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Phytoextraction of Zinc, Cadmium and Lead from Contaminated Soil by Vetiver Grass

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ABSTRACT

Phytoextraction of heavy metals is an environmental remediation method that uses plants to transport and accumulate the metals into harvestable growth. The ability of two vetiver ecotypes for phytoextraction of heavy metals (Zn, Cd and Pb) was studied using a 2 × 4 factorial pot experiment in a completely randomized design with three replications. The two factors were the vetiver ecotype and heavy metal concentration level in the soil. The Ratchaburi vetiver ecotype (*Chrysopogon nemoralis*) and the Surat Thani vetiver ecotype (*Chrysopogon zizanioides*) were grown in contaminated soils with four levels of Zn, Cd and Pb. The results indicated that vetiver could grow well in the contaminated soil without any heavy metal toxicity symptoms. The Ratchaburi ecotype had a significantly better plant height and shoot dry weight than the Surat Thani ecotype. Vetiver plants grown in soil highly contaminated with heavy metals decreased in growth and accumulated more heavy metals. Two vetiver ecotypes gave different translocation factors of heavy metals. In general, vetiver accumulated more heavy metals in the roots than in the shoots. However, the Ratchaburi ecotype accumulated significantly more Zn in its shoots than roots, indicating its potential for use in the phytoextraction of Zn from contaminated soil.
Keywords: vetiver, heavy metals, zinc, cadmium, lead, phytoremediation

INTRODUCTION

Heavy metal contamination in soils is one of the major environmental problems as it has significant risk to human health and the ecosystem. In Thailand, rapid industrial expansion and population growth have resulted in an increasing degree of heavy metal contamination so that it is becoming one of the serious environmental problems. Solving this issue has been restricted to mechanical and chemical treatments which are costly and complex. Phytoremediation, an emerging green technology, is considered as an innovative, economical and environmentally compatible method for cleaning up contaminated sites. (Cunningham *et al*., 1995) It utilizes plants to remove, transfer, degrade or immobilize environmental pollutants with the aim of restoring contaminated sites to a useable condition. Phytoextraction is a mechanism involved in phytoremediation that uses plants to remove contaminants from soils, sediments or water and the contaminants are accumulated in harvestable plant biomass. Plant species used in this process must tolerate a high level of toxic pollutants, be fast growing and have a high capacity for uptake

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of pollutants. For heavy metal remediation, using hyperaccumulator plants can increase metal uptake, as they are capable of accumulation a hundredfold greater than non-accumulator plants (Lasat, 1996). However, most metal hyperaccumulators such as alpine pennycress (*Thlaspi caerulescens*), Indian mustard (*Brassica juncea*) and Chinese brake fern (*Pteris vittata* L.) are slow growing and produce little biomass. The suitability of some nonaccumulator plants with heavy metal tolerance and high biomass production characteristics should be considered for phytoextraction.

Vetiver grass, a versatile plant in agriculture, is widely known for its effectiveness in erosion and sediment control. It was introduced for soil and water conservation in Thailand in 1991. Following discovery of the tolerance of vetiver to extreme climatic variation, soil conditions and heavy metals (Truong 1996; Roongtanakiat and Chairoj, 2001a, 2001b), the concept of using vetiver for phytoremediation has been realized. Later, Truong (1999), Greenfield (2002) and Roongtanakiat (2006) reported that vetiver is a nonhyperaccumulator. However, the unique characteristics of vetiver compensate for and overcome its phytoremediation limitation. The strong, deep root system of vetiver does not restrict its use to sites with shallow contamination. Vetiver is both a xerophyte and hydrophyte; therefore, it can be applied to soil and water decontamination. It is not a weedy plant species and can survive in harsh environments. Due to its nonhyperaccumulator characteristic, vetiver does not pose a risk to grazing animals and harvested biomass should not be classified as hazardous waste. There are two species of vetiver in Thailand—namely, *Chrysopogon nemoralis* (Balansa) Holttum and *Chrysopogon zizanioides* (L.) Roberty. Both species have distinct ecological characteristics that allow them to adapt to different habitats and there are many ecotypes (Roongtanakiat, 2009). For phytoextraction purposes, the vetiver ecotype used must grow well in contaminated soils, accumulate high concentrations of pollutants in the aerial parts of the grass and should have a high biomass. Therefore, the ability of two vetiver ecotypes to phytoextract Zn, Cd and Pb was studied. The concentration and uptake of these metals in vetiver shoots and roots were also investigated.

MATERIALS AND METHODS

A pot experiment was conducted using a 2 × 4 factorial experiment in a completely randomized design with three replications. The two factors were the vetiver ecotype and the heavy metal concentration level in the soil. Two popular vetiver ecotypes, the Ratchaburi ecotype (*Chrysopogon nemoralis*) and the Surat Thani ecotype (*Chrysopogon zizanioides*) were used in this study. Uncontaminated soil (A1), used as the control treatment, was collected from the Huai Sai Royal Development Study Center, Cha-am district, Phetchaburi province. Contaminated soils with three levels of Zn, Cd and Pb (A2, A3 and A4) were collected near an operational zinc mine in Mae Sod district, Tak province. The concentrations of heavy metals and nutrients in the soils as well as chemical and physical soil properties were analyzed before growing the vetiver (Tables 1). Two vetiver plantlets were grown in each pot containing 8 kg of soil for 120 d. The height of the vetiver plants and the number of new shoots were recorded before harvest at 120 d after planting. After harvest, the shoot and root parts were separated for dry matter measurement and heavy metal analysis using atomic absorption spectrophotometry (Baker and Amacher, 1982; Burau, 1982). The uptake of heavy metal in the vetiver shoots and roots were calculated by multiplying the heavy metal concentration by the dry biomass. Analysis of variance and mean comparisons were performed with statistically significant differences tested using the 0.05 level of probability.

RESULTS AND DISCUSSION

Vetiver growth and biomass

At harvest (120 d after planting), the average plant height of the Ratchaburi ecotype was significantly higher than that of the Surat Thani ecotype (Table 2). Vetiver grew well in uncontaminated soil (A1) and in the soils with low-

Table 1 Properties of soil used in the study.

Soil property	Soil sample[a]				Reference for analysis method
	A1	A2	A3	A4	
pH	6.29	5.89	7.35	7.41	Soil Conservation Service (1982)
CEC (cmole kg^{-1})	4.39	6.31	8.62	7.92	Jackson (1958)
Organic matter (g kg^{-1})	11.70	20.40	24.40	23.70	Walkley and Black (1934)
Soil texture	Sandy loam	Sandy loam	Sandy clay loam	Loam	Day (1965)
Sand (%)	72	67	57	51	
Silt (%)	15	20	20	28	
Clay (%)	12	13	23	21	
Total P(%)	0.03	0.01	0.03	0.03	Yoshida *et al*. (1971)
Total K (%)	0.21	0.36	0.71	0.76	Dept. of Agriculture (2001)
Total Ca (%)	0.11	0.12	0.69	0.74	Dept. of Agriculture (2001)
Total Mg (%)	0.35	0.09	0.28	0.78	Dept. of Agriculture (2001)
Available P (mg kg^{-1})	98	8	4	4	Bray and Kurtz (1945)
Extractable K (mg kg^{-1})	78	37	52	48	Pratt (1965)
Extractable Ca (mg kg^{-1})	395	575	2,333	2,839	Pratt (1965)
Extractable Mg (mg kg^{-1})	71	132	298	263	Pratt (1965)
Heavy metal					Baker and Amacher (1982); Burau (1982)
Total Zn (mg kg^{-1})	14	221	1,984	6,462	
Total Cd (mg kg^{-1})	0.002	3	48	277	
Total Pb (mg kg^{-1})	0.001	14	105	345	
Extractable Zn (mg kg^{-1})	1	61	174	397	
Extractable Cd (mg kg^{-1})	Trace	1	21	35	
Extractable Pb (mg kg^{-1})	Trace	5	18	30	

[a] A1= uncontaminated soil, A2 = low contaminated soil, A3 = moderately contaminated soil, A4 = highly contaminated soil.

Table 2 Average plant heights of Ratchaburi and Surat Thani vetiver ecotypes grown in soil with four levels of heavy metals.

Soil[1/]	Plant height (cm)		
	Ratchaburi ecotype	Surat Thani ecotype	Average[2/]
A1	128	100	114 [a]
A2	125	107	116 [a]
A3	126	102	114 [a]
A4	119	95	107 [b]
Average[2/]	124 [a]	101 [b]	

[1/] A1= uncontaminated soil, A2 = low contaminated soil, A3 = moderately contaminated soil, A4 = highly contaminated soil.
[2/] Figures in the same column or row with a common letter are not significantly different at the 0.05 probability level.

to-moderate contamination (A2 and A3), and plant height was significantly higher in these treatments than for plants in the highly contaminated soil. The heavy metals in the contaminated soils (A2–A4) caused a noticeable tillering reduction of 50% in the Rachaburi ecotype; however, only the Surat Thani ecotype grown in highly contaminated soil (A4) had a significant level of tillering reduction (Figure 1).

For biomass, the Ratchaburi ecotype had better growth in the aerial parts compared to the roots, which gave a significantly higher shoot dry weight but lower root dry weight compared to the Surat Thani ecotype (Tables 3 and 4). Heavy metals, especially in the highly contaminated soil, caused a reduction in the vetiver biomass (shoot and root dry weight). Nevertheless, there was no significant difference in biomass among vetiver plants grown in the low-to-highly contaminated soils (A2–A4). Troung (1999) reported that the threshold levels in the soil for vetiver growth of Zn, Cd and Pb are greater than 700, 60 and greater than 1500 mg kg^{-1}, respectively. In the current study, only the concentration of Zn in soils with moderate (1,984 mg kg^{-1}) and high (6,462 mg kg^{-1}) contamination were above the threshold level. Moreover, the levels of available P in the contaminated soil were very low (4-8 mg kg^{-1});

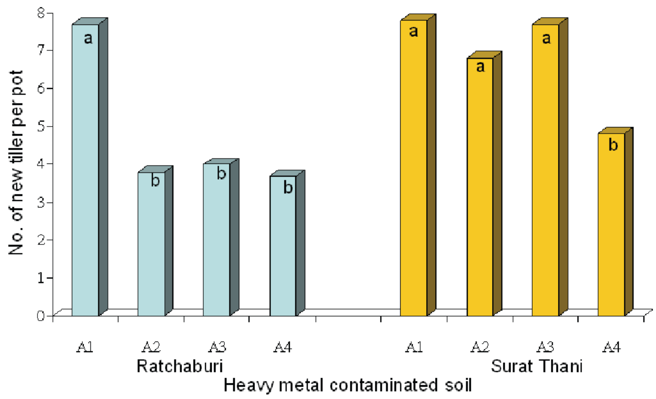

Figure 1 Average number of new shoots for Ratchaburi and Surat Thani vetiver ecotypes grown in soils with four levels of heavy metal contaminated soils. A1 = uncontaminated soil; A2 = low contaminated soil; A3 = moderately contaminated soil; and A4 = highly contaminated soil. Bars associated with a common letter are not significantly different at the 0.05 probability level.

Table 3 Average shoot dry weight of Ratchaburi and Surat Thani vetiver ecotypes grown in soils with four levels of heavy metals.

Soil[1/]	Shoot dry weight (g pot^{-1})		
	Ratchaburi ecotype	Surat Thani ecotype	Average[2/]
A1	31.1	27.3	29.2 [a]
A2	26.2	22.7	24.5 [b]
A3	24.8	25.7	25.3 [b]
A4	25.6	19.1	22.4 [b]
Average[2/]	26.9 [a]	23.7 [b]	

[1/] A1= uncontaminated soil; A2 = low contaminated soil; A3 = moderately contaminated soil; and A4 = highly contaminated soil.
[2/] Figures in the same column or row with a common letter are not significantly different at the 0.05 probability level.

this may inhibit vetiver growth, as Ghannoum and Conroy (2007) revealed that P deficiency reduced leaf growth, tillering and plant dry mass in three grasses (*Panicum laxum*, *P. coloratum* and *Cenchrus ciliaris*). Therefore, a high concentration of Zn and low available P in contaminated soils may cause a reduction in vetiver growth. However, all vetiver plants were healthy and had no heavy metal toxicity symptoms.

Heavy metal concentration in vetiver shoots and roots

The average concentrations of Zn, Cd and Pb in the shoot and root parts of both vetiver ecotypes grown in soils contaminated with low- to high- levels of heavy metals (A2–A4) were significantly higher than those of plants grown in uncontaminated soil (A1), as shown in Tables 5 and 6. The concentrations of heavy metals in the vetiver shoots and roots increased as the heavy metal concentrations in the soil increased; these results were similar to those reported by Roongtanakiat and Chairoj (2001a) and Chen *et al*. (2004).

In general, the average concentrations of heavy metals in the two vetiver ecotypes were not significantly different, except for the concentration of Zn in the shoots and Pb in the roots of the

Table 4 Average root dry weight of Ratchaburi and Surat Thani vetiver ecotypes grown in soil with four levels of heavy metals.

Soil1/	Root dry weight (g pot^{-1})		
	Ratchaburi ecotype	Surat Thani ecotype	Average2/
A1	12.1	19.5	15.8 a
A2	9.9	15.6	12.8 b
A3	9.1	16.4	12.8 b
A4	10.0	12.6	11.3 b
Average2/	10.3 b	16.0 a	

1/ A1= uncontaminated soil; A2 = low contaminated soil; A3 = moderately contaminated soil; and A4 = highly contaminated soil.
2/ Figures in the same column or row with a common letter are not significantly different at the 0.05 probability level.

Table 5 Average concentrations of Zn, Cd and Pb in shoots of Ratchaburi and Surat Thani vetiver ecotypes grown in four heavy metal levels.

Heavy Metal	Vetiver ecotype	Concentration of heavy metals (mg kg^{-1}) in shoots of vetiver grown in soil type1/				
		A1	A2	A3	A4	Average2/
Zn	Ratchaburi	16.1	145.9	228.9	250.2	160.3 a
	Surat Thani	14.1	83.4	125.9	204.5	107.0 b
	Average2/	15.1 d	114.7 c	177.4 b	227.4 a	
Cd	Ratchaburi	0.003	3.832	3.686	5.783	3.326 a
	Surat Thani	0.005	2.747	3.325	5.796	2.968 a
	Average2/	0.004 c	3.290 b	3.506 b	5.790 a	
Pb	Ratchaburi	0.005	2.473	4.186	4.215	2.720 a
	Surat Thani	0.005	2.296	3.547	5.995	2.961 a
	Average2/	0.005 d	2.385 c	3.867 b	5.105 a	

1/ A1= uncontaminated soil; A2 = low contaminated soil; A3 = moderately contaminated soil; and A4 = highly contaminated soil.
2/ Figures in the same column or row with a common letter are not significantly different at the 0.05 probability level.

Ratchaburi ecotype that were significantly higher than those of the Surat Thani ecotype. The average concentrations of all the heavy metals in the roots were higher than those in the shoots; these results were in good agreement with those reported by Yang *et al*. (2003) and Roongtanakiat *et al*. (2008; 2009). The average translocation factors (the ratio of metal concentration in the shoots to that in the roots) of Zn, Cd and Pb varied between the two vetiver ecotypes, with values of 0.69, 0.25 and 0.27, respectively, for the Ratchaburi ecotype and 0.43, 0.27 and 0.46, respectively, for the Surat Thani ecotype. However, all of the translocation factor values were less than one. The results were in agreement with previous reports when vetiver was grown in iron ore tailings (Roongtanakiat *et al*., 2008) and in soil from a zinc mine (Roongtanakiat *et al*., 2009). The Ratchaburi vetiver ecotype had the highest translocation factor for Zn (0.69) which was higher than the values reported by Yang *et al*. (2003) and Wong *et al*. (2007) of 0.05–0.14 and 0.2–0.6, respectively. This might have been due to the differences in the vetiver cultivars and the properties of the soil used in the studies.

Heavy metal uptake in vetiver shoots and roots

The uptake of heavy metals in vetiver shoots and roots is shown in Figure 2. Vetiver grown in uncontaminated soil had the lowest uptake of total Zn, Cd and Pb with levels of 0.77, 0.0011 and 0.0012 mg/pot, respectively. Uptake increased as the level of heavy metal contamination in the soil increased. The highest uptake was observed in the highly contaminated soil; concentrations of Zn, Cd and Pb in this treatment were 10.19, 0.451 and 0.311 mg/pot, respectively. The Ratchaburi ecotype could take up Zn and Pb in higher amounts than the Surat Thani ecotype, while both vetiver ecotypes showed no difference in their uptake of Cd. This result confirmed the reports of Roongtanakiat and Chairoj (2001a, 2001b) and Roongtanakiat *et al*. (2007) that the vetiver ecotype is one of the important factors affecting heavy metal uptake.

In general, vetiver accumulated more heavy metals in its roots than shoots; therefore it is suitable for phytostabilization as suggested by Yoon *et al*. (2006) and Roongtanakiat *et al*. (2008; 2009). However, Zn uptake in the shoots of the Ratchaburi ecotype was significantly higher than

Table 6 Average concentrations of Zn, Cd and Pb in roots of Ratchaburi and Surat Thani vetiver ecotypes grown in four heavy metal levels.

Heavy metal	Vetiver ecotype	Concentration of heavy metals (mg kg^{-1}) in roots of vetiver grown in[1/]				
		A1	A2	A3	A4	Average[2/]
Zn	Ratchaburi	17.1	211.8	260.0	435.2	231.0 [a]
	Surat Thani	22.1	236.2	279.3	455.1	248.2 [a]
	Average[2/]	19.6 [d]	224.0 [c]	269.7 [b]	445.1 [a]	
Cd	Ratchaburi	0.07	4.12	18.89	30.75	13.46 [a]
	Surat Thani	0.06	4.01	13.02	27.00	11.02 [a]
	Average[2/]	0.07 [d]	4.07 [c]	15.96 [b]	28.88 [a]	
Pb	Ratchaburi	0.06	5.59	11.32	22.88	10.05 [a]
	Surat Thani	0.04	3.33	8.49	13.63	6.37 [b]
	Average[2/]	0.05 [d]	4.64 [c]	9.91 [b]	18.26 [a]	

[1/] A1= uncontaminated soil; A2 = low contaminated soil; A3 = moderately contaminated soil; and A4 = highly contaminated soil.
[2/] Figures in the same column or row with a common letter are not significantly different at the 0.05 probability level.

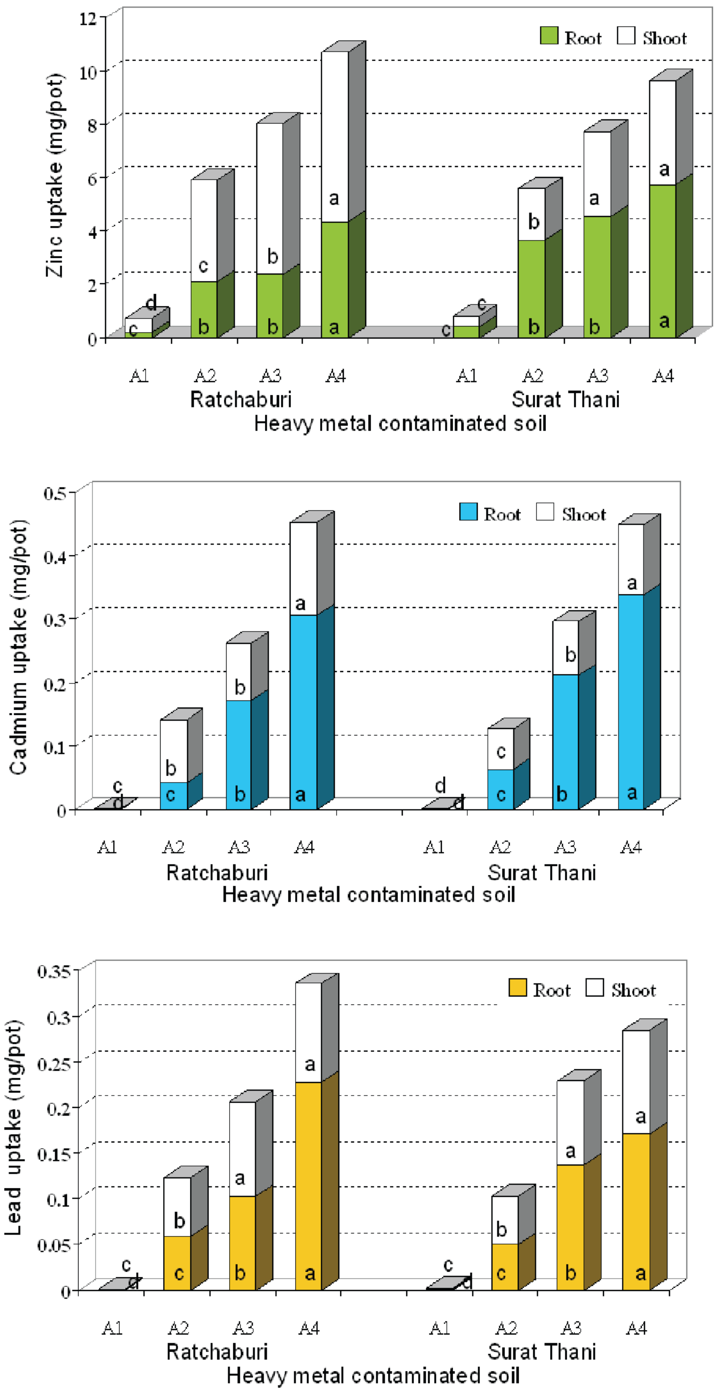

Figure 2 Uptakes of Zn, Cd and Pb in shoots and roots of Ratchaburi and Surat Thani vetiver ecotypes grown in soil with four levels of heavy metals. A1 = uncontaminated soil; A2 = low contaminated soil; A3 = moderately contaminated soil; and A4 = highly contaminated soil. Bars associated with a common letter are not significantly different at the 0.05 probability level.

that of the Surat Thani ecotype due to a higher shoot biomass and the highest translocation factor of Zn (0.69). Therefore the Ratchaburi ecotype has considerable potential for phytoextraction of Zn from contaminated soil.

Vetiver is a C4 plant with a high efficiency in converting solar radiation to biomass. Its biomass production (dry weight) usually ranges from 20 to 40 t ha^{-1} y^{-1} (Vieritz *et al.*, 2003). In southeast China, an average yield of 99 t ha^{-1} y^{-1} was reported (Zhang, 1998, as cited by Chen *et al.*, 2004). A biomass yield of 100 t ha^{-1} y^{-1} on an irrigated farm in Texas was also reported (Zarotti, 2002 as cited by Vieritz, e*t al.*, 2003). Owing to its high biomass, vetiver has great potential for phytoremediation even though vetiver is not a hyperaccumulator. Chen *et al.* (2000) reported that because of the high biomass of vetiver, its total above ground uptake of Cd was greater than that of *Thlaspi caerulescens,* a hyperaccumulator plant. Moreover, the long deep roots of vetiver can penetrate to clean up the deep soil layer. These unique characteristics of vetiver can compensate and overcome the limitations of normal hyperaccumulator plants—such as, slow growing, low biomass and shallow decontamination.

CONCLUSION

The Ratchaburi and the Surat Thani vetiver ecotypes grew well in soil contaminated with Zn, Cd and Pb. The Ratchaburi ecotype had significantly higher plant height and shoot dry weight than those of the Surat Thani ecotype. Both vetiver ecotypes took up Zn, Cd and Pb in high amounts when they grew in a soil highly contaminated with these heavy metals. They accumulated more heavy metals in their roots than shoots and gave different values for the heavy metal translocation factor. However, the Ratchaburi vetiver ecotype accumulated more Zn in its shoots than its roots, with the highest translocation factor of 0.86. Therefore, it should have potential for phytoextraction of Zn from contaminated soil. By growing vetiver and harvesting its shoots periodically for regrowth, the heavy metals will gradually be removed from the soil.

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