MITIGATING THE HARM CAUSED BY COPPER POLLUTION IN SEDIMENT TO SUBMERGED MACROPHYTES BY ADDING BIOCHAR TO WATER

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Abstract. Heavy metal-contaminated sediment may release harmful ions into the water column and seriously affect the growth of aquatic organisms. Biochar is a good adsorbent for water pollutants. In this study, a simulated water ecosystem with copper (Cu)-contaminated sediment was established to study the effects of biochar addition (CK: 0 g/L, T1: 3 g/L, T2: 6 g/L, T3: 12 g/L) on Cu content in water and the growth and accumulation of Cu in a submerged macrophyte, *Vallisneria natans*. Compared with the CK group, Cu contents in water decreased by 43.65%, 33.86%, and 54.10% in the T1, T2, and T3 groups, respectively. Cu contents in the aboveground part of *V. natans* decreased by 56.03%, 61.90%, and 52.38% in T1, T2, and T3, respectively, while in the belowground part Cu contents decreased by 17.46%, 11.94%, and 55.90%, respectively. The plant height, fresh weight, and chlorophyll content significantly increased with the increase of biochar addition, showing that biochar promoted the growth of *V. natans*. This study demonstrated that the addition of biochar in water could significantly reduce the Cu content in water and the accumulation of Cu in *V. natans*, thus reducing the harm caused by Cu to *V. natans*. This study provides a method for the remediation and management of Cu-polluted sediment.
Keywords: *heavy metal pollution, water quality, Vallisneria natans, phytoremediation, phytoextraction*

Introduction

Human activities, such as the mining of metal minerals, the use of agricultural chemicals, and the discharge of chemical plants, are important sources of serious heavy metal pollution in soil and sediment worldwide (Decou et al., 2019; Khalid et al., 2020, 2016). Studies have found that more than 99% of heavy metals would be stored in sediment in various forms after entering water bodies. However, these heavy metals are likely to be slowly released into water bodies over time, posing a risk to human health through bioaccumulation (Peng et al., 2009; Corzo Remigio et al., 2021). Cu is one of the most common heavy metal pollutants in sediment around the world (Ugwu and Agunwamba, 2020). When the Cu content is too high, it inhibits the growth of plants and damage plant metabolic systems, resulting in toxicity symptoms (Shabbir et al., 2020; Decou et al., 2019; Delmail et al., 2011).

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Phytoremediation is an environmentally friendly in situ remediation technology that uses plants to enrich and stabilize heavy metal elements in sediments. This technique has received widespread attention in recent decades (Chen et al., 2014; Corzo Remigio et al., 2021; Shi et al., 2017). Submerged macrophytes grow underwater, and their roots, stems, and leaves can accumulate heavy metals, making macrophytes useful in the remediation of water bodies that are mildly polluted by heavy metals (Rezania et al., 2016). Plants used for restoration accumulate a high concentration of heavy metal elements through their roots. These heavy metals are then transferred to other tissues and enriched (Paz-Ferreiro et al., 2014; Roubeau Dumont et al., 2022; Krayem et al., 2018). Xue et al. (2010) found that Cu mainly accumulated in the aboveground part of submerged macrophytes, and the roots could transfer Cu from the belowground to the aboveground tissues. Venkateswarlu et al. (2019) found that the removal rates of Cu content in water were 69% and 33.35% for *Hydrilla verticillata* and *Pistia stratiotes*, respectively, at 2 mg/L and 7 mg/L Cu concentrations, respectively, making it possible to remove heavy metals from the sediment through multiple harvests. However, Paz-Ferreiro et al. (2014) have suggested that phytoremediation is not suitable for areas with high heavy metal pollution because the toxic effects of heavy metals on plants limit plant growth and reduce the uptake of heavy metals by plants. Copper pollution has a significant impact on plant chromosomes and photosynthesis, as well as normal cellular metabolism and physiological processes (Decou et al., 2019; Delmail et al., 2011). Therefore, slow growth and low biomass under Cu contamination are major concerns when using phytoremediation.

Biochar is a carbon-rich product created by cracking biomass materials at high temperatures (<700 °C) under hypoxia or closed conditions. Biochar, as an effective sediment amendment, can effectively adsorb heavy metals due to its high porosity and a large number of lignocellulosic materials (Ugwu and Agunwamba, 2020; Chen et al., 2011; Xu et al., 2016; Wang et al., 2021). Aydin et al. (2008) studied the Cu adsorption capacity of wheat husk and rice husk at three different temperatures and found that the adsorption capacity was the highest at 313 K, at 16.077 mg/g for wheat husk and 17.422 mg/g for rice husk. Yuan et al. (2011) have reported that biochar is mostly alkaline, and can neutralize the acidity of water and sediment and increase the pH value of sediment. The pH and basicity of biochar increases as the pyrolysis temperature rises. Peng et al. (2009) have concluded that pH is one of the key factors affecting the re-release of Cu from sediment to the overlying water. When the pH value of sediment drops to 4, Cu and other heavy metal elements are released in large quantities. Therefore, it can be speculated that the addition of biochar can inhibit the release of Cu from sediment to the overlying water and adsorb Cu from overlying water.

Biochar has both positive and negative effects on the growth of submerged plants. Chi and Liu (2016) speculated that such effects may be caused by factors such as biochar type, dosage, and sediment characteristics. Currently, there is insufficient research on how biochar affects the growth of submerged plants. In this study, different doses of biochar were added to Cu-contaminated sediment to adsorb Cu released from the sediment, thereby reducing heavy metal toxicity to submerged plants. The objectives of the current study were: 1) to investigate the effects of biochar addition on the Cu accumulation and growth of submerged plants; and 2) to provide a reference for more effective phytoremediation and the use of adsorbent–plant combined remediation to solve the problem of heavy metal pollution in sediment.

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Materials and methods

Material processing

Rice husk is a resource-rich agricultural waste. In China, about 100 million tons of rice husk are produced each year, accounting for 50% of the world's total output (Shafigh et al., 2014). Pyrolysis can be used to convert rice husk into biochar, which can help reduce agricultural waste. Biochar made from rice husk has a special pore structure. When the lignin and cellulose in rice husk are degraded, carbon attaches to the skeleton, thus making it a good adsorbent (Yuan et al., 2011). In this study, biochar made from rice husk was used as the adsorbent for Cu pollution. The rice husk biochar was purchased from Ruike New Energy Co., LTD. Shandong. The rice husk biochar was prepared under hypoxia at 400 °C.

V. natans is a large submerged vascular plant with good pollution resistance, a wide distribution, and strong adaptability. *V. natans* was selected as the experimental material in this study and collected from the Poyang Lake Model Experimental Research Base in Gongqing city, Jiangxi province, China. The *V. natans* seedlings used in this study were of uniform size, exhibited normal growth, and had no branches; the length of the aboveground and belowground parts of the *V. natans* seedlings were 12 cm and 3 cm, respectively. The initial fresh weight of each *V. natans* seedling was 1.53 ± 0.10 g.

In May 2018, about 20 L of sediment were collected from Poyang Lake using bottom grab and mixed evenly after removing stones and debris. The collected sediment was dried under natural conditions, ground, and then screened through a 100-mesh sieve for later use. The organic matter and total nitrogen and phosphorus contents in the sediment were 22.6, 1.3, and 0.9 mg/g, respectively.

Experimental design

Cu-contaminated sediment was prepared by thoroughly mixing the collected uncontaminated sediment with Cu sulfate solution. According to the literature (Ji et al., 2018), the Cu contents in the sediments of rivers or lakes polluted by heavy metals range from 37.92 mg/kg to 709.28 mg/kg in Poyang Lake. The planned addition amount of Cu content in the sediment is 500 mg/kg, and the measured copper content after adding copper sulfate is 664 mg/kg. Therefore, it can be inferred that the original Cu content in the sediment is about 164 mg/kg. The Cu-contaminated sediment was added to 12 rectangular plastic boxes (19 cm in length, 13.5 cm in width, and 5 cm in height). Each box contained 650 g of Cu-contaminated sediment and was used to plant six *V. natans* seedlings. Each box was slowly placed into a glass tank (28 cm in length, 21 cm in width, and 23 cm in height) filled with 10 L of aerated tap water. The ammonia nitrogen, nitrate-nitrogen, and total nitrogen and phosphorus contents in the water were 0.011 mg/L, 1.832 mg/L, 1.915 mg/L, and 0.007 mg/L, respectively.

To facilitate the recycling of biochar and avoid turbidity caused by biochar dispersion and suspension in water, rice husk biochar with a few stones was loaded into permeable non-woven bags and hung in the water (*Fig. 1*.). A photo of the experimental culture and equipment is shown in the *Appendix* (*Fig. A1*). The dose gradients of the biochar added to the water in this experiment were set at 0, 3, 6, and 12 g/L, which were labeled as CK, T1, T2, and T3, respectively. Each treatment had three replicates. A plant growth lamp with a power of 9 W was used for each glass tank to provide supplementary lighting for *V. natans* from 7:00 a.m. to 7:00 p.m. every day during the experiment. The water temperature was 25-28 °C. The light

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intensity at the water surface is about 17,000 lx. Other water quality indicators are in the Results part. The experiment lasted two weeks, and the water quality index was measured every three days.

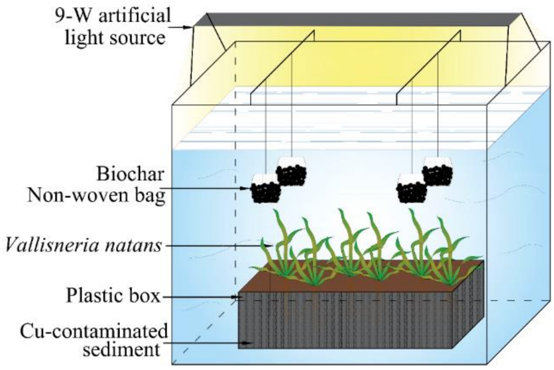

Figure 1. *Schematic diagram of experimental equipment*

Parameter determination

During the experiment, water temperature (T), dissolved oxygen (DO), conductivity (COND), oxidation-reduction potential (ORP), and pH were measured by a multi-parameter water quality analyzer (HQ40D, Hach, USA). *V. natans* were harvested at the end of the experiment. After the experiment, the macrophyte samples were carefully washed with distilled water at least three times and separated into aboveground and belowground parts. After the harvesting, the macrophyte heights and weights of the aboveground and belowground parts of *V. natans* were measured. The measured heights correspond to the maximal value of the main leaf/root. A chlorophyll meter was used to determine the chlorophyll content (SPAD) (SPAD-502Plus, Japan) of the leaves of *V. natans*. The samples were then placed in an oven for 1 h at 105 °C followed by 48 h at 75 °C. All plant samples were then ground into a fine powder and homogenized with mortar and pestle. The sediment was naturally air-dried and sifted through a 100-mesh sieve for chemical analysis. The samples of *V. natans* were digested by nitric acid and perchloric acid at a ratio of 4:1, and the samples of sediment were digested by hydrochloric acid, nitric acid, hydrofluoric acid, and perchloric acid at a ratio of 1:2:1:1. The Cu contents of the plant digestion solution, sediment digestion solution, and water sample were determined using granite furnace atomic absorption spectrometry (AAnalyst700, PerkinElmer, USA).

Statistical analysis

Bioconcentration Factor (BCF) is the concentration of Cu uptake by biota from sediment. It is used to determine a plant’s capacity for the accumulation of metals. It can be calculated by the following equation:

$$BCF = C_B \ / C_S \qquad \text{(Eq.1)}$$

where C_B represents the average concentration of Cu in the certain tissue (mg/g of dry weight), and C_S concentration of Cu in sediment (mg/g of dry weight).

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Translocation Factor (TF) is used to determine a plant's potential for the translocation of metals. It can be calculated by the following equation:

$$TF = C_{AG} / C_{BG} \tag{Eq.2}$$

where C_{AG} and C_{BG} represent the average concentration of Cu in the aboveground and belowground tissues of *V. natans* (mg/g of dry weight).

Data processing and analysis were completed using SPSS software (SPSS Inc., USA) and Microsoft Excel (Microsoft Corporation, Washington, USA). One-way analysis of variance method (one-way ANOVA) and least significant difference method (LSD) were used to test the significance of differences of each factor among treatment groups. The results of these test are shown in the *Appendix* (*Tables A1, A2, A3*). All data were tested for normality and homogeneity before performing comparison. Data were logarithmically transformed to obtain normality and/or homogeneity if they did not meet the basic assumptions. The threshold for significant differences among groups was at the level of $P < 0.05$. Pearson correlation analysis was used to analyze the relationships between different factors. The threshold for significant differences among groups was at the level of $P < 0.05$. Plotting was completed using the R-4.1.2 package 'corrplot' (Wei and Simko, 2021) and OriginPro 8.0 (OriginLab, Corporation, Northampton, MA, USA).

Results

Changes in water quality

COND in the four experimental groups gradually increased and then leveled off after one week. At the end of the experiment, the COND in T1, T2, and T3 increased by 8.56%, 16.22%, and 42.64%, respectively, compared with CK (*Fig. 2*. (a)). The DO content of water increased rapidly in all four experimental groups from the seventh day, and the DO content of the CK group was the lowest at the end of the experiment (*Fig. 2*. (b)). The water ORP values of the four groups showed an initial upward trend, and then decreased as time progressed (*Fig. 2c*). The pH value of water increased with different biochar addition amounts. On the first day, T1, T2, and T3 increased by 0.34, 0.91, and 1.68 compared with CK, and T3 reached 9.3, showing significant differences among the four groups ($P < 0.05$). Over time, the pH of the four experimental groups increased overall. On day 14, there were significant differences between treatment groups (*Fig. 2d*).

The changes in Cu contents in water

The Cu content in the CK group gradually increased over time, reaching 12.44 g/L at the end of the experiment. There was no significant difference in Cu content between the treatment and control groups on the first day of biochar addition ($P > 0.05$). There was a significant difference between CK and the treatment groups from the fourth day to the end of the experiment ($P < 0.05$). The Cu content in T1, T2, and T3 decreased by 43.65%, 33.86%, and 54.10%, respectively, compared to CK at the end of the experiment. The Cu content of T1 increased after reaching the minimum value on the fourth day in the treatment group, while the Cu contents of T2 and T3 increased after reaching the minimum value on the tenth day (*Fig. 3*).

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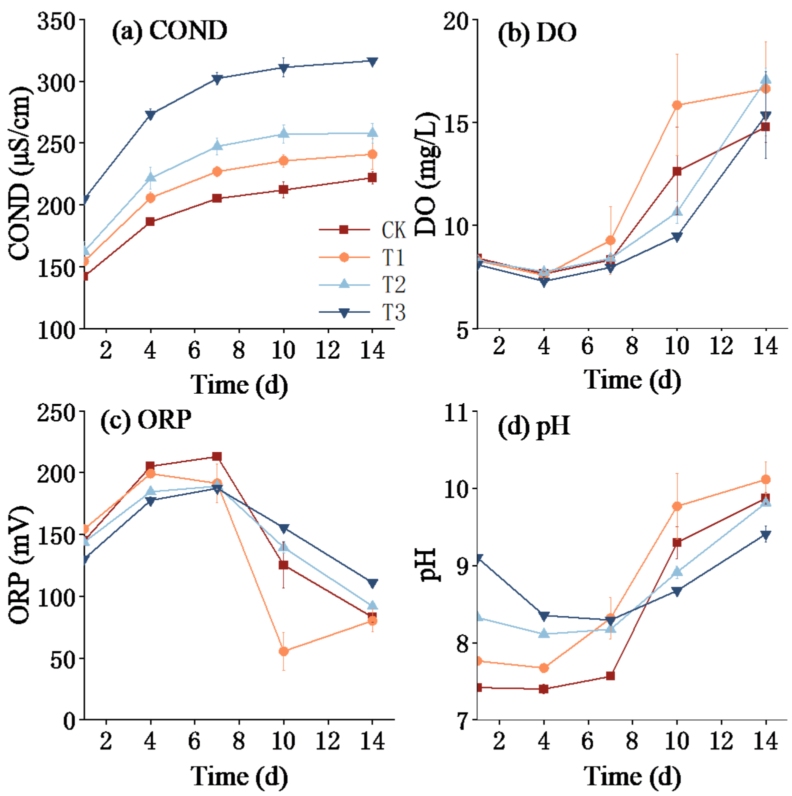

Figure 2. *Changes in the conductivity (COND) (a), dissolved oxygen (DO) (b), oxidation-reduction potential (ORP) (c), and pH (d) of water in different treatments over time, Data are shown as the mean ± SE, n = 3. CK: 0 g/L biochar, T1: 3 g/L biochar, T2: 6 g/L biochar, T3: 12 g/L biochar. Error bars represent standard error*

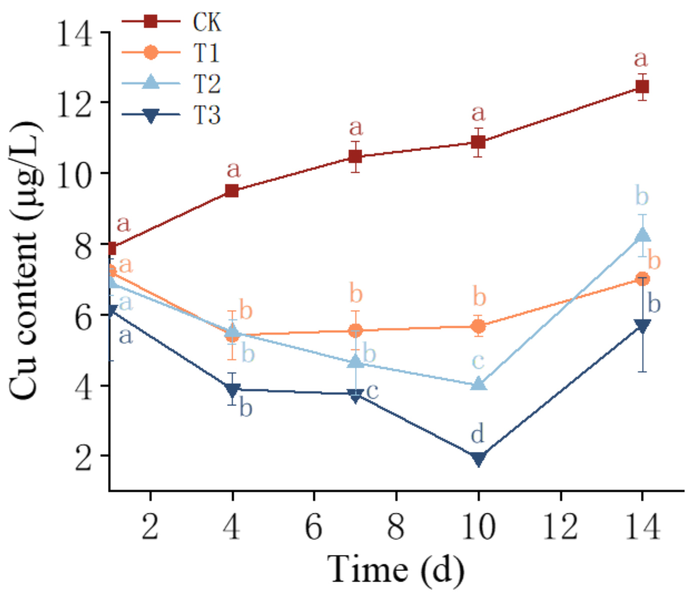

Figure 3. *Change in the Cu content of water in different treatments over time, Data are shown as the mean ± SE, n = 3. CK: 0 g/L biochar, T1: 3 g/L biochar, T2: 6 g/L biochar, T3: 12 g/L biochar. Different lowercase letters at same sampling day mean significant difference of Cu content among different treatments at p < 0.05. Error bars represent standard error*

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The changes of Cu contents in sediment

The Cu contents in the sediment of the CK, T1, T2, and T3 groups after the experiment were 662.43, 627.26, 626.23, and 656.03 mg/kg, respectively. The Cu contents in the sediment of the T1, T2, and T3 groups decreased by 5.38%, 5.58%, and 1.96%, respectively, when compared to the CK group. However, there was no statistically significant difference between the four experimental groups ($P > 0.05$).

Accumulation of Cu content in V. natans

Cu content in the aboveground tissues of the *V. natans* treatment group differed significantly from the CK group ($P < 0.05$). The Cu contents in the aboveground tissues were 630, 340, 240, and 300 mg/kg in the CK, T1, T2, and T3 groups, respectively. The Cu contents in the T1, T2, and T3 groups were 46.03%, 61.90%, and 52.38% lower than in the CK group, respectively. The Cu content of the T2 group was significantly lower than that of the T1 and T3 groups. In the belowground tissues, the Cu content in the T3 group was 56.17% lower than that in the CK group, and there was no significant difference between the CK group and T1 and T2 groups, or between the T1, T2, and T3 groups ($P < 0.05$) (*Fig. 4*).

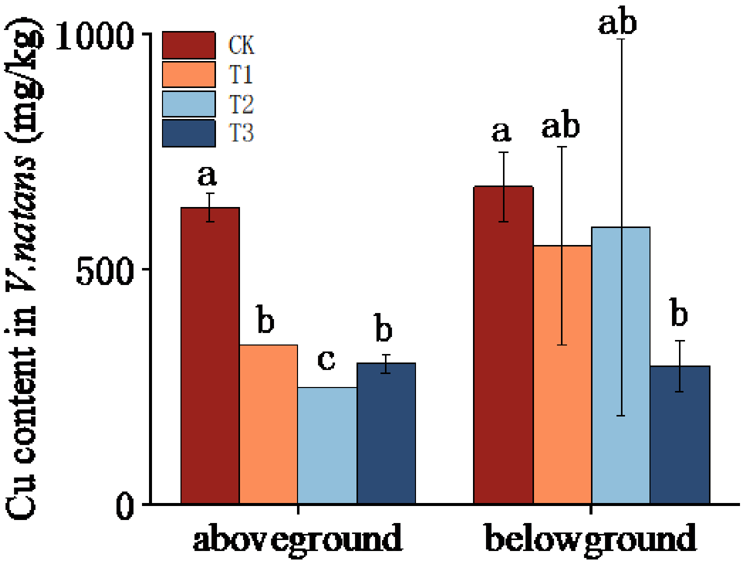

Figure 4. *The final Cu content of V. natans in different treatments in aboveground and belowground tissues. CK: 0 g/L biochar, T1: 3 g/L biochar, T2: 6 g/L biochar, T3: 12 g/L biochar. Different lowercase letters represent significant differences among experimental conditions (LSD test after one-way ANOVA), Data are shown as the mean ± SE, n = 3. Error bars represent standard error*

Changes in chlorophyll contents of V. natans

The amount of biochar used increased the change in chlorophyll content in *V. natans* leaves. The SPAD values were 7.45, 8.92, 10.39, and 10.92 in the CK, T1, T2, and T3 groups, respectively. The SPAD values of the T1, T2, and T3 groups were 19.73%, 39.46%, and 46.58% higher than those of the CK group, respectively. There was a significant difference between the CK group and the T1, T2, and T3 groups. There was no statistically significant difference between the T2 and T3 groups ($P > 0.05$) (*Fig. 5*).

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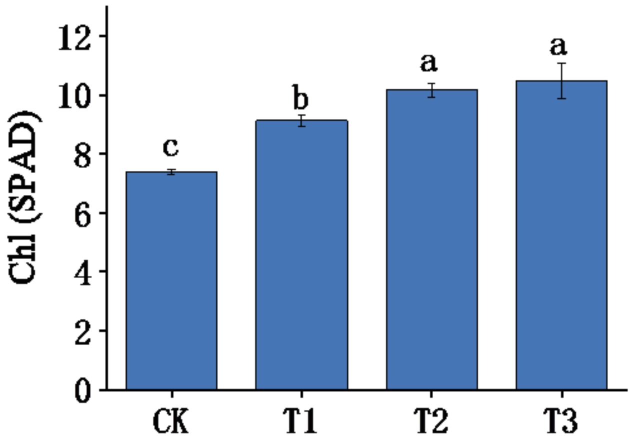

Figure 5. *The final chlorophyll contents (SPAD) of V. natans in different treatments. CK: 0 g/L biochar, T1: 3 g/L biochar, T2: 6 g/L biochar, T3: 12 g/L biochar. Different lowercase letters represent significant differences among experimental conditions (LSD test after one-way ANOVA), Data are shown as the mean ± SE, n = 3. Error bars represent standard error*

Growth status of V. natans

The aboveground part lengths of *V. natans* in the CK, T1, T2, and T3 groups were 22.13, 24.52, 25.04, and 26.73 cm, respectively. In the T1, T2, and T3 groups, the lengths were increased by 10.83%, 13.18%, and 20.83%, respectively, when compared to the CK group. There were statistically significant differences between the control and treatment groups, significant differences between the T1 and T3 groups, and no significant difference between the T2 and T1 groups ($P > 0.05$). There was no significant difference in the length of the belowground part between the treatment and control groups, and the length of the belowground part was approximately 4.77 cm in all four groups (*Fig. 6a*).

The fresh weight of the aboveground part of *V. natans* in the CK, T1, T2, and T3 groups was 1.86, 2.11, 2.29, and 2.5 g, respectively. When compared to the CK group, the fresh weight of the T1, T2, and T3 groups increased by 13.18%, 23.11%, and 34.36%, respectively. There was no statistically significant difference between the CK and T1 groups, and no difference between treatment groups ($P > 0.05$). There was no significant difference between the belowground fresh weight of the treatment groups and the CK group, but the belowground fresh weight of the T1 group was less than that of the T2 group, and the difference between the two groups was significant (*Fig. 6b*).

Bioconcentration Factor (BCF) and Translocation Factor (TF)

With the addition of biochar, BCF decreased to varying degrees. The BCF (shoot/sediment) in the T1, T2, and T3 groups, the BCF were decreased by 42.76%, 59.25%, and 51.80%, respectively, when compared to the CK group. The BCF (root/sediment) in the T1, T2, and T3 groups, the lengths were decreased by 18.01%, 12.53%, and 56.19%, respectively, when compared to the CK group. When compared to the CK group, TF in the T1 and T2 groups decreased by 21% and 34%, respectively. Interestingly, when biochar was added at 12 g/L, T3 > CK (*Table 1*).

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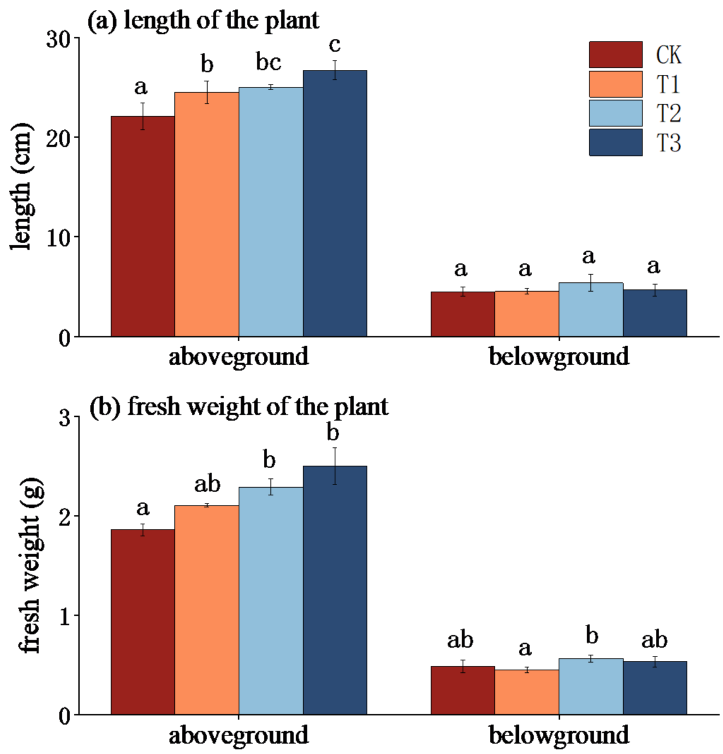

Figure 6. *The length (a) and fresh weight (b) of V. natans in different treatments. CK: 0 g/L biochar, T1: 3 g/L biochar, T2: 6 g/L biochar, T3: 12 g/L biochar. Different lowercase letters represent significant differences among experimental conditions (LSD test after one-way ANOVA), Data are shown as the mean ± SE, n = 3. Error bars represent standard error*

Table 1. *Bioconcentration factor (BCF) and translocation factor (TF) in different treatments*

Treatment group	BCF		TF
	Shoot/sediment	Root/sediment	
CK: 0 g/L biochar	0.945	1.018	0.928
T1: 3 g/L biochar	0.541	0.882	0.613
T2: 6 g/L biochar	0.385	0.942	0.409
T3: 12 g/L biochar	0.456	0.450	1.011

Pearson correlation analysis

Biochar addition positively correlated with chlorophyll ($r = 0.78$, $P < 0.01$), aboveground length ($r = 0.85$, $P < 0.01$), and aboveground fresh weight ($r = 0.87$, $P < 0.01$) in *V. natans*. Significant positive correlations were found between sediment Cu content and the Cu content of *V. natans* aboveground ($r = 0.88$, $P < 0.01$) and belowground ($r = 0.80$, $P < 0.01$) tissues. This study found a highly significant negative correlation between water Cu content and chlorophyll ($r = -0.81$, $P < 0.01$), aboveground length ($r = -0.82$, $P < 0.01$), and aboveground fresh weight ($r = -0.78$, $P < 0.05$) in *V. natans*. The Cu content of *V. natans* aboveground tissues had significant negative influences on chlorophyll ($r = -074$, $P < 0.05$), aboveground length ($r = -0.79$, $P < 0.01$), and aboveground fresh weight ($r = -0.70$, $P < 0.01$) (*Fig. 7*).

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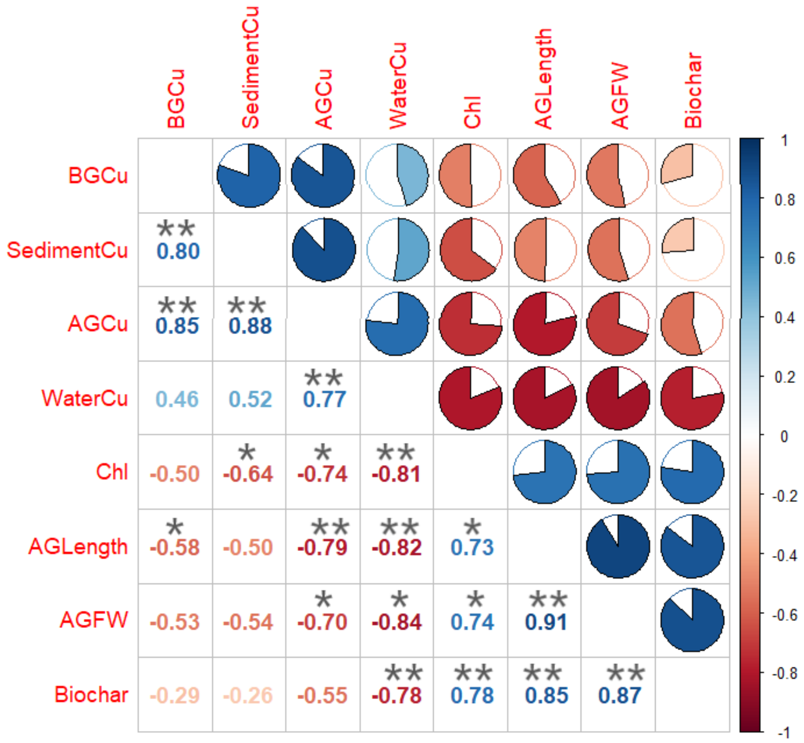

Figure 7. *Pearson correlation analysis among different indicators (AGCu, Cu content of aboveground part of V. natans; BGCu, Cu content of belowground part of V. natans; AGLength, length of the aboveground part of V. natans; AGFW, fresh weight of aboveground part of V. natans). The pie in this graph represents the change in the correlation coefficient, and the asterisk (*) represents the significance level ("*" for P < 0.05 and "**" for P < 0.01)*

Discussion

This study demonstrated that adding biochar to the water column of an aquatic ecosystem with Cu-contaminated sediment could reduce the Cu content in water as well as the effect of Cu on the growth of the submerged macrophyte, *V. natans*. The Cu in the water in this experiment mainly came from the release of Cu from sediment. In this experiment, the calculation of copper content in sediment, water, and bitter grass shows that Cu in sediments is mainly adsorbed by biochar, and a small part is enriched in *V. natans*. Biochar has been demonstrated to have a strong adsorption capacity for Cu (Cheng et al., 2020). Adding biochar to the water column could continuously absorb the Cu released from sediment to the water, thus maintaining low Cu content in the water. Submerged macrophytes have also been shown to have strong abilities to absorb and accumulate heavy metals (Krayem et al., 2021; Lu et al., 2018). However, high concentrations of Cu would inhibit plant photosynthetic activity by decreasing leaf chlorophyll content via oxidative damage, and could even lead to death. The present study showed that the Cu content in water was significantly negatively correlated with the fresh weight, length, and chlorophyll content of the aboveground parts of *V. natans* ($P < 0.05$), indicating that Cu in water inhibited the growth of *V. natans*. The supplemental amount of biochar in water had a significant positive correlation with the fresh weight, length, and chlorophyll content of the aboveground parts of *V. natans* ($P < 0.01$), suggesting that the addition of biochar promoted the growth of *V. natans*.

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Biochar addition may reduce Cu availability by *V. natans* through surface sorption reactions. In addition, unwashed biochar may retain inorganic elements, such as nitrogen, phosphorus, and potassium (Tan and Lagerkvist, 2011), which may be released into the water. Those elements from biochar may promote the growth of submerged macrophytes. The rice husk biochar used in this study was untreated, which could also be a reason why biochar promoted the growth of *V. natans*. In conclusion, the beneficial effects of biochar addition to *V. natans* were mainly due to decreased Cu uptake by *V. natans* and increased nutrients availability.

The present study showed that the aboveground biomass of *V. natans* accounted for 81.97% of the total biomass. The accumulation of Cu in the aboveground tissues of *V. natans* accounted for 71.89% of the total accumulation. Therefore, Cu pollution in sediment could be reduced by harvesting the aboveground parts of *V. natans*. This study demonstrates that biochar combined with submerged macrophytes has good application prospects in the remediation of heavy metal pollution in sediment. By adding biochar, the heavy metal content in the water was reduced, and the inhibition of heavy metals on the growth of submerged macrophyte was alleviated, thus increasing the potential of plant accumulation of more heavy metals.

Nutrient elements remain in the pores of biochar during the preparation of biochar (Qian et al., 2013; Ippolito et al., 2020). Therefore, the direct use of untreated biochar in water may potentially increase eutrophication. Residual nutrients may also clog up the pores in biochar, thus reducing its Cu adsorption capacity. In addition, adding untreated biochar directly to water may raise the pH of the water because biochar is generally alkaline (Yuan et al., 2011). Therefore, biochar is generally modified by water washing, pickling, or other methods before being used as adsorption material for water pollutants (Xiang et al., 2020). However, the biochar used in this study did not undergo any modification, mainly for the following reasons. One was to reduce the cost of using biochar; second, the nutrient elements left in biochar could have promoted the growth of submerged macrophytes, thus increasing their resistance to heavy metal pollution; and third, the increase of pH could have reduced the release of heavy metals from sediment (Shabbir et al., 2020). The results of this study showed that untreated biochar also had good Cu removal potential and could promote the growth of the submerged macrophyte *V. natans*. These results indicate that untreated biochar can be used for the remediation of heavy metal pollution in water and sediment and has good environmental and economic benefits.

The Cu content in the sediment of the treatment groups decreased slightly after the experiment, but there was no significant difference between the treatment and control groups. This may have been related to the fact that the addition of biochar increases sedimental pH, cation exchange capacity and functional groups, thereby potentially decreasing Cu element solubility, mobility and phyto-availability (Natasha et al., 2021), and that the strong retention ability of Cu in the sediment (Peng et al., 2009). In this study, the main reason for the insignificant reduction of sedimental Cu content was that the water column maintained a high pH value. It may also be because bioremediation is a slow process, and it is difficult to significantly reduce heavy metal pollution in a short time. During the experiment, the Cu content in the water of the treatment groups dropped to its lowest point on the tenth day and then increased. This may have been because of the continuous release of copper from the sediment to the water, which reduces the sorption capacity on biochar surfaces, suggesting that biochar placed into water needs to be recycled regularly.

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Conclusion

The present study demonstrated the feasibility of combining biochar and submerged macrophytes for the remediation of heavy metal pollution in sediment. It was found that biochar could indirectly reduce the harm caused by heavy metals to submerged macrophytes by absorbing heavy metals in water. BCF, TF and different plant parameters showed that the benefit of biochar addition at 12 g/L to mitigate *V. natans* harm was the most obvious. Furthermore, submerged macrophytes could not only accumulate heavy metals but also improve and stabilize water quality and reduce the potential negative effects of biochar on water. Although both biochar and submerged macrophytes could be used independently in the remediation of heavy metal pollution, their synergies could more effectively repair heavy metal pollution and have a good application prospect. However, the situation of natural lakes and rivers are more complex, and environmental factors such as water circulation, turbidity, and hydrochemistry may affect the release of pollutants from sediments, thus affecting the implementation effect of ecological restoration measures. Therefore, the results obtained from the model experiment in the present study need to be verified in natural water bodies.

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APPENDIX

Table A1. *Results from the one-way ANOVA and LSD in Cu content of water in different treatments over time*

Treatments		Day1	Day4	Day7	Day10	Day14
CK (0 g/L)	T1	0.573	0.003	0.002	0.000	0.007
	T2	0.415	0.003	0.003	0.000	0.017
	T3	0.177	0.001	0.000	0.000	0.003
T1 (3 g/L)	CK	0.573	0.003	0.002	0.000	0.007
	T2	0.781	0.892	0.427	0.010	0.321
	T3	0.363	0.076	0.050	0.001	0.294
T2 (6 g/L)	CK	0.415	0.003	0.003	0.000	0.017
	T1	0.781	0.892	0.427	0.010	0.321
	T3	0.506	0.065	0.021	0.005	0.080
T3 (12 g/L)	CK	0.177	0.001	0.000	0.000	0.003
	T1	0.363	0.076	0.050	0.001	0.294
	T2	0.506	0.065	0.021	0.005	0.080

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Table A2. *Results from the one-way ANOVA and LSD in the final Cu content of V. natans in aboveground and belowground tissues, and chlorophyll contents (SPAD) of V. natans in different treatments*

Treatments		Aboveground	Belowground	Chl (SPAD)
CK (0 g/L)	T1	0.000	0.728	0.006
	T2	0.000	0.814	0.000
	T3	0.000	0.309	0.000
T1 (3 g/L)	CK	0.000	0.728	0.006
	T2	0.022	0.909	0.062
	T3	0.169	0.473	0.022
T2 (6 g/L)	CK	0.000	0.814	0.000
	T1	0.022	0.909	0.062
	T3	0.118	0.413	0.517
T3 (12 g/L)	CK	0.000	0.309	0.000
	T1	0.169	0.473	0.022
	T2	0.118	0.413	0.517

Table A3. *Results from the one-way ANOVA and LSD in the length and fresh weight of V. natans in different treatments*

Treatments		Length (cm)		Fresh weight (g)	
		Aboveground	Belowground	Aboveground	Belowground
CK (0 g/L)	T1	0.019	0.984	0.169	0.376
	T2	0.007	0.105	0.042	0.086
	T3	0.001	0.762	0.012	0.268
T1 (3 g/L)	CK	0.019	0.984	0.169	0.376
	T2	0.544	0.109	0.274	0.020
	T3	0.027	0.777	0.054	0.066
T2 (6 g/L)	CK	0.007	0.105	0.042	0.086
	T1	0.544	0.109	0.274	0.020
	T3	0.073	0.169	0.224	0.466
T3 (12 g/L)	CK	0.001	0.762	0.012	0.268
	T1	0.027	0.777	0.054	0.066
	T2	0.073	0.169	0.224	0.466

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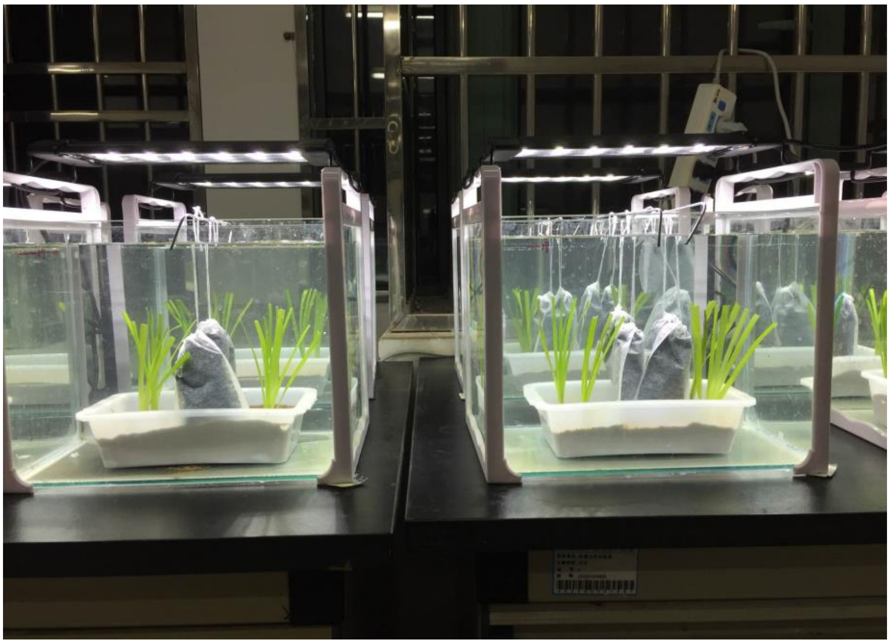

Figure A1. *Photo of the experimental culture and equipment*

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