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Effects of Combined Pb and Cu Pollution on the Growth and Activities of Plant Antioxidant Enzymes and Rhizospheric Soil Enzymes of *Miscanthus floridulus*

ABSTRACT

Phytoremediation of mine wastes is a promising approach for the restoration of contaminated soils. Miscanthus floridulus is a perennial herbaceous grass with high productivity and tolerant to a variety of heavy metals. Sixty-day pot experiments were carried out to investigate the effects of combined Pb-Cu contamination on some physio-biochemical markers of M. floridulus and rhizospheric soil enzyme activities. M. floridulus was tolerant to a combination of 200 mg kg^{-1} Pb and 100 mg kg^{-1} Cu stress as indicated by invisible foliar injury, increased contents of photosynthetic pigments (increased by 17.2% to 22.8% compared to the control), enhanced accumulation of soluble sugar contents by 33.5% to 52.8% in response to all treatments except the Pb100Cu50, decreased contents of malondialdehyde by 5.18% at 100 mg kg^{-1} Pb and 50 mg kg^{-1} Cu. As a response to combined Pb-Cu stress, the antioxidant enzymes (catalase, peroxidase and superoxide dismutase) activities in M. floridulus were reduced by any of the Pb-Cu treatments used in the present study. Although the growth parameters such as plant height, root number, maximal root length, and the dry biomass of aboveground parts and root were all lowered by Pb-Cu treatments, the rhizospheric soil invertase, catalase, urease and phosphatase enzyme activities of M. floridulus kept increasing when the concentration of Pb and Cu below 400 mg kg^{-1} Pb and 200 mg kg^{-1} Cu.

Keywords: *Combined Pb-Cu pollution, Miscanthus floridulus, antioxidant enzymes, rhizospheric soil enzymes, phytoremediation*

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INTRODUCTION

Since the beginning of the industrial revolution, the contamination of the biosphere by heavy metals has increased dramatically. Among the different sources of pollutants, industrial, mining, agricultural activities and automobile exhaust have largely contributed to metal contamination in surrounding soils. According to the data heavy metal contaminated area in China is 8,100,000 ha (*Evangelou et al. 2012*). The management of metal-polluted soils is now of major concern for most industrialized countries because of the ubiquity of the heavy metals, their environmental persistence and hazardous effects on environment and human health. Although various remediation methods such as landfilling, fixation and leaching may contribute to restoring metal-polluted soils, these methods however are either expensive or even unfortunately induce adverse effects on the biological activity, the structure and fertility of soils (*Mulligan et al. 2001; Conesa et al. 2012*). In contrast, the use of plants to stabilize or remove pollutants from soils, generally defined as phytoremediation, offers the great advantage of being inexpensive, environmental-friendly and not altering soil matrix (*Gardea-Torresdey et al. 2005; Conesa et al. 2012*). In recent years, advances in tripartite analysis have provided novel strategies for the remediation of organically contaminated soils (*Tartaglia et al. 2022a, b*).

Hyper-tolerant and hyper-accumulator plants can be used for phytoremediation, however, most of these plants are usually featured by slow growth, less biomass, limited root system extension depth, selective absorption of trace elements and insufficient translocation factor (*Nsanganwimana et al. 2014*). The foregoing properties practically reduce the phytoremediation efficiency and prolong the phytoremediation time, and as a result, the potential for large-scale bioremediation is limited. Therefore choosing appropriate plants for revegetation needs to consider their ability to produce high biomass, to tolerate organic/inorganic pollutants, to avoid health risks and to provide ecosystem services (*Henry et al. 2013*).

Miscanthus plants are C4 perennial energy plants (*Liu et al. 2013*) with high productivity of biomass, commonly native in East Africa and Asia (*Zhou et al. 2012*). Their relatively high yield of biomass and photosynthetic efficiency, significantly lower concentrations of moisture and ash, as well as fast-growing property, make them available for forage grass, clothing, shelter, liquid fuels and chemicals (*Meng et al. 2012*). *Miscanthus* spp can be successfully grown on heavily contaminated soil with Zn, Cd, Pb and Cu (*Pavel et al. 2014; Yang et al. 2014*). At an abandoned mining dump in Guangdong Province, China, the coverage of *M. floridulus* is above 80% after about 20 years of succession (*Zhang et al. 2009*). In field conditions, *M. floridulus* reduced heavy metal availability by enhancing their conversion to chelated fractions or sediments, and concomitantly, bacterial performance for cellulose decomposition, phenol transformation, nitrogen fixation, ammonification, nitrification and organic phosphorus decomposition was enhanced (*Zhang et al. 2009*). At some sites contaminated by combined heavy metals *M. floridulus* has even developed metal-tolerant ecotypes (*Qin et al. 2011a, b*). These ecotypes are more tolerant to Pb and Cd than conspecific plants collected from uncontaminated sites (*Hsu and Chang 1992*). According to the inclusion criteria suggested by *Boularbah et al.* (*2006*), *M. floridulus* is a hyper-tolerant plant species because the biotransfer factor of specific heavy metal is above one. In addition, *M. floridulus* can accumulate > 400 mg kg^{-1} Zn in the harvestable parts (*Wang et al. 2012*), and in the aboveground parts of *M. floridulus* the concentration of As and Pb exceeds 300 mg kg^{-1} and 200 mg kg^{-1}, respectively (*Leung et al. 2007*).

M. floridulus has a cross-tolerance to many heavy metals, such as Mn, Ni, As, Pb and Zn (*Ren et al. 2006; Sun et al. 2006*), and it is even tolerant to phytotoxic levels of Cd and Mn (*Liu et al. 2014*). The absorbed heavy metal is mainly confined in roots and less is translocated to the aboveground parts (*Qin et al. 2011a*). t was previously reported that *M. floridulus* is tolerant to the individual heavy metal, Cd (*Yang et al. 2014*). Thus, *M. floridulus* is considered a good plant candidate for the rehabilitation of the mine tailings. This study examined the effects of combined Pb and Cu pollution on the physiology of *M. floridulus*, and the effects of the growth of *M. floridulus* on soil fertility parameters of artificially polluted brown soil. The researchers will prove that *M. floridulus* is a plant species tolerant to Pb and Cu pollution and is suitable for the phytomanagement of mine wastes. Result of the study will supply more information on the phytoremediation potential in mine wastes.

MATERIALS AND METHODS

Material and Experimental Design

Plants. Seedlings of *M. floridulus* were collected at the mining area in Tongling, Anhui Province, China and then were acclimatized at the Botanical Garden of Anhui Normal University for two weeks. Seedlings of similar height and biomass were transplanted into plastic pots for experimentation.

Soil. The test soil was collected at Zheshan, a hill where Anhui Normal University is situated. The soil was air dried and passed through a screen. This study also listed the basic physiochemical properties of the soil (**Table 1**).

Experimental design. Each plastic pot (diameter = 20 cm) contains two kg of test soil. The chemical pure $Pb(NO_3)_2$ and $Cu(NO_3)_2$ were directly drooped to the test soil to simulate Pb and Cu pollution, respectively. The final concentrations of Pb and Cu in the soil were Pb0Cu0, Pb100Cu50 (100 mg kg^{-1} Pb and 50 mg kg^{-1} Cu), Pb200Cu100 (200 mg kg^{-1} Pb and 100 mg kg^{-1} Cu), Pb400Cu200 (400 mg kg^{-1} Pb and 200 mg kg^{-1} Cu) and Pb800Cu500 (800 mg kg^{-1} Pb and 500 mg kg^{-1} Cu). The contaminated soil was allowed to stabilize at room temperature for one week, and then two *M. floridulus* seedlings were transplanted into each pot. Every treatment had 6 replicates. The pots transplanted with *M. floridulus* were arranged in the botanical garden and *M. floridulus* were grown according to natural day time rhythm. Water content of soil was maintained at 70%. On the 60th day, *M. floridulus* were harvested for analysis.

Methods

Growth parameters of *M. floridulus*. The entire *M. floridulus* plants were harvested for the determination of growth parameters. Root number, maximal root length, plant height, fresh and dry root weight as well as the fresh and dry weight of aboveground parts were all determined.

Observation of apparent injury. The degree of apparent injury was classified into four levels according to *Qin et al.* (*1997*), i.e., no visible injury, slight injury as indicated

Table 1. The physiochemical properties of test soils.

pH	Organic matter (g kg^{-1})	Total N (g kg^{-1})	Total P (g kg^{-1})	EC (μs cm^{-1})	Total K (g kg^{-1})	Available K (mg kg^{-1})	Total Cu (mg·kg^{-1})	Total Pb (mg kg^{-1})
6.85	13.80	0.24	1.55	1.37	7.81	192.00	18.31	3.67

Note:N - nitrogen; P - phosphorous; EC - electricity conductivity; K - potassium by chlorosis at the center of leaves, moderate injury as indicated by chlorosis both at the center and pericenter of leaf blades, and severe injury as indicated as severe chlorosis.

Determination of chlorophyll pigment and contents of malondialdehyde and soluble sugar. Chlorophyll content: 0.2 g of fresh leaves was extracted in 80% acetone and absorbance was read at 663 nm, 645 nm and 430 nm (*Li 2000*).

MDA content: the level of lipid peroxidation was measured by estimating malondialdehyde (MDA) content using thiobarbituric acid (TBA) as a substrate (*Heath and Packer 1968*). The absorbance of resulting MDA was read at 532 nm and corrected for non-specific absorbance at 600 nm. MDA content was calculated using an extinction coefficient of 155 mM^{-1}, cm^{-1} and expressed as μmol g^{-1}, FW.

Soluble sugar: the total amount of soluble sugar was estimated by anthrone reagent. Absorbance was read at 525 nm and the content of soluble sugar was calculated from a standard curve. The content of total sugar was expressed as μmol·g^{-1} FW (*Li 2000*).

Measurement of antioxidant enzyme activities in *M. floridulus*. The antioxidant enzyme activities were measured based on *Li* (*2000*). Superoxide dismutase (SOD) activity was assayed by determining the inhibition of photochemical reduction of nitroblue tetrazolium. One unit of SOD was defined as the enzyme activity that inhibited the photoreduction of nitroblue tetrazolium to blue formazan by 50%. SOD activity was expressed as ΔOD_{560} g^{-1} FW·min^{-1}. Catalase (CAT) activity was determined using a titrimetric method according to *Huang et al.* (*2000*). Five hundred microliter enzyme extract was used for the determination of CAT activity which was defined as the amount of $KMnO_4$ consumed during titration. CAT activity was expressed as mL $KMnO_4$ mg^{-1} FW. Peroxidase (POD) activity was measured by following the increase of absorbance at 470 nm owing to the guaiacol oxidation. One unit of POD activity was defined as the amount of enzyme required to convert 1 μmol of H_2O_2 to H_2O and O_2. POD activity was expressed as OD470 g^{-1} FW min^{-1}.

Measurement of rhizospheric soil enzyme activities. Soil enzyme activities were determined based on *Huang et al.* (*2000*). Invertase activity was measured by using $Na_2S_2O_3$ titration method and the activity was expressed as the amount of 0.1 mM of $Na_2S_2O_3$ usedduring titration (Glucose mg g^{-1} $24h^{-1}$). Soil catalase (CAT) activity was measured using $KMnO_4$ titration method. CAT activity was expressed as the amount of 0.1 M $KMnO_4$ consumed per gram of soil sample (0.1 M $KMnO_4$ mL^{-1}). The indophenol colorimetric method was used to measure the NH_4^+ released by enzymatic hydrolysis of urea. Indophenol was determined colorimetrically at 578 nm and urease activity was expressed as mg $NH_{3-}N$ g^{-1} soil d^{-1} (NH_3-N mg g^{-1} $24h^{-1}$). Phosphatase activity was measured colorimetrically in the presence of disodium phenyl phosphate and the activity was expressed as mg (P_2O_5)-1g (soil) as mg P_2O_5 $100g^{-1}$ soil $2h^{-1}$.

Data analysis. Data reported in this study were the mean value of 3 replicates. All data were analyzed by SPSS 17.0 software package, followed by Tukey's multiple comparison. Statistical significance was tested at $p < 0.05$.

RESULTS AND DISCUSSION

Effects of Combined Pb-Cu Stress on the Growth Parameters of *M. floridulus*

With the increasing concentrations of combined Pb-Cu stress and prolonged duration of exposure, the growth parameters of *M. floridulus* were all affected (**Table 2**). After sixty-day exposure to combined Pb-Cu stress, Pb100Cu50 treatment did not cause any apparent injury to *M. floridulus* leaves. When the combined concentration of Pb-Cu was above Pb200Cu100 mg kg^{-1}, more and more severe apparent injury at *M. floridulus* leaves was observed. The severe injury occurred when combined concentration of Pb-Cu was Pb400Cu200 mg kg^{-1} and Pb800Cu500 mg kg^{-1}.

Plant height, root number, maximal root length, dry biomass of aboveground part and root of *M. floridulus* were all significantly depressed compared with control (**Table 2**, $P < 0.05$). Pb800Cu500 treatment had the most significant effects on the foregoing parameters. In particular, plant height, root number, maximal root length and dry biomass of aboveground part and root were reduced by 75.45%, 93.9%, 92.82%, 78.73% and 93.53%, respectively, than control.

Effects of Combined Pb-Cu Stress on the Contents of Photosynthetic Pigments of *M. floridulus*

The contents photosynthetic pigments increased at lower Pb-Cu treatments, but decreased at higher Pb-Cu treatments (**Table 3**). In general, Pb100Cu50 and Pb200Cu100 treatments potentiated the synthesis of photosynthetic pigments. The total chlorophyll content and carotenoid content under these two treatments were

Table 2. Effects of combined Pb-Cu treatments on *M. floridulus* growth.

	Combined Pb-Cu concentrations (mg kg^{-1})				
	Control	**Pb100Cu50**	**Pb200Cu100**	**Pb400Cu200**	**Pb800Cu500**
Apparent injury	No visible injury	No visible injury	Slight injury	Moderate injury	Severe injury
Plant height (cm)	29.33±2.59a	25.93±0.91b	21.27±2.47c	11.40±0.26d	7.20±1.47e
Root number	27.33±1.15a	16.33±0.58b	9.00±0.58c	5.00±0.00d	1.67±1.15e
Maximal root length (cm)	35.3±1.20a	24.4±1.37b	12.60±1.37c	7.60±2.72d	2.53±1.53e
Dry biomass of aboveground part (g)	12.18±0.17a	9.27±0.43b	8.05±0.43c	5.43±0.20d	2.59±0.45e
Dry biomass of root (g)	4.91±0.17a	3.54±0.14b	2.07±0.14c	1.15±0.25d	0.32±0.11e

Note: data in the table are mean ± SD, different letters in the same row represent significance at P < 0.05.

significantly higher than control and other two treatments, Pb400Cu200 and Pb800Cu500 (P < 0.05). On the contrary, Pb400Cu200 and Pb800Cu500 have inhibited the synthesis of photosynthetic pigments of *M. floridulus*.

Effects of Combined Pb-Cu Treatments on MDA Content of *M. floridulus*

The MDA content in *M. floridulus* leaves decreased at lower combined Pb-Cu treatment, but increased at higher Pb-Cu concentration (P < 0.01) (**Figure 1**). In general, MDA content in *M. floridulus* leaves was slightly reduced by Pb100Cu50 treatment (P > 0.05). When the combined Pb-Cu concentrations were above Pb200Cu100, MDA content was increased with increasing concentration of combined Pb-Cu treatments. MDA content at the Pb800Cu500 was 2.38 times higher than that of control.

Effects of Combined Pb-Cu Treatments on Soluble Sugar Content in *M. floridulus*

Soluble sugar content is an important parameter in response to environmental stress. The soluble sugar content of *M. floridulus* leaves reduced at lower Pb-Cu concentration but increased at higher Pb-Cu concentration (P < 0.01) (**Figure 2**). Soluble sugar content in *M. floridulus* slightly decreased at Pb100Cu50 treatment, but those at Pb200Cu100, Pb400Cu200 and Pb800Cu500 were significantly increased compared with the control and Pb100Cu50 treatment. Pb800Cu500 caused thesignificant increase of the soluble sugar content in *M. floridulus*, which was 1.53 times higher than the control.

Effects of Combined Pb-Cu Treatment on the Antioxidant Enzyme Activities of *M. floridulus*

Antioxidant enzyme system consists of a series of enzymes that scavenge reactive oxygen species resulting from bioactive or abiotic stress. The combined Pb-Cu stress had a significant effect on the antioxidant enzyme activities in *M. floridulus* leaves (P < 0.05) (**Table 4**). The SOD and POD activities in *M. floridulus* decreased with increasing Pb-Cu concentrations. The lowest SOD and POD activities occurred at Pb800Cu500 treatment, which was 82.59% and 54.10% of the control. CAT activity increased at Pb100Cu50 treatment, but the activity decreased with increasing Pb-Cu concentration(P < 0.05). At Pb800Cu500 treatment, CAT activity was decreased by 69.13% compared with the control (P < 0.05).

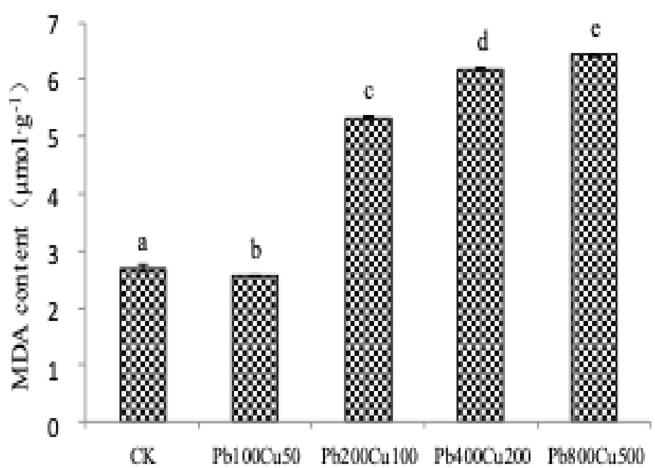

Figure 1. Effects of combined Pb-Cu treatment on MDA content in *M. floridulus*.

Table 3. Effects of combined Pb-Cu treatments on photosynthetic pigment content of *M. floridulus*.

Photosynthetic pigment	Combined Pb-Cu concentrations (mg kg^{-1})				
	CK	**Pb100Cu50**	**Pb200Cu100**	**Pb400Cu200**	**Pb800Cu500**
Total Chlorophyll (mg kg^{-1})	1.45±0.18b	1.78±0.00a	1.70±0.00a	0.75±0.01c	0.61±0.00d
Carotenoid (mg kg^{-1})	0.26±0.08b	0.39±0.01a	0.35±0.00a	0.20±0.00c	0.01±0.00d

Note: data in the table are mean ± SD, different letters in the same row represent significance at P < 0.05.

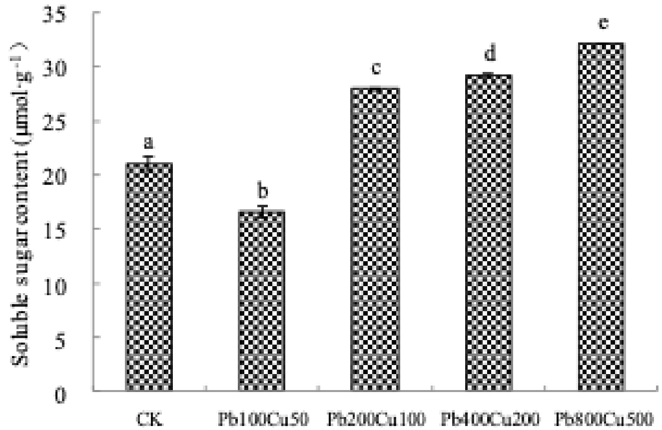

Figure 2. Effects on combined Pb-Cu stress on soluble sugar content in *M. floridulus*.

Effects of Combined Pb-Cu Stress on Rhizospheric Soil Enzyme Activities of *M. floridulus*

The activities of rhizospheric soil enzymes (phosphatase, invertase, urease and catalase) increased at lower Pb-Cu treatments, but decreased at higher Pb-Cu treatments (**Table 5**). The rhizospheric soil enzyme activities were negatively correlated with Pb-Cu concentrations ($P < 0.01$). In general, the correlation coefficients for CAT, phosphatase, invertase and urease were -0.876, -0.895, -0.908 and -0.909, respectively. The activities of phosphatase, invertase and urase increased at Pb400Cu200 treatment, but decreased at Pb800Cu500 treatment. CAT activity was the highest at Pb100Cu50 treatment, and the activities at other treatments were all significantly lower than the control.

Based on the results, Pb200Cu100 stress caused slight foliar injury in *M. floridulus*. Although foliar injury was enhanced at other higher Pb-Cu treatments, *M. floridulus* could survive through the sixty-day Pb-Cu exposure, suggesting that this plant is tolerant, to a certain degree of combined Pb-Cu contamination. Y*ang et al.* (*2014*) has reported that *M. floridulus* was tolerant to 200 mg kg^{-1} of Cu. Others also reported that *M. floridulus* could accumulate and was tolerant to Cd, Pb, Zn, Ni and Mn (*Ren et al. 2006; Sun et al. 2006*).

Photosynthetic pigments are fundamentals upon which plants grow and survive. The study found that at Pb100Cu50 and Pb200Cu100 treatments the total contents of chlorophyll and carotenoid in *M. floridulus* were statistically higher than control, but the photosynthetic pigments content decreased at higher Pb-Cu concentrations. The study thus demonstrated that *M. floridulus* can grow on moderately contaminated sites. *M. floridulus* is a heavy metal-tolerant plant species. *Qin et al.* (*2010*) reported that the net photosynthetic rate and actual photosynthetic rate for metal-tolerant ecotypes of *M. floridulus* decreased but slower than the conspecific intolerant plants in the case of environmental stress, and the structure and function of the photosynthetic apparatus was only slightly damaged. The decreased chlorophyll contents of *M. floridulus* may be due to lipid peroxidation of chloroplast and thylakoid membranes and chlorophyll degradation mediated by excess Pb and Cu stress (*Vinti-Dunand et al. 2002*).

Despite reduced total chlorophyll content and small shoot dry weight as suggested by the results, roots and rhizomes grew quite well and were able to support and enhance nitrification processes. Similarly, in the

Table 4. Effects of combined Pb-Cu stress on plant antioxidant enzyme activities of *M. floridulus*.

Antioxidant enzymes	Combined Pb-Cu concentrations (mg kg^{-1})				
	Pb0Cu0	**Pb100Cu50**	**Pb200Cu100**	**Pb400Cu200**	**Pb800Cu500**
SOD (g^{-1} FW min^{-1})	453.43±5.14a	420.24±5.10b	398.39±6.78c	384.35±6.46d	374.48±6.70d
POD (g^{-1} FW min^{-1})	101.95±6.60a	97.60±0.23a	79.85±0.68b	61.20±1.73c	55.15±0.53d
CAT mL $KMnO_4$ mg^{-1} FW	0.05±0.01ab	0.06±0.01a	0.04±0.00b	0.03±0.00c	0.02±0.00d

Note: data in the table are mean ± SD, different letters in the same row represent significance at $P < 0.05$.

Table 5. Effects of combined Pb-Cu treatment on rhizospheric soil enzyme activities.

Soil enzymes	Combined Pb-Cu concentrations (mg kg^{-1})				
	Pb0Cu0	**Pb100Cu50**	**Pb200Cu100**	**Pb400Cu200**	**Pb800Cu500**
Catalase activity	3.84±0.01e	4.65±0.02a	4.42±0.02c	4.48±0.03b	4.12±0.02d
phosphatase activity	22.13±0.02d	24.74±0.07c	25.65±0.05b	32.94±0.16a	21.49±0.37e
Invertase activity	6.36±0.02d	7.72±0.03c	8.39±0.04b	8.92±0.02a	6.30±0.07d
Urease activity	5.13±0.03d	6.26±0.21c	8.31±0.52b	9.44±1.79a	5.13±0.03d

Note: data in the table are mean ± SD, different letters in the same row represent significance at $P < 0.05$. Catalase activity/(0.1mol $L^{-1}KMnO_4$ mL^{-1}), phosphatase activity/(mg $100g^{-1}$ $2h^{-1}$), Invertase activity/(Glucose mg g^{-1} $24h^{-1}$), Urease activity/(NH_3^+-N mg g^{-1} $24h^{-1}$).

monitoring study of a Cu mining and tailing site in China, plant communities including *M. floridulus* and *M. sinensis* were able to restore several parameters (increased pH, soil structure, and microbial diversity including nitrogen-fixing proteobacteria, organic matter, and water contents) of heavy metal-contaminated soils (*Zhan and Sun 2011*).

The MDA is an reliable biomarker of membrane peroxidation and its content reflects the degree of membrane damage. Pb100Cu50 stress has enhanced *M. floridulus* tolerance to environmental stress, i.e. slight reduction of MDA content at lower Pb-Cu stress. But high Pb-Cu concentrations could destroy plant membrane integrity as shown by high MDA content. (*Hui et al. 2013*) found that long-term Cd exposure had increased the degree of electrolyte leakage and MDA content of *Zea mays,* var CT38. Also MDA content was consistent with the enhanced degree of apparent foliar injury of *M. flordulus* in this study.

The study object is a metal tolerant ecotype collected at trace-element enrichment mine tailings. These plants have well-developed tolerance mechanisms to abiotic stress, i.e., low translocation factor (TF) and low bioconcentration factor (BCF). According to *Liu et al.* (*2014*), the TF of Pb and Cu in *M. floridulus* is 0.90 and 2.10, respectively and the BCF for Pb and Cu is 0.44 and 0.41. It was also reported that Pb content in the aboveground part and root of *M. floridulus* collected at Pb/Zn tailings were 1102.43 mg kg^{-1} and 1225.74 mg kg^{-1}, respectively (*Wang et al. 2010*). This may explain why *M. floridulus* can survive after 60-day Pb-Cu exposure. Therefore, *M. floridulus* is believed to be a super-tolerant plant species suitable for the management of a trace element-enriched system (*Wang et al. 2010; Qin et al. 2011b*).

Abiotic stress usually induces changes in the soluble sugar contents of plants. Thus, the soluble sugar content is an important index of plant response to environmental stress. This study found out that Pb100Cu50 treatment decreased the soluble sugar content in *M. floridulus* leaves, but other Pb-Cu treatments had primed for the synthesis of soluble sugar content. This finding agrees with previous studies that about the response of *M. floridulus* to Cu stress (*Yang et al. 2014*). Populations of *M. floridulus* collected from contaminated sites are more tolerant to Pb and Cd than those collected from uncontaminated sites (*Hsu and Chang 1992*). This may explain the enhanced production of soluble sugar content in *M. floridulus* leaves at high Pb-Cu treatments.

In plants, the production of a large amount of reactive oxygen species and the resulting generation of antioxidant enzymes are among the best-known and earliest aspects of heavy metal toxicity. An activation of antioxidant enzymes (catalase, superoxide dismutase and peroxidase) can be observed both in the roots and leaves of the challenged plants. Avoidance of metal accumulation and distribution may constitute a homeostatic mechanism to avoid acute toxicity. The phytotoxic symptoms in *M. floridulus* induced by Cd include oxidative stress as indicated by the activation of antioxidant enzymes and lipid peroxidation (*Yang et al. 2014*). Similar results were also observed at lower Pb-Cu treatment in the present study. But high Pb-Cu concentration may destroy the antioxidant enzyme system of plants.

The colonization of *M. floridulus* at Pb-Zn tailings can improve the content of organic carbon, total N and P, available P, stability of soil granule, maximal water-holding capacity and soil enzyme activities (*Zhang et al. 2009*). Pb100Cu50 and Pb200Cu100 have induced the activities of rhizospheric urease, invertase and phosphatase, but their activities were reduced at Pb400Cu200 treatment (**Table 5**).

The inhibition of rhizospheric soil enzyme activities at high Pb-Cu treatment (Pb800Cu500) may be explained by the inhibited growth and reproduction of bacteria, the inactivation of the active site of soil enzymes or both. The results were consistent with the findings reported by *Tang et al.* (*2022*) and *Shi and Ma* (*2017*). Similarly, an ecoenzymatic stoichiometry analysis showed a close relation between microbial metabolism with heavy metals contamination (*Duan et al. 2022*).

Cell wall binding, vacuolar compartmentalization, accumulation and sequestration in biologically less active organelles are the primary tolerance mechanism of metallophytes to Cd (*Qin et al. 2009*), similar to all of the hyper-accumulator plants. These mechanisms are possibly involved with the formation of inert metal-organic acid complexes in apoplasts and vacuoles (*Lyubenova et al. 2013*). Also, the well-developed root system of *M. floridulus* is associated with increased rhizodeposition, which supplies nutrients and energy source for some trace element-tolerant microorganisms, thereby increasing the heavy metal-binding surface within the rhizosphere (*Neagoe et al. 2013*). Therefore, *M. floridulus* is favorable for phytostabilization and phytodegradation of contaminated sites.

CONCLUSIONS AND RECOMMENDATIONS

M. floridulus is a moderate tolerant plant to Cu and Pb in terms of its photosynthetic pigments, antioxidant enzyme activities and its orchestration with soil enzymes. Considering its fast growth, high biomass, tolerance to multiple heavy metals and well-developed root, *M. floridulus* is candidate plant for the phytostabilization and rehabitation of heavy metal contaminated sites.

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