EFFECTS OF SOIL AMENDMENT ON GROWTH AND LEAD ACCUMULATION IN GRASSES *Vetiveria zizanioides* AND *Thysanolaena maxima* GROWN IN LEAD-CONTAMINATED SOIL

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Abstract

A glasshouse study was conducted to compare growth performance, metal tolerance and metal uptake by two grasses, *Thysanolaena maxima* (Roxb.) O. Kuntze and four ecotypes of *Vetiveria zizanioides* (L.) Nash (Surat Thani, Songkhla, Kamphaeng Phet, and Sri Lanka) and to study the effects of pig manure (20 and 40% w/w) and inorganic fertilizer (75 and 150 mg kg^{-1}) amendments to this lead mine soil. The results showed that both *T. maxima* and *V. zizanioides* (Surat Thani and Songkhla) could tolerate high Pb concentrations in soil (10 750 mg kg^{-1}) and had very good growth performance. Application of pig manure increased electrical conductivity (EC) and reduced DTPA-extractable Pb concentration in the soils. Pig manure application improved the growth of vetiver, but not *T. maxima* because they could not tolerate high EC values. The uptake by roots and transport of Pb to shoots of both species was reduced when soils were amended with pig manure. Application of inorganic fertilizer did not improve growth of vetiver but did improve that of *T. maxima*. Fertilizer application did not have any great influence on the Pb uptake in vetiver while *T. maxima* took up more Pb as a result of the fertilizer enhancing its biomass yield. Both species transported low Pb concentrations to shoots (8.3-180 mg kg^{-1}) and accumulated higher concentrations in roots (107-911 mg kg^{-1}). In summary, both species well suited for phytostabilization in tropical lead mine areas.

Keywords: Phytostabilization; Lead mine soils; Soil amendment; Grass; Lead tolerance

1. Introduction

Selection of appropriate plant species is very important to ensure a self-sustaining vegetation cover (Wong, 2003). Two species of grass were selected in the present study, vetiver grass [*Vetiveria zizanioides* (L.) Nash] of different ecotypes and a native grass commonly discovered in the lead mine area in Thailand, *Thysanolaena maxima* (Roxb.) O. Kuntze. Vetiver grass is a fast-growing plant that tolerates various extreme environments, including soil pH values between 3.0 and 10.5 and temperature from -10 to 48^{o}C (Dalton et al., 1996; Wong, 2003). It also effectively controls erosion of soil by water (Truong and Baker, 1998). The previous studies have shown that vetiver grass can grow well in soils contaminated with multiple elements at high concentrations such as those found at coal, cadmium, and gold mining sites (Truong and Baker 1998; Roongtanakiat and Chairoj, 2002) and can accumulate relatively high concentrations of lead (Chantachon et al., 2004). *T. maxima* is one of the most widely distributed species in Bo Ngam lead mine ($PbCO_3$) in Thong Pha Phum District, Kanchanaburi Province, Thailand. It can attain a very good height (2-3 m) and produces large biomass even when growing in extremely high lead content (10 to 100 g Pb kg^{-1}) soils. However, the use of *T. maxima* in phytoremediation technology is not widely recognized owing to the lack of detailed investigations of its capacity to absorb contaminants, its tolerance and practical field application. As for vetiver, although researches in Australia, China and Thailand have established its tolerance to high level of heavy metals in the soil and its effectiveness in phytoremediation work in contaminated land but vetiver has not been studied specifically on lead contamination alone and on a lead mine area.

Soil amendment is a major requirement for the successful establishment of vegetation in metal-contaminated soils. The addition of amendments such as fly ash, pig manure, sewage sludge, is effective in lowering the metal toxicity of soil and provides a slow release of nutrient sources such as N, P, K to support plant growth (Wong, 2003; Chiu et al., 2006). Cow manure, poultry manure and pig manure were found to be effective in reducing lead availability to plants, leading to lower uptake of lead (Scialdone et al., 1980; Wong and Lau, 1985; Ye et al., 1999). They are commonly used as tailings amendments because the addition of organic matter can significantly improve the physical characteristics and the nutrient status of mine soil (Ye et al., 1999). In addition, fertilizers are an essential ingredient for successful restoration of mine wastes (Bradshaw and Chadwick, 1980).

Since the revegetation process is ongoing at Bo Ngam lead mine, it is very important to develop experiments (both laboratory and field) that will aid in establishing the restoration technology at this important site in Thailand. Hence, the main objective of the present study was to evaluate the effects of fertilizer and pig manure amendments on lead mine soil, using vetiver grass (*V. zizanioides*) and the native grass, *T. maxima* in a pot experiment. It was hoped that this research would aid in establishing suitable application dosage of these materials for amending the lead mine soil.

2. Materials and Methods

The study comprised two experiments: Experiment I was designed to assess lead tolerance and accumulation in *V. zizanioides* and *T. maxima* grown in soil spiked with various concentrations of lead and inorganic fertilizer. Experiment II was designed to test the effects of pig manure and inorganic fertilizer amendments on lead mine soil using *V. zizanioides* and *T. maxima.*

2.1. Plant materials

Four ecotypes of *V. zizanioides* (three from Thailand: Surat Thani, Songkhla, Kamphaeng Phet, and one from Sri Lanka) were obtained from the Land Development Department, Kanchanaburi Province, Thailand. They were grown in the nursery at Mahidol University, Kanchanaburi, Thailand. The native grass, *T. maxima* were collected from the field site about 60 km from Bo Ngam lead mine, Kanchanaburi, Thailand.

2.2. Soil preparation

Experiment I

Natural soil was collected from 0-20 cm depth from a suburban area of Kanchanaburi, where native grasses including *T. maxima* were growing. It was air-dried and sieved through a 2 mm mesh sieve. Standard soil characterizations were performed (Department of Soil Science, Ministry of Agriculture and Cooperatives, Bangkok, Thailand). Lead was added to give nominal concentrations of 100, 1000 and 10 000 mg Pb kg^{-1} by mixing with 0.125, 1.25 and 12.5 g of $2PbCO_3{\cdot}Pb(OH)_2$ per kg soil. Soil without addition of lead served as the control. The fertilizers applied to the soil consisted of 120 mg N kg^{-1} of dry soil as NH_4NO_3, 80 mg P kg^{-1} of dry soil and 100 mg K kg^{-1} of dry soil as KH_2PO_4. The total and DTPA-extractable concentration of lead in the soil, electrical conductivity (EC) and soil pH after four weeks' equilibration were determined.

Soils were measured for pH and EC before the addition of lead using a glass-electrode pH meter and EC meter respectively. Organic matter was determined by Walkley-Black titration (Walkley and Black, 1934), total N by the Kjedhal method (Black, 1965), total P by Bray II method (Bray and Kurtz, 1945), total K by atomic absorption spectrophotometer after digestion with Na_2CO_3 and texture was assessed using the hydrometer method (Allen et al., 1974) (Table 1). Total concentrations of lead and other metals in soil were determined using 0.5 g soil sub-samples digested with HNO_3 (APHA, 1998). After digestion, lead concentration was determined by flame atomic absorption spectrophotometry (FAAS Varian Spectra AA 55B) and other metals (Fe, Zn, Mn, Cu, Cd and Ni) were determined by ICP–OES (ERAN 6000). DTPA-extractable lead content was determined by FAAS (ICARDA, 2001) (Tables 1, 2).

Experiment II

Lead mine soil was collected from the open pit mine area at Bo Ngam lead mine, Kanchanaburi at 0-20 cm depth. Soil was air-dried, sieved through a 2 mm mesh sieve and mixed well. Pig manure was supplied from a pig farm in Kanchanaburi, air-dried for 4 weeks and sieved through a 2 mm mesh. Soil and pig manure characterizations were investigated using similar methods as described in Experiment I (Tables 1, 2).

2.3. Plant growth experiment

Experiment I

A glasshouse pot trial was designed to test the lead accumulation and growth of *T. maxima* and the four ecotypes of *V. zizanioides.* Soil was placed in plastic pots (17 cm in diameter, 20 cm in height) which had two pieces of plastic screen at the bottom to retain the soil. Plants were selected, pruned (shoots were 20 cm and roots were 5 cm in length) and then transplanted into the pots (2 plants/pot) containing various concentrations of lead: 113, 192, 707, 10 750 mg kg^{-1} of dry soil (Table 2). There were four replicates for each treatment. The pots were placed in the glasshouse under controlled conditions and the soil moisture content was maintained at 40% of the water holding capacity by weighing and adding deionized water every two days (temperature 20-30 ^{o}C, day length 13-14 h). After 60 d, the plants were harvested. Plant samples were washed thoroughly with tap water, rinsed with deionized water and divided into shoots and roots. They were oven-dried at 60 ^{o}C for 48 h to a constant weight and the dry weight yield was recorded.

Experiment II

A glasshouse experiment was designed to test the growth and accumulation of *T. maxima* and the four ecotypes of *V. zizanioides* in soil amended with fertilizer or pig manure as follows: Treatment 1: Soil only (S), Treatment 2: Soil + 20% pig manure (S+20), Treatment 3: Soil + 40% pig manure (S+40), Treatment 4: Soil + fertilizer 75 mg kg^{-1} (S+F1), Treatment 5: Soil + fertilizer 150 mg kg^{-1} (S+F2).

Pig manure was mixed with soil at different percentages (20% and 40% w/w). In addition, soil was also amended with two levels of fertilizer addition (N:P:K = 15:15:15): 75 and 150 mg kg^{-1} of dry soil. Soil without fertilizer and pig manure served as the control. The total and diethylenetriamine-pentaacetic acid (DTPA)-extractable concentration of lead in the soil, EC and soil pH after four weeks' equilibration were determined. The DTPA-extractable of lead contents were determined using flame atomic absorption spectrophotometry (FAAS) after extraction with 0.005 M DTPA (1.79 g DTPA + 79.06 g NH_4HCO_3 + 2 ml NH_4OH) dissolved in 800 ml deionized H_2O, and made up to 1 l, pH 7.6) (ICARDA, 2001). The procedures for planting, harvest and analysis of lead were similar to those described in Experiment I.

2.4. Growth and metal accumulation

Plant growth and survival were determined as percentage survival, plant height and dry biomass. To determine lead accumulation, dried plant samples were ground to a fine powder and sieved through nylon mesh. The concentrations of lead in shoots and roots of plants were determined using 0.5 g plant sub-samples digested with HNO_3 (APHA, 1998). Lead concentration was measured by FAAS.

The root/shoot ratio is a highly representative indicator of environmental stress that is encountered by plants (Chiu et al., 2006). It was calculated as follows:

$$\text{Root/shoot ratio} \equiv \frac{\text{Dry weight of root}}{\text{Dry weight of shoot}}$$

2.5. Statistical analysis

The data for height, biomass, heavy metal concentrations of soil and plant and root/shoot ratios of plants under different treatments were analyzed using the SPSS statistical package by one-way analysis of variance (ANOVA) to compare the means of different treatments. Where significant *F* values were obtained, differences between individual means were tested using the least significant different test (LSD) at the $p = 0.05$ significance level.

3. Results

3.1. Experiment I: Lead tolerance and accumulation in T. maxima and V. zizanioides

3.1.1. Soil characteristics

The general properties of the soil are presented in Table 1. It was near neutral pH (6.8), with an EC value of 0.41 dS m^{-1}. The soil texture was clay with low organic matter content (0.5%). The soil contained low levels of total N, P and K (400, 400 and 900 mg kg^{-1}). Background total lead concentration in the soil was slightly elevated, 113 mg kg^{-1}. After the application of lead, total and DTPA-extractable lead concentrations were in the range of 113-10 750 mg kg^{-1} and 16.5-1065 mg kg^{-1}, respectively (Table 2). The results showed that the addition of lead to the soils decreased pH, especially at the highest application dosage.

Table 1

Physical and chemical properties of soil, lead mine soil and pig manure used for the pot experiment

Parameter	Soil	Lead mine soil	Pig manure
pH	6.75	7.07	6.5
EC(dS m^{-1})	0.42	0.23	4.1
Organic Matter (%)	0.5	0.2	29.65
Soil Texture	Clay	Loam	
Element concentrations		Total	
N (mg kg^{-1})	400	200	22 400
P (mg kg^{-1})	400	300	34 000
K (mg kg^{-1})	900	600	8300
Ca (mg kg^{-1})	1300	1100	31 800
Mg (mg kg^{-1})	400	300	11 300
Fe (mg kg^{-1})	33 592	22 584	314
Zn (mg kg^{-1})	<208	<208	843
Mn (mg kg^{-1})	503	1238	449
Cu (mg kg^{-1})	27.5	62.09	82.6
Cd (mg kg^{-1})	0.43	0.55	0.5
Ni (mg kg^{-1})	24.35	20.51	10.1
Pb (mg kg^{-1})	113	9017	10

Table 2
pH, EC, total and extractable of Pb (mean ± sd, n = 3) in the different treatments before plant growth experiment

Treatment	pH	EC ($dS\ m^{-1}$)	Total Pb ($mg\ kg^{-1}$)	Extractable Pb ($mg\ kg^{-1}$)
Experiment I				
1 (0 $mg\ kg^{-1}$)	6.75 ± 0.05	0.41 ± 0.03	113 ± 17.6	16.5 ± 0.2
2 (100 $mg\ kg^{-1}$)	6.4 ± 0.15	0.38 ± 0.02	192 ± 28.4	43.7 ± 0.6
3 (1000 $mg\ kg^{-1}$)	6.3 ± 0.3	0.39 ± 0.02	707 ± 15.3	253 ± 6
4 (10 000 $mg\ kg^{-1}$)	5.3 ± 0.2	0.37 ± 0.02	10 750 ± 624	1065 ± 13.2
Experiment II				
S	7.07 ± 0.12	0.23 ± 0.02	9020 ± 954	263 ± 11.5
S+20	7 ± 0	3.13 ± 0.03	8730 ± 983	183 ± 2.9
S+40	7 ± 0	3.96 ± 0.06	5650 ± 522	143 ± 2.9
S+F1	7.13 ± 0.15	1.04 ± 0.14	10 600 ± 1500	367 ± 17.6
S+F2	7.03 ± 0.06	1.76 ± 0.06	11 920 ± 1290	403 ± 5.8

3.1.2. Growth performance

The growth performance data (survival rate, height and biomass) of *T. maxima* and *V. zizanioides* are shown in Table 3. *T. maxima*, *V. zizanioides* (Surat Thani) and *V. zizanioides* (Songkhla) showed the best survival (100% in all treatments). *V. zizanioides* (Kamphaeng Phet) had the lowest survival. The height of the plants was significantly decreased with increases in lead concentration ($p \leq 0.05$). *T. maxima* attained the highest biomass in 100 mg Pb kg^{-1} treatment and remained rather similar with increase in lead while in *V. zizanioides*, there was 50-60%, reduction in biomass when lead concentration was increased.

3.1.3. Root/ shoot ratio

Root/shoot ratio is an indicator of environmental stress. The root/ shoot ratios of *T. maxima* and *V. zizanioides* grown in different treatments are presented in Fig. 1. *T. maxima* showed the highest values in all treatments. However, statistical analysis (LSD test) showed that means were not significantly different for each group ($p \geq 0.05$).

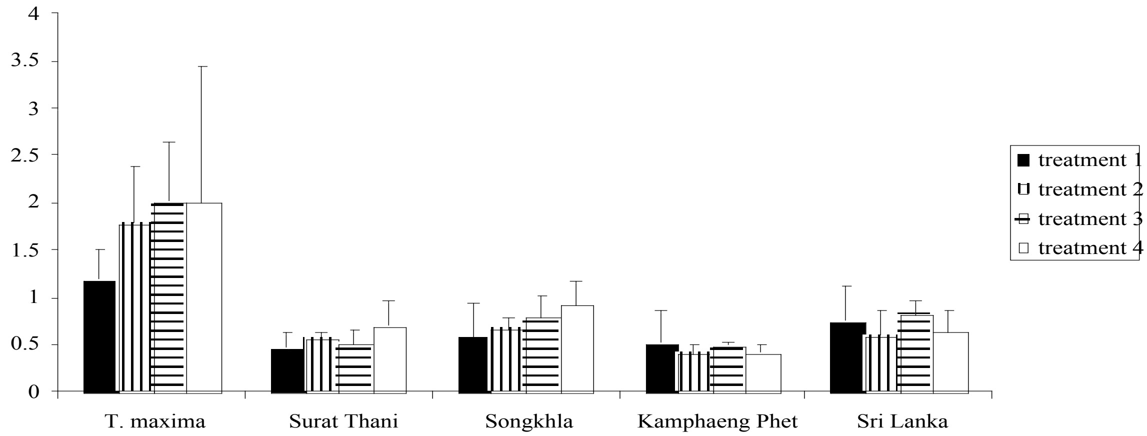

Fig. 1. Effects of application of lead on the root/shoot ratio for *T. maxima* and four ecotypes of *V. zizanioides*. The root/shoot ratio was not significantly different in each group at 5% level according to LSD test (n = 4).

3.1.4. Lead accumulation in plants

Lead accumulation in shoots and roots of *T. maxima* and four ecotypes of *V. zizanioides* was similar (Table 4). Plants accumulated higher lead concentrations in roots (4100-5900 mg kg^{-1}) than shoots (248-422 mg kg^{-1}). Lead concentrations in the same part (shoot or roots) of plants under different treatments were generally in the descending order of 10 000 > 1000 > 100 ≈ 0 mg kg^{-1} treatment.

3.2. Experiment II: Effects of pig manure and inorganic fertilizer amendments on V. zizanioides and T. maxima

3.2.1. General properties of lead mine soil and pig manure

The general properties of lead mine soil and pig manure are presented in Table 1. The lead mine soil and pig manure were near neutral (pH 7.1 and 6.5, respectively). The EC value of lead mine soil was low (0.23 dS m^{-1}), while that of pig manure was high (4.1 dS m^{-1}). The pig manure contained much higher levels of organic matter, total N, P and K (140, 110, 110 and 10 times, respectively, higher than those in the lead mine soil). In terms of heavy metals, total concentrations of Mn, Cd, Ni and Pb in lead mine soil were higher while total Zn and Cu were higher in pig manure.

The addition of pig manure or fertilizer to lead mine soil did not change the pH value but significantly increased the EC value, especially at the highest application dosage of pig manure (S+40, EC = 3.96 dS m^{-1}) (Table 2). The pig manure reduced total and extractable lead, while these were increased with fertilizer application.

3.2.2. Growth performance

The growth performance (survival rate, height and biomass) of *T. maxima* and *V. zizanioides* is presented in Table 5. *V. zizanioides* (Surat Thani) and *V. zizanioides* (Songkhla) showed best survival (100% in all treatments) while *V. zizanoides* (Kamphaeng Phet) had the lowest survival. The addition of pig manure decreased the survival of *T. maxima* and *V. zizanioides* (Sri Lanka). The results in terms of height and biomass of *T. maxima* showed that they were significantly decreased with the application of pig manure ($p \leq 0.05$) in comparison to those grown in pure lead mine soil or amended with fertilizer. For *V. zizanioides*, application of pig manure significantly increased the height and biomass of plants ($p \leq 0.05$). *V. zizanioides* (Sri Lanka) showed the maximum height and biomass in the 20% pig manure treatment. However, the addition of fertilizer did not significantly increase the height and biomass of vetiver grass ($p > 0.05$).

3.2.3. Root/ shoot ratio

Figure 2 shows the effects of pig manure and fertilizer application on the root/ shoot ratio. *T. maxima* plants grown in lead mine soil amended with pig manure showed the highest root/shoot ratio (2.33), indicating that they had suffered the greatest stress while in fertilizer application, they were significantly lower ($p \leq 0.05$). In contrast, *V. zizanioides* (Surat Thani) grown in soil amended with fertilizer showed the lowest ratio.

3.2.4. Lead accumulation in plants

Lead concentrations in roots and shoots of *T. maxima* and *V. zizanioides* are presented in Table 6. In the unamended soil, Surat Thani and Songkhla ecotypes of vetiver showed the highest lead accumulation in shoot, while the Kamphaeng Phet ecotype showed the highest lead accumulation in root. Both grass species accumulated highest lead concentrations in the roots.

The results showed that lead concentrations in both the shoot and root tissues of *T. maxima* and vetiver in soil amended with pig manure were significantly decreased ($p \leq 0.05$) (Table 6). *T. maxima* grown in soil amended with 20% fertilizer showed the maximum lead accumulation both in shoot and root tissues. In vetiver, the application of 40% fertilizer significantly increased the lead concentrations in root tissues ($p \leq 0.05$).

4. Discussion

From the present study, at the highest application dosage of lead (10 750 mg kg^{-1} as Pb), *T. maxima* and *V. zizanioides* (Surat Thani and Songkhla) could grow well and showed no toxicity symptoms indicating that they could tolerate the high lead concentrations in the soil, especially *T. maxima* which had the better growth performance. Lead concentrations in shoots and roots of both species were in the range of 248-422 mg kg^{-1} and 4100-5900 mg kg^{-1}, respectively. The results on tolerance and accumulation of lead by vetiver grass were rather similar to the previous studies. Chen et al. (2004) and Chantachon et al. (2004) had shown that *V. zizanioides* could grow well at 5000-10 000 mg Pb kg^{-1} as $Pb(NO_3)_2$ and there were no visual signs of phytotoxicity. *V. zizanioides* has a massive finely structured and deep root system and it has been known for its effectiveness in erosion control and the ability to tolerate extreme soil conditions including heavy metal contamination (Wong, 2003). Truong and

Baker (1998) have reported the successfully use of *V. zizanioides* to stabilize mining overburden and highly saline, sodic, magnesic and alkaline (pH 9.5) tailings of coal mine and highly acidic (pH 2.7) and high arsenic tailings of gold mines in Australia. Nowadays vetiver grass has been widely used as an alternative method for rehabililation of mine tailings in several countries, including China (Pang et al., 2003).

Our previous study has shown *T. maxima* is one of the most widely distributed species at Bo Ngam lead mine (Rotkittikhun et al., 2006). It has the ability to withstand very high soil lead concentrations (up to 100 000 mg kg^{-1}). Similar to vetiver grass, lead was found to accumulate more in roots (10 720 mg kg^{-1}) than in shoots (370 mg kg^{-1}). In the present study, *T. maxima* showed better growth performance than vetiver grass. In addition, in the field, *T. maxima* has a very fast growth rate, is very large in size and possesses a very deep root system, and can tolerate extremely high lead concentrations. The only draw back is they commonly grow well at high elevation from about 2500 to 5000 feet (Rhind, 1945). Nevertheless, *T. maxima* can be another alternative for phytostabilization of lead mine soil.

In mine lands, there are some major constraints to plant growth and the success of restoration is dependent upon overcoming these problems (Bradshaw, 1997). The present study indicated that Bo Ngam lead mine soil contained high levels of total and DTPA-extractable lead and low levels of organic matter and major nutrients (N, P, K). Therefore, the application of pig manure or inorganic fertilizer should improve these soil properties. The application of pig manure to lead mine soils resulted in a significant increase in EC value because pig manure contained large amount of soluble salts such as Fe and K ions. However, it reduced the total and DTPA-extractable lead in the soil. This may be due to chelation, complexation, and adsorption between metals in soils and the organic matter that is contained in organic amendments and also the dilution effect when they are mixed with soils (Friedland, 1989; Lozano-cerezo et al., 1999; Chiu et al., 2006). Similar results were obtained when applying lime, manure compost and sewage sludge to Pb/Zn mine tailings (Chiu et al., 2006; Ye et al., 1999). In contrast, the application of fertilizer increased the total and DTPA-extractable lead in our lead mine soils.

The present study demonstrated that the application dosage of 20% pig manure was the most effective in improving the biomass of vetiver grown in lead mine soils. However, the application of inorganic fertilizer did not effectively improve vetiver growth. These results suggested that pig manure amendment could provide enough nutrients for the growth of vetiver in lead mine soils and may improve their physical conditions. Analysis of pig manure in this study showed 100 times more organic matter, N and P, and 14 times more K than in the lead mine soil. These could enhance growth in vetiver. Similar results were reported by Ye et al. (1999); adding inorganic fertilizer alone did not significantly improve the growth of *Agropyron elongatum* or *Trifolium repens*. On the contrary, *T. maxima* had high biomass when grown in lead mine soils amended with 150 mg kg^{-1} inorganic fertilizer and very poor when amended with pig manure. This may be due to the increase in EC value by pig manure amendment (from 0.23 to 3.96 dS m^{-1}). In general, most plant species survive in the EC range of 0-2 dS m^{-1} and sensitive species are affected by EC of 4-8 dS m^{-1}, while only tolerant species can achieve satisfactory growth when the EC is greater than 8 dS m^{-1} (Shu et al., 2001). The results indicated that vetiver is more salt tolerant than *T. maxima.*

The addition of pig manure to the lead mine soil reduced the total and extractable lead while inorganic fertilizer amendment resulted in the increase of lead in the soil. Hence, both *T. maxima* and *V. zizanioides* showed a decrease in lead uptake when grown in soil amended with pig manure On the other hand, higher applications of inorganic fertilizer (150 mg kg^{-1})

caused an increase in lead uptake in these grass species, while lower additions (75 mg kg^{-1}) did not have any significant effect on lead uptake. Several other studies have reported that refuse, pig manure and $CaCO_3$, cow manure, manure compost and sewage sludge effectively reduce lead availability to plants leading to lower lead uptake (Sciadone et al., 1980; Wong and Lau, 1985; Ye et al., 1999; Chiu et al., 2006). It was suggested that soil had a relatively large capacity for the immobilization of ionic lead and that the precipitation of lead was primarily through the fixation by organic matter (Alloway, 1995). Hence, the high organic carbon content of amended soil caused the lower uptake of lead by these grass species.

5. Conclusions

Both grass species, *T. maxima* and *V. zizanioides* (Surat Thani and Songkhla) are good choices for phytostabilization of lead mine soil. They show very high tolerance to lead and could accumulate much more lead in roots than in shoots.

The applications of pig manure and inorganic fertilizer to the lead mine soil could increase nutrients for plant growth. *T. maxima* showed the best growth when grown in lead mine soil amended with 150 mg kg^{-1} inorganic fertilizer while the amendment with 20% pig manure resulted in the best growth of *V. zizanioides*. In addition, the use of pig manure also resulted in decreased pH and lead concentrations, and increases in EC and organic matter.

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