

Copper accumulation process and rhizosphere mechanism under different water regime in riparian wetland of Poyang Lake, China

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Research Article

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Abstract

Purpose Conducting research about the metal accumulation process of wetland plants and relevant rhizosphere mechanism can better understand the biogeochemical behavior of metals in riparian wetland. However, little relative information is available for riparian wetland.

Methods In this study, we explored the copper accumulation process of dominant plant of *Artemisia* and rhizosphere mechanism under different water regime (flooding condition [FC], dry condition [DC] and alternate dry and flooding condition [DFC]) in riparian wetland of Poyang Lake.

Results The result indicated that continuous or intermittent flooding may impede the accumulation and transformation of Cu by *Artemisia*. The correlation and multivariate analysis indicated that rhizosphere pH, Cu fraction and iron plaque on root surface can significantly influence Cu accumulation process. The transformation of exchangeable fraction to carbonate bound fraction and organic fraction under DFC and FC treatments may impede the accumulation of Cu in *Artemisia*. Formation of iron plaque under DFC and DC treatments can enhance the Cu accumulation in roots, but impede the translocation of Cu to aboveground tissues. Higher rhizosphere pH under DFC and FC treatments may impede the translocation of Cu by enhancing formation of iron plaque. Additionally, the growth status, bioaccumulation factor, translocation factor and root/aerial Cu content indicated that *Artemisia* can accumulate quantitative Cu in roots, but not suitable for phytoextracting Cu contaminated soil because of significant decrease of biomass with Cu addition, especially under DFC and FC treatments.

Conclusion Altogether, our results indicate that Cu accumulation processes and ability is impeded by DFC and FC treatments.

Introduction

Riparian wetland, located in the transition of aquatic and terrestrial ecosystem, plays an important role maintaining nutrients, keeping biodiversity, reducing erosion and exchanging materials (Liu et al., 2019; Zhang et al., 2020; Mir et al., 2022). However, the riparian zone has been intensively contaminated by heavy metals because of anthropogenic activities including agricultural and industrial development (Rajeshkumar et al., 2018; Zhang et al., 2021). With the characteristics of toxicity, persistence and transitivity, metals entering the system can threaten the function and health of the riparian zone (Hussain et al., 2021; Kumar et al., 2021). Riparian sediment, as the sink of pollutants, can accumulate most metals entering the system by adsorption (Pavlović et al., 2019; Zhao et al., 2021). However, metals in the riparian sediment can resuspend to the waterbody due to environmental change (Liu et al., 2019; Zhao et al., 2021). Therefore, it's of great significance to analyze the bio-geochemical behavior of metals in the riparian sediment to provide basis for keeping the health of riparian zone.

Wetland plants, a key composition of the riparian wetland, play a key role in changing the bio-geochemical behavior of pollutants in the sediment (Ou et al., 2019; Xu et al., 2021). Previous researches indicated that wetland vegetation can accumulate large amounts of metals in the roots or aboveground part because of their well-developed root ecosystem, fast growth rate and large biomass, thus reducing metals in the sediment (Wang et al., 2018; Zhang et al., 2021; Yan et al., 2022). Many wetland plants, including *Phragmites australis* (Eid et al., 2021), *Schoenoplectus lacustris* (Duman et al., 2007) and *Typha latifolia* (Amir et al., 2020) have high ability of accumulating metals. However, the metal accumulation processes and ability change with environmental condition, including water regime and sediment properties (Jha et al., 2016; Yang et al., 2017; Kumar et al., 2021). Hence, it's of great significance to pay attention to the metal accumulation of wetland plants with environmental change.

Bioavailability of metals, namely the availability of metals to plants, determines the efficiency of the metal accumulation process (Cao et al., 2019; Cao et al., 2020). Located along lake or river ecosystem, riparian wetland has the most important characteristics of periodical hydrological change, which can significantly influence the availability of metals by affecting the soil characteristics, like pH and redox environment (Boostani et al., 2021; Yan et al., 2021). Rhizosphere, the millimeter zone around the plant roots, have apparent different soil properties with the non-rhizosphere because of the roots of plants (Xu et al., 2021). In riparian wetland, influenced by the interaction of plant roots and water regime, rhizosphere environment may be more complex. For example, wetland plants may generate oxygenated rhizosphere environment via radial oxygen loss (ROL) and exudate organic matter more complex under flooding condition, thus changing the rhizosphere properties (including pH, Eh, organic matter, iron plaque, microbial community et al) and subsequent metal availability and accumulation processes (Xiao et al., 2020; Wei et al.,

2021). Many studies have been conducted to explore metal availability, but few information is available for the rhizosphere availability and subsequent accumulation under the influence of wetland plants and hydrology in riparian wetland.

Poyang Lake, the largest freshwater lake in China, has been reported polluted by metals to different extent (Niu et al., 2019; Yan et al., 2019; Xu et al., 2021). Additionally, affected by the anthropogenic activities including mineral mining and agricultural development, copper (Cu) has been one of the most polluted metals in the Poyang Lake area (Wang et al., 2019; Yan et al., 2019). Influenced by the hydrological change caused by subtropical climate, large area of riparian wetland is distributed along the lake (Wu et al., 2021). And the dominant plants including *Artemisia* mainly grow on these riparian wetlands (Xu et al., 2020). Studies have been conducted to analyze the metal accumulation of plants in riparian wetland of Poyang Lake (Xu et al., 2020), but few are aware of the rhizosphere influence on metal accumulation processes considering the influence of hydrological regime.

Considering above, we studied the accumulation processes of Cu by *Artemisia* and the rhizosphere mechanism under different hydrological condition in the riparian wetland of Poyang Lake. This study aims to: (1) analyze the accumulation processes of Cu by *Artemisia* with the influence of hydrological regime; (2) illustrate the rhizosphere effects on Cu uptake by *Artemisia* with the effect of hydrological regime; (3) make clear the Cu accumulation ability of *Artemisia* under different water condition. This study can provide new idea for the health of aquatic ecosystem.

Materials And Methods

Experimental design and sampling

Soil and plants for experiments were gotten from a riparian wetland of Poyang Lake with few Cu. The soil (0–20 cm) sampled, dried and then sieved before used for experiments. Plants with similarly growth characteristics (mainly height and biomass) were collected.

The experiment was performed in the green house of the Poyang Laboratory for Wetland Ecosystem Research, Chinese Academy of Sciences (116°3'4"E, 29°26'54"N). Polyethylene plastic buckets (height: 27 cm, upper diameter: 24.5 cm and lower diameter: 19.5 cm) were used for experiment. According to the pollution status of the Poyang Lake drainage, the Cu ($\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$) concentration was set to 0, 60, 120 and 240 mg/kg in this study. Three water condition of dry (DC), flooding (FC), alternate dry and flooding (DFC) were set. The detailed information of water condition can be seen in our previous study of (Xu et al., 2021). Six seedlings were planted in each bucket and three replicates were set for each setting. The experiment lasted for 3 months.

Pretreatment and analysis on soil and plant samples

At the end of the experiment, plant samples were obtained by a stainless shovel. The rhizosphere soil was gotten by the shaking method (Wang et al., 2009). Soil samples were air dried, ground and sieved before analysis. Soil pH were analyzed with a portable pH. Soil total organic carbon (TOC) were measured by an Elementar TOC analyzer (EA3000, EuroVector, Italy). Soil Cu fraction (exchangeable fraction (F1), carbonate fraction (F2), Fe/Mn oxide bound fraction (F3), Organic fraction (F4), residual fraction (F5)) was extracted by sequential extraction method and measured by inductively coupled plasma-mass spectrometry (ICP-MS, 7700x, Agilent; US), which can reveal the bioavailability of soil metals.

Plant samples were cleaned, divided (as roots, aboveground parts), dried (70°C), weighted, and then grinded to sieve for Cu concentration measurement. The iron plaque on fresh plant root was extracted with the improved dithionite–citrate–bicarbonate (DCB) method (Liu, 2015). The Cu, Fe, Mn concentrations in iron plaque ($\text{Cu}_{\text{plaque}}$, $\text{Fe}_{\text{plaque}}$ and $\text{Mn}_{\text{plaque}}$) were analyzed. Metal concentrations were all measured by the method of ICP-MS.

Metal accumulation process and ability

The Cu accumulation processes can be expressed by bioaccumulation factor (BAF, the ration of Cu concentration in roots to rhizosphere soils) and translocation factor (TF, the ratio of Cu concentrations in aboveground parts to roots or ratio of Cu in leaves to shoots). The BAF and TF can also indicate the ability of accumulation, $\text{BAF} > 1$ or $\text{TF} > 1$ suggests that *Artemisia* has high ability of phytostabilization (accumulates higher metals in roots) or phytoextraction (accumulates higher metals in shoots), respectively (Xu et al., 2020). Cu content in *Artemisia* were calculated to further explain the phytoremediation ability. The potential of

phytostabilization is addressed as: root Cu content = root Cu concentration × root biomass; the potential of phytoextraction was calculated as: aerial metal content = shoot metal concentration × shoot biomass + leaf metal concentration × leaf biomass (Yang et al., 2019).

Statistical analysis

One-way or Two-way ANOVA was used for the inter-group differences, and Turkey's multiple comparisons test was used for inner-group differences. Correlation and redundancy analysis (RDA) were performed to elucidate the influence of rhizosphere environment on Cu uptake by *Artemisia*. Before RDA, Shapiro-Wilk and Leven's test were conducted to ensure the normality and homogeneity of data, respectively. Data that were not normal were transformed logarithmically or exponentially. All analysis were conducted using R v3.5.1.

Results

Plant growth under different water condition

Water condition significantly influenced plant growth ($p < 0.001$) (Table 1). Generally, the biomass and height of *Artemisia* was lower under DFC and FC treatment, and the difference was particularly significant when Cu addition lower than 120 mg/kg. Cu addition significantly decreased biomass and height of *Artemisia* ($p < 0.05$). For example, shoot biomass was significantly ordered as control > T60 > T120 > T240 under DC condition.

Table 1
The growth condition of *Artemisia* under the influence of Cu and flooding

Cu addition	Water treatment	Root Biomass (g. DW ⁻¹)	Shoot Biomass (g. DW ⁻¹)	Leaf Biomass (g. DW ⁻¹)	Height (cm)
Control	DC	4.04a ± 0.68	12.12a ± 0.47	2.74a ± 0.37	108.25a ± 35.00
	DFC	1.54cb ± 0.28	7.3b ± 0.54	0.81c ± 0.14	72.4b ± 22.64
	FC	1.36dcb ± 0.32	4.73c ± 0.87	0.26d ± 0.04	100.4a ± 17.07
T60	DC	1.98b ± 0.73	4.99c ± 1.38	1.26b ± 0.69	100.06a ± 18.31
	DFC	1.09edc ± 0.3	3.49d ± 0.78	0.5cd ± 0.12	60.21c ± 16.13
	FC	0.65fe ± 0.2	2.36e ± 0.18	0.15d ± 0.09	84.13b ± 8.56
T120	DC	0.76fed ± 0.42	1.96e ± 0.26	0.21d ± 0.11	65.3a ± 15.53
	DFC	0.32f ± 0.01	0.89f ± 0.09	0.13d ± 0.03	57.47a ± 8.12
	FC	0.39f ± 0.1	0.94f ± 0.17	0.1d ± 0	55.64b ± 9.23
T240	DC	0.32f ± 0.21	0.74f ± 0.25	0.17d ± 0.06	30.4b ± 8.09
	DFC	0.42f ± 0.06	0.6f ± 0.01	0.08d ± 0	35.8b ± 8.13
	FC	0.25f ± 0.06	0.67f ± 0.04	0.08d ± 0.03	41.2a ± 7.03

Cu accumulation and translocation in *Artemisia* under different water condition

Water condition showed significant influence on Cu concentrations in *Artemisia* tissues in certain condition (Fig. 3, $p < 0.05$ or $p < 0.01$). Generally, Cu in *Artemisia* tissues were lower under DFC and FC treatment compared with DC treatment. For example, Cu in both root and leaf was significantly lower under DFC and FC treatment when Cu addition at 240 mg/kg ($p < 0.05$). And Cu

concentrations in *Artemisia* leaf was significantly lower under DFC and FC treatment with all Cu addition condition ($p < 0.01$). Besides, Cu concentrations in all tissues were significantly influenced by Cu addition ($p < 0.01$). Cu concentration in different tissues with Cu addition was generally higher than control.

BAF was only higher than 1 in the control under DC treatment (Fig. 4). Generally, BAF values was lower under DFC and FC treatment compared with DC treatment, but the difference was only significant under control ($p < 0.05$). Besides, BAF values was significantly influenced by Cu addition, which was significantly lower at all Cu addition than control under DC treatment ($p < 0.01$). $TF_{\text{root-shoot}}$ values were all lower than 1. Generally, $TF_{\text{root-shoot}}$ was lower under DFC and FC treatment than DC treatment when Cu addition lower than 240 mg/kg. But the influence of water regime on $TF_{\text{root-shoot}}$ was not statistically significant ($p > 0.05$). The $TF_{\text{root-leaf}}$ values were generally higher than 1 when Cu addition lower than 240 mg/kg ($p < 0.05$). $TF_{\text{root-leaf}}$ was significantly lower under DFC and FC treatments when Cu addition were no more than 60 mg/kg. Cu addition had significant influence on $TF_{\text{root-leaf}}$ ($p < 0.01$), with its values generally decreased with Cu addition. $TF_{\text{shoot-leaf}}$ values were all higher than 1 except Cu addition 240 mg/kg. Despite the generally higher values under DFC treatment, water condition did not exert a significant influence on $TF_{\text{shoot-leaf}}$ ($p > 0.05$). Cu addition had a significant influence on $TF_{\text{shoot-leaf}}$, with its values particularly lower at Cu addition of 240 mg/kg compared with control ($p < 0.01$).

Rhizosphere influence on Cu availability and accumulation process under different water conditions

Rhizosphere characteristics

Rhizosphere pH was significantly influenced by water condition (Table 2, $p < 0.01$). Generally, rhizosphere pH was significantly higher under DFC and FC treatments than DC treatment, especially when Cu concentration was lower than 240 mg/kg. TOC was only significantly higher under FC treatment than DFC and DC treatments when Cu concentration was 120 mg/kg (Table 2, $p < 0.05$).

Table 2
Rhizosphere soil property change under different water regime

Cu	Water condition	pH	TOC
Control	DC	4.72b ± 0.06	0.036a ± 0.0025
	DFC	4.88c ± 0.04	0.039a ± 0.0015
	FC	5.15a ± 0.08	0.036a ± 0.0015
T60	DC	4.66a ± 0.13	0.037a ± 0.0015
	DFC	5.02b ± 0.13	0.038a ± 0.0012
	FC	5.24b ± 0.08	0.038a ± 0.001
T120	DC	4.3c ± 0.07	0.038b ± 0.0021
	DFC	4.74b ± 0.07	0.037b ± 0.0006
	FC	5.06a ± 0.1	0.045a ± 0.0047
T240	DC	4.59b ± 0.42	0.039a ± 0.003
	DFC	4.94ab ± 0.18	0.038a ± 0.002
	FC	5.28a ± 0.08	0.039a ± 0.001

Water condition significantly affected Fe and Mn concentrations in iron plaque (Fig. 1, $p < 0.01$). Fe and Mn in iron plaque was generally ordered as FC > DFC > DC. Fe concentrations was significantly higher under DFC and FC treatment at all Cu addition. While Mn concentrations was particularly higher under DFC and FC treatment when Cu addition at 60 and 120 mg/kg. Besides, Fe and Mn in iron plaque increased with Cu addition ($p < 0.01$). For example, the Fe in iron plaque at Cu addition of 60, 120, 240 mg/kg

was 1.23, 1.70 and 1.73 times higher than control treatment under DFC treatment, and the difference was significant when Cu addition at 240 mg/kg.

Generally, Cu in iron plaque was ordered as FC > DFC > DC, and it was significantly higher under FC treatment when Cu addition higher than 120 mg/kg ($p < 0.05$). Cu addition also significantly influence Cu in iron plaque ($p < 0.01$). Generally, Cu in iron plaque increased under all Cu addition compared with control, and the increase was particularly significant when Cu addition at 240 mg/kg.

Rhizosphere Cu fraction

Water condition had a significant influence on F1 proportion of rhizosphere Cu fraction (Fig. 2, $p < 0.05$). The proportion of F1 fraction was significantly lower under FC and DFC treatment compared with DC treatment. Proportion of F2 fraction was generally higher under DFC and FC treatment and proportion of F3 fraction was lower under FC treatment. Proportion of F4 fraction was higher under DFC and FC treatment when Cu addition higher than 60 mg/kg. But the proportion change of F2, F3 and F4 fraction was not statistically significant ($p > 0.05$).

The proportion of F1, F3 and F4 fraction was significantly different among Cu addition ($p < 0.01$). Proportion of F1 fraction was apparently higher at Cu addition of 240 mg/kg than control ($p < 0.01$). And proportion of F3 fraction at all Cu addition was significantly higher than control ($p < 0.01$). While proportion of F4 fraction at all Cu addition was significantly lower than control ($p < 0.01$).

Correlation among all influencing factors

The influence of rhizosphere characteristics on rhizosphere Cu fraction was analyzed by Pearson correlation (Fig. 5). Proportion of F1 and F3 was significantly and positively related with Cu_{DCB} ($r = 0.42$ and 0.52 , $p < 0.05$ and $p < 0.01$). F2 and F4 was positively correlated with DCB_{Mn} , DCB_{Cu} , DCB_{Fe} ($r = 0.45-0.81$, $p < 0.05$ or $p < 0.01$). pH was positively correlated with DCB_{Mn} , DCB_{Fe} and DCB_{Cd} ($r = 0.39-0.67$, $p < 0.05$ or $p < 0.01$).

Correlation and RDA analysis of factors influencing Cu accumulation

The influence of rhizosphere characteristics on Cu accumulation was explored by correlation analysis (Table 3). The results indicated that Cu_{root} was positively correlated with Mn_{DCB} , Cu_{DCB} , and Cu_{DCB} ($r = 0.42-0.62$, $p < 0.01$), while Cu_{leaf} , $TF_{root-shoot}$ and $TF_{root-leaf}$ was negatively correlated with Mn_{DCB} , Cu_{DCB} or Cu_{DCB} ($r = -0.69- -0.30$, $p < 0.05$ or $p < 0.01$). F1, F2, F3 and F4 positively influenced Cu_{root} ($r = 0.74-0.93$, $p < 0.01$), while negatively influence $TF_{root-shoot}$ and $TF_{root-leaf}$ ($r = -0.76- -0.37$, $p < 0.05$ or $p < 0.01$). Cu_{shoot} and $TF_{shoot-leaf}$ was positively correlated with F1, F2 and F3 ($r = 0.35-0.58$, $p < 0.05$ or $p < 0.01$). BAF showed negative correlation with F2 and F4 ($r = -0.30$ and -0.34 , $p < 0.05$). Cu_{leaf} showed significant negative correlation with pH ($r = -0.54$, $p < 0.01$).

Table 3

Correlation analysis of the effect of all influencing factors on the Cu accumulation and transformation by *Artemisia*

	Mn _{DCB}	Cu _{DCB}	Cu _{DCB}	F1	F2	F3	F4	F5	pH	TOC
Cu _{root}	0.62**	0.43**	0.42**	0.80**	0.74**	0.93**	0.84**	0.94**	-0.222	0.132
Cu _{shoot}	-0.07	0.001	-0.10	-0.02	0.18	0.12	0.06	0.11	-0.065	0.106
{\text{C}\text{u}}_{\text{leaf}}	-0.19	-0.18	-0.30*	0.52**	0.55**	0.36*	0.23	0.47**	-0.536**	0.085
BAF	-0.26	-0.12	-0.15	-0.16	-0.30*	-0.28	-0.34*	-0.31*	-0.105	-0.293
{\text{T}\text{F}}_{\text{root-shoot}}	-0.57	-0.30*	-0.37*	-0.50**	-0.37*	-0.57**	-0.51**	-0.55**	-0.089	-0.138
{\text{T}\text{F}}_{\text{root-leaf}}	-0.69**	-0.44**	-0.48**	-0.52**	-0.60**	-0.76**	-0.74**	-0.75**	-0.223	-0.290
{\text{T}\text{F}}_{\text{shoot-leaf}}	0.26	0.07	0.14	0.58**	0.40**	0.35*	0.24	0.44**	0.069	-0.134

Notes: ** indicates the correlation was significant at $p < 0.01$ level; * indicates the correlation was significant at $p < 0.05$ level.

RDA analysis was conducted to further identify the influence of rhizosphere characteristics on metal accumulation processes under different water condition (Fig. 6). The results indicated that all environmental factors explained 86.20% of the total variance, with the first two axis significantly illustrated 82.34% of the total variance ($p < 0.01$ or $p < 0.05$). pH showed apparent negative correlation with TF_{root-shoot} and Cu_{leaf}. Cu_{DCB} showed significant positive correlation with Cu_{root} and Cu_{shoot}, while negative correlation with TF_{root-leaf}, and TF_{shoot-leaf}. Fe_{DCB} was also negatively related with TF_{root-leaf} and TF_{root-shoot}. TOC and Mn_{DCB} showed weak relationship with TF_{root-leaf}. Different Cu fractions showed significant positive correlation with Cu_{root} and Cu_{shoot}, while negative correlation with TF_{root-leaf} and TF_{shoot-leaf}.

Accumulation ability

Cu content in *Artemisia* under different water condition is shown in Table 5. Water condition had significant influence on root and aerial Cu content ($p < 0.01$). Root Cu content was significantly higher under DFC and FC treatment when Cu addition at 0, 120 and 240 mg/kg. Aerial Cu content was lower under DFC and FC treatment, which is particularly significant when Cu addition at 0 and 60 mg/kg. Besides, root and aerial Cu content was not significantly different among Cu addition ($p > 0.05$). Although generally higher under DFC and FC treatment, root/aerial Cu content was not significantly different among different water condition ($p > 0.05$). While root/aerial Cu content was significantly different among Cu addition, which was particularly higher when Cu addition at 240 mg/kg ($p > 0.01$).

Table 5
Root and aerial Cu content under different water regime

Cu treatment	water condition	root Cu content	aerial Cu content	root/aerial Cu content
Control	DC	120.26ba ± 38.64	334.3a ± 47.89	0.38d ± 0.18
	DFC	52.06dc ± 10.18	68.42c ± 6.88	0.78dcb ± 0.24
	FC	37.11d ± 9.88	37.12c ± 1.63	1dcb ± 0.29
T60	DC	97.59cba ± 13.28	238.52b ± 97.96	0.44dc ± 0.12
	DFC	59.02dc ± 18.95	47.65c ± 17.32	1.26cb ± 0.2
	FC	40.56dc ± 13.57	45.92c ± 38.07	1.13dcb ± 0.5
T120	DC	74.2dcb ± 41.01	71.72c ± 4.28	1.03dcb ± 0.55
	DFC	23.13d ± 2.07	25.56c ± 5.66	0.93dcb ± 0.2
	FC	34.66d ± 8.45	26c ± 3.36	1.37ba ± 0.46
T240	DC	130.73a ± 79.3	70.29c ± 25.29	1.78ba ± 0.64
	DFC	61.71dc ± 10.64	25.14c ± 1.36	2.45a ± 0.34
	FC	42.4dc ± 9.5	45.68c ± 2.17	0.94dcb ± 0.26

Discussion

Plant growth

Plant biomass can reflect the phytotoxicity caused by heavy metals (Wei et al., 2021). Heavy metals can damage the physiological system and antioxidant system, thus influencing plant biomass and height (Kumar et al., 2021). Cu is an essential element for plant growth, but it will be toxic to plant when present in excessive amount in plant tissues (30 mg/kg dry weight) (Cao et al., 2019; Kumar et al., 2021). In this study, plant biomass and height were significantly reduced by Cu addition, with Cu concentrations in root and leaf tissues higher than 30 mg/kg, indicating the toxicity of Cu to *Artemisia*.

Prolonged or periodic flooding can induce the change of plant biomass. Yang et al (2019 and 2020) indicated that *Salix* genotype showed increase of belowground biomass upon flooding. Yang et al (2017) also indicated the remarkably higher biomass of 10 wetland plants under flooding condition than non-flooding condition (Yang et al., 2017). However, in this study, both aboveground and belowground biomass of *Artemisia* significantly reduced under DFC and FC treatment compared with DC treatment. This indicated that *Artemisia* may be more sensitive to water regime than other plants in previous studies. Generally, wetland plants may grow rhizome for reproduction or aquatic adventitious root for breathing under continuous or periodic flooding (Yang et al., 2019). The observed less rhizome roots under DFC and FC treatments in this study further testified the sensitivity of *Artemisia* to continuous or intermittent flooding. Besides, continuous or periodic flooding may reduce energy provision by disturb the photosynthesis processes in leaf and adenosine triphosphate (ATP) formation in root, thus hindering *Artemisia* growth (Yang et al., 2019; Yang et al., 2020).

Uptake and translocation of Cu by *Artemisia*

Metal addition in soil can influence their concentration in plants. In our study, Cu concentrations in all plant tissues were higher than control and the values in roots and shoots increased with Cu addition (Fig. 3). This is coincident with previous studies (Liu et al., 2008; Ubeynarayana et al., 2021). Metal addition can usually increase root exudates of plants, thus increasing metal availability and subsequent accumulation by plants (Ubeynarayana et al., 2021). This is supported by the increase tendency of F1 fraction with Cu addition (Fig. 2). However, the BAF values with Cu addition were lower than control, and $TF_{\text{root-leaf}}$ values decreased with Cu addition (Fig. 4). The growth of *Artemisia* may be impeded by the damage of root structure, xylem and plant system because of

increasing Cu addition (Huang et al., 2019), which may then decrease the ability of *Artemisia* to accumulate and translocate Cu. This was further testified by the significant decrease of *Artemisia* biomass with Cu addition (Table 1).

Water regime is an important factor influencing metal accumulation process by plants (Tang et al., 2020; Yang et al., 2020; Boostani et al., 2021). In this study, our results indicated that Cd concentrations in the tissues of *Artemisia* were generally lower under DFC and FC treatments compared with DC treatment. And the lower BAF, $TF_{\text{root-shoot}}$, $TF_{\text{root-leaf}}$ under DFC and FC treatments indicated that continuous or intermittent flooding may impede the accumulation and transformation of Cu by *Artemisia*. This was coincided with previous studies (Cao et al., 2019; Yang et al., 2019; Tang et al., 2020). For example, Cao et al (2019) indicated that flooding significantly decreased Cd concentrations in all plant tissues of *Salix* and impeded the transfer of Cd to the aerial part (Cao et al., 2019). The metal transfer process is energy consuming and largely depends on the transpiration of wetland plants (Yang et al., 2019; Yang et al., 2020). According to previous studies, biomass can positively reflect the oxygenated condition of wetland plants (Xu et al., 2021). Therefore, the lower biomass under DFC and FC treatments may indicate the less oxygenated environment in the rhizosphere of *Artemisia*. The less aerobic environment under DFC and FC treatments may hinder the photosynthesis in leaf, ATP production in root and the transpiration pull via xylem (Ashraf et al., 2018; Zhang et al., 2019), thus reducing the Cu allocation in aerial part of *Artemisia*.

Rhizosphere factors influencing Cu accumulation process

Rhizosphere Cu fraction

F1 fraction and other fractions can transfer interactively when environment changes (Wang et al., 2018; Kumar et al., 2021). In this study, the proportion of F1 fraction was significantly lower under DFC and FC treatments compared with DC treatment (Fig. 2). Our previous study and other studies also showed similar results (Wan et al., 2019; Xu et al., 2021). The proportion of F1 and F2 showed significant positive correlation (Fig. 5), indicating that the transfer of F1 to F2 may lead to the decrease of F1 under DFC and FC treatments. This was coincided with the increase of the proportion of F2 fraction under DFC and FC treatments (Fig. 2). Previous studies showed that the carbonate bound fraction is most sensitive to the change of pH, and the pH increase may induce the formation of F2 fraction (Li et al., 2017; Di et al., 2019; Hussain et al., 2021). However, F1 and F2 showed no significant relationship with pH (Fig. 5). Further researches are needed to explore the potential mechanism. Additionally, despite that water regime did not significantly change soil organic matter in this study (Table 2), intermittent or continuing flooding can enhance the transfer of F1 to F4 by changing the composition of organic matter (Zhou et al., 2020). Concludingly, the transfer of F1 to F2 and F4 may elucidate the decrease of F1 fraction under DFC and FC treatments.

Metal fraction, indicating the bioavailability of metals, is the most important factor determining metal accumulation processes (Zhang et al., 2021). In this study, F1 fraction showed significant positive correlation with Cu_{root} (Table 3), indicating the significance of F1 fraction in enhancing metal Cu accumulation in *Artemisia*. Additionally, the lower BAF, $TF_{\text{root-shoot}}$ and $TF_{\text{root-leaf}}$ indicated that the decrease of F1 fraction may weak the accumulation processes of Cu by *Artemisia*. This may explain the lower accumulation and transfer values of BAF, $TF_{\text{root-shoot}}$, $TF_{\text{root-leaf}}$ under DFC and FC treatment.

Rhizosphere pH

pH is a key environmental factor influencing soil metal accumulation by wetland plants. In this study, rhizosphere pH was higher under FC and DFC treatment. This was coincided with our previous study of Xu et al (2021). Zhang et al (2019) and Tang et al (2020) also indicated the significant increase of rhizosphere pH under flooding condition (Zhang et al., 2019; Tang et al., 2020). Biomass is reported positively reflecting the ROL in the rhizosphere of wetland plants (Yang et al., 2020). Hence, the significant less total biomass under DFC and FC treatments compared with DC treatment may reveal their less oxygenated environment (Table 1). The more anions in less oxygenated environment under DFC and FC treatments in comparison with that under DC treatment may explain the higher rhizosphere pH (Wan et al., 2019).

Rhizosphere pH showed significant negative relationship with Cu_{leaf} and $TF_{\text{root-shoot}}$ in this study, revealing that the higher pH under DFC and FC treatments may impede the translocation of Cu by *Artemisia*. This was coincided with our previous study of Xu et al (2021). Existing research indicated that the increase of rhizosphere pH can decrease metal availability by ions adsorption, thus impeding the metal accumulation process (Zhang et al., 2019). Whereas, rhizosphere pH showed weak correlation with Cu

fraction in this study (Fig. 5). The weak influence of pH on Cu fraction may be due to little change of pH (Yang et al., 2017). The positive correlation of rhizosphere pH with Cu, Fe and Mn in iron plaque may help illustrate the negative influence of pH on Cu translocation process (Fig. 5). The following discussion will describe the mechanism clearly.

Rhizosphere iron plaque

Iron plaque is often formed under continuous or intermittent flooding condition because of the oxygenated root environment, provide of Fe^{2+} and Mn^{2+} from non-rhizosphere and increase of Fe and Mn-oxidizing bacteria (Peng et al., 2018; Wei et al., 2021). In this study, the significant lower ROL under DFC and FC treatment than under DC treatment as discussed above indicating that that iron plaque on root may be formed less under DFC and FC treatments. However, the amount of iron plaque under DFC and FC condition were higher than that under DC treatment (Fig. 1). Previous studies showed that the acidophilic Fe-oxidizing bacteria may enhance the oxidation of Fe(II), thus promoting the formation of iron plaque (Nordstrom and Southam, 1997; David et al., 1999). Therefore, the higher rhizosphere pH under DFC and FC treatments may promote the formation of iron plaque in this study. This was testified by the significant positive relationship between Fe and Mn in iron plaque and rhizosphere pH (Fig. 5).

Iron plaque on plant roots can usually reduce metal accumulation by immobilizing metals (Xiao et al., 2020; Xiao et al., 2021). In this study, the significantly higher Cu concentrations in iron plaque under DFC and FC condition indicated that continuous or intermittent flooding may impede Cu accumulation process by *Artemisia* through iron plaque formation. This is confirmed by the apparent positive correlation between Fe, Mn and Cu concentrations in iron plaque (Fig. 5). Whereas, in this study, Mn_{DCB} , Cu_{DCB} , or Cu_{DCB} showed positive correlation with Cu_{root} , while negative correlation with Cu_{leaf} , $\text{TF}_{\text{root-shoot}}$ and $\text{TF}_{\text{root-leaf}}$, indicating that the formation of iron plaque induced the accumulation of Cu in the root and impeded the transfer of Cu in aboveground tissues (Fig. 5 and Fig. 6). This was in line with Shen et al (2021) and Yang et al (2014) (Yang et al., 2014; Shen et al., 2021). The metal absorption of iron plaque is closely related with the amount of iron plaque, the excess iron plaque on root may impede the accumulation processes of metals by plants (Wan et al., 2019; Zhang et al., 2019). This may explain the less Cu accumulated in the tissues of *Artemisia* under DFC and FC treatment in this study. Besides, iron plaque may provide Fe^{2+} for plant cells in aboveground tissues to compete adsorption site with Cd ions (Peng et al., 2018), thus reducing the transfer of Cu to aerial part of *Artemisia* under DFC and FC treatments. Further researches are needed to explain the influence of iron plaque on the Cu accumulation processes by *Artemisia*, especially under the influence of flooding.

Rhizosphere TOC

Generally, water regime and metal addition can promote the secretion of root exudates of wetland plants, thus changing the content and fraction of soil organic matter (Li et al., 2016). However, TOC did not show significant change under the influence of water regime and water condition in this study (Table 2). In this study, DFC and FC treatments significantly decreased biomass of *Artemisia* compared with DC treatment, Cu addition also significantly decreased the biomass. The decrease of *Artemisia* biomass under the environmental stress (including intermittent and continuous flooding, Cu addition in this study) may decreased the ability of *Artemisia* to secrete root exudates (Duan et al., 2020), thus decreasing their influence on soil organic matter. This may explain the weak influence of soil organic matter on metal accumulation processes under different water regime. Additionally, soil organic matter in this study did not show significant correlation with metal fraction (Fig. 5). This is in accordance with previous studies (Golestanifard et al., 2020). Generally, it's the composition rather than the total concentration of soil organic matter that decides its influence on metal fraction and subsequent accumulation processes (Zhou et al., 2020). The only measurement of total amount of soil organic matter may not explain the weak relationship between TOC and Cu accumulation in this study (Table 3 and Fig. 5). Further researches are needed to deeply explore the influence of soil organic matter on Cu fraction and accumulation processes with the consideration of organic matter composition.

Accumulation ability

Wetland plants are reported with high metal accumulation capacity (). The accumulation ability is closely related with both metal concentrations in plant tissues and plant biomass (Yang et al., 2019). Therefore, it's necessary to consider both the metal accumulation process and metal content in plant tissues when assessing the accumulation ability of wetland plants (Xu et al., 2020); Yang et al., 2019). In this study, the BAF values were generally lower than 1, indicating that *Artemisia* was not suitable for phytostabilizing Cu under different water condition. The $\text{TF}_{\text{root-leaf}}$ and $\text{TF}_{\text{shoot-leaf}}$ values were generally higher than 1 and

$TF_{\text{root-leaf}}$ value was lower under DFC and FC treatments. This indicated that *Artemisia* is suitable for the phytoextracting Cu, but the ability was weakened by intermittent or continuing flooding. Previous studies also showed that flooding can weaken the phytoextraction ability of wetland plants (Chen et al., 2012; Yang et al., 2019). This is confirmed by the lower aerial Cu content and generally higher root/aerial Cu content under DFC and FC treatments compared with DC treatment in this study (Table 5). Whereas, it's worth noting that *Artemisia* had its biomass decreased with Cu addition significantly, especially under DFC and FC treatment. This indicated that *Artemisia* cannot grow in soils polluted by Cu over a long period of time, and is not suitable for phytoextracting Cu polluted soil, especially under DFC and FC treatment.

Limitation and future work

Water regime can significantly influence the metal accumulation processes and capacity of wetland plants. Analyzing the Cu accumulation process by *Artemisia* and the potential rhizosphere mechanism with the influence of water regime can help better understanding the biogeochemical process of metals in the riparian wetland of Poyang Lake ecosystem. Whereas, there are still many limitations of this study: (1) The potential mechanism of the influence of pH, TOC and iron plaque in rhizosphere on Cu uptake has not been explained clearly; (2) Key rhizosphere factors, including microorganism, is not considered in this study; (3) The transferring mechanism inside the plant is not considered in this study. Our future research will pay attention to make clear the influence of rhizosphere characteristics, including microorganism, on metal transferring process by considering the subcellular distribution of metals in wetland plants.

Conclusion

The results of this study indicated that DFC and FC treatments can impede the accumulation processes of Cu by *Artemisia* by influencing the rhizosphere Cu fraction, pH and iron plaque on root surface. Additionally, the comprehensive analysis through BAF, TF, root/aerial Cu content and biomass indicated that *Artemisia* is not suitable for phytoextracting Cu polluted soil because of the decrease of biomass with Cu addition, especially under DFC and FC treatments. The results of this study can help better understand biochemical behavior of metals in riparian system and provide basis for protecting the health of riparian wetland. However, the key rhizosphere factors, including microorganism and transferring mechanism inside the plants are not considered. Further researches are needed for making clear the potential mechanism of the biochemical behavior of Cu in riparian wetland.

Declaration

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Statements and Declarations

The authors declare that there are no competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Table

Table 4 is not available with this version.

Figures

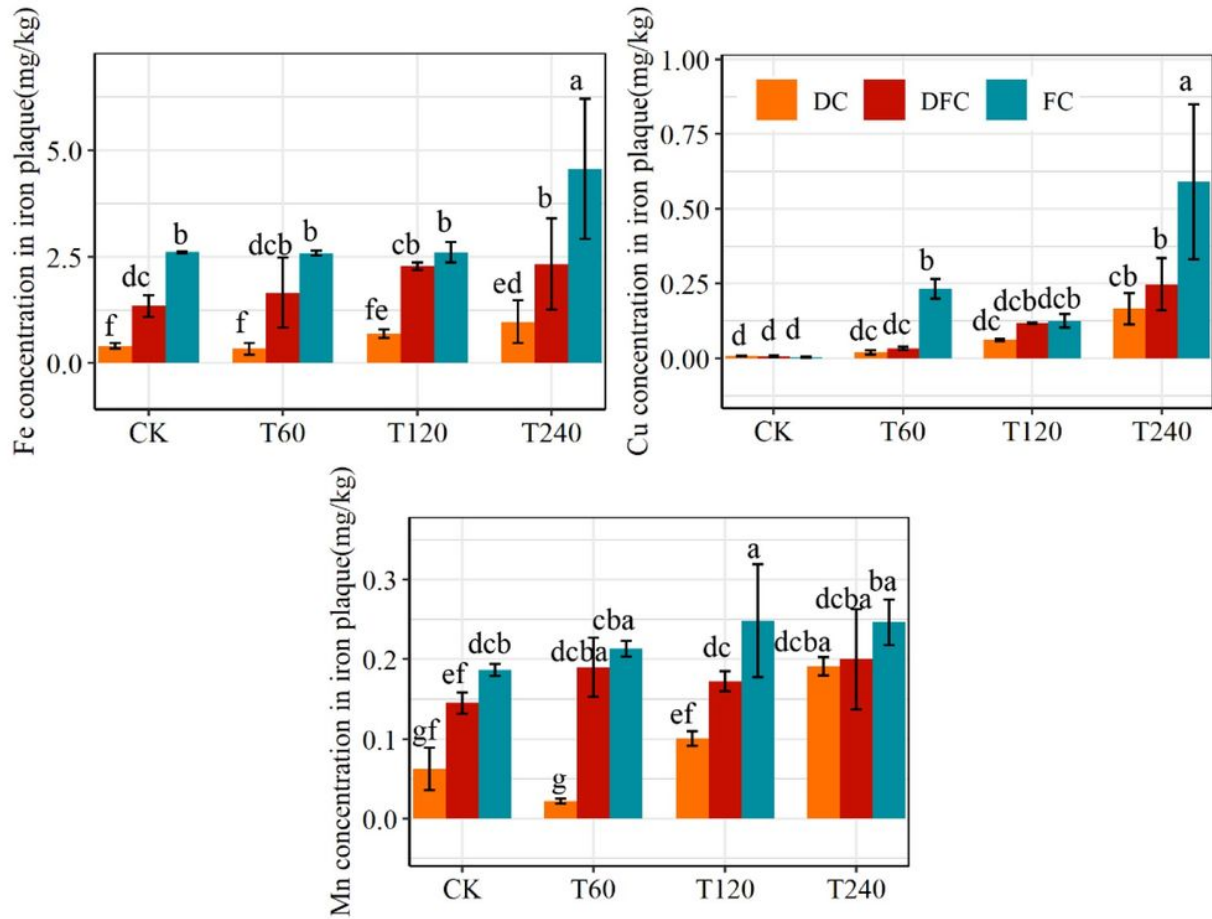


Figure 1

Metal concentrations in iron plaque

Note: Different letters indicate significant difference ($p < 0.05$) among the treatments

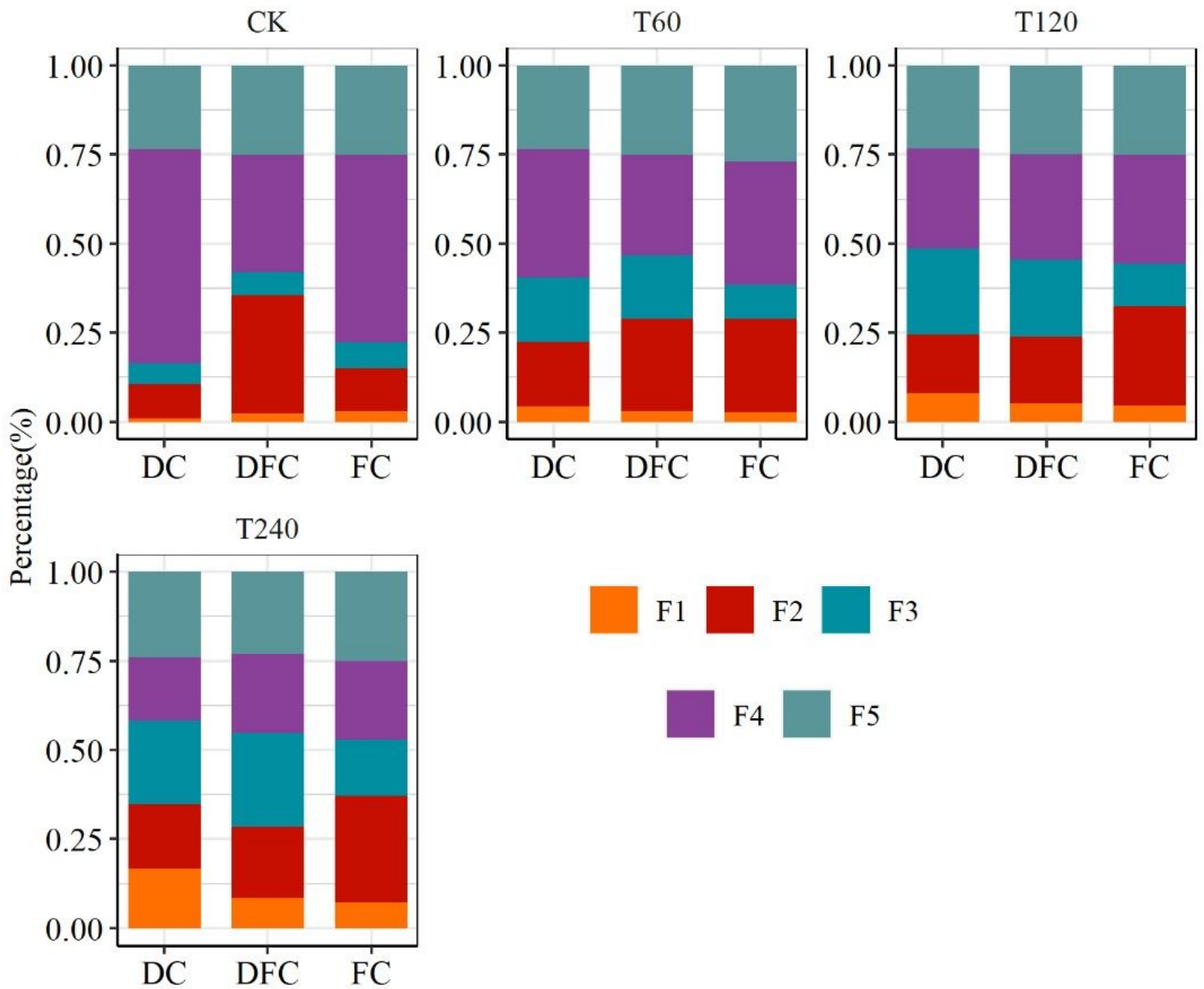


Figure 2

Metal speciation in rhizosphere soil under the influence of Cu and flood stress (F1, F2, F3, F4, F5 represent exchangeable fraction, carbonate bound fraction, Fe/Mn oxide bound fraction, organic fraction and residual fraction, respectively)

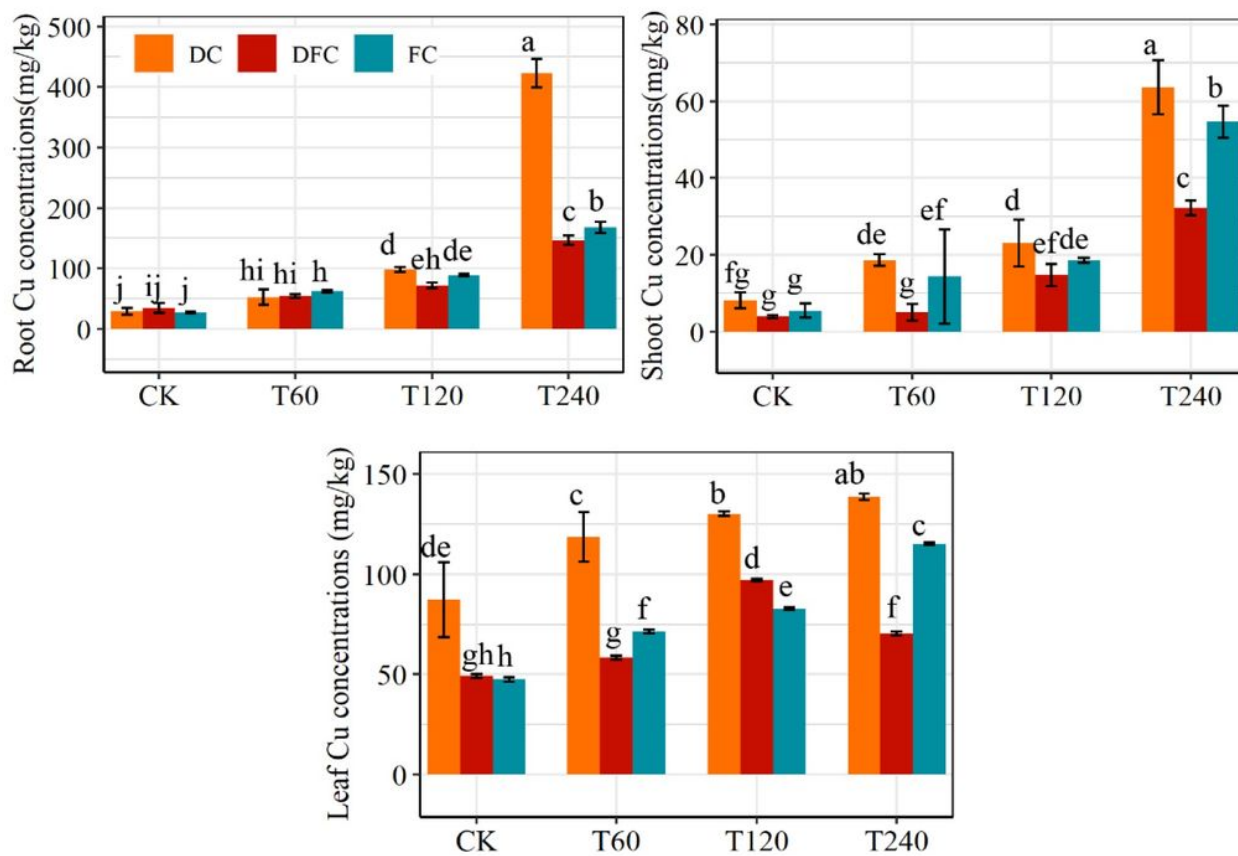


Figure 3

Cu concentrations in plant tissues under different water condition

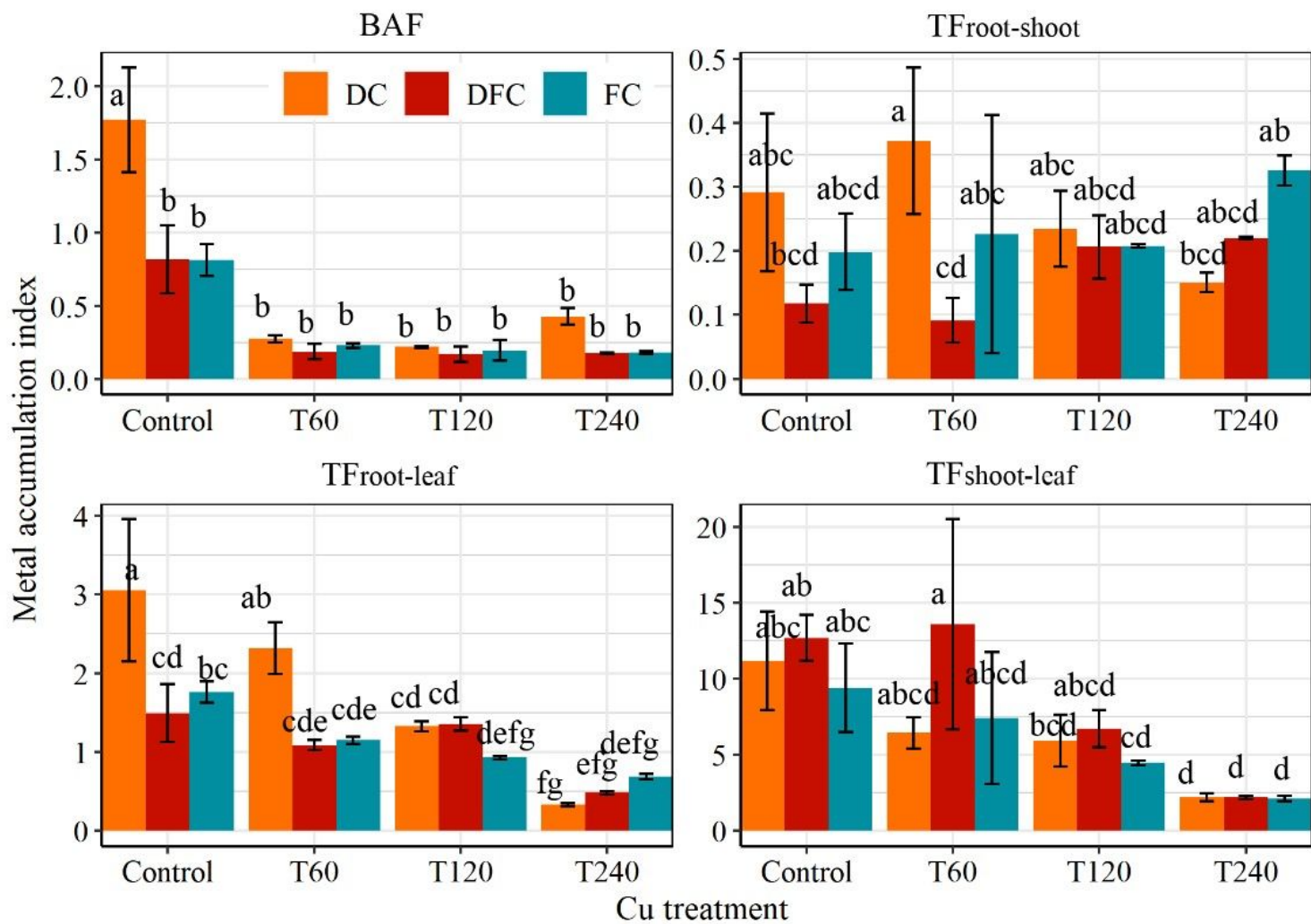


Figure 4

Metal accumulation and translocation characteristics

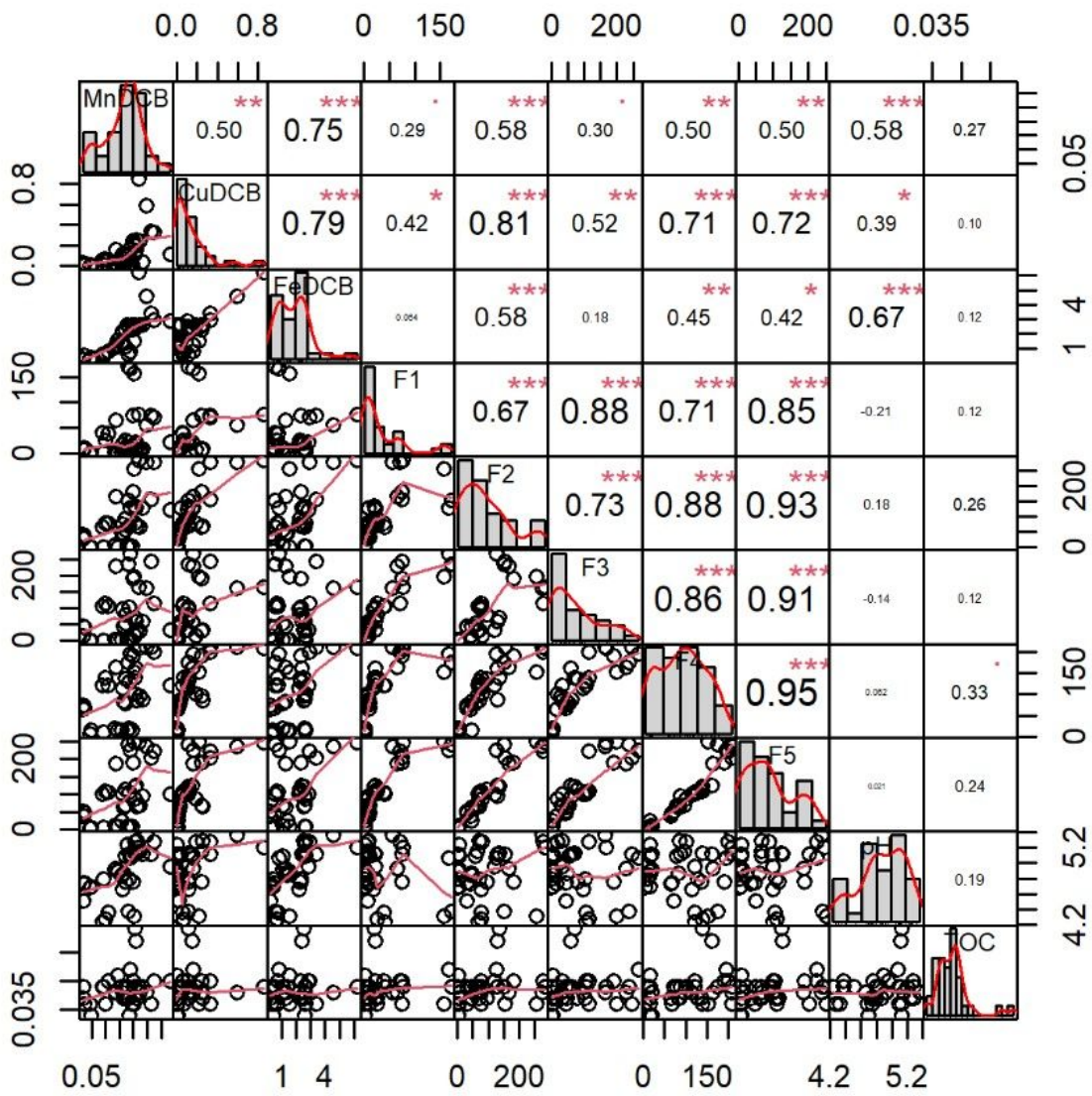


Figure 5

The correlation all influencing factors on Cu availability

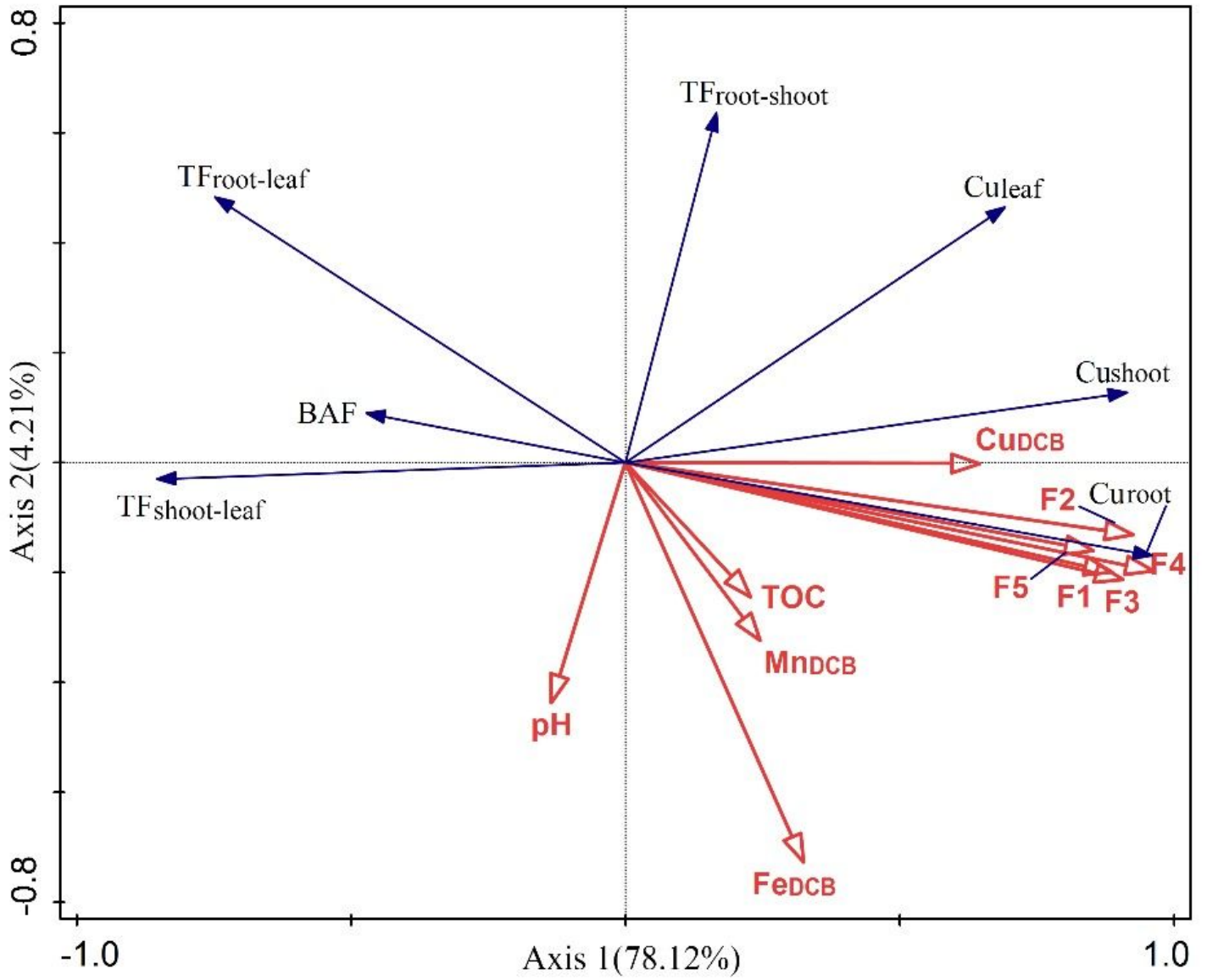


Figure 6

The RDA analysis of Cd accumulation by *Artemisia* under different water condition