



# Co-contamination of Cu and Cd in paddy fields: Using periphyton to entrap heavy metals



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## HIGHLIGHTS

- Periphyton was capable of simultaneously entrapping Cu and Cd from paddy fields.
- Cu and Cd bioavailability decreased with time after exposure to periphyton.
- Periodic adsorption–desorption was the main mechanism used to remove Cd and Cu.
- Periphyton was able to adapt to steady accumulation of Cu and Cd.
- The inclusion of periphyton will help entrap heavy metals in paddy fields.

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## ABSTRACT

The ubiquitous native periphyton was used to entrap Cu and Cd from paddy fields. Results showed that Cu- and Cd-hydrate species such as  $\text{CuOH}^+$ ,  $\text{Cu}_2(\text{OH})_2^{2+}$ ,  $\text{CdOH}^+$ , and  $\text{Cu}_3(\text{OH})_4^{2+}$  decreased with time in the presence of periphyton. When the initial concentrations of Cu and Cd were 10 mg/L, the heavy metal content in the periphyton fluctuated from 145.20 mg/kg to 342.42 mg/kg for Cu and from 101.75 mg/kg to 236.29 mg/kg for Cd after 2 h exposure. The concentration of Cd in periphytic cells varied from 42.93 mg/kg to 174 mg/kg after 2 h. The dominant periphyton microorganism species shifted from photoautotrophs to heterotrophs during the exposure of periphyton to Cu and Cd co-contamination. Although Cu and Cd could inhibit periphyton photosynthesis and carbon utilization, the periphyton was able to adapt to the test conditions. Cu and Cd accumulation in rice markedly decreased in the presence of periphyton while the number of rice seeds germinating was higher in the periphyton treatments. These results suggest that the inclusion of native periphyton in paddy fields provides a promising buffer to minimize the effects of Cu and Cd pollution on rice growth and food safety.

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## 1. Introduction

There has been increasing pollution of cropland by heavy metals around the world, especially in China. Generally, combined heavy metal pollution (e.g., Zn, Pb, Cd, Cu and As) occurs in intensive agricultural areas such as paddy fields [1,2]. Heavy metal contamination in croplands can result in dietary exposure through the soil–plant–food chain transfer, causing elevated levels of toxic metals in human organs. The combined toxicity of multiple heavy metals [3] might pose a higher potential risk to organisms and ecosystem health than a single heavy metal [4–6]. For instance,

the combined effect of Cu and Cd is more toxic to animals such as *Hemigrapsus crenulatus* [7] and humans [8], than each metal individually.

Many approaches have been proposed to remove heavy metals from the environment [9–11]. These can be divided into three general groups. The first group immobilizes (inactivates) heavy metals and subsequently reduces their bioavailability using specific materials such as biochar and rice straw ash [12–14]. The second group “consumes” or “takes up” heavy metals using hyper-accumulating plants which are then removed from the land [15,16].

The third group employs native specific functional microbial communities such as algae [17], fungi [18], and bacteria [19] to immobilize heavy metals in the environment. Although this group has proven a promising approach for remediation of heavy metal contamination [12], the removal process is dominated by single

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process-adsorption [20–22]. These adsorbed heavy metals could be released into environment again under certain conditions. Moreover, microbial communities dominated by a single species or group are generally vulnerable to shifts in environmental conditions such as pH, temperature, nutrients and multiple contaminants [23].

Compared with single microbial communities, microbial aggregates (e.g., periphyton), contain many organisms and have more complex structures [24]. There are many micro-porous structures of various sizes in the periphyton [25,26], which allow the transformation of nutrient substrates such as phosphorus and nitrogen, and contaminants such as heavy metals in the cavities [27–29]. Different nutrient and contaminant (microcystin-RR) adsorption and desorption processes might occur due to the different active sites on the periphyton surface [30,31].

The main chemical compositions of periphyton are metal oxides (e.g., iron, manganese and aluminum oxide), organic matter (e.g., sugar, protein) and other inorganic chemical compounds (e.g., calcium, phosphorus and silicon) [25,26]. The periphyton communities are enclosed in a matrix of extracellular polymeric substances (EPS) [28], comprised of protein, polysaccharides and other unknown organic matter [32], which may chelate heavy metals. As periphyton is ubiquitous in paddy fields, it is possible to employ native periphyton in the effective removal of heavy metals in these areas [30].

Previous studies have investigated the response of microbial communities to heavy metal pollution. For example, the photosynthesis of *Penicillium chrysogenum* and *Bacillus licheniformis* ceased due to pollution by heavy metals such as Co, Cd, and Pb [33,34]. Periphyton, with more complex components however, contain many microbial species such as microalgae, cyanobacteria, bacteria, fungi, actinomycetes and protozoa [35,36], forming a relatively stable microecosystem of hierarchical structure (i.e., producers, consumers, decomposers). As periphyton is sensitive to habitat quality and can respond quickly to any changes in its habitat, its response to heavy metal contamination may differ from that of a single microbial community.

While a large number of heavy metals have been found contaminating cropland and crops in China, the latest data published by China EPA [37], reports that Cd and Cu contamination in soil is significant, accounting for 7% and 2.1% of all sites sampled. In many sites Cd and Cu occur simultaneously. The interactions between Cu and Cd could alter the absorption kinetics and dynamics of metals [38] thereby exacerbating the toxic impacts on specific organisms [38,39]. As Cd and Cu often coexist in soil and water in paddy fields [39], the development of a multi-functional *in-situ* remediation technology for combined heavy metal pollution in paddy fields is critical.

The objectives of this study were to investigate (i) the capacity of periphyton to entrap Cu and Cd from the aqueous phase, (ii) the response of periphyton to Cu and Cd in paddy fields, (iii) the relationship between heavy metals (Cu and Cd) and the community structure properties of periphyton, and (iv) the effect of Cu and Cd on rice seed germination and heavy metal accumulation in rice seed in the presence of periphyton. The findings present an attractive option for simultaneously entrapping Cu and Cd at the soil–water interface in paddy fields.

## 2. Materials and methods

### 2.1. Periphyton sample collection and culture

Periphyton was collected from six paddy fields in Changshu City in the southeast of Jiangsu province (120°33′–121°03′E, 31°33′–31°50′N). To reduce bias and error, all sampling sites had

similar physical and chemical conditions (such as light, shade, substrata, water depth and flow velocity). Periphyton was peeled off rock surfaces using a sterilized brush and mixed together for indoor culture. The mixed periphyton was transferred into a spiral glass pipe for culture in the laboratory. The external and internal diameters of the spiral glass pipe were 4.0 mm and 2.0 mm, respectively. The total length of the pipe was 76.4 cm.

Prior to experiments, the periphyton was colonized in glass tanks with tap water in an open system at ambient temperature (10–25 °C) for three weeks. The periphyton was then transferred into six spiral glass pipes (length: 76.4 cm, internal diameter: 2.0 mm) with Woods Hole culture medium (WC media). The periphyton in the spiral pipes was cultured in an incubator with light (power: 1800 lux), a light/dark regime of 12 h/12 h, and air temperature (25 ± 1 °C). When the periphyton matured, i.e., the color turned to deep green, they were used in experiments.

### 2.2. Accumulation experiment

To investigate the accumulation of Cu and Cd by mature periphyton, a series of experiments were conducted under different conditions in the spiral glass pipes previously described. The mixture solution containing 10 mg/L Cu and 10 mg/L Cd was prepared by dissolving  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$  and  $\text{Cd}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$  into deionized water. The mixture solution was then pumped into the spiral glass pipe at a velocity of 0.01 mL/s. The influent and effluent samples were collected at 0, 2, 18, 30, 42, 54, 72, 102, 144, 168 and 240 h for the determination of Cu and Cd in aqueous phase. The spiral glass assembly with periphyton was placed in the incubator with light (power: 1800 lux), light/dark regime of 12 h/12 h, and air temperature (25 ± 1 °C).

### 2.3. Rice seed germination experiment

The rice germination experiment was conducted to investigate the effect of periphyton on rice submerged in a mixed solution of Cu and Cd. Whole, similar sized rice seeds were selected for this experiment. The rice seeds were immersed in a 1% NaClO solution for 20 min to sterilize the causative agent adhered to the seeds then washed with deionized water to remove the residual NaClO. The seeds were placed on filter paper in a Petri dish for culturing under different conditions. There were 20 rice seeds in each dish.

There were three different groups in the experiment. The treatment group was cultured in Petri dishes containing 5 g periphyton and 15 mL of the mixed Cu and Cd solution. The first control group was cultured in 15 mL deionized water only while the second control group was cultured in 15 mL of the Cu and Cd solution but without periphyton. The experiment was conducted in triplicate. The Petri dishes were transferred to an artificial incubator with temperature (25 ± 1 °C) and a light/dark regime of 12 h/12 h. To avoid drying out in the incubator, 15 mL of the corresponding solution was added to each dish every day. The number of germinating rice seeds was counted every day. The concentrations of Cu and Cd in rice and the liquid phase were measured by inductively coupled plasma mass spectrometry (ICP-MS) after seven days.

### 2.4. Sampling and analysis

The concentrations of Cu and Cd in the influent and effluent of the spiral glass pipes were measured by ICP-MS after filtration. The speciation of Cu and Cd in the sample was obtained using the visual MINTEQ 3.1 software [40]. The accumulated Cu and Cd in periphyton were calculated using the following equation:

$$Q_t = V(C_0 - C_t)/B_t$$

where  $Q_t$  is the entrapped content of heavy metal (Cu/Cd) at time  $t$ ;  $V$  is the total volume (L) of heavy metal solution pumped into spiral glass tube until time  $t$ ;  $C_0$  is the original concentration of the Cu/Cd in the influent (mg/L);  $C_t$  is the concentration of Cu/Cd in the effluent (mg/L), and  $B$  is the biomass (g) of periphyton at time  $t$ .

To evaluate the amount of heavy metals (Cu and Cd) accumulated in periphytic cells, 4 M ethylenediaminetetraacetate (EDTA) was added to desorb the Cu and Cd adsorbed to the surface of the periphyton. The accumulated metals (Cu or Cd) in the periphytic cells were calculated using the following equation:

$$Q_{t,c} = V(C_0 - C_{t,c})/B_t$$

where  $Q_{t,c}$  is the accumulated content (mg/kg) of heavy metal (Cu or Cd) at time  $t$ ;  $C_{t,c}$  is the concentration (mg/L) of Cu or Cd in the effluent when EDTA was added to the influent.

To avoid destroying the community structure of the periphyton on the inner surface of the spiral glass tube, the periphyton and the glass pipe were placed in an oven at 60 °C for 30 min after air drying at 20–25 °C for 6 h. The net biomass of periphyton was calculated from the difference between the total weight of glass pipe containing periphyton and the glass pipe only.

The periphyton samples were collected at the beginning and the end of the experiments. The community structure of the periphyton was observed by optical microscope and scanning electron microscope (SEM). The maximum electron transfer rate ( $ETR_{max}$ ), total chlorophyll, and the algal species and their chlorophyll in the periphyton were determined using a Phyto-PAM fluorescence analyzer (Waltz, Gernmen). The biomass of each species of algae was calculated by the concentration of its algal chlorophyll multiplied by the volume of the periphyton in the spiral glass tube. The biomass of the heterotrophs was calculated as the difference between the total biomass of the periphyton and the biomass of all algae.

Carbon source utilization by periphyton was assayed using the commercially available Biolog<sup>TM</sup> ECO Microplates (Hayward, CA, USA). The detailed analysis process is described in our previous study [41]. The functional diversities of the periphyton were obtained based on the utilization of different carbon sources.

Statistical Package for Social Science (SPSS Version 17) was used for analyses of the Biolog data. The linear relationships between Cu and Cd in periphyton (cells), and between AWCD and the content of Cu and Cd were analysed using regression analyses in Excel 2010. Statistically significant differences between the data were evaluated using One-Way ANOVAs.  $\alpha$  was set at 0.05 for all analyses.

### 3. Results and discussion

#### 3.1. Heavy metal removal and their speciation distribution

Cu was more easily accumulated by the periphyton than Cd. When the initial concentrations of Cu and Cd were 10 mg/L each, the removal rates of Cu and Cd from aqueous phase fluctuated from 39.2% to 68.8% and 22.5% to 49.9%, respectively (Fig. 1a).

During the experiment, the aquatic environment, especially the pH value, changed with periphyton growth and influenced the Cu and Cd speciation distribution [42–47]. Different Cu- and Cd-hydrates such as  $Cd2OH^+$ ,  $Cu_2(OH)_2^{2+}$ ,  $CdOH^+$ , and  $Cu_3(OH)_4^{2+}$  were analyzed in the experiment. The toxicity of the Cu- and Cd-hydrates affected the substance transportation between cells and habitat [42,43]. The results of the heavy metal speciation analysis shows that  $Cd2OH^+$  and  $Cu_2(OH)_2^{2+}$  were the dominant forms of the Cu- and Cd- hydrates after 2 h, accounting for 19% of the total heavy metals (Fig. 1b). They sharply decreased to 6.26% and 0.58% of the total content at 18 h and then remained stable until increasing to 2.9% at 102 h.

$Cu_3(OH)_4^{2+}$  and  $Cu_2OH^{3+}$  were only detected at 2 h and 102 h.  $CdOH^+$  was the only species of Cd-hydrate observed, making up 0.03% of the total  $Cd^{2+}$  amount (Fig. 1b–d).  $Cu(OH)_2$  (aq) was very low during the whole experiment, only nearing 0.08% of the total Cu(II) at 2 h and 0.01% at 18 h (Fig. 1b–d).

#### 3.2. Cu and Cd accumulation in periphytic surfaces and cells

Periphyton can entrap Cu and Cd through surface adsorption and accumulation inside cells. The surface adsorption occurred through extracellular polymeric substances (EPS) which can absorb metal ions as the first defensive line protecting the community system [48–50]. The heavy metal contents entrapped by the periphyton fluctuated from 145.20 mg/kg to 342.42 mg/kg for Cu and from 101.75 mg/kg to 236.29 mg/kg for Cd after 2 h. The concentrations of Cd accumulated in the periphytic cells (heavy metal in periphyton extracted by 4 M EDTA) varied from 42.93 mg/kg to 174 mg/kg after 2 h (Fig. 2). More Cu than Cd was observed on the periphyton surface but no Cu was detected in the periphytic cells after 54, 192 and 240 h. These results showed that the periphytic cells were more inclined to accumulate Cd than Cu.

The relationships between Cu and Cd content on the periphyton surface and in the cells were studied during periphyton culture in the mixed solution of Cu and Cd. Significant relationships between Cu and Cd content on the periphyton surface and in cells were as follows:

$$Cd(mg/kg) = 0.5884 \times Cu(mg/kg) + 23.561 (R^2 = 0.80, p < 0.05) \quad (1)$$

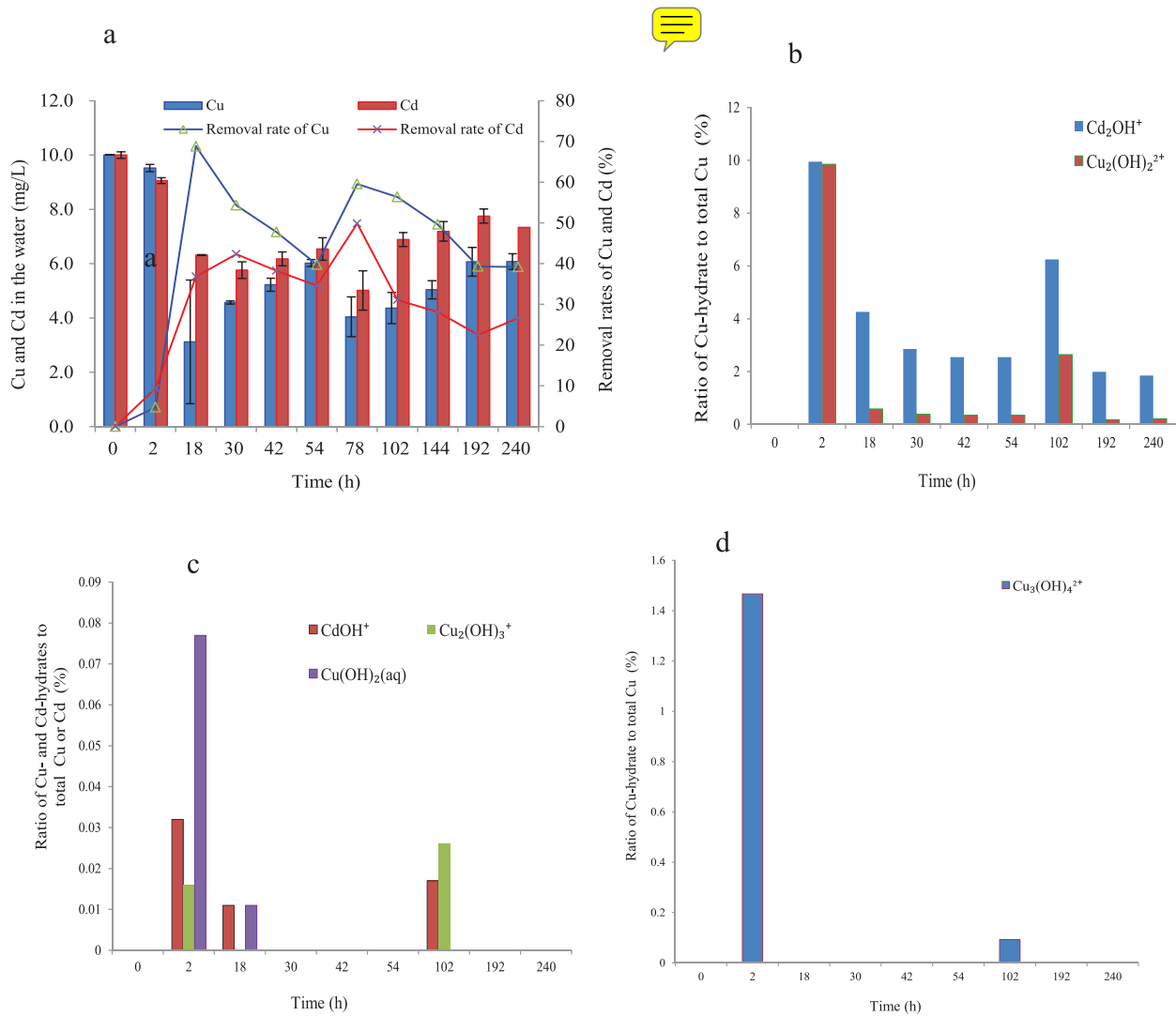
$$Cd(mg/g) = 0.7809 \times Cu(mg/g) + 50.775 (R^2 = 0.47, p < 0.05) \quad (2)$$

The correlation coefficient between Cu and Cd in the cell was lower than that on the periphyton surface. These results demonstrate that Cu and Cd could be simultaneously entrapped by periphyton, but that the presence of Cd had negative effects on Cu accumulation by periphytic cells.

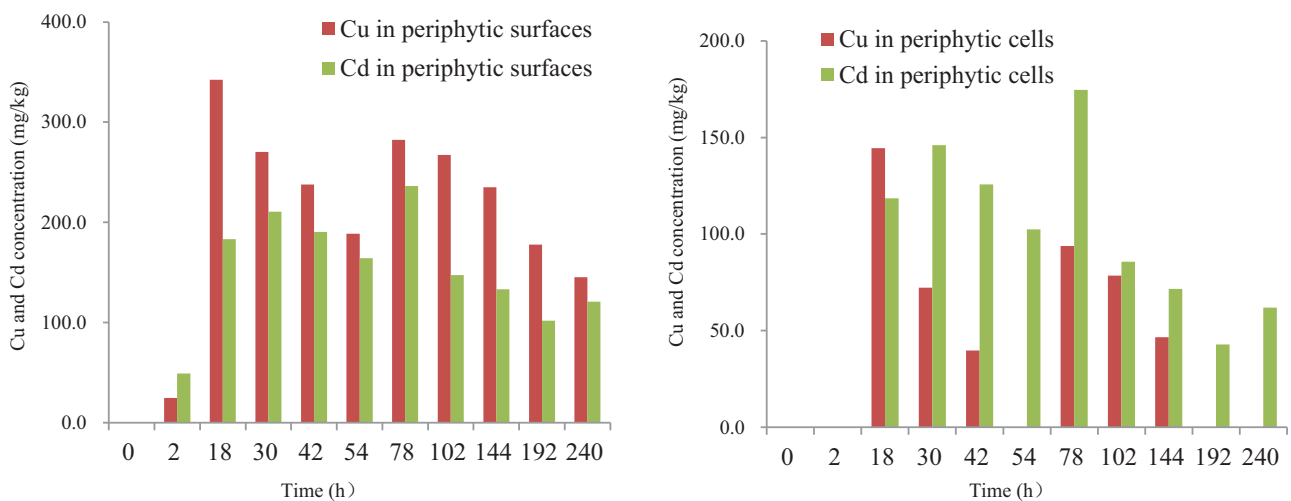
#### 3.3. Effect of heavy metals on periphyton growth

To determine the effect of heavy metal contamination on periphyton growth, total periphyton chlorophyll was measured in the presence and absence of heavy metals (Fig. 3). During the first 2 h after exposure to heavy metals, no variation in total chlorophyll was observed. After 2 h, the total chlorophyll decreased sharply from 2.08 mg/L at the beginning of experiment to 0.13 mg/L at the end of experiment. The total chlorophyll in the control treatment increased from 2.77 mg/L to 5.61 mg/L during the same period. This indicates that the presence of Cu and Cd inhibited the growth of periphyton. A slight increase in chlorophyll was observed in the middle stages (from 18 to 54 h) and the late stages (from 78 to 240 h) in the presence of heavy metals, implying that the periphyton is able to self-regulate.

The maximum electron transfer rate ( $ETR_{max}$ ) is a critical parameter for characterizing the rate of photosynthesis of microbial aggregates, such as multiple algal communities, and the state of algal growth [51]. Results showed that the  $ETR_{max}$  of periphyton in the control treatment fluctuated between  $0.205 \mu\text{mol}/(\text{m}^2 \text{ s})^{-1}$  and  $0.379 \mu\text{mol}/(\text{m}^2 \text{ s})^{-1}$  during the experiment. The  $ETR_{max}$  after exposure to heavy metal pollution however, sharply decreased from  $\sim 0.268 \mu\text{mol}/(\text{m}^2 \text{ s})^{-1}$  in the beginning to



**Fig. 1.** (a) The concentration profiles of Cu/Cd and their corresponding removal rates. (b), (c) and (d) The contributions of the different species of Cu- and Cd-hydrates to the total Cu(II) and Cd(II) in the aqueous phase during the removal of Cu and Cd by periphyton.



**Fig. 2.** The contents of Cu and Cd on periphyton surfaces and in periphyton cells.

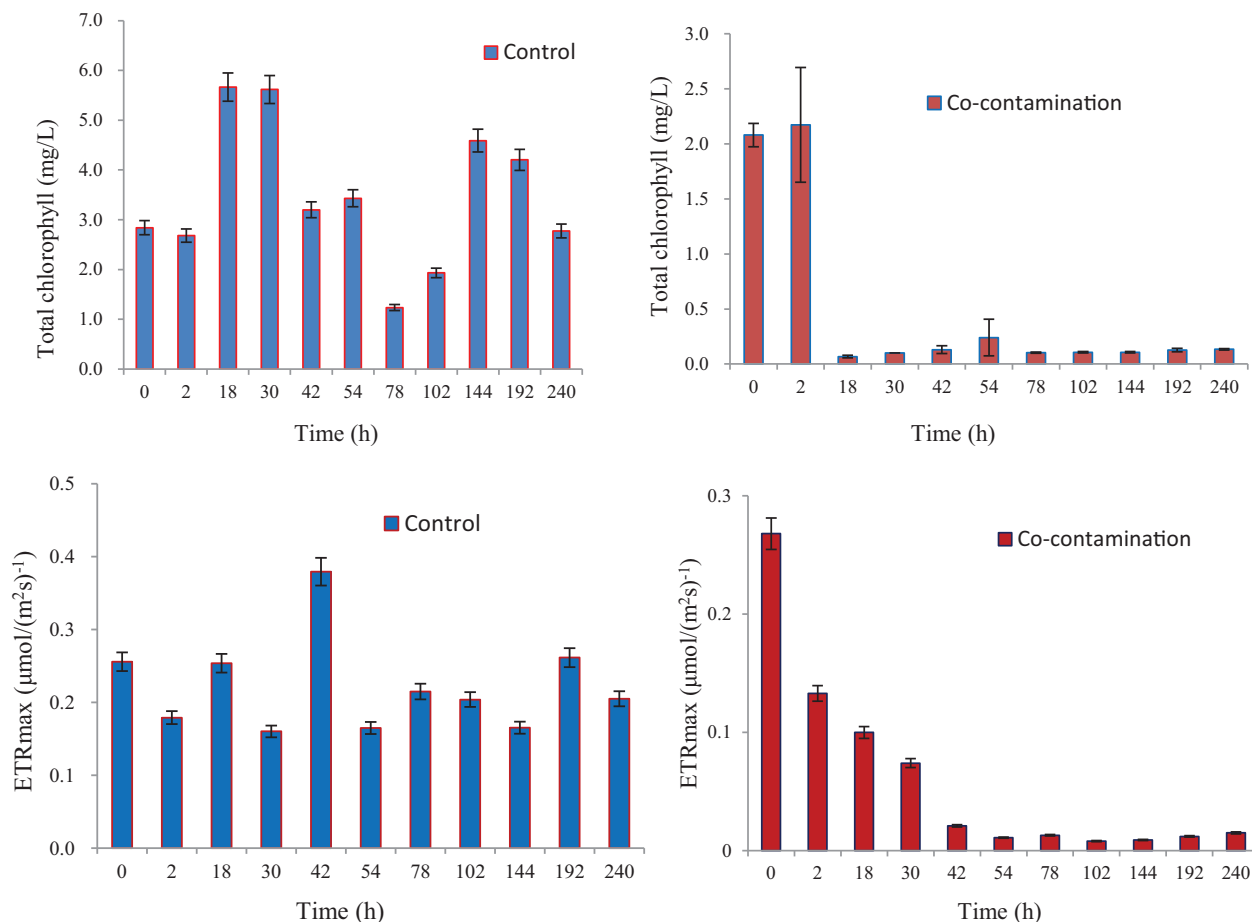


Fig. 3. Variation in the total chlorophyll and maximum electron transport rate ( $ETR_{max}$ ) of the periphyton in the presence and absence of heavy metal contamination.

$0.008 \mu\text{mol}/(\text{m}^2 \text{s})^{-1}$  at 102 h (Fig. 3). Interestingly, a slight increase in  $ETR_{max}$  of  $0.008$  to  $0.015 \mu\text{mol}/(\text{m}^2 \text{s})^{-1}$  was observed between 102 h and 240 h, with another increase to  $0.105 \mu\text{mol}/(\text{m}^2 \text{s})^{-1}$  at 960 h (data not shown in Fig. 3). These increases may be due to the periphyton adapting to the contaminated conditions.

#### 3.4. The evolution of the periphyton community

Algae (such as *Cyanobacteria*, diatoms), bacteria (such as *antimonias* and *Nitrobacteria*), fungi (such as *trichobacteria* and *Candida*) and protozoa were the dominant taxa in the periphytic communities at the beginning of the experiment (Fig. 4). After exposure to heavy metal pollution the periphyton community changed with algae becoming the dominant species.

Blue algae (*Cyanobacteria*), green algae and brown algae were the dominant algae identified by chlorophyll content (Fig. 5a and b). The biomass of these three algal groups rapidly declined over time with brown algae becoming the main component of the periphyton in the later stages (from 102 to 240 h) in the presence of heavy metal contamination. In the control treatment, green and brown algae were the dominant species after 18 h. These results suggest that only the brown algae could withstand the combined pollution of Cu and Cd, though it was also inhibited to some degree. The biomass of heterotrophs was calculated as the difference between total biomass (represented by total chlorophyll) and autotrophic organism biomass (represented by green, blue and brown algal chlorophyll).

The results showed that the biomass of heterotrophs in the control treatment fluctuated periodically due to the aging-

regeneration shifts of the periphyton. In the treatment with Cu and Cd, the biomass of heterotrophs sharply increased from 8.23 to 21.43 mg after 2 h (Fig. 5c). These results show that the dominant periphyton species changed from autotrophic organisms (i.e. algae) to heterotrophs to adapt to the contaminated conditions. The ability of microorganisms to utilize amines and amino acids increased in the periphyton compared to the control (Fig. 5d), suggesting that the proportion of heterotrophs in the periphyton increased gradually [41].

#### 3.5. Mechanism used in heavy metal removal by periphyton

Microbial metabolism activity based on the utilization of carbon sources is usually characterized by average well-color development (AWCD) determined using Biolog EcoPlates™ [41]. In the control, the AWCD significantly increased from the beginning to the middle of the experiment ( $p < 0.05$ ) and then slightly decreased in the later stages (Fig. 6). The AWCD values increased with incubation time at each period of the experiment (the middle stage and the end of the experiment) in the contaminated treatment. There was a positive linear relationship between AWCD and the contents of Cu and Cd in the periphyton as follows:

$$\begin{aligned} \text{AWCD} &= 0.0219 \times (\text{Cu and Cd, mg/kg}) + 0.0384(R^2 \\ &= 0.70, p < 0.05) \end{aligned} \quad (3)$$



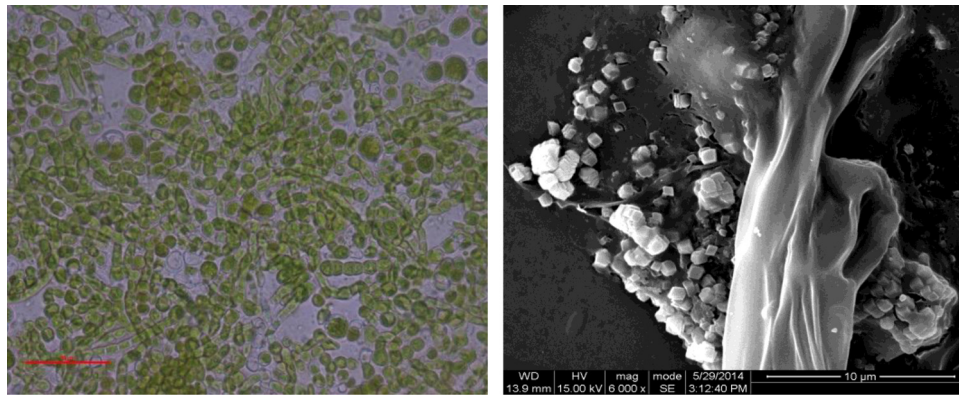


Fig. 4. Periphyton composition observed by optical microscope and scanning electron microscope (SEM) at the experiment beginning.

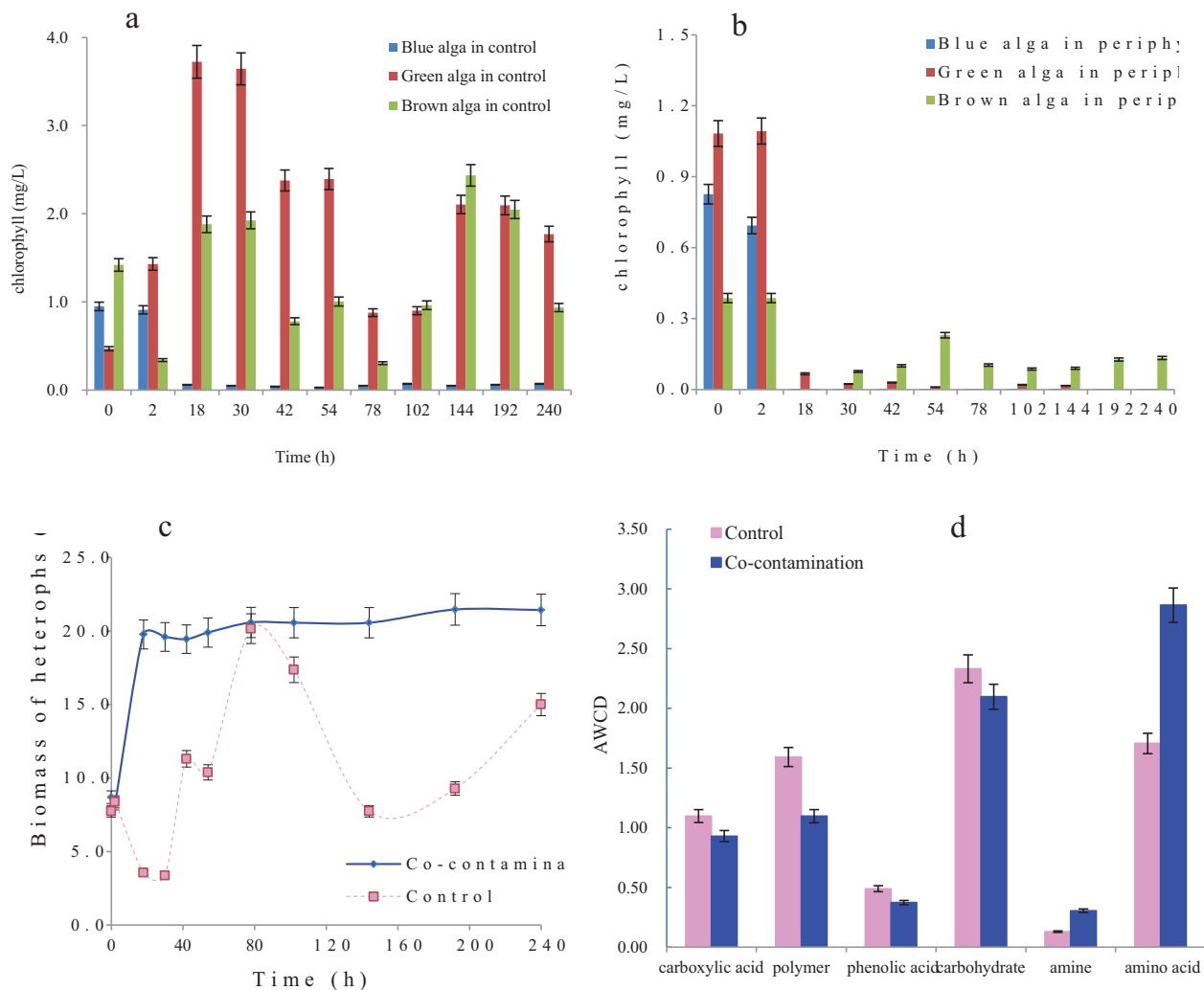


Fig. 5. (a) Biomasses represented by chlorophyll contents in different algae in the control and (b) in the treatment with Cu and Cd solution; (c) biomass of heterotrophs in the periphyton during culture in Cu and Cd solution, and (d) the ability of microorganisms in the periphyton to utilize different carbon sources at the end of the experiment.

These results showed that although the presence of Cu and Cd decreased the ability of the periphyton to utilize carbon, the periphyton had the ability to adapt to the contaminated conditions.

Diversity indices (Simpson, Shannon, McIntosh and Pielou indices) calculated based on AWCD values were used to characterize the function of periphyton [41,52]. Although the Shannon, Simpson and Pielou indices decreased with incubation time in the

presence of Cu and Cd, the average McIntosh index increased from 1.98 at the beginning to 2.01 at the middle stage of the experiment (Fig. 6). This implies that the periphyton had experienced a self-adjustment period to maintain the evenness of periphytic communities [41]. This result is similar to previous results demonstrating that the dominant species changed from photoautotrophs to heterotrophs.

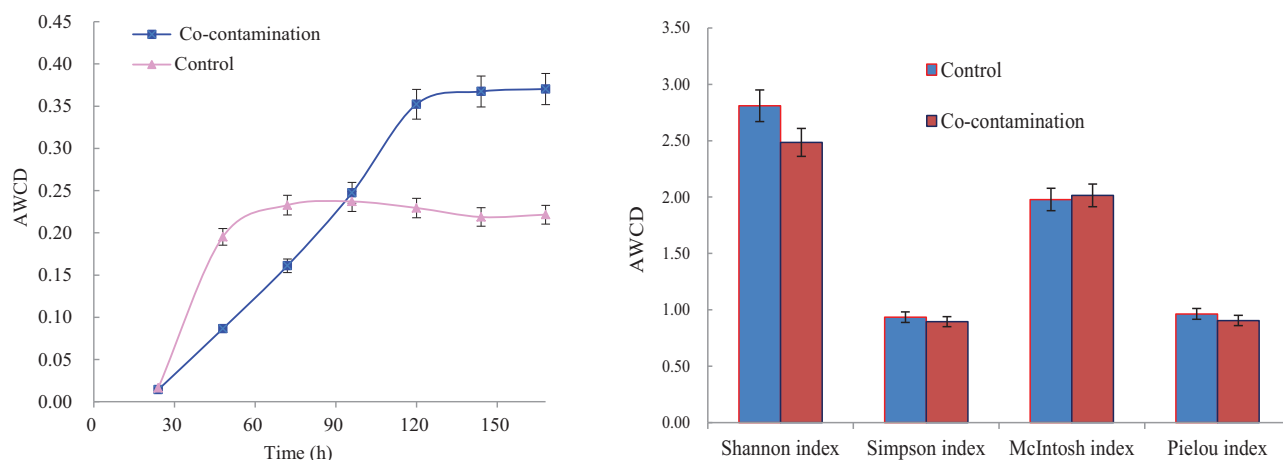


Fig. 6. Microbial metabolism activity and functional diversity calculated from the utilization of carbon sources.

Extracellular polymeric substance (EPS) is the main chemical component on the periphyton surface [36], and has a strong affinity for heavy metal ions. High molecular organic matter such as proteins and polysaccharides in the EPS might change the adsorption–desorption processes of Cu and Cd. In addition, the negatively charged protein groups containing amino, amide and carboxyl group in the EPS might lead to competitive adsorption of coexisting metal ions such as  $\text{Cu}^{2+}$  and  $\text{Cd}^{2+}$  [11,53,54]. Consequently, the amount of Cd accumulated on the periphyton surface and in the cells differed from the amount of Cu accumulated. The capacity of the periphyton to accumulate heavy metals is higher than in some previous studies. For instance, the maximum capacity of Cd entrapment by the Cd-hyperaccumulator *Solatum nigrum* L. is 124.6 mg/kg [15], which is lower than our results (Cd capacity up to 174 mg/kg). Moreover, using periphyton permits the simultaneous removal of Cd and Cu while the specific heavy metal hyperaccumulator can only accumulate single species heavy metals [12,15].

Due to the diverse compositions of periphyton, the periphyton forms a self-regulating hierarchical microecosystem [36]. Due to this self-adjusting ability, the use of periphyton has potential for in-situ remediation of heavy metal contaminated paddy fields (Figs. 5 and 7). In addition, the complex structure of the periphyton is beneficial for entrapping multiple heavy metals. In this study, the Cu and Cd were first adsorbed by periphyton and then desorbed (Fig. 1a). Compared to single microbial communities such as algae [17], fungi [18], and bacteria [19], the heavy metal contents desorbed from the periphyton were low. A Cu and Cd removal efficiency of approximately 22.5% to 68.8% was observed with initial concentrations of 10 mg/L (Fig. 1a).

### 3.6. The accumulation of Cd and Cu in rice seed

The rice germination experiments were conducted to investigate the effects of periphyton on heavy metal accumulation in rice and rice germination. The results showed that when water (without heavy metals and periphyton) was used to culture rice, the concentration of Cu and Cd in rice was very low, 0.49 mg/L for Cd and 10.08 mg/L for Cu; and the number of rice seeds germinating increased with time, implying that the rice seeds were healthy.

The concentration of Cu and Cd accumulated in the rice in the treatment (with heavy metals and periphyton) was lower than those in the control (with heavy metals but without periphyton, Fig. 7). Cu was more easily accumulated by rice seed than Cd in the different treatments. Although the number of rice seeds germinating increased with incubation time under the different conditions,

the number of rice seeds germinating in the control with heavy metals only was lower than in the treatment with heavy metals and periphyton. These results suggest that periphyton reduces the toxic impacts of Cu and Cd on rice.

### 3.7. Implications for in-situ remediation of heavy metal contamination in paddy fields

Due to increasing mining activity and the overuse of chemical fertilizers on paddy fields, more attention has been paid to the remediation of heavy metal contaminated paddy fields. The reduction of heavy metal bioavailability and the accumulation of heavy metals by hyper-accumulators have proved to be two effective measures [12]. The immobilization and accumulation of Cu and Cd by environmentally friendly materials such as rice straw ash and biochar [13,14] or hyper-accumulators such as *S. nigrum* L. [15] and *Commelina communis* [16] have been widely employed.

The additional materials such as rice straw ash and biochar however, might adsorb and/or desorb other contaminants including Cd, Cu, Pb and Zn [13,14,55] or nutrients such as phosphorus [56], leading to shifts in the balance of the element biogeochemical cycles in paddy fields. Additionally, the in-situ remediation of contaminated paddy fields using hyper-accumulators might be impractical at large scales.

In this study, the removal process of Cu and Cd by periphyton was a periodic cycling of adsorption–desorption. While the use of periphyton is a stable method for the removal of heavy metals such as Cu and Cd from paddy fields over the long term, the periphyton containing the accumulated Cu and Cd must be removed from the paddy fields before the heavy metals are desorbed. This periphyton removal should be done in non-planting seasons.

The toxicity of Cu and Cd in paddy fields poses an ecological risk to rice safety and human health [57]. These multiple contaminants also cause heavy damage to periphyton in the early stages. Although the periphyton was stressed by Cu and Cd, the activity and functionality of the periphyton recovered to some degree after adaption. The periphyton in this study exhibited an ability to adapt to the combined pollution of Cu and Cd through changes in photosynthesis, microbial composition, utilization of carbon sources and functional diversity (Figs. 3, 5 and 6).

In the study, the pH decreased from 6.63 to 5.6 during the introduction of periphyton, which is needed to keep the heavy metals dissolvable at the early stage of the experiment (Fig. 1). As these pH levels are similar to those occurring naturally in rice paddy fields,

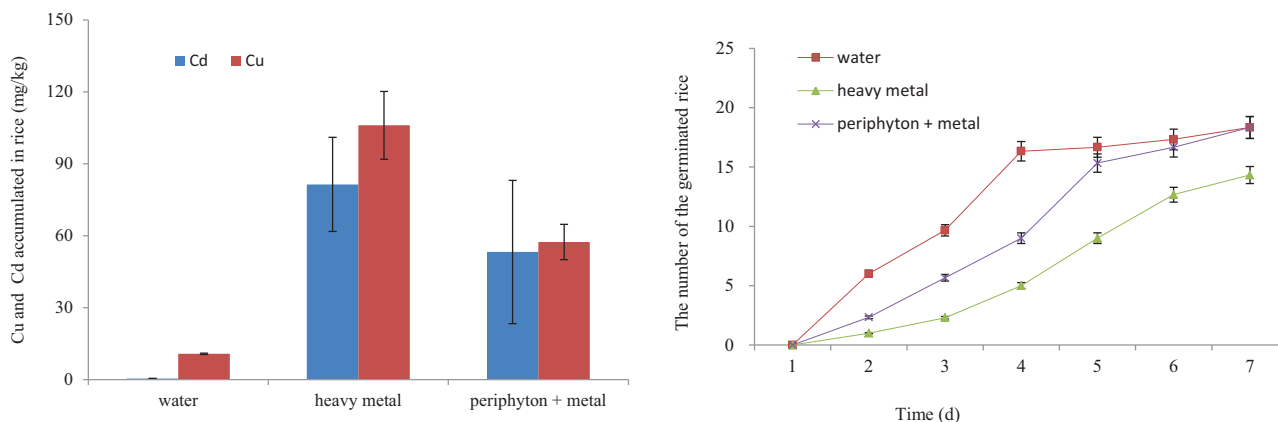


Fig. 7. Variation in heavy metal accumulation in rice and the number of rice seeds germinating under different conditions.

little modification will need to be made in relation to periphyton performance in natural pH conditions.

From an ecological point of view, the microorganisms in periphyton have the ability to self-acclimate and thus can adapt to various contamination levels [24,30,36], as seen in this study (Figs. 3, 6 and 7), leading to a relatively steady entrapment/accumulation of heavy metals by periphyton. In the paddy field environment, sediments provide a habitat for native microorganisms, which in turn supply microbial species for the aggregation of periphyton on the surface of the sediments, thus facilitating the decontamination of heavy metals.

#### 4. Conclusions

In this study, native periphyton was proposed to entrap Cu and Cd in paddy fields. The periphyton in paddy fields kept Cu and Cd-dissolvable and then accumulated the heavy metals from the aqueous phase. The periphyton had a high capacity to accumulate Cu and Cd from water. As a result, the Cu and Cd contents in rice were low and the number of rice seeds germinating was high compared to the control (without periphyton). Cd was accumulated more by the periphytic cells than Cu. Although Cu and Cd negatively affected periphyton growth, the periphyton exhibited significant potential to recover its photosynthesis ability, metabolic activity and functionality by altering its community structure. The findings in this study provide a promising and environmentally benign bioremediation to simultaneously entrap Cu and Cd from paddy fields, and also offer some valuable insights into the adaption of periphyton to ecosystems contaminated by heavy metals.

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