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Phytotoxic effects of Cu, Cd and Zn on the seagrass *Thalassia hemprichii* and metal accumulation in plants growing in Xincun Bay, Hainan, China

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Abstract

Seagrasses play an important role in coastal marine ecosystems, but they have been increasingly threatened by human activities. In recent years, seagrass communities have rapidly degenerated in the coastal marine ecosystems of China. To identify the reasons for the decline in seagrasses, the phytotoxic effects of trace metals (Cu, Cd and Zn) on the seagrass *Thalassia hemprichii* were investigated, and the environmental contents of the metals were analyzed where the seagrass grows. The results showed that leaf necrosis in *T. hemprichii* exposed to 0.01–0.1 mg L^{-1} of Cu^{2+} for 5 days was more serious than that in plants exposed to the same concentrations of Cd^{2+} and Zn^{2+}. The chlorophyll content in *T. hemprichii* declined in a concentration-dependent manner after 5 days of exposure to Cu^{2+}, Cd^{2+} and Zn^{2+}. The evident reduction in $\Delta F/F_m$' in *T. hemprichii* leaves was observed at day 1 of exposure to 0.01–1.0 mg L^{-1} of Cu^{2+} and at day 3 of exposure to 0.1–1.0 mg L^{-1} of Cd^{2+}. The antioxidant enzyme activities (SOD, POD and CAT) in *T. hemprichii* leaves exposed to the three metal ions also showed significant changes. In seawater from Xincun Bay (Hainan, China), where *T. hemprichii* grows, Cu had reached a concentration (i.e., 0.01 mg L^{-1}) that could significantly reduce chlorophyll content and $\Delta F/F_m$' in *T. hemprichii* leaves. Our results indicate that Cu influences the deterioration of seagrasses in Xincun Bay.

Keywords Chlorophyll fluorescence · Metals · Seagrass · *Thalassia hemprichii*

Introduction

Seagrasses, a group of marine flowering plants represented by approximately 65 species from four families, occur widely in the coastal areas of temperate and subtropical regions, including open coasts, bays and estuaries (Duarte 1999; Green and Short 2004). Seagrasses have high primary productivity in coastal marine ecosystems (Wilkes et al. 2017); provide spawning, nursery, refuge and feeding ground for a variety of fish and shellfish (Duffy 2006); support detrital and grazing food webs (Lewis and Devereux 2009); stabilize sediments that provide essential shoreline protection (Short et al. 2011); and maintain the microbial diversity in marine sediments (Jiang et al. 2015). Importantly, seagrass ecosystems also play a critical role in carbon and nutrient cycling (Liu et al. 2016).

However, because of their proximity to the coastline, seagrasses are vulnerable to a variety of anthropogenic threats (Ambo-Rappe 2014), including organic and inorganic pollutants derived from industry, agriculture, fishery, transportation, urbanization and tourism (Jackson et al. 2001; Vörösmarty et al. 2010; Bazzano et al. 2014). Due to anthropogenic influences, seagrasses have experienced considerable global declines over the last 40 years (Orth et al. 2006; Unsworth and Cullen 2010). Globally, 29% of the known areal extent of seagrass has disappeared since seagrass areas were initially recorded in 1879 (Waycott et al. 2009), and 14% of seagrass species are at an elevated risk of extinction (Short et al. 2011). In China, seagrasses

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have rapidly declined in recent years, and the distribution area of seagrasses decreased from 1.64 km^2 to just 0.5 km^2 along the south coast of Hainan Island between 2008 and 2014 (Chen et al. 2015). The loss of seagrass species and damage to seagrass ecosystems will have devastating consequences for marine biodiversity and the human population relying on the ecosystem services provided by seagrasses. Anthropogenic impacts on seagrasses will intensify with the rapid human population growth occurring near the coastal areas and the increased carbon emissions per person.

As a consequence of anthropogenic activities, the increased input of trace metals into coastal marine ecosystems has gained attention worldwide (De et al. 2004; Yuan et al. 2004; Alina et al. 2012). Trace metals play a critical role in the functioning of marine ecosystems (Sánchez-Quiles et al. 2017; Bonanno and Orlando-Bonaca 2018); furthermore, some trace metals are toxic to organisms (e.g., Cd, Hg, Pb), while others are basic micronutrients (e.g., Co, Cu, Fe, Mn, Ni, Zn) working as cofactors in a number of enzymes and metabolic pathways (e.g., redox reactions, chlorophyll synthesis and photosynthetic electron transfer) (Hänsch and Mendel 2009). If the concentrations of trace metals exceed a threshold value of toxicity, the essential metals will also have negative effects on seagrass growth (Bonanno and Orlando-Bonaca 2017), such as inhibiting photosynthetic electron transport (Ciscato et al. 1999), limiting photosynthetic efficiency (Clijsters and Van 1985; Prange and Dennison 2000), damaging chlorophyll pigments (Macinnis-NG and Ralph 2002; Macinnis-Ng and Ralph 2004), or even killing seagrasses. Unlike organic pollutants, trace metals cannot be removed from aquatic ecosystems by natural processes (Jain 2004); once they accumulate in plant tissues, they begin to move up the food chain, often bio-magnifying at higher trophic levels and ultimately threatening human health (Timmermans et al. 1989; Barwick and Maher 2003; Rainbow 2007; Roach et al. 2008).

Thalassia hemprichii is a dominant seagrass species found along the coast of Hainan Island, China. In the past 5 years, our field study found that the distribution of this seagrass was still undergoing rapid loss, and the health of existing seagrasses was deteriorating. To reveal whether trace metals contributed to the decline in seagrasses, the phytotoxic effects of Cu, Cd and Zn on *T. hemprichii* were investigated under control conditions by monitoring the changes in photosynthetic pigment content, chlorophyll fluorescence and antioxidase activity. Additionally, the contents of these metals were analyzed in seawater and sediments associated with *T. hemprichii* in Xincun Bay (Hainan, China) by inductively coupled plasma mass spectrometry (ICP-MS).

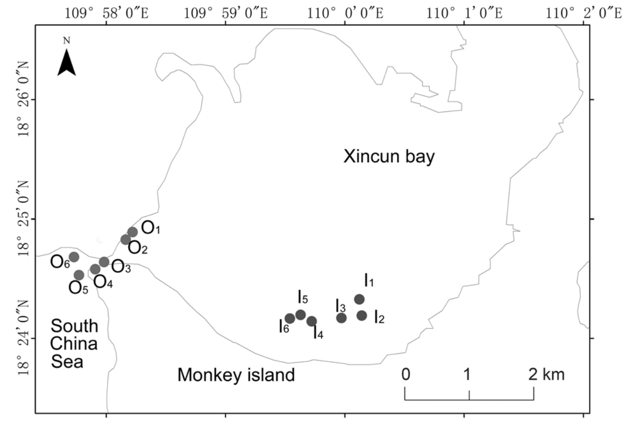

Fig. 1 Seagrass, seawater and sediment sampling sites in Xincun Bay region, Hainan, China. ● sampling sites, O seawater and sediment sampling sites in the outfall, I seagrass, seawater and sediment sampling sites in the interior

Materials and methods

Sampling site

Individuals of *T. hemprichii* were collected from the intertidal seagrass beds during low tide in Xincun Bay (18°24′–18°27′N, 109°58′–110°2′E) in June 2014, submerged in seawater and taken to a lab for further culture. Xincun Bay is one of the major seagrass habitats in China, and Xincun Bay is located next to Nanwan Monkey Island, a famous tourist attraction, in the southeast coastal region of Hainan Island. The bay is nearly closed, with only one narrow channel allowing the exchange of water with the open sea (Fig. 1). With the rapid development of cage aquaculture in recent years, the bay has become one of the key fish farms in China. Fish farming and pollution discharge have caused a rapid decline in seagrasses (Yang and Yang 2009). An investigation by Chen et al. (2015) showed a 44% loss of seagrasses from 2004 to 2013.

Plant culture and treatment

Seawater was prepared at 30 practical salinity units (PSU) of salinity using sea salt in transparent cuboid methacrylate aquaria (20 cm length, 22 cm width, 20 cm height). A 5-cm-thick layer of nutrition soil, i.e., a mixture of peat soil, forest humus soil and ash soil (25:15:9), was added to the bottom of each aquarium and covered with a 5-cm-thick layer of clean sand. *T. hemprichii* was grown on the sand layer in the aquaria and placed in a greenhouse with 20% of full sunlight at 25–28 °C. Air was pumped into each aquarium at 900 mL min^{-1}. After two weeks of growth, *T. hemprichii* pants were separately exposed to 0.01, 0.1 and 1.0 mg L^{-1} of Cu^{2+} and Cd^{2+}, and 0.1, 1.0 and 5.0 mg L^{-1} of Zn^{2+} for

5 days and then returned to normal conditions. A control group was not exposed to any metals. Experimental treatments were randomly placed and each had three replicates.

Estimation of leaf necrosis

The incidence of necrosis in leaves was estimated according to the method of Pagès et al. (2010). In each aquarium, leaves of seagrasses were counted and classified into one of three categories: "green" (i.e., no evident necrosis spots and chlorosis),"spotted" (i.e., obvious necrosis spots or chlorosis, but a surface less than 75% necrotic) and "black" (i.e., surface more than 75% necrotic). Leaf necrosis incidence was expressed as the percentage of each category relative to the total number of leaves present in each treatment.

Determination of chlorophyll fluorescence

Chlorophyll fluorescence on *T. hemprichii* leaves was measured by using a portable pulse amplitude modulation fluorometer (PAM-2100, Walz, Germany). The steady-state fluorescence (F_s) and the maximum fluorescence (F_m') were determined on the second youngest leaves at 150 µmol (photon) m^{-2} s^{-1} actinic light. The effective quantum yield of PSII ($\Delta F/F_m'$) was calculated as: $(F_m' - F_s)/F_m'$.

Determination of photosynthetic pigments

Fresh leaf samples (0.1 g) were homogenized using a mortar and pestle in 10 mL of 80% acetone, and then samples were centrifuged at 4000 g for 10 min. The absorbance of supernatant was detected at 663, 646 and 470 nm relative to an 80% acetone blank. The contents of Chl *a*, Chl *b*, total Chl and carotenoids (Car) were calculated according to Wellburn (1994).

Determination of antioxidase activity

Fresh samples (0.1 g) were ground with liquid nitrogen and homogenized in 50 mM cold potassium phosphate buffer (pH 7.8) containing 0.1% (V/V) Triton X-100, 2% (W/V) polyvinylpyrollidone (PVP) and 0.1 M EDTA (pH 8.0). The mixture was centrifuged at 12,000 g for 20 min at 4 °C, and the supernatant was used as the raw extract for enzyme assays.

Superoxide dismutase (SOD) activity was assayed by monitoring the inhibition of photochemical reduction of nitroblue tetrazolium (NBT) by superoxide radicals to blue-colored formazan (Beauchamp and Fridovich 1971). The reaction mixture contained 2 mL of phosphate buffer (50 mM, pH 7.8), 0.3 mL of L-methionine (130 mM), 0.3 mL of NBT solution (750 µM), 0.3 µL of EDTA (0.1 M), 0.1 mL of enzyme and 0.3 mL of riboflavin (20 µM) and was delivered to small glass tubes, illuminated at an intensity of 50 µmol (photon) m^{-2} s^{-1} for 15 min, and then the absorbance was determined at 560 nm relative to a mixture that did not experience a light reaction. One unit of SOD activity (U) was defined as per minute and per gram of fresh sample required to cause 50% inhibition of the reduction of NBT.

Peroxidase (POD) activity was assayed by the guaiacol method (Bestwick et al. 1998). The reaction mixture consisted of 1.89 mL of sodium phosphate buffer (50 mM, pH 7.0) and contained 1% guaiacol, 0.01 mL of enzyme extract, and 1 mL of H_2O_2 solution (30 mM). The reaction was initiated by the addition 1 mL of H_2O_2 and the increase in absorbance at 470 nm for 180 s was measured. Catalase (CAT) activity was estimated by the method described by Upadhyaya et al. (1985). POD and CAT activity (U) was defined as the reduction in absorbance by 0.01 per min and mg of fresh sample.

Determination of Cu, Cd and Zn

For analyzing metal concentrations in the seagrass habitat of *T. hemprichii*, seawater and sediments were sampled from six randomly selected sites in the seagrass bed in the southern region of Xincun Bay (Fig. 1) when the tide was beginning to ebb in June 2017. In addition, seawater and sediments were sampled from another six randomly selected sites in the outfall. At each sampling site, ten individual seagrass plants were collected, washed immediately in seawater to remove the adhering shells, epiphytes, and sediments, and then placed in sealed plastic bags and stored on ice. Seawater was sampled at the depth of ~0.5 m and stored in acid-washed bottles at 4 °C. Sediments were collected from the top 10 cm of the surface, stored in separate polyethylene bags and placed at 4 °C until further processing.

In the laboratory, the seagrass tissues collected from the bay were washed again using deionized water to adequately remove any attached materials. Each seagrass sample was separated into aboveground tissues (leaves) and underground tissues (roots and rhizomes), placed in paper bags and air-dried at room temperature to a constant weight. The dried tissues were ground with a grinder (MDJ-D4072, Bear, Foshan, China) and then passed through a 500-µm diameter sieve. Sediment samples were dried in the same way as the seagrass tissues and then passed through a 1-mm diameter sieve. Dried tissue or sediment sample (0.2 g) was put into a 100-mL digestive tube, 10 mL of concentrated HNO_3 was added, and the sample was digested in a microwave oven (COOLPEX, Preekem, Shanghai, China) at three ascending temperature steps, i.e., 120, 150 and 190 °C, for 5 min each. To facilitate the digestion of sediments, 2 mL of 30% H_2O_2 was added to the samples prior to the addition of concentrated HNO_3. After digestion, the plant and sediment samples were diluted with deionized water to a final volume of 25 mL. Seawater samples (50 mL) were filtrated using nylon membrane (0.45 μm) and acidified with 1.5 mL of concentrated HNO_3. The contents of Cu, Cd and Zn were determined in the digested samples and seawater samples by using an Agilent 7700 × ICP-MS (Agilent Technologies, Santa Clara, USA). Indium (In) was used as internal standard. The accuracy of the method was tested using reference water standards and mean recovery was 96 ± 11%. Content of the metals was calculated as: $(\rho*V*f)/(m*1000)$, where ρ is the metal concentration (μ g L^{-1}); V is the final volume (mL); f is the dilution ratio; m is the weight or volume of the sample (g dried weight or mL); and the coefficient 1000 is the scaling factor.

The bio-concentration factor (BCF) was calculated as C_a/C_b, where C_a is the content of metal in seagrass tissues (μg g^{-1} DW), and C_b is the content of metal in seawater (mg L^{-1}) or sediments (μg g DW) (Lewis et al. 2007).

Data analyses

All statistical analyses were performed by using the IBM SPSS Statistics 19.0 (IBM, Armonk, NY, USA). The results of the antioxidase activity assays were analyzed by one-way ANOVA to test for significant differences among different groups of metal concentrations. Tukey's test of multiple comparisons was used to compare group means at the level $P = 0.05$. Prior to performing ANOVA, data were checked for normality and homogeneity of variances, and where these assumptions were not met, data were log-transformed to correct deviations from the assumptions. The results of metal measurements were analyzed by two-tailed t tests to test for significant differences between the seagrass above-ground tissue and underground tissue and between the interior of the bay and its outfall. All quantitative results were presented as the mean of 4–6 replicates and the standard error (SE).

Results

Change in phenotype in seagrass *T. hemprichii*

After exposure to Cu, Cd and Zn for 5 days, the leaves of *T. hemprichii* displayed symptoms of chlorosis and necrosis (Figs. 2 and 3). The leaf necrosis incidence of *T. hemprichii* exposed to Cu^{2+} was higher than that in plants exposed to Cd^{2+} and Zn^{2+} at both low and high concentrations. After exposure to 0.01 mg L^{-1} of Cu^{2+}, *T. hemprichii* leaf necrosis incidence was twice as high as the control. For those exposed to 0.1 and 1.0 mg L^{-1} of Cu^{2+}, leaf necrosis incidence accounted for approximately 50 and 100% of total leaves, respectively. In contrast, leaf necrosis incidence was slightly increased in *T. hemprichii* exposed to various concentrations of Cd^{2+} and Zn^{2+}. Under low concentrations of Cd^{2+} (0.01 mg L^{-1}) and Zn^{2+} (0.1–1.0 mg L^{-1}), the

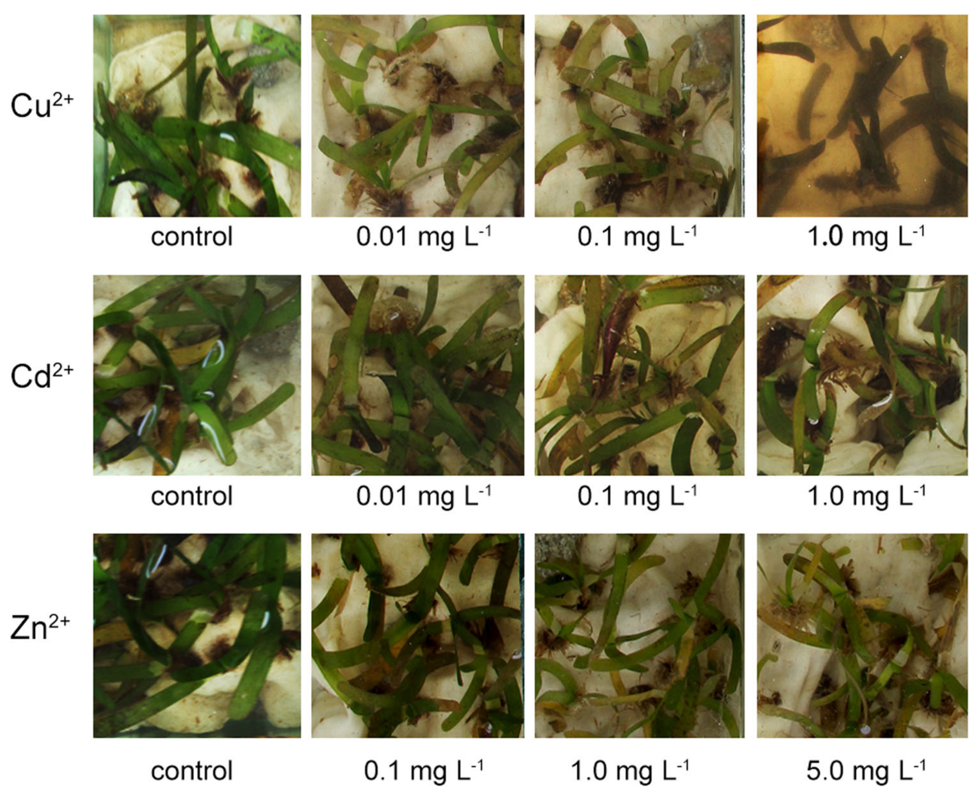

Fig. 2 Appearance of *Thalassia hemprichii* after 5 days of exposure to copper, cadmium and zinc ions

percentage of *T. hemprichii* leaves still displaying the normal green color was the same as the control; however, under high concentrations of Cd^{2+} ($1\ mg\ L^{-1}$) and Zn^{2+} ($5\ mg\ L^{-1}$), approximately 30% of *T. hemprichii* leaves stayed the typical green color.

Change in chlorophyll pigments

The content of chlorophyll (Chl) *a* and *b* in *T. hemprichii* decreased after 5 days of exposure (DOE) to Cu^{2+}, Cd^{2+} and Zn^{2+} in a concentration-dependent manner, and the decrease in Chl *a* was more rapid than the decrease in Chl *b* along a concentration gradient of the metals (Fig. 4). In contrast, the content of carotenoids increased slightly in *T. hemprichii* under treatments with 0.01–0.1 $mg\ L^{-1}$ of Cu^{2+}, 0.01 $mg\ L^{-1}$ of Cd^{2+} and 0.1–1.0 $mg\ L^{-1}$ of Zn^{2+}, and then the content of carotenoids decreased under treatments with higher concentrations of these metals. The amplitude of the decrease in carotenoids content in *T. hemprichii* exposed to 1.0 $mg\ L^{-1}$ of Cu^{2+} was greater than the amplitudes in those exposed to the same concentrations of Cd^{2+} or Zn^{2+}.

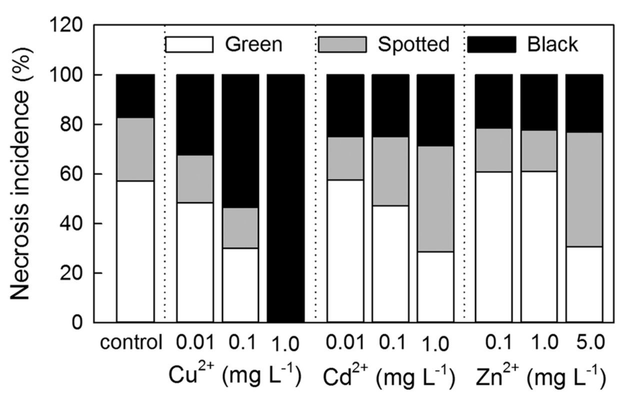

Fig. 3 Leaf necrosis incidence in *Thalassia hemprichii* after 5 days of exposure to copper, cadmium and zinc ions

Change in chlorophyll fluorescence

Effective quantum yield ($\Delta F/F_m'$) in *T. hemprichii* exposed to different metal ions was inhibited to different degrees (Fig. 5). The evident reduction of $\Delta F/F_m'$ in *T. hemprichii* leaves was observed at day 1 of exposure to 0.01–1.0 $mg\ L^{-1}$ of Cu^{2+} and at day 3 of exposure to 0.1–1.0 $mg\ L^{-1}$ of Cd^{2+}. The decline of $\Delta F/F_m'$ in *T. hemprichii* exposed to 1.0 $mg\ L^{-1}$ of Cu^{2+} was very dramatic. However, little inhibitory effect on $\Delta F/F_m$' was observed in *T. hemprichii* exposed to 0.1–5.0 $mg\ L^{-1}$ of Zn^{2+} for 5 days. After individuals were removed from 0.01–0.1 $mg\ L^{-1}$ of Cu^{2+} and 0.1–0.1 $mg\ L^{-1}$ of Cd^{2+}, $\Delta F/F_m'$ in *T. hemprichii* was restored to the levels of the control within 5 days. Because 1.0 $mg\ L^{-1}$ of Cu^{2+} was lethal to *T. hemprichii*, $\Delta F/F_m'$ could not be restored after 5 days of recovery (DOR).

Antioxidant enzyme activities

Antioxidant enzyme activities in *T. hemprichii* after 5 days of exposure to metals and after 5 days of recovery is shown in Fig. 6. The SOD activity declined in *T. hemprichii* leaves exposed to high concentrations of the metal ions compared with that in the control. After 5 days of recovery, The SOD activity was restored to various levels; it was restored to control levels in the 0.1 $mg\ L^{-1}$ of Cu^{2+} and Cd^{2+} and 0.1–5.0 $mg\ L^{-1}$ of Zn^{2+} treatments but was still lower than control levels in treatments with higher concentrations of Cu^{2+} and Cd^{2+}. In contrast, the POD activity in *T. hemprichii* leaves exposed to various concentrations of the three metal ions (with the exception of those treated with 1.0 mg L^{-1} of Cu^{2+}) increased by 1–2-fold of the control, and POD activity was still maintained at levels higher than the control after 5 days of recovery. The CAT activity was significantly lower in *T. hemprichii* leaves exposed to 0.01–1.0 $mg\ L^{-1}$ of Cu^{2+} than in the control, but not many differences were

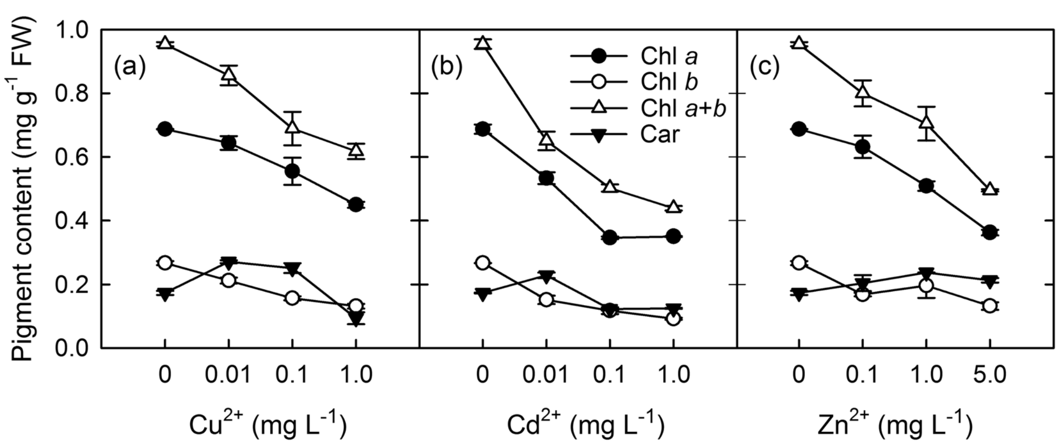

Fig. 4 Content of chlorophyll (Chl) *a*, Chl *b* and carotenoids (Car) in leaves after 5 days of exposure to copper (**a**), cadmium (**b**) and zinc ions (**c**). Data are the means of 4–5 replicates ± SE

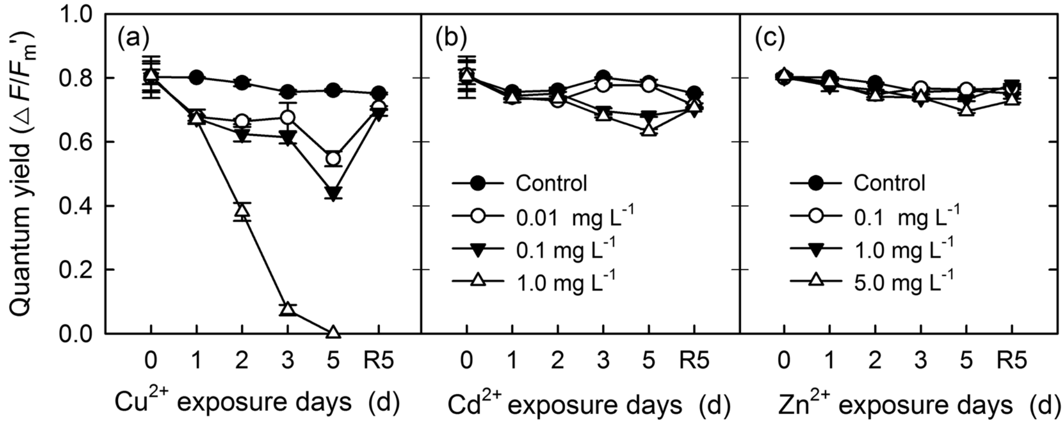

Fig. 5 Effective quantum yields ($\Delta F/F_m$') in *Thalassia hemprichii* over 5-d exposure and 5-day recovery periods to copper (**a**), cadmium (**b**) and zinc ions (**c**). Data are the means of 4–5 replicates ± SE

observed between those exposed to 0.01–1.0 mg L^{-1} of Cd^{2+} or 0.1–5.0 mg L^{-1} of Zn^{2+} and the control. After recovery from the 0.01–0.1 mg L^{-1} of Cu^{2+}treatment, the CAT activity was restored to levels close to the control. However, a significant reduction in CAT activity was observed in *T. hemprichii* leaves after 5 days of recovery in the plants treated with the high concentrations of Cd^{2+} (0.1–1.0 mg L^{-1}) and Zn^{2+} (5.0 mg L^{-1}).

Investigation of Cu, Cd and Zn in field sampling

The contents of Cu, Cd and Zn were determined in seawater and sediments sampled from both the interior and the outfall of Xincun Bay as well as in *T. hemprichii* sampled from the interior (Table 1). Both seawater and sediment in the interior of Xincun Bay had higher concentrations of Cu, Cd and Zn than did the seawater and sediment in the outfall. Specifically, the concentrations of Cu and Zn in seawater from the interior were approximately 1- and 1.5-fold higher than those from the outfall, respectively. Cu, Cd and Zn were significantly enriched in *T. hemprichii* leaves. As a result, the BCF for Zn (6479) was greater than that of Cu (5766) and Cd (4028) in leaves versus seawater. The contents of Cu and Cd in leaves were 1-fold greater than those in roots and rhizomes, and the content of Zn was 30% greater in leaves than in roots and rhizomes. The contents of Cu, Cd and Zn in roots and rhizomes were lower, comparable and greater, respectively, than those in sediments.

Discussion

Metal pollutants can greatly influence organisms at different organizational levels. Necrosis is a visual symptom of *T. hemprichii* that has been exposed to high concentrations of Cu^{2+}, Cd^{2+} and Zn^{2+} for 5 days (Figs. 2 and 3) and is consistent with the symptoms observed in seagrasses suffering severe nutrient deficiencies (van der Heide et al. 2008) and hyper-salinity (Pagès et al. 2010; Sandoval-Gil et al. 2012). Among the three tested metals, Cu^{2+} was the most toxic to *T. hemprichii*. This result is consistent with previous observations in other seagrasses (Prange and Dennison 2000; Macinnis-NG and Ralph 2002). Under the 1.0 mg L^{-1} of Cu^{2+} treatment, *T. hemprichii* rapidly turned black within 5 days, accompanied by the dramatic reduction of photosynthetic pigments and the decline in effective quantum yield (Figs. 4 and 5). High concentrations of Cu^{2+} might disturb functioning of Ca^{2+}, Fe^{2+}, Mg^{2+} and K^{+} in *T. hemprichii*, resulting in malnutrition that induced the progressive senescence of the plant, as previous shown in *Koeleria splendens* (Ouzounidou 1995). Moreover, Cu^{2+} can also alter the chloroplast membrane ultrastructure, resulting in K^{+} and Cu^{2+} leakage into the chloroplast, and this could aggravate the inhibition of the PSII electron transport (Shioi et al. 1978; Ouzounidou 1994).

Although Cd^{2+} and Zn^{2+} were less toxic to *T. hemprichii* relative to Cu^{2+}, Chl contents were obviously reduced in *T. hemprichii* individuals exposed to various concentrations of Cd^{2+} and Zn^{2+}, and $\Delta F/F_m'$ decreased in individuals exposed to 0.1–1.0 mg L^{-1} of Cd^{2+}. High levels of metals can inhibit the uptake of nutrient elements through competition for binding sites (Wang et al. 2009). Thus, the reduction in Chl content in *T. hemprichii* exposed to Cu^{2+}, Cd^{2+} and Zn^{2+} might be because these metal ions disturbed the uptake of Mg^{2+}, thereby inhibiting the turnover of Chl. Additionally, Cu^{2+}, Cd^{2+} and Zn^{2+} might also change the redox state in *T. hemprichii* leaves, which caused damage to the Chl and photosynthetic apparatus. Changes in redox state in *T. hemprichii* exposed to metal ions can be reflected by changes in antioxidant enzyme activities, including POD, SOD and CAT (Fig. 5).

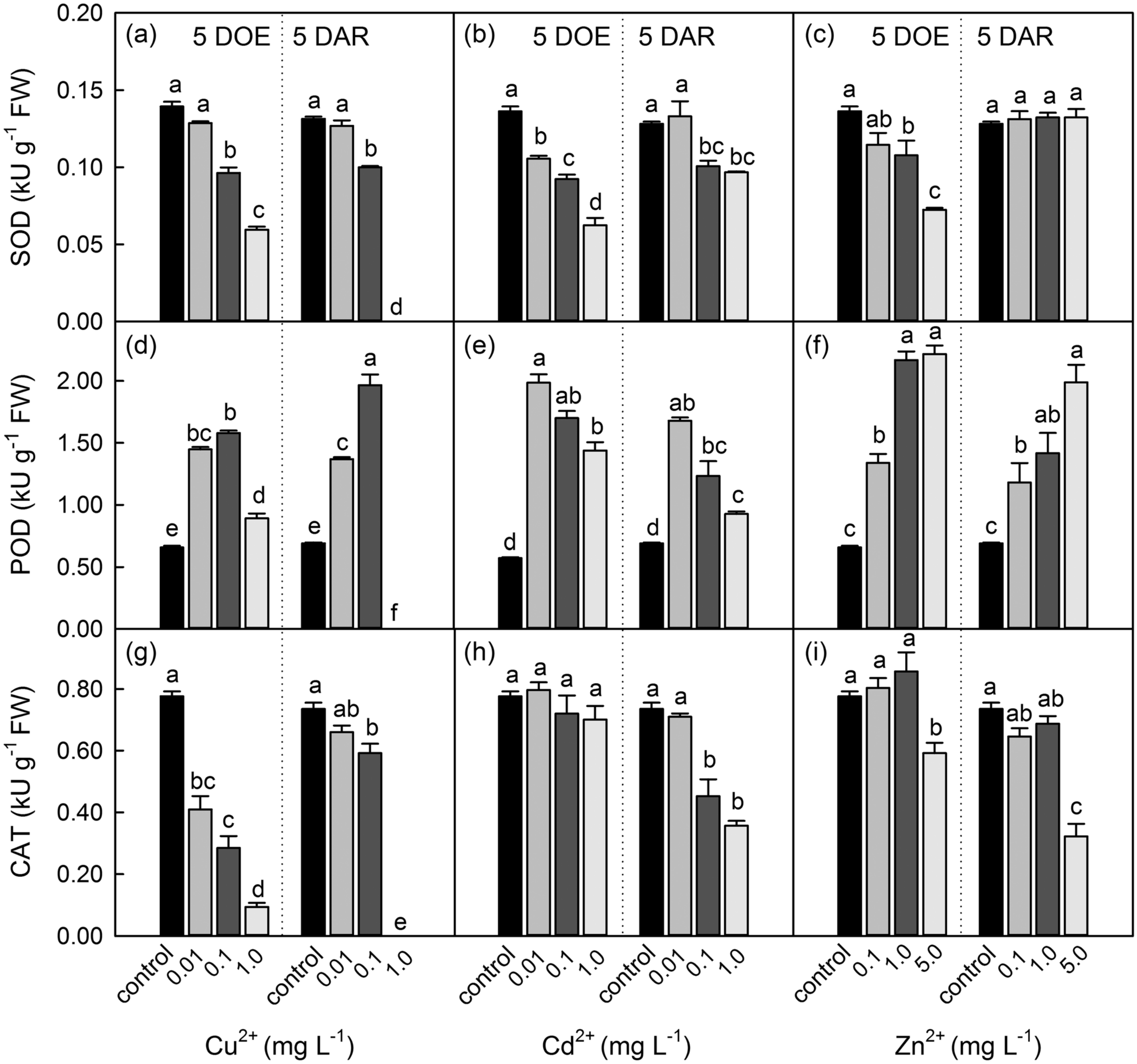

Fig. 6 Activity of superoxide dismutase (SOD, **a–c**), guaiacol peroxidase (POD, **d–f**) and catalase (CAT, **e–i**) in *Thalassia hemprichii* after 5 DOE to copper, cadmium and zinc ions and after recovery at normal conditions. Data are means ± SE (n = 4–5). Different letters above bars indicate statistical significance ($P < 0.05$). DOE, days of exposure. DAR, days after recovery

The mechanism explaining why *T. hemprichii* has a higher tolerance to Cd^{2+} and Zn^{2+} relative to Cu^{2+} is still unclear. The aquatic plant *Ipomoea aquatic* can withstand high levels of Cu^{2+} by sequestering large quantities of Cu^{2+} in its necrotic parts (Chanu and Gupta 2014). In addition, excluding or sequestering Cd^{2+} into the structural components of the leaf tissues, such as the vacuole, can also serve as a mechanism for seagrasses to maintain a high resistance to Cd^{2+} (Ward 1989). Whether *T. hemprichii* has mechanisms to withstand Cd^{2+} and Zn^{2+} similar to those that have been previously reported is still unknown. Carotenoids and antioxidant enzymes play an important role in protecting photosystems in leaves against photooxidation in plants experiencing stressed conditions (Schützendübel and Polle 2002; McElroy and Kopsell 2009; Sinha et al. 2010). After 5 days of exposure to 1.0–5.0 mg L^{-1} of Zn^{2+}, *T. hemprichii* leaves had higher carotenoids content and POD activity than did those exposed to 1.0 mg L^{-1} of Cu^{2+} and Cd^{2+}, suggesting that enhanced photoprotection played a role in the tolerance of *T. hemprichii* to Zn^{2+}.

The metal contents in seawater (Cu 11.43; Cd 0.43; Zn 35.49 μg L^{-1}) and sediments (Cu 40.03; Cd 0.93; Zn 98.74 μg g^{-1}) associated with seagrass in Xincun Bay were lower than the maximum levels measured in the seawater

Table 1 Concentrations of copper, cadmium and zinc in field sampling from Xincun Bay and bio-concentration factors for the three metals in *Thalassia hemprichii*

Field sampling	Cu	Cd	Zn
Concentration			
Seawater in the interior (μg $L^{-;1}$)	11.13 ± 0.02	0.43 ± 0.03	35.49 ± 0.64
Seawater in the outfall (μg L^{-1})	5.83 ± 0.01	0.36 ± 0.01	13.82 ± 0.03
Sig. (Student's-test)	($P < 0.001$)	($P = 0.164$)	($P < 0.001$)
Sediment in the interior (μg g^{-1} DW)	40.03 ± 0.91	0.93 ± 0.03	98.74 ± 2.24
Sediment in the outfall (μg g^{-1} DW)	25.93 ± 1.30	0.88 ± 0.05	89.86 ± 2.68
Sig. (Student's-test)	($P < 0.001$)	($P = 0.364$)	($P = 0.064$)
T. hemprichii leaves (μg g^{-1} DW)	64.18 ± 2.44	1.75 ± 0.04	229.97 ± 2.53
T. hemprichii roots/rhizomes (μg g^{-1} DW)	28.66 ± 6.48	0.85 ± 0.09	173.06 ± 18.67
Sig. (Student's-test)	($P < 0.01$)	($P < 0.001$)	($P < 0.05$)
Bio-concentration factor			
Leaves/seawater	5766	4028	6479
Roots and rhizomes/seawater	2575	1956	4876
Leaves/sediments	1.60	0.17	2.33
Root and rhizome/sediments	0.72	0.08	1.75

Data are mean ± SE (n = 6)

(Cu 123; Cd 16.0; Zn 51.0 μg L^{-1}) and sediments (Cu 397; Cd 87.0; Zn 7000 μg g^{-1}) of Mediterranean seagrass ecosystems (Bonanno and Orlando-Bonaca 2018). The metal contents in *T. hemprichii* (Cu 64.18; Cd 1.75; Zn 229.97 μg g^{-1}) from Xincun Bay were also evidently lower than the maximum levels measured in Mediterranean seagrasses (Cu 148; Cd 85.7; Zn 787 μg g^{-1}) (Bonanno and Orlando-Bonaca 2018), but they were still higher than the global mean content in seagrasses (Cu 9.88; Cd 0.99; Zn 39.6 μg g^{-1}) (Sánchez-Quiles et al. 2017). Compared with another *T. hemprichii* habitat on the coast of Ambon Island, Indonesia, the seagrass ecosystem of Xincun Bay also had higher Cd content in sediments (0.93 μg g^{-1} versus 0.19 μg g^{-1}) and plant tissues (0.85–1.75 μg g^{-1} versus 0.275–0.363 μg g^{-1}) (Tupan and Uneputty 2017). Thus, metal contamination in the seagrass ecosystem of Xincun Bay was within the intermediate level of those found around the world. Since there is no industry around Xincun Bay, high-intensity cage aquaculture, domestic wastewater, and tourism may be the main sources of metals.

According to the phytotoxic effects of the metals (Figs. 2–5), the current contents of Cd and Zn in seawater were safe for *T. hemprichii*, but the current content of Cu in seawater has reached harmful levels (Table 1). Therefore, it was concluded that Cu is at least partially responsible for the deterioration of seagrasses in Xincun Bay. Cu may interact with other organic and inorganic pollutants, thereby exacerbating the decline of seagrasses. Future analysis of responses of seagrasses to interactive effects between Cu and other metals or organic pollutants may contribute to the understanding of the deterioration of seagrasses. More importantly, seagrass habitats in China are in urgent need of protection.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

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