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Accumulation of heavy metals in four grasses grown on lead and zinc mine tailings

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Abstract: A field experiment was conducted to compare the growth and metal accumulation of *Vetiveria zizanioides*, *Paspalum notatum*, *Cynodon dactylon* and *Imperata cylindraca* var. *major* on the tailings, amended with 10 cm domestic refuse + complex NPK fertilizer(Treatment A), 10 cm domestic refuse(Treatment B) and complex NPK fertilizer(Treatment C) respectively, and without any amendment used as control(Treatment D). The results indicated that *V. zizanioides* was a typical heavy metal excluder, because the concentrations in shoots of the plants were the lowest among the four plants tested. The most of metal accumulated in *V. zizanioides* distributed in its root, and transportation of metal in this plant from root to shoot was restricted. Therefore, *V. zizanioides* was more suitable for phytostabilization of toxic mined lands than *P. notatum* and *C. dactylon*, which accumulated a relatively high level of metals in their shoots and roots. It was also found that *I. cylindraca* var. *major* accumulated lower amounts of Pb, Zn and Cu than *C. dactylon* and *P. notatum*, and could also be considered for phytostalilisaton of tailings. Although the metal(Pb, Zn and Cu) concentrations in shoots and roots of *V. zizanioides* were the lowest, the total amounts of heavy metals accumulated in shoots of *V. zizanioides* were the highest among the four tested plants due to the highest dry weight yield of it. The results indicated that *V. zizanioides* was the best choice among the four species used for phytoremediation(for both phytostabilization and phytoextraction) of metal contaminated soils.

Keywords: Pb/Zn tailings; heavy metals; phytoremediation; *Vetiveria zizanioides*; *Paspalum notatum*; *Cynodon dactylon*; *Imperata cylindraca* var. *major*

Introduction

One developing alternative remediation technique for metal-contaminated sites is phytostablization, also called "inplace inactivation" or "phytorestoration". It is a type of phytoremediation technique that involves stabilizing heavy metals with plants in contaminated soils. To be a potentially cost-effective remediation technique, plants selected must be able to tolerate high concentrations of heavy metals, and stabilize heavy metals in soils by roots of plants and some organic or inorganic amendments, such as domestic refuse, fertilizer, and others. Revegetation of mining wastes is one of the congest practiced and well-documented approaches for stabilization of heavy metals in mining wastes (Bradshaw, 1980).

Mining activities produce a large quantity of waste materials (such as tailings), which frequently contain excessive concentrations of heavy metals. These mining activities and waste materials have created pollution problem and generated land dereliction, without vegetation coverage. Phytoremediation of metalliferous mine tailings is necessary for long-term stability of the land surface, or removal of toxic metals. The success of reclamation schemes is greatly dependent upon the choice of plant species and their methods of establishment(Bradshaw, 1980; Johnson, 1994). There are some important considerations when selecting plants for phytostablization. Plants should be tolerant of the soil metal levels as well as the other initial site conditions(e.g., soil pH, salinity, soil structure, water content, lack of major nutrients and organic materials). Plants chosen for phytostablization should also be poor translocators of metal contaminants to aboveground plant tissues that could be consumed by humans or animals. Additionally, the plants must grow quickly to establish ground cover, have dense rooting systems and canopies, and have relatively high transpiration rates to effectively dewater the soil (Raskin, 2000).

Since 1999, a field trial has been conducted at Lechang Pb/Zn Mine, Lechang City of Guangdong Province of China, aimed at comparing the growth of the four grasses(*Vetiveria zizanioides*, *Paspalum notatum*, *Cynodon dactylon* and *Imperata cylindraca* var. *major*) on Lechang Pb/Zn mine tailings with different amendments, for screening the most useful grass and the most effective measure for revegetation of

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tailings. Chemical analyses indicated that Lechang Pb/Zn mine tailings contained high concentrations of heavy metals (Pb, Zn, Cu and Cd) and low levels of major nutrient elements (N, P and K) and organic matter. Field experiment indicated that the domestic refuse and NPK fertilizer improved plant growth, and the combination use of domestic refuse and NPK fertilizer achieved the best growth. After six months, *V. zizanioides* growing on treatment A had coverage of 100% and dry weight yield of 2111 g/m^2. Its biomass and metal tolerance index were significantly greater than the other three grasses growing under the same treatment and might be the best choice for revegetation of Pb/Zn mine tailings (Shu, 2002a). However, the metal accumulation in the four grasses kept unknown, therefore, the experiment presented here aims at investigating the extent of heavy metals (Pb, Zn, Cu and Cd) accumulation in the four tested plants for further assessing their different role of the plants in phytoremediation.

1 Materials and methods

1.1 Site description and revegetation experiment

The Lechang Pb/Zn Mine is located at about 4 km east of Lechang City in the most northern part of Guangdong Province, China. The climate is sub-tropical and the annual rainfall is about 1500 mm. It is a conventional underground mining operation covering an area of 1.5 km^2, and produces approximately 30000 t of tailings annually, with a dumping area of 60000 m^2 (Shu, 1997). Lechang Pb/Zn mine tailings contained high concentrations of heavy metals (Pb, Zn, Cu and Cd) and low levels of major nutrient elements (N, P and K) and organic matter (Table 1). Heavy metal toxicity and extreme infertility might be the major constraints on phytoremediation (Ye, 2002).

Table 1 Physico-chemical properties of the tailings and domestic refuse (mean ± *sd*, *n* = 6)

Parameters	Units	Tailings	Domestic refuse
pH		7.13 ± 0.11	6.65 ± 0.21
EC	DS/m	2.09 ± 0.28	0.52 ± 0.07
Organic matter	%	0.57 ± 0.17	1.58 ± 0.31
Total N	mg/kg	507 ± 83	743 ± 129
Total P	mg/kg	619 ± 75	2184 ± 363
Total K	mg/kg	2023 ± 425	3035 ± 457
Pb Total	mg/kg	3123 ± 407	209 ± 57
Extractable	mg/kg	98 ± 24	4.88 ± 1.97
Zn Total	mg/kg	3418 ± 652	107 ± 20
Extractable	mg/kg	101 ± 35	3.26 ± 1.12
Cu Total	mg/kg	174 ± 31	53 ± 12
Extractable	mg/kg	4.28 ± 2.03	1.99 ± 0.42
Cd Total	mg/kg	22 ± 4.76	2.25 ± 0.42
Extractable	mg/kg	0.79 ± 0.26	0.12 ± 0.04

The tailings pond was tilled to a depth of 20 cm, and then divided into 12 plots of 16 m^2 (4 m × 4 m), each plot was further divided into four subplots of 4 m^2 (2 m × 2 m) for planting different grasses. There were four treatments with three replicates each arranged in a completely randomized block. The 4 plants were planted on tailings with 4 treatments: tailings amended with 10 cm domestic refuse + complex NPK fertilizer (Treatment A); tailings amended with 10 cm domestic refuse (Treatment B) and tailings applied with complex NPK fertilizer (Treatment C) respectively, and tailings without any amendment used as control (Treatment D). The amended domestic refuse was covered at the surface of the tailings. For treatments A and C, NPK fertilizer (N:P:K = 15:15:15) was applied at a total amount of 225 kg N/hm^2 in three separate occasions in April, July and September, respectively. Four grasses, including *Vetiveria zizanioides*, *Paspalum notatum*, *Cynodon dactylon* and *Imperata cylindraca* var. *major*, were planted on each subplot respectively in April 1999. The first stage experiment showed that *V. zizanioides* growing on treatment A had coverage of 100% and dry weight yield of 2111 g/m^2 after six months growth (Table 2). Its biomass and metal tolerance index were significantly greater than the other three grasses growing under the same treatment, although *C. dactylon* had higher tolerance to Pb, Zn and Cu (Shu, 2002a; 2002b).

Table 2 Biomass of *Vetiveria zizanioides*, *Pasaplum notatum*, *Imperata cylindraca* var. *major* and *Cynodon dactylon* growing on tailings with different treatments (g/m^2, dry weight, mean ± *sd*, *n* = 4)

Treatment	A	B	C	D
V. zizanioides				
Shoot	1845	1354	975	418
Root	266	218	131	56
Total	2111	1571	1106	474
P. notatum				
Shoot	831	490	257	136
Root	259	174	109	44
Total	1090	665	366	180
I. cylindraca				
Shoot	645	427	180	121
Root	44	31	16	12
Total	689	458	196	132
C. dactylon				
Shoot	827	502	237	54
Root	71	59	24	7
Total	898	562	260	61

Notes: Treatment A: tailings amended with 10 cm domestic refuse + complex NPK fertilizer; Treatment B: tailings amended with 10 cm domestic refuse; Treatment C: tailings applied with complex NPK fertilizer; Treatment D: tailings without any amendment

1.2 Plant sample collection and chemical analysis

After 6 months' growth, a 0.25 m^2 quadrat (0.5 m × 0.5 m) was randomly placed on each subplot for sampling. Shoots of the plants were clipped at 5 mm above ground, then roots were excavated as completely as possible. Plant materials were washed with distilled water, oven-dried at 80℃ for 24 h, then milled and passed through 2 mm sieve. Approximately 0.4 g milled plants were placed into 100 ml digest tubes, added with 5 ml 16 mol/L HNO_3 and 1 ml 12 mol/L $HClO_4$, then digested at 180℃ until the samples were completely digested. The concentrations of Pb, Zn and Cu in the digestates were determined by AAS (Allen, 1989). The shoot/root metal concentration quotients (M_S/M_R) in different species are calculated by following equation:

$$M_S/M_R = \frac{\text{Metal concentration in shoot part of plant}}{\text{Metal concentration in root part of plant}}.$$

The total amounts of heavy metals accumulated in plants are calculated by the concentration of heavy metal in plant times the dry weight of plant.

1.3 Statistical analysis

Least significant difference (*LSD*) was used to compare heavy metal contents in the four grasses in the same treatment or the same species growing on different treatments.

2 Results

2.1 Heavy metal (Pb, Zn and Cu) concentrations in plants

The concentrations of Pb, Zn and Cu in shoots and roots of the four species are shown in Table 3. Metal concentrations exhibited similar trends among species, organs and treatments. For the four grasses, the metal concentrations in shoot and root of plants grown under different treatments were in the descending order of D > C > B > A. It was also found that roots always accumulated significantly higher metals than shoots ($p < 0.05$). In general, plants accumulated much higher Pb and Zn than Cu. *C. dactylon* always accumulated the highest concentrations of Pb, Zn and Cu, while *V. zizanioides* accumulated the least concentrations of these metals. For example, the Pb, Zn and Cu concentrations in shoots of *C. dactylon* grown on treatment D were 68.1, 175.6 and 17.4 mg/kg, while those in shoots of *V. zizanioides* grown on the same treatment were 26.1, 30.2 and 6.4 mg/kg, respectively (Table 3).

Table 3 Pb, Zn and Cu concentrations in shoot and root of *Vetiveria zizanioides*, *Pasaplum notatum*, *Imperata cylindraca* var. *major* and *Cynodon dactylon* growing on tailings with different treatments (mg/kg, dry weight, mean ± *sd*, *n* = 4)

Treatment		Shoot				Root			
		A	B	C	D	A	B	C	D
Pb	*V. zizanioides*	19.4 ± 2.6^{bc}	18.5 ± 3.1^{c}	24.4 ± 2.0^{ab}	26.1 ± 3.8^{a}	119.4 ± 27.9^{b}	102.1 ± 11.7^{b}	143.3 ± 25.7^{b}	183.7 ± 15.6^{a}
	P. notatum	25.8 ± 5.5^{c}	30.5 ± 3.0^{c}	36.8 ± 0.6^{b}	63.5 ± 11.6^{a}	68.4 ± 8.5^{c}	105.5 ± 27.3^{bc}	151.7 ± 31.9^{ab}	177.9 ± 20.1^{a}
	I. cylindraca	6.8 ± 1.8^{d}	10.8 ± 2.0^{c}	27.4 ± 5.7^{b}	59.4 ± 10.0^{a}	55.3 ± 8.4^{b}	75.0 ± 24.2^{b}	163.7 ± 8.7^{a}	236.2 ± 58.7^{a}
	C. dactylon	13.3 ± 1.0^{c}	14.4 ± 2.2^{c}	30.8 ± 5.3^{b}	68.1 ± 6.7^{a}	78.9 ± 13.0^{c}	144.5 ± 24.9^{b}	366.7 ± 59.9^{a}	458.8 ± 59.1^{a}
Zn	*V. zizanioides*	22.1 ± 2.9^{b}	26.3 ± 4.8^{ab}	23.9 ± 4.7^{ab}	30.2 ± 1.6^{a}	148.3 ± 34.0^{c}	175.4 ± 41.1^{bc}	150.8 ± 26.1^{c}	219.2 ± 38.1^{ab}
	P. notatum	44.0 ± 3.7^{b}	34.4 ± 2.9^{c}	44.6 ± 6.5^{b}	72.3 ± 3.4^{a}	109.0 ± 14.8^{c}	124.6 ± 26.6^{bc}	138.5 ± 14.6^{b}	222.1 ± 42.0^{a}
	I. cylindraca	38.7 ± 3.7^{c}	39.1 ± 2.2^{bc}	49.8 ± 6.9^{b}	73.7 ± 4.0^{a}	204.7 ± 9.6^{b}	225.6 ± 6.5^{ab}	206.0 ± 13.6^{b}	289.6 ± 34.2^{a}
	C. dactylon	76.0 ± 8.0^{c}	86.2 ± 7.0^{bc}	96.5 ± 7.2^{b}	175.6 ± 28.6^{a}	205.3 ± 62.4^{c}	283.2 ± 53.0^{bc}	365.2 ± 85.4^{ab}	494.5 ± 27.2^{a}
Cu	*V. zizanioides*	5.1 ± 0.6^{b}	4.9 ± 1.4^{b}	4.7 ± 0.6^{b}	6.4 ± 0.5^{a}	26.8 ± 11.5^{a}	34.4 ± 7.0^{a}	23.5 ± 3.4^{a}	29.5 ± 12.6^{a}
	P. notatum	7.3 ± 0.8^{b}	6.0 ± 1.1^{b}	10.2 ± 1.2^{a}	9.3 ± 1.2^{a}	36.0 ± 6.0^{c}	49.3 ± 3.5^{b}	67.3 ± 2.8^{a}	78.3 ± 6.6^{a}
	I. cylindraca	9.1 ± 1.5^{a}	7.1 ± 0.7^{a}	9.5 ± 1.8^{a}	9.2 ± 1.4^{a}	57.8 ± 4.2^{a}	67.0 ± 3.8^{a}	60.8 ± 5.7^{a}	66.4 ± 13.0^{a}
	C. dactylon	11.9 ± 1.0^{c}	12.8 ± 2.3^{bc}	13.8 ± 0.8^{b}	17.4 ± 1.5^{a}	29.2 ± 5.1^{c}	44.2 ± 7.2^{b}	50.3 ± 9.4^{ab}	64.6 ± 7.4^{a}

Note: Data in the same horizontal column and the same plant tissues with different letters indicate a significant difference at 5% level according to *LSD* test (Please refer to Table 2 for explanation of treatments)

2.2 Distribution of heavy metals in shoots and roots

For each plant species, the shoot/root metal concentration quotients (M_S/M_R) are listed in Table 4. For given species, the quotients showed slight variations between different treatments. However, the quotients seemed to be species and metal specific. For Pb and Zn, *P. notatum* had relatively higher quotients than other species; for Zn and Cu, *C. dactylon* had higher quotients. *V. zizanioides* and *I. cylindraca* always had stable and lower quotients compared with *C. dactylon* and *P. notatum*.

2.3 Total amounts of metals (Pb, Zn and Cu) accumulated in shoot of plants

The total amounts of Pb, Zn and Cu accumulated in shoots of the four species are illustrated in Fig.1. In general, plants grown under Treatment A accumulated the highest amounts of metals while those grown under Treatment D accumulated the least amounts. *V. zizanioides* accumulated the highest amount of Pb, *C. dactylon* the highest amount of Zn, while the two plants accumulated similar and highest amount of Cu. The levels of Pb, Zn and Cu accumulated in *P. notatum* and *I. cylindraca* var. *major* were constantly lower than those in *V. zizanioides* and *C. dactylon*.

Table 4 The shoot/root metal concentration quotients (M_S/M_R) of *Vetiveria zizanioides*, *Paspalum notatum*, *Imperata cylindraca* var. *major* and *Cynodon dactylon* growing on tailings with different treatments

Treatment		A	B	C	D
Pb	*V. zizanioides*	0.16	0.18	0.17	0.14
	P. notatum	0.38	0.29	0.24	0.36
	I. cylindraca	0.12	0.14	0.17	0.25
	C. dactylon	0.17	0.10	0.08	0.15
Zn	*V. zizanioides*	0.15	0.15	0.16	0.14
	P. notatum	0.40	0.28	0.32	0.33
	I. cylindraca	0.19	0.17	0.24	0.25
	C. dactylon	0.37	0.30	0.26	0.36
Cu	*V. zizanioides*	0.19	0.14	0.20	0.22
	P. notatum	0.11	0.08	0.28	0.19
	I. cylindraca	0.16	0.11	0.16	0.14
	C. dactylon	0.41	0.29	0.27	0.27

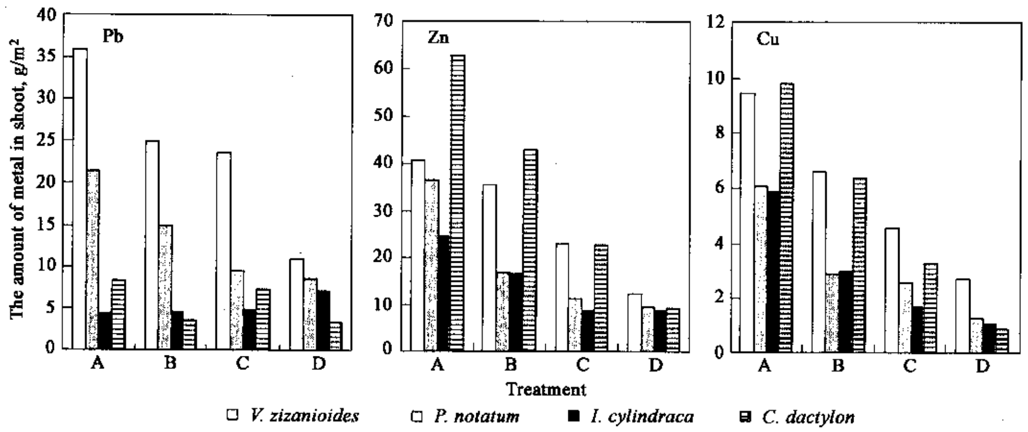

Fig.1 The amounts of Pb, Zn and Cu accumulated in shoots of *Vetveria zizanioides*, *Paspalum notatum*, *Imperata cylindraca* and *Cynodon dactylon* grown on tailings with different treatments(g/m^2, refer to Table 2 for explanation of treatments)

3 Discussion

Phytoremediation of mine wastelands such as mine tailings at least included two aspects: phytostabilisation and phytoextraction. (1) Phytostabilisation, in which metal tolerant plants were used to reduce the mobility of metals, thereby reducing risks of further environmental degradation, by leaching into the ground-water or by airborne(Bradshaw, 1980; Williamson, 1982); (2) phytoextraction, in which metal accumulating plants are used to extract metals from soils and concentrate them into the harvestable parts of roots or above-ground shoots (Baker, 1994; Salt, 1995; Vangronsveld, 1998; Khan, 2000). Therefore, the requirement for selection of plant species for phytostablization and phytoextraction is different. For phytostablization, plants should have higher tolerance to heavy metals, furthermore, also be poor translocators of metal contaminants to aboveground plant tissues for reducing the risk of metal entering food chain. Phytoextraction is based on the use of hyperaccumulators with exceptional metal-accumulating capacity, and the ability to accumulate metals in their shoots.

It was long recognized that the uptake of metals by plants were metal species and plant species depended (Adriano, 1986). Our present study also demonstrated that the strategy of heavy metal taken up by the four plants were different. In general, concentrations of Pb, Zn and Cu in shoots and roots of *V. zizanioides* were significantly less than other three species (Table 3), and the shoot/root metal concentration quotients(M_S/M_R, Table 4) for Pb, Zn and Cu were also lower than that of other three species, which indicated that *V. zizanioides* was an excluder of heavy metals. Firstly, roots of the species accumulated a low level of metals by avoidance or restriction of uptake, and secondly, shoots of the species accumulated many low concentrations of metals by restriction of transport. Judging from the metal concentrations in plant tissues, *V. zizanioides* was more suitable for phytostabilization of toxic mined lands than *P. notatum* and *C. dactylon*, which accumulated a relatively high level of metals in their shoots and roots. It was also noted that *I. cylindraca* var. *major* accumulated lower amounts of Pb, Zn and Cu than *C. dactylon* and *P. notatum*, and could also be considered for phytostabilization of tailings.

Two approaches have been proposed for phytoextraction of heavy metals, namely continuous or natural phytoextraction and chemically enhanced phytoextraction(Salt, 1998). The first is based on the use of natural hyperaccumulator plants with exceptional metal-accumulating capacity, however, many hyperaccumulator plants tend to be slow-growing and produce low biomass, and long period is needed to clean up a contaminated site(Lombi, 2001; Liu, 2003). Some metals such as Pb are largely immobile in soil and their extraction rate is limited by solubility and diffusion to root surface, and also limit the efficiency of continuous phytoextraction of metals from soils. Chemically enhanced phytoextraction has been developed to overcome these problems(Huang, 1997). This approach makes use of high-biomass, and high-metal tolerance crops that are induced to take up large amounts of metals when their mobility in soil is enhanced by chemical treatments, such as citric acid, EDTA, CDTA, DTPA, EGTA, EDDHA, and NTA.

In present study, although the metal contents in shoots of *V. zizanioides* were significantly lower than other three grasses, the total amount of metals (Pb, Zn and Cu) accumulated in shoots were the highest among the four tested plants(Fig. 1), due to its highest biomass. The amounts of metals accumulated in harvest parts of plant were the most important for phytoextraction of metal contaminated soils (Khan, 2000). *V. zizanioides* is a fast growing perennial species with a large biomass, and its shoot could be harvested 3—5 times per year (Xia, 1999). These characteristics enabled *V. zizanioides* remove metals from soils more effectively than other grass species in terms of phytoextraction. During the past ten years, great attention has been paid to the use of hyperaccumulators in phytoremediation of metal contaminated soils, but most hyperaccumulators of Pb, Zn, Cu and Cd have slow growth with a small biomass and therefore not suited for phytoextraction (Brooks, 1998). Perhaps, the addition of appropriate synthetic chelates, such as EDTA, DTPA, to enhance metal dispersion form soil to soil solution, improvement of metal uptake and increase transfer of metal from roots to shoots (Huang, 1997) of *V. zizanioides* could be explored.

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