

RESTORATION OF Cu²⁺-CONTAMINATED PURPLE SOIL BY APPLYING YEASRACT AND INTERPLANT

LI, W. B.^{1,2*} – HE, H. X.¹ – DENG, H. Y.^{1,2} – KANG, L.¹ – YANG, D. Q.¹ – ZHUANG, Q.¹ – MENG, Z. F.^{3,4} – LI, X. L.¹ – LI, Y. X.^{1,2}

¹College of Environmental Science and Engineering, China West Normal University, No.1, Shida Road, Shunqing District, Nanchong, Sichuan 637009, China

²Institution of Environmental Science, China West Normal University, Nanchong, Sichuan 637002, China

³Key Laboratory of Plant Nutrition and Agri-Environment in Northwest China, Ministry of Agriculture, Yangling 712100, China

⁴Department of Natural Resource and Environment, Northwest A&F University, Yangling 712100, China

*Corresponding author

e-mail: lwb062@hotmail.com; phone: +86-0817-2568-455; fax: +86-0817-2568-455

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Abstract. Beer yeast powder was added to purple soil (PS) at a 1‰ mass ratio to prepare mixed soil samples (PS_B) to investigate the enhancement effect of fungus in the restoration of Cu²⁺-polluted PS by sudangrass (*Sorghum sudanense* (Piper) Stapf.) and the intercropping of sudangrass and calliopsis (*Cosmos bipinnata* Cav.). Then, indoor potted experiments were performed to explore the physiological indices and Cu²⁺ accumulation in sudangrass and calliopsis in different Cu²⁺-polluted PS and PS_B. Three key results were observed. (1) PS_B promoted the germination rate of sudangrass but inhibited that of calliopsis; the germination rates of sudangrass planted alone were higher than those of the intercropped ones under the same Cu²⁺ pollution concentration. Sudangrass grew well on PS_B, and its height was higher than that of calliopsis under intercropping conditions. (2) The aboveground and underground biomasses of sudangrass planted in PS_B by monoculture were higher than those by intercropping. The correlation of plant physiological indices in PS_B was higher than that in PS. (3) Cu²⁺ content in roots was higher than that in cornus. Soil polluted with 75 mg/kg Cu²⁺ was beneficial to the enrichment of plant, and the translocation coefficient was the largest in PS_B.

Keywords: beer yeast powder, plant intercropping, phytoremediation, physiological characteristics, contamination accumulation

Introduction

The discharge of livestock raising, industrial, and agricultural wastewater has caused serious heavy metal pollution of soil (Li et al., 2017; Barsova et al., 2019). The pollution control work became a difficult and important topic in the current research (Deng et al., 2020). Soil microbes can not only promote plant growth but also enhance the absorption of heavy metal ions by pollution-repairing plants (Liu et al., 2013). Therefore, adding microbes to the soil to improve the plants' remediation ability bears significance for the research of combined plant-microorganism remediation of soil pollution.

Phytoremediation has been the focus of studies and applied by many researchers due to its low cost and easy operation (Zheng and Yuan, 2017; Acosta et al., 2018). Seed germination rate and plant growth are promoted under low-concentration copper pollution (Li et al., 2018), but when Cu²⁺ levels in the soil exceeds a certain load, the growth of

plants will be seriously affected (Jin et al., 2012; Fu et al., 2016). *Elsholtzia splendens* Nakai and *Commelina communis* L. show high tolerance to Cu²⁺-contaminated soil, whereas *Commelina communis* L. can normally grow in soil with a Cu²⁺ concentration of 500 mg/kg (Liu et al., 2006, 2014). In the phytoremediation of Cu²⁺-contaminated soils, biomass directly determines the efficiency of restoration (Peng and Yang, 2005), whereas root exudates promote the root absorption of Cu²⁺ (Xu et al., 2017). Sudangrass (*Sorghum sudanense* (Piper) Stapf.) grows vigorously, has well-developed roots, and shows strong adaptability and resilience (Jia et al., 2014); thus, it is widely used in soil remediation and pollution control. The Cu²⁺ accumulation of sudangrass root reaches 673 mg kg⁻¹ (Wang, 2014). Cropping patterns have observable effect on plant biomass and its adsorbability to heavy metals (An et al., 2011; Singh et al., 2012; Li et al., 2013; Wei et al., 2015), whereas intercropping can change the types and quantity of rhizospheric microorganisms (Nai et al., 2013), soil enzyme activity in the rhizosphere (Zhang et al., 2015), plant growth, and nutrient conditions and ultimately influence the absorption effect of heavy metals by plants (Su et al., 2010). Therefore, adjusting the planting pattern is one of the important ways to improve the effects of phytoremediation.

Microbial remediation technology has become an important topic in the treatment of heavy metal pollution (Xue et al., 2012; Jacob et al., 2018). Microorganisms present a good adsorption effect on heavy metal ions with the advantages of large specific surface area, rapid reproduction, and strong metabolic capacity (Cao et al., 2016), and their repair mechanisms mainly include biosorption and biotransformation (Xue et al., 2012). Cao and Cheng (2004) reported that at low Cu²⁺ concentration (≤ 5 mg/L) in the soil environment, microorganisms exhibited a good repair performance for Cu²⁺ pollution, and the removal (consolidation) rate reached 25%–60%. Stanila et al. (2016) noted that beer yeast has a high adsorption capacity and adsorption rate for copper ions, and the adsorption equilibrium can be reached in 10 min. Studies demonstrated that the plant-microbial repair system is beneficial to the remediation of heavy-metal contaminants in soil (Li et al., 2015).

The phytoremediation, microbial remediation, and combined repair of Cu²⁺ in soil all achieve good outcomes. The advantage of intercropping can be further exploited if the combined microorganism–plant repair is optimized. The repair effect of Cu²⁺ can be improved to a great extent due to the no-increase repair cost. Minimal exploration was conducted in this area. In this paper, beer yeast was used to strengthen the intercropping of sudangrass and calliopsis (*Cosmos bipinnata* Cav.) for repairing different degrees of Cu²⁺ pollution of purple soil (PS). By measuring the changes in physiological characterization of sudangrass and calliopsis under different Cu²⁺ conditions and analyzing the combined repair effect of applying yeasract and interplant on Cu²⁺ pollution, this study can provide a reference for microorganism–plant intercropping to repair heavy metal-contaminated soil.

Materials and methods

Experimental materials

Potted soil (PS; a kind of soil formed on weathered materials of purple rock, and it maintains the physical and chemical properties of the parent material) was collected from the test field of China West Normal University. After multi-point sampling, the soil samples were mixed evenly, dried, and crushed, and the physicochemical properties of the soil samples were measured. *Table 1* shows the results.

Table 1. Basic properties of the test soil

Soil layer (cm)	pH value	CEC (mmol/kg)	TOC content (g/kg)	Cu ²⁺ content (mg/kg)
0~30	8.08	288.46	16.66	18.60

The fungus powder was tested with beer yeast, which is a yellow-brown powder and granule with more than 60 million active bacteria per gram and less than 10% water content. The beer yeast was purchased from Guangzhou Pengxiang Agriculture Co., Ltd.

The test plants were sudangrass and calliopsis. The seeds with impurities, worm corrosion, and low maturity were discarded. A total of 960 full seeds of sudangrass and 120 full seeds of calliopsis were selected and soaked in 1% potassium permanganate solution for 15 min, rinsed with tap water, and three times by deionized water, and finally dried with a filter paper.

Copper pollution concentration was expressed as Cu²⁺; the reagent was analytically pure CuSO₄·5H₂O and purchased from Xilong Chemical Industry Co., Ltd.

Experimental design

Beer yeast powder (B) was mixed with PS at a mass ratio of 1‰ to form the amended soil sample (PS_B). Three groups of indoor pot experiment of PS-S (single sudangrass planted on PS), PS_B-S (single sudangrass planted on PS_B), and PS_B-M (sudangrass and calliopsis intercropping planted on PS_B) were implemented. Among PS_B-M, PS_B-MS (sudangrass in sudangrass–calliopsis intercropping) and PS_B-MC (the calliopsis in sudangrass–calliopsis intercropping) were measured separately.

The Cu²⁺ content of soil in the pot experiments was set up for four treatments: 0, 75, 100 and 125 mg/kg soil. Each experiment was repeated thrice.

Plastic pots with a diameter of 43 cm, a width of 15.5 cm, and a height of 11 cm were loaded with 2.0 kg soil samples. Then, 500 mL Cu²⁺ solutions of different concentrations (0, 300, 400, and 500 mg/L) were added to the 36 pots in order. After the Cu²⁺ solution was permeated uniformly, 30 plant seeds (the number of sudangrass and calliopsis were 20 and 10 in the intercropping experiment, respectively) were sown uniformly at a depth of 2 cm below the soil surface of each pot. Then, the pots were watered at 100 mL volume per week after germination (four times in total). During the growth period, the germination rate and plant height of sudangrass and calliopsis were measured, and the biomasses of aboveground and underground parts were measured after harvest.

Experimental method

Determination of germination rate and plant height

The germination rates of sudangrass and calliopsis seeds and the number of seed germination was recorded every day. When the number of germinating seeds remained unchanged for two consecutive days, the germination was considered complete, and the germination rate was computed. The formula for germination rate is:

$$\text{Germination rate} = (\text{germination number of seeds} / \text{total number of seeds}) \times 100\% \quad (\text{Eq.1})$$

The average plant height of sudangrass and calliopsis was measured every 3 days after germination (10 times in total). The plant height of all plants (sudangrass and calliopsis)

was measured separately. Then, the highest and lowest 10% scores were removed, and the average value of the remaining plants was calculated to obtain the average plant height.

Determination of biological indicator

All the plants were harvested after 30 days of growth. Then, the plant samples were divided into aerial (stems and leaves) and underground parts (roots). The soil and dirt that attached to the plant samples were thoroughly rinsed off with tap water, followed by rinsing with distilled water for three times. Then, the water was filtered out, and the fresh weight was determined. After weighing, the samples were individually loaded into a kraft bag and cured in an oven at 105 °C for 30 min and then dried to constant weight at 85 °C. The dry weight of the aerial parts and root parts was determined.

Determination of Cu²⁺ in plants

The leaves and root samples of the dried plant were grinded and passed through a 100-mesh nylon sieve. Cu²⁺ contents in plant samples were determined via Hitachi Z-5000 (Japan) flame atomic absorption spectrophotometry, and background absorption was corrected through Zeeman effect.

Data processing

SPSS 16.0 statistical analysis software was used to process the experimental data for variance and correlation analysis. SigmaPlot 10.0 software was adopted to improve data plotting. The data were expressed as the means with standard deviation, and different letters indicate significant differences among various amendments. Analysis of variance was performed to determine the effects of amendments, followed by Tukey's honestly significant difference test. Differences of $p < 0.05$ were considered significant.

Results and discussion

Effect of Cu²⁺ on the germination rate

Figure 1 shows the germination rate of plant seeds under different Cu²⁺-contaminated soils. With the increase in Cu²⁺ concentration, the seed germination rates decreased in PS-S and PS_B-MC, whereas it increased first and then decreased in PS_B-S and PS_B-MS. When the concentration of Cu²⁺ was 0 mg/kg, the germination rate of PS-S was the highest (90%), that is, it was 1.13 times higher than that of other treatments. When the concentration of Cu²⁺ increased to 75 mg/kg, the seed germination rate of PS_B-S and PS_B-MS increased by 10.00% and 5.00% compared with 0 mg/kg, respectively. Meanwhile, the PS-S and PS_B-MC decreased by 20.00% and 10.00% compared with 0 mg/kg, respectively. When the concentration of Cu²⁺ was 100 mg/kg, the PS_B soil showed a strong promoting effect on the seed germination of sudangrass (PS_B-S and PS_B-MS), and the germination rate increased by 16.67% and 15.00% compared with that of 0 mg/kg. However, the germination rate decreased under the 125 mg/kg Cu²⁺ treatment.

Under the same Cu²⁺ concentration, the plant germination rate was expressed as PS_B-S > PS_B-MS > PS-S > PS_B-MC. When Cu²⁺ concentration was 0–125 mg/kg, the germination of sudangrass was promoted, but calliopsis was inhibited in PS_B. In general, PS_B soil samples had a promoting effect on the seed germination of sudangrass and inhibiting effect on calliopsis.

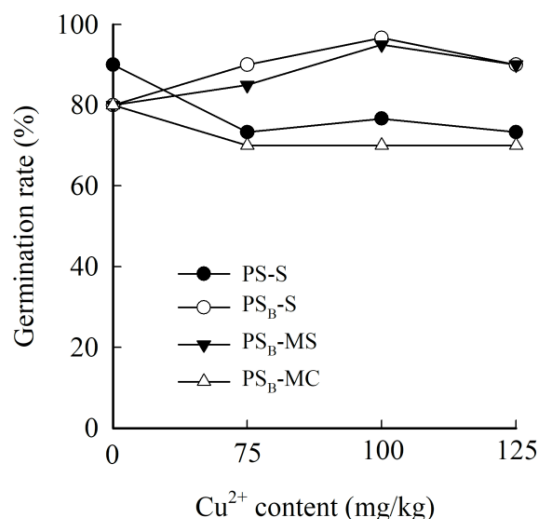


Figure 1. Effect of Cu²⁺ content on the germination rate of the studied plants

The germination of plant seeds is affected by pollution stress. Low concentration of pollution stress can improve the germination rate of plant seeds. With the increase in pollution concentration, the inhibiting effect of seed germination will gradually increase until germination cannot be achieved (Salian et al., 2018; Li et al., 2018). The addition of beer yeast can increase the microbial activity in soil, and at the same time, microorganisms can metabolize certain pollutants (Riaz-ul-Haq and Shakoory, 2000; Liu et al., 2013), thus reducing the inhibitory effect of pollutants on seed germination rate. Therefore, for PS-S treatment, 75 mg/kg Cu²⁺ treatment inhibited the germination rate of sudangrass, whereas for PS_B-S and PS_B-MS, the germination rate increased continuously with the Cu²⁺ content from 0 mg/kg to 100 mg/kg and reached the maximum under 100 mg/kg treatment, indicating that the addition of beer yeast played a role in promoting seed germination. Cu²⁺ inhibited the seed germination rate of calliopsis planted by intercropping, indicating the existence of inter-species survival competition (Sofa et al., 2013), which is consistent with the results of previous studies.

Effect of Cu²⁺ on plant height

Figure 2 shows that after germination (3 days later), the plant height gradually increased with prolonged growth period. The plant height of PS-S increased rapidly within 6 days after germination, with an average daily growth of 1.64 cm and a maximum of 1.98 cm. However, the growth rate was slow at 9–30 days and remained stable after the 15th day. The growth rate of PS_B-S increased the fastest with the increase in growth days and reached a maximum of 26.2 cm. For PS_B-M, the height of PS_B-MS was significantly higher than that of PS_B-MC. The plant height treatments were higher in 0 and 75 mg/kg Cu²⁺ than that in 100 and 125 mg/kg Cu²⁺. At different concentrations of Cu²⁺, sudangrass grew well on PS_B soil samples, and the height of calliopsis was lower than that of sudangrass under intercropping conditions, which indicates that beer yeast can promote the growth of sudangrass. The plant height and germination rate of the two plants had a similar relationship. After the addition of beer yeast to soil samples, the plant height of sudangrass significantly increased, but that of intercropping plants was inhibited.

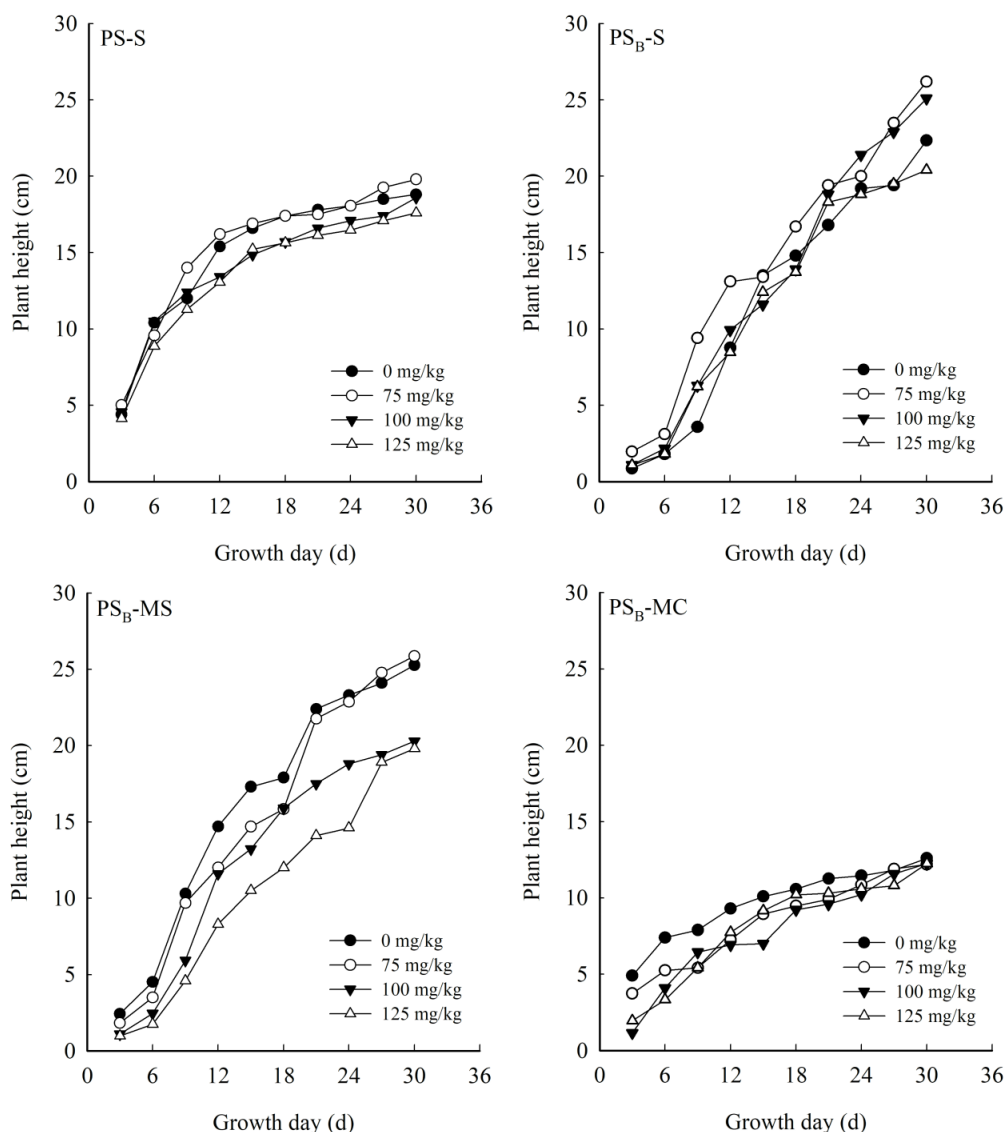


Figure 2. Average plant height of sudangrass and calliopsis in 30 days

Fresh and dry weights of aerial parts

The fresh and dry weights of PS-S, PS_B-MS and PS_B-MC increased first and then decreased with the increase in Cu²⁺ content of potted soil. The values reached the maximum at 75 mg/kg Cu²⁺ and 1.34–1.36 (PS-S), 1.43–1.58 (PS_B-MS), and 2.11–2.40 (PS_B-MC) times higher than that of 0 mg/kg Cu²⁺ (Fig. 3). The fresh and dry weights of PS_B-S showed a fluctuating trend with the increase in Cu²⁺ content. When the content of Cu²⁺ was 100 mg/kg, the fresh and dry weights of PS_B-S were the highest at 4.97 and 0.64 g, respectively, and significantly differed from those of other treatments ($p < 0.05$).

For the single-planting experiments, PS_B promoted the aboveground biomass growth of sudangrass, and the maximum biomass was reached at the Cu²⁺ content of 100 mg/kg. For the intercropping plants, the maximal promotion of the aboveground biomass was recorded at the Cu²⁺ content of 75 mg/kg. In summary, the aboveground biomass of sudangrass planted in PS_B by monoculture was higher than that planted by intercropping.

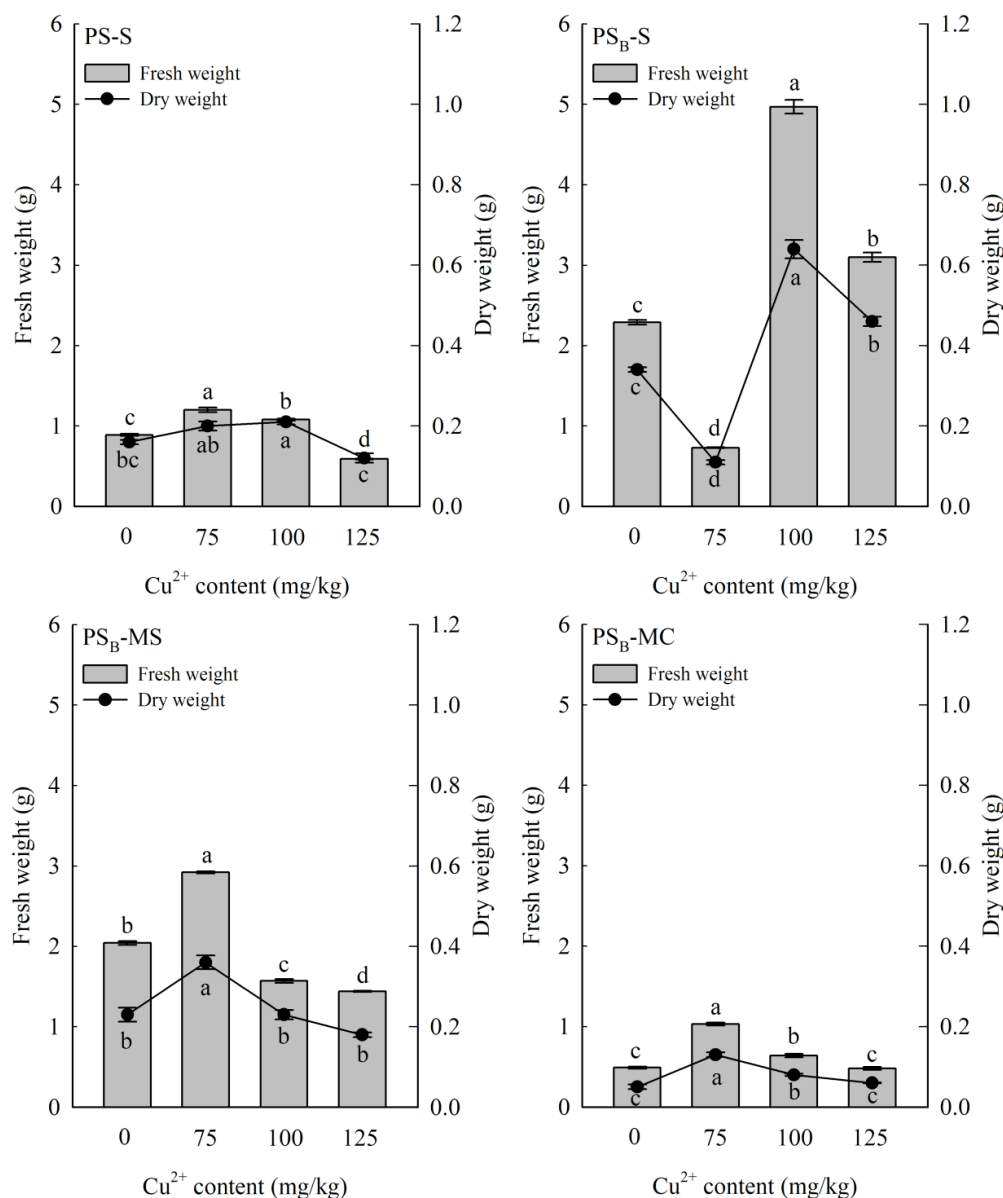


Figure 3. Fresh and dry weights of aboveground parts of sudangrass and calliopsis. Note: Different lowercase letters indicate significant difference under various treatments at 0.05 levels (the same meaning of different lowercase letters in the following figures)

Plant biomass is closely related to the overall growth status. Except for PS_B-S, the biomass of plants under 75 mg/kg treatment was high on the whole, indicating that low-concentration pollution stress is conducive to the accumulation of plant biomass. The biomass of PS_B-S was the lowest at 75 mg/kg Cu²⁺ and increased significantly at 100 mg/kg Cu²⁺. This result is related to the tolerance of microorganisms to pollutants, and it is consistent with the research results by Boiko et al. (2020).

Fresh and dry weights of underground parts

With the increase in Cu²⁺ content, the root fresh and dry weights of PS-S and PS_B-S increased, reached the peak value at 100 mg/kg Cu²⁺, and then decreased at 125 mg/kg

Cu²⁺ (Fig. 4). The fresh and dry weights of roots of PS_B-MS and PS_B-MC were the highest under the 75 mg/kg Cu²⁺ treatment. A corresponding relationship was observed between the plant's root fresh and dry weights at different Cu²⁺ concentration treatments. The fresh and dry weights of intercropped sudangrass were significantly higher than those of calliopsis, indicating that sudangrass dominated the competition, and its root system had strong adaptability to Cu²⁺-polluted PS. However, the plant roots grew poorly under Cu²⁺ concentration of 100–125 mg/kg, indicating that high concentrations of Cu²⁺ inhibited plant root growth. At 75 mg/kg Cu²⁺, the intercropped plants showed better growth and stronger adaptability, but their underground biomass was still inhibited compared with the monoculture of sudangrass.

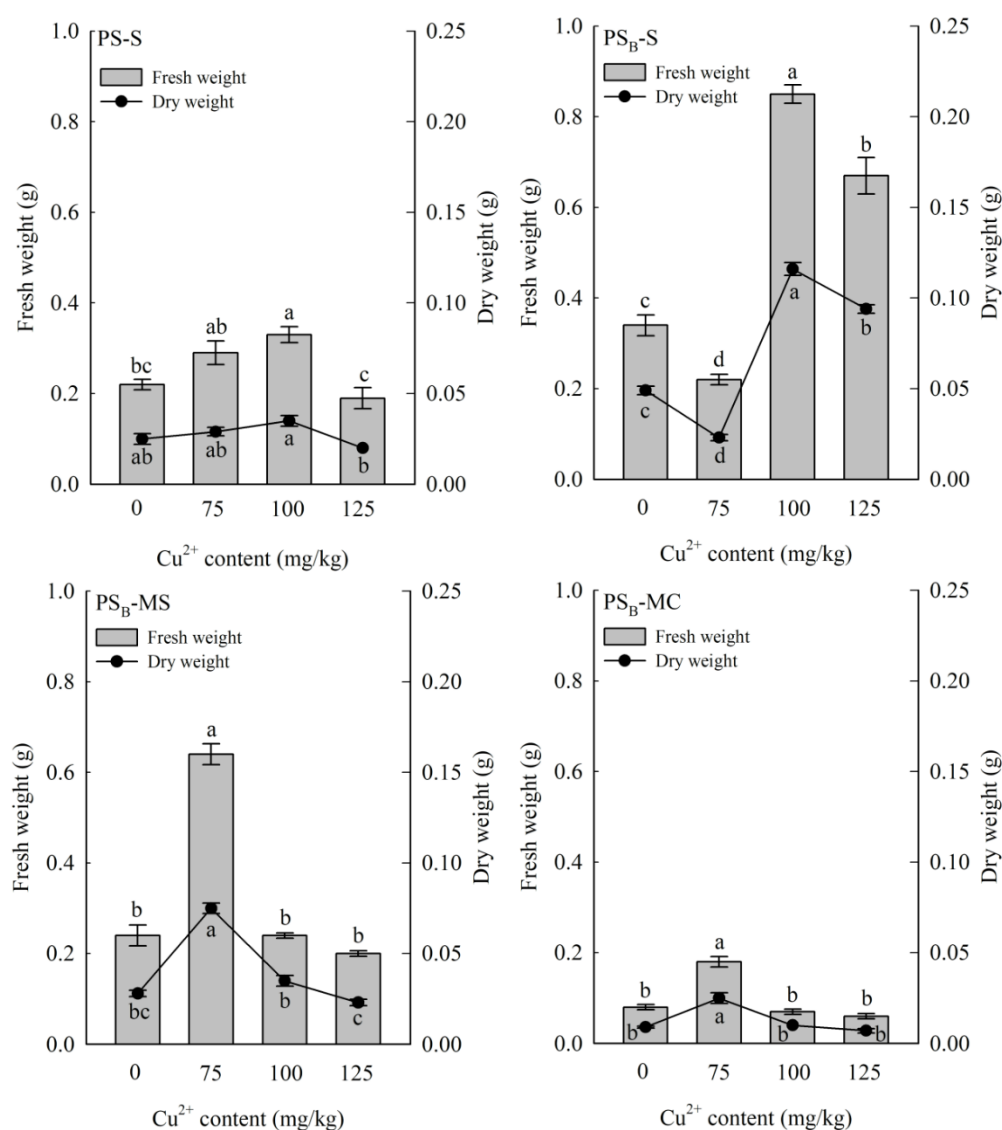


Figure 4. Fresh and dry weights of roots of sudangrass and calliopsis

Compared with the aboveground plant parts, plant roots are directly exposed to pollutants and have a higher tolerance to pollution. The root biomass was the largest under the treatment of 100 mg/kg Cu²⁺ for sudangrass monocultures. For the intercropped plants,

the two kinds of plant roots were influenced not only by pollution tolerance but also their competition relationship. The root biomass of intercropped plants was the largest under 100 mg/kg Cu²⁺ treatment.

Correlation analysis of physiological indices

Table 2 shows the positive correlation between various physiological indicators of sudangrass and calliopsis. No significant correlation was observed between plant height and other indicators, whereas a significant correlation was noted between dry and wet biomass. As for PS-S, the aboveground dry weight was significantly related to the root fresh and dry weights. For PS_B-S, PS_B-MS and PS_B-MC, a significant correlation existed between any two of the aboveground fresh weight, aboveground dry weight, root fresh weight, and root dry weight. An extremely significant correlation was recorded between the aboveground and root dry weights of PS_B-MS and between the aboveground fresh weight and root dry weight of PS_B-MC. The overall correlation of plant physiological indices in PS_B-S was higher than that in PS. For the same plant, the change in root and aboveground biomass was closely related to plant pollution tolerance. Therefore, compared with PS, PS_B showed a higher correlation between the aboveground and underground plant parts, which also indicates that microorganisms promoted the tolerance of plants to pollution.

Table 2. Correlation analysis of physiological indexes of sudangrass and calliopsis

Correlation coefficients		Plant height (cm)	Above ground fresh weight (g)	Above ground dry weight (g)	Root fresh weight (g)	Root dry weight (g)
PS-S	Plant height (cm)	1	0.5547	0.2513	0.0904	0.0593
	Above ground fresh weight (g)		1	0.9010*	0.7372	0.6442
	Above ground dry weight (g)			1	0.9525*	0.8885*
	Root fresh weight (g)				1	0.9560*
	Root dry weight (g)					1
PS _B -S	Plant height (cm)	1	0.1585	0.2455	0.1209	0.1926
	Above ground fresh weight (g)		1	0.9861**	0.9156*	0.9316*
	Above ground dry weight (g)			1	0.9198*	0.9531*
	Root fresh weight (g)				1	0.9897**
	Root dry weight (g)					1
PS _B -MS	Plant height (cm)	1	0.5340	0.3605	0.2220	0.2122
	Above ground fresh weight (g)		1	0.9032*	0.8807*	0.8271*
	Above ground dry weight (g)			1	0.9509*	0.9725**
	Root fresh weight (g)				1	0.9778**
	Root dry weight (g)					1
PS _B -MC	Plant height (cm)	1	0.0497	0.1243	0.0010	0.0033
	Above ground fresh weight (g)		1	0.9795**	0.9231*	0.9706**
	Above ground dry weight (g)			1	0.8508*	0.9109*
	Root fresh weight (g)				1	0.9865**
	Root dry weight (g)					1

Note: ** or * indicates that the correlation coefficient is significant at $p=0.01$ or $p=0.05$ level ($r=0.959$ or $r=0.878$ when the degree of freedom $f=3$)

Changes in Cu²⁺ Content of Plant Samples

Table 3 shows the content of Cu²⁺ in plant cormus (leaves and stems) and roots under different soil treatments. Cu²⁺ content in the cormus and roots of plants increased with the increase in soil Cu²⁺ concentration, with the maximum values reaching 8.82 (cormus in PS_B-MC) and 30.15 mg/kg (roots in PS_B-MC). Cu²⁺ content in the root was higher than that in the cormus, which indicates that the plant root system was more likely to enrich Cu²⁺; this result was consistent with the findings of Wang et al. (2014). The enrichment coefficient of cormus and roots was the largest under the soil treatment of 75 mg/kg Cu²⁺, indicating that the 60-day-grown plants were more capable of enriching low-concentration pollution. The enrichment coefficient showed the trend of PS_B-MC > PS_B-MS > PS_B-S > PS-S of cormus, whereas the trend was PS_B-MC > PS_B-S > PS_B-MS > PS-S for the roots. The largest translocation coefficient was observed on PS_B-MC, indicating that calliopsis was more capable of Cu²⁺ transport than sudangrass.

Table 3. Remediation effect of Cu²⁺ on potted plants

Treatment mg/kg		Overground part (cormus)		Underground part (roots)		Translocation coefficient (%)
		Cu ²⁺ content (mg/kg)	Enrichment coefficient (%)	Cu ²⁺ content (mg/kg)	enrichment coefficient (%)	
PS-S	75	2.60	3.47	14.82	19.76	17.54
	100	2.71	2.71	18.90	18.90	14.34
	125	2.76	2.21	19.69	15.75	14.02
PS _B -S	75	4.85	6.47	20.60	27.47	23.54
	100	5.05	5.05	21.81	21.81	18.57
	125	5.88	4.70	23.48	18.78	16.52
PS _B -MS	75	6.30	8.40	20.76	27.68	30.35
	100	6.56	6.56	24.74	24.74	24.09
	125	7.35	5.88	30.15	24.12	17.74
PS _B -MC	75	7.31	9.75	18.86	25.14	39.13
	100	8.07	8.07	23.68	23.68	34.12
	125	8.82	7.06	27.33	21.86	28.61

The above results were mainly due to the direct contact of root system to Cu²⁺. Thus, the Cu²⁺ content of the root system was higher than that of the cormus. The enrichment and transport coefficients of intercropped plants were higher than that of monocultures and mainly caused by the competition between sudangrass and calliopsis. The competition involved the nutrients and pollution elements.

Conclusions

In this paper, the enhancement effect of fungus in the restoration of Cu²⁺-polluted PS by sudangrass and the intercropping of sudangrass and calliopsis was studied by indoor potted experiments. The main conclusions are summarized as follows: PS_B promoted the germination rate of sudangrass, and the germination rates of sudangrass planted alone were higher than that of those planted by intercropping. Sudangrass grew well on PS_B, and the height of calliopsis was lower than that of sudangrass under intercropping conditions. The biomass of sudangrass planted in PS_B by monoculture was higher than

that by intercropping. Cu²⁺ content in roots was higher than that in the cormus. The enrichment coefficient of cormus and roots was the largest under the soil treatment of 75 mg/kg Cu²⁺.

The results of this study provide a reference for microorganism–plant intercropping to repair heavy metal-contaminated soil. Based on the conclusions obtained in this study, future research can be further discussed in the following aspects: (1) determination of optimum microbial dosage and its combination with plants; (2) collocation and selection of intercropped plants; (3) effects of microorganisms on plant rhizosphere and their combined effects on contaminants.

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