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Influence of Heavy Metals and Soil Amendments on Vetiver (*Chrysopogon zizanioides*) Grown in Zinc Mine Soil

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ABSTRACT

Soil amendments, such as compost and chelating agents, are often required to improve phytoremediation efficiency. In this study, the Ratchaburi vetiver ecotype was grown in soil uncontaminated by heavy metal and in zinc mine soil amended with compost and chelating agents (EDTA and DTPA). It was found that the concentration of Zn in the mine soil was about 5.6 times higher than the toxic level in soil. It inhibited vetiver growth as well as causing leaf chlorosis which was a symptom of either Zn toxicity or iron deficiency. The application of compost had no influence on growth performance, primary nutrient uptake and heavy metal uptake. EDTA could enhance the concentration and uptake of Zn, Mn and Cu, but not Fe, while DTPA increased the concentration of these heavy metals, but not their uptake. The compost and chelating agents did not affect Mn or Zn translocation. However, they could elevate Fe and Cu translocation, especially when compost was applied together with chelating agents of which EDTA and DTPA gave significantly-different results. In summary, EDTA was superior for soil amendment in a zinc mine soil with high concentrations of multi-heavy metals.
Key words: phytoremediation, vetiver, compost, soil amendment, zinc mine

INTRODUCTION

Soil pollution by heavy metals occurs as a consequence of manufacture, the disposal of waste and mining activity. The demand for minerals has been increasing as a result of industrialization and urbanization.

In Thailand, mineral value production increased from 29,656.5 in 2002 to 39,480 million baht in 2006 (Department of Mineral Resource, 2008). Heavy metal contamination from mining activities may eventually lead to a deterioration of groundwater quality. This problem not only affects the environment, but also has a severe impact on human health.

Phytoremediation is a green technology for cleaning up contaminated soil and water. It is attractive because of its lower cost and lower maintenance compared to other remediation techniques. Many researchers have shown that phytoremediation approaches often require soil amendment using compost and a chelating agent. Organic matter can have important effects on both the physical property and the nutrient status of soil. It can be used to modify metal bioavailability, speciation, plant uptake and translocation

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processes (Chami *et al.,* 2007). Ye *et al.,* (2000) reported that organic matter resulted in successful revegetation of mine soil. In addition, organic matter could reduce heavy metal toxicity to plants from complexing metals (Wong and Lau, 1985; Chami *et al.*, 2007). Chelating agents, such as ethylenediaminetetraacetic acid (EDTA), diethylenetriaminepentaacetic acid (DTPA) and nitrilotriacetic acid (NTA) are effective in enhancing the solubility of heavy metals and other element cations in soil (Huang and Cunningham, 1996; Robinson *et al.*, 1999). The application of chelating agents could increase heavy metal concentration and uptake (Wenger *et al.*, 2003; Lai and Chen, 2004; Chiu *et al.*, 2006), while some other authors have reported that chelating agents did not enhance (Liphadzi *et al.*, 2003) or even decreased heavy metal uptake (Huebert and Shay, 1992). However, there are few reports on the influence of soil amendment on plants grown in soil with high concentrations of multi-heavy metals. Therefore, a greenhouse experiment was conducted to study the effect of heavy metals and their toxicity on vetiver grown in zinc mine soil contaminated with high concentrations of multi-heavy metals. The influence of compost and the chelating agents, EDTA (ethylenediaminetetraacetic acid) and DTPA (diethylenetriminepentaacetic acid) on growth performance, primary nutrient (N, P and K) uptake, as well as heavy metal (Fe, Zn, Mn and Cu) translocation and their uptake by vetiver were also investigated.

Vetiver grass (*Chrysopogon zizanioides* (L.) Roberty), a perennial grass of the Poaceae and native to India, was selected because of its deep root system, fast growth rate and high biomass, as well as its high tolerance to heavy metals (Truong and Baker, 1998). Moreover, its potential has been recognized for the decontamination of heavy metals from soil (Truong and Baker, 1998; Roongtanakiat and Chairoj, 2001a; 2001b), garbage leachate (Xia *et al.*, 2000; Roongtanakiat *et al.*, 2003) and wastewater (Kong *et al.,* 2003; Roongtankiat *et al.,* 2007). It was also hoped that the present experiment could yield information that would be useful for the remediation of contaminated sites by vetiver and for further study.

MATERIALS AND METHODS

Soil sample and characteristics

Zinc mine soil was collected at a depth of 0-15 cm from a mining area in Tak province. Hupkaphong-series sandy soil was also collected from the Huai Sai Royal Development Center, Phetchaburi province and served as the soil control with no heavy metal contamination. Individual soils were air-dried, passed through a 2 mm sieve and mixed well. Standard soil properties were analyzed and the results are shown in Table 1. For organic matter determination, the method described by Walkley and Black (1934) was used,

Table 1 Some properties of zinc mine soil and uncontaminated soil used in the experiment.

Parameter	Uncontaminated soil	Zn mine soil
pH	7	8
OM (%)	2	0.1
Total N (mg kg^{-1})	102	200
Available P (mg kg^{-1})	15	2
Extractable K (mg kg^{-1})	12	11
Zn (mg kg^{-1})	814	5,039
Mn (mg kg^{-1})	48	587
Fe (mg kg^{-1})	26	6,759
Cu (mg kg^{-1})	0.5	5

while total N was determined by the Kjeldahl method and total P by the colorimetric method (Yoshida *et al*., 1971). The concentrations of K, Mn and Cu in the tailings were determined by digestion with concentrated HNO_3 and $HClO_4$ (3:1) and analyzed with an ICP spectrometer (Wallace and Barrett, 1981). A quantitative analysis of Fe and Zn in the soil samples used the neutron activation method (Linihan *et al*., 1972; Ehman and Vance, 1991).

Plant growth and analysis

A pot experiment was conducted using a completely randomized design with seven treatments and three replications. The treatments and their descriptions are shown in Table 2. Three plantlets of Ratchaburi vetiver ecotype were planted in each pot containing 8 kg of uncontaminated soil or zinc mine soil mixed with soil amendment, compost and/or the chelating agents, EDTA and DTPA. Plant height was measured at 30, 60, 90 and before harvest at 120 days. After harvest, shoot and root parts were separated for dry matter measurement and also for primary nutrient and heavy metal analysis. The Kjeldahl method was used for total N determination and total P was determined by the colorimetric method (Yoshida *et al*., 1971). Total K, Fe, Zn, Mn and Cu were analyzed with an ICP spectrometer (Wallace and Barrett, 1981).

Statistical analysis and calculation

The data on vetiver height and biomass, in addition to the concentrations of primary nutrients and heavy metals, as well as the uptake of primary nutrients and heavy metals were analyzed using analysis of variance and Duncan's multiple range test for mean comparisons. A probability level of $p<0.05$ was considered for significant difference testing. The translocation factor of heavy metals in the vetiver plants was determined as the ratio of metal concentration in the shoot part to that in the root part.

RESULTS AND DISCUSSION

Vetiver growth

The Ratchaburi vetiver grew well and looked healthy in the heavy metal uncontaminated soil, while severe chlorosis and a light yellowish to white color appeared on young leaves of vetiver grown in Zn mine soil (Figure 1). These may have been the symptoms of Zn toxicity due to the concentration of Zn in soil being as high as 5,039 mg kg^{-1} which was very much higher than the toxic concentration level (900 mg kg^{-1}) in soil (Alloway, 1995). The heavy metal toxicity caused a significant decrease in plant height of vetiver grown in zinc mine soil (T1-T3) compared to that of the control treatment (T0) (Table 3).

Table 2 Treatments used in the experiment.

Treatment	Description
T0	Uncontaminated soil (control)
T1	Zn mine soil
T2	Zn mine soil + compost[1]
T3	Zn mine soil + EDTA[2]
T4	Zn mine soil + DTPA[2]
T5	Zn mine soil + EDTA + compost
T6	Zn mine soil + DTPA + compost

[1] Compost (33 % OM) at the rate of 10 g kg^{-1} soil
[2] Chelating agent at the rate of 1 g kg^{-1} soil

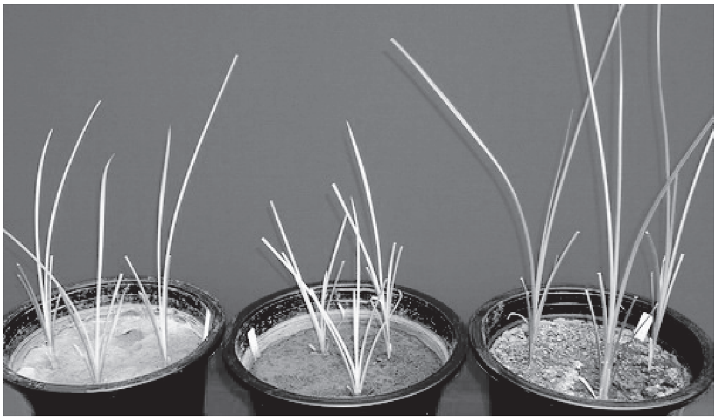
T4 T6 T1

Figure 1 Chlorosis caused by heavy metal toxicity in vetiver grown in zinc mine soil (T1), amended with DTPA (T4) and amended with a combination of compost and DTPA (T6).

Table 3 Average height of vetiver grown in uncontaminated soil and zinc mine soil at 30, 60, 90 and 120 days after planting.

Treatment[1]	Height (cm) after planting[2]			
	30 days	60 days	90 days	120 days
T0	82 a	98 a	115 a	119 a
T1	61 b	74 b	53 b	54 b
T2	57 bc	69 bc	54 b	54 b
T3	51 cd	62 c	51 b	51 b
T4	31 e	32 d	26 c	26 c
T5	46 d	62 c	50 b	50 b
T6	36 e	37 d	31 c	31 c

[1] : See description at Table 2.

[2] : Figures in the same column with a common letter are not significantly different at 0.05 probability based on DMRT.

At 90 and 120 days after planting, the vetiver samples grown in soil treated with compost (T2) or EDTA (T3) or a combination (T5) were not significantly different from those in the non-amended zinc mine soil (T1) in average plant height. These were significantly higher than those of the DTPA treatment (T4) and the combination with compost treatment (T6).

The response of vetiver biomass to heavy metal and soil amendments had a similar trend to plant height (Table 4). Vetiver grown in uncontaminated soil (T0) had a shoot dry weight higher than the root dry weight, while those grown in zinc mine soil (T1-T6) had a shoot dry weight lower than the root dry weight, probably due to chlorosis reducing the leaf mass. This could also have indicated that the vetiver shoot was more susceptible to heavy metal than its root.

From the vetiver height and biomass data, it could be concluded that EDTA and compost did not significantly affect the growth performance of vetiver grown in zinc mine soil, while DTPA produced a negative effect. This result was different from former studies (Roongtanakiat *et al.*, 2008), which showed that vetiver could grow well in heavy-metal contaminated soil and the chelating agents (EDTA and DTPA), especially in combination with compost, could enhance the growth of vetiver. The differences in results might have been due to a difference in the mine soil used and the very high concentration of heavy metal in the Zn mine soil used in this study. The adverse effect of chelating agents was similar to the findings of Lai and Chen (2004), where an application of EDTA slightly decreased the biomass of vetiver. Amending the soil with compost did not significantly increase plant growth, probably due to heavy metal toxicity, the low rate of compost application and the slow decomposition of the compost in the zinc mine soil.

Primary nutrient concentrations and uptakes

The concentrations of primary nutrient (N, P and K) in the shoot and root parts of vetiver are presented in Figure 2. Nitrogen concentrations in the vetiver shoots and roots grown in uncontaminated soil (T0) were 4.47 and 5.69%, respectively. These were significantly higher than those of other treatments, due to poor plant growth caused by the high levels of heavy metals in the zinc mine soil. However, there was no significant difference in the P and K concentrations in the vetiver shoot and root samples among the treatments. Soil amendment application had no effect on the primary concentration in both vetiver parts. This result agreed with a former finding (Roongtanakiat *et al*., 2008) and the study of Chiu *et al*. (2006).

The highest N, P and K uptake in shoots and roots occurred with vetiver grown in uncontaminated soil (Table 5). Among the zinc

Table 4 Average dry weight of vetiver grown in uncontaminated soil and zinc mine soil.

Treatment[1]	Dry weigh of vetiver[2] (g)		
	Shoot	Root	Total dry weight
T0	34.5 a	25.7 a	60.3 a
T1	3.5 b	4.7 b	8.2 b
T2	3.6 b	5.2 b	8.8 b
T3	3.4 b	3.6 c	7.0 b
T4	1.6 c	2.4 c	4.0 c
T5	3.0 b	4.2 b	7.2 b
T6	2.1 c	2.9 cd	5.0 c

[1] : See description at Table 2.

[2] : Figures in the same column with a common letter are not significantly different at 0.05 probability based on DMRT.

Table 5 Primary nutrient uptake in the shoots and roots of vetiver grown in zinc mine soil.

Treatment[1]	Plant nutrient uptake (mg pot^{-1}) in vetiver[2]					
	N		P		K	
	Shoot	Root	Shoot	Root	Shoot	Root
T0	1542 a	1463 a	150 a	82 a	475 a	199 a
T1	74 b	146 c	15 b	15 b	45 b	35 b
T2	87 b	174 a	17 b	18 b	49 b	43 b
T3	75 b	115 d	15 b	12 c	42 b	24 b
T4	36 b	76 e	7 c	7 d	20 c	17 b
T5	74 b	138 c	14 b	14 bc	41 b	33 b
T6	53 b	99 d	9 bc	10 d	29 bc	24 b

[1] : See description at table 2.

[2] : Figures in the same column with a common letter are not significantly different at 0.05 probability based on DMRT.

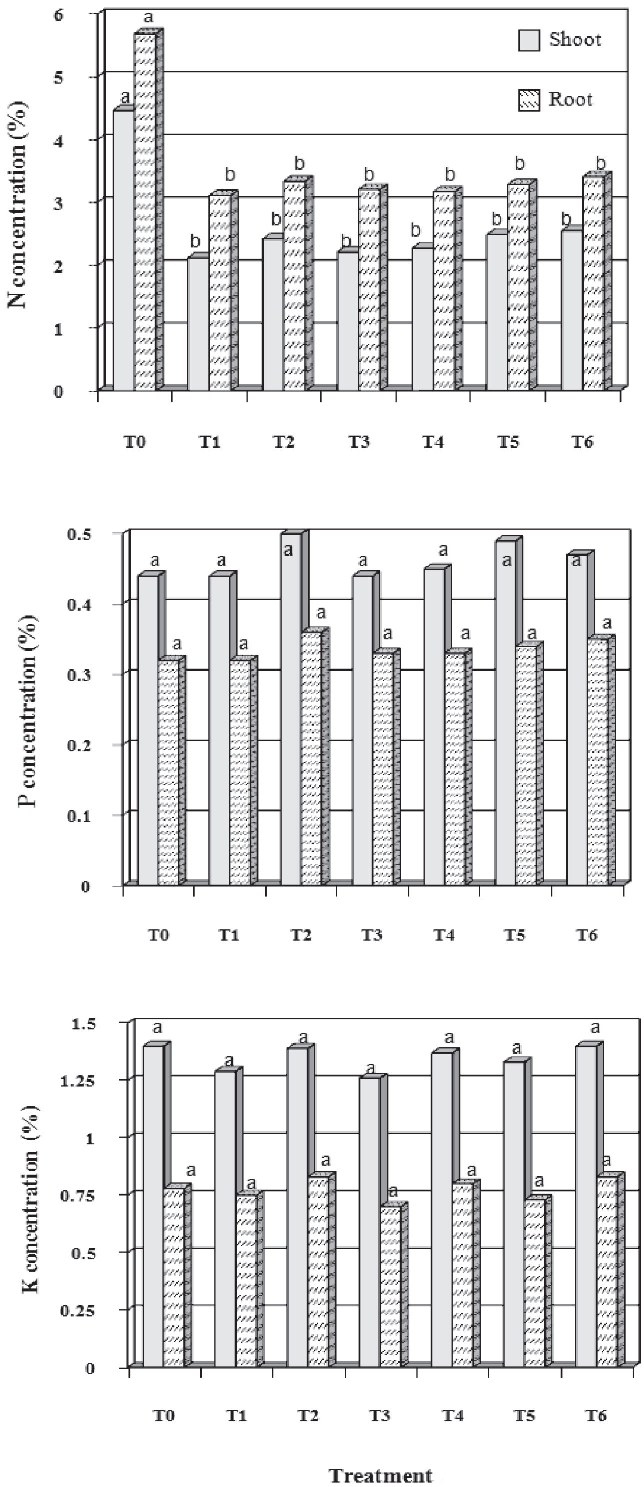

Figure 2 Concentration of N, P and K in the shoots and roots of vetiver grown in uncontaminated and zinc mine soil. [Bars associated with a common letter were not significantly different and T0-T6 are defined as in Table 2].

mine soil treatments, there was no difference in the N uptake by the vetiver shoots. An application of only compost (T2) tended to increase the uptake, which showed a significantly-higher N uptake in roots than that of the non-amended soil treatment (T1). The amended zinc mine soil with EDTA (T3) yielded higher primary nutrient uptakes than the DTPA amendment (T4, T6), with the application of DTPA alone (T4) giving the lowest uptake. Contrary to the report of Roongtanakiat *et al.* (2008), chelating agents improved N, P and K uptakes in the shoots and roots of vetiver grown in iron mine tailings. This may have been due to a different type of heavy metal concentration in the mine soil.

Heavy metal concentrations in vetiver plant

The concentration of heavy metals (Mn, Fe, Zn and Cu) in the shoots and roots of vetiver is presented in Figure 3. The results showed that vetiver grown in uncontaminated soil (T0) had the lowest content of Mn and Zn. However, they were within the normal range of 20-300 mg kg^{-1} and 20-100 mg kg^{-1} for Mn and Zn, respectively (Davies, 1980). The concentrations of Mn and Zn in both the shoot and root tissue of the vetiver in the zinc mine soil treatments were significantly increased, especially when amended with chelating agents. They were higher than the toxicity levels for plant growth of 300 mg kg^{-1} for Mn and 400 mg $kg^{-1}$for Zn, respectively (Davies, 1980). Truong (1999) reported that the Zn toxic threshold level in vetiver shoots was above 880 mg kg^{-1}. However, this study revealed that the vetiver grown in the unamended zinc mine soil with 793 mg Zn kg^{-1} in shoots and 1038 mg Zn kg^{-1} in roots showed mild Zn toxic symptoms.

The Fe concentrations in the vetiver shoots and roots grown in uncontaminated soil were 90 and 158 mg kg^{-1}, respectively. In comparison with the zinc mine soil, the concentration of Fe in the vetiver shoots and roots varied within the range of 11-19 and 19-37 mg kg^{-1}, respectively, which was lower than the critical level (50 mg kg^{-1}) for plant growth (Davies, 1980), which may have been caused by a lower solubility of Fe in mine soil with a high pH of 8. Amending the soil with chelating agents significantly decreased the Fe concentration in both vetiver shoots and roots. This was probably due to the high concentration of Zn in mine soil acting in a strongly-antagonistic way with the Fe (Osotsapar, 2003).

The highest concentration of Cu was found in vetiver grown in uncontaminated soil which was within the normal range of 5-20 mg Cu kg^{-1} (Agricultural and Environmental Service Laboratory, 2008). In the zinc mine soil treatments, vetiver had a low concentration of Cu, especially in the shoot parts which was lower than the deficiency level (5 mg kg^{-1}). Amending the soil with chelating agents (T3-T6) resulted in a significantly-increased Cu content in both vetiver shoots and roots. This result was similar to a reports by: Jiang and Yang (2004), which found that EDTA addition could increase the potential and efficiency of Cu phytoextraction by *E. splendens* in polluted soils; and Luo *et al.* (2006), who reported that EDTA-treated soil had a significant ability to enhance the concentration of Cu and Pb in the shoots of *Zea mays* L.

From the study of Roongtanakiat *et al.* (2008), it was found that the application of EDTA or DTPA gave equal results in enhancing Mn, Fe, Zn and Cu concentrations in vetiver plant grown in iron mine tailings. However, this present study revealed that the DTPA application produced a better result than EDTA in Mn and Cu absorption, while they had an equal effect on the absorption of Fe and Zn. Even though the addition of chelating agents could increase the Zn and Cu concentration in the plant, they produced negative results for Fe, which may have been due to antagonism between Fe and Zn. Therefore, chlorotic symptoms of vetiver plants grown in zinc mine soil with a high pH and Zn concentration, especially in the

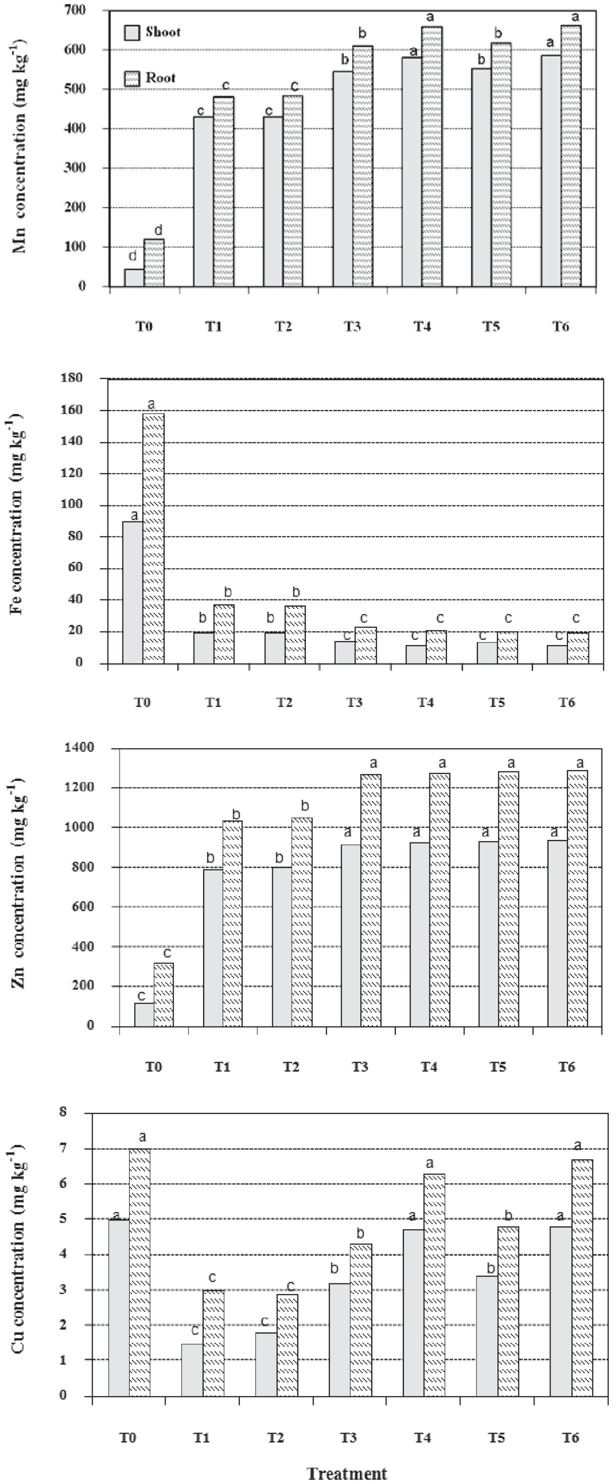

Figure 3 Concentration of Mn, Fe, Zn and Cu in the shoots and roots of vetiver grown in uncontaminated and zinc mine soil. [Bars associated with a common letter were not significantly different and T0-T6 are defined as in Table 2].

chelating agent application treatments, might have been due to Fe-deficiency chlorosis, which resembles Zn toxicity-induced chlorosis (Rufner and Barker, 1984).

Some researchers have reported that compost could reduce heavy metal toxicity (Chemi *et al.,* 2007; Rotkittikhum *et al.,* 2007). Chiu *et al*. (2006) concluded that both the Cu and Zn content tended to increase in the shoots of vetiver grown on Pb/Zn and Cu mine tailings amended with manure compost and sewage sludge. The present results showed that the application of compost had no influence on heavy metal toxicity, as well as their concentrations in vetiver plants grown in zinc mine soil, similar to those reported by Roongtanakiat *et al*. (2008) in iron ore tailings. This may have been due to the low rate of compost application (1 g kg^{-1} soil).

Translocation factor

In all treatments, vetiver roots always had a higher heavy metal content than shoots which was a similar result to that reported by Yang *et al.* (2003) and Roongtanakiat *et al.* (2007, 2008). The ability of vetiver to translocate heavy metals from the shoots to the roots could be explained by a translocation factor (TF) as shown in Figure 4. The average TF for Mn, Zn, Fe and Cu in vetiver grown in uncontaminated soil was: 0.375, 0.377, 0.570 and 0.714, respectively. In zinc mine soil (T1), the TF for Mn and Zn significantly increased to 0.894 and 0.764, respectively. This may have been due to the high content of Mn and Zn in the mine soil. A contrary result was observed for Fe and Cu with a TF of 0.514 and 0.5, respectively, as their translocation may have been interfered with by the Zn, especially for Fe which is considerably immobile.

In all the zinc mine soil treatments (T1-T6) studied, vetiver translocated the highest amount of Mn from the roots to the shoots, which confirmed previous results (Roongtanakiat *et al.*, 2007, 2008). The compost and chelating agents did not affect the Mn and Zn translocation. However, they could elevate the Fe and Cu translocation amounts, especially when compost was applied with chelating agents, but EDTA and DTPA did not give significantly different results. This positive result of a chelating agent on heavy metal translocation was similar to those reported by Piechalak *et al.* (2003), Wenger *et al.* (2003) and Chen *et al.* (2004). Even the TF of Cu was increased by 50% with the EDTA application, it was still less than one. This was in agreement with

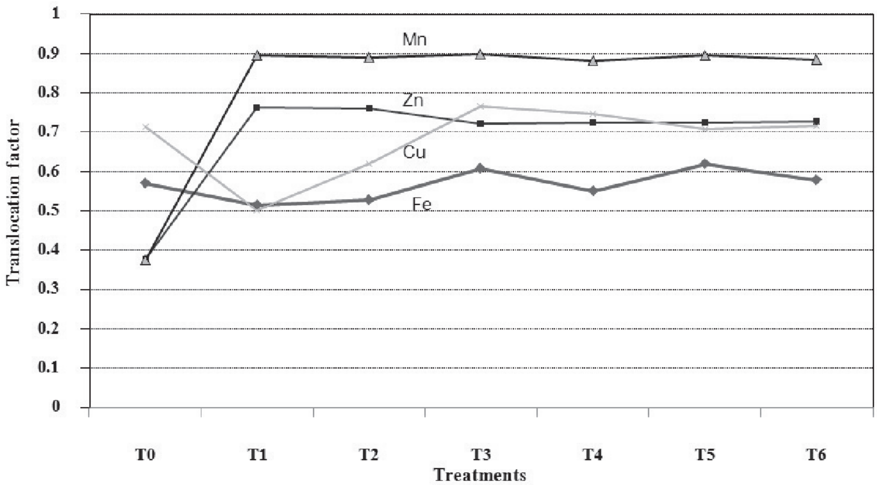

Figure 4 Translocation factors of Mn, Zn, Cu and Fe for vetiver grown in uncontaminated soil and zinc mine soil amended with chelating agent and compost. [T0-T6 are defined as in Table 2].

a previous report (Roongtanakiat *et al.*, 2008), where none of the TF values for Mn, Zn, Fe or Cu were greater than one when vetiver was grown in iron ore tailings. The results confirmed that vetiver is suitable for phytostabilization (Roongtanakiat *et al.*, 2008).

Heavy metal uptake

The amount of heavy metal uptake by vetiver is shown in Table 6. As expected, vetiver grown in uncontaminated soil had the best growth performance and could uptake the highest amount of Mn, Zn, Fe and Cu in shoots and roots. In the zinc mine soil samples, vetiver had the highest Zn uptake, while the lowest uptake was for Fe, due to the antagonism between Fe and Zn. This result, in conjunction with the information on the Zn and Fe concentration in Figure 3, confirmed that the strong leaf chlorosis in vetiver was caused by either Zn toxicity or Fe deficiency.

The application of only compost to zinc mine soil did not affect the uptake of any of the heavy metals by vetiver, which was a similar result to a study on iron ore tailing (Roongtanakiat *et al.*, 2008). However, the combination of compost and chelating agent, especially DTPA, significantly reduced the Zn and Mn uptake.

With regard to the influence of EDTA and DTPA, this study revealed that a sole EDTA application could increase Zn, Mn and Cu uptake in both vetiver shoots and roots. This positive effect of EDTA on heavy metal uptake was similar to that observed by Huang and Cunningham (1996), Piechalak *et al.* (2003), Chen *et al.* (2004), Jiang and Yang (2004) and Hsia *et al.* (2007). The EDTA was significantly better than DTPA in Zn and Mn uptake, even though DTPA could enhance heavy metal concentration in vetiver, especially for Mn and Cu (Figure 3). This was caused by the poor growth and low biomass of vetiver from the DTPA treatment. For phytoremediation purposes, good biomass is an important factor for heavy metal uptake, therefore, it could be concluded that EDTA is better than DTPA for heavy metal remediation in zinc mine soil by vetiver.

CONCLUSIONS

Vetiver developed well and looked healthy in uncontaminated soil. In zinc mine soil, vetiver growth was limited by high concentrations of heavy metals. Leaf chlorosis occurred as a symptom of Zn toxicity or Fe deficiency in vetiver grown in all the mine soil treatments in this study. The application of compost had no influence on growth performance, primary nutrient uptake and heavy metal uptake. With regard to chelating agents, EDTA enhanced the concentration and uptake of Zn, Mn and Cu, but not Fe due to antagonism between the Zn and Fe. While DTPA

Table 6 Uptake of Fe, Zn, Mn and Cu in the shoots and roots of vetiver grown in zinc mine tailings.

Treatment[1]	Uptake of heavy metals in vetiver[2] (mg/pot)							
	Fe		Zn		Mn		Cu	
	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root
T0	3.10 a	4.05 a	4.16 a	8.23 a	1.55 a	3.08 a	0.172 a	0.179 a
T1	0.06 b	0.17 b	2.78 c	4.88 b	1.51 a	2.27 b	0.005 b	0.014 b
T2	0.06 b	0.18 b	2.89 c	5.48 b	1.56 a	2.52 b	0.006 b	0.015 b
T3	0.04 b	0.08 c	3.12 b	4.56 b	1.86 a	2.20 bc	0.010 b	0.015 b
T4	0.02 b	0.04 c	1.48 e	3.06 b	0.93 c	1.58 d	0.007 b	0.015 b
T5	0.03 b	0.08 c	2.73 c	5.36 b	1.66 a	2.60 b	0.010 b	0.020 b
T6	0.02 b	0.05 c	1.98 d	3.75 b	1.23 b	1.92 cd	0.010 b	0.019 b

[1] : See description at table 2.

[2] : Figures in the same column with a common letter are not significantly different at 0.05 probability based on DMRT.

could increase heavy metal concentrations, it resulted in poor plant growth and biomass. Compost and chelating agents could increase Fe and Cu translocations, but did not affect Zn and Mn translocation.

The translocation factors for the heavy metals were all less than one. Vetiver, a plant with a long, deep root system, was considered suitable for heavy metal immobilization (phytostabilization). Before planting vetiver for remediation and revegetation of zinc mine soil with very high concentrations of heavy metals, the application of EDTA and a capping of fertile soil may be needed in order to enhance the heavy metal uptake and provide a better substrate for plant growth.

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