Effects of soil amendment on growth and lead accumulation on grasses *Vetiveria zizanioides* and *Thysanolaena maxima* grown in lead-contaminated soil

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Kanchanaburi

Bo Ngam lead mine

What are the problems in revegetation of mine land?

- Extreme soil properties.
- Low amount of nutrients.
- Toxicity of heavy metals.

The key in successful revegetation

- Selection of suitable metal-tolerant plant species.
- The amendment of soil.

Thysanolaena maxima (Roxb.) O. Kuntze

- *T. maxima* can grow up to 3.5 m tall.
- Grow well at high elevation from about 2500-5000 feet.
- Tolerate extremely high lead concentration.
- Inflorescences are used in making brooms.

Vetiveria zizanioides Nash.

Why did we use soil amendment?

- Lowering the metal toxicity of soil.
- Providing nutrient source to support plant growth.
- Reducing heavy metals availability to plant.
- Improving the physical characteristics of mine soil.

Objectives

- To compare growth performance, metal tolerance and metal uptake by two grasses, *T. maxima,* and four ecotypes of (*V. zizanioides*) in a pot experiment.

- To evaluate the effects of fertilizer and pig manure amendments on lead mine soil.

Materials and Methods

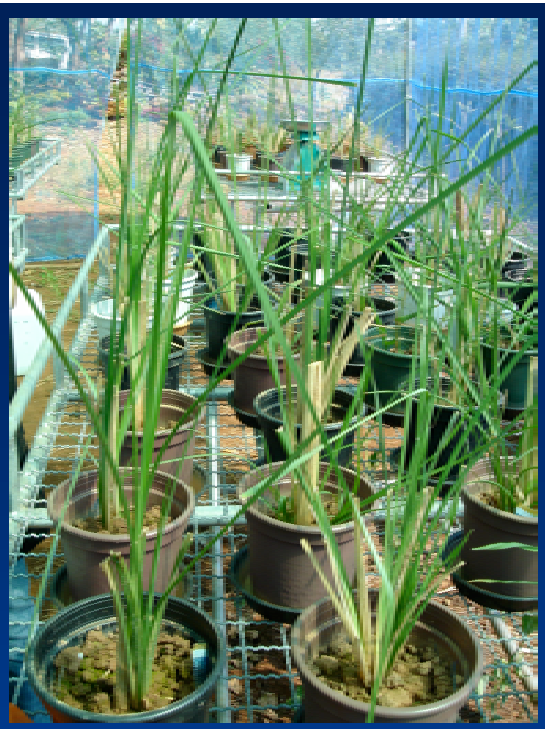
- There were 2 parts in this experiment

1. Growth and lead accumulation by *T. maxima* and vetiver grass grown in soil with various lead concentration.

2. Growth and lead accumulation by grasses grown in lead-contaminated soil amended with pig manure and fertilizer.

Plant materials

1. *V. zizanioides*
   - Surat Thani
   - Songkhla
   - Kamphaeng Phet
   - Sri Lanka
2. *T. maxima*



Soil preparation

Experiment I

- Local soil (from Kanchanaburi) was spiked with various lead concentrations ($2PbCO_3 \cdot Pb(OH_2)$): 100, 1000 and 10,000 mg kg^{-1}.

- The fertilizer 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.

Experiment II

- Lead-contaminated soil (from Bo Ngam lead mine) (~10,000 mg kg^{-1}) was amended with various treatments.

Treatment	
1	Soil only (S)
2	Soil + 20% pig manure (S+20)
3	Soil + 40% pig manure (S+40)
4	Soil + fertilizer 75 mg kg^{-1} (S+F1)
5	Soil + fertilizer 150 mg kg^{-1} (S+F2)

Plant growth experiment

- Soil was placed in plastic pots (17 cm in diameter, 20 cm in height).
- Plants were selected, pruned (shoots were 20 cm and roots were 5 cm in length) and then transplanted into the pots (2 plants/pot).
- There were four replicates for each treatment.
- The soil moisture content was maintained at 40% of WHC.
- After 60 days, the plants were harvested.

Growth and metal accumulation

- Plant growth and survival were determined as percentage survival, plant height and dry biomass.
- Lead accumulation in shoots and roots of plants were determined.
- The root/shoot ratio is a highly representative indicator of environmental stress that is encountered by plants (Chiu et al., 2006).

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

Results

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

- Experiment II: Effects of pig manure and inorganic fertilizer amendments on lead mine soil using *T. Maxima* and *V. zizanioides*

Table 1 Physical and chemical properties of soil used for the pot experiment

Parameters	Soil
pH	6.75
EC(dS m^{-1})	0.42
Organic Matter (%)	0.5
Soil Texture	Clay

Element concentrations	Total
N (mg kg^{-1})	400
P (mg kg^{-1})	400
K (mg kg^{-1})	900
Ca (mg kg^{-1})	1300
Mg (mg kg^{-1})	400
Fe (mg kg^{-1})	33 592
Zn (mg kg^{-1})	<208
Mn (mg kg^{-1})	503
Cu (mg kg^{-1})	27.5
Cd (mg kg^{-1})	0.43
Ni (mg kg^{-1})	24.35
Pb (mg kg^{-1})	113

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})
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 ± 6.0
4 (10 000 mg kg^{-1})	5.3 ± 0.2	0.37 ± 0.02	10 750 ± 624	1065 ± 13.2

Table 3 Survival (%) of *T. maxima* and four ecotypes of *V. zizanioides* grown on different lead concentrations for a period of 60 days

Treatment	*T. maxima*	*V. zizanioides* (Surat Thani)	*V. zizanioides* (Songkhla)	*V. zizanioides* (Kamphaeng Phet)	*V. zizanioides* (Sri Lanka)
1	100	100	100	100	100
2	100	100	100	75	100
3	100	100	100	50	100
4	100	100	100	50	75

Treatment 1= Control; Treatment 2= 100 mg kg^{-1}; Treatment 3= 1000 mg kg^{-1}; Treatment 4= 10 000 mg kg^{-1}

Table 4 Height (cm), and biomass (g dry weight/ pot) (mean ± sd, *n* = 4) of *T. maxima* and four ecotypes of *V. zizanioides* grown on different lead concentrations

Treatment		*T. maxima*	*V. zizanioides* (Surat Thani)	*V. zizanioides* (Songkhla)	*V. zizanioides* (Kamphaeng Phet)	*V. zizanioides* (Sri Lanka)
Height	1	37.8 ± 12.2 a*-d#	59.5 ± 6.1 a-c	69 ± 24.4 a-b	54.8 ± 7 b-c	89.5 ± 15.0 a-a
	2	28.1 ± 14.3 b-d	57.3 ± 3.7 a-c	68.5 ± 13.9 a-b	60.7 ± 14.0 a-c	84.4 ± 18.7 a-a
	3	26.8 ± 5.1 b-c	44.4 ± 5.8 b-b	45.88 ± 25.3 b-b	54.5 ± 9.2 b-a	48.3 ± 23.8 b-ab
	4	24 ± 4.6 b-d	39.3 ± 6.9 c-b	46.5 ± 6.8 b-a	26.3 ± 3.2 c-cd	28.3 ± 8.7 c-c
Biomass	1	6.5 ± 0.8 b-c	7.0 ± 1.4 a-c	8.1 ± 1.5 a-b	12.5 ± 3.9 a-a	8.9 ± 1.2 a-b
	2	7.4 ± 1.9 a-c	7.0 ± 1.6 a-c	8.6 ± 1.3 a-b	12.0 ± 5.1 a-a	8.6 ± 2.3 a-b
	3	6.3 ± 2.4 b-b	4.0 ± 1.4 b-c	5.6 ± 2.2 c-b	7.7 ± 2.4 b-a	7.6 ± 1.5 b-a
	4	6.4 ± 1.3 b-a	4.8 ± 2.4 b-b	6.7 ± 2.7 b-a	7.3 ± 3.1 b-a	4.5 ± 1.9 c-b

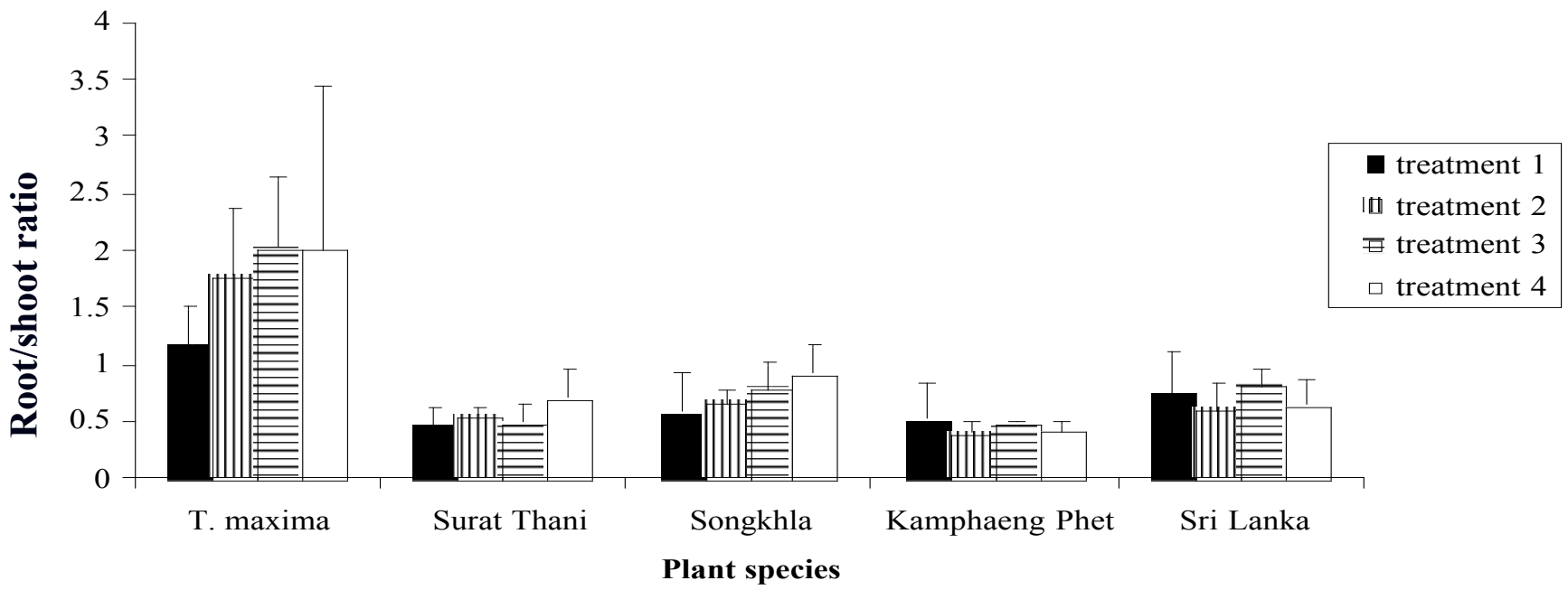


Fig. 1. Effects of application of lead on the root/shoot ratio for *T.maxima* and four ecotypes of *V. zizanioides*.

Table 5 Lead concentrations (mean ± SD, n = 4) in plants of *T. maxima* and four ecotypes of *V. zizanioides* grown at different lead concentrations for a period of 60 days

	Metal concentration (mg kg^{-1})			
	Treatment 1	**2**	**3**	**4**
T. maxima				
Shoot	**16.2 ± 9.5 c**	**20 ± 4.1 c**	**140 ± 118 b**	**357 ± 61.3 a**
Root	**45.0 ± 23.51 c**	**106 ± 75.4 c**	**509 ± 268 b**	**4290 ± 719 a**
V. zizanioides (Surat Thani)				
Shoot	**12.5 ± 5.0 c**	**16.2 ± 9.4 c**	**142 ± 54.5 b**	**359 ± 2601 a**
Root	**18.7 ± 2.5 c**	**41.1 ± 9.4 c**	**474 ± 74.3 b**	**4940 ± 1080 a**
V. zizanioides (Songkhla)				
Shoot	**12.5 ± 2.9 c**	**23.7 ± 8.5 c**	**214 ± 143 b**	**422 ± 387 a**
Root	**39.9 ± 30.3 c**	**55.0 ± 7.1 c**	**569 ± 242 b**	**4170 ± 827 a**
V. zizanioides (Kamphaeng Phet)				
Shoot	**11.2 ± 6.3 c**	**15.0 ± 5.0 c**	**102 ± 25.1 b**	**287 ± 53.0 a**
Root	**39.8 ± 20.3 c**	**48.3 ± 7.8 c**	**775 ± 24.9 b**	**5950 ± 1200 a**
V. zizanioides (Sri Lanka)				
Shoot	**16.2 ± 7.5 bc**	**15.0 ± 4.1 c**	**69.8 ± 7.0 b**	**248 ± 271 a**
Root	**63.6 ± 32.9 c**	**56.0 ± 10.2 c**	**628 ± 310 b**	**5770 ± 1390 a**

Results

- Experiment II: Effects of pig manure and inorganic fertilizer amendments on lead mine soil using *T. Maxima* and *V. zizanioides*

Table 6 Physical and chemical properties of lead mine soil and pig manure used in the experiment

Parameters	Lead mine soil	Pig manure
pH	7.07	6.5
EC(dS m^{-1})	0.23	4.1
Organic Matter (%)	0.2	29.65
Soil Texture	Loam	

Element concentrations	Lead mine soil	Pig manure
N (mg kg^{-1})	200	22 400
P (mg kg^{-1})	300	34 000
K (mg kg^{-1})	600	8300
Ca (mg kg^{-1})	1100	31 800
Mg (mg kg^{-1})	300	11 300
Fe (mg kg^{-1})	22 584	314
Zn (mg kg^{-1})	<208	843
Mn (mg kg^{-1})	1238	449
Cu (mg kg^{-1})	62.09	82.6
Cd (mg kg^{-1})	0.55	0.5
Ni (mg kg^{-1})	20.51	10.1
Pb (mg kg^{-1})	9017	10

Table 7 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 ($mg\ kg^{-1}$)
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

S = Soil only, S+20 = Soil + 20% pig manure, S+40 = Soil + 40% pig manure, S+F1 = Soil + fertilizer 75 mg kg^{-1}, S+F2 = Soil + fertilizer 150 mg kg^{-1}.

Table 8 Survival (%) of *T. maxima* and four ecotypes of *V. zizanioides* grown on Bo Ngam lead mine soil in different treatments

Treatment	*T. maxima*	*V. zizanioides* (Surat Thani)	*V. zizanioides* (Songkhla)	*V. zizanioides* (Kamphaeng Phet)	*V. zizanioides* (Sri Lanka)
S	100	100	100	50	100
S+20	75	100	100	100	100
S+40	75	100	100	25	75
S+F1	100	100	100	75	100
S+F2	100	100	100	50	100

Table 9 Height (cm), and biomass (g dry weight/ pot) (mean ± sd, *n* = 4) of *T. maxima* and four ecotypes of *V. zizanioides* grown in lead mine soil with different treatment

	Treatment	*T. maxima*	*V. zizanioides* (Surat Thani)	*V. zizanioides* (Songkhla)	*V. zizanioides* (Kamphaeng Phet)	*V. zizanioides* (Sri Lanka)
Height	**S**	**35.1 ± 9.9 b*-bc#**	**36.6 ± 9.7 d-bc**	**37.9 ± 10.4 c-b**	**33.8 ± 3.9 d-c**	**63.5 ± 6.0 b-a**
	S+20	**23.8 ± 2.5 c-c**	**63.3 ± 3.9 b-b**	**66.0 ± 8.0 a-b**	**60.0 ± 20.2 b-b**	**86.0 ± 17.4 a-a**
	S+40	**23.3 ± 3.1 c-c**	**69.6 ± 3.1 a-b**	**68.8 ± 13.7 a-b**	**83.5 ± 0 a-a**	**80.5 ± 16.2 a-a**
	S+F1	**35.5 ± 14.7 b-c**	**49.1 ± 8.5 c-b**	**53.5 ± 13.8 b-ab**	**52.2 ± 13.6 c-ab**	**56.9 ± 13.3 b-a**
	S+F2	**40.5 ± 2.7 a-c**	**59.1 ± 17.6 b-a**	**43.5 ± 14.0 c-bc**	**48.8 ± 3.9 c-b**	**60.3 ± 19.0 b-a**
Biomass	**S**	**5.7 ± 1.4 c-ab**	**3.5 ± 1.1 b-c**	**4.6 ± 1.4 c-bc**	**6.3 ± 0.3 c-ab**	**6.7 ± 1.3 c-a**
	S+20	**5.5 ± 1.1 c-b**	**7.8 ± 2.1 a-ab**	**8.0 ± 2.0 a-ab**	**9.2 ± 3.4 a-a**	**8.9 ± 1.4 a-a**
	S+40	**4.5 ± 1.1 d-a**	**7.5 ± 1.3 a-a**	**7.0 ± 3.5 b-a**	**5.4 ± 0 d-a**	**8.0 ± 5.1 ab-a**
	S+F1	**7.0 ± 2.5 b-a**	**4.2 ± 1.5 b-b**	**5.5 ± 0.9 c-ab**	**7.9 ± 0.9 b-a**	**7.1 ± 2.4 bc-a**
	S+F2	**8.1 ± 3.7 a-a**	**3.9 ± 2.0 b-c**	**5.1 ± 0.7 c-bc**	**6.9 ± 0.4 c-ab**	**6.9 ± 2.3 bc-ab**

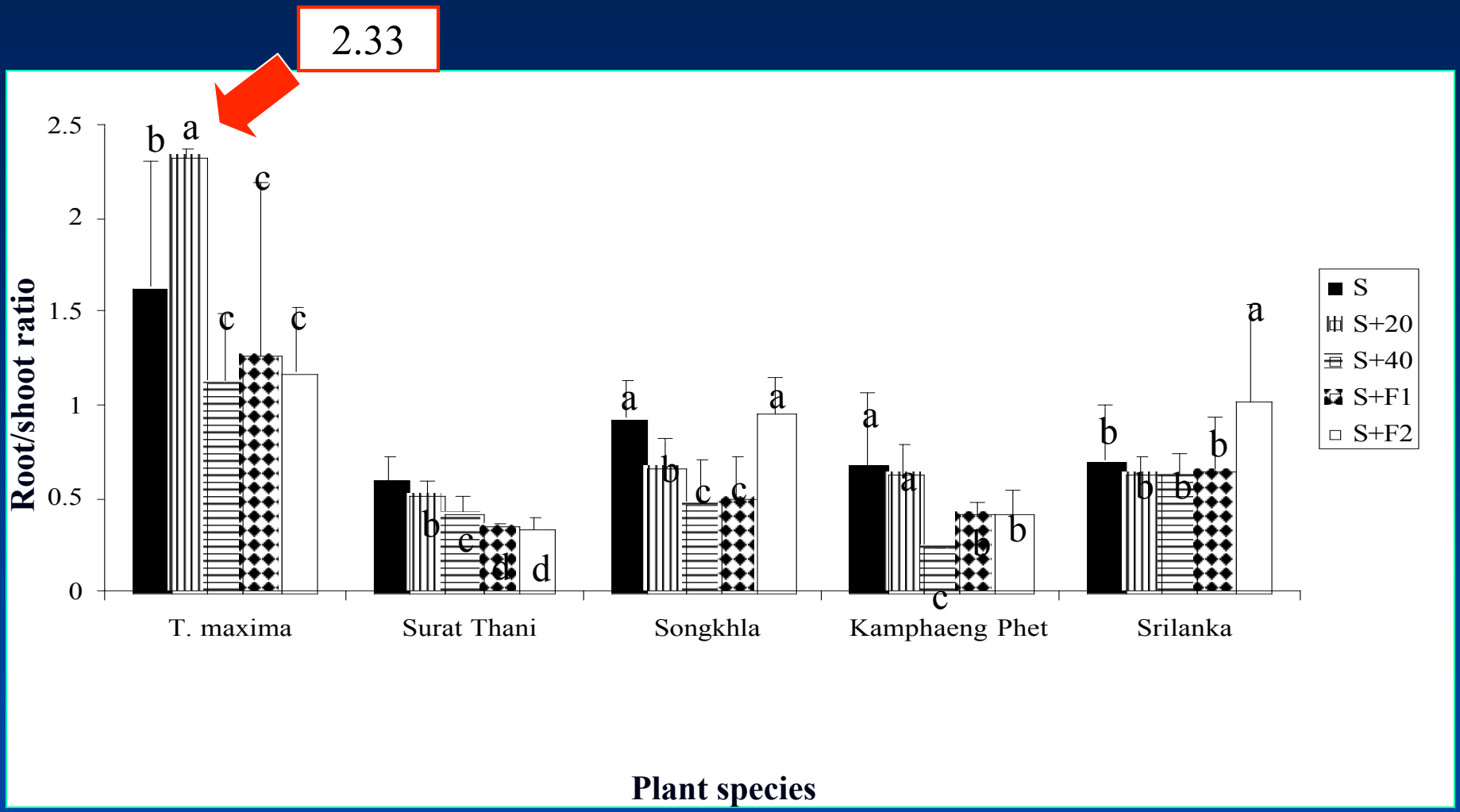


Fig. 2. Effects of application of manure and fertilizer on the root/shoot ratio for *T. maxima* and four ecotypes of *V. zizanioides* in lead mine soil.

Table 10 Lead concentrations (mean ± sd, *n*=4) in plants of *T. maxima* and four ecotypes of *V. zizanioides* grown on lead mine soil under different treatments for a period of 60 days

	Metal concentration (mg kg^{-1})				
	S	**S+20**	**S+40**	**S+F1**	**S+F2**
T. maxima					
Shoot	**19.9 ± 4.1 c**	**13.3 ± 2.9 c**	**15.0 ± 0.1 c**	**56.1 ± 50.1 a**	**37.4 ± 9.6 b**
Root	**271 ± 84.8 b**	**115 ± 75.3 c**	**146 ± 28.8 c**	**565 ± 410 a**	**277 ± 18.8 b**
V. zizanioides (Surat Thani)					
Shoot	**128 ± 61.5 a**	**11.2 ± 2.5 d**	**15.0 ± 7.0 d**	**39.9 ± 10.8 c**	**59.8 ± 20.3 b**
Root	**347 ± 120 c**	**236 ± 221 d**	**126 ± 49.6 e**	**475 ± 179 b**	**705 ± 188 a**
V. zizanioides (Songkhla)					
Shoot	**124 ± 75.9 b**	**18.7 ± 4.7 c**	**23.7 ± 8.5 c**	**43.6 ± 4.7 c**	**179 ± 200 a**
Root	**388 ± 154 b**	**234 ± 51.5 c**	**200 ± 83.8 c**	**193 ± 47.7 c**	**712 ± 538 a**
V. zizanioides (Kamphaeng Phet)					
Shoot	**90.2 ± 106 a**	**33.6 ± 13.1 c**	**54.8 ± 0 b**	**102 ± 62.6 a**	**54.8 ± 35.1 b**
Root	**759 ± 509 b**	**462 ± 310 c**	**214 ± 0 d**	**759 ± 226 b**	**911 ± 6.7 a**
V. zizanioides (Sri Lanka)					
Shoot	**34.9 ± 10.8 c**	**26.2 ± 7.5 c**	**8.3 ± 2.9 d**	**59.9 ± 27.4 b**	**138 ± 57.8 a**
Root	**459 ± 386 b**	**138 ± 63.3 c**	**107 ± 20.5 c**	**504 ± 155 b**	**686 ± 195 a**

Conclusions

- Both grass species, *T. maxima* and *V. zizanioides* (Surat Thani and Songkhla) are good choices for phytostabilization of lead mine soil.
- High tolerance to lead and could accumulate much more lead in roots than in shoots.
- *T. maxima* showed the best growth when grown in lead mine soil amended with 150 mg kg^{-1} inorganic fertilizer.
- Amendment with 20% pig manure resulted in the best growth of *V. zizanioides*.

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