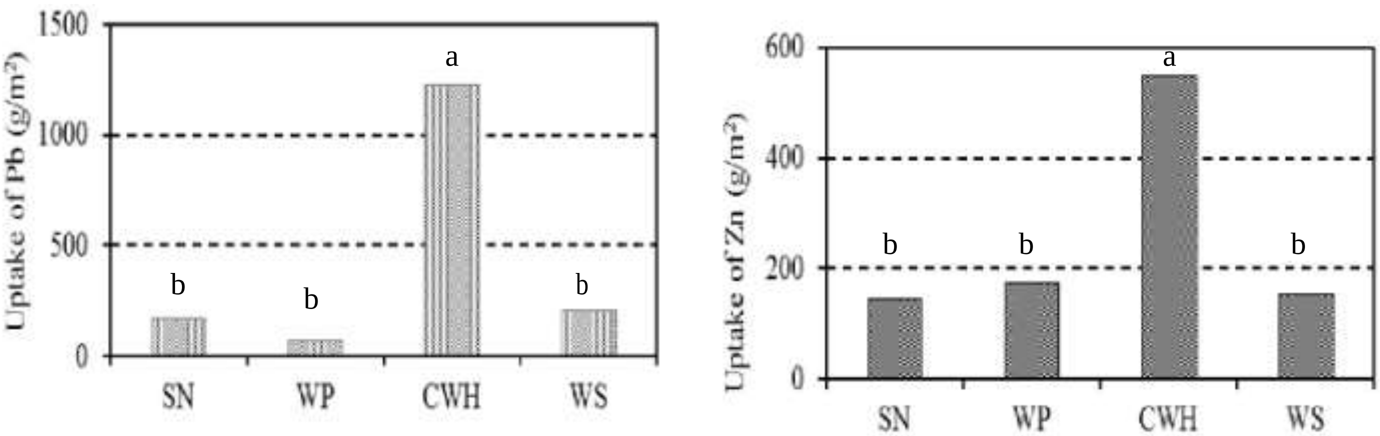

Figure 3. Uptake of Pb and Zn by the shoots for the four plant species. Each uptake value is the mean of plots of the same species at sites 1 and 2. Different letters above each column indicate values that are significantly different at the 0.05 or 0.01 levels according to simple main-effect test.

Zn were not significantly different by the multiple comparison test. The differences in uptake by the roots of plant species were not observed ($F_{3, 12}$=1.62, MSe=1,789, P>0.05), and no significant interactions between heavy metal types and plant species were observed (F6, 12=1.05, MSe= 1,156, P>0.05). In contrast, uptake by shoots showed significant differences by heavy metal type and plant species ($F_{2, 12}$=18.7, MSe=194,623, P<0.01 and F3, 12=43.3, MSe=451,249, P<0.01, respectively). A significant interaction between heavy metal type and plant species was also observed ($F_{6, 12}$=10.8, MSe=112,479, P<0.01). For the uptake of Pb and Zn by shoots, significant differences were found among the plant species by the simple main effect test (Figure 3). Uptake of Cu by shoots was not found to differ among plant species by the same test, though data not shown in Figure 3. The uptake of Pb and Zn was the greatest in CWH (Figure 3). However, uptake of Pb and Zn did not significantly differ among SN, WP and WS. Figure 4 shows the uptake of Pb, Zn and Cu by the shoots of CWH, for which a significant difference was elucidated by the simple main effect test. The uptake by shoots was in order of Pb > Zn > Cu. No differences in uptake by shoots were observed for other three plant species.

Tlustos et al. (2006) showed that maximum uptake of Pb and Zn by shoots of high biomass-producing crops (sweet clover, red clover, curled mallow, safflower and hemp) to be 120 and 104 mg/m^2, respectively. According to Dai et al. (2007), the uptake of Pb by roots plus shoots

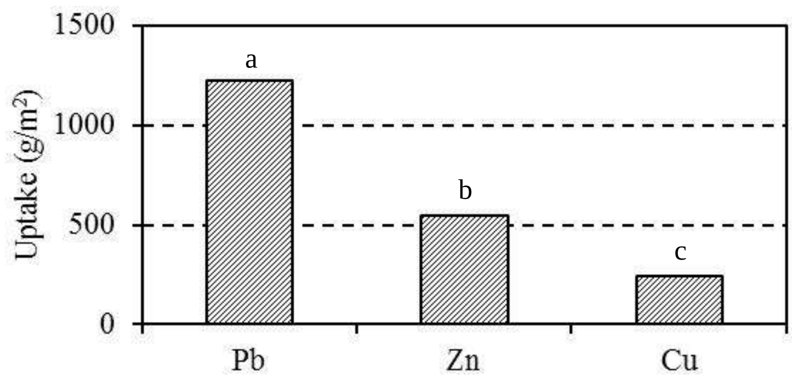

Figure 4. Uptake of Pb, Zn and Cu by the shoots in CWH. Each value is the mean of the plots of CWH at Sites 1 and 2. Different letters above the column indicate that the values are significantly different at the 0.05 or 0.01 level according to the simple main-effect test.

of maize was 465 mg/m^2. In comparison, uptake of Pb and Zn by shoots (nearly 1,200 and 500 mg/m^2, respectively) and the uptake of Pb by roots plus shoots (nearly 1,400 mg/m^2) for CWH in our study are far greater.

From these results, we can conclude that CWH has superior characteristics for the uptake of heavy metals, particularly of Pb, and can be effectively utilized for phytoremediation in this study area.

Conclusions

1. To evaluate the phytoremediation capacity, the uptake by plants per unit land area was compared. Uptake per unit land area by roots differed by heavy metal type, and uptake of Pb was the largest while uptakes of Zn and Cu were low with no difference from each other.

2. The uptake was higher in shoots than in roots for each plant species, due to the greater biomass of shoots than roots.

3. The uptake by shoots differed by both plant species and heavy metal type. Uptakes of Pb and Zn were greatest in CWH, while that of Cu did not differ among plant species. A difference in uptake among the heavy metal type was observed only in CWH, where the uptake was in order of Pb > Zn > Cu.

4. From the aforementioned higher uptake of heavy metals by the shoots of CWH, CWH has superior phytoremediation capacity, particularly, for the uptake of Pb.

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