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Bioaccumulation of heavy metal uptake by two different Vetiver grass (*Vetiveria zizanioides* and *Vetiveria nemoralis*) species

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The efficiency of Cd, Pb and Zn uptake of upland vetiver grass (*Vetiveria nemoralis*) ecotypes as Kamphaeng Phet1, Prajuab Kirikhun, and Ratchaburi and lowland vetiver grass (*Vetiveria zizanioides*) ecotypes consisting of Kamphaeng Phet2, Mae Hongson, and Surat Thani were compared under sterile condition for interferent repudiation. The results showed that the total Cd accumulation of *V. zizanioides* was in the order of Kamphaeng Phet1 > Ratchaburi > Prajuab Kirikhun ecotype and in *V. nemoralis* was Mea Hongson approximately Kamphaeng Phet2 > Surat Thani ($p < 0.05$). All the vetiver grass ecotypes preferentially accumulated Pb in roots than shoots, with the rate of translocation less than 1. However, the efficiency of Cd and Zn translocation was different, depending on each vetiver ecotype. In addition, all vetiver ecotypes, except Kamphaeng Phet1, displayed high capability of Zn uptake in both shoots and roots after 7 days. Therefore, the vetiver plant can be considered a good "hyperaccumulator" only for Zn.

Key words: *Vetiveria zizanioides*, *Vetiveria nemoralis*, total accumulation rate (TAR), transport factor (TF).

INTRODUCTION

Environmental heavy metal contamination is a major global concern due to the high persistency, potential toxicities and bioaccumulation of metals in living organism (Gardea-Torresdey et al., 2004; Singh et al., 2004). The remediation technologies of heavily heavy metal contaminated soils are generally extremely complicated and expensive. Phytoremediation is an alternative emerging technology utilizing plants to reduce, remove, degrade or immobilize xenobiotics from contaminated environment (Suthersan, 1999). The advantages of phytoremediation are relatively simple, eco-friendly and cost effective compared to other conventional strategies (Burd et al., 2000; Glick, 2003; McGrath et al., 1997). However, in order to survive and be established at the high level of metal-polluted sites, plants must acquire the capabilities to grow, produce high biomass and evolve to tolerate metal toxicities as well as other hostile environment conditions. Having a deep and dense root system, vetiver is a good candidate for phytoremedial work. Vetiver grass which can be taxonomically classified into 2 dominant species namely *Vetiveria nemolaris* as upland vetiver and *Vetiveria zizanioides* as lowland vetiver is well recognized for its effectiveness in soil erosion and sediment control, high tolerance to extreme environmental variations including prolonged drought, flood, extreme fluctuations of temperature (22-60°C), soil pH (3.0-10.5), and most importantly high tolerance to heavy metal stress

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(Truong and Baker, 1998). The aims of this study were to compare the uptake efficiency of Cadmium (Cd), Lead (Pb) and Zinc (Zn) on 2 species of vetiver grass, *Vetiver zizanioides* and *Vetiveria nemoralis*, in axenic condition. In addition, the pattern of metal distribution and translocation in plant parts were also investigated.

MATERIALS AND MEDTHODS

Plant cultures

One month tissue culture of *V. zizanioides* (Kamphaeng Phet1, Prajuab Kirikhun, and Ratchaburi ecotypes) and *V. nemoralis* (Kamphaeng Phet2, Mae Hongson, and Surat Thani ecotypes) were cultured in sterile 30 cm length glass tubes containing 0.7% agar in Musashige and Skoog medium (as ppm of 1,650.0 NH_4NO_3, 6.20 H_3BO_3, 332.20 $CaCl_2{\cdot}2H_2O$, 0.0250 $CoCl_2{\cdot}6H_2O$, 0.0250 $CuSO_4{\cdot}5H_2O$, 37.260 $Na_2EDTA{\cdot}2H_2O$, 27.80 $FeSO_4{\cdot}7H_2O$, 2.0 Glycine, 180.70 $MgSO_4{\cdot}7H_2O$, 16.90 $MnSO_4{\cdot}4H_2O$, 100.0 myo-Inositol, 0.50 nicotinic acid, 0.830 KI, 1,900.0 KNO_3, 170.0 KH_2PO_4, 0.50 Pyridoxine hydrochloride, 0.250 $Na_2MoO_4.2H_2O$, 0.10 Thiamine hydrochloride, 8.60 $ZnSO_4{\cdot}7H_2O$) in the absence or presence of one heavy metal (20 ppm Cd or 500 ppm Zn or 500 ppm Pb). These heavy metal concentrations were chosen as they are the metal tolerance levels in soil of vetiver grass (Truong, 2000). Tubes were closed with sterile cottons and kept in a plant growth chamber at 23 to 28°C for 7 days. Each treatment variable was conducted in triplicates.

Heavy metal extraction

At the end of experiment, vetivers were weighed for biomass determination, and then heavy metal concentration was determined as described by Farwell et al. (2007). Briefly, plants were extensively rinsed with several changes of 0.01 M EDTA and followed by distilled water to remove some non-specifically bound heavy metal. Afterwards, the separated shoots and roots of grass were dried in a hot air oven at 65°C for 1 day and were finely cut. 30 mg dried plant samples were digested with 0.8 ml of purified HNO_3 in an incubator at 65°C for 3 h. The supernatants were determined for heavy metals by Graphite Furnace Atomic Absorption Spectrophotometry (GFAAS) model Perkin Elmer AAnalyst 100.

The plant heavy metal uptakes were expressed as total accumulation rate (TAR) (Zhu et al., 1999) and transport factor (TF) as described previously (Marchiol et al., 2004) and were calculated by the following formula:

$$TAR = \frac{(\text{Shoot dry weight (g)} \times \text{Shoot HM conc } (\mu g/g)) + (\text{Root dry weight (g)} \times \text{Root HM conc } (\mu g/g))}{((\text{Shoot dry weight (g)} + \text{Root dry weight (g)}) \times \text{days of harvest})}$$

$$TF = \left[\frac{\text{Shoot heavy metal conc } (\mu g/g)}{\text{Root heavy metal conc } (\mu g/g)}\right]$$

HM conc. = heavy metal concentration

Statistical analysis of data

The results were expressed as mean ± SD from three biological replicates. Data were analyzed by one-way analysis of variance (ANOVA) with means separated using Duncan's multiple range test. $P < 0.05$ was considered statistically significant. All data were analyzed by SPSS 16.0 software.

RESULTS

Heavy metal concentration in vetiver

The total Cd accumulation of *V. zizanioides* was in the order of Kamphaeng Phet1 > Ratchaburi > Prajuab Kirikhun ecotypes and for *V. nemoralis* was Mea Hongson approximately Kamphaeng Phet2 > Surat Thani ecotypes ($p < 0.05$). For over all comparison, Kamphaeng Phet1, Mea Hongson and Kamphaeng Phet2 exhibited almost equally highest efficiency of Cd uptake up to 600 ppm (Figure 1). In addition, most of the tested plants stored more Cd in shoots. The total Pb uptake by the tested plants ranges from 381 to 606 ppm. Though Kamphaeng Phet1, Surat Thani, Kamphaeng Phet2 and Mae Hongson ecotypes showed the trend of higher Pb accumulation (>500 ppm), the bioaccumulation levels were not significantly different from Ratchaburi and Prajuab Kirikhun. Notably, the root Pb concentrations of all tested plants were at least 4 times higher than in shoots. The capacity of Zn uptake was in the range of 8,714 to 23,285.4 ppm. All tested plants except Kamphaeng Phet1 were able to uptake and accumulate Zn higher than 10,000 ppm.

Heavy metal translocation in vetiver

Translocation factor (TF) is the ratio that indicates the relative transportation of metals from roots to shoots of the plants (Mellem et al., 2012). TF values of greater than 1 indicate the greater translocation of metals from root to the shoot part of the plant. In contrast, TF values of less than 1 mean that metals are largely store in the root part of plants (Mellem et al., 2012; Rezvani and Zaefarian, 2011). According to Table 1, ecotypes Mae Hongson, Prajuab Kirikun and Ratchaburi were capable of accumulating Cd higher in the shoot part than in the root part, with the maximum translocation capability (TF > 1) observed in the Ratchaburi ecotype (TF = 1.92±0.02). However, the total metal accumulation rate as indicated by the TAR values in Table 1 suggested no differences in the total accumulation per day of both Cd and Pb in all tested vetiver ecotypes. For Pb, the TF value of less than 1 indicated that all tested ecotypes preferentially accumulated Pb in roots rather than shoots (Table 1). In addition, Surat Thani exhibited higher intrinsic capability of Pb translocation than Ratchaburi and Mae Hongson ecotypes. Kamphaeng Phet2, Ratchaburi and Surat Thani showed bulk Zn contents in shoots rather than roots (TF > 1) suggesting their high potential of Zn translocation. The Ratchaburi and Surat Thani significantly displayed higher potential of Zn translocation than the rest of tested ecotypes ($p < 0.05$). The total Zn

Table 1. Heavy metal concentration in different vetiver grass ecotypes grown under heavy metal stress.

Vetiver	20 ppm Cd		500 ppm Pb		500 ppm Zn	
	TF*	TAR**	TF	TAR	TF	TAR
Kum Phangphet1	0.61±0.12[a]	2.547±0.255[a(c)]	0.26±0.06[ab]	0.03±0.013[a(a)]	0.42±0.08[bc]	1.081±0.552[a(b)]
Kum Phangphet2	0.87±0.25[a]	2.296±0.559[a(c)]	0.28±0.08[ab]	0.034±0.001[a(a)]	2.47±0.83[b]	1.087±0.045[a(b)]
Mae Hongson	1.41±0.83[b]	2.293±0.622[a(c)]	0.20±0.06[a]	0.033±0.007[a(a)]	0.70±0.33[ab]	1.557±0.458[b(b)]
Prajuab Kirikun	1.80±0.74[b]	1.702±0.603[a(c)]	0.29±0.13[ab]	0.021±0.007[a(a)]	0.31±0.26[a]	0.847±0.474[a(b)]
Ratchaburi	1.92±0.02[c]	1.992±0.591[a(b)]	0.21±0.16[a]	0.028±0.001[a(a)]	4.41±0.27[c]	1.884±0.596[b(b)]
Surat Thani	0.77±0.13[a]	2.612±1.059[a(c)]	0.45±0.07[b]	0.028±0.011[a(a)]	3.79±1.39[c]	1.197±0.428[a(b)]

Value in the same column with different superscript are significantly different at $p < 0.05$. Value in parentheses ( ) in the same TAR roll with different superscript are significantly different at $p < 0.05$.

$$*\mathrm{TF} = \left[\frac{\text{Shoot heavy metal conc } (\mu g/g)}{\text{Root heavy metal conc } (\mu g/g)}\right]$$

$$**\mathrm{TAR} = \frac{(\text{Shoot dry weight (g)} \times \text{Shoot HM conc } (\mu g/g)) + (\text{Root dry weight (g)} \times \text{Root HM conc } (\mu g/g))}{((\text{Shoot dry weight (g)} + \text{Root dry weight (g)}) \times \text{days of harvest})}$$

accumulation rate (TAR) of Ratchaburi was more effective than Kamphaeng Phet1, Kamphaeng Phet 2 and Prajuab Kirikun. There were no differences of Zn total accumulation rate among Surat Thani and Mae Hongson ecotypes (Table 1). All the tested vetiver surprisingly showed the best Cd accumulation rate (TAR) followed by Zn and Pb, respectively ($p < 0.05$) as shown in Table 1.

DISCUSSION

A raising concern regarding human health risks and environmental consequences associated with heavy metal pollution have created a need for efficient and effective remediation strategies. Phytoremediation has recently attracted a great attention as a highly promising strategy because it is relatively inexpensive, environmentally-friendly, and more aesthetically pleasing compared to the other remediation technologies. This study chose upland (*V. zizanioides*) and lowland (*V. nemoralis*) vetiver grass as the test plants for phytoremediation due to their fast growth, high biomass, deep massive and fibrous root system, versatile adaptability under various harsh environmental conditions, and most importantly, high resistance to heavy metal toxicities (Chomchalow, 2011; Roongtanakiat and Chairoj, 2001; Truong and Baker, 1998). At present, there are still very few studies on the potential of vetiver for the use in phytoremediation especially the comparison of metal remediation efficiency among different vetiver ecotypes (Punamiya et al., 2010; Roongtanakiat and Chairoj, 2001, 2002; Roongtanakiat et al., 2007). Therefore, the present study explored the capability and compared the efficiency of heavy metal uptake, translocation and accumulation among different ecotypes of upland and lowland vetivers. The experiments were intentionally conducted under axenic condition to avoid un-estimated effects of other environmental factors that might affect the metal uptake efficiency.

Different ecotypes of vetiver were ranked in descending order of the capability to uptake Cd as follows: Kamphaeng Phet1 ~ Mea Hongson ~ Kamphaeng Phet2 > Ratchaburi ~ Surat Thani > Prajuab Kirikhun (Figure 1). In contrast, Roongtanakiat and Chairoj (2002) reported that there were significantly higher concentrations of cadmium in Ratchaburi ecotype compared to those found in Surat Thani and Kamphaeng Phet ecotypes in the pot experiment. Variations of each vetiver ecotype, soil properties, the existing soil microorganisms (Truong, 1999) and season of planting (Devies, 1997) are among the factors that can affect the heavy metal uptake efficiency. These probably explained the different results obtained in the present study which was conducted under axenic condition. Therefore, the different results observed from the axenic and pot experiments might be caused by various properties of the media and soil which are different under axenic and pot conditions. A variation of plant species also widely exhibited different ability of heavy metal accumulation (Baker and Senft, 1997).

All tested ecotypes in both species of vetiver grass displayed equal capability of Pb uptake when cultured under axenic media supplemented with 500 ppm Pb (Figure 1B), and the uptake was in the range of 400 to 600 ppm Pb. Similarly, Punamiya et al. (2010) studied Pb accumulation by vetiver grass [*Chrysopogon zizanioides* (L.)] in hydroponic solutions supplemented with 2,070, 4,140 and 8,280 ppm Pb. The results found that the corresponded Pb uptakes by the vetiver grass were 600, 900 and 1,400 ppm, respectively. The present study demonstrated that almost all tested vetiver ecotypes, except Kamphaeng Phet1, could be regarded as Zn hyperaccumulator as all displayed a capacity of Zn

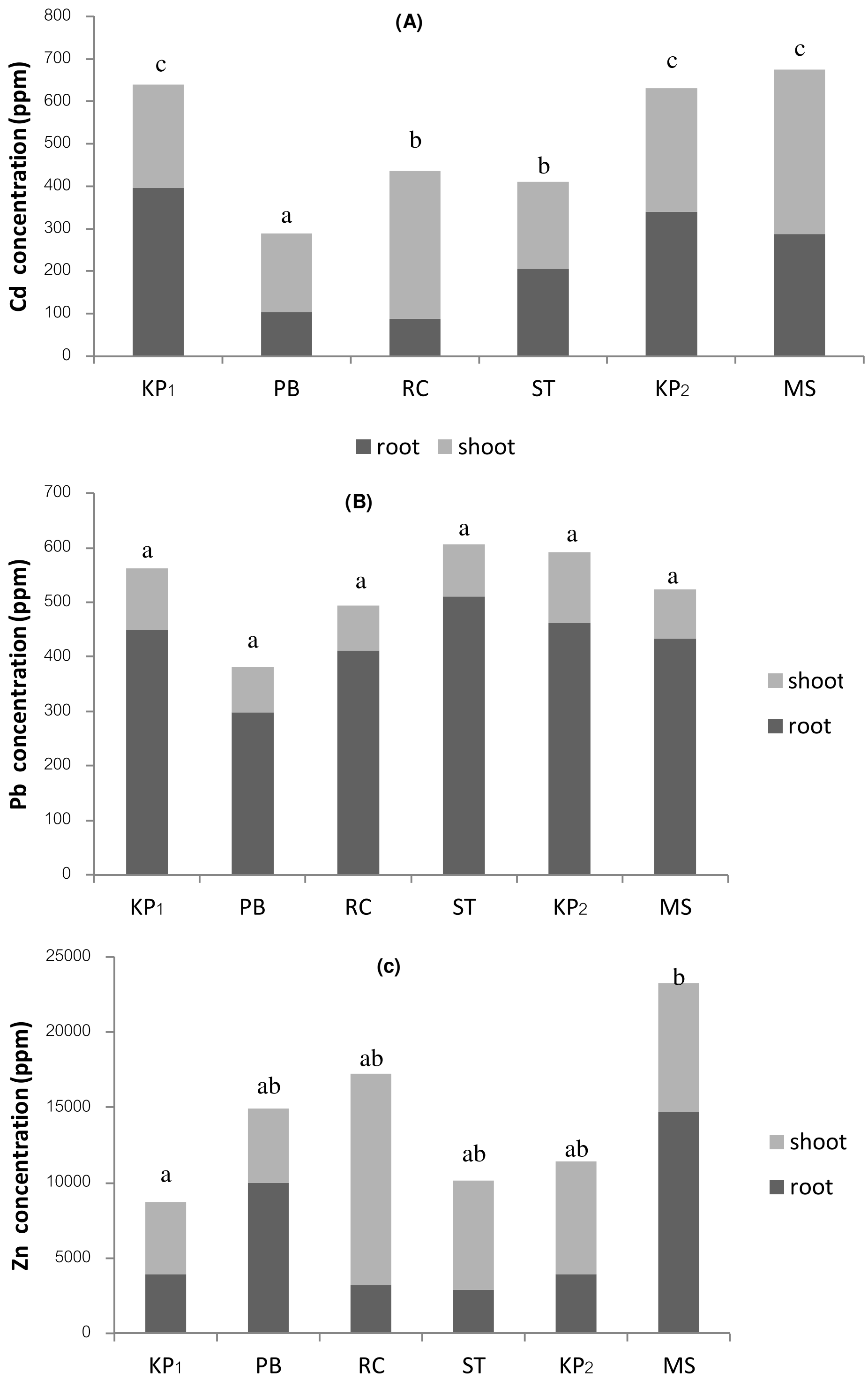

Figure 1. Total heavy metal uptake of *V. zizanioides* (Kumphang Phet1 (KP1), Prajuab Kirikhun (PB), and Ratchaburi (RC)) and *V. nemoralis* (Kumphang Phet2 (KP2), Mae Hongson (MS), and Surat Thani (ST)) ecotypes grown under Cd (A), Pb (B), or Zn (C). Bars of each ecotype with different superscript are significantly different at $p < 0.05$.

uptake more than 1% of their dry weight. This agrees to the term defined by Baker and Brooks (1989) who referred plants with a capability to accumulate Zn more than 1% of their dry weight as “hyperaccumulators”. The result in Figure 1C also suggested that Mae Hongson ecotype is the best Zn hyperaccumulator while

Kamphaeng Phet1 is the least. In addition, the results showed that the increasing TAR value corresponded with the increasing Zn uptake in vetiver grass in accordance with Kosesakal et al. (2011). They reported that higher metal application rate causes the increasing metal uptake and significantly increase TAR tend. Similar to our results significantly showed the higher TAR value of Cd and Zn application than Pb application. Notably, the capacities of Pb uptake by the vetiver in the study of Punamiya et al. (2010) were all much lower than the supplemented concentrations of metals. On the contrary, the present study demonstrated that all tested ecotypes of both vetiver species could concentrate Cd, Pb and Zn many folds higher than their surrounding (supplemented) metal concentrations.

The capability of vetiver grass to concentrate higher amount of metals than its surrounding environment concentrations and preferentially accumulate higher level of Zn than Pb were also supported by other investigators. Antiochia et al. (2007) reported that *V. zizanioides* could accumulate 6,000 ppm Pb and 9,500 ppm, Zn after 8 days of exposure to 621 ppm Pb and 653 ppm Zn of supplemented soil in the pot study. They concluded that *V. zizanioides* is an effective hyperaccumulator for both Pb and Zn. Ratchaburi ecotype exhibited the highest translocation of Cd from roots to shoots ($p < 0.05$) while Mae Hongson and Prajuab Kirikun showed a trend of higher Cd translocation than the rest of tested ecotypes (Table 1). Similarly, Rezvani and Zaefarian (2011) exhibited the translocation factor of Cd more than one by *Aeluropus littoralis* in pot experiments. Roongtanakiat and Chairoj (2002) also reported that Ratchaburi ecotype preferentially accumulated Cd higher in shoots rather than roots while there were no differences in Pb translocation capability among tested vetiver ecotypes, Ratchaburi and Surat Thani significantly displayed the highest translocation of Zn from the roots to the shoot parts (Table 1). Results from the present study suggested that all tested vetiver ecotypes could effectively act as phytostabilizer for Pb which the metal were mainly retained in the root part of the plants with the TF values of less than 1 (Figure 1B and Table 1). The low metal translocation to their aerial parts or mainly restrict metal in their root call metal excluders (Ghosh and Singh, 2005). In agreement with the present study, Chen et al. (2004) also reported the low translocation of Pb from roots to shoots in *V. zizanioides.* Tang et al. (2009) also pronounced the TF value of *Arabis paniculata* Franch less than one when was supplemented with range of Pb concentration (9-267 µM). Consequently, vetiver grass might be a remediated plant of choice for immobilization of Pb at the contaminated sites. In addition, Ratchaburi ecotype which exhibited the highest Cd translocation could be a good candidate for phytoextraction of Cd-contaminated soils. All tested ecotypes of vetiver except Kamphaeng Phet1 are only good hyperaccumulator for Zn, but not for Cd and Pb. Therefore, different ecotypes of vetiver grass possess distinct capability and efficiency in remediating certain heavy metals through various phytoremedating mechanisms under axenic culture. These differences should be taken under consideration to warranty for a fruitful exploitation of vetiver as remediating plants for heavy metals in further pot and field studies.

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