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Experimental Research on the Remediation Ability of Four Wetland Plants on Acid Mine Drainage

Aijing Wu [1], Yongbo Zhang [1,*], Xuehua Zhao [1], Hong Shi [2], Shuyuan Xu [3], Jiamin Li [1], Guowei Zhang [1] and Lina Guo [1]

[1] College of Water Resources Science and Engineering, Taiyuan University of Technology, Taiyuan 030024, China; wuaijing0077@link.tyut.edu.cn (A.W.); zhaoxuehua@tyut.edu.cn (X.Z.); lijiamin0404@link.tyut.edu.cn (J.L.); zhanguowei0421@link.tyut.edu.cn (G.Z.); guolina0069@link.tyut.edu.cn (L.G.)

[2] College of Environmental Science and Engineering, Taiyuan University of Technology, Taiyuan 030024, China; sx_hosh196@sina.com

[3] Department of Geology and Surveying and Mapping, Shanxi Institute of Energy, Jinzhong 030600, China; xushuyuan0062@link.tyut.edu.cn

* Correspondence: zhangyongbo2021@sina.com

Abstract: In order to study the economical, efficient, and environmentally friendly techniques for the treatment of acid mine drainage (AMD), this paper investigated the effects of watering with AMD on the growth condition, the resilience of four wetland plants, as well as the uptake and transport of pollutants by plants. The results showed that *Typha orientalis* was more resistant to AMD (irrigation with AMD increased its catalase activity and glutathione content and promoted its growth), so it was suitable for treating high concentrations of AMD (SO_4^{2-} ≈ 9400 mg/L); *Cyperus glomeratus* was suitable for treating medium concentrations of AMD (SO_4^{2-} ≈ 4600 mg/L); and *Scirpus validus* and *Phragmites australis* could be used to treat low concentrations of AMD (SO_4^{2-} ≈ 2300 mg/L). All four plants could be used for phytoextraction for Mn-contaminated water (TF > 1). *Phragmites australis* could be used for phytoextraction for Zn-contaminated water, and the other three plants could be used for phytostabilisation for Zn-contaminated water (TF < 1); the microbial biomass in the soil was affected not only by the concentration of AMD but also by plant species. This study provides a scientific basis for the phytoremediation technology of AMD.

Keywords: acid mine drainage (AMD); phytoremediation; wetland plants; pot experiment; transfer factor

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1. Introduction

Acid mine drainage (AMD) is a product of serious harm to the health of the ecological environment produced by mine development and utilization [1,2], which has the characteristics of a low pH value and a high concentration of sulfate and heavy metal ions [3,4]. The water pollution caused by AMD makes the problem of water shortages more serious. What is more, its discharge will destroy the growing environment of fish, algae, and microorganisms and damage the various functions of water; in addition, the pollutant entering the food chain will also threaten human health [5–7]. Therefore, there is an urgent need for the treatment of AMD.

At present, the common treatment processes of AMD are neutralization, constructed wetland, and microbial methods. Neutralization is a method of removing sulfate by converting it into gypsum using a neutralizing reagent containing calcium ions, but it has the disadvantage of high operating costs and a large amount of sludge [8]. Although there is no secondary pollution in the microbial method, there is a problem of microbial culture, and the process is complex. The constructed wetland uses natural plants for decontamination, which has the advantages of low investment, low operating cost, simple technological process, ornamental value, and so on [9,10]. It has been widely used in

wastewater treatment [11] and has unique advantages in the treatment of acidic water and heavy metals [12].

As an important part of the constructed wetland system, plants play a very important role in the wastewater treatment process. The main mechanisms of heavy metal removal by plants include the chemical precipitation of metals and their adsorption on sediments promoted by aquatic plants; the retention of metals in plant tissues through filtration, adsorption, cation exchange, and inter-root induction; the retention and precipitation of heavy metals during symbiosis with inter-root bacteria (e.g., formation of iron oxide layers can adsorb other metals); and direct metal uptake by plant roots [13,14]. Thus, screening wetland plants with strong stress resistance and good treatment effect is a necessary link in constructing the constructed wetland system for wastewater treatment. Plant roots can enhance the function of the plant–microbial–filler complex structure by adsorbing, filtering, and modifying the environment [15,16]. Leung, et al. [17] evaluated the current status of heavy metals in constructed wetland in Shaoguan (Guangdong, China) and found that *Typha latifolia* accumulated the highest amount of heavy metals compared to *Phragmites australis* and *Cyperus malaccensis*. Oustriere, et al. [18] evaluated the ability of *Arundo donax* L., *Cyperus eragrostis* Lam., *Iris pseudacorus* L., and *Phalaris arundinacea* L. to accumulate Cu after incubating 7-month-old plants in different gradients of Cu solutions for 2 months and showed that *Iris pseudacorus* L. and *Phalaris arundinacea* L. grew well and accumulated more Cu in the organism. Miloskovic, et al. [19] examined the concentrations of heavy metals in five species of plants (*Typha angustifolia*, *Iris pseudacorus*, *Polygonum amphybium*, *Myriophyllum spicatum,* and *Lemna gibba*) and found that among the five plants, I *Iris pseudacorus* and *Polygonum amphybium* had the highest levels of Cd and As. In addition, Brisson and Chazarenc [20] reviewed 35 experimental studies on the effect of plant selection on pollutant removal in subsurface flow constructed wetland systems and found that the removal efficiency of different plant species varied significantly for one or more pollutants, indicating that the choice of plant species is crucial.

Therefore, through literature review and field survey in the mining area, four wetland plants adapted to the mining environment were selected as the subjects of this study. The growth condition, resilience, and the uptake and transport of sulfate, Mn, and Zn by each plant under AMD irrigation were investigated through pot experiments to investigate the remediation ability of the four wetland plants for AMD. The use of wetland plants for AMD remediation helps to achieve the treatment and comprehensive use of AMD, which is of great significance to solve the problem of water shortage and drainage pollution in mining areas.

2. Materials and Methods

2.1. Materials

Phragmites australis (*P. australis*), *Typha orientalis* (*T. orientalis*), *Cyperus glomeratus* (*C. glomeratus*), and *Scirpus validus* (*S. validus*) were purchased from Anxin County, Baoding City, Hebei Province, China.

AMD used in the experiment was collected from the mine area (38°01′35″ N, 113°30′52″ E) in the Shandi River basin of Yangquan City, Shanxi Province, China (Figure 1), it was transported back to the lab in several 25 L polyethylene plastic drums and sealed and stored under refrigeration at 4 °C. Yangquan city is one of the main coal-producing areas in Shanxi Province. In recent years, with the closure of a large number of coal mines and the cessation of mining, a large amount of AMD has been generated, which poses a threat to the local residents' water sources for living and production. The basic physicochemical properties of AMD are shown in Table 1.

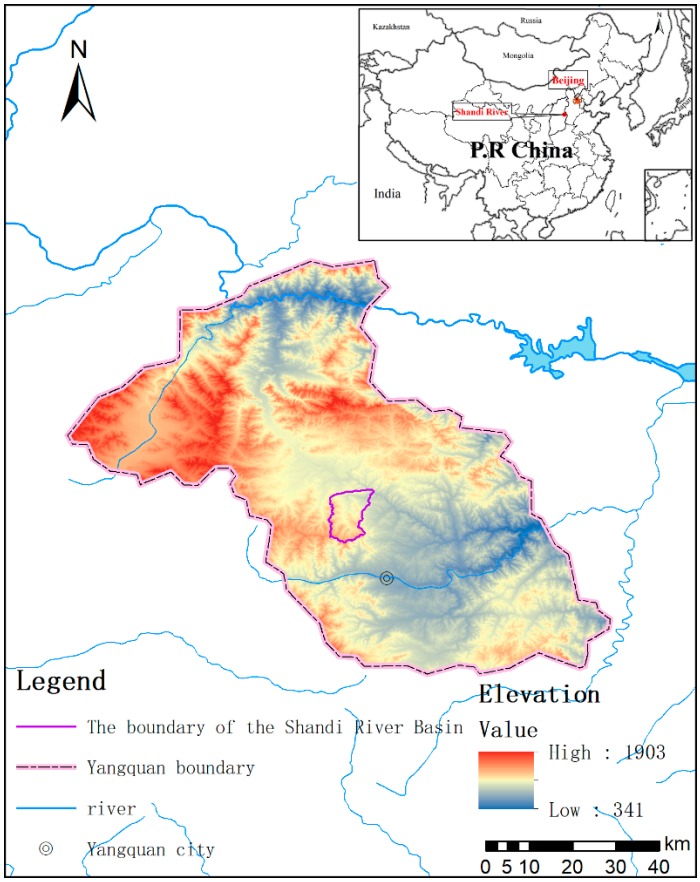


Figure 1. The location of the Shandi River Basin.

Table 1. Basic physicochemical properties of AMD.

pH Value	Conductivity /(mS/cm)	SO_4^{2-}	Fe	Mn	Zn	Ca^{2+}	K^+	Na^+	Pb	Cl^-	F^-	NO_3^-	NO_2^-
							/(mg/L)						
3.62	7.44	9398.53	117.98	24.42	8.45	303	0.52	30.4	0.003	92.9	13	0.81	0.15

The soil was taken from a farmland area (37°42′4″ N, 112°55′22″ E) in Dongzhao Township, Yuci District, Jinzhong City, Shanxi Province, China. The basic physicochemical properties of the soil are shown in Table 2, which shows the average measurements of three randomly selected soil samples.

Table 2. Basic physicochemical properties of the soil.

Organic Matter	Total N	Total K	Total P	Total S	Mn	Zn	pH	CEC
g/kg				mg/kg				mmol/kg
11.11	0.78	18.97	0.84	153.27	227.07	150.35	8.14	102.63

2.2. Experimental Design

The experiment was carried out using 16 rectangular sets of pots (Figure 2). The inner pots were 71 cm long, 50 cm wide, 43 cm high, containing 40 cm deep soil with holes at the bottom. The outer pots were 73 cm long, 52 cm wide, and 48.5 cm high, and the bottom of the inner pots was paved with a layer of fine sand mesh to prevent the loss of soil. Four irrigation gradients were set for each plant (the preparation method of experimental water samples is shown in Table 3), i.e., four sets of pots for each plant. Four plants with consistent growth were screened out and transplanted into each set of pots, and each set of

pots was planted with 30 strain plants. The plants were irrigated with experimental water samples every 2 days, 1 L each time. Plants were harvested after planting for two months.

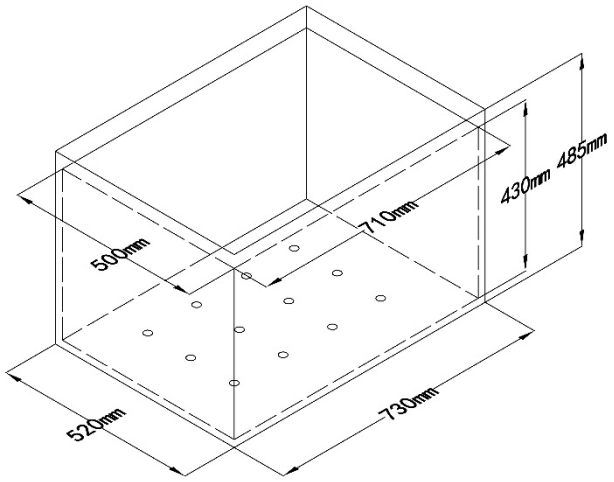


Figure 2. Schematic diagram of the experimental device.

Table 3. Preparation and composition of experimental water samples.

Code	Treatment	Distilled Water: AMD	SO_4^{2-}	Mn mg/L	Zn
CK	Control group	1:0	0	0	0
C1	Low concentration AMD	3:1	2315.51	6.24	2.34
C2	Medium concentration AMD	1:1	4605.32	11.25	4.33
C3	High concentration AMD	0:1	9398.53	24.42	8.45

2.3. Test Methods

Three plants were randomly selected from each treatment. The harvested plant samples were washed with running water and then with distilled water to eliminate the interference of minerals on the plant. The plant height was measured with a ruler and the average plant height was calculated; then, the plants were separated into shoots and roots parts. They were placed in a constant temperature oven at 105 °C for half an hour and dehydrated to constant weight at 80 °C. The dried samples were ground to fine-textured powder with a grinder, and then one gram of the powder was digested using a tri-acid mixture (HNO_3, $HClO_4$, and H_2SO_4; 5:1:1) at 80 °C until the solution became clear. The digested samples were filtered and then analyzed for metals by flame (acetylene) ionization using an atomic absorption spectrophotometer (TAS-990, Persee, Beijing, China), and sulfur was determined by ion chromatography (883 Basic IC plus, Metrohm, Herisau, Switzerland).

The activity of plant catalase (CAT) and the content of glutathione (GSH) were determined using fresh plant samples. Five grams of plant tissue were ground in liquid nitrogen in phosphate buffer (20 mM L^{-1}, pH 7.4), and the supernatant was then acquired by centrifugation at 4 °C. The CAT activity was measured using the assay kit purchased from Nanjing Jiancheng Bioengineering Institute (Nanjing, China). GSH content was determined using the method of Ding, et al. [21]. Soil microbial biomass was assayed according to the reference of GB/T 39228-2020 [22].

Transfer factor (TF) of the plant is defined as the ratio of the metal concentration of the aboveground plant tissues to that of the belowground plant tissues. It is given by Equation (1) [23]:

$$TF = \frac{C_a}{C_b} \quad (1)$$

where C_a ($mg \cdot g^{-1}$) and C_b ($mg \cdot g^{-1}$) represent the metal concentrations of aboveground plant tissues and belowground plant tissues, respectively.

2.4. Statistical Analysis

A one-way ANOVA was performed to identify significant differences among treatments, and, when detected, a post hoc Duncan's Multiple Range Test was performed using the SPSS 26.0 statistical software. Differences between the two treatments were analysed using a *t*-test (SPSS 26.0). The differences were considered significant when $p < 0.05$.

3. Results and Discussion

3.1. Physiological Indicators of Four Plants

3.1.1. CAT Activity

In the process of plant growth and development, the production and consumption of reactive oxygen species are in a state of equilibrium, and when disturbed by adverse external factors, the state of equilibrium is destroyed, which increases the number of reactive oxygen species and poses a threat to plant cells. There is a reactive oxygen defence system within plants, and catalase (CAT) is one of the most critical antioxidant enzymes in this system [24].

The CAT activity in four plants under different concentrations of AMD irrigation is shown in Figure 3a. The CAT activity of *S. validus* and *C. glomeratus* decreased gradually with the increase in the AMD concentration, and the CAT activity of *T. orientalis* increased gradually, while the CAT activity of *P. australis* increased first and then decreased and reached the highest in the C1 treatment. This was due to the fact that when *C. glomeratus* and *S. validus* were irrigated with AMD for a long period of time, the plant cells in them were kept in high O^{2-} concentration, and the active substances, including enzymes, were damaged. Therefore, the CAT activity decreased. However, under the stress of AMD, the synthetic expression of antioxidant enzymes in *T. orientalis* increased, and the activity of CAT gradually increased, indicating that *T. orientalis* was more resistant to AMD stress. In addition, AMD contains high levels of Mn and Zn, which are required for enzyme activation and stress resistance according to Guo and Chi [25]. The CAT activity of *P. australis* increased first and then decreased, indicating that the antioxidant enzyme had a tolerance limit. When the pollutant content exceeded its tolerance limit, the enzyme activity would decrease.

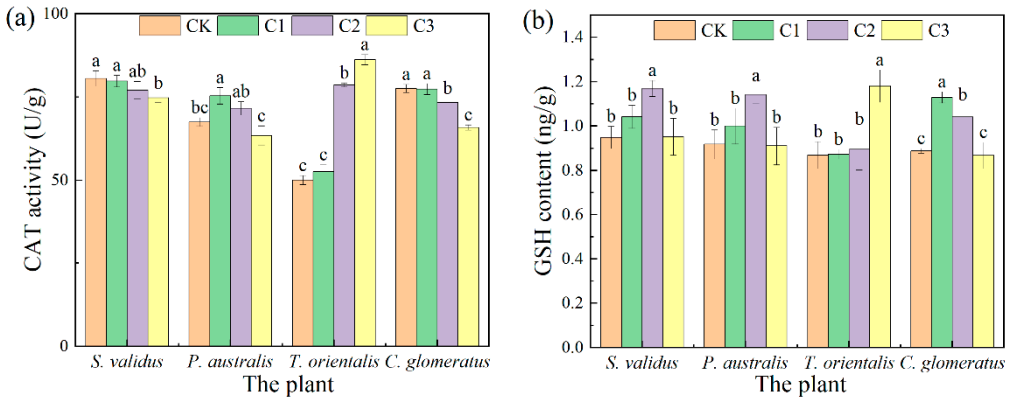


Figure 3. *Cont.*

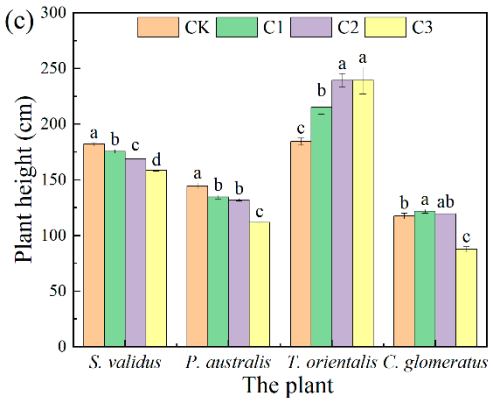


Figure 3. Effect of different concentrations of AMD irrigation on (**a**) CAT activity; (**b**) GSH content; (**c**) plant height of four plants. Bars and error bars represent the mean ± SD of three replicates. The same letter in the histogram of a certain plant represents no significant difference at the level of 0.05 (Duncan's Multiple Range Test).

3.1.2. GSH Content

Glutathione (GSH), which is widely distributed in living organisms, helps to maintain normal immune system function and has antioxidant and integrative detoxification effects [26]. In recent years, GSH has attracted more and more attention because of its multiple roles in plants under stress [27,28]. Heavy metal ions derived from municipal wastewater, industrial wastewater, and pesticide spraying can accumulate in water and soil and enter plants through roots, which have extremely high potential toxicity to plants [29]. The detoxification of exogenous and heterologous toxic substances mainly depends on GSH and its dependent enzyme system, which can reduce the polarity and toxicity of toxic electrophilic substances through nucleophilic substitution and addition [30].

Figure 3b shows the effect of different concentrations of AMD irrigation on the GSH content of four plants. With the increase in the AMD concentration, the GSH content of *S. validus*, *P. australis*, and *C. glomeratus* increased first and then decreased. Under the treatment of C2, the GSH content of *S. validus* and *P. australis* reached the highest, and under the treatment of C1, the GSH content of *C. glomeratus* was the highest. The GSH content of *T. orientalis* increased gradually with the increase in the AMD concentration. The results showed that under C2 treatment, GSH was synthesized in *S. validus* and *P. australis* to relieve the toxicity, but with the increase in the pollutant concentration, the ability to synthesize GSH was inhibited, and the content of GSH decreased. The same was true for the change in the GSH content in *C. glomeratus*. With the increase in the AMD concentration, the content of GSH in *T. orientalis* increased, which was a mechanism of the plant's resistance to stress, indicating that *T. orientalis* had good resistance to AMD stress.

3.1.3. The Plant Height

Figure 3c shows the effect of different concentrations of AMD irrigation on the plant height of four species. It can be seen that with the increase in the AMD concentration, the plant height of *S. validus* and *P. australis* decreased gradually, and all of them were lower than that under CK treatment. The plant height of *T. orientalis* in different concentrations of AMD treatment was higher than that in CK treatment, and with the increase in the AMD concentration, the plant height of *T. orientalis* rose first and then remained stable. There was no significant difference in the plant height of *C. glomeratus* between the C1, C2, and CK treatments; however, the plant height of *C. glomeratus* in the C3 treatment was significantly lower than that in the CK treatment.

The experimental results showed that irrigation with different concentrations of AMD inhibited the growth of *S. validus* and *P. australis*, and the higher the concentration, the stronger the inhibition; irrigation with AMD could promote the growth of *T. orientalis*; irrigation with the low concentration of AMD had no significant effect on the growth of

C. glomeratus, but irrigation with the high concentration of AMD had an inhibitory effect on its growth. Such results were closely related to the CAT activity, GSH content, and the ability of the four plants to resist AMD stress.

3.2. Plant Uptake of Sulfur, Mn, Zn

Sulfur (S) is one of the indispensable nutrients for plant growth and development. Part of the sulfur absorbed by plants is used to synthesize organic sulfur to meet their growth needs, and the rest is stored in the vesicles in the form of sulfate ions [31], so this paper used the total sulfur content in plants as an indicator to reflect the uptake of SO_4^{2-} in AMD by plants. From Figure 4a, it could be seen that the total sulfur content in the four plants under AMD treatment was higher than that under CK treatment. *S. validus* and *T. orientalis* had the highest sulfur contents under C2 treatment, 27.41 mg/g and 16.34 mg/g, respectively. As the AMD concentration continued to increase, the sulfur content decreased. *P. australis* had the highest sulfur content under the C1 treatment, with a sulfur content of 10.43 mg/g, and then the sulfur content decreased with the increase in the AMD concentration. The highest sulfur content in *C. glomeratus* was 13.25 mg/g under C3 treatment. There was no significant difference in sulfur content in *C. glomeratus* between the C1 and C2 treatments.

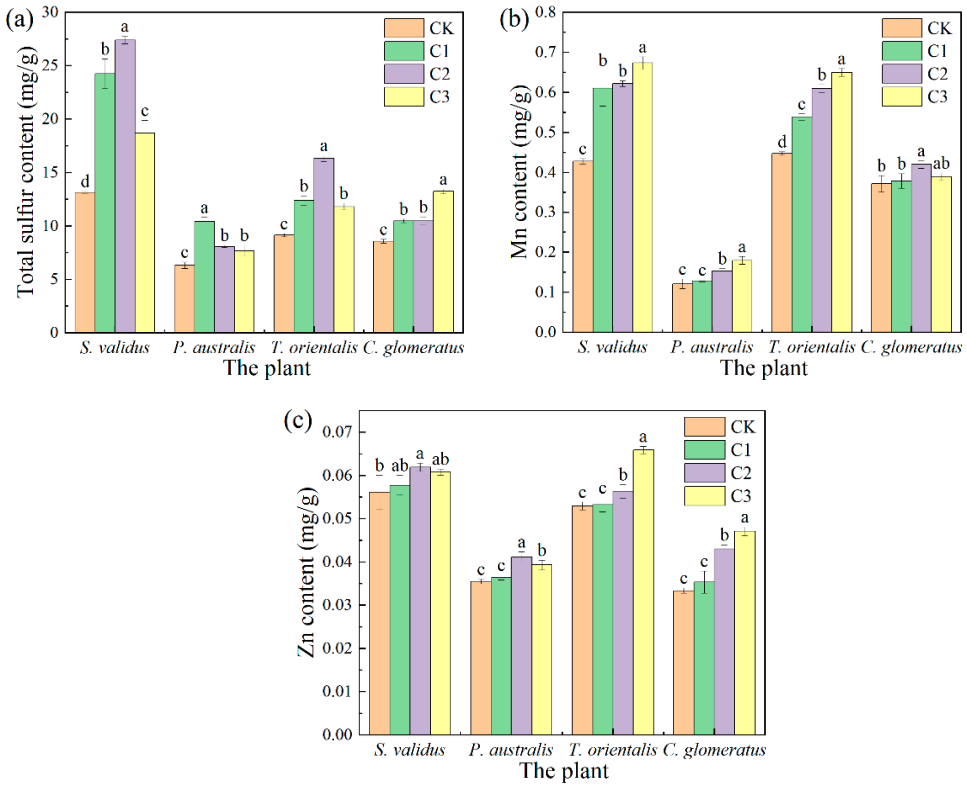


Figure 4. Effect of different concentrations of AMD irrigation on (**a**) total sulfur content; (**b**) Mn content; (**c**) Zn content in four plants. Bars and error bars represent the mean ± SD of three replicates. The same letter in the histogram of a certain plant represents no significant difference at the level of 0.05 (Duncan's Multiple Range Test).

It can be seen from Figure 4b that with the increase in the AMD concentration, the Mn content of *S. validus*, *P. australis*, and *T. orientalis* showed an increasing trend. Under the C3 treatment, the Mn contents of *S. validus*, *P. australis*, and *T. orientalis* were 0.67 mg/g, 0.18 mg/g, and 0.65 mg/g, respectively. The Mn content of *C. glomeratus* was highest under the C2 treatment at 0.42 mg/g.

From Figure 4c, it can be seen that the Zn contents in the four plants under the AMD treatment were higher than that under the CK treatment. The effects of different AMD concentrations on the Zn content of *S. validus* and *P. australis* were similar, and their Zn contents

peaked at 0.06 mg/g and 0.04 mg/g under the C2 treatment, respectively. With the increase in the AMD concentration, the Zn contents in *T. orientalis* and *C. glomeratus* showed an upward trend, peaking at 0.07 mg/g and 0.05 mg/g under the C3 treatment, respectively.

The ratios of the content of pollution elements (sulfur, Mn, Zn) in four plants under different concentrations of the AMD treatment to that under the CK treatment are shown in Table 4. Under C1 treatment, *S. validus* showed the strongest extraction of sulfur and Mn, which was 1.85 and 1.43 times that of CK, and *C. glomeratus* showed the strongest extraction of Zn, which was 1.06 times that of CK. Under C2 treatment, *S. validus* showed the strongest extraction of sulfur and Mn, which was 2.09 and 1.45 times that of CK, and *C. glomeratus* showed the strongest extraction of Zn, which was 1.29 times that of CK. Under C3 treatment, *S. validus* showed the best extraction effect on Mn, which was 1.57 times that of CK, and *C. glomeratus* showed the best extraction effect on sulfur and Zn, which was 1.54 and 1.41 times that of CK. The results indicated that all four plants showed extraction effects on sulfate, Mn, and Zn under AMD treatment, Rana and Maiti [32] also demonstrated the effectiveness of aquatic macrophytes for the bioremediation of aquatic environments affected by toxic elements, while *C. glomeratus* and *S. validus* showed stronger extraction.

Table 4. Ratios of the content of pollution elements (sulfur, Mn, Zn) in four plants under different concentrations of AMD treatment to that under CK treatment.

Pollution Element	Treatment	*S. validus*	*P. australis*	*T. orientalis*	*C. glomeratus*
Sulfur	CK	1.00	1.00	1.00	1.00
	C1	1.85	1.65	1.35	1.22
	C2	2.09	1.28	1.78	1.22
	C3	1.42	1.21	1.29	1.54
Mn	CK	1.00	1.00	1.00	1.00
	C1	1.43	1.06	1.20	1.02
	C2	1.45	1.26	1.36	1.13
	C3	1.57	1.48	1.45	1.05
Zn	CK	1.00	1.00	1.00	1.00
	C1	1.03	1.03	1.01	1.06
	C2	1.10	1.16	1.06	1.29
	C3	1.08	1.11	1.25	1.41

3.3. Transfer Factor of Plants to Metals

The transfer factors (TFs) of heavy metals by plants reflect the ability of plants to transfer heavy metals from underground parts (roots) to aboveground parts (stems, leaves, fruits) after absorbing them from the environment. Plants with higher TFs can absorb heavy metals from the soil around the roots and transfer and accumulate most of the heavy metals to the stems, leaves, and fruits of the plant to protect the roots from toxicity and maintain their normal life activities [33]. Therefore, in the process of phytoremediation, we can gradually reduce the pollution of heavy metals in the soil by planting and harvesting these plants with strong transfer ability several times. Thus, the purpose of treating contaminated soil can be achieved.

Figure 5 shows the metal contents of aboveground and belowground parts of each plant under different treatment and TFs of each plant. From Figure 5a, it could be seen that Mn in the four plants mainly accumulated in the aboveground part (TF > 1), so all four plants could be used to remediate Mn contamination in the water, among which the TFs of *T. orientalis* and *C. glomeratus* were larger, indicating that they had a stronger ability to transfer Mn. Figure 5b shows that Zn in *P. australis* mainly accumulated in the aboveground part (TF > 1), which is consistent with the results of previous studies [34,35]. Meanwhile, Zn in *S. validus*, *T. orientalis*, and *C. glomeratus* mainly accumulated in the belowground part (TF < 1), indicating that *P. australis* had a stronger ability to transfer Zn than the other three plants. Yoon, et al. [36] suggested that plants with a TF greater

than one could be used for phytoextraction, and plants with a TF less than one could be used for phytostabilisation. Therefore, all four plants could be used for phytoextraction for Mn-contaminated water, *P. australis* could be used for phytoextraction for Zn-contaminated water, and *S. validus*, *T. orientalis*, and *C. glomeratus* could be used for phytostabilisation for Zn-contaminated water.

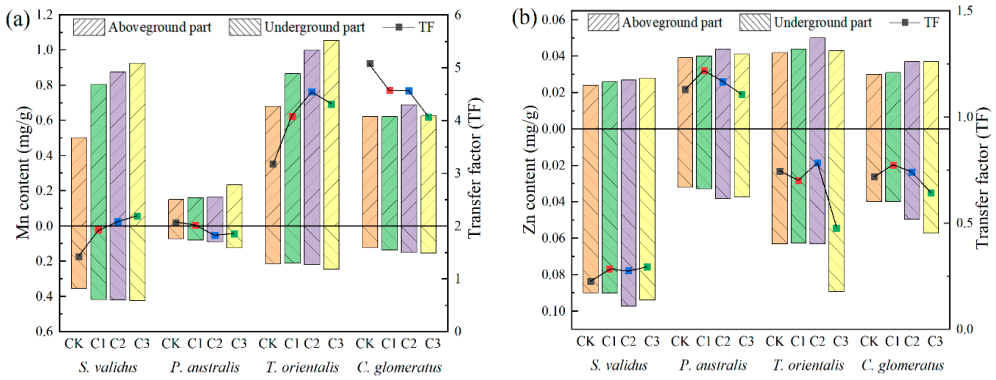


Figure 5. (**a**) Mn and (**b**) Zn content in aboveground and underground parts of four plants and transfer factor (TF).

3.4. Microbial Biomass in Soil

Since the metabolic processes of microorganisms play an important role in the treatment of AMD, a lot of research on microorganisms has been conducted by scholars worldwide [37–39]. The microbial biomass in soil mainly refers to the number of microorganisms involved in regulating soil nutrient cycling and organic matter conversion. The entry of heavy metal pollutants into the soil affects the growth and reproduction of soil microorganisms and their metabolic processes [40]. Figure 6 shows the effect of different concentrations of AMD irrigation on microbial biomass in soil. From Figure 6, it could be observed that the microbial biomass in the soil planted with *S. validus*, *P. australis*, and *T. orientalis* was greater under low concentrations of AMD treatment than that under the CK treatment, and less under high concentrations of AMD treatment than that under the CK treatment. This indicated that the microbial biomass in soil increased when the AMD concentration was low, but there was a toxic effect on soil microorganisms when the concentration exceeded a certain level. However, the microbial biomass in the soil planted with *C. glomeratus* was smaller under all concentrations of the AMD treatment than that under the CK treatment, which indicated that the microbial biomass in the soil was affected not only by the concentration of pollutants but also by plant species.

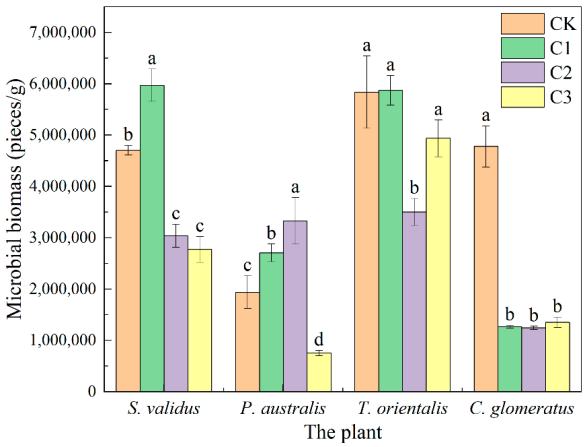


Figure 6. Microbial biomass in soil under different experimental treatments. Bars and error bars represent the mean ± SD of three replicates. The same letter in the histogram of a certain plant represents no significant difference at the level of 0.05 (Duncan's Multiple Range Test).

4. Conclusions and Suggestion

In this paper, we investigated the growth condition, stress tolerance, and the uptake and translocation of sulfate, Mn, and Zn by four species of plants under AMD irrigation conditions and explored the remediation potential of four wetland plants for AMD through pot experiments. The main conclusions we reached are as follows:

(1) The growth of *S. validus* and *P. australis* was inhibited under various concentrations of AMD treatment; the growth of *C. glomeratus* was inhibited under high concentrations of AMD treatment; and the growth of *T. orientalis* thrived as the concentration of AMD increased, indicating that *T. orientalis* was more resistant to AMD. therefore, *T. orientalis* was suitable for treating high concentrations of AMD ($SO_4^{2-} \approx 9400$ mg/L); *C. glomeratus* was suitable for treating medium concentrations of AMD ($SO_4^{2-} \approx 4600$ mg/L); and *S. validus* and *P. australis* could be used to treat low concentrations of AMD ($SO_4^{2-} \approx 2300$ mg/L).

(2) All four plants had certain extraction effects on SO_4^{2-}, Mn, and Zn, among which *C. glomeratus* and *S. validus* showed stronger extractions. Based on the TFs of each plant for heavy metals, it was known that all four plants could be used for phytoextraction for Mn-contaminated water; *P. australis* could be used for phytoextraction for Zn-contaminated water; and *S. validus*, *T. orientalis*, and *C. glomeratus* could be used for phytostabilisation for Zn-contaminated water.

This study provided a scientific basis for the screening of suitable wetland plants for the treatment of AMD; however, due to the limitations of the experimental conditions, further research is still needed. It is known that the removal of metals in AMD is easy, while the removal of sulfate has been a challenge in the treatment of AMD. Currently, one of the most promising methods for sulfate removal is the microbial method, and scholars mainly focus on sulfate-reducing bacteria (SRB). Therefore, based on the results of this study, future research can be conducted on the comprehensive treatment effect of plant–microbial–substrate triple system on AMD through the construction of constructed wetland systems.

Author Contributions: Conceptualization, A.W. and Y.Z.; methodology, Y.Z.; software, A.W. and L.G.; investigation, A.W., J.L., and G.Z.; writing—original draft preparation, A.W.; writing—review and editing, H.S., S.X. and X.Z.; supervision, Y.Z.; project administration, Y.Z. and H.S.; funding acquisition, Y.Z. and H.S. All authors have read and agreed to the published version of the manuscript.

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