



Comparison of Lead Tolerance and Accumulation Characteristics of Fourteen Herbaceous Plants

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ABSTRACT

To compare lead (Pb) tolerance and accumulation characteristics, 14 herbaceous plants were treated with different concentrations of lead ($0 \text{ mg}\cdot\text{kg}^{-1}$, $500 \text{ mg}\cdot\text{kg}^{-1}$, $1000 \text{ mg}\cdot\text{kg}^{-1}$, $1500 \text{ mg}\cdot\text{kg}^{-1}$, $2000 \text{ mg}\cdot\text{kg}^{-1}$) through an indoor pot experiment. Results indicated that the shoot dry weights (DWs), tolerance index (TI) and root tolerance index (RTI) of 14 herbaceous plants decreased with the increase of lead concentration. After comprehensive evaluation, *Campsis grandiflora*, *Polygonum lapathifolium*, *Lolium perenne*, and *Poa annua* were confirmed as tolerant plants to be cultivated in lead-zinc mining area. Moreover, shoots of the *Rudbeckia hirta* could effectively absorb the lead (Pb) with the bioconcentration factor (BCF) of 2.29. The translocation factor (TF) of 6 herbaceous plants were larger than 1.0. They are: *Polygonum lapathifolium* (3.04) > *Medicago sativa* (2.49) > *Rudbeckia hirta* (1.72) > *Talinum paniculatum* (1.44) > *Capsicum annuum* cv. 276 (1.36) > *Trifolium repens* (1.21). Finally, after integration the BCF, TF and repair potential indices, we found that *Rudbeckia hirta* had a good restoration potential and its lead cumulation in the shoot was the highest (2.576 mg per plant) when the concentration was up to $1000 \text{ mg}\cdot\text{kg}^{-1}$. Therefore, *Rudbeckia hirta* could be identified as a pioneer species of Pb-hyperaccumulator.

INTRODUCTION

Lead is the most prevalent heavy metal contaminant and a human carcinogen (Ozkan et al. 2005, Guo et al. 2016). Over the past 50 years, approximately 783 thousand tons of lead have been reported to be entering the environment, especially the soil (Cui et al. 2013). Soil lead pollution disrupts the normal function of the ecosystem and poses a huge risk to human health. Cleaning up contaminated soil is a major challenge in environmental engineering.

Despite soil remediation techniques are numerous, most of them require high cost, intensive labour and may cause irreversible soil disturbances (Bhargava et al. 2012). Phytoremediation can provide efficient, cost-effective, and environmentally friendly remediation methods for the decontamination of heavy metal-polluted soils. Therefore, screening hyperaccumulators and tolerant species is a key step in the phytoremediation of soils (Mahdavian et al. 2017). Nowadays scientists found that the hyper-accumulators are mostly small biomass plants, and the most suitable hyper-accumulators are often the dominant plants in contaminated areas (Gao et al. 2014, Qin et al. 2013). Some studies show that the families of Gramineae, Compositae, Leguminosae, Cruciferae, *Cyperus*, and Pteridaceae in metal mining areas are prominent in accumulation and translocation (Nie et al. 2004). *Sesbania drummondii* (Sahi et al. 2002, Sharma et al.

2004), *Hemidesmus indicus* (Chandra et al. 2005), *Arabis paniculata* (Tang et al. 2009), and *Plantago orbignyana* (Bech et al. 2011) have been successfully used for phytoremediation of lead-zinc mines in some areas (Srivastava et al. 2014, Li et al. 2015). However, only a few studies focus on lead tolerant plants in southwestern China. As the distribution of plant resources is regional and temporal, screening out hyperaccumulators of high biomass and strong resistance for phytoremediation become practical.

We have selected 14 plant species in the southwest of China based on previous research to carry out seed germination indoor and experiment on plants' response to lead stress. The objectives of the study included: (1) evaluate and compare the effect of different application rates of lead on the growth; and (2) finding more hyperaccumulators through the evaluation and comparison of lead tolerance and accumulation traits of 14 plant species.

MATERIALS AND METHODS

Pot Experiment

Seeds of fourteen plant species were collected from areas surrounding Xiangbao mountain (Lat. $26^{\circ}11' - 27^{\circ}22'N$, Long. $106^{\circ}07' - 107^{\circ}11'E$) in Guiyang city because there is a decades-year-old coal mine. The species sampled were

from 6 families and 14 genera, including *Cynodon dactylon* (L.) Pers., *Lolium perenne* L., *Poa annua* L., *Aster ageratoides* Turcz. var. *laticorymbus* (Vant.) Hand.-Mazz., *Rudbeckia hirta* L., *Cosmos sulphureus* Cav., *Gynura bicolor* L., *Capsicum annuum* cv.276, *Trifolium repens*, *Medicago sativa* L., *Calendula officinalis*, *Bidens pilosa* L., *Polygonum lapathifolium* L. and *Talinum paniculatum* (Jacq.) Gaertn. Seeds were germinated in an incubator at 25 ± 1 . After 7 days, seedlings were planted in individual pots. Soils were taken from the nearby farmland of Guizhou Normal University. Soil properties were as follows, pH: 7.36, organic matter: $31.24 \text{ g}\cdot\text{kg}^{-1}$, nitrogen (N): $213.52 \text{ mg}\cdot\text{kg}^{-1}$, phosphorus (P): $4.53 \text{ mg}\cdot\text{kg}^{-1}$ and potassium (K): $4.27 \text{ mg}\cdot\text{kg}^{-1}$. Fertilization was not necessary during the growth stage. Five seedlings were planted in a $15 \text{ cm} \times 18 \text{ cm}$ pot with 1 kg of soil, which contains lead (heavy metal) acetate [$\text{Pb}(\text{CH}_3\text{COO})_2 \cdot 3\text{H}_2\text{O}$; 0, 500, 1000, 1500 and $2000 \text{ mg}\cdot\text{kg}^{-1}$]. After 90 days of treatment, plant samples were drawn for observations.

Chemical Analysis

The plant samples were divided into roots and shoots before rinsed thoroughly with tap water and distilled water to remove adhering soil particles and sewage. Shoot height (the distance between the base of the tallest leaf and the tip of the lamina) and root length were measured. For dry weight determination, the cleaned samples were oven-dried at 105°C for 15 min and 70°C until constant weight. After their weight was recorded, dried plant samples were ground to pass a 1 mm mesh sieve, and wet digested in an $\text{HNO}_3/\text{HClO}_4$ (5:1) mixture (Wu et al. 2010). The samples were analysed on an atomic absorption spectrophotometer, model ZEEnit 700P. The accuracy of the method was verified by analysing certified reference material (GBW 07604 - Poplar leaves) from the National Centre for Standard Materials (Beijing China).

Data Processing

One-way analysis of variance (ANOVA) was used to test the effects of the different valuables on the measured factors. Duncan's multiple range test was used to compare means when a significant variation was highlighted by the analysis of variance. SPSS 22.0 software and Origin 9.1 software were used for data processing. Bioconcentration factor (BCF), translocation factor (TF), single index of lead tolerance factor (SILTF) and metal accumulation in the shoot of plants (MASP) were calculated based on the following formulas:

$$\text{BCF} = [\text{Metal}]_{\text{shoot}} / [\text{Metal}]_{\text{soil}} \quad \dots(1)$$

$$\text{TF} = [\text{Metal}]_{\text{shoot}} / [\text{Metal}]_{\text{root}} \quad \dots(2)$$

$$\text{SILTF} = [\text{Determination of indicators}]_{\text{average}} / [\text{Control determination}] \quad \dots(3)$$

$$\text{MASP} = [\text{Metal}]_{\text{shoot}} \times [\text{Biomass}]_{\text{shoot}} \quad \dots(4)$$

Evaluation Methods

A comprehensive evaluation of lead tolerance of plants was conducted with Membership Function Method and Standard Deviation Coefficient (Li et al. 2009, Li et al. 2015). Related formulas can be expressed as follows:

- (1) Using membership function to standardize the indicators:

$$\mu(X_{ij}) = (\bar{X}_{ij} - X_{j\min}) / (X_{j\max} - X_{j\min}) \quad \dots(5)$$

where $\mu(X_{ij})$ is the membership function of index j of class i ; \bar{X}_{ij} is the average measured value of index j of class i , $X_{j\min}$ and $X_{j\max}$ and denote the minimum and maximum values of index j .

- (2) Determination of weight:

$$V_{ij} = \sqrt{\sum_{i=1}^m [\mu(X_{ij}) - \mu(\bar{X}_{ij})]^2 / \mu(\bar{X}_{ij})} \quad \dots(6)$$

$$W_{ij} = V_{ij} / \sum_{j=1}^n V_{ij} \quad \dots(7)$$

Where, V_{ij} is the standard deviation coefficient of each index; W_{ij} is the weight coefficient of each index.

- (3) The calculation of D, the comprehensive evaluation value:

$$D = \sum_{j=1}^n [\mu(X_{ij}) \cdot W_{ij}] \quad \dots(8)$$

Where, D refers to the comprehensive evaluation value of all indices. It is used to measure the lead tolerance.

RESULTS

Tolerance Analysis of Pb

Different shoot dry weights (DWs) under changing lead concentrations are given in Table 1. Under the lead concentration of $500 \text{ mg}\cdot\text{kg}^{-1}$, DWs of 14 plant species do not change significantly, indicating that the plants were free of this level of poison. The shoot biomass of *Rudbeckia hirta*, *Cosmos sulphureus*, *Gynura bicolor*, *Capsicum annuum* cv.276 and *Bidens pilosa* decreased considerably under 1,000, 1,500 and $2,000 \text{ mg}\cdot\text{kg}^{-1}$ of lead concentrations. The shoot biomass of *Cynodon dactylon*, *Lolium perenne*, *Poa annua*, *Aster ageratoides* and *Trifolium repens* decreased substantially under 1,500 and $2,000 \text{ mg}\cdot\text{kg}^{-1}$ of lead concentrations. In contrast, no significant change was observed in the shoot biomass of *Medicago sativa*, *Calendula officinalis*, *Polygonum lapathifolium* and *Talinum paniculatum* under all lead concentrations.

The tolerance index (TI) is the ratio of shoot dry weights of the treatment group to the control group. TI greater than

Table 1: Shoot dry weights under different lead concentrations.

Species	Shoot dry weight under different lead concentrations (g·pot ⁻¹)				
	CK	500 mg·kg ⁻¹	1000 mg·kg ⁻¹	1500 mg·kg ⁻¹	2000 mg·kg ⁻¹
<i>Cynodon dactylon</i>	3.23 ± 0.08 ^a	3.06 ± 0.25 ^a	3.18 ± 0.21 ^a	2.17 ± 0.13 ^b	1.97 ± 0.27 ^b
<i>Lolium perenne</i>	2.73 ± 0.04 ^a	2.97 ± 0.16 ^a	2.31 ± 0.25 ^a	1.89 ± 0.32 ^b	1.39 ± 0.21 ^c
<i>Poa annua</i>	1.88 ± 0.45 ^a	1.82 ± 0.13 ^a	1.53 ± 0.12 ^a	0.89 ± 0.19 ^b	0.67 ± 0.10 ^b
<i>Aster ageratoides</i>	2.31 ± 0.25 ^a	2.07 ± 0.12 ^a	1.82 ± 0.13 ^b	0.98 ± 0.31 ^c	-
<i>Rudbeckia hirta</i>	3.97 ± 0.23 ^a	2.19 ± 0.04 ^{ab}	1.32 ± 0.12 ^b	1.61 ± 0.17 ^b	1.13 ± 0.18 ^c
<i>Cosmos sulphureus</i>	2.03 ± 0.02 ^a	1.86 ± 0.16 ^a	1.21 ± 0.06 ^b	1.09 ± 0.24 ^b	1.31 ± 0.07 ^b
<i>Gynura bicolor</i>	1.51 ± 0.09 ^a	1.36 ± 0.17 ^a	1.25 ± 0.03 ^{ab}	1.1 ± 0.26 ^b	0.96 ± 0.19 ^b
<i>Capsicum annuum cv.276</i>	3.02 ± 0.30 ^a	2.78 ± 0.35 ^a	2.36 ± 0.12 ^b	1.73 ± 0.11 ^c	-
<i>Trifolium repens</i>	1.98 ± 0.17 ^a	2.17 ± 0.36 ^a	1.85 ± 0.23 ^a	1.3 ± 0.15 ^b	0.95 ± 0.18 ^c
<i>Medicago sativa</i>	1.77 ± 0.16 ^a	1.58 ± 0.17 ^a	1.35 ± 0.03 ^a	1.27 ± 0.23 ^{ab}	1.15 ± 0.22 ^b
<i>Calendula officinalis</i>	2.56 ± 0.24 ^a	2.1 ± 0.31 ^a	1.83 ± 0.06 ^a	1.95 ± 0.28 ^a	1.69 ± 0.32 ^b
<i>Bidens pilosa</i>	5.53 ± 0.39 ^a	5.41 ± 0.32 ^a	6.33 ± 0.45 ^b	5.1 ± 0.27 ^a	4.17 ± 0.19 ^c
<i>Polygonum lapathifolium</i>	1.87 ± 0.03 ^a	1.85 ± 0.24 ^a	0.99 ± 0.15 ^b	1.32 ± 0.09 ^{ab}	1.27 ± 0.06 ^{ab}
<i>Talinum paniculatum</i>	0.61 ± 0.04 ^a	0.55 ± 0.12 ^a	0.6 ± 0.15 ^a	0.65 ± 0.11 ^a	0.63 ± 0.06 ^a

Note: Different letters indicate a significant difference between the different treatments of the same plant (mean±S.D, p < 0.05, n = 5).

0.5 indicates the plant grows well and has a good tolerance (Wu et al. 2017). The root tolerance index (RTI) is the ratio of the average root length of each treatment group to the control group. Generally, RTI greater than 0.9 indicates that the root growth of the plant is not significantly inhibited (Chehregani et al. 2009). Therefore, TI and RTI can be used as an important index of plant heavy metal tolerance.

According to the TI and RTI values under different lead concentration (Table 2), it is shown that *Cynodon dactylon*, *Lolium perenne*, *Cosmos sulphureus*, *Gynura bicolor*, *Calendula officinalis* and *Polygonum lapathifolium* had good tolerance for lead stress, and their TI and RTI values were greater than 0.5 and 0.9 in all treatments. As the lead concentration increased in the treatment group, both TI and RTI of these 14 plants gradually declined. TI of *Poa annua*, *Rudbeckia hirta* and *Trifolium repens* gradually decreased with the increase of lead concentrations to 0.5. But most of the plants showed significant damage at the lead concentration of 2000 mg·kg⁻¹, and the RTI values gradually began to be less than 0.9; furthermore, *Aster ageratoides*, *Capsicum annuum cv.276* were killed at the lead concentration of 2000 mg·kg⁻¹. When evaluated from DW, TI and RTI together, the *Polygonum lapathifolium* showed a strong tolerance and no obvious signs of damage under all treatments.

Characteristics of Lead Accumulation

The lead concentration in shoot and root of 14 herbaceous plants were analysed by variance analysis (Fig. 1). The aerial part results demonstrated that most plants were significantly affected by the increase in lead concentrations. However, the

lead concentration in shoots of *Rudbeckia hirta*, *Trifolium repens* and *Polygonum lapathifolium* increased and then stabilise before slowly declining. Therefore, the lead concentration in shoots did not maintain growth but slowed down or even declined when it exceeded the tolerance concentration, due to inhibition effects. At the initial concentration, no significant difference was observed on the lead concentration in shoots and roots. As lead concentrations increased, most roots have higher lead concentration than shoots.

Under the treatments of 1000 mg·kg⁻¹ and 1500 mg·kg⁻¹, no significant change of lead concentration in shoots was observed, except for *Cynodon dactylon*, *Cosmos sulphureus*, *Capsicum annuum cv.276*, and *Medicago sativa*. When the treatment exceeded 500 mg·kg⁻¹, *Cynodon dactylon*, *Aster ageratoides*, *Rudbeckia hirta*, *Gynura bicolor*, *Capsicum annuum cv.276* and *Trifolium repens* would have a substantially higher lead concentration in shoots compared with the control group. When the treatment exceeded 1000 mg·kg⁻¹, *Lolium perenne*, *Poa annua*, *Cosmos sulphureus*, *Medicago sativa* and *Calendula officinalis* would have a substantially higher lead concentration in shoots compared with the control group. It is not until the treatment exceeding 1500 mg·kg⁻¹ when *Bidens pilosa* and *Polygonum lapathifolium* started to show significantly higher lead concentration in shoots than that in the control. The lead accumulation in shoots of the *Rudbeckia hirta* and *Capsicum annuum cv.276* passed the critical value of 1000 mg·kg⁻¹ when they were treated with 1,000 mg·kg⁻¹ of lead concentration. It was noted that the lead concentration in the shoots of *Rudbeckia hirta* had already exceeded the critical value when it was under the

Table 2: Tolerance index and root tolerance index of 14 species of plants under different lead gradient.

Species	Tolerance index and Root tolerance index							
	500/mg·kg ⁻¹		1000/mg·kg ⁻¹		1500/mg·kg ⁻¹		2000/mg·kg ⁻¹	
	TI	RTI	TI	RTI	TI	RTI	TI	RTI
<i>Cynodon dactylon</i>	0.95	0.96	0.98	0.96	0.67	0.94	0.61	0.92
<i>Lolium perenne</i>	1.09	1	0.85	0.96	0.69	0.96	0.51	0.94
<i>Poa annua</i>	0.97	0.97	0.81	0.97	0.47	0.92	0.36	0.92
<i>Aster ageratoides</i>	0.90	0.98	0.79	0.98	0.42	0.93	-	-
<i>Rudbeckia hirta</i>	0.55	1	0.33	0.96	0.41	0.93	0.28	0.89
<i>Cosmos sulphureus</i>	0.92	1.02	0.60	1.05	0.54	0.96	0.65	0.91
<i>Gynura bicolor</i>	0.90	0.99	0.83	0.98	0.73	0.95	0.64	0.95
<i>Capsicum annuum cv.276</i>	0.92	1.02	0.78	1.04	0.57	1	-	-
<i>Trifolium repens</i>	1.10	1	0.93	1.02	0.66	0.93	0.48	0.86
<i>Medicago sativa</i>	0.89	0.93	0.76	0.98	0.72	0.95	0.65	0.88
<i>Calendula officinalis</i>	0.82	1	0.71	0.96	0.76	0.95	0.66	0.92
<i>Bidens pilosa</i>	0.98	1	1.14	1.03	0.92	1	0.75	0.86
<i>Polygonum lapathifolium</i>	0.99	0.99	0.53	0.99	0.71	0.99	0.68	0.97
<i>Talinum paniculatum</i>	0.90	1	0.98	0.94	1.07	0.91	1.03	0.85

500 mg·kg⁻¹ treatment, with the highest average lead concentration of 1783 mg·kg⁻¹.

The bioconcentration factor (BCF) and translocation factor (TF) of 14 plants under different concentrations are shown in Fig. 2. The results indicated that *Lolium perenne*, *Aster ageratoides*, *Bidens pilosa* and *Polygonum lapathifolium* showed no significant change in BCF under all treatments. Except for *Cynodon dactylon* and *Capsicum annuum cv.276*, BCF of other plants decreased with the increase of lead treatment. TF of *Poa annua* and *Aster ageratoides* showed no significant difference at all concentrations. However, TF of *Lolium perenne*, *Rudbeckia hirta*, *Cosmos sulphureus*, *Gynura bicolor* and *Trifolium repens* decreased with the increase of lead treatment.

Among 14 plants, only *Rudbeckia hirta*'s BCF was more than 1.0 at both the concentrations of 500 mg·kg⁻¹ and 1000 mg·kg⁻¹. When the *Capsicum annuum cv.276* was treated with 1500 mg·kg⁻¹ lead concentration, *Medicago sativa* was treated at 1000 mg·kg⁻¹, and *Rudbeckia hirta*, *Trifolium repens* and *Talinum paniculatum* were treated at 500 mg·kg⁻¹, their TFs were more than 1.0, indicating that lead transport capacity of plants was closely related to their tolerance.

Different capital letters meant a significant difference of translocation factors at 0.05 level among treatments

Comprehensive Evaluation of Lead Tolerance for 14 Plants

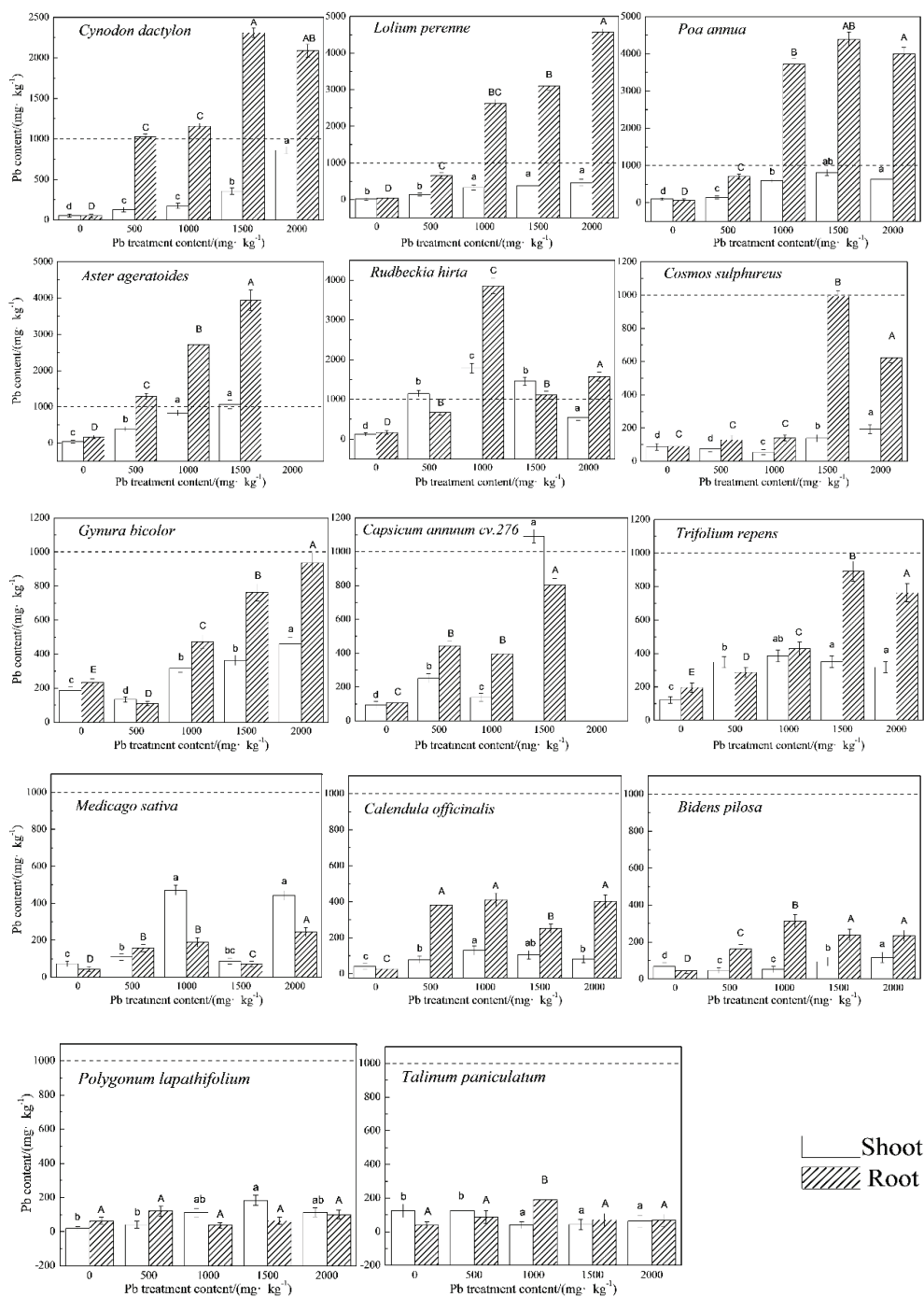
The lead tolerance of 14 plant species was sorted according to the standard deviation coefficient. Six indicators of plant

height, root length, shoot and root biomass and lead absorption were considered. Besides, lead tolerance coefficients, subordination and D value of comprehensive evaluation were calculated (Table 3). Based on the results, the comprehensive lead tolerance for 14 plants were as follows: *Trifolium repens* > *Polygonum lapathifolium* > *Lolium perenne* > *Poa annua* > *Aster ageratoides* > *Bidens pilosa* > *Cosmos sulphureus* > *Cynodon dactylon* > *Medicago sativa* > *Gynura bicolor* > *Capsicum annuum cv. 276* > *Rudbeckia hirta* > *Calendula officinalis* > *Talinum paniculatum*.

Among 14 herbaceous plants, *Rumex acetosa* showed a strong tolerance. Shi et al. (2007) used four indicators, including lead concentration in shoots, lead concentration in roots, RTI and BCF, to evaluate the lead tolerance of 3 gramineous forages. They reached the following conclusion of lead tolerance orders: *Lolium perenne* > *Poa annua* > *Cynodon dactylon*. According to the comprehensive evaluation results, the uptake of lead in the root systems of *Trifolium repens* and *Polygonum lapathifolium* was low at various concentrations. They showed a strong resistance to lead. In contrast, the advantages of *Lolium perenne* and *Poa annua* included higher capacity in lead absorption and adaptation. Therefore, these plants with exclusion and accumulation characteristics could be cultivated together as tolerant plants in the lead-zinc mining area.

DISCUSSION

Tolerance mechanisms for heavy metals vary in different plants. Even the same species of plants will react in differ-

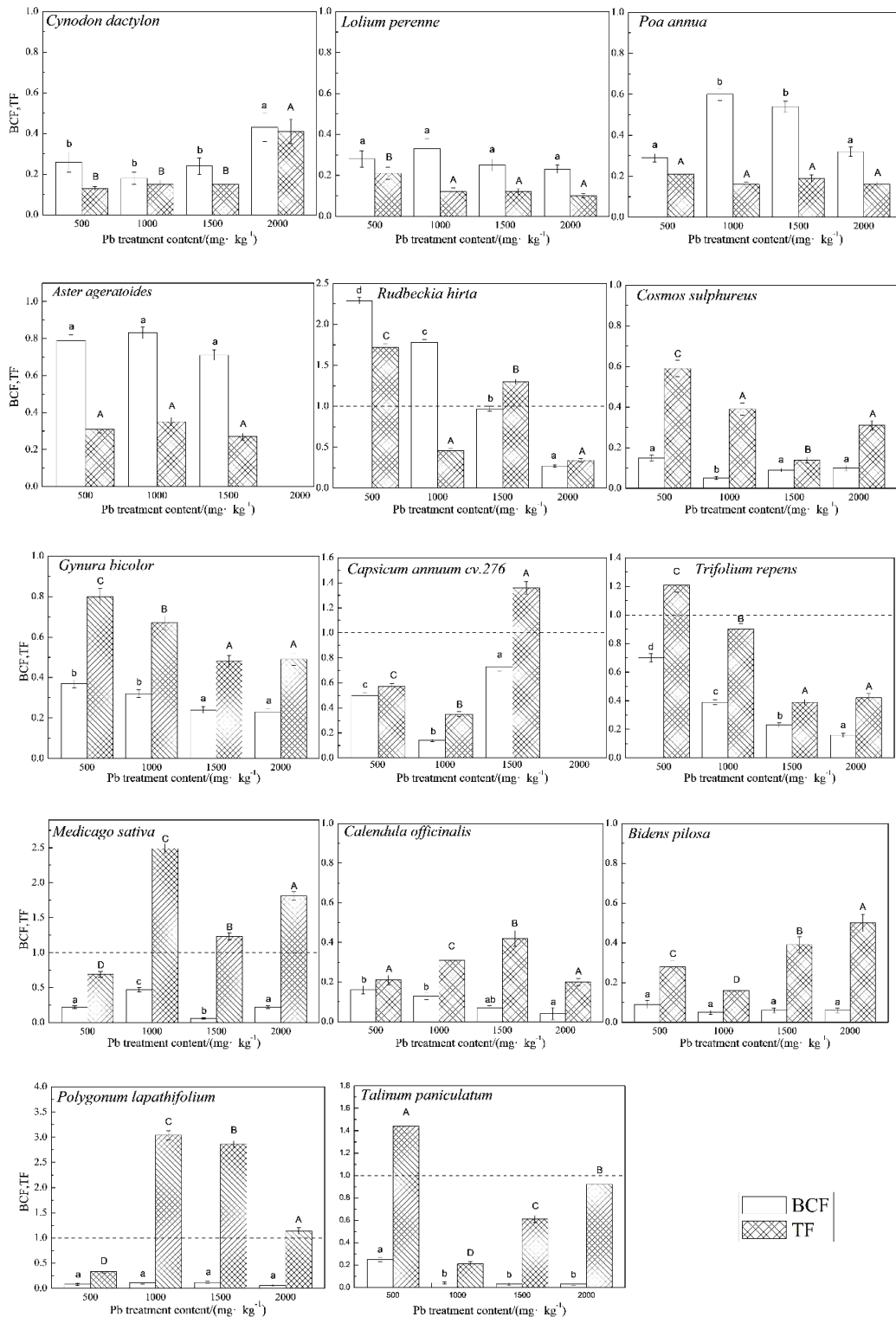


Note: Different small letters meant a significant difference of shoot lead content at 0.05 level among treatments; Different capital letters meant a significant difference of root lead content at 0.05 level among treatment

Fig. 1: Shoot and root lead accumulation under different lead concentrations.

ent ways at different concentrations of heavy metals. The biomass of *Trifolium repens* and *Lolium perenne* increased at 500 mg·kg⁻¹ of lead treatment, indicating that low lead concentration could promote growth. Liu et al. (2006) have

also found that lead concentrations below 200 mg·kg⁻¹ will promote the root growth of cucumber seedlings. It might be caused by organic acids excreted by the plants under the stimulus of heavy metals at low concentrations. Organic



Note: Different small letters meant a significant difference in shoot bioaccumulation factors at 0.05 level among treatments;

Fig. 2: Shoot bioaccumulation and translocation factors under different Pb concentrations.

Table 3: The single lead tolerances coefficient, subordination value and D value of comprehensive evaluation.

Plants	Resistance to each index lead coefficient						Membership function value						D values
	Plant height	Root length	Shoots bio-mass	Root bio-mass	Shoots lead absorption	Root lead absorption	$\mu(1)$	$\mu(2)$	$\mu(3)$	$\mu(4)$	$\mu(5)$	$\mu(6)$	
<i>Cynodon dactylon</i>	0.87	0.70	0.80	0.89	5.71	26.03	0.639	0.450	0.597	0.625	0.323	0.576	0.504
<i>Lolium perenne</i>	0.89	0.72	0.78	0.83	22.28	56.98	0.722	0.533	0.549	0.555	0.559	0.477	0.568
<i>Poa annua</i>	0.96	0.70	0.65	0.82	3.15	37.46	0.576	0.440	0.569	0.564	0.501	0.581	0.542
<i>Aster ageratoides</i>	0.85	0.70	0.76	0.97	3.26	3.21	0.574	0.560	0.442	0.582	0.411	0.486	0.486
<i>Rudbeckia hirta</i>	0.96	0.70	0.79	0.86	2.50	2.63	0.480	0.628	0.574	0.587	0.694	0.457	0.569
<i>Cosmos sulphureus</i>	0.86	0.70	0.39	0.54	8.48	8.99	0.625	0.600	0.322	0.331	0.535	0.355	0.450
<i>Gynura bicolor</i>	0.84	0.71	0.74	0.58	2.11	11.11	0.435	0.550	0.436	0.172	0.524	0.697	0.523
<i>Capsicum annuum</i> cv.276	0.95	0.73	0.67	1.18	1.27	4.39	0.591	0.880	0.264	0.273	0.398	0.338	0.411
<i>Trifolium repens</i>	1.00	0.73	0.95	0.87	1.12	4.53	0.516	0.671	0.527	0.619	0.410	0.573	0.528
<i>Medicago sativa</i>	0.94	0.72	0.77	0.60	1.56	2.16	0.400	0.500	0.502	0.459	0.487	0.476	0.474
<i>Calendula officinalis</i>	0.84	0.64	0.70	0.75	12.95	12.00	0.533	0.500	0.612	0.610	0.527	0.492	0.531
<i>Bidens pilosa</i>	0.85	0.74	0.73	0.90	3.52	3.18	0.364	0.600	0.534	0.585	0.495	0.661	0.557
<i>Polygonum lapathifolium</i>	1.26	0.68	0.76	0.74	4.17	4.05	0.698	0.417	0.576	0.521	0.300	0.474	0.454
<i>Talinum paniculatum</i>	0.85	0.68	1.00	1.24	0.63	2.33	0.584	0.600	0.580	0.356	0.462	0.343	0.444
Weights							0.10	0.08	0.08	0.12	0.30	0.32	

acids will stimulate the root growth of plants. However, when lead concentration becomes high, the plant biomass begins to decline due to growth inhibition. In this experiment, when the lead concentration of the treatment reached 2000 mg·kg⁻¹, the biomass of *Cynodon dactylon*, *Poa annua*, *Trifolium repens*, *Rudbeckia hirta*, *Calendula officinalis*, *Bidens pilosa* and *Gynura bicolor* dropped sharply to 63.68%, 43.70%, 53.54%, 32.22%, 64.34%, 68.87% and 51.97% of the biomass of CK, respectively. Studies show that when the soil contains a high concentration of lead, the uptake of lead in plants will reach saturation. As the lead absorbed by roots is difficult to transport to the shoots due to the root adsorption, passivation or precipitation in cells, the toxicity accumulate and inhibit the absorption of macroelements such as K, Ca, Mg, and trace elements, such as Fe, Cu, Zn. As a result, plants grow slowly or die from the lack of nutrients (Seregin et al. 2008, Akinci et al. 2010, Wang et al. 2010 and Liu et al. 2016).

A hyperaccumulator is a plant capable of growing in

soils with very high concentrations of metals, absorbing these metals through their roots, and concentrating extremely high levels of metals in their tissues (Brooks et al. 1997, Sun et al. 2008). According to Baker (1981), there are three indicators to define a Pb-hyperaccumulator: (a) the threshold value of metal accumulated in the shoots of the plant is up to 1000 mg·kg⁻¹; (b) both bioconcentration factor (BCF) index, the proportion in the shoot of the plant to the soil, and translocation factor (TF) index, the proportion of metal concentration in shoots to roots, are greater than 1; and (c) the hyperaccumulator should have great tolerance capability; the shoot biomass of a hyperaccumulator should not decrease significantly when the concentration of heavy metals reach the critical value. Among 14 plants tested in this experiment, only *Rudbeckia hirta* meet the three conditions. Current hyperaccumulator plants have disadvantages such as small biomass, slow growth and low translocation capacity of heavy metals. Therefore, the total accumulation of heavy metals at the part of the plant above ground is a key factor

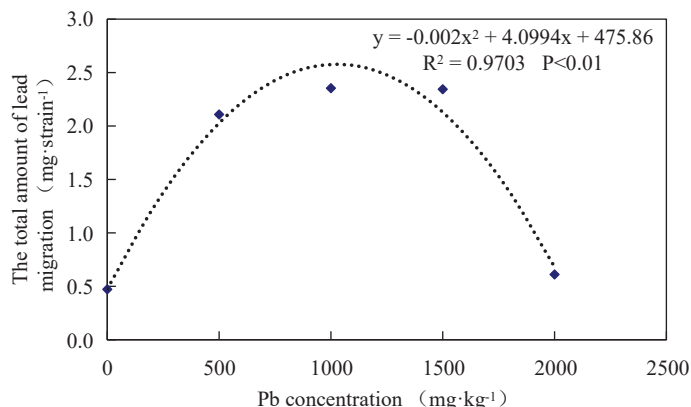


Fig. 3: Total lead concentration accumulated in shoots of *Rudbeckia hirta* versus different treatments.

to evaluate the potential of a hyperaccumulator (Monni et al. 2000, Lasat et al. 1988).

It can be seen from Fig. 3 that the total amount of lead in shoots of the *Rudbeckia hirta* is as per the binomial fitting curve and $R^2 = 0.9703$. According to the fitting curve, there was a sharp increase in the total accumulation of lead in shoots of the *Rudbeckia hirta* and then the percentage gradually went down. In the vicinity of $1000 \text{ mg}\cdot\text{kg}^{-1}$, the cumulative total reached a saturated state, and after reaching the critical value, the cumulative amount begins to decrease. According to the fitting equation, when the soil lead concentration was $1025 \text{ mg}\cdot\text{kg}^{-1}$, the lead concentrations in shoots of the *Rudbeckia hirta* was the highest at $2.576 \text{ mg}\cdot\text{strain}^{-1}$, which was significantly higher than that of the control. Wang et al. (2005) have studied the total concentration of lead in *Bidens maximowicziana* Oett. at the same concentration. They found that the total amount of lead in the ground above was $0.3262 \text{ mg}\cdot\text{strain}^{-1}$. Our experiment result on *Rudbeckia hirta* is 7.8 times than that of *Bidens maximowicziana* Oett, indicating that the tolerance and absorption of *Rudbeckia hirta* are better than that of *Bidens maximowicziana* Oett.

This study first proposes *Rudbeckia hirta* as a pioneer hyperaccumulator. We need to further confirm its function in the restoration of contaminated land and reduction of heavy metals' impacts on human health. Further research needs to be done for better understanding of the tolerance mechanism and restoration ability of *Rudbeckia hirta*.

CONCLUSIONS

Among fourteen herbaceous plants in this experiment, the lead tolerance ability of *Trifolium repens* was the strongest and *Calendula officinalis* was the worst; the bioconcentration factor of *Rudbeckia hirta* was above 2.29, indicating that it can effectively absorb lead in soil; the translocation factor

of the six plants were greater than 1.0, and their transport capacity was: *Polygonum lapathifolium* (3.04) > *Medicago sativa* (2.49) > *Rudbeckia hirta* (1.72) > *Talinum paniculatum* (1.44) > *Capsicum annuum* cv.276 (1.36) > *Trifolium repens* (1.21).

Through the comprehensive evaluation of lead tolerance, *Trifolium repens*, *Polygonum lapathifolium*, *Lolium perenne*, *Poa annua* can be used as a tolerant plant to cultivate in the lead-zinc mining district. *Rudbeckia hirta* satisfies the requirements of the hyperaccumulator plant, which is a pioneer species of Pb-hyperaccumulator plant. According to the repair potential index, *Rudbeckia hirta* has the best remediation potential. When the soil lead concentration was about $1000 \text{ mg}\cdot\text{kg}^{-1}$, the total amount of lead accumulation in shoots was $2.576 \text{ mg}\cdot\text{strain}^{-1}$.

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