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Pb, Zn and Cu Tolerance and Accumulation in Populations of *Paspalum distichum* *

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Abstract: An investigation was conducted into the multi-element tolerance of the grass *Paspalum distichum* growing on Fankou (FK) and Lechang (LC) Pb/Zn mine tailings at Shaoguan, Guangdong Province. Population of the grass growing on uncontaminated pasture was used as a control. Two tailings sites contained higher levels of Pb, Zn and Cu, as a consequence, the heavy metal contents (Pb, Zn and Cu) in the plant tissues collected from FK and LC tailings sites were significantly higher than those of plant samples collected from the uncontaminated pasture. Root elongation test indicated that populations of FK and LC had significantly higher index of tolerance and ED_{50} to the three metals than the control population, indicating that the populations of FK and LC have evolved to Pb, Zn and Cu tolerant ecotypes.

Keywords: heavy metal; accumulation; tolerance; *Paspalum distichum*

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The vegetation growing on tailings is subjected to a harsh environment especially the various adverse edaphic factors including the elevated heavy metals, therefore, the natural colonizers of mine wastes often had evolved metal tolerance. An economic and effective technique with potential for reclaiming these toxic wastes is to make use of metal tolerant plant. Various tolerant species of grasses and legumes are used for initial coverage on disturbed land[1]. There has been considerable interest in finding native tolerant species, which are able to colonize metal-enriched soils for wasteland reclamation[2]. Ecological survey of natural colonization of plants on five Pb/Zn mine tailings in South China indicated that *Paspalum distichum* was one of five dominant species of the natural vegetation of the tailings, suggesting that this grass may have evolved heavy metal tolerance and therefore has some potential use in revegetation of Pb/Zn tailings. Therefore, the objectives of the present experiments are to study: ① lead, zinc and copper tolerance in different populations of the grass, ② accumulation of Pb, Zn and Cu in different populations of the grass.

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1 Materials and Methods

1.1 Sample Collection

Samples of *P. distichum* were collected from the waterlogged areas of Fankou (FK) and Lechang (LC) lead/zinc mine tailings respectively. The control population was sampled from an uncontaminated pasture at the campus of Zhongshan University (ZS), Guangzhou, located approximately 200 km from LC and FK tailings. Soil or tailings samples were also collected from rhizosphere of *P. distichum* at the three study sites.

1.2 Soil and plant analyses

The soil samples were air-dried and pass through a 2 mm mesh sieve. They were then determined for pH, electrical conductivity (EC), total and DTPA extractable concentrations of Pb, Zn and Cu. The contents of Pb, Zn and Cu in plants were analyzed after being digested with HNO_3.

1.3 Root elongation test

Root elongation of tillers of the grasses collected from three different sites was tested using the method described by Wilkins[3]. The working concentrations of the metals were 5, 10, 20, 30 and 40 $mg \cdot L^{-1}$ for Pb; 2.5, 5, 10, 20 and 30 $mg \cdot L^{-1}$ for Zn; 0.25, 0.50, 1 and 2 $mg \cdot L^{-1}$ for Cu. The tillers were kept under natural 16 h/8 h cycle at temperature of (25±5) ℃. The solutions were changed every three days. The root lengths of the tillers were measured at the end of the experiment of 14 d. Culture solution containing 0.5 $g \cdot L^{-1}$ $Ca(NO_3)_2$ without metal was used as control and there were triplicates for each treatment. Tolerant index was calculated by following equation:

$$\text{Tolerant index (TI)} = \frac{\text{Mean length of the longest root in solution with metal}}{\text{Mean length of the longest root in solution without metal}} \times 100\%$$

2 Results and Discussion

2.1 Soil analyses

The pH value of LC tailings collected from the waterlogged area was 7.38 compared with pH 5.32 of the normal soil from the pasture of Zhongshan University. The total and extractable contents of Pb, Zn and Cu were higher in both tailings sites than the control site ($P < 0.05$). EC values of two tailings sites were also one magnitude higher than that of the normal soil. Judging from the levels of these metals in the tailings, it is assumed that plants growing there must be able to withstand the toxic effect of the metals (Tab.1).

Tab.1 The pH, EC and metal contents (mean±sd, n=4, $mg \cdot kg^{-1}$) of FK tailings, LC tailings and the uncontaminated soil (ZS soil)1)

		FK tailings	LC tailings	Uncontaminated soil
pH		7.25±0.24 a	7.38±0.61 a	5.32±0.36 b
EC/($dS \cdot m^{-1}$)		2.13±0.18 a	2.36±0.16 a	0.56±0.09 b
Pb	Total	5686±621 a	2335±1233 b	119.16±24.50 c
	Extractable	219.43±47.29 a	269.49±55.94 a	6.82±1.32 b
Zn	Total	3009±78 b	7607±2088 a	79.03±20.83 c
	Extractable	257.93±78.26 a	325.56±135.14 a	3.79±1.07 b
Cu	Total	99±6.4 b	190.89±33.47 a	2.55±0.56 c
	Extractable	10.15±2.59 a	7.38±3.26 a	1.10±0.09 b

1) Different letters indicate a significant difference at 5% level according to LSD test

2.2 Metal contents in plants

Plant samples collected from FK and LC tailings contained significantly higher metal concentrations in their tissues than that collected from ZS pasture (Tab. 2, $P < 0.05$). Higher metal contents were also observed in the root than the shoot of all the plants collected from different sites, except the Cu concentrations in *P. distichum* collected from FK tailings. The roots of *P. distichum* growing on waterlogged area of LC tailings accumulated the highest amount of Pb and Zn. It was a common phenomenon that higher metal concentrations were observed in the root than the shoot. The results also indicated that there was a close relationship between the metal contents in plant tissues and those in soil.

Tab. 2 The contents of Pb, Zn and Cu of three populations of *Paspalum distichum* collected from Fankou, Lechang tailings and pasture at Zhongshan University 1)

	Shoot			Root		
	FK	LC	Soil	FK	LC	Soil
Pb	79.08±23.63 c	705.5±131.1 b	15.24±2.45 c	610.4±142.8 b	1899±173 a	26.08±3.14 c
Zn	116.22±30.10 c	816.58±55.44 b	78.38±14.79 c	663.9±267.9 b	1864±301 a	121.54±13.90 c
Cu	32.27±14.43 a	8.79±1.83 c	13.35±3.40 bc	21.48±2.90 b	21.38±2.29 b	20.30±6.69 b

1) mean±sd, $n=4$, dry weight, $mg \cdot kg^{-1}$

Different letters indicate a significant difference at 5% level according to LSD test

2.3 Root test

2.3.1 Root elongation

It was obvious that plants collected from the uncontaminated area were more sensitive to the three metals and thus had relatively shorter roots than those collected from contaminated areas (Fig. 1). When only $Ca(NO_3)_2$ was added into the solution, the root length of the normal (soil) population was significantly longer than that of the two corresponding populations from FK and LC tailings. However, when more than 20 $mg \cdot L^{-1}$ Pb, 10 $mg \cdot L^{-1}$ Zn and 0.25 $mg \cdot L^{-1}$ were added to $Ca(NO_3)_2$ solution respectively, the root lengths of the normal (soil) population were severely inhibited. Distinctive population differences were found when the tillers subjected to higher concentrations of the three metals.

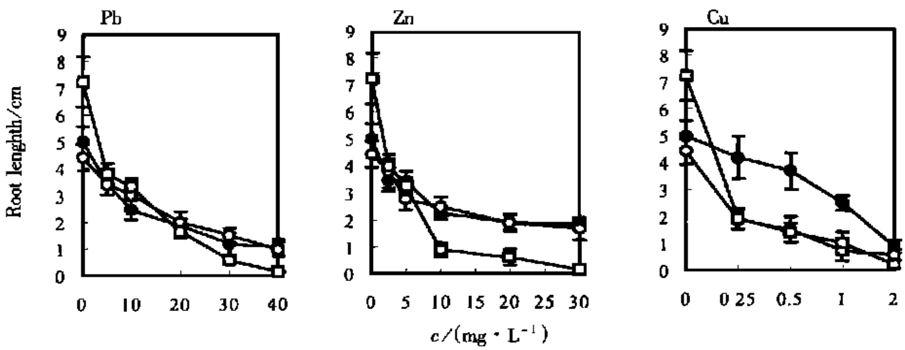

Fig 1 Root growth of different populations of *P. distichum* at different concentrations of Pb, Zn and Cu for a period of 14 d
($n=8$, ○ LC population, ● FK population, □ Normal population)

2.3.2 Index of tolerance

Table 3 lists the index of tolerance (TI) of different populations of *P. distichum* at various concentrations of Pb, Zn and Cu. In all the metal concentrations used, TIs of FK and LC populations were significantly higher than the normal population. In general, LC population was less sensitive to Pb and Zn than FK population, while FK population was the less sensitive to Cu. TIs of FK population in all Cu treatments were significantly greater than those of LC population ($P < 0.05$). It might be due to the rather high concentration of Cu of the FK tailings (Tab. 1).

Because different root growing performance in different metal concentrations, TI value was concentration dependent. The present study indicated that 30 mg·L^{-1} Pb, 10 mg·L^{-1} Zn and 0.25 ~ 0.50 mg·L^{-1} Cu were suitable for measuring the metal tolerance of different populations of *P. distichum* (Tab. 3).

Tab. 3 The values of index of tolerance (TI) of different populations of *Paspalum distichum* subjected to different concentrations of Pb, Zn and Cu (mg·L^{-1})[1)]

	Pb				Zn				Cu		
	FK	LC	Soil		FK	LC	Soil		FK	LC	Soil
5	69.5 b	91.5 a	53.0 c	2.5	69.5 b	86.1 a	55.4 c	0.25	84.2 a	43.2 b	27.1 c
10	50.1 b	69.0 a	46.0 c	5	68.5 a	62.7 a	45.3 b	0.5	74.1 a	34.6 b	19.7 c
20	38.2 a	45.7 a	22.9 b	10	45.2 b	56.7 a	12.1 c	1.0	50.5 a	22.6 b	18.7 c
30	24.4 b	35.0 a	8.2 c	20	38.6 a	43.4 a	8.3 b	2.0	18.2 a	13.5 b	2.9 c
40	21.3 a	22.6a	2.3 b	30	37.2 a	38.1 a	2.1 b				

1) Different letters in the same metal and row indicate a significant difference at 5% level according to LSD test

2.3.3 ED_{50} of the three populations

The 14 d ED_{50} values of soil, LC and FK populations were 7.8, 22 and 12.5 mg·L^{-1} for Pb; 4.2, 17.8 and 9.5 mg·L^{-1} for Zn, 0.19, 0.22 and 1.1 mg·L^{-1} for Cu, respectively (Tab. 4). The

Tab. 4 The concentrations of (mg·L^{-1}) of Pb, Zn and Cu in solutions which produced 50% of normal root growth in *Paspalum distichum* compare with studies of other species

	Species	ED_{50}	Growing Sites	References
Pb	*Paspalum distichum* (FK)	12.5	Fankou Pb/Zn tailings	Present study
	Paspalum distichum (LC)	22.0	Lechang Pb/Zn tailings	Present study
	Paspalum distichum (Soil)	7.8	Normal soil	Present study
	Phragmites australis	15.5 ~ 20	Fankou Pb/Zn tailings	Ye et al., 1997[4]
	Typha latifolia	8.4	Fankou Pb/Zn tailings	Ye et al., 1997[4]
Zn	*Paspalum distichum* (FK)	9.5	Fankou Pb/Zn tailings	Present study
	Paspalum distichum (LC)	17.8	Lechang Pb/Zn tailings	Present study
	Paspalum distichum (Soil)	4.2	Normal soil	Present study
	Phragmites australis	2.2 ~ 4.3	Fankou Pb/Zn tailings	Ye et al., 1997[4]
	Typha latifolia	3.3	Fankou Pb/Zn tailings	Ye et al., 1997[4]
Cu	*Paspalum distichum* (FK)	1.1	Fankou Pb/Zn tailings	Present study
	Paspalum distichum (LC)	0.22	Lechang Pb/Zn tailings	Present study
	Paspalum distichum (Soil)	0.19	Normal soil	Present study
	Festuca rubra	0.21	(Tolerant genotypes)	Karataglis, 1982[5]
	Agrostis tenuis	0.6	Cu mine	Jowett, 1958[6]

results indicated that *P. distichum* collected from FK tailings had the highest tolerance to Cu while LC population had the highest tolerance to Pb and Zn among all the tested populations. Tab. 4 compares the ED_{50} values with studies of other species. Although statistic analysis could not be used to compare the difference of ED_{50} values of difference species, it was obvious that *P. distichum* was one of the most tolerant ecotypes, because *P. distichum* showed more tolerant to Pb, Zn and Cu than these known tolerant genotypes such as *Agrostis tenuis*, *Festuca rubra* and *Typha latifolia* (Tab. 4).

The overall results reveal that FK and LC populations of *P. distichum* are better able to withstand the rather high concentrations of the three metals than ZS populations of the same species, suggesting that the co-tolerant (multi-tolerant) ecotypes have evolved in these two populations. Because the tailings often contain more than one metal at toxic level, metal co—tolerant genotypes have a higher application value.

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双穗雀稗种群对铅、锌和铜的积累和耐性研究

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摘　要：以正常草地的双穗雀稗（*Paspalum distichum*）种群作对照，研究了生长于广东韶关凡口和乐昌铅锌尾矿的 2 个双穗雀稗种群的重金属耐性．凡口和乐昌铅锌尾矿都含有极高浓度的总量和有效态的重金属（Pb，Zn 和 Cu），与之相应，采自两个铅锌尾矿的植物体内的铅、锌和铜的含量显著高于对照点．根伸长的耐性实验表明，凡口和乐昌铅锌尾矿的 2 个双穗雀稗种群对 Pb，Zn 和 Cu 的耐性指数和 ED_{50} 值均显著高于对照种群，这两个种群已形成对 Pb，Zn 和 Cu 的多重耐性．

关键词：重金属；积累；耐性；双穗雀稗

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