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Effects of Soil Amendment on Growth and Heavy Metals Content in Vetiver Grown on Iron Ore Tailings

Nualchavee Roongtanakiat[1]*, Yongyuth Osotsapar[2] and Charoen Yindiram[3]

ABSTRACT

A greenhouse experiment was conducted to evaluate the effects of soil amendment on growth, performance and the accumulation of primary nutrients as well as Fe, Zn, Mn and Cu in vetiver. Ratchaburi vetiver ecotype plantlets were planted on iron ore tailings amended with compost and chelating agents (EDTA and DTPA). The results indicated that iron ore tailings contained high concentrations of heavy metals with total Fe, Zn, Mn and Cu concentrations of 63,920, 190, 3,220 and 190 mg kg^{-1}, respectively and low contents of primary nutrients and organic matter. The combination of soil amendment materials, especially DTPA and compost, was more effective than sole chelating agents and sole compost in enhancing vetiver growth, nutrient and heavy metals uptake. The soil amendments used in this study did not affect Fe and Zn translocation from vetiver roots to shoots. However, chelating agent amendment could increase Cu translocation, especially in combination with compost, while it slightly decreased Mn translocation. The average mean translocation factors of Mn, Fe, Zn and Cu were 0.86, 0.71, 0.69 and 0.55, respectively. These results indicated that vetiver is a potential plant for phytostabilization and rehabilitation of iron ore mine areas.

Key words: phytoremediation, phytostabilization, vetiver, compost, soil amendment, EDTA, DTPA

INTRODUCTION

Minerals have played an important role in the economic growth of many countries, including Thailand. Over 40 minerals have been developed and exploited in the country and the value to local consumption of these minerals has been consistently rising (Leepowpanth *et al.*, 1990). Undeniably, exploitation of mineral resources has resulted in extensive deterioration of land. This has caused many serious environmental problems, especially soil erosion, soil degradation and heavy metal pollution.

Several methods are available to remediate soil that has been contaminated by heavy metals such as physical/chemical treatment, burying or removal of the contaminated soil. However, these methods are costly and unsustainable. Recently, several authors pointed out that phytoremediation using certain plant species for cleaning the heavy metal contaminated soil was an economical and environmentally efficient method (Cunningham *et al.*, 1995; Chaney *et al.,* 1997; Leblanc *et al.,* 1999).

1 Department of Applied Radiation and Isotopes, Faculty of Science, Kasetsart University, Bangkok 10900, Thailand.

2 Department of Soil Science, Faculty of Agriculture, Kasetsart University Kamphaeng Saen Campus, Nakhon Pathom 73140, Thailand.

3 Thailand Institute of Nuclear Technology (Public Organization), Chatuchak, Bangkok 10900, Thailand.

* Corresponding author, e-mail: fscincr@ku.ac.th

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Phytoremediation can be specified for many applications including: phytoextraction in which plants take up heavy metals, translocate and store in an above ground part of their structure which then can be harvested and removed from the site. Phytostabilization involves the use of plants to minimize heavy metal mobility in contaminated soil, while in phytovolatilization, plants extract volatile metals from the soil and volatilize them from the foliage (Cunningham *et al.,* 1995).

Vegetation is an important factor in all phytoremediation applications. It is necessary to use plants that can tolerate high levels of heavy metals. Vetiver grass (*Vetiveria zizanioides,* recently reclassified as *Chrysopogon zizanioides*) is widely known for its effectiveness in erosion and sediment control. In addition, previous studies have shown that vetiver could grow well in soil contaminated with multiple elements (Truong and Baker, 1998; Roongtanakiat and Chairoj, 2001a; Roongtanakiat and Chairoj, 2001b; Roongtanakiat *et al.,* 2003).

For phytoremediation of tailings, soil amendment materials such as organic matter and chelating agents are often required to establish successful vegetation and metal uptake. Organic matter application significantly improved soil physical characteristics and nutrient availability, while a chelating agent could modify metal bioavailability, plant uptake and translocation (Robinson *et al.*, 1999; Walker *et al.*, 2003; Chami *et al.,* 2007). However, these effects can differ among chelating agents as well as tailings. Therefore, a pot experiment was conducted to investigate the effects of compost and the chelating agents, EDTA (ethylenediaminetetraacetic acid) and DTPA (diethylenetriminepentaacetic acid), on the growth and performance of vetiver grown on iron ore tailings. Primary nutrients and heavy metal accumulation in the shoots and roots of vetiver were also analyzed. Information obtained from this study was expected to be useful in using vetiver to remediate heavy-metal-contaminated land.

MATERIALS AND METHODS

Iron ore tailings were collected from the mining area at Nakhon Sawan province from a depth of 0 to 15 cm, then air-dried, sieved through a 2-mm sieve and mixed well. Samples of tailings and compost were collected for chemical analysis. The following parameters were analyzed: pH, organic matter (OM), total N (nitrogen), P (phosphorus), K (potassium) and total Fe (iron), Zn (zinc), Mn (manganese) and Cu (copper). Organic matter was determined using the method described by Walkley and Black (1934), 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 tailings were determined by digestion with conc. 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 tailings was carried out using the neutron activation method (Linihan *et al.*, 1972; Ehman and Vance, 1991).

A pot experiment was conducted using a completely randomized design with six treatments and three replications. Details of the treatments and their descriptions are shown in Table 1. Three plantlets of Ratchaburi vetiver ecotype were planted in each pot containing eight kg of iron ore tailings mixed with compost and/or chelating agents. Plant height was measured at 30, 60, 90 and 120 days. The number of tillers was recorded before harvest at 120 days. After harvest, shoot

Table 1 Treatments used in the experiment.

Treatment	Description
F1	Iron ore tailings (control)
F2	Tailing + compost[1]
F3	Tailing + EDTA[2]
F4	Tailing + DTPA[2]
F5	Tailing + EDTA + compost
F6	Tailing + DTPA + compost

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

and root parts were separated for dry matter measurement, nutrient and heavy metal analysis. Determination of total N and P followed the procedure mentioned above. Total K, Fe, Zn, Mn, and Cu were analyzed with an ICP spectrometer (Wallace and Barrett, 1981). Data was statistically 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.

RESULTS AND DISCUSSION

General properties of iron ore tailings

The iron ore tailings were infertile with low organic matter (1%) content, a pH of 7, 0.1% total N, 0.05% total P and 0.17% total K. Total Fe, Zn, Mn and Cu were 63,920, 190, 3,220 and 190 mg kg^{-1}, respectively, indicating high heavy metal concentrations in the tailings, especially for Cu and Mn. These were higher than the threshold values for contaminated soil of 60 mg kg^{-1} and 500 mg kg^{-1}, respectively (Truong, 1999).

Growth performance

The Ratchaburi vetiver grew well on iron ore tailings. The plant height increased until harvest at 120 days (Table 2). In the early to middle growth periods, the vetiver treated with the combination of chelating agent (EDTA or DTPA) and compost, had higher plant height than those in the other treatments. At the later growth stage (90-120 days), the vetiver in the treatments receiving sole chelating agents (F3, F4) and in combination with compost (F5, F6) were not significantly different in height. They were significantly higher than those in the control treatment (F1), and the compost treatment (F2).

The combination of compost and either one of the chelating agents also resulted in a significantly higher number of vetiver tillers than the other treatments (Table 3). The highest dry

Table 2 Average height of vetiver grown on iron ore tailing at 30, 60, 90 and 120 days after planting.

Treatment[1]	Height (cm) after planting[2]			
	30 days	60 days	90 days	120 days
F1	76 b	97 c	101 b	101 b
F2	78 b	96 c	103 b	103 b
F3	65 c	110 b	124 a	125 a
F4	75 bc	111 b	121 a	121 a
F5	92 a	118 a	123 a	124 a
F6	99 a	126 a	128 a	130 a

[1] See description in Table 1.

[2] Mean values in a column with a common letter are not significantly different at 0.05 probability by DMRT.

Table 3 Average number of tillers and dry weight of vetiver grown in iron ore tailings.

Treatment[1]	No. of tiller[2]	Dry weight of vetiver[2] (g)	
		Shoot	Root
F1	3.3 b	13.3 d	6.0 c
F2	4.0 b	14.4 d	6.3 c
F3	4.0 b	23.5 c	6.9 c
F4	3.7 b	23.7 c	7.2 c
F5	6.0 a	36.8 b	12.8 b
F6	6.3 a	55.5 a	18.7 a

[1] See description in Table 1.

[2] Mean values in a column with a common letter are not significantly different at 0.05 probability by DMRT.

weights of shoots (55.5 g/pot) and roots (18.7 g/pot) were obtained in the vetiver grown in tailings receiving the combination of DTPA and compost (F6). The combination of EDTA and compost (F5) resulted in higher shoot and root dry-weights than the application of a sole chelating agent (F3, F4). Both EDTA and DTPA applications had an equal effect on vetiver biomass, which was significantly higher than in the compost application and control treatments. The positive result from including a chelating agent differed from the study by Lai and Chen (2004), who reported that the application of EDTA slightly decreased the biomass of vetiver. For vetiver dry matter, amended tailings with compost (F2) showed no significant difference from the control treatment (F1) which had the lowest yield. Chiu *et al*. (2006) reported that manure compost application increased the yield of vetiver in Pb/Zn mine tailings, but not in Cu mine tailings. Moreover, improvement of vetiver growth by pig manure and domestic refuse was also reported by Rotkittikhun *et al*. (2007) and Yang *et al*. (2003), respectively. The effect of compost on vetiver growth was not as good as expected. This may have been due to the slow decomposition in mine tailings with less soil microorganisms.

Primary nutrients content

The contents of N, P and K in the shoots and roots of vetiver are presented in Table 4. The ranges of N, P and K concentrations in vetiver shoots were 5.31 to 5.42, 0.45 to 0.50 and 1.27 to 1.46%, respectively. They were higher (except for K) than those reported by the Land Development Department (1994) (2.5, 0.17 and 1.5%, for N, P and K, respectively). These difference may have been due to differing maturity rates in the plants.

Vetiver grown on tailings treated with the combination of DTPA and compost (F6) had the highest primary nutrient concentrations in both shoots and roots, except for nitrogen in the roots. However, statistical analysis indicated no significant difference among the treatments. These results are similar to the study by Chiu *et al.* (2006) who reported that the application of manure compost or sewage sludge did not increase N, P, or K concentrations in vetiver.

The effect of the chelating agents and compost on N, P and K uptake in vetiver shoots and roots is illustrated in Figure 1. Chelating agents and compost improved the uptake of N, P and K in vetiver shoots and roots, especially in combination, as they improved the vetiver biomass. The combination of DTPA and compost resulted in the highest uptake in both shoots and roots, while the lowest uptake occurred in the control treatment, which was not significantly different from that of the compost treatment. The three nutrient uptakes of vetiver under different treatments were F6>F5>F4=F3=F2=F1.

Table 4 Concentration of primary nutrients in shoots and roots of vetiver grown on iron ore tailings.

Treatment[1]	Concentration of primary nutrients (%) in vetiver[2]					
	N		P		K	
	Shoot	Root	Shoot	Root	Shoot	Root
F1	5.31 a	6.34 a	0.45 a	0.27 a	1.27 a	0.77 a
F2	5.38 a	6.61 a	0.48 a	0.29 a	1.40 a	0.84 a
F3	5.33 a	6.32 a	0.47 a	0.28 a	1.30 a	0.82 a
F4	5.34 a	6.38 a	0.47 a	0.28 a	1.36 a	0.84 a
F5	5.39 a	6.70 a	0.49 a	0.29 a	1.42 a	0.87 a
F6	5.42 a	6.65 a	0.50 a	0.30 a	1.46 a	0.89 a

[1] : See description in Table 1.

[2] Mean values in a column with a common letter are not significantly different at 0.05 probability by DMRT.

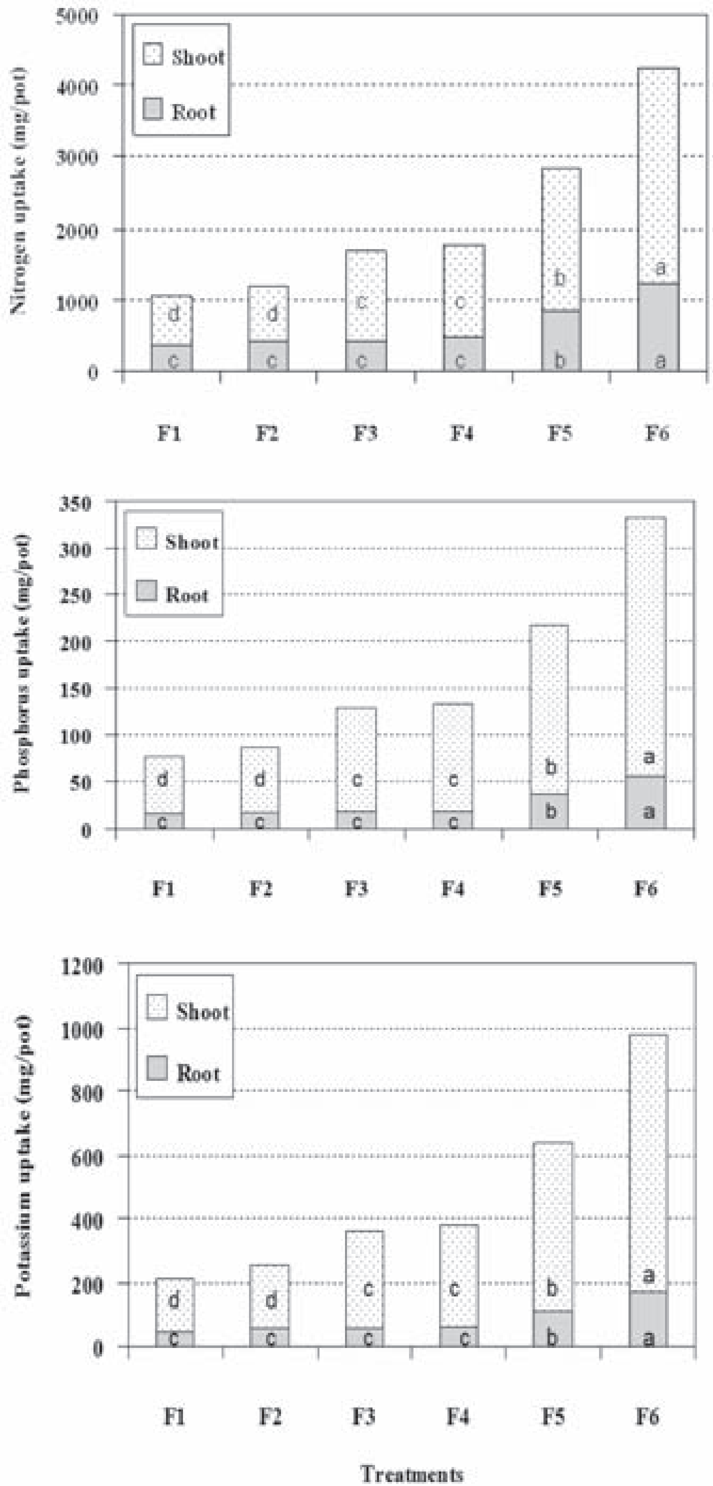

Figure 1 Amount of N, P and K uptake in shoots and roots of vetiver grown on iron ore tailings [Bars associated with a common letter were not significantly different at 0.05 probability level by DMRT and F1-F6 are as in Table 1].

Concentration of heavy metals in vetiver shoots and roots

The concentrations of Fe, Zn, Mn and Cu in shoots and roots of vetiver are shown in Table 5. In general, heavy metal concentrations exhibited similar trends among treatments. Vetiver accumulated high concentrations of Fe, Zn and Mn and a low concentration of Cu. Additionally, roots accumulated higher concentrations than shoots as reported by Yang *et al*. (2003) and Roongtanakiat *et al*. (2007). Heavy metal concentrations in vetiver shoots grown in different treatments ranged from 545 to 1197, 302 to 531, 415 to 648 and 13 to 66 mg kg^{-1} for Fe, Zn, Mn and Cu, respectively. Even though these concentrations were quite high, the vetiver did not show any toxic symptoms to heavy metals, especially with the high concentrations of Cu in shoots (47 mg kg^{-1}) and in roots (66 mg kg^{-1}) which were higher than the reported threshold level of Cu for vetiver growth (13 to 15 mg kg^{-1}) (Truong, 1999). This finding agreed with that of Roongtanakiat *et al*. (2003) who reported that vetiver could tolerate Cu at higher than the previously reported threshold levels.

Amending iron ore tailings with the combination of chelating agents and compost (F5 and F6) resulted in significantly higher heavy metal concentrations in both vetiver shoots and roots than for those with either chelating agent alone, compost or the control treatments, with the exception of Zn concentration, which showed no significant difference between the sole chelating agent and the combination treatments. The increase of heavy metal concentrations in vetiver plants supplied with a chelating agent may have been due to the increased solubility of heavy metals caused by the chelating agent (Lai and Chen, 2004)

This study revealed that compost did not enhance heavy metal concentrations in vetiver. This differed from other studies which reported that pig manure and domestic refuse, applied to Pb mine tailings, could decrease Pb, Zn and Cu concentrations in vetiver (Rotkittikhun *et al*., 2007 and Yang *et al*., 2003). This may be the result of differing chemical properties in the mine tailings used.

The ability of plants to translocate metals from roots to shoots is measured by the translocation factor (TF), which is defined as the ratio of metal concentrations in the shoots to the roots (Yoon *et al.,* 2006). Many reports have refered to this as "distribution" of metal in the shoots and the roots (Truong 1999; Roongtanakiat *et al*., 2001b; 2007). Vetiver grown on iron ore tailings treated with soil amendments had different amounts of heavy metals translocations as shown in Figure 2. The chelating agents and compost did not affect Fe and Zn translocation, while chelating agents could increase Cu translocation, especially when combined with compost. Contrary to the trend, the Mn translocation showed a slightly negative response to the chelating agents.

Table 5 Concentration of Fe, Zn, Mn and Cu in shoot and root of vetiver grown on iron ore tailings.

Treatment[1]	Concentration of heavy metals in vetiver[2] (mg kg^{-1})							
	Fe		Zn		Mn		Cu	
	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root
F1	545 c	813 c	302 b	435 b	415 c	465 c	13 c	39 c
F2	556 c	822 c	301 b	438 b	423 c	472 c	14 c	39 c
F3	810 b	1123 b	355 a	506 a	520 b	624 b	31 b	54 b
F4	819 b	1135 b	359 a	523 a	528 b	630 b	34 b	57 b
F5	859 a	1180 a	356 a	510 a	545 a	641 a	45 a	62 a
F6	871 a	1197 a	362 a	531 a	552 a	648 a	47 a	66 a

[1] : See description in Table 1.

[2] In a column, means with a common letter are not significantly different at 0.05 probability by DMRT.

However, the Mn had the highest translocation ability, similar to that reported by Roongtanakiat *et al*. (2007). Translocation was greatest in Mn, followed by Fe, Zn and Cu, with the average TF being 0.86, 0.71, 0.69 and 0.55, respectively. Since none of the TF values were greater than one, vetiver was considered unsuitable for phytoextraction (Yoon *et al*., 2006).

Heavy metal uptake in vetiver

Heavy metal uptake by vetiver was expressed as metal content accumulated in vetiver shoots and roots, and is shown in Figure 3. In general, vetiver could take up and accumulate a greater amount of all the studied heavy metals in its shoots than its roots, even though the metal concentration in the roots was higher than in the shoots. This could be explained by a higher shoot biomass than root biomass. The amounts of heavy metal in vetiver plants were in the order of Fe>Mn>Zn>Cu. By using chelating agent amendment with EDTA and DTPA, it was possible to increase heavy metal uptake in both vetiver shoots and roots. The combination of DTPA and compost amendment had the highest metal uptake, which was significantly higher than the combination of EDTA and compost. EDTA and DTPA gave an equal result in metal uptake and both had a significantly higher uptake than either of the compost or control treatments. The positive results of a chelating agent on heavy metal uptake were also studied by Lai and Chen (2004), who reported that EDTA significantly increased the total uptake of Pb in shoots. Chiu *et al*. (2006) also reported that an application of NTA in As-amended soil and HEIDA in Cu-amended soil increased three to four fold the amount of As and Cu in shoots, whereas NTA increased one to five fold the amount of Zn accumulation in shoots of vetiver and maize.

The sole compost application did not enhance heavy metal uptake as it insignificantly increased the heavy metal concentration and biomass of vetiver. Improvement of growth and metal uptake by compost amendment might differ between composts and soils. However, compost application not only increased organic matter, but also improved the poor physical properties and microbial activities of tailings.

CONCLUSION

Vetiver could tolerate high concentrations of heavy metal and grew well in iron ore tailings.

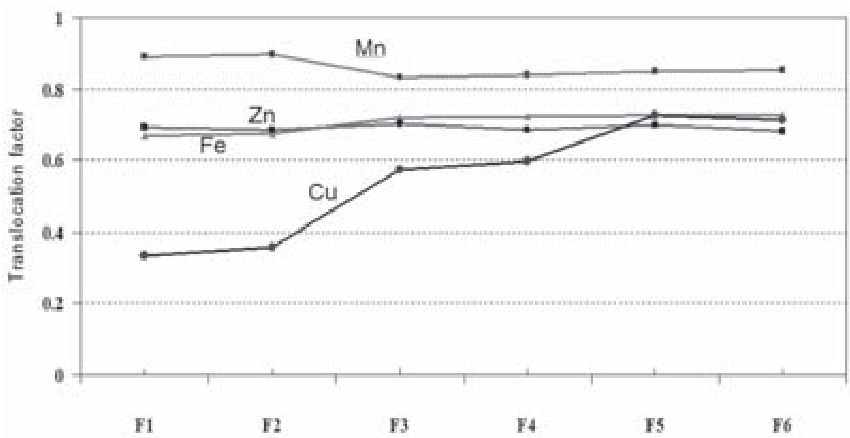

Figure 2 Translocation factors of Fe, Zn, Mn and Cu for vetiver grown on iron ore tailings amended with chelating agent and compost.
[F1-F6 are as in Table1]

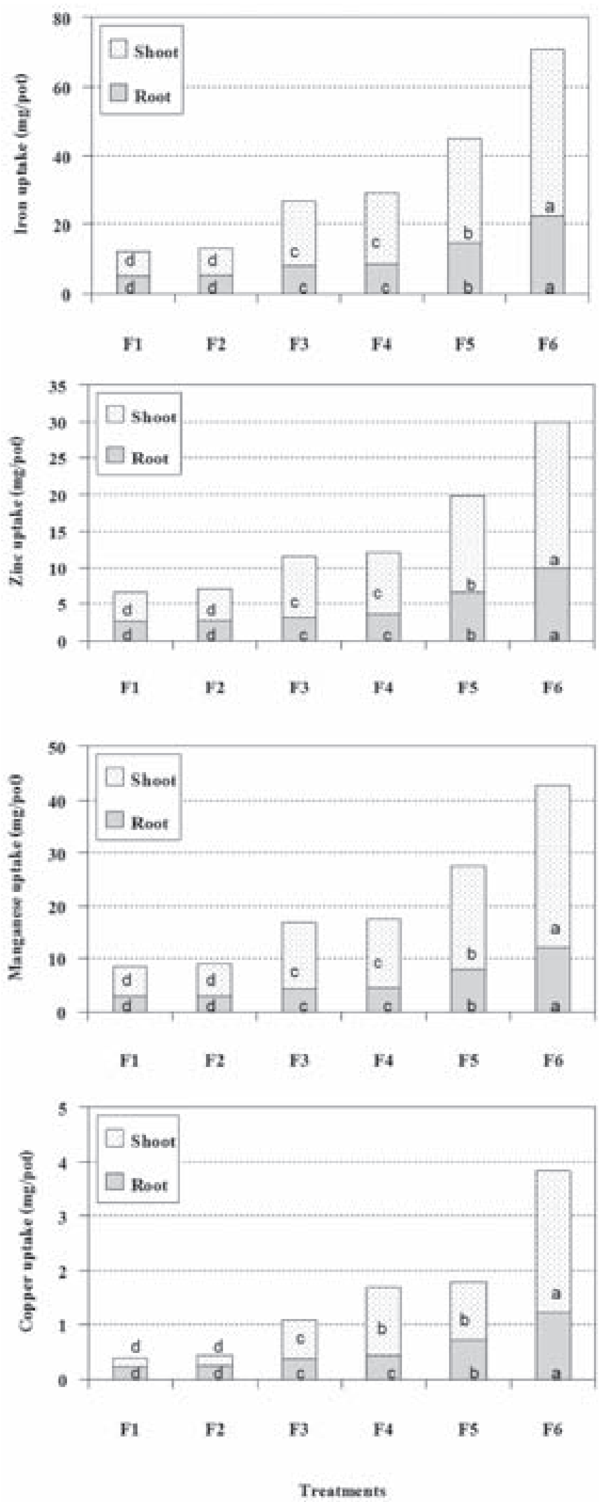

Figure 3 Uptake of Fe, Zn, Mn and Cu in vetiver shoots and roots grown on iron ore tailings [Bars associated with a common letter were not significantly differnet at 0.05 proability level by DMART and F1-F16 are as in Table 1].

The soil amendments used in this study, EDTA, DTAP and compost, did not significantly affect the concentration of primary nutrients in vetiver. The application of the chelating agents, EDTA and DTPA, could enhance growth and heavy metal uptake of vetiver. Using compost amendment did not show a positive effect. The best growth and highest metal uptake were obtained when chelating agents were applied. Chelating agent application increased Cu translocation, while Mn had the highest translocation factor. Vetiver was not considered a phytoextractor because the translocation factors of the heavy metals were less than 1. Owing to its unique characteristics of long, deep roots and a high tolerance to extreme soil conditions, including heavy metal contamination, vetiver was considered a potential plant for phytostabilization and rehabilitation of iron ore mine areas.

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