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RESEARCH ARTICLE

Effects on *Eichhornia crassipes* under Zn stress

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Abstract

Eichhornia crassipes is a macrophyte widely used in phytoremediation, demonstrating a high ability to remove metals from water. The aim of this work was to evaluate its enzymatic detoxification strategies and metal accumulation when it is exposed to different Zn concentrations (0, 2, 4, 6, and 9 ppm) for periods of 24, 48, and 72 h. Zn concentration in roots was significantly higher than in aerial parts. Independently of the treatment, in the first 48 h, concentrations of photosynthetic pigments were not affected. However, a significant increase (between 19 and 34%) in Chl-*b* concentrations for all treatments was observed at 72 h. Carotenoid concentration was not affected during the first 48 h, while at 72 h, there was a significant increase regarding the control (between 11 and 24%) for all treatments. Malondialdehyde concentration in aerial parts and roots was not affected during the experiment. Nonetheless, a significant increase in the enzymatic activity of the antioxidant system was observed. Results suggest that Zn could have potential antioxidant properties, which may result in the activation of different antioxidant enzymes involved in the protection against metal stress.

Keywords Macrophytes · Metals · Plant stress · ROS scavenging · Lipid peroxidation

Introduction

Zn is one of the most essential micronutrients for plant growth (Broadley et al. 2007). It constitutes part of metalloenzymes and is a cofactor of various enzymes as anhydrases, dehydrogenases, oxidases, and peroxidases (Hewitt 1983). In addition, it contributes to the regulation of nitrogen metabolism, cell multiplication, photosynthesis, and auxin synthesis (Shier 1994). Zn deficiency has been reported as a stimulant of increased membrane permeability and exudation of metabolites (Cakmak 2000; Cakmak and Marschner 1988; Li et al. 2013), suggesting an important function in membrane stabilization products. However, Zn can be toxic to plants when concentrations exceed thresholds, reducing rooting and photosynthetic capacity and causing the chlorosis of leaves (Tewari et al. 2008; Cambrollé et al. 2012). Furthermore, Zn stress increases the generation of reactive oxygen species (ROS), which are highly reactive molecules that interact with various cellular components leading to oxidative damage, causing lipid peroxidation, and altering antioxidant enzyme activity in plants (Artetxe et al. 2002; Tripathi and Gaur 2004). Nevertheless, Zn plays a dual role in the ROS-induced oxidative stress in plants. Zn excess leads to ROS production, while Zn is also an important cofactor of superoxide dismutase (SOD), which catalyzes the dismutation of superoxide radical. SOD is an enzyme of the complex enzymatic antioxidant system which includes guaiacol peroxidase (POD), catalase (CAT), and glutathione reductase (GR), among others.

A number of studies have investigated Zn uptake, accumulation, distribution, and detoxification in terrestrial plant species as *Kandelia obovate* (Hu and Wenjiao 2015),

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Phyllostachys pubescens (Peng et al. 2015) and *Cistus monspeliensis* (Arenas-Lago et al. 2016), and aquatic species such as *Hydrilla verticillata* (Srivastava and Shrivastava 2016; Wang et al. 2009; Xu et al. 2013) and *Lemna minor* (Radić et al. 2010). However, studies on the physiological and biochemical changes in *Eichhornia crassipes* due to a Zn exposure have not been reported. *E. crassipes* can adapt easily to different environmental conditions and is highly efficient for the accumulation of metals from water (González et al. 2015a, b; Hadad et al. 2009, 2011; Módenes et al. 2013). The aim of this study was to assess the enzymatic Zn detoxification strategies and the metal accumulation in *E. crassipes* tissues. This species was chosen since it was the dominant floating macrophyte in a wetland constructed for the effluent treatment at a metallurgical industry (Maine et al. 2009). Zn was studied for being one of the contaminants found in the treated effluents at this constructed wetland.

Materials and methods

Plant material and growth conditions

E. crassipes plants and water were collected from an unpolluted pond of the Middle Paraná River floodplain. Collected plants were acclimated for 7 days in the laboratory under controlled conditions, temperature of 23 ± 2 °C, light intensity of 1400 μmol photons m^{-2} s^{-1} from natural sunlight at 11 h/13 h day/night cycle, and relative humidity of 54 ± 10%. After acclimation, only young healthy plants of a uniform size and similar fresh weight (30–40 g) were selected for the experiment. One plant and 2 L of pond water were disposed in plastic experimental pots.

The used pond water showed the following chemical composition (mean ± standard deviation): pH = 8.0 ± 0.2, conductivity = 122 ± 1 μS cm^{-1}; dissolved oxygen = 7.7 ± 0.10 mg l^{-1}; soluble reactive phosphorus = 0.033 ± 0.002 mg l^{-1}; N-NH_4^+ = 0.551 ± 0.019 mg l^{-1}; N-NO_3^- = 0.649 ± 0.005 mg l^{-1}; N-NO_2^- = 0.007 ± 0.001 mg l^{-1}; Ca^{2+} = 10.5 ± 0.8 mg l^{-1}; Mg^{2+} = 3.5 ± 0.5 mg l^{-1}; Na^+ = 13.1 ± 1.0 mg l^{-1}; K^+ = 3.50 ± 0.5 mg l^{-1}; Cl^- = 10.4 ± 1.3 mg l^{-1}; SO_4^{2-} = 7.8 ± 1.8 mg l^{-1}; HCO_3^- = 52.3 ± 0.8 mg l^{-1}, Fe = 4 μg l^{-1}, Zn = non-detected (detection limit = 5 μg l^{-1}).

Zn stock solutions were prepared by dissolving an appropriate amount of $ZnCl_2 \cdot 6H_2O$ (99.9% Sigma-Aldrich, China) in deionized water. Then, dilutions were made to obtain concentrations of 2, 4, 6, and 9 mg l^{-1}. These concentrations were chosen based on the results of previous works that studied the tolerance of *E. crassipes* exposed to different metals (González et al. 2015a, b; Hadad et al. 2011). The experiment was conducted by triplicate over 3 days, taking samples after 24, 48, and 72 h. A control without Zn addition was used.

At the end of the experiment, plants were collected, washed with distilled water, air-dried, and separated in aerial parts and roots. Then, samples were frozen in liquid nitrogen for storage at − 80 °C.

Metal accumulation

Plant samples were dried, ground, and digested with a HNO_3/HCl mixture (USEPA 1994) and analyzed with atomic absorption spectrophotometer (Perkin Elmer AAnalyst 200). Translocation factor (TF) was calculated as the ratio of Zn concentrations between aerial parts and roots (Baker and Brooks 1989).

Measurement of photosynthetic pigments

Measurement of photosynthetic pigments were carried out as previously reported by González et al. (2015a). Photosynthetic pigments were reported as chlorophyll *a* (Chl-*a*), chlorophyll *b* (Chl-*b*), and carotenoids and calculated according to Wellburn (1994).

Enzyme extraction

Enzyme extracts from aerial parts and roots were carried out as previously reported by González et al. (2015a). Total soluble protein was estimated according to Bradford (1976).

Antioxidant enzyme assays

Catalase (CAT), guaiacol peroxidase (POD), superoxide dismutase (SOD), and glutathione reductase (GR) total activities were assayed as described by González et al. (2015a).

Estimation of lipid peroxidation

For the estimation of lipid peroxidation, the method of Heath and Packer (1968) was employed. It was measured in terms of malondialdehyde (MDA) concentration. It was calculated according to Hodges et al. (1999).

Statistical analysis

All assays and measurements were performed in triplicate. The results are expressed as mean ± standard deviation. Analysis of variance (ANOVA) was applied. The assumptions of normality and homoscedasticity were verified. Duncan's multiple range test was used to determinate the significant differences among treatments. Differences at $p < 0.05$ were considered significant.

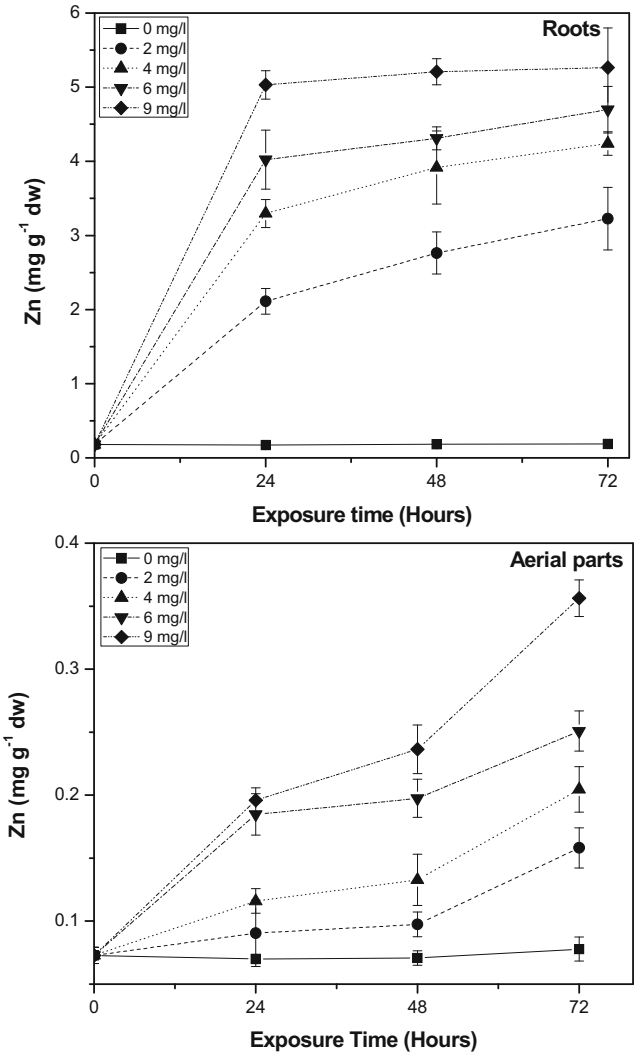

Fig. 1 Zn accumulation in aerial parts and roots of *E. crassipes* at different concentrations and exposure times. All values are mean of triplicates ± SD

Results

Metal accumulation

Zn concentration increased in aerial parts and roots both in terms of exposure times and initial metal concentration in water (Fig. 1). The highest Zn concentrations were observed in roots reaching concentrations 50 times higher than in aerial parts. In aerial parts, the highest Zn accumulation was observed during the first 24 h for all treatments. While at 48 h,

Table 1 Translocation factors for Zn accumulation in plants

Zn concentrations (mg l^{-1})	Exposure time (h)		
	24	48	72
2	0.043	0.035	0.049
4	0.035	0.034	0.048
6	0.045	0.044	0.047
9	0.039	0.045	0.067

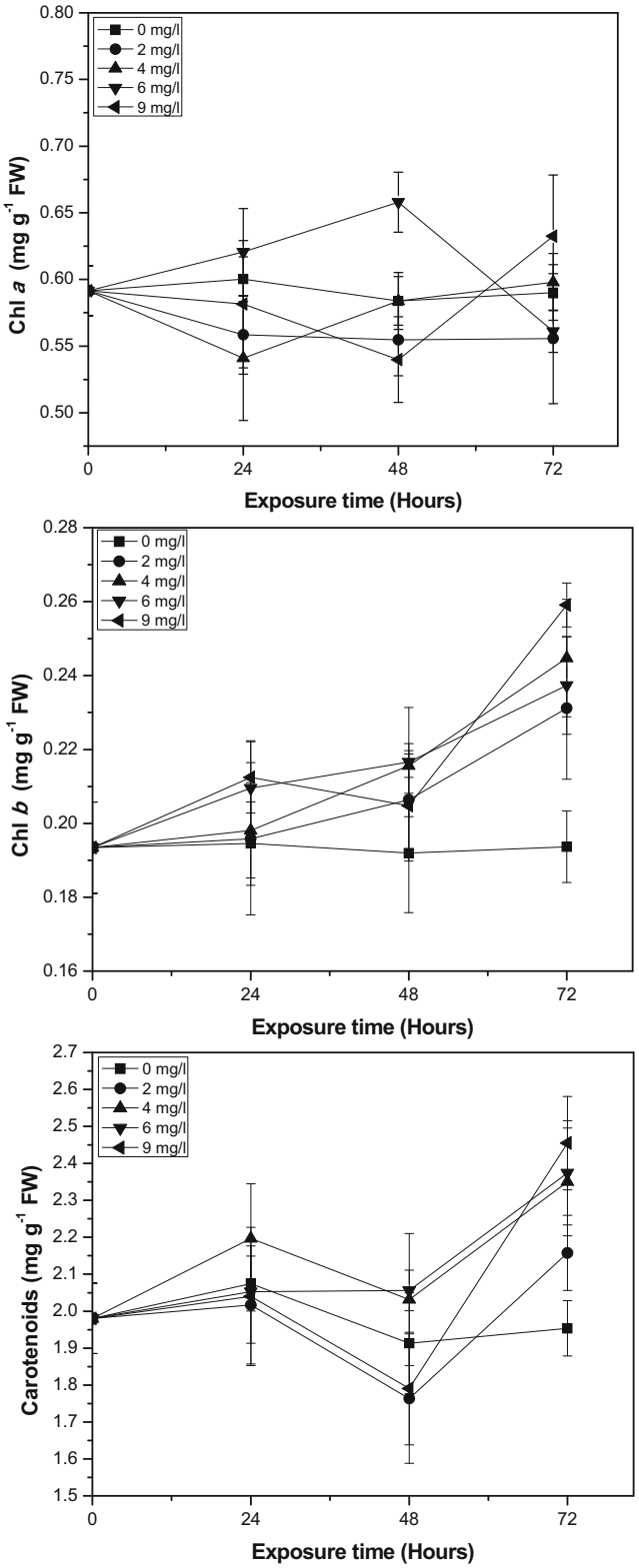

Fig. 2 Effects of Zn on photosynthetic pigments in the leaves of *E. crassipes*. All values are mean of triplicates ± SD

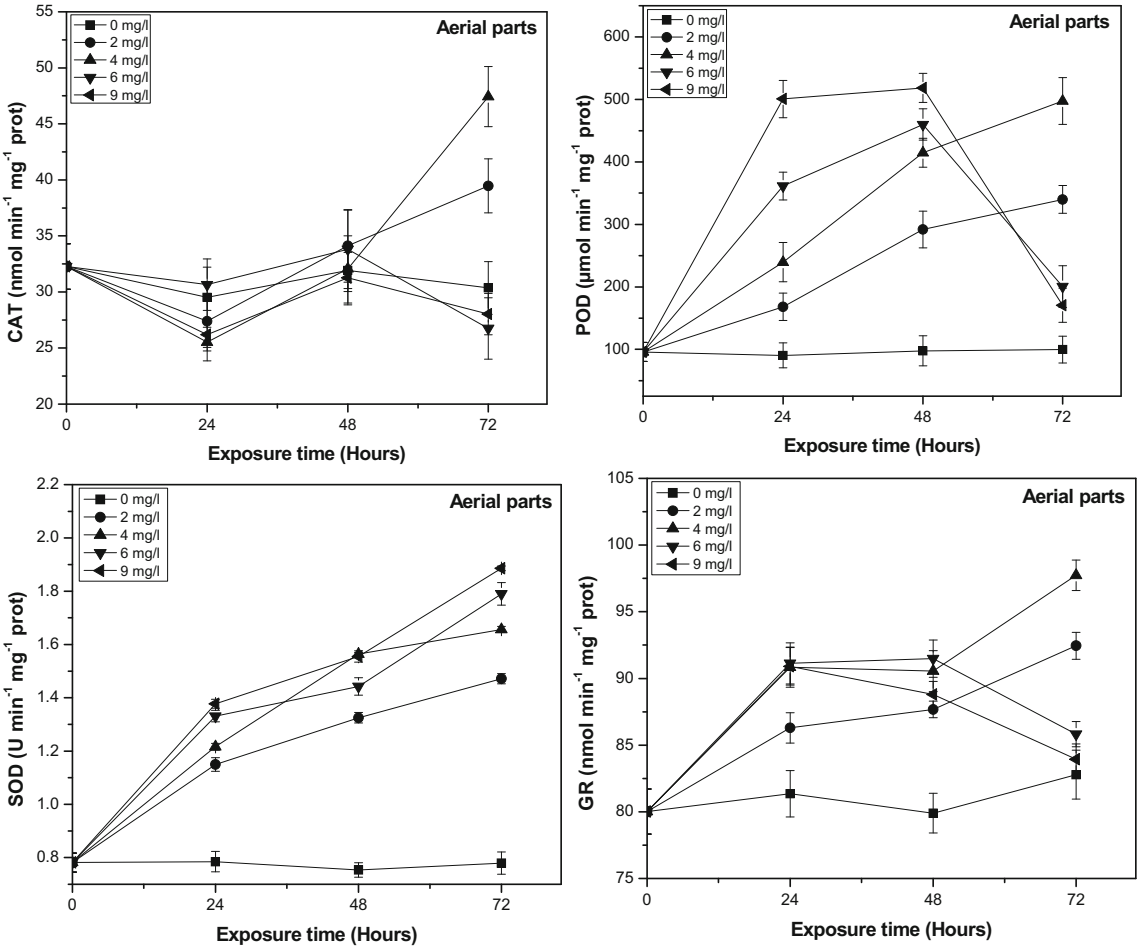

Fig. 3 Effects of Zn on antioxidant enzyme activities of *E. crassipes*, in aerial parts. All values are mean of triplicates ± SD

there were no significant changes in accumulation, at 72 h, a significant increase was observed. In roots, the highest Zn accumulation occurred in the first 24 h for all treatments, and no further increase was detected over time for the two highest Zn concentrations. According to the TFs calculated (between 0.03 and 0.06), Zn was not translocated to aerial parts (Table 1).

Effects of Zn on photosynthetic pigments

No significant differences were observed in Chl-*a* concentrations during treatments, except for exposure to 6 mg l^{-1} Zn at 48 h (11% of increase) (Fig. 2). On the other hand, no significant differences were observed in Chl-*b* concentrations at 24 and 48 h compared to the control. However, a significant increase (between 19 and 34%) in Chl-*b* concentrations for all treatments was observed at 72 h. Carotenoid concentration was not affected during the first 48 h, while at 72 h, there was a significant increase regarding the control (between 11 and 24%) for all treatments.

Effects of Zn on antioxidant enzyme activity

Antioxidant enzyme activity was affected by Zn exposure both in roots and in aerial parts (Figs. 3 and 4). In aerial parts (Fig. 3), CAT activity was not significantly different from the control during the first 48 h. However, at 72 h, enzyme activities showed a significant increase (between 22 and 47%) for the two lowest Zn concentrations. This was not the case for the two highest Zn concentrations, which showed no significant differences compared to the control. A significant increase in POD activity (between 75 and 440%) was observed in all Zn treatments and exposure times, compared to the control. It is important to note that, for the two highest Zn concentrations at 72 h, a decrease in POD activity was observed compared to the previous samplings, whereas for the two lowest Zn concentrations, POD activities remained increasing over time. SOD activity in aerial parts increased significantly (between 47 and 141%) for all Zn treatments and exposure times studied. A significant increase was observed in GR activities during the first 48 h for all treatments, compared to the control, whereas at 72 h for the two lowest Zn concentrations, a

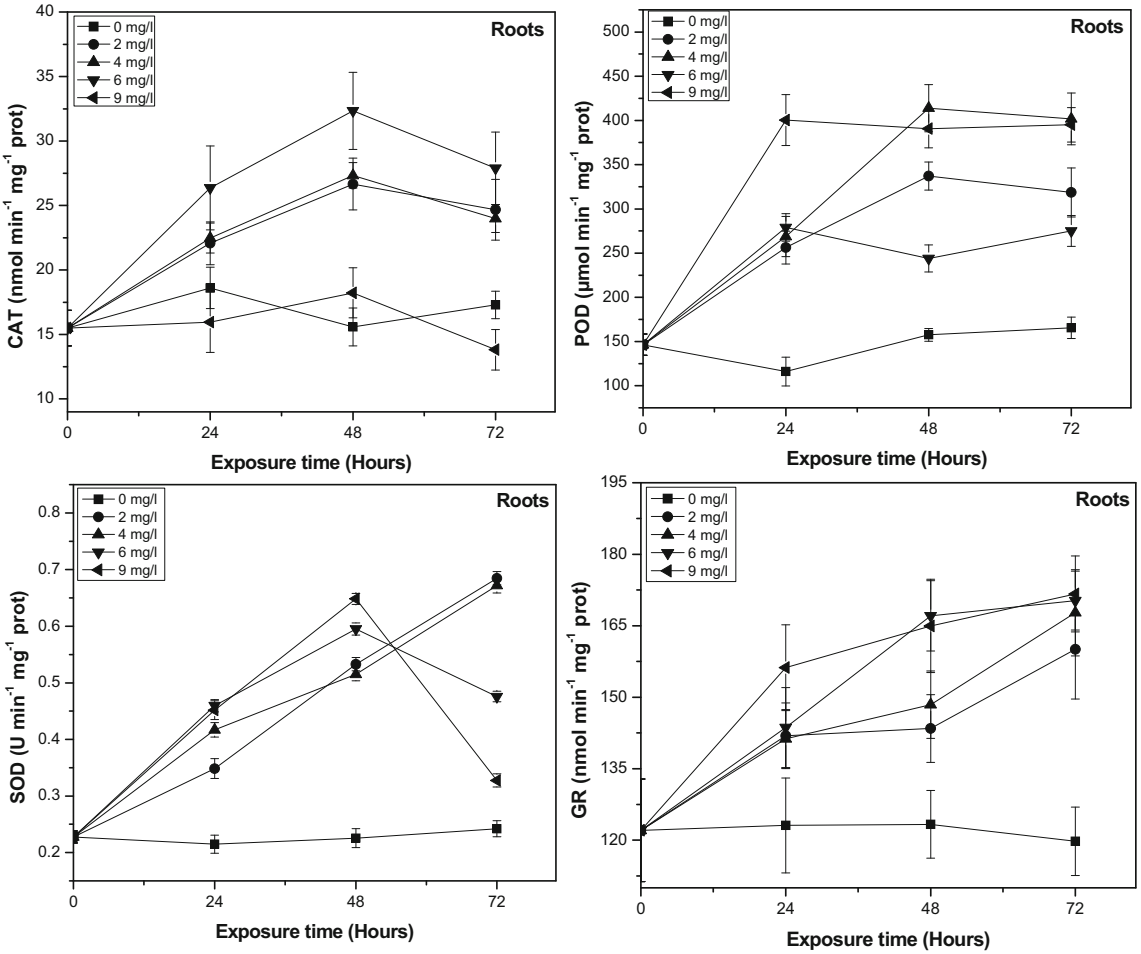

Fig. 4 Effects of Zn on antioxidant enzyme activities of *E. crassipes*, in roots. All values are mean of triplicates ± SD

significant increase (15 and 22%) in GR activity was observed. No significant differences were recorded for the two highest Zn concentrations.

In roots (Fig. 4), CAT activity increased significantly (between 42 and 108%) for 2, 4, and 6 mg l^{-1} Zn treatments and all exposure times, while it showed a significant decrease in their activity in 9 mg l^{-1} Zn treatment at 72 h. POD activity was significantly higher (between 75 and 182%) for all Zn concentrations and exposure times compared to the control. SOD activity increased significantly (between 44 and 200%) for all treatments and exposure times compared to the control until 48 h. While SOD activities continued increasing over time for the two lowest Zn concentrations, it was observed that, for the two highest Zn concentrations, SOD activities showed a decrease at 72 h. GR activity increased significantly (between 15 and 40%) for all treatments and exposure times.

Effects of Zn on lipid peroxidation

Zn effects on MDA concentration are showed in Fig. 5. In aerial parts, no significant differences in the MDA concentrations regarding the control were observed during the first 2 days of exposure, except for 9 mg l^{-1} Zn treatment at 48 h, where a significant increase (15%) was observed. At 72 h for the two highest Zn concentrations, a significant decrease (25 and 17%) in MDA concentrations was observed. In roots, MDA concentration was not affected by the treatments.

Discussion

E. crassipes showed significantly higher Zn concentrations in roots than in aerial parts, with a scarce translocation, in agreement with literature (Deng et al. 2004; Hadad et al. 2011; Hasan et al. 2007; Miretzky et al. 2004; Mishra and Tripathi 2009; Yapoga et al. 2013). Poor Zn translocation could be due to metal binding to the root cell walls (Wainwright and Woolhouse 1977), forming metal complexes with soluble compounds (organic acids and amino acids) (Turner and Marshall 1972) or binding to specific proteins, thus contributing to the plant tolerance.

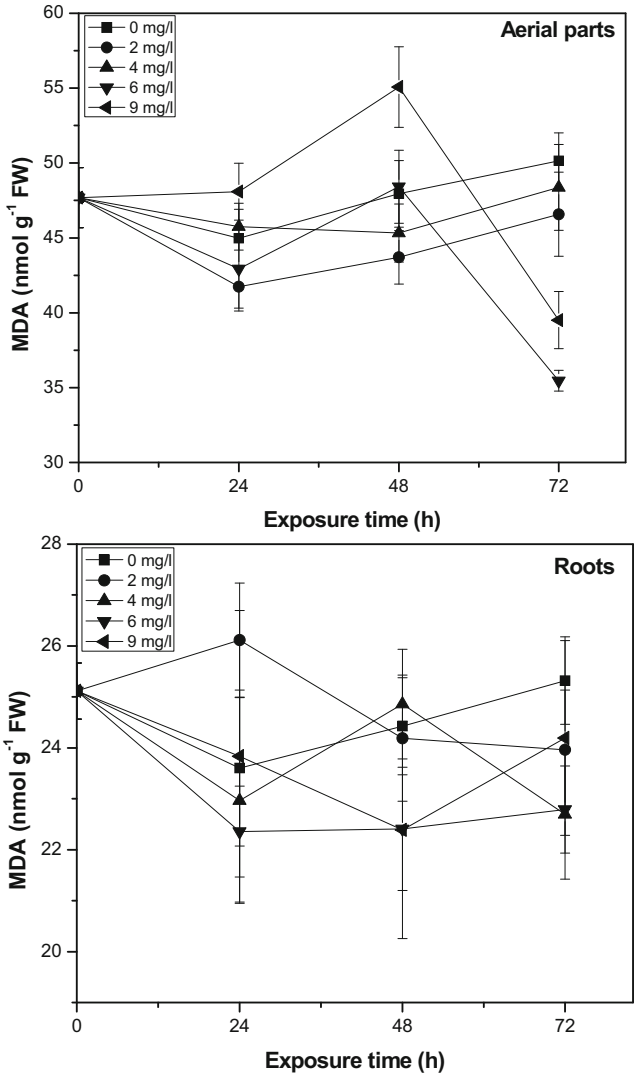

Fig. 5 Effects of Zn on lipid peroxidation of *E. crassipes*, in aerial parts and roots. All values are mean of triplicates ± SD

It has been observed that Zn preferentially is accumulated in chloroplasts (Van Assche and Clijsters 1986), which can interact directly with thylakoid membranes (Szalontai et al. 1999). This interaction can inhibit chlorophyll synthesis, causing a reduction in pigment concentrations. Mishra and Tripathi (2009) observed a decrease in chlorophyll concentration due to Zn accumulation in *E. crassipes*, after a period of 7 days of incubation. Hadad et al. (2011) reported a significant reduction in chlorophyll concentrations when *E. crassipes* was exposed to 1 mg l^{-1} Zn during 30 days. In our study, Zn concentrations did not affect photosynthetic pigment concentrations in the first 48 h of experiment. However, it is important to note that after 72 h, all Zn treatments showed a significant increase in Chl-*b* and carotenoids. This response may be due to the presence of Zn in aerial parts, generating ROS production and causing oxidative stress, as reported by other studies (Artetxe et al. 2002; Cuypers et al. 2001; Madhava Rao and Sresty 2000; Tewari et al. 2008). Therefore, considering that carotenoids are not only essential components of photosynthetic apparatus but also essentially protective against photooxidative damage, acting as free radical scavengers preventing lipid peroxidation (Hou et al. 2007), this response was expected. Wang et al. (2009) observed an increase in total chlorophyll concentration in leaves of *H. verticillata*, when exposed to 0.05 and 0.5 mg l^{-1} Zn for a period of 7 days. However, in the same experiment, a decrease in total chlorophyll concentration and necrotic symptoms were observed in leaves when exposed to concentrations higher than 10 mg l^{-1} Zn.

A Zn excess can generate oxidative stress by increasing ROS production, causing lipid peroxidation and altering antioxidant enzyme activity in terrestrial plants (Cuypers et al. 2001; Madhava Rao and Sresty 2000), aquatic organisms such as algae (Tripathi et al. 2006), and aquatic plants (Artetxe et al. 2002; Radić et al. 2010; Wang et al. 2009). In this context, oxidative stress produced by Zn excess increases lipid peroxidation and membrane permeability, reducing the content of sulfhydryls (Tripathi and Gaur 2004). In general, in our experiment, a significant increase in antioxidant enzyme activities was observed. Yuan et al. (2009) observed that SOD and CAT activities increased significantly in leaves of *Alternanthera philoxeroides*, exposed to Zn concentrations from 0.25 to 5 mM for a period of 5 days. Similar results were also observed in *H. verticillata* exposed to Zn concentrations from 5 to 30 mg l^{-1} for a period of 7 days (Wang et al. 2009). There are also reports of increased activity of antioxidant enzymes in terrestrial plants (Prasad et al. 1999; Tewari et al. 2008; Tripathi et al. 2006). This can be considered as a circumstantial evidence for an increase in free radical production under Zn stress in *E. crassipes*. However, the increase observed in antioxidant enzyme activities involved in the antioxidant system of *E. crassipes* favored the tolerance to Zn. This was reflected in lipid peroxidation, measured as MDA concentration, which evidenced no significant difference compared to the control for all treatments and exposure times in roots. In aerial parts, MDA concentrations did not change compared to the control during the first 48 h, highlighting a significant decrease for the two highest Zn concentrations. This duality has a positive effect on antioxidant enzyme activities and does not cause lipid peroxidation. This fact would allow us to affirm that Zn is a metal with potential antioxidant properties. Literature suggests that Zn plays an important role in protecting DNA and membranes from damage caused by reaction with ROS (Cakmak 2000). Finally, it has been demonstrated that Zn exposure may protect plants against oxidative stress induced by other heavy metals (Aravind and Prasad 2003, 2004, 2005; Aravind et al. 2009; Cherif et al. 2011; Tkalec et al. 2014).

Conclusions

- Zn accumulation was observed to be higher in roots than in aerial parts, where it occurred mainly during the first 24 h.
- Zn accumulation caused a significant increase in Chl-*b* and carotenoid at 72 h of treatment in *E. crassipes*. Such response was expected because these photosynthetic pigments have a protective character against photooxidative damage.
- An increase in MDA concentrations in aerial parts was observed only for 9 mg l^{-1} Zn at 48 h, while a decrease in MDA concentrations at 72 h was observed for the two highest Zn concentrations. In roots, MDA concentrations were not significantly affected.
- A rapid increase in antioxidant defense response, in aerial parts and roots, was observed in all Zn treatments. This response ensured the redox homeostasis.
- *E. crassipes* tolerated all Zn treatments during short exposure times by the stimulation of its antioxidant enzymatic defense system.
- Zn acted as a metal with potential antioxidant properties. Further work is needed to understand the role of Zn as a stimulator of complex enzymatic antioxidant system.

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