The Difference of Lead Accumulation and Transport in Different Ecotypes of Miscanthus floridulus

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Article

The Difference of Lead Accumulation and Transport in Different Ecotypes of *Miscanthus floridulus*

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Abstract: *Miscanthus floridulus* is a plant with a high biomass and heavy metal tolerance, which is a good candidate for phytoremediation. Pot experiments were conducted to compare the growth response, Pb enrichment ability, and the effect on Pb speciation of two ecotypes of *M. floridulus* from the Dabaoshan Mining Area and the non-mining area of Boluo County, Huizhou, in soils with different Pb contents. The results showed that two ecotypes of *M. floridulus* had different growth responses to Pb concentrations in soil. Under a low concentration of Pb (100 mg·kg^{-1}) treatment, the aboveground biomass of the non-mining area plant ecotype was significantly affected, while the plants with the mining area ecotype were not significantly affected. When the concentration of Pb increased, the aboveground biomass of the non-mining ecotype was 30.2–41.1% of the control, while that of the mining ecotype was 57.8–65.0% of the control. The root biomass of the non-mining ecotype decreased with the increase of treatment concentration, accounting for 57.8–64.2% of the control, while that of the mining ecotype increased significantly, accounting for 119.5–138.6% of the control. The Pb content in the shoots and roots of the mining ecotype *M. floridulus* increased rapidly with the increase of the Pb treatment concentration in the soil, and the increase in speed was obviously faster than that of the non-mining ecotype. The total amount of Pb accumulated in the roots of the ecotype from the mining area was much greater than that of the ecotype from the non-mining area, and increased significantly with the increase of Pb concentration in the soil ($p < 0.05$). With the aggravation of Pb stress, the transfer coefficient and tolerance index of the two ecotypes decreased by different degrees. The transfer coefficient and tolerance index of the mining ecotype were significantly higher than those of the non-mining ecotype. Pearson correlation analysis showed that root biomass was positively correlated with shoot biomass, and shoot biomass was negatively correlated with Pb content in both root and shoot, indicating that Pb accumulation in root and shoot was toxic to plants and inhibited the growth of *M. floridulus*. The mining ecotypes showed stronger tolerance to and enrichment of Pb.

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1. Introduction

Heavy metal contamination in soil or sediment can constitute a selective driving force for plant evolution [1]. Environmental pollution brings a new living environment to plants. In this case, some plant ecotypes undergo a process of selection and ecotype reconstruction. Ecotypes have undergone major changes in physiological, biochemical, and genetic characteristics, resulting in gradient groups and ecotypes [1,2]. Due to its long-term survival in a polluted environment, *M. floridulus* in metal mining areas may have undergone resistance evolution adapted to the polluted environment and formed resistant ecotypes [3–5]. Studying the physiological and ecological differences between resistant ecotypes and sensitive ecotypes is an important way to understand the mechanism of plant resistance and increase the efficiency of technology for phytoremediation of heavy metal pollution [6,7]. Previous studies have shown that non-mining ecotypes of *Elsholtzia Splenden* are more seriously harmed by peroxidation under copper stress than that of mining ecotypes [8,9]. Similarly, the tolerance of *Pteris vittata* L. to lead was higher in mining ecotypes than in non-mining ecotypes [10]. The differences in the resistance mechanisms of different ecotypes of plants to heavy metal stress may be caused by the variations in genetic mechanisms, the role of the antioxidant enzyme system, heavy metal avoidance, cell regionalization and chelation detoxification of heavy metals, or other factors, but there is no consistent conclusion at present [11–13].

Miscanthus floridulus is a perennial herb plant of the *Miscanthus*, which is widely distributed in southern China and has strong adaptability [14,15]. It is an ideal plant for phytoremediation due to its rapid growth, large biomass and well-developed root system, which can accumulate heavy metals and reduce their mobility and availability [16,17]. There have been some reports on the relationship between *M. floridulus* and heavy metals. Some scholars have investigated plants in Diaojiang Basin of the Guangxi and Anhui nonferrous metal mining area, and found the *M. floridulus* to have a large capacity for absorption of manganese, nickel, arsenic, and zinc [18]. Sun Jian et al. investigated heavy metal pollution in soil and plants of a lead–zinc mining area in Chenzhou, Hunan Province, and found that *M. floridulus* has a large absorption and transport capacity for lead and zinc [19]. Li Qin et al. found that *M. floridulus* has a strong tolerance for Cu, Zn, Pb, and Cd, and that the order of accumulation of the four heavy metals is Cd < Cu < Pb = Zn [5]. Because of its long-term survival in the polluted environment, *M. floridulus* from the metal mining area may have undergone resistance evolution, forming a resistant ecotype [20]. Studying the physiological and ecological differences between the resistant ecotype and the sensitive ecotype is an important way to reveal the mechanism of plant resistance and allow the phytoremediation technology of heavy metal pollution to be widely applied [21–23].

Dabaoshan mine, located at the junction of Qujiang County and Wengyuan County, Shaoguan City, Guangdong Province, is a large iron polymetallic sulfide-associated deposit [24]. Fu Shanming et al. [24] analyzed the total amount and morphology of heavy metals in the soil profile of Dabaoshan Mountain and showed that Pb, Zn, Cu, and Cd had different degrees of pollution, of which Cd and Pb were the most serious. Qin Jianqiao et al. [25,26] conducted a large number of studies on vegetation restoration and biocommunity reconstruction, soil enzyme activities, and plant growth in Dabaoshan's tailings pond. The results showed that *M. floridulus* is a suitable grass pioneer species for vegetation restoration construction of the metal mining area, and can be conducive to accelerating the ecological restoration process of abandoned tailings land. Previous studies have shown that *M. floridulus* can grow normally in the seriously polluted soil of the Dabaoshan mining area, its roots can absorb a large amount of Pb, and its biomass is large, so it is a good candidate for phytoremediation [27,28]. This study used the pot experiment method to compare the growth reaction, Pb enrichment ability, and effect on chemical forms of Pb in soil of two ecotypes of *M. floridulus*, one each from the Dabaoshan mining area in Shaoguan and a non-mining area in Boluo County, Huizhou, in soil with a different Pb content in order to further explore the Pb tolerance ability and mechanism of *M. floridulus*. This research also provides more theoretical basis for the practice of plant reclamation in metal mining areas in the future.

2. Materials and Methods

2.1. Test Materials

The experimental material was *M. floridulus* seedlings. New seedlings of the current year were collected in early March. The mining ecotype *M. floridulus* was collected from Dabaoshan Mining area, Shaoguan, Guangdong (24°33′36.6″ N, 113°43′14.0″ E), while the non-mining ecotype *M. floridulus* was collected from the hills and mountains of Boluo

County, Huizhou, Guangdong (23°08′57.8″ N, 114°21′10.0″ E), in the same subtropical monsoon climate zone (Figure 1). The two kinds of *M. floridulus* from different origins can be distinguished by the appearance of their leaves. The leaves of *M. floridulus* from mining areas have large edges with deep serrations, while the leaves from non-mining areas have relatively small edges with shallow serrations. The sodden seedlings were taken back to the greenhouse, their roots were washed with tap water, and they were set aside.

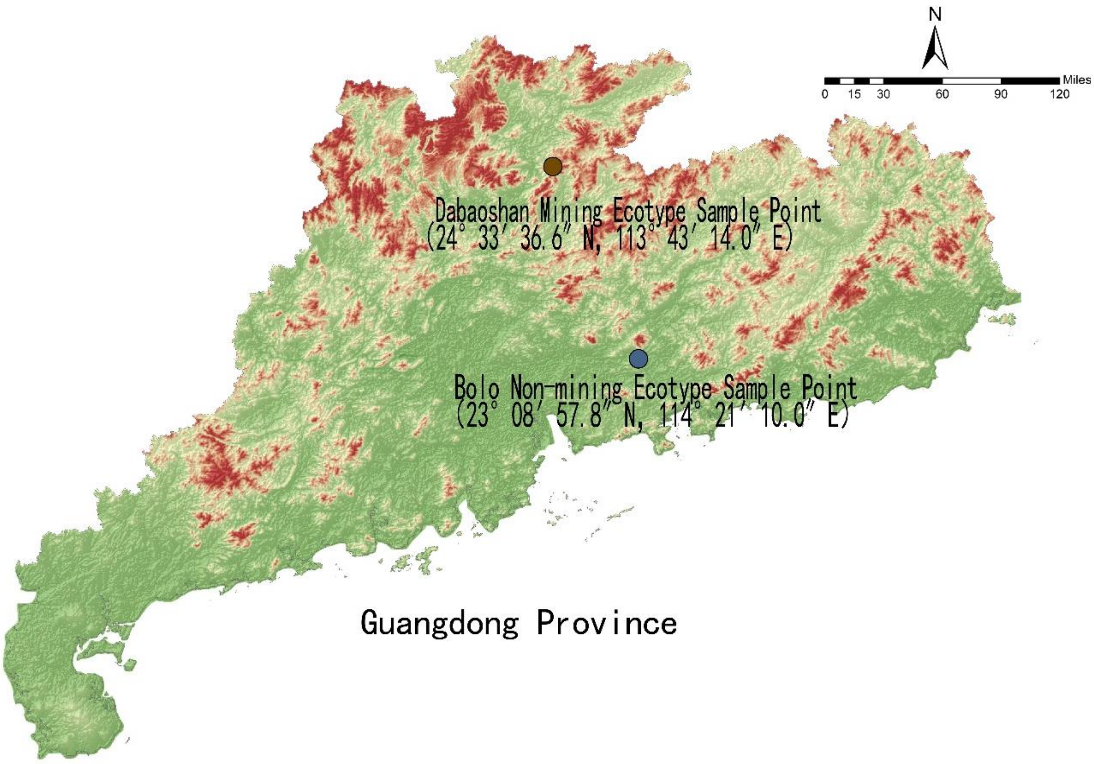

Figure 1. Location of sampling points.

2.2. Test Soil

The test soil was collected from the unpolluted *M. floridulus* grassland (24°29′56.1″ N; 113°49′19.6″ E) in Suoyikeng, Xinjiang town, Shaoguan City, Guangdong. The air-dried and 2 mm screened soil was put into plastic pots, with 1.2 kg per pot. The base fertilizer standard was 100 mg N kg^{-1} dry soil, added with H_2NCONH_2, and 80 mg P kg^{-1} and 100 mg K kg^{-1} were added as KH_2PO_4. This was mixed well and set aside.

The total amounts of Zn, Pb, Cu, and Cd in the soil were determined by digestion using HCl, HF, and perchloric acid, and then by ICP-OES (Optima5300DV, Perkin-Elmer, Sheldon, CT, USA) [29]. The basic chemical properties of the soil were determined using soil agrochemical analysis methods [30]: the soil pH value was measured using a pH meter after the water and soil were mixed at 2.5:1; organic matter was determined using the potassium dichromate volumetric method; alkaline hydrolysis N was determined using the alkaline hydrolysis diffusion method; after extracting soil samples with 0.5 mol^{-1} sodium bicarbonate were extracted, the available P was measured using molybdenum blue colorimetry.

The basic chemical properties and heavy metal contents of the soil at the plant sample collection site and the test soil are shown in Table 1.

Table 1. The basic chemical properties of the soil at the sampling points of two ecotypes of *M. floridulus* and the soil for the pot experiment.

Soil Sample Point	Organic C ($mg\cdot kg^{-1}$)	Available P ($mg\cdot kg^{-1}$)	Available N ($mg\cdot kg^{-1}$)	Heavy Metal Contents ($mg\cdot kg^{-1}$)			
				Zn	Pb	Cu	Cd
Dabaoshan Mining Area	14.7 ± 0.9 b	32.2 ± 2.0 b	30.2 ± 1.9 b	1768.7 ± 91.1 a	1253.3 ± 71.3 a	1701.3 ± 77.5 a	9.1 ± 0.9 a
Boluo County	13.8 ± 0.9 b	26.6 ± 1.8 b	28.4 ± 2.7 b	135.2 ± 13.1 b	242.6 ± 44.1 b	48.4 ± 9.5 b	1.1 ± 0.2 b
Soil samples tested	36.2 ± 1.1 a	60.5 ± 9.9 a	61.5 ± 10.9 a	60.5 ± 9.9 b	35.2 ± 7.2 c	6.3 ± 1.7 b	0.13 ± 0.1 b

Note: Data in the table are means ± SD (n = 3), different letters in the same vertical column indicate significant difference according to SSR test ($p < 0.05$), the same below.

2.3. Test Design

The levels of Pb stress treatment were: CK (control), 100 $mg\cdot kg^{-1}$, 300 $mg\cdot kg^{-1}$, 500 $mg\cdot kg^{-1}$, 1000 $mg\cdot kg^{-1}$, and 2000 $mg\cdot kg^{-1}$. Pb was added in the form of $Pb(NO_3)_2$. After soil treatment, it was mixed well and kept stable for two weeks. After the soil was stabilized, the seedlings of *M. floridulus* were transplanted into it. Plants of the mining ecotype and non-mining ecotype were separated. Plants with the same weight and height were selected and randomly assigned to each concentration treatment. Each treatment of each ecotype was planted with 3 pots, and each pot was planted with 3 plants. After transplanting, the soil water content was maintained at 60–70% of the field capacity by weight.

2.4. Sample Analysis

After 180 days of Pb stress treatment, the tested plants were harvested. Each sample was divided into root, overground, and soil. The root and overground were separated and washed with tap water to remove the soil and dirt adhered to the sample, and then washed with deionized water. After the plant samples were drained to remove water, they were first dried in an oven (DHG-9070G, Shanghai, China) at 105 °C for 30 min, and then dried in an oven (DHG-9070G, Shanghai, China) at 80 °C to a constant weight. First, the dry weight of the dried samples was determined using a balance (Hirp JA2003N, Shanghai, China), and then the samples were crushed with a plant shredder and mixed evenly into the labeled paper bags for testing. The collected soil samples were dried naturally. They were then ground through a 100-mesh sieve (DXR302, Hebei, China) and put into labeled paper bags for testing.

Soil NH_4OAc-extracted Pb was determined with this process: weigh 10.00 g of air-dried soil, add 50 mL of 1.0 $mol\cdot L^{-1}$ NH_4OAc solution with pH value of 7.0, shake at room temperature for 2 h, filter, and determine the Pb content in the filtrate.

Determination of lead content in plant samples: first, the prepared plant samples were digested using the HNO_3-$HClO_4$ (3:1) method, and then the Pb content was determined with ICP-OES (Optima5300DV, Perkin-Elmer, Sheldon, CT, USA). Each sample was repeated three times [31]. Soil reference materials (gbw07388) and parallel samples were inserted during digestion and analysis of soil samples for quality control of accuracy. The recovery rate of reference material analysis is 75–110%, indicating that the analysis method is reliable.

2.5. Data Processing and Statistical Analysis

(1) Tolerance index (*TI*), According to the method laid out by Metwall et al. [32], the tolerance index was calculated to evaluate the tolerance degree of *M. floridulus* to Pb. *TI* is defined as:

$$TI = \text{Biomass of treatment}/\text{Biomass of control} \quad (1)$$

(2) Translocation factor (*TF*), This represents the capability for Pb transport from root system to stem and leaf [33]. *TF* is defined as:

$$TF = \text{Pb content in shoot/Pb content in root} \quad (2)$$

Statistical analysis of data was performed using a combination of Microsoft Excel 2003 and SPSS 16.0 software, and the significance of differences between means was analyzed using Duncan's multiple comparisons (SSR test, $p < 0.05$).

3. Results

3.1. Pb Content in the Body of *M. floridulus* under Pb Stress

Shown in Figure 2, under soil culture conditions, the Pb content in the body of different ecotypes of *M. floridulus* treated with different concentrations of Pb was significantly different. The Pb content in the shoots of the non-mining ecotype *M. floridulus* increased slowly with the increase of soil Pb concentration, and reached its maximum at the highest Pb concentration (2000 mg·kg^{-1}), which was 109.2 mg·kg^{-1}. The Pb content in the roots also increased slowly with the increase of soil Pb concentration in the range of 0–500 mg·kg^{-1}. When the concentration of Pb treatment increased to 1000 mg·kg^{-1}, the content of Pb in the roots significantly increased ($p < 0.05$). Under the highest concentration of Pb treatment (2000 mg·kg^{-1}), the content of Pb in the roots reached the maximum, which was 214.8 mg·kg^{-1}. This may be due to the fact that under the high concentration of Pb treatment, the plant roots were poisoned by Pb and the cell permeability increased, thus passively absorbing a large amount of Pb.

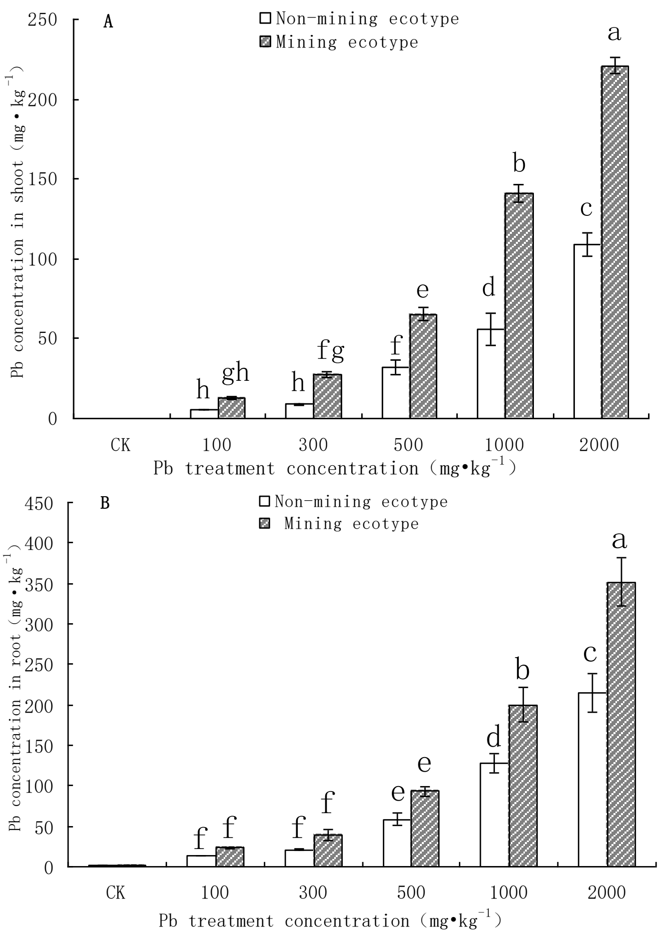

Figure 2. Pb concentrations in root and shoot of two ecotypes of *M. floridulus* under different Pb treatment ((**A**) in shoot, (**B**) in root). Note: Error bars indicate standard deviation; Different letters in the same group indicate significant difference at $p < 0.05$ according to Duncan's multiple range tests; the same below.

With the increase of soil Pb concentration, the Pb content in the shoots and roots of the mining ecotype *M. floridulus* increased rapidly, and the rate of increase was significantly faster than that of the non-mining ecotype (Figure 2). Under the treatment of 2000 mg·kg^{-1} Pb, the Pb content in the shoots reached the maximum, 221.0 mg·kg^{-1}, which is 2.02 times of the non-mining ecotype under the same treatment concentration. The Pb content in the roots of the mining ecotype *M. floridulus* also increased significantly with the increase of Pb concentration ($p < 0.05$). Under the highest Pb concentration (2000 mg·kg^{-1}), the Pb content in the roots reached the maximum (351.8 mg·kg^{-1}), which was 1.65 times that of the non-mining ecotype under the same treatment concentration.

3.2. Aboveground and Root Biomass of M. floridulus under Pb Stress

The effects of Pb treatment on shoot and root biomass of two ecotypes of *M. floridulus* are shown in Figure 3. As can be seen from the figure, compared with the control, the aboveground biomass of non-mining ecotype plants was significantly affected by the low concentration of Pb (100 mg·kg^{-1}) ($p < 0.05$), while the mining ecotype plants were not significantly affected ($p > 0.05$). When the concentration of Pb increased, the biomass of *M. floridulus* from the mining area (Dabaoshan) was less affected under different concentrations of Pb stress, while the biomass of *M. floridulus* from the non-mining area (Boluo) decreased significantly with the increase in concentration. Specifically, with the increase of Pb stress concentration, shoot biomass of both populations decreased. Under 500 mg·kg^{-1}, 1000 mg·kg^{-1}, and 2000 mg·kg^{-1} Pb stress, the biomass of mining ecotype and non-mining ecotype plants were 65.0%, 61.1%, and 57.8%, and 41.1%, 37.4%, and 30.2% of the control, respectively. Obviously, the biomass of mining ecotype plants decreased less under higher Pb treatments, while that of non-mining ecotype plants decreased significantly. In particular, the aboveground biomass of mining ecotype plants was 2.03 times higher than that of non-mining ecotype plants treated with 2000 mg·kg^{-1}.

As can be seen from Figure 3, under the treatment of different concentrations of Pb, the root biomass of *M. floridulus* plants of mining ecotypes and non-mining ecotypes showed different trends: the non-mining ecotype plants decreased with the increase of treatment concentration. Under 1000 mg·kg^{-1} and 2000 mg·kg^{-1} Pb stress, the root biomass decreased significantly, to 62.2% and 55.8% of the control, respectively. However, the root biomass of mining ecotype plants increased significantly with the increase of Pb concentration ($p < 0.05$), and reached the maximum value when the Pb concentration was 1000 mg·kg^{-1}, which was 138.6% of the control. Root biomass decreased when the Pb concentration was 2000 mg·kg^{-1}, but was still 119.5% of the control. The results showed that the effect of Pb stress on root biomass was much greater for non-mining ecotypes than mining ecotypes. The results showed that the roots of the mining ecotype *M. floridulus* had a certain Pb tolerance built up in an environment of heavy metal stress for a long time.

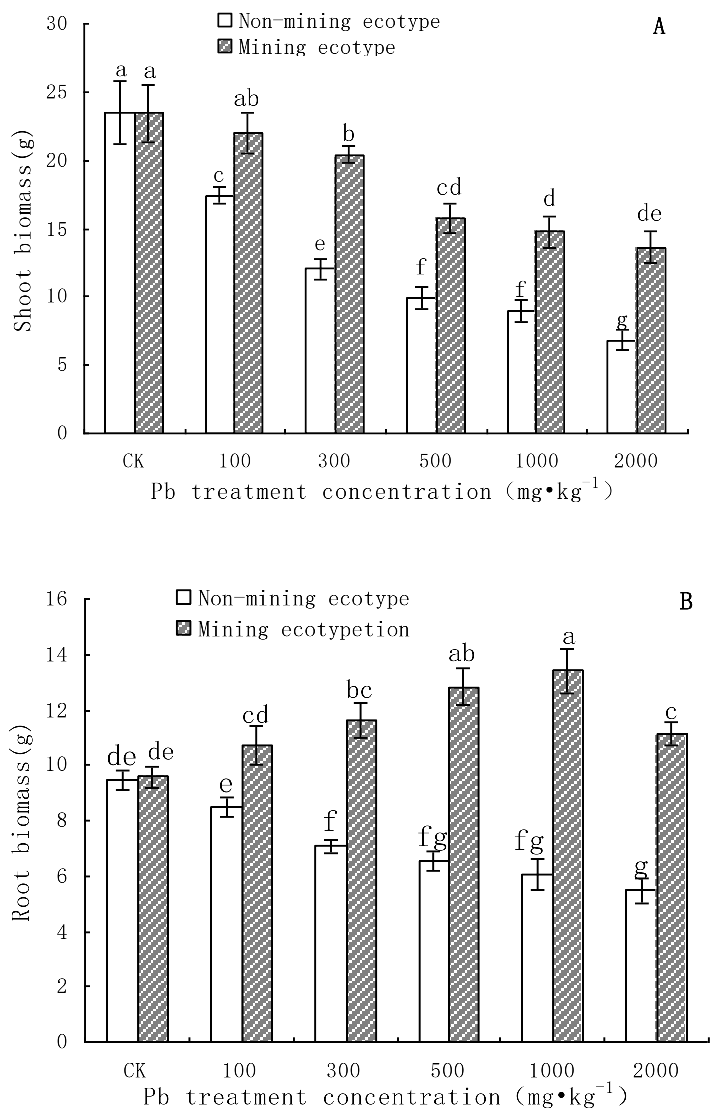

Figure 3. Root biomass and shoot biomass of two ecotypes of *M. floridulus* under different Pb treatment ((**A**) is shoot biomass, (**B**) is root biomass). Note: Error bars indicate standard deviation; different letters in the same group indicate significant difference at $p < 0.05$ according to Duncan's multiple range tests.

3.3. Pb Accumulation in M. floridulus under Pb Stress

The efficiency of phytoremediation of heavy metal-contaminated soil not only depends on the metal content of the shoot, but is also closely related to its biomass of shoots. Therefore, the total accumulation of Pb in the shoots of *M. pentanthus* can be obtained by the biomass × Pb content of the shoots, which can represent the ability of *M. pentanthus* to remove soil Pb. It can be seen from Figure 4 that under the low Pb treatment level (<500 mg·kg^{-1}), with the increase of Pb concentration in the soil, the accumulation of Pb in the aboveground parts of the two ecotypes of *M. floridulus* increased slowly, but from the treatment of 1000 mg·kg^{-1}, the mining ecotype *M. floridulus* increased significantly ($p < 0.05$). Compared with the two ecotypes, the accumulation of Pb in the aerial parts of the mining ecotype was significantly higher than that of the non-mining ecotype ($p < 0.05$). In the range of 500–2000 mg·kg^{-1} Pb concentration, the higher the soil Pb concentration, the greater the difference between the two ecotypes. Under the treatment of 2000 mg·kg^{-1} Pb, the mining ecotype plants' accumulation of Pb was 3.78 times that of the non-mining ecotype plants.

It can be seen from Figure 4 that the total amount of Pb accumulated in the root of mining ecotype *M. floridulus* is much greater than that in the non-mining ecotype, and it increases significantly with the increase of Pb addition in the soil ($p < 0.05$). Under the stress of 1000 mg·kg^{-1} and 2000 mg·kg^{-1} Pb, the Pb accumulation in the root of mining ecotype *M. floridulus* was 3.09 times and 3.35 times of that in non-mining ecotype, respectively. This indicates that the mining ecotype *M. floridulus* has stronger Pb enrichment ability than non-mining ecotype *M. floridulus*.

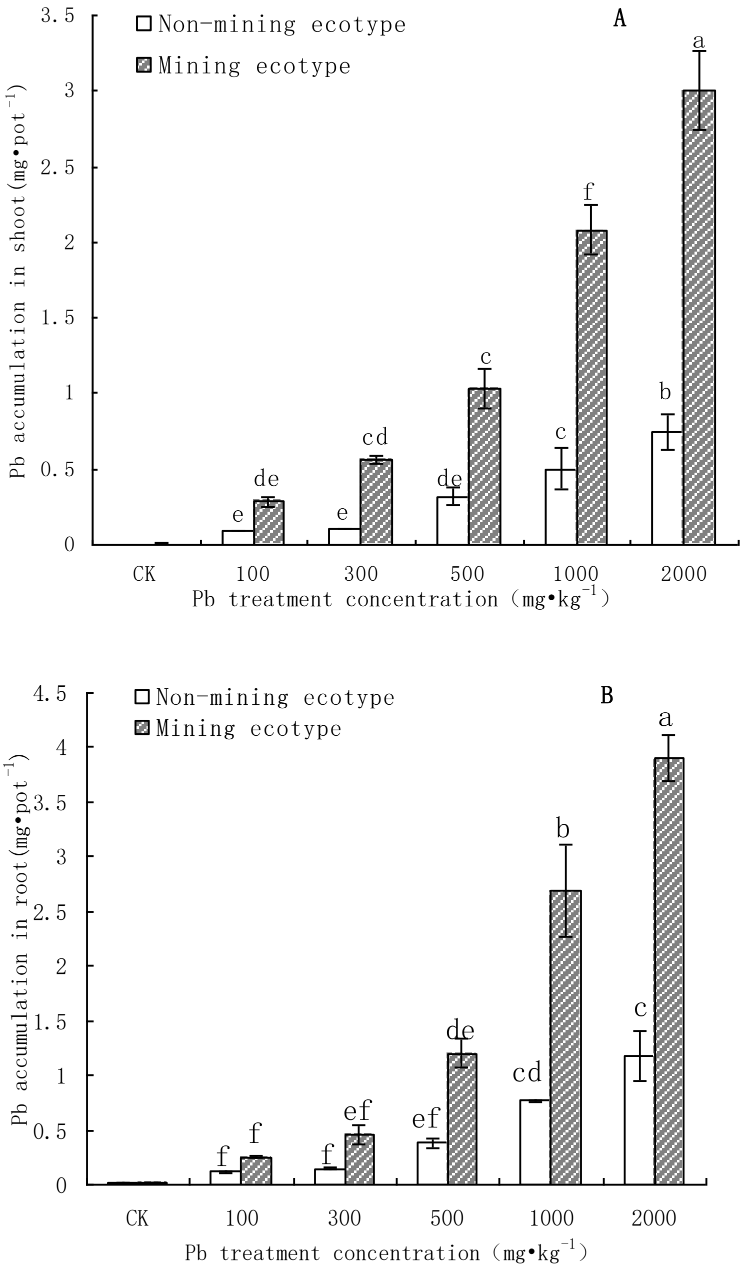

Figure 4. Pb accumulation in root and shoot of Two ecotypes of *M. floridulus* under different Pb treatment ((**A**) in shoot, (**B**) in root). Note: Error bars indicate standard deviation; different letters in the same group indicate significant difference at $p < 0.05$ according to Duncan's multiple range tests.

3.4. Transfer Coefficient and Tolerance Index of M. floridulus under Pb Stress

Translocation factor (*TF*) refers to the ratio of the content of elements above ground and the content of the same elements in the roots of plants, which is used to evaluate the transport and enrichment ability of heavy metals from the underground to the ground.

The greater the transfer coefficient, the stronger the transport capacity of heavy metals from root to shoot organs. As can be seen from Figure 5, with the increase of Pb stress, the two ecotypes of *M. floridulus* increased to different degrees, but the *TF* value increased differently among different treatments. The translocation factor (*TF*) of *M. floridulus* in the mining ecotype was significantly higher than in the non-mining ecotype ($p < 0.05$). The *TF* value of the mining ecotype is 1.28 times that of the non-mining ecotype.

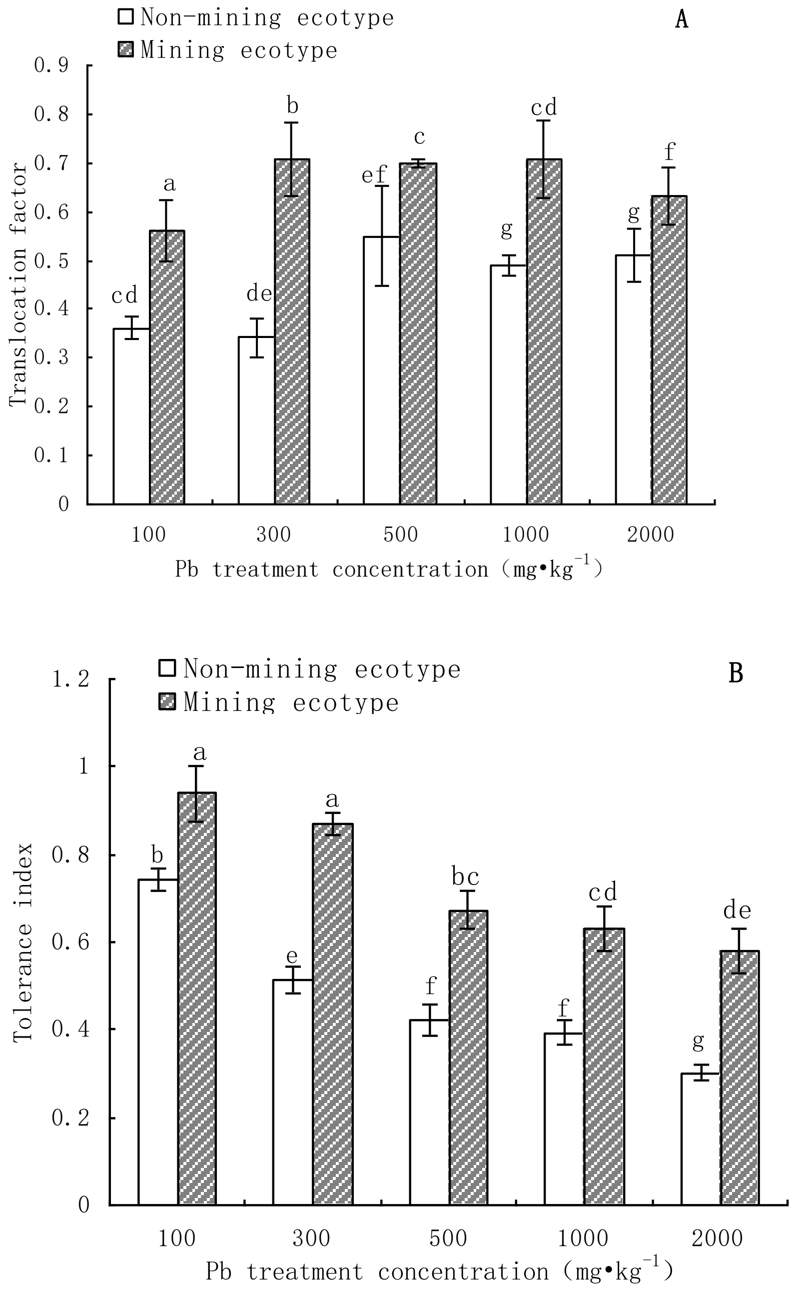

Figure 5. Translocation factor and tolerance of Two ecotypes of *M. floridulus* under different Pb treatment ((**A**) is *TF*, (**B**) is *TI*). Note: Error bars indicate standard deviation; different letters in the same group indicate significant difference at $p < 0.05$ according to Duncan's multiple range tests.

The tolerance index (*TI*) of the two ecotypes under Pb stress treatment is shown in Figure 5. The larger the tolerance index, the stronger the tolerance to Pb. As can be seen from the figure, under mild Pb stress (100 mg·kg^{-1}, 300 mg·kg^{-1}), the tolerance index of non-mining ecotypes of *M. floridulus* decreased significantly ($p < 0.05$), while the tolerance index of mining ecotypes of *M. floridulus* did not decrease significantly ($p > 0.05$). When the concentration of Pb was increased to 1000 mg·kg^{-1} and 2000 mg·kg^{-1}, the tolerance index of the non-mining ecotype continued to decrease significantly ($p < 0.05$), while the tolerance index of the mining ecotype did not decrease significantly ($p > 0.05$), which was 1.55 and 1.80 times of that of the mining population, respectively.

3.5. Variation of Pb Content of NH_4OAc Extraction in Soil

Chemical extracts are widely used to evaluate the availability of heavy metals to plants. Lead extracted by NH_4OAc is mainly derived from water-soluble lead, exchangeable lead, and some loosely bound lead in soil, and its content mainly depends on the conversion of bound lead to exchangeable lead in soil and the absorption of water-soluble lead by plants [34]. It is a commonly used extractant to evaluate the availability of heavy metals to plants [35,36]. After growing on Pb treated soil for 6 months, the two ecotypes of *M. floridulus* plants had a significant effect on the content of NH_4OAc-extracted Pb in the soil (Table 2). After harvesting the plants, the NH_4OAc-extracted Pb in the soil of each treatment was significantly lower than before transplanting ($p < 0.05$). Comparing the content of NH_4OAc-extracted Pb in the soil planted with two ecotypes of *M. floridulus* plants, it was found that the content with mining ecotype plants was significantly lower than that with non-mining ecotype plants ($p < 0.05$). This may be due to the strong Pb absorption capacity of mining ecotype plants, which absorbed more Pb from the soil. The Pb absorption capacity of non-mining ecotype plants was relatively small.

Table 2. Concentration of ammonium acetate-extractable Pb (NH_4OAc-Pb) in soils before and after planting *Miscanthus floridulus*(mg·kg^{-1}).

Pb Treatment Concentration (mg·kg^{-1})	Before Planting	After Harvest	
		Non-Mining Ecotype	Mining Ecotype
CK	1.209 ± 0.101 a	0.13 ± 0.01 b	0.12 ± 0.000 b
100	30.060 ± 0.77 a	12.098 ± 0.199 b	9.799 ± 0.300 c
300	120.094 ± 6.032 a	63.163 ± 6.205 b	53.509 ± 4.160 c
500	152.877 ± 8.093 a	78.396 ± 8.105 b	66.66 ± 5.103 c
1000	317.817 ± 18.166 a	159.500 ± 11.127 b	127.785 ± 11.100 c
2000	788.833 ± 30.955 a	442.530 ± 20.951 b	254.674 ± 38.852 c

Note: Data in the table are means ± SD (n = 3), different letters in same column indicate a significant difference according to SSR test ($p < 0.05$).

3.6. Correlation Analysis of Biomass, Pb Content, TF and TI of M. floridulus

It can be seen from Table 3 that there is a significant positive correlation between the root biomass and the aboveground biomass ($p < 0.01$), indicating that the accumulation of root biomass has a positive effect on the accumulation of aboveground biomass. However, the aboveground biomass showed a significant negative correlation with Pb content in roots and aboveground parts ($p < 0.01$), reflecting that Pb accumulation in roots and aboveground parts has toxicity to plants and inhibited the growth of *M. floridulus*. The Pb content in the shoot is positively correlated with the Pb content and *TF* in the root ($p < 0.01$), which indicates that Pb is absolutely transferred to the shoot after being absorbed by the root system. The accumulation of Pb in the root system is the determinant of *TF*, and both determine the accumulation of Pb in the shoot. The tolerance index *TI* of *M. floridulus* was negatively correlated with Pb content in roots and shoots ($p < 0.01$), which also reflected that Pb toxicity to plants inhibited the accumulation of biomass.

Table 3. Pearson correlation coefficients of biomass, Pb content in root and shoot, *TF* and *TI* of the tested *M. floridulus*.

Item	Shoot Biomass	Root Biomass	Pb Content in Shoot	Pb Content in Root	Pb Accumulation in Shoot	Pb Accumulation in Root	Translocation Factor (*TF*)
Root biomass	0.551 **	1					
Pb content in shoot	−0.481 **	0.194	1				
Pb content in root	−0.508 **	0.109	0.967 **	1			
Pb accumulation in shoot	−0.299	0.428 **	0.952 **	0.919 **	1		
Pb accumulation in root	−0.329	0.369 *	0.945 **	0.949 **	0.983 **	1	
Translocation factor (*TF*)	0.936 **	0.459 **	0.548 **	−0.598 **	−0.393 *	−0.418 *	1
Tolerance index (*TI*)	0.993 **	0.558 **	−0.483 **	−0.505 **	−0.299	−0.316	0.943 **

Note: * and ** indicate significance under $p < 0.05$ and $p < 0.01$, respectively.

4. Discussion

From an evolutionary perspective, species interact with the environment. When the environment changes, the changed environmental factors will select genetic variations caused by random mutation and recombination. When the selection pressure and action time reach a certain degree, genetic variants that cannot adapt to environmental changes will be eliminated, and genetic variants that adapt to environmental changes will be retained [37,38]. For example, under the special selection pressure of environmental pollution, some plant populations cannot adapt, their viability decreases, and they gradually withdraw from the pollution zone. Some plant populations can still survive and reproduce, but they have undergone great changes in physiological, biochemical, and genetic characteristics, resulting in gradients and ecotypes that can tolerate or accumulate excessive pollutants [39,40]. According to Xiong Z.T. [41], a resistant ecotype grows better than a non-resistant ecotype in the polluted environment, while the latter grows better than the former in the pollution-free environment. Macnair [42] suggested that the effect of increasing pollution levels on resistant ecotypes was smaller than on non-resistant ecotypes. Due to the strong selective pressure of heavy metal stress and the dominant character of metal stress tolerance in plants, metal stress tolerance differentiates rapidly among populations, and the populations growing on different mines or heavy metal polluted soils have different abilities to tolerate metal stress or accumulate metal [43,44].

The results of a local culture experiment showed that under Pb stress, the mining ecotype *M. floridulus* grew well and the plant biomass was large. However, the non-mining ecotype *M. floridulus* grew less well and the plant biomass was small. In addition, the impacts on the aboveground and root biomass of the mining ecotype were significantly smaller than on those of the non-mining ecotype. Therefore, it was concluded that the mining ecotype was more tolerant to Pb stress than the non-mining ecotype, and was a kind of Pb-stress-resistant ecotype.

The rejection mechanism of plants to heavy metals usually includes two aspects: to reduce the absorption of heavy metals in roots, and to restrict the transfer of heavy metals to shoot using compartmentalization and preservation in roots [45]. The most important feature of the exclusion plants is that the heavy metal content of the plant body, especially the shoots, is low. The exclusion plant is an ideal remediation plant for the stable remediation of heavy metal-contaminated soil [46]. In contrast to hyper accumulating plants, rejection plants reduce their transfer to the shoot with in vitro antibodies as the dominant mechanism [47]. The results of this study showed (Figures 2 and 3) that there were significant differences in the capacity of uptake, transport, and accumulation of Pb between the two ecotypes. Under the same Pb treatment level, the content and accumulation of Pb in the shoot and root of the mining ecotype were significantly higher than those of the non-mining ecotype. At the same time, the Pb content of the roots was higher than that of the shoots, and the Translocation factor (*TF*) was less than 0.7, which indicated that the ecotypes in the mining area not only had a strong ability to absorb soil Pb but also could retain a large amount of Pb in the roots.

When exogenous heavy metals are added to the soil, they will undergo various physical, chemical, and biological reactions with soil components (especially clay minerals and organic matter) and exist in different forms in the soil [48,49]. The bioavailability of heavy metals in soil is not only related to the total amount, but largely depends on the presence of different forms of heavy metals in soil. The bioavailability of different speciation of heavy metals varies greatly; among these, the water-soluble form is the direct source for plant absorption, and its availability is the highest. The exchangeable state accounts for a large proportion of heavy metals in soil; it has high activity, which plays a decisive role in plant absorption [50,51]. It was found in this study (Table 2) that after planting plants, the NH_4OAc-extracted Pb content in soil further decreased. This may be due to the effect of plant roots and microorganisms, which promoted the adsorption, chelation, or precipitation of some of the available Pb through soil components. Alternatively, the available Pb content in soil decreased due to the uptake and removal by plant roots. From the changes of NH_4OAc-extracted Pb content in the soil after harvesting plants, the NH_4OAc-extracted Pb content in the soil planted with the mining ecotype *M. floridulus* decreased significantly, while the NH_4OAc-extracted Pb content in the soil planted with non-mining ecotype plants decreased slightly. The reason may be that the roots of the mining ecotype *M. floridulus* have a strong ability to absorb Pb, which leads to the decrease of the available Pb content in the soil.

5. Conclusions

(1) The aboveground and root biomass of the mining ecotype *M. floridulus* was significantly less affected than that of the non-mining ecotype by Pb concentration in soil. There were also significant differences in Pb uptake, transport, and accumulation between the two ecotypes of *M. floridulus*. Under the same Pb treatment level, the Pb content and accumulation in the shoots and roots of ecotypes in mining areas were significantly higher than in ecotypes in non-mining areas; the mining ecotype plants were more tolerant to environmental Pb stress than the non-mining ecotype, which is a Pb-stress-tolerant ecotype.

(2) The Pb content in roots was higher than that in shoots, and the translocation factor (*TF*) was less than 0.7, which indicates that the two ecotypes of *M. floridulus* not only have a strong ability to absorb Pb from soil, but also can retain a large amount of Pb in the roots. Only a small amount of Pb absorbed by the roots is transferred to the upper part of the ground, thus reducing the toxicity of Pb to the plant.

(3) The amount of NH_4OAc-extracted Pb in the soil planted with the mining ecotype plants *M. floridulus* decreased significantly, which may be due to the strong ability of the roots of this ecotype to absorb Pb, leading to the decrease of the content of available Pb in the soil.

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