Acta Oceanol. Sin., 2012, Vol. 31, No. 3, P. 98-108
DOI: 10.1007/s13131-012-0210-3
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Photosynthetic activity and antioxidative response of seagrass *Thalassia hemprichii* to trace metal stress

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Received 24 January 2011; accepted 31 March 2012

Abstract
This study concerned the accumulation of trace metals in tissues of seagrass (*Thalassia hemprichii*) exposed to various concentrations of Zn^{2+}, Cd^{2+}, Pb^{2+} and Cu^{2+} for 10 d, and the effect of excessive metals on quantum yield ($\Delta F/F'_{\rm m}$), photosynthetic pigments and antioxidative enzymes like superoxide dismutase (SOD), guaiacol peroxidase (POD) were also examined. Cadmium was the most highly accumulated metal. Meanwhile, high metals levels led to a remarkable breakdown of photosynthetic parameters. Especially, $\Delta F/F'_{\rm m}$, chlorophyll and carotenoid were significantly low during prolonged Cu exposure. Besides, $\Delta F/F'_{\rm m}$ was more severely depressed by Cu and Zn than Pb and Cd. However, *T. hemprichii* had positive response by increasing the activity of SOD and POD. The results indicate that *T. hemprichii* is the most sensitive to Cu, and the antioxidative protection mechanisms of *T. hemprichii* are more efficiently activated to avoid damage of Zn, Cd and Pb stress. Finally, due to the high Cd-accumulation and strong Cd-tolerance capacity, *T. hemprichii* can be used for phytoremediation in Cd-contaminated areas.

Key words: *Thalassia hemprichii*, metal stress, photosynthetic pigments, quantum yield, antioxidant enzymes

1 Introduction

Seagrass beds, having great ecological and commercial values, house diverse communities in intertidal zones of tropical, subtropical and temperate coastal areas where anthropogenic activity is the most intense. However, many seagrass habitats around the world are vulnerable to degradation and many of them have been completely destroyed with the expansion of developmental activities in the coastal areas. Trace metal contamination is an important aspect of destruction of seagrass habitat (Huang et al., 2006). Similar to other wetlands (e.g., mangrove and salt marsh ecosystems), seagrasses sequester trace metals from the marine environment via both blades and root-rhizomes and these concentrations can be correlated with the water column and sediments, respectively (Tiller et al., 1989). Many studies suggest that seagrasses possess a remarkable capacity to retain metals and tolerate relatively high levels of trace metal stress, but in excessive metal contamination, seagrass may initiate a variety of subcellular response which may cause metabolic turbulence and other damage at cellular levels (Hamoutene et al., 1996; Ralph and Burchett, 1998; Prange and Dennison, 2000; Macinnis-Ng and Ralph, 2002). Although micro-nutrient metals such as Cu and Zn are required in a number of enzyme systems, yet excess of them can lead to senescence, chlorosis or even death of plants (Ebbs and Kochian, 1997).

The toxicity of metals is generally thought to be

Foundation item: The Chinese Nature Science Foundation (CNSF) Project under contract Nos 40776086 and 41076069; Forefront Program of the Knowledge Innovation Project, South China Sea Institute of Oceanology, Chinese Academy of Sciences under contract No. LYQY200706; the National 908 Special Project under contract No. GD908-02-08.
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caused by oxidative stress which is due to uncontrolled and excessive production of reactive oxygen species (ROS), such as superoxide anion radicals ($O_2^{\cdot-}$), singlet oxygen ($^1O_2$), hydrogen peroxide (H_2O_2) and hydroxyl radicals (OH·) (Salin, 1988; Apel and Hirt, 2004). Excessive accumulation of ROS may be the consequence of disruption of the balance between their production and the scavenging of antioxidative system, which is composed of antioxidant enzymes such as superoxide dismutase (SOD, EC 1.1.5.1.1), guaiacol peroxidase (POD, EC 1.11.1.7) and catalase (CAT, EC 1.11.1.6), and non-enzymic scavengers, e.g., carotenoids, ascorbate and glutathione (Vangronsveld and Clijsters, 1994). The imbalance causes lipid peroxidation, enzyme inactivation and DNA damage, resulting in dramatic reduction of growth and productivity or even plant death (Stobrawa and Lorenc-Plucińska, 2008).

Chlorophyll a fluorescence was reported as a rapid, non-destructive way for the assessment of the physiological effects of metal stress on photosynthesis of seagrass (Schreiber et al., 1994). Among all the variables (F_m, F_o, F_v/F_m and $\Delta F/F'_m$), quantum yield of photosystem II (PS II) ($\Delta F/F'_m$) is the most sensitive and rapidly responding variable in terms of monitoring the toxic effect of metals on plants (Ralph and Burchett, 1998). In seagrasses, the previous studies have concentrated on seagrass morphology, taxonomy and distribution (den Hartog and Yang, 1990, Huang et al., 2006), or distribution and accumulation of metals in seagrass tissues (Malea and Haritonidis, 1995) and effect of metals on photosynthesis of *Zostera capricorni* and *Halophila ovalis* in Australia (Ralph and Burchett, 1998; Prange and Dennison, 2000; Macinnis-Ng and Ralph, 2002). Hamoutene et al. (1996) and Ferrat et al. (2002a, b) investigated the effects of Cd and Hg on oxidative metabolism (CAT or glutathione S-transferase activity) in *Posidonia oceanica* in the Mediterranean area. To our knowledge, little information is found on photosynthetic activity and antioxidative response of tropical seagrasses under metals stress especially for *Thalassia hemprichii* (Ehrenb.) Aschers. *T. hemprichii* is one of the most widely distributed tropical seagrasses in the western Pacific (Mukai, 1993). Also, it is one of the two dominant seagrass species in the southeast of the Hainan Island, the South China Sea (Huang et al., 2006). Hence, it is necessary to study photosynthetic activity and antioxidative enzymes responses of tropical seagrass *T. hemprichii* treated with trace metals.

In the present study, *T. hemprichii* was exposed to elevated levels of Zn, Cd, Pb and Cu concentrations and then physiological response and antioxidant defences of the seagrass to trace metals were determined. Especially, $\Delta F/F'_m$ and photosynthetic pigments values were used to assess the physiological changes of photosynthetic activity. Also the enzymatic activities of SOD and POD in the leaves of *T. hemprichii* were detected to evaluate the antioxidant defences of *T. hemprichii*.

2 Materials and methods

2.1 *Seagrass material and trace metal aquarium treatment*

Plants of *T. hemprichii* were obtained from the Xincun Bay, the Hainan Island, South China. The whole cores including seagrass and rhizosphere sediment were collected to ensure that the samples were complete and undamaged. Then the intact samples were transferred to 1 L glass beakers and sealed with plastic bags to avoid excess water loss during transportation to the laboratory. Intact samples of *T. hemprichii* were placed in aerated glass aquaria, filled with 15 L of seawater from the Xincun Bay. Plants of equal size were placed in each of the 18 aquaria. Five hundred milliliters of 10% Hoagland's solution (Hoagland and Arnon, 1950) was taken in each aquarium. Before metals treatment, seagrass samples were acclimatized for one week in laboratory conditions [light intensity of 250 μmol·m^{-2}·s^{-1} at the temperature of (25±2)℃]. For concentration-dependent experiments, seagrass plants were treated with concentrations of Zn^{2+}, Cd^{2+} (0, 10, 100, 300 μmol/L), Pb^{2+} and Cu^{2+} (0, 10, 50, 100, 150 μmol/L), maintained in seawater in aerated glass aquaria under the above mentioned laboratory conditions for a period of 1, 2, 4 (5), 7 and 10 d. Seagrasses in aquaria without treated metals kept with each set of experiment as control. After harvesting, epiphytes were removed from leaves by scraping and then plants were washed with double distilled water and used for the study of various parameters. All glassware, sample containers and experimental chambers were cleaned with anionic detergent, then soaked in a 2 mol/L HNO_3 acid-bath for 24 h, and finally rinsed with double distilled H_2O.

2.2 *Chlorophyll fluorescence analyses*

Chlorophyll fluorescence was determined by PAM fluorometer (Walz, Germany). Steady-state chloro-

phyll fluorescence analysis was measured at ambient temperature, using dark-adaptation clips (Walz, Germany). $\Delta F/F'_{\mathrm{m}}$ analysis was performed at a standardized position on the second leaf from the meristem, approximately in the middle of the adaxial surface. Fluorescence measurements were made over a discontinuous time-scale; and after the initial exposure, samples were measured at hourly intervals for 10 h, and then daily (10:00 am) for the following 10 d.

2.3 *Determination of photosynthetic pigments*

Fresh leaves were homogenized using a chilled pestle and mortar in 8 ml 80% acetone (Arnon, 1949) in a dark room. The homogenate was centrifuged at 4 000 r/min at 4℃ and the supernatant liquid was used for chlorophyll a (Chl a), chlorophyll b (Chl b) and carotenoid estimation at 663, 646 and 470 nm, respectively and the results were expressed as mg/g fresh weight (FW).

2.4 *Estimation of Zn, Cd, Pb and Cu*

After being washed three times with double distilled H_2O, the harvested samples were immediately blotted and oven-dried at 105℃ for 20 min and then kept at 80℃ for 72 h. The dried material was ground. The powdered samples were digested with HNO_3-$HClO_4$ solution and heated at 100–200℃ until a white fume evolved. The contents of Zn, Cd, Pb and Cu were determined by a Varian Spectr AA 220 flame atomic absorption spectrometer (AAS). Plant reference materials (GBW 08517, China) were used to confirm the detection accuracy.

2.5 *Extraction and assays of enzymes*

The 0.5 g fresh weight leaves were homogenized in 50 mmol/L cold sodium phosphate buffer (pH 7.8) including 0.1 mmol/L EDTA and 8.75% (w/v) polyvinylpyrrolidone (PVP, molecular weight 10 000) using a prechilled mortar and pestle in an ice bath. The homogenate was centrifuged at 10 500 r/min for 30 min at 4℃ and the supernatant was used for enzymes assays.

SOD activity was assayed by monitoring the inhibition of photochemical reduction of nitro-blue tetrazolium (NBT) according to the method of Beauchamp and Fridovich (1971). The 3-ml reaction mixture contained 50 mmol/L potassium phosphate buffer (pH 7.8), 13 mmol/L methionine, 75 μmol/L NBT, 0.1 mmol/L EDTA, 2 μmol/L riboflavin and 0.2 ml of enzyme extract. The reaction mixtures were illuminated at an intensity of 4 500 lx for 20 min. The absorbance of the supernatant at 560 nm was measured. One unit of SOD activity was defined as the amount of enzyme required to cause 50% inhibition of the reduction of NBT.

POD activity was determined as oxidation of guaiacol by H_2O_2 (Upadhyaya et al., 1985). The reaction mixture was 3 ml of 200 mmol/L potassium phosphate buffer (pH 6.0), 0.18% (v/v) H_2O_2, 1% guaiacol and 0.5 ml of enzyme extract. The oxidation of guaiacol was measured by the increase in absorbance at 470 nm every 30 s [extinction coefficient 26.6 L/(mmol·cm)]. One unit of POD activity was defined as units per min and mg of protein.

2.6 *Statistical analysis*

The analysis of variance (ANOVA) was done to confirm the variability of data and validity of results, and Duncan's multiple range test (DMRT) was performed to determine whether the differences between treatments were significant as compared with control. Data are presented as means±standard error (SE) of three replicates.

3 Results

3.1 *Accumulation of metals in seagrass tissues*

The Zn, Cd, Pb and Cu accumulations in different parts of *T. hemprichii* after 10-d exposure are shown in Table 1. The accumulations of four metals in the aboveground and underground tissues were proportional with the increasing concentrations of metals in seawater. In the treatments with low concentration of Zn and Cd (10 μmol/L), metals concentrations in seagrass tissues were not significantly higher than that of control. However, with the increasing concentration of metals, significant increase in accumulation was observed at the treatment of Cu and Pb (>10 μmol/L), Cd and Zn (>100 μmol/L) as compared with control (Table 1). In all treatments, the concentrations of four metals in aboveground tissues were significantly higher than that in underground tissues, with the ratio of aboveground/underground concentrations for Zn, Cd, Pb and Cu varying between 1.7 to 4.7, 1.8 to 5.2, 1.1 to 1.7 and 1.2 to 3.8, respectively. Especially, Cd accumulated in aboveground tissues of *T. hemprichii* was more than 7 000 times higher than control after 10-d exposure.

Table 1. Accumulation of metal in different parts of *T. hemprichii* after 10 d of growth under different concentrations of that metal stress

Tissues	Treatments/μmol·L^{-1}	Accumulation of metals/mg·kg^{-1}			
		Zn	Cd	Pb	Cu
Aboveground	control	81.03^{a}±1.05	0.28^{a}±0.02	14.80^{a}±2.11	8.98^{a}±0.23
	10	186.05^{a}±7.05	201.87^{a}±16.90	111.40^{b}±5.12	119.30^{b}±6.32
	50			111.80^{b}±4.60	350.81^{c}±19.63
	100	1 144.97^{b}±89.03	1 182.37^{b}±205.02	107.00^{b}±8.60	503.42^{d}±31.36
	150			108.90^{b}±5.60	491.63^{d}±25.32
	300	1 452.24^{c}±110.03	1 969.93^{c}±208.04		
Underground	control	40.01^{ad}±1.30	0.21^{ad}±0.05	12.03^{a}±5.51	9.25^{a}±0.86
	10	53.69^{d}±4.30	33.37^{d}±2.30	70.20^{c}±7.20	71.83^{e}±4.36
	50			95.11^{bc}±8.42	136.71^{f}±6.31
	100	245.88^{e}±89.01	229.02^{d}±12.30	97.06^{bc}±8.91	252.30^{g}±8.96
	150			91.20^{dc}±9.82	328.22^{h}±6.37
	300	519.83^{f}±46.30	1 073.66^{e}±99.30		

Notes: All the values are mean of triplicates±SE. ANOVA significant at $p \leqslant 0.05$. Different letters indicate significantly different values at a particular duration (DMRT, $p \leqslant 0.05$).

3.2 *Effect of metals on quantum yield* ($\Delta F/F'_{\mathrm{m}}$)

Zinc exposure resulted in an obvious change to $\Delta F/F'_{\mathrm{m}}$. It rapidly declined in all treatments during the initial 4-h period. After 10-h exposure, the quantum yield continued to decline only under high concentrations of Zn treatment (Fig. 1, Zn).

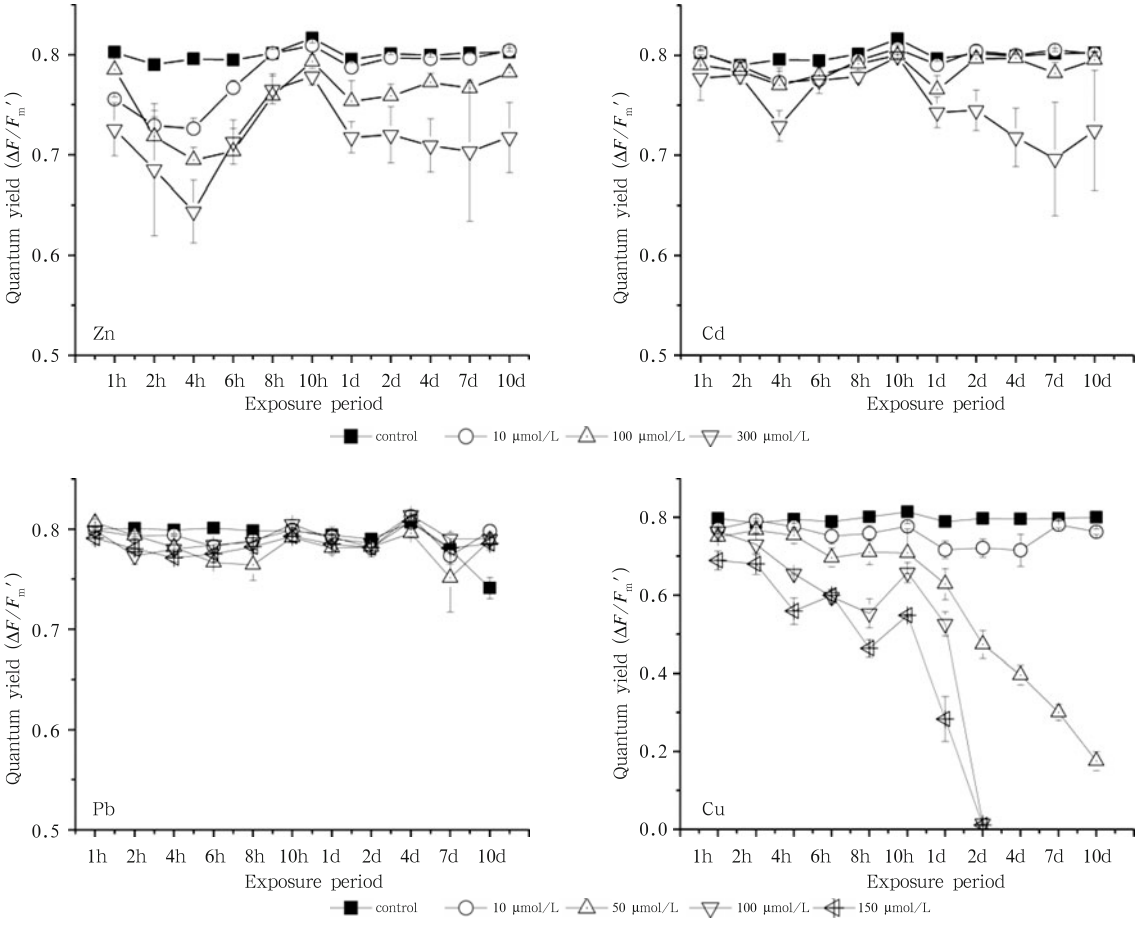

Fig.1. Effective quantum yields ($\Delta F/F'_{\mathrm{m}}$) for *T. hemprichii* over a 10-d exposure period to Zn, Cd, Pb and Cu. Error bars indicate the mean of triplicates±SE.

T. hemprichii showed a limited photosynthetic stress when exposed to Cd (10–300 μmol/L) for 10 d. The $\Delta F/F'_{\mathrm{m}}$ for all three Cd treatments declined during the initial 4-h period, with respect to the control response. However, at 10 and 100 μmol/L treatments, it remained relatively stable, and the strongest response was observed at the 300 μmol/L during the 10-d period (Fig. 1, Cd).

No significant effect on $\Delta F/F'_{\mathrm{m}}$ was observed when exposed to Pb (10 to 150 μmol/L) (Fig. 1, Pb).

Exposure to Cu, from concentration 10 to 150 μmol/L, had a lethal effect on *T. hemprichii*. $\Delta F/F'_{\mathrm{m}}$ demonstrated a moderate decline in all Cu concentrations in the initial 10 h. After 10-h exposure, the 10 μmol/L Cu treatment remained relatively stable during the next 9 d exposure, while the 100 and 150 μmol/L treated samples showed rapid decline in $\Delta F/F'_{\mathrm{m}}$. Leaves under 100 and 150 μmol/L treatments prematurely senesced from the second day and so fluorescence measurement could not be performed for those treatments (Fig. 1, Cu).

3.3 *Effect on photosynthetic pigments*

Photosynthetic pigment analysis of Zn treatment shows that Chl a and b content of all three treatments were lower than the control in the initial 24 h. After 2-d exposure, the Chl a and b content remained stable at 10 μmol/L, whilst at 100 and 300 μmol/L treatments continued declining (Figs 2a and b). All Zn treatments showed elevated carotenoid content and the 10 μmol/L treatment had the highest level, however, no significant difference was observed when compared with the control (Table 2).

Chl a and b content of all three Cd treatments declined rapidly during the initial 2-d exposure. After that, it remained stable for 10 and 100 μmol/L treatments, while at 300 μmol/L treatments it continued declining. After 7-d exposure, at 10 and 100 μmol/L treatments, it returned to the same levels as the controls (Figs 2c and d). Total carotenoid increased

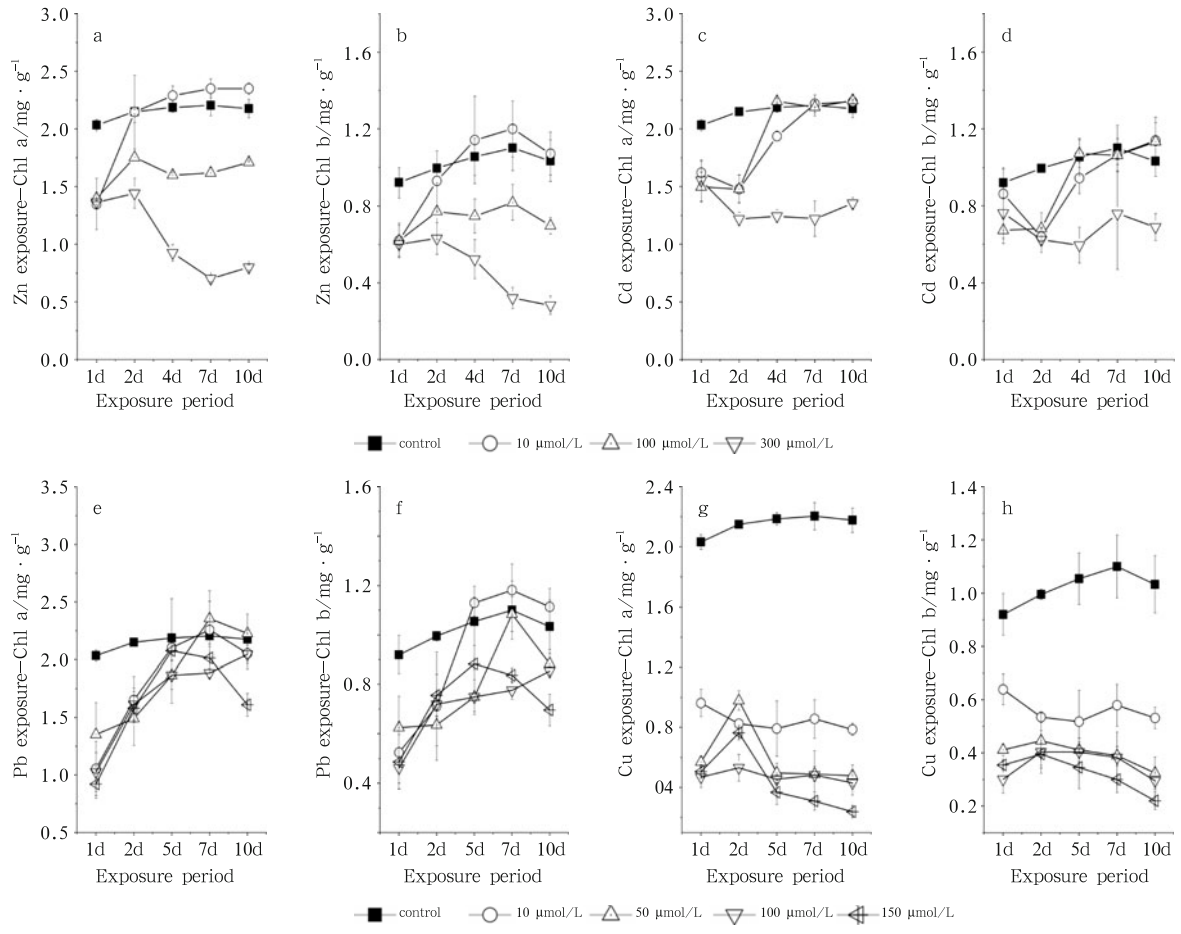

Fig.2. The content of chlorophyll (Chl a and Chl b) in the leaves of *T. hemprichii* exposed to different concentrations of four trace metals. Error bars indicate the mean of triplicates±SE.

Table 2. Total carotenoids concentrations (mg/g) in leaves of *T. hemprichii* treated with different concentrations of metals

Exposure Period/d	Zn			
	Control	10 μmol/L	100 μmol/L	300 μmol/L
1	$0.198^{a}\pm0.011$	$0.212^{a}\pm0.045$	$0.226^{a}\pm0.021$	$0.237^{a}\pm0.007$
2	$0.194^{a}\pm0.017$	$0.343^{a}\pm0.051$	$0.305^{a}\pm0.067$	$0.294^{a}\pm0.009$
4	$0.215^{a}\pm0.009$	$0.324^{a}\pm0.045$	$0.264^{a}\pm0.020$	$0.279^{a}\pm0.018$
7	$0.200^{a}\pm0.017$	$0.225^{a}\pm0.020$	$0.283^{a}\pm0.014$	$0.242^{a}\pm0.008$
10	$0.245^{a}\pm0.045$	$0.281^{a}\pm0.016$	$0.285^{a}\pm0.003$	$0.254^{a}\pm0.006$
	Cd			
1	$0.195^{a}\pm0.032$	$0.196^{a}\pm0.010$	$0.236^{a}\pm0.028$	$0.260^{a}\pm0.046$
2	$0.191^{a}\pm0.014$	$0.268^{b}\pm0.016$	$0.285^{b}\pm0.013$	$0.186^{a}\pm0.013$
4	$0.168^{a}\pm0.015$	$0.300^{bc}\pm0.011$	$0.374^{c}\pm0.014$	$0.181^{ab}\pm0.013$
7	$0.162^{a}\pm0.014$	$0.165^{a}\pm0.003$	$0.186^{a}\pm0.011$	$0.222^{a}\pm0.044$
10	$0.186^{a}\pm0.024$	$0.248^{a}\pm0.011$	$0.306^{a}\pm0.013$	$0.324^{b}\pm0.004$
	Pb			
	Control	10 μmol/L	100 μmol/L	150 μmol/L
1	$0.192^{a}\pm0.030$	$0.197^{a}\pm0.050$	$0.204^{a}\pm0.016$	$0.116^{a}\pm0.006$
2	$0.266^{a}\pm0.048$	$0.280^{a}\pm0.036$	$0.289^{a}\pm0.028$	$0.345^{a}\pm0.010$
5	$0.278^{a}\pm0.039$	$0.270^{a}\pm0.011$	$0.284^{a}\pm0.025$	$0.327^{a}\pm0.071$
7	$0.300^{a}\pm0.055$	$0.323^{a}\pm0.028$	$0.301^{a}\pm0.005$	$0.350^{a}\pm0.005$
10	$0.280^{a}\pm0.036$	$0.336^{a}\pm0.078$	$0.325^{a}\pm0.030$	$0.270^{a}\pm0.002$
	Cu			
1	$0.176^{a}\pm0.031$	$0.075^{b}\pm0.008$	$0.037^{b}\pm0.009$	$0.029^{b}\pm0.005$
2	$0.156^{a}\pm0.022$	$0.068^{b}\pm0.008$	$0.016^{bc}\pm0.003$	$0.006^{c}\pm0.004$
5	$0.181^{a}\pm0.018$	$0.047^{b}\pm0.011$	$0.001^{b}\pm0.001$	$0.000^{b}\pm0.000$
7	$0.197^{a}\pm0.020$	$0.072^{b}\pm0.018$	$0.000^{c}\pm0.000$	$0.000^{c}\pm0.000$
10	$0.164^{a}\pm0.007$	$0.080^{b}\pm0.016$	$0.000^{c}\pm0.000$	$0.000^{c}\pm0.000$

Notes: All the values are mean of triplicates±SE. ANOVA significant at $p \leqslant 0.05$. Different letters indicate significantly different values at a particular duration (DMRT, $p \leqslant 0.05$).

under all Cd treatments as compared with the control and significant increase was detected under the 10 and 100 μmol/L treatments during the 2-4 d exposure period (Table 2).

Lead treatment from 10 to 150 μmol/L had an obvious effect on chlorophyll in the initial 1-d exposure. And then Chl a and b stably increased from the 2nd to 5th day, and they decreased in the last three days (Figs 2e and f). Carotenoid content was also increased but no significant effect was observed (Table 2).

Exposure of the experimental material from 10 to 150 μmol/L of Cu had a severe effect on photosynthetic pigments. Chl a and b declined rapidly at 50, 100 and 150 μmol/L during the initial 24 h. After 2-d period, all treatments had a stable decrease and a significantly lower level than the control was detected (Figs 2g and h). All Cu treatments had a significantly lower content of carotenoids than the control after 24-h exposure (Table 2).

3.4 *Response of antioxidant enzymes*

Different concentrations of Zn and Cd treatments (from 10 to 300 μmol/L) caused an increase of the endogenous SOD activity in *T. hemprichii* leaves during 10-d period (Fig. 3, Zn and Cd). Under Zn treatment at 100 and 300 μmol/L, SOD activity was significantly higher than control during 1 to 10 d while at 10 μmol/L significant increase was observed only after the 7th d. Meanwhile, significant increase was detected under all Cd treatments from the 4th d and the highest response was observed after 10-d exposure as compared with the control.

Under Pb and Cu treatments (from 10 to 150 μmol/L), SOD activity in *T. hemprichii* leaves exhibited a different response as compared with Zn and Cd. Under Pb treatments, SOD activity increased in the initial two days. After 2-d exposure, SOD activity rapidly decreased. However, during all exposure period, SOD activity was lower than the control and significantly lower activity was observed from 5 to10 d. Under Cu stress, significant increase of SOD activity was observed during 2-d exposure, and then a rapid decrease was observed during the following eight days, of which, significant decrease was observed during 7 to 10 d (Fig. 3, Pb and Cu).

POD activity in *T. hemprichii* leaves was higher

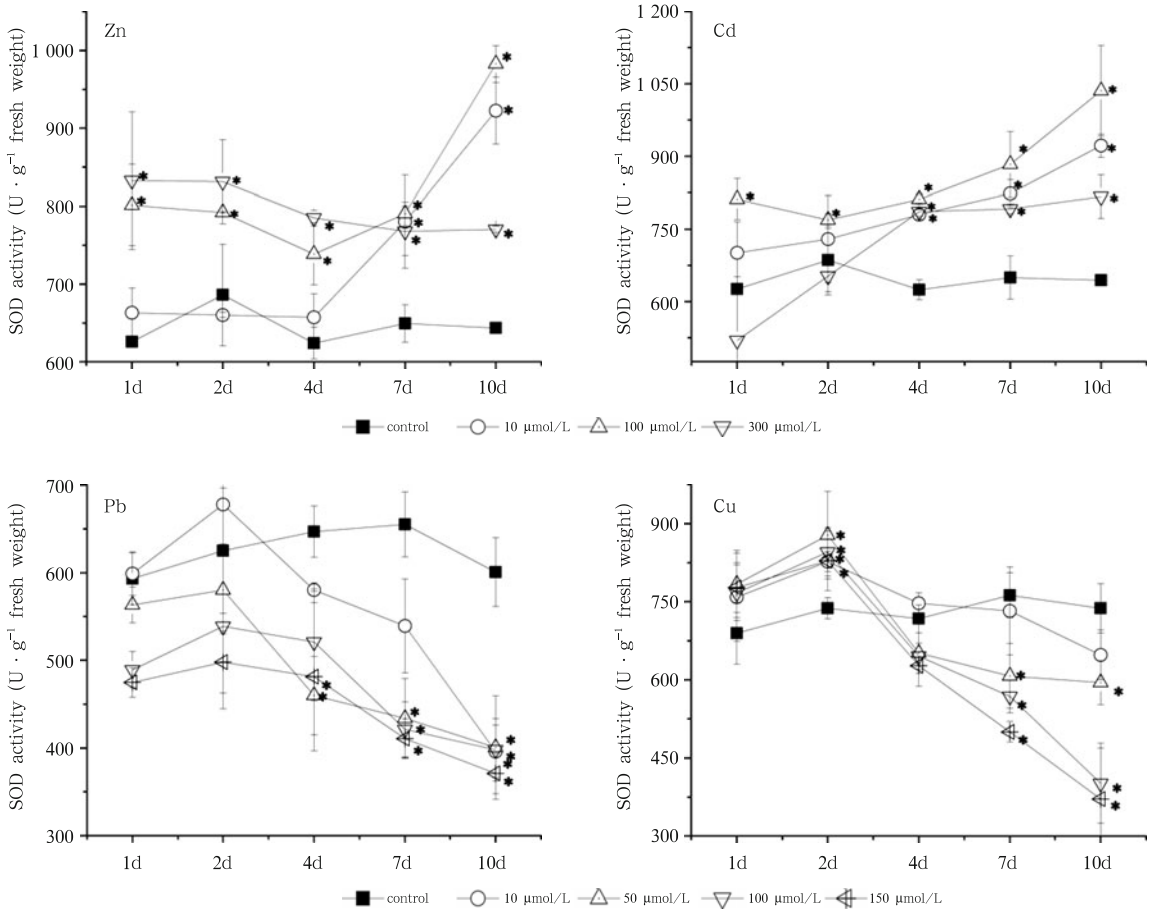

Fig.3. Effect of different concentrations of Zn, Cd, Pb and Cu on activity of superoxide dismutase (SOD) in leaves of *T. hemprichii*. Error bars indicate the mean of triplicates±SE. Asterisks (*) indicate that mean values are significantly different between metals treatments and controls (p <0.05).

than the control under all treatments of Zn, Cd and Pb (5 to 10 d). Significantly higher POD activity was observed under Zn and Cd treatments after 2-d exposure. Meanwhile, all Pb treatments showed significantly elevated POD activity during 7 to 10 d as compared with the control (Fig. 4, Zn, Cd and Pb). Besides, a very different response of POD activity under Cu treatments was detected. It had already been restrained even at 10 μmol/L during the initial hours. In the following 9 d, POD activity of all treatments was lower than control (Fig. 4, Cu).

4 Discussion

T. hemprichii accumulated high amount of Zn, Cd, Pb and Cu depending on the concentration and duration. The uptake of four metals was proportional with its concentration in the aquarium and this coincided with earlier studies obtained by Zn, Cd, Pb and Cu treatments (Catalado et al., 1983; Caspi et al., 2000; MacFarlane and Burchett, 2001; Liu et al., 2010). This study shows that Zn, Cd, Pb and Cu are preferentially accumulated in aboveground tissues (blades) of *T. hemprichii*. The previous study reported that Cd and Pb were preferentially accumulated in blades of *P. oceanica* (Lafabrie et al., 2008). This result allows us to collect the samples (the blades) for the metal concentration analyses without removing the entire *T. hemprichii* plants in future studies.

Among all chlorophyll a fluorescence parameters, quantum yield was considered as the most sensitive parameter for measuring metal-induced stress (Ralph and Burchett, 1998). Estimation of $\Delta F/F'_{\mathrm{m}}$ is an indicator to detect responses of metal stress within 1 h of exposure. In the present study, a rapid decline of $\Delta F/F'_{\mathrm{m}}$ was observed during four hours of Cu and Zn exposure in all concentrations. Especially, under high concentrations of Cu treatments, a rapid decline of

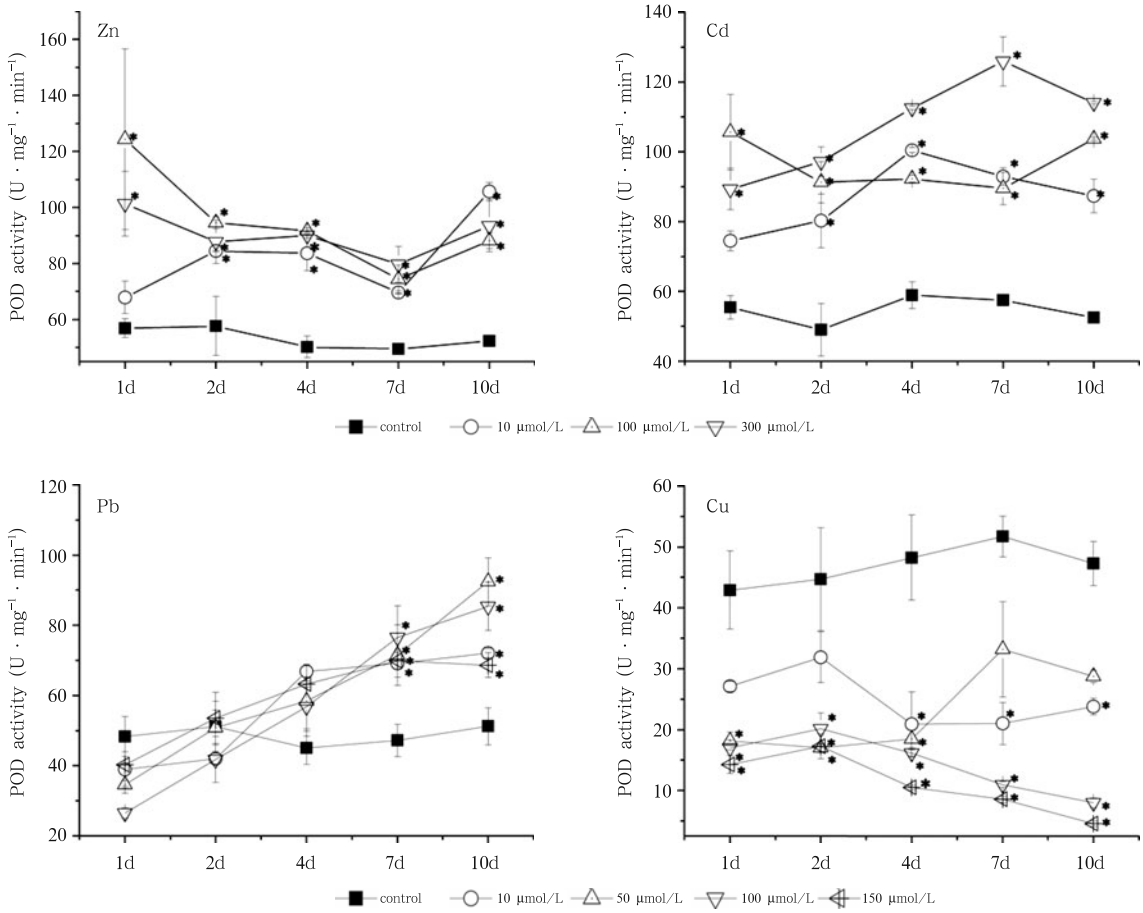

Fig.4. Effect of different concentrations of Zn, Cd, Pb and Cu on activity of guaiacol peroxidase (POD) in leaves of *T. hemprichii*. Error bars indicate the mean of triplicates±SE. Asterisks (*) indicate that mean values are significantly different between metals treatments and controls (p <0.05).

$\Delta F/F'_{\mathrm{m}}$ was continued during 2 to 10 d exposure. This indicates that Cu was the most toxic metal tested. Our result consists with the previous studies in *Zostera capricorni* exposed to different concentrations of metals (Macinnis-Ng and Ralph, 2002). Although Cu is an essential microelement and a constituent of plastocyanins and plays important roles in electron transport system (Prasad and Strzalka, 1999), high concentration of Cu can attributed to an overall inhibition of PS II, due to the alterations in the chloroplast membrane ultrastructure, resulting in the K^+ and Cu^{2+} leakage into the chloroplast (Ouzounidou, 1994). In this study, $\Delta F/F'_{\mathrm{m}}$ remained stable after 2-d Zn exposure, so Zn has a less phytotoxic effect on *T. hemprichii* than Cu. Zinc may interact with the donor side of PS II and inhibit photosynthetic CO_2 fixation and O_2 release in the Hill reaction (Prasad and Strzalka, 1999). Cadmium and lead as nonessential elements displayed only limited effect on $\Delta F/F'_{\mathrm{m}}$ especially in the initial 24-h period in this study. Seagrass may actively exclude or sequester Cd and Pb into structural components of the leaf tissues (e.g., vacuole), therefore preventing them from affecting the more sensitive and important photosynthetic metabolic processes (Ward, 1989). In addition, the lower solubilities of Cd and Pb may lead to greater chelation and adsorption by colloidal particles in seawater.

As a visible symptom, the reduced chlorophyll content can be used to monitor metals induced damage in seagrass leaves. In the present study, the levels of Chl a and Chl b decreased and to a lesser degree carotenoids increased in a dose-dependant manner with increasing exposure levels of Zn, Cd and Cu. This indicates that Zn, Cd and Cu stress could significantly reduce chlorophyll contents. The previous studies reported that excess Zn, Cd and Cu could damage chloroplast, induce the loss of chlorophyll, cause chloroplast disorganization and reduce the number of

thylakoid and grana (Ebbs and Kochian, 1997). In addition, high concentrations of Zn, Cd and Cu could inhibit the uptake of nutrient elements through competition for binding sites (Wang et al., 2009). These nutrient elements are normally involved in many important biological processes. For example, Mg is an important component of chlorophyll. Iron and Manganese play important roles in synthesis or stability of chlorophyll (Marschner, 1995). Carotenoid, a non-enzymatic antioxidant, apart from functioning as accessory light harvesting photosynthetic pigments, plays an important role in the protection of chlorophyll pigments under metals stress by quenching photodynamic reactions and replacing peroxidation (Sinha et al., 2010). The previous studies reported increase (Drążkiewicz and Baszyński, 2005), decrease (Huang and Wang, 2010) or no change (Mishra et al., 2006b) in the content of carotenoid in plants under different concentrations of various metal stresses. In this study, carotenoid was significantly increased under low Cd concentration treatment but decreased when treated with high concentrations of Cd and all concentrations of Cu. The lowest carotenoid content observed under Cu treatment in this study may add to the stress-induced damage of Cu to seagrass leaves. Because this could lead to a diminished capacity to protect photosystems in leaves against photooxidation and resulted in more oxidative stress and senescence of leaves (Di Toppi et al., 2009). Lead was reported to be less damaging to the photosynthetic apparatus, and no significant inhibitory effect of Pb on photosynthetic pigments was observed after long term exposure especially under low Pb treatments (<50 μmol/L) in this study which was consistent with the previous study (Ahmed and Tajmir-Riahi, 1993).

Apart from damage to chloroplast and inducing the loss of chlorophyll, the excessive uptake of trace metals had also been reported to interfere in cellular metabolism through production of ROS by autooxidation and Fenton reaction (Schützendübel and Polle, 2002). ROS can lead to the response of plants to oxidative stress, including enhancement or suppression of activities of antioxidative enzymes (Apel and Hirt, 2004). SOD acts as the first line of defense against ROS, and catalyses $O_2^{\cdot-}$ to H_2O_2. In the present study, SOD activity was higher than the control under all Zn and Cd treatments during the whole experiment while it decreased and was much lower than the control after long-term exposure to Pb and Cu. This suggests that SOD in *T. hemprichii* is more tolerant to Zn and Cd than Cu and Pb. The increased SOD activities in leaves of *T. hemprichii* peaked at high concentration of Zn and Cd exposure indicated that SOD played an important role in protecting seagrass against Zn and Cd stress. Enhanced SOD activity was also reported in leaves of *T. caerulescens* under Zn and Cd stress (Wójcik et al., 2006). The decreased SOD activities in the present study after long term exposure to high concentrations of Cu and Pb consisted with the report by Mishra et al. (2006a). The reason for the decrease might be inactivation of SOD by H_2O_2 or binding of metals to active center of the enzyme (Fatima and Ahmad, 2005). POD is another principal enzyme involved in the elimination of ROS, and is mainly located in cytoplasm, vacuole, membrane and cell wall. It is considered as stress marker enzyme having broad specificity for phenolic compounds and higher affinity for H_2O_2 than CAT. The previous researches also reported that an increase in the level of metals could bring with it an increase in enzyme activities, which could be used as environmental biomarkers (Pergent-Martini and Pergent, 2000). POD can break down H_2O_2 into H_2O. The previous study reported that POD activity in *Hydrilla verticillata* (L.f.) Royle increased till 7 d at 25 μmol/L Cu exposure (Srivastava et al., 2006). In this study, POD activity increased under Zn, Cd and Pb stress but decreased under Cu stress as compared with the control. The decreased POD activities also suggest that *T. hemprichii* is very sensitive to Cu and is very easy to be severely damaged by Cu stress. Meanwhile, decreased POD activities of *T. hemprichii* under Cu stress might result in accumulation of H_2O_2 which could cause disorder of metabolism including low photosynthesis efficiency and the decreased content of protein and sugar or even death of plants (Cao et al., 2004). Elevated POD activity in *T. hemprichii* under Zn, Cd and Pb stress might contribute to enhancing the ability to cope with metal stress with a stiffened cell wall by producing phenoxy compounds that are polymerized to produce lignin (Reddy et al., 2005).

Finally we concluded that *T. hemprichii* was very sensitive to Cu: the photosynthetic metabolic organ could be severely damaged and the plant might even die. *T. hemprichii* had positive response by increasing the activity of SOD and POD. Especially, POD was the most important ROS scavenging enzyme in protecting seagrass from metals stress, and the antioxidative protection mechanisms of *T. hemprichii* were more efficiently activated to avoid damage of Zn, Cd

and Pb stress. Besides, the seagrass *T. hemprichii*, in view of its high accumulation potential of Cd, high efficiency of avoiding damage to the photosynthetic systems of leaves and high activities of SOD and POD induced by Cd, seems to be suitable candidate to decontaminate inshore sea areas moderately contaminated with Cd. Although metals concentrations in this study might be higher than the bioavailable metals in seagrass ecosystems, the information presented might help us understand the physiological detoxification strategies that seagrasses adapt to metals stress.

Acknowledgements

The authors wish to thank Mr. Zhang Jingping, Jiang Zhijian, Peng Bo and Miss Fan Minling for sampling collections. We also would like to thank the editors and reviewers for comments and suggestion to the manuscript.

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