RESEARCH ARTICLE

Cadmium-induced Oxidative Stress and Antioxidative Enzyme Response in Water Hyacinth and Salvinia

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ABSTRACT

The reactive oxygen species generation, lipid peroxidation and antioxidative enzyme response of water hyacinth and salvinia to Cd were evaluated. Cadmium was absorbed/accumulated mainly in the roots, but significant amounts also translocated to the leaves. No Cd effect on dry weight was detected, although toxicity symptoms were visible. Superoxide and H_2O_2 concentrations increased, in addition to lipid peroxidation in both species, especially in the leaves of salvinia. In general, antioxidative enzyme activities were reduced in both species following Cd treatment, especially in salvinia. Glutathione peroxidase (GPX, EC 1.11.1.9) activity decreased in water hyacinth but increased in salvinia. Glutathione S-transferase (GST, EC 2.5.1.18) activity increased in the leaves but decreased in the roots of both species. So, Cd induced ROS generation/accumulation, but the antioxidative enzymes were not able to combat the Cd-induced oxidative injury in these two species. Nevertheless, water hyacinth consistently showed a higher tolerance to Cd than salvinia.

Keywords: aquatic plants, *Eichhornia crassipes*, metal toxicity, oxidative stress, *Salvinia auriculata*

Abbreviations: APX, ascorbate peroxidase; CAT, catalase; GPX, glutathione peroxidase; GR, glutathione reductase; GST, glutathione S-transferase; POX, peroxidase; ROS, reactive oxygen species; SOD, superoxide dismutase.

INTRODUCTION

Cadmium is a toxic metal that is present at low concentrations in rocks, soils and water and usually does not pose health risks to humans and wildlife. The concentrations of heavy metals (e.g., Cd) in some areas, however, is becoming increasingly high due to anthropogenic activities, including mining and industrial processes and the utilization of heavy metals in agriculture as herbicides, fungicides and phosphate fertilizers (Davis, 1984). Unfortunately, most Cd and other pollutants eventually enter the aquatic environment via the natural weathering of rocks and industrial effluents or agricultural run-off (Singh et al., 2006).

Cadmium is easily taken up by plant roots and, once accumulated at toxic levels, may interfere with a number of metabolic processes, including photosynthesis and respiration (Hasan et al., 2009). Toxicity may result from the binding of metal to sulfhydryl groups involved in the catalytic action or structural integrity of enzymes. Metals can also act by inducing the deficiency or substitution of essential ions in metalloproteins (Van Assche and Clijsters, 1990). Furthermore, cadmium can induce oxidative stress by generating reactive oxygen species (ROS) (Romero-Puertas et al., 2007). Reactive oxygen species are considered to be unavoidable by-products of normal aerobic metabolism; however, many stresses can disrupt cellular homeostasis, thus enhancing the production

of ROS (Mittler, 2002). These ROS react with lipids, proteins, pigments and nucleic acids, causing lipid peroxidation, membrane damage and enzyme inactivation, thus affecting cellular metabolism (Romero-Puertas et al., 2007). Plants use a diverse array of antioxidative enzymes, including superoxide dismutase (SOD), ascorbate peroxidase (APX), peroxidase (POX) and catalase (CAT), in addition to low molecular weight antioxidants, such as reduced glutathione and ascorbic acid. These agents scavenge different types of reactive oxygen intermediates, thereby protecting cells against injury and potential damage (Mittler, 2002). Some enzymes from the glutathione metabolic pathway may also be involved in the plant tolerance to oxidative stress induced by metals. Two of the most important enzymes for this function are glutathione S-transferase, which catalyzes the conjugation of glutathione to xenobiotics (Davis and Swanson, 2001), and glutathione reductase, which is involved in H_2O_2 decomposition via the ascorbate-glutathione cycle (Pereira et al., 2002).

Oxidative stress appears to be involved in Cd toxicity in different plant species (Romero-Puertas et al., 2007). The enzyme response to oxidative stress induced by metals, however, is dependent on the metal type and experimental conditions (Hegedus et al., 2001), such as metal concentration, exposure time and plant species (Hegedus et al., 2001; Pereira et al., 2002). Therefore, the contribution of antioxidative enzymes to a plant's tolerance to Cd is still largely unknown (Liu et al., 2007).

The aquatic species *Eichhornia crassipes* (Mart.) Solms (water hyacinth) and *Salvinia auriculata* Aubl. (salvinia) are able to uptake and accumulate considerable amounts of Cd from contaminated water without significant reductions in growth and/or apparent visual toxicity symptoms (Vestena et al., 2007). Therefore, they have been suggested as potential plants for the phytoremediation or biological indication of waters polluted with Cd or other metals (Hasan et al., 2007). Several aspects of Cd uptake, accumulation and distribution (Vestena et al., 2007) and the involvement of sulfur acquisition and metabolism in Cd tolerance (Oliveira et al., 2009) in these species have been studied; however, little is known about their responses to oxidative stress induced by toxic levels of cadmium.

The objective of this work was to evaluate the influence of Cd on the induction of oxidative stress and the antioxidative enzyme response in two tropical aquatic species: water hyacinth and salvinia.

MATERIAL AND METHODS

Plant material and growth conditions: Aquatic plants of *Eichhornia crassipes* (Mart.) Solms (water hyacinth) and *Salvinia auriculata* Aubl. (salvinia), collected in ponds of the Universidade Federal de Viçosa (Viçosa, MG, Brazil) that were free of laboratory or household wastewater discharge, were used in this experiment. Plants were selected for uniformity in size and shape. Before any treatment, their surfaces were sterilized with 1% (v/v) sodium hypochlorite for 1 min and immediately washed in running tap water. They were then rinsed and kept in deionized water for 24 h. Next, the plants were transferred to a growth chamber at 25±1ºC on a 16 h light/8 h dark cycle with a photon flux density of 230 μmoles m^{-2} s^{-1} for 3 days.

After this adaptation period, plants were transferred to polyethylene pots with 2.5 L of Hoagland's nutrient solution (Hoagland and Arnon, 1950) at pH 7.0 with 1/5 of the original ionic strength and exposed to cadmium (Cd) at concentrations of 0 and 5 μM applied in the form of $Cd(NO_3)_2.4H_2O$. The nutrient solution was renewed after 3 days of growth, and the experiment was ended on the sixth day. Plants were washed in running tap and deionized water and were immediately processed for the determination of enzyme activity.

Hydrogen peroxide, superoxide anion and lipid peroxidation determination: Hydrogen peroxide levels in the roots and leaves were determined, according to method described by Gay and Gebicki (2000). Tissue was ground in liquid nitrogen and homogenized in 2 mL of 50 mM potassium buffer (pH 6.5), containing 1 mM hydroxylamine and centrifuged at 10,000 *x* g for 15 min at 4°C. Supernatant aliquots of 100 μL were added to a reaction médium consisting of 250 μM ferrous ammonium sulfate, 25 mM sulfuric acid, 250 μM xylenol orange and 100 mM sorbitol for a final volume of 2 mL. After 30 min in the dark, the absorbance was read at 560 nm. The hydrogen peroxide concentration was estimated, using a standard curve prepared with known concentrations of H_2O_2.

To determine the superoxide anion levels, the roots and leaves were cut into small segments and placed in 2

mL of a reaction mixture, consisting of 100 μM Na_2 EDTA, 20 μM NADH and 20 mM sodium phosphate buffer (pH 7.8) (Mohammadi and Karr, 2001). The reaction was initiated by adding 100 μL of 25.2 mM epinephrine (freshly prepared in 0.1 N HCl). The samples were shaken for 5 minutes at 28°C on a rotary shaker. The tissue was then removed, and the slope/min at 480 nm was measured over a period of 5 min and the amount of adrenochrome produced was estimated, using a molar extinction coefficient of 4.0 10^3 M^{-1}.

The levels of lipid peroxidation in the roots and leaves were estimated as the malondialdehyde (MDA) concentration after a reaction with thiobarbituric acid (TBA), according to Cakmak and Horst (1991). Tissue was homogenized in 1% (w/v) trichloroacetic acid (TCA) and centrifuged at 12,000 *x* g for 15 min at 4°C. Supernatant aliquots were added to 1.5 mL of 0.5 % (w/v) thiobarbituric acid (TBA) in 20% TCA and incubated in a shaking water bath at 95ºC. After 30 min, the reaction was stopped, samples were centrifuged at 10,000 *x* g for 10 min and the absorbance was measured at 532 nm. The non-specific absorption at 600 nm was subtracted. The amount of MDA-TBA complex was calculated from the molar extinction coefficient of 155 mM^{-1} and expressed as nmol (MDA) g^{-1} fresh weight.

Enzyme extraction and assays: To assess enzyme activities, leaf and root fresh weight samples were ground in liquid nitrogen and homogenized in the following buffer media: a) Superoxide dismutase (SOD, EC 1.15.1.1), peroxidase (POX, EC 1.11.1.7) and catalase (CAT, EC 1.11.1.6): 0.1 M potassium phosphate buffer (pH 6.8), 0.1 mM ethylenediamine tetraacetic acid (EDTA), 1 mM phenylmethanesulfonyl fluoride (PMSF) and 1% (w/v) polyvinylpolypyrrolidone (PVPP) (Peixoto et al., 1999) ; b) ascorbate peroxidase (APX, EC 1.11.1.11): 50 mM potassium phosphate buffer (pH 7.0), 1 mM EDTA, 1 mM ascorbic acid, 1 mM PMSF, 2 mM dithiothreitol (DTT) and 1% (w/v) PVPP (Peixoto et al., 1999); c) glutathione S-transferase (GST, EC 2.5.1.18): 0.2 M Tris-HCl buffer (pH 7.8), 1 mM EDTA, 1 mM DTT, 0.1 mM PMSF and 5% (w/v) PVPP (Habig and Jakoby, 1981); d) glutathione reductase (GR, EC 1.6.4.2): 0.1 M potassium phosphate buffer (pH 7.0), 1 mM EDTA, 0.02% (w/v) triton X-100, 2 mM DTT, 1 mM PMSF and 1% (w/v) PVPP (Carlberg and Mannervik, 1985); e) glutathione peroxidase (GPX, EC 1.11.1.9): 0.1 M Tris-HCl buffer, pH 7.5, 1 mM EDTA and 10 mM $MgCl_2$ (Nagalakshmi and Prasad, 2001). In all cases, after being filtrated through four layers of cheesecloth, the homogenates were centrifuged at 12,000 *x* g for 15 min at 4ºC, and the supernatant was used as the source of crude enzyme.

Enzyme activities were determined by adding 0.1 mL of the crude enzyme extract to: a) POX: 2.9 mL of a reaction medium, consisting of 0.1 M potassium phosphate buffer (pH 6.8), 20 mM pyrogallol and 20 mM H_2O_2 ; b) CAT: 2.9 mL of a reaction medium, consisting of 50 mM potassium phosphate buffer (pH 7.0) and 12.5 mM H_2O_2; c) APX: 2.9 mL of a reaction medium, consisting of 50 mM potassium phosphate buffer (pH 6.0), 0.8 mM ascorbic acid and 1 mM H_2O_2 (Cakmak and Host, 1991); d) GST: 0.9 mL of a reaction medium, consisting of 0.2 M potassium phosphate buffer (pH 6.5), 20 mM GSH and 0.1 mM 1-chloro-2,4-dinitrobenzene (Habig and Jakoby, 1981); e) GR: 0.9 mL of a reaction medium, consisting of 50 mM tris-HCl buffer (pH 7.5), 1 mM oxidized glutathione (GSSG) and 0.1 mM NADPH (Carlberg and Mannervik, 1985); f) GPX: 0.9 mL of a reaction medium, consisting of 50 mM potassium phosphate buffer (pH 7.0), 1 mM EDTA, 0.1 M NaCl, 1 mM GSH and 0.2 mM NADPH, 0.25 mM H_2O_2 and 1 unit of glutathione reductase (Nagalakshmi and Prasad, 2001). In all cases, the mixtures were incubated at 30ºC, and the absorbances were measured during the first minute of the reaction. Enzyme activities were estimated using the following molar extinction coefficients: POX (420 nm; ε: 2.47 mM^{-1} cm^{-1}); CAT (240 nm, ε: 36 M^{-1} cm^{-1}); APX (290 nm, ε: 2.8 mM^{-1} cm^{-1}); GST and GPX (340 nm, ε: 9.6 mM^{-1} cm^{-1}; and GR (340 nm, ε: 6.22 mM^{-1} cm^{-1}).

The activity of SOD was determined by adding the root or leaf crude enzymatic extract to a reaction mixture, consisting of 50 mM potassium phosphate buffer (pH 7.8), 13 mM methionine, 0.1 mM EDTA, 75 μM nitroblue tetrazolium (NBT) and 2 μM riboflavin. The reaction was carried out in a chamber with a 15 W fluorescent lamp at 25ºC. After 5 min of illumination, the blue formazan was measured at 560 nm (Giannopolitis and Ries, 1977). All rates were corrected for non-enzymatic activity. One unit of SOD activity was defined as the amount of enzyme required to cause a 50% inhibition of the rate of NBT reduction.

Total and soluble cadmium determination: Oven-dried (80ºC) plant materials, which were finely ground in a stainless steel electric grinder, were digested in a nitric-perchloric acid mixture (2:1) or extracted in a buffer mixture consisting of 0.1 M Tris-HCl, 1 mM EDTA and 1% (w/v) ascorbic acid at

pH 8.0. Cadmium levels in the extracts were determined by atomic absorption spectrophotometry (Shimadzu, model AA-6701FG).

Statistical design: The experimental design was a completely randomized factorial design performed in triplicates. Data were subjected to an analysis of variance (ANOVA), and the means were statistically compared by Tukey's test at a 5% probability.

RESULTS

The dry mass did not change in the leaf or root of water hyacinth and salvinia after 6 days of exposure to 5 μM Cd (Figure 1A). Despite this, symptoms of Cd toxicity were observed in both species, especially in salvinia. In the Cd-treated salvinia plants, darkening of the roots and leaves became evident after only 3 days, while in the water hyacinth, only slight internerval chlorosis and root darkening were observed after 5 days of Cd treatment.

Cd-treated water hyacinth plants yielded 3.5-times more dry mass and accumulated about 5-times more Cd than salvinia (Figure 1B). Most of this Cd was retained by the roots: about 62% in water hyacinth and only 36% in salvinia. In both cases, a substantial amount of the metal was transferred from the roots to the leaves, especially in salvinia. In the roots of Cd-treated water hyacinth plants, the Cd concentration was about 38% higher than in salvinia. In the leaves, however, the Cd concentration in salvinia was about 17% higher. Over 50% of Cd was in the free form, which was easily extracted with the buffer mixture used. In the roots, the two species had about the same percentage of free Cd (about 63%), but in the leaves, salvinia had significantly more free Cd (66%) than water hyacinth (52%).

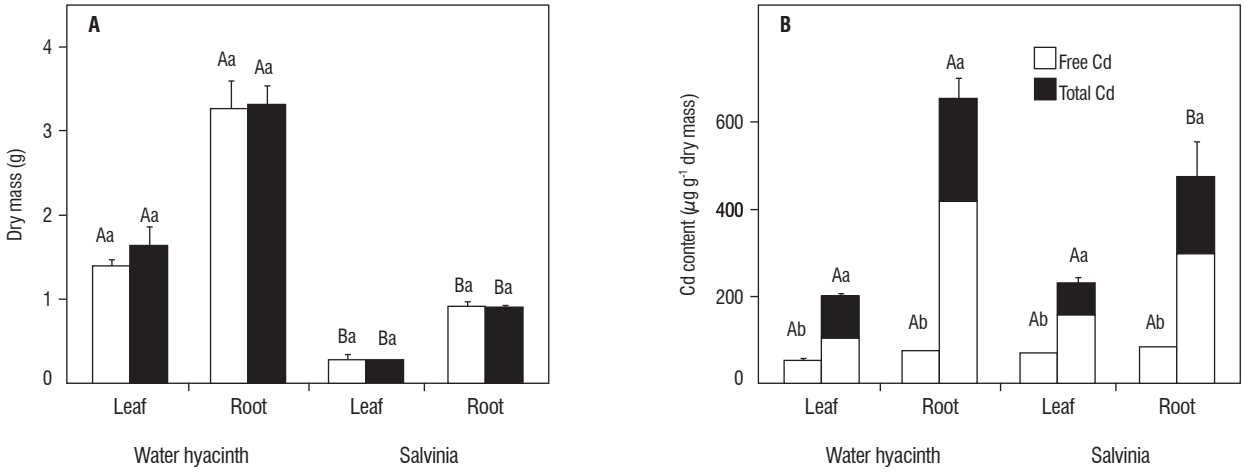

Figure 1. Dry mass and Cd concentration in leaves and roots of water hyacinth and salvinia after exposure to 0 (□) and 5 μM (■) Cd for 6 days. Means followed by the same capital letter between species for each plant part and by the same small letter between Cd concentrations for each plant part within species did not differ ($p \leq 0.05$). Bars represent standard deviations (n=3).

The leaf superoxide anion ($O_2^{\cdot-}$) concentrations also increased in the two species following Cd treatment (Figure 2A). In the roots, however, a reduction of about 65% in water hyacinth, as well as an increase of about 61% in salvinia, was observed. The $O_2^{\cdot-}$ concentrations in both the leaves and roots of salvinia were always higher than in water-hyacinth, independent of the Cd treatment.

Leaf hydrogen peroxide (H_2O_2) concentrations strongly increased in both species after Cd treatment, especially in salvinia (Figure 2B). The leaves of salvinia always exhibited higher H_2O_2 concentrations, independent of the Cd treatment. In the roots, the H_2O_2 concentration was slightly higher in water hyacinth, but it did not change with the Cd treatment.

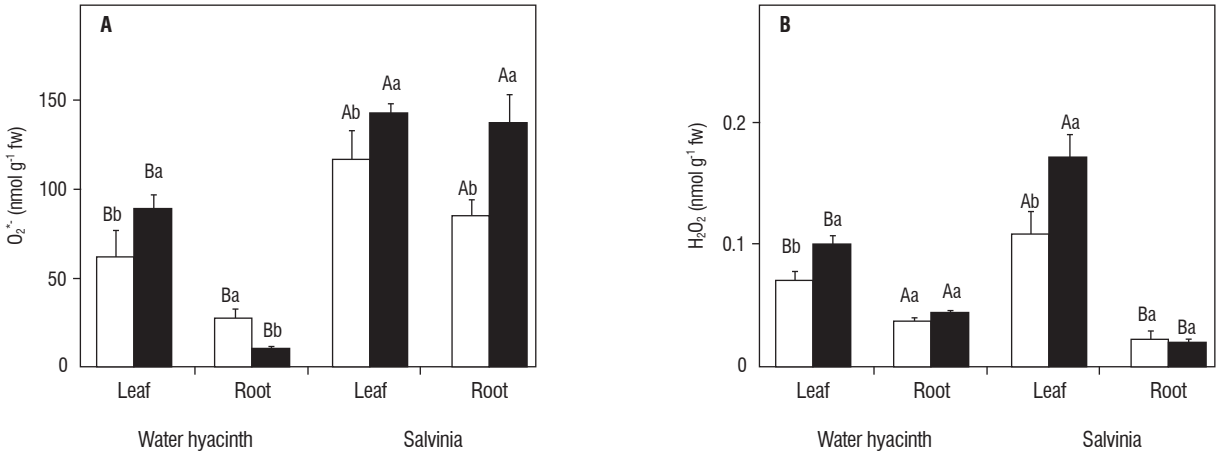

Figure 2. Hydrogen peroxide (H_2O_2) and superoxide ($O_2^{\bullet-}$) concentrations in leaves and roots of water hyacinth and salvinia after exposure to 0 (□) and 5 μM (■) cadmium for 6 days. Means followed by the same capital letter between species for each plant part and Cd concentration and by the same small letter between Cd concentrations for each plant part within species did not differ ($p \leq 0.05$). Bars represent standard deviations (n=3).

Leaf lipid peroxidation after plant exposure to Cd increased only in salvinia (Figure 3). In the roots, lipid peroxidation increased at about same intensity in both species following Cd treatment. In salvinia, most of the lipid peroxidation was observed in the leaves.

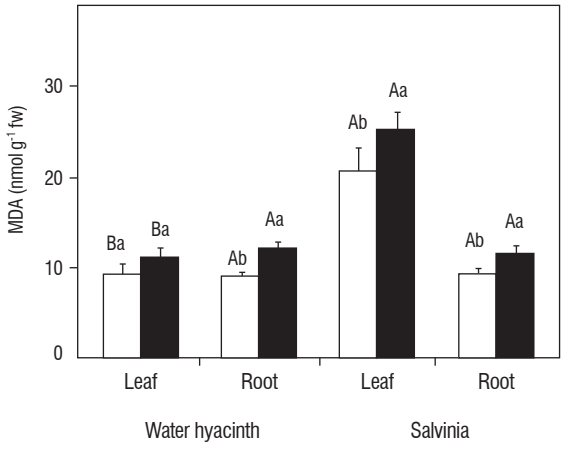

Figure 3. Lipid peroxidation (MDA) in leaves and roots of water hyacinth and salvinia after exposure to 0 (□) and 5 μM (■) Cd for 6 days. Means followed by the same capital letter between species for each plant part and Cd concentration and by the same small letter between Cd concentrations for each plant part within species did not differ ($p \leq 0.05$). Bars represent standard deviations (n=3).

In the leaves, superoxide dismutase (SOD) activity did not change in water hyacinth but increased about 26% in salvinia, following Cd treatment (Figure 4A). In the roots, however, SOD activity increased in water hyacinth but decreased in salvinia. Total SOD activity in water hyacinth Cd-treated plants (leaf + root) was about 30% higher than in salvinia.

Peroxidase (POX) and ascorbate peroxidase (APX) activities were much higher in water hyacinth, independent of the Cd treatment or plant organ (Figure 4B, D). Following Cd treatment, the activities of these enzymes decreased in both roots and leaves of the plants of the two species. However, the reductions were much stronger in the roots of both species.

Catalase activity (CAT) in the leaves of the water hyacinth was much higher than in the roots or the two organs of salvinia (Figure 4C). The cadmium treatment reduced the activity of this enzyme in the leaves of both species. Moreover, the CAT activity reduction in the salvinia leaves was about 8-times higher than in water hyacinth.

Glutathione peroxidase (GPX) activity decreased in both parts of the water hyacinth but increased in both parts of salvinia when the plants were exposed to Cd (Figure 4E). The activity of this enzyme, however, always remained higher in water hyacinth, independent of the plant part analyzed. Water

hyacinth showed a much higher GPX activity in its leaves, independent of Cd treatment.

Glutathione reductase (GR) activity decreased in water hyacinth leaves; however, GR activity decreased in both parts of the salvinia plants that were exposed to Cd (Figure 4F). In the roots of water hyacinth, a small increase of about 13% was observed. The reduction in enzyme activity in salvinia leaves was about 74% stronger than in water hyacinth.

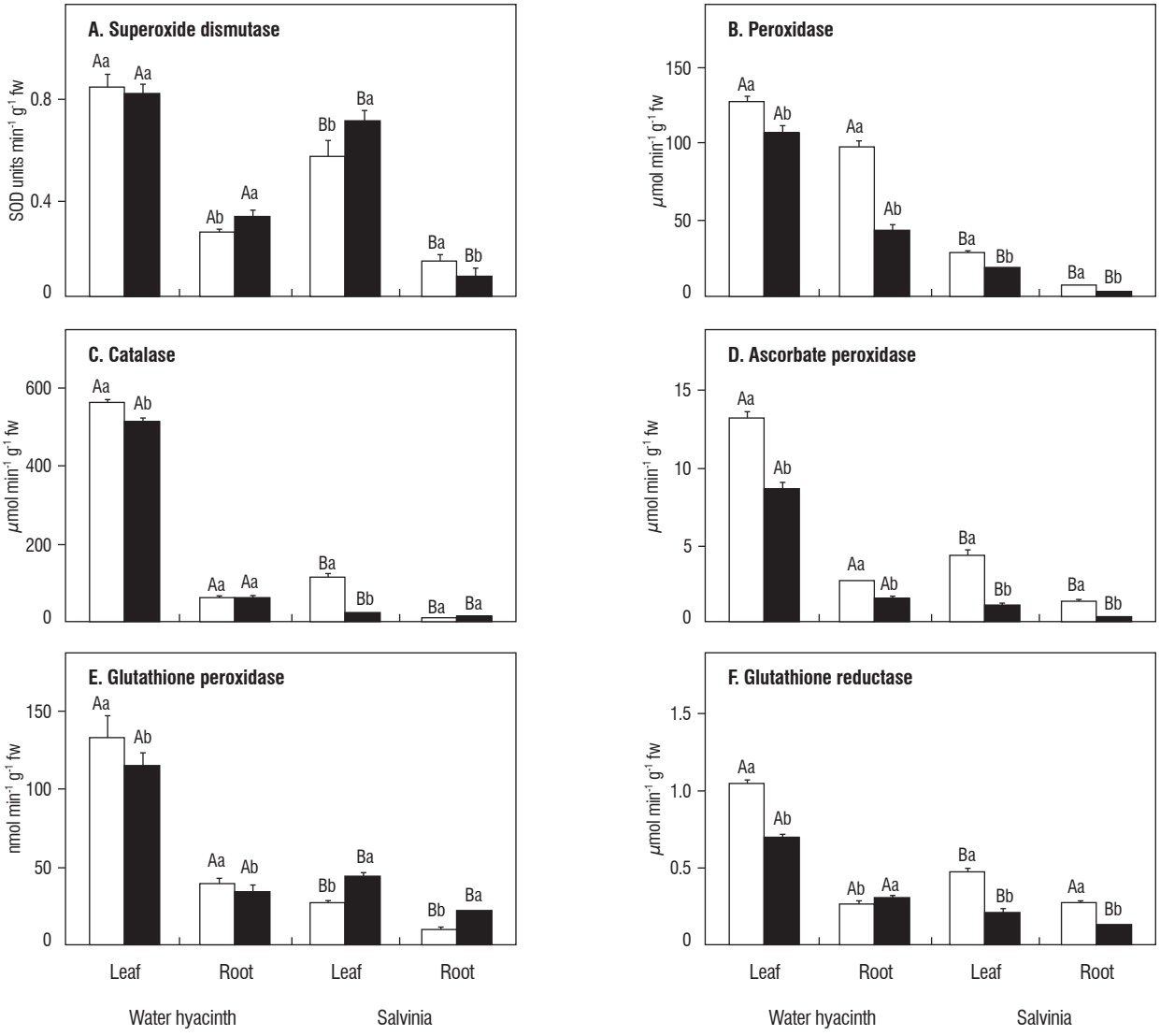

Figure 4. Activities of antioxidative enzymes in leaves and roots of water hyacinth and salvinia after exposure to 0 (□) and 5 μM (■) Cd for 6 days. Means followed by the same capital letter between species for each plant part and Cd concentration and by the same small letter between Cd concentrations for each plant part within species did not differ ($p \leq 0.05$). Bars represent standard deviations (n=3).

Glutathione S-transferase (GST) activity increased in the leaves but decreased in the roots of both species treated with Cd (Figure 5). Salvinia showed a much higher GST activity in the roots, independent of the Cd treatment.

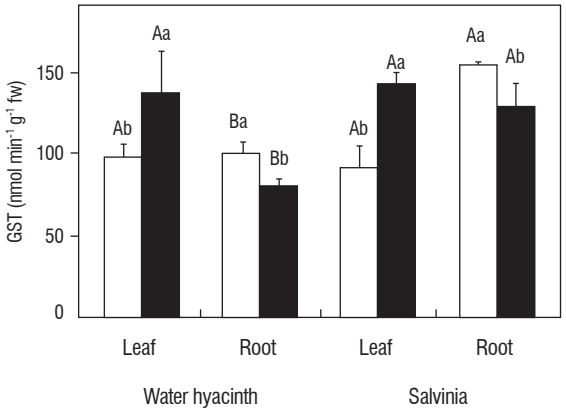

Figure 5. Glutathione S-transferase activities in leaves and roots of water hyacinth and salvinia after exposure to 0 (□) and 5 μM (■) Cd for 6 days. Means followed by the same capital letter between species for each plant part and Cd concentration and by the same small letter between Cd concentrations for each plant part within species did not differ ($p \leq 0.05$). Bars represent standard deviations (n=3).

DISCUSSION

Cadmium (Cd) is a non-essential element that negatively affects the growth and development of plants (Hasan et al., 2009). Nevertheless, cadmium is taken up rapidly by the roots and can accumulate in plants. The amount of Cd that accumulates in roots or translocates to leaves and the intensity of its effects on plants differ considerably among species and are dependent on several factors, including the concentration and duration of metal exposure (Rodríguez-Serrano et al., 2006). In some experiments, Cd is used in extremely high concentrations (up to 1 mM), leading to unrealistic interpretations (Milone et al., 2003). In the present experiment, plants were exposed to only 5 μM Cd, and after 6 days, plants did not show any changes in dry weight. Nevertheless, Cd concentrations in treated plants were much higher than in control plants, and toxicity symptoms in the leaves were visible in both species. Normally, Cd ions are retained in the roots with only small amounts being transported to the leaves (Cataldo et al., 1983). The two species studied here were aquatic plants, and therefore, during the experiment, the leaves maintained direct contact with the nutrient solution. The leaves likely contributed to an increase in Cd absorption, especially in salvinia. Corroborating this hypothesis, in an experiment where leaf contact with the nutrient solution was not allowed, a significant decrease in leaf Cd concentration was observed in salvinia but not in water hyacinth (Oliveira et al., 2001). For this reason, salvinia leaves probably showed higher Cd concentrations. Additionally, the leaves of this species showed higher free Cd concentrations. Thus, although the water hyacinth absorbed more Cd, most of it was retained in the roots and/or was kept in a bound and less toxic form than in salvinia.

Several studies have demonstrated that the excessive absorption of heavy metals by plants induces the production of reactive oxygen species (ROS) in plant tissues (Singh et al., 2006). In both aquatic species, there were strong increases in H_2O_2 and $O_2^{\cdot-}$, increasing lipid peroxidation, especially in the leaves of salvinia. These ROS cause imbalances in the antioxidative defenses of plants and induce oxidative stress (Edreva, 2005). Plant antioxidative defenses against ROS may involve antioxidative enzymes and nonenzymatic antioxidants, including ascorbate and glutathione (GSH) in addition to tocopherol, flavonoids, alkaloids and carotenoids (Apel and Hirt, 2004).

Enzymatic ROS scavenging mechanisms in plants may include superoxide dismutase (SOD), catalase (CAT), peroxidase (POX) and enzymes of the ascorbate-glutathione cycle and the glutathione peroxidase cycle (Apel and Hirt, 2004). Superoxide dismutase acts as the first line of defense against ROS, dismutating superoxide to H_2O_2 and APX, GPX and CAT and subsequently detoxifying H_2O_2.

The superoxide dismutase in the leaves of water hyacinth was not able to keep the $O_2^{\cdot-}$ level down, while in the roots, the SOD activity increased, causing a strong reduction in $O_2^{\cdot-}$ levels in this part of the plants. In salvinia, however, despite an increase in SOD activity in the leaves, the $O_2^{\cdot-}$ levels increased in both organs of the plant.

Peroxidase (POX) and ascorbate peroxidase (APX) activities decreased in both parts of the plants, while catalase (CAT) activity decreased only in the roots of both species. Usually, activity increases for these antioxidative enzymes are expected, and this is taken as an indication of their role in the detoxification of Cd-induced ROS. Such increases have been observed in radish (Vitória et al., 2001), *Crotalaria juncea* (Pereira et al., 2002) and rice (Shah et al., 2001). In other cases, however, decreases in enzyme activities have been observed, such as in pea that was treated with Cd at concentrations ranging from 0 to 50 μM (Sandalio et al., 2001). Reductions in antioxidative

enzyme activities, such as those observed here, are indicative of very strong oxidative stress conditions, which may affect enzyme biosynthesis and/or the assembly of enzyme subunits (Singh et al., 2006).

Glutathione peroxidase (GPX), like APX, detoxifies H_2O_2 to H_2O, but uses GSH directly as the reducing agent. The regeneration of GSH is made possible by the reduction of GSSG by glutathione reductase (GR), closing the GPX cycle (Apel and Hirt, 2004). In general, GR activity increases in plants under oxidative stress. This has been observed in *Raphanus sativus* (Vitória et al., 2001), *Crotalaria juncea* (Pereira et al., 2002), *Beta vulgaris* and *Beta maritima* (Bor et al., 2003), especially in the leaves. This tendency, however, was not confirmed by our experiment, except for a small increase observed in GR activity in the roots of water hyacinth. In all other situations, strong reductions were found in the activity of this enzyme. The observed inhibition of GR in Cd-treated plants was paralleled with a decrease in GSH concentration (data not published), lowering the amount of substrate available for GPX. The expected reduction in GPX activity, however, was observed only in water hyacinth. In salvinia, on the contrary, despite a reduction in GSH concentration, GPX activity increases of over 60% were observed in Cd-treated plants. GPX appears to be capable of using reduced substrates other than GSH, including lipid hydroperoxides, as suggested by Herbette et al. (2002). This possibility and the great variety of GPX isoenzymes (Eshdat et al., 1997) may explain, at least in part, the difference in species responses to Cd that was observed here. Nevertheless, GPX activity was always higher in water hyacinth, indicating a higher capacity of this species to scavenge the ROS induced by Cd.

Although ROS are continuously formed in normal metabolism, cells must be able to quickly and efficiently scavenge these reactive species to reach a homeostasis. When ROS generation is not adequately regulated, its accumulation may cause oxidative damage to cells. Our results indicate that there is incomplete enzyme scavenging of $O_2^{\cdot-}$ and H_2O_2, which may be used to generate the much more reactive hydroxyl free radical (Edreva, 2005) and/or other destructive species such as lipid peroxides, usually associated with lipid peroxidation and eventually cell death (Noctor and Foyer, 1998).

Glutathione S-transferase (GST) is an enzyme with a determinant function in the detoxification processes. It catalyzes the conjugation of several xenobiotics to reduced glutathione (GSH) (Davis and Swanson, 2001). This enzyme may also protect plants from oxidative injury, functioning as the glutathione peroxidase, by using glutathione to reduce organic hydroperoxides (Dixon et al., 2002) produced by the oxidative degradation of membrane lipids and/or nucleic acids. Thus, the observed increase in GST activity in the leaves of both plants exposed to Cd (Figure 5) may be considered indicative of an oxidative stress induced by these metals. In the roots, however, the enzyme was inhibited in both species, likely due to the higher Cd concentrations accumulated there. Cadmium at high concentrations may have a direct effect on protein biosynthesis (Mazhoudi et al., 1997). It may also induce the accumulation of hydroperoxides or other by-products of ROS action, leading to GST inhibition (Nagalakshmi and Prasad, 2001), as was observed here in the roots of both species.

Most of the antioxidative enzymes analyzed, with the exception of GST in the leaves of both species and GPX in both salvinia parts, showed decreased activities in plants exposed to Cd. In all cases, enzyme activity reductions were much lower in water hyacinth, indicating that this species is much more tolerant to Cd than salvinia.

Observed enzyme activity reductions are indicative of a limited protection against oxidative stress (Schutzendubel and Polle, 2002). The intensity and direction of the antioxidative response to Cd appeared to be dependent on the plant species, tissue analyzed, metal identity (Rout and Shaw, 2001), duration of metal exposure (Hegedus et al., 2001) and stage of plant development (Rout and Shaw, 2001). The explanation for such antioxidative enzyme activity reductions is not fully known yet. Apparently, excessive metal accumulation can inhibit enzymes by binding to catalytic active groups or causing protein denaturation (Das et al., 1997). Furthermore, toxic metals can induce ROS production and accumulation, which can cause protein oxidation and enzyme inhibition (Mittler, 2002).

In conclusion, cadmium induced ROS generation/accumulation, causing lipid peroxidation and the inhibition of most of the antioxidative enzymes. Apparently, scavenging of the produced ROS by antioxidative enzymes does not seem to be a major mechanism of Cd tolerance in these two aquatic species. Nevertheless, water hyacinth always showed higher tolerance to Cd than salvinia.

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REFERENCES

Apel K, Hirt A (2004) Reactive oxygen species: metabolism, oxidative stress, and signal transduction. Annu. Rev. Plant Biol. 55:373–399.

Bor M, Özdemir F, Türkan I (2003) The effect of salt stress on lipid peroxidation and antioxidants in leaves of sugar beet *Beta vulgaris* L. and wild beet *Beta maritima* L. Plant Sci. 164:77-84.

Cakmak I, Horst WJ (1991) Effect of aluminium on lipid peroxidation, superoxide dismutase, catalase, and peroxidase activities in root tips of soybean (*Glycine max*). Physiol. Plant. 83:463-468.

Carlberg C, Mannervik B (1985) Glutathione reductase. Methods Enzymol. 113:488-495.

Cataldo DA, Garland TR, Wildung RE (1983) Cadmium uptake kinetics in intact soybean plants. Plant Physiol. 73:844-848.

Das P, Samantaray S, Rout GR (1997) Studies on cadmium toxicity in plants: a review. Environ. Pollut. 98:29-36.

Davis DG, Swanson HR (2001) Activity of stress-related enzymes in the perennial weed leafy spurge (*Euphorbia esula* L.). Environ. Exp. Bot. 46:95-108.

Davis RD (1984) Cadmium-a complex environmental problem: cadmium in sludge used as fertilizer. Experientia 40:117–126.

Dixon DO, Lapthorn A, Edwards R (2002) Plant glutathione transferases. Genome Biol. 3:1-10.

Edreva A (2005) Generation and scavenging of reactive oxygen species in chloroplasts: a submolecular approach. Agr. Ecosyst. Environ. 30:119–133.

Eshdat Y, Holland D, Faltin Z, Ben-Haygim G (1997) Plant glutathione peroxidases. Physiol. Plant. 100:234-240.

Gay C, Gebicki JM (2000) A critical evaluation of the effect of sorbitol on the ferric-xylenol orange hydroperoxide assay. Anal. Biochem. 284:217-220.

Giannopolitis CN, Ries SK (1977) Superoxide dismutases: Occurrence in higher plants. Plant Physiol. 59:309–314.

Habig WH, Jakoby WB (1981) Assay for differentiation of glutathione S-transferases. Methods Enzymol. 77:398-405.

Hasan SA, Fariduddin Q, Ali B, Hayat S, Ahmad A (2009) Cadmium: toxicity and tolerance in plants. J. Environ. Biol. 30:21-31.

Hasan SH, Talat M, Rai S (2007) Sorption of cadmium and zinc from aqueous solutions by water hyacinth (*Eichhornia crassipes*). Bioresource Technol. 98:918-928.

Hegedüs A, Erdei S, Horváth G (2001) Comparative studies of H_2O_2 detoxifying enzymes in green and greening barley seedlings under cadmium stress. Plant Sci. 160:1085-1093.

Herbette S, Lenne C, Leblanc N, Julien JL, Drevet JR, Roeckel-Drevet P (2002) Two GPX-like proteins from *Lycopersicon esculentum* and *Helianthus annuus* are antioxidant enzymes with phospholipid hydroperoxide glutathione peroxidase and thioredoxin peroxidase activities. Eur. J. Biochem. 269:2414-2420.

Hoagland DR, Arnon DI (1950) The water-culture method for growing plants without soil. Calif. Agric. Exp. Stn. Cir. 347:1–39.

Liu Y, Wang X, Zeng G, Qu D, Gu J, Zhou M, Chai L (2007) Cadmium-induced oxidative stress and response of the ascorbate–glutathione cycle in *Bechmeria nivea* (L.) Gaud. Chemosphere 69:99-107.

Mazhoudi S, Chaou A, Ghorbal MH, El Ferjani E (1997) Response of antioxidant enzymes to excess copper in tomato (*Lycopersicon esculentum*, Mill.). Plant Sci. 127:129-137.

Milone MA, Sgherri C, Clijsters H, Navari-Izzo F (2003) Antioxidative responses of wheat treated with realistic concentration of cadmium. Environ. Exp. Bot. 50:265-274.

Mittler R (2002) Oxidative stress, antioxidants and stress tolerance, Trends Plant Sci. 7:405-410.

Mohammadi M, Karr AL (2001) Superoxide anion generation in effective and ineffective soybean root nodules. J. Plant Physiol. 158:1023-1029.

Nagalakshmi N, Prasad MNV (2001) Responses of glutathione cycle enzymes and glutathione metabolism to copper stress in *Scenedesmus bijugatus*. Plant Sci. 160:291-299.

Noctor G, Foyer CH (1998) Ascorbate and glutathione: Keeping active oxygen under control. Annu. Rev. Plant Physiol. Plant Mol. Biol. 49:249–79.

Oliveira JA, Cambraia J, Oliva MA, Jordão CP (2001) Absorção e acúmulo de cádmio e seus efeitos sobre o crescimento relativo de plantas de aguapé e de salvínia. Rev. Bras. Fisiol. Veg. 13:329-341.

Oliveira JA, Cambraia J, Souza MV, Oliva MA (2009) Sulphate uptake and metabolism in water hyacinth and salvinia during cadmium stress. Aquatic Botany 91:257–261.

Peixoto PHP, Cambraia J, Sant'Anna R, Mosquim PR, Moreira MA (1999) Aluminum effects on lipid peroxidation and on the activities of enzymes of oxidative metabolism in sorghum. Rev. Bras. Fisiol. Veg. 11:137-143.

Pereira GJG, Molina SMG, Lea PJ, Azevedo RA (2002) Activity of antioxidant enzymes in response to cadmium in *Crotalaria juncea*. Plant Soil 239:123-132.

Rodríguez-Serrano M, Romero-Puertas MC, Zabalza A, Corpas FJ, Gómez M, Del Río LA, Sandalio LM (2006) Cadmium effect on oxidative metabolism of pea (*Pisum sativum* L.) roots. Imaging of reactive oxygen species and nitric oxide accumulation *in vivo.* Plant Cell Environ. 29:1532-1544.

Romero-Puertas MC, Corpas FJ, Rodríguez-Serrano M, Gómez M, Del Río LA, Sandalio LM (2007) Differential expression and regulation of antioxidative enzymes by cadmium in pea plants. J. Plant Physiol. 164:1346-1357.

Rout NP, Shaw BP (2001) Salt tolerance in aquatic macrophytes: possible involvement of the antioxidative enzymes. Plant Sci. 160:415-423.

Sandalio LM, Dalurzo HC, Gómez M, Romero-Puertas MC, Del Rio LA (2001) Cadmium induced changes in the growth and oxidative metabolism of pea plants. J. Exp. Bot. 52:2115-2126.

Schützendübel A, Polle A (2002) Plant responses to abiotic stresses: heavy metal-induced oxidative stress and protection by mycorrhization. J. Exp. Bot. 53:1351-1365.

Shah K, Kumar RG, Verma S, Dubey RS (2001) Effect of cadmium on lipid peroxidation, superoxide anion generation and activities of antioxidant enzymes in growing rice seedlings. Plant Sci. 161:1135-1144.

Singh S, Eapen S, Souza SFD (2006) Cadmium accumulation and its influence on lipid peroxidation and antioxidative system in an aquatic plant, *Bacopa monnieri* L. Chemosphere 62:233-246.

Van Assche F, Clijsters H (1990) Effects of metals on enzyme activity in plants. Plant Cell Environ. 13:195-200.

Vestena S, Cambraia J, Oliva MA, Oliveira JA (2007) Cadmium accumulation by water hyacinth and salvinia under different sulfur concentrations. J. Braz. Soc. Ecotoxicol. 2:269-274.

Vitória AP, Lea PJ, Azevedo RA (2001) Antioxidant enzymes responses to cadmium in radish tissues. Phytochem. 57:710-715.