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Effect of Lead Toxicity on Aquatic Macrophyte *Elodea canadensis* Michx.

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Abstract Effects of Pb accumulation on the contents of chlorophylls (a and b), carotenoid, ascorbic acid (AsA), non-protein SH groups and protein were investigated in aquatic macrophyte *Elodea canadensis*. Pb accumulation in *E. canadensis* tissues increased with increasing metal concentrations. The increases at 1, 10 and 100 mg/L Pb are about 12.0, 44.6 and 71.1 times greater than control, respectively. Contents of chlorophylls, carotenoid and protein were adversely affected by Pb accumulation. Induction of non-protein SH groups and AsA showed that Pb accumulation caused oxidative stress. It is also possible that increased non-protein SH groups by Pb accumulation may be due to their role in Pb detoxification.

Keywords Ascorbic acid · Non-protein SH · Protein · Chlorophyll

According to the Environmental Protection Agency (EPA), Pb is the most common heavy metal contaminant in the environment (Watanabe 1997). It is one of the hazardous heavy metal pollutants of the environment that originates from various sources like mining and smelting of lead-ores, burning of coal, effluents from storage battery industries, automobile exhausts, metal plating and finishing operations, fertilizers, pesticides and from additives in pigments and gasoline (Eick et al. 1999).

Lead has not been shown to be essential in plant metabolism, although it occurs naturally in all plants (Kabata-Pendias and Pendias 1984). Responses of plants to Pb exposure include decrease in root elongation and biomass (Fargasova 1994), inhibition of chlorophyll biosynthesis (Miranda and Ilangovan 1996), and induction or inhibition of several enzymes (Van Assche and Clijsters 1990).

When Pb enters inside the cells even in small amounts it produces a wide range of adverse effects on physiological processes. One of the phytotoxic effects of Pb appears to be induction of oxidative stress in growing plant parts due to enhanced production of reactive oxygen species (ROS) resulting in an unbalanced cellular redox status. A number of different ROS, including the superoxide anion ($O_2^{\bullet -}$), singlet oxygen ($^1O_2$), hydrogen peroxide (H_2O_2) and the hydroxyl radical ($^{\bullet}OH$) are produced during normal oxidative metabolism in aerobic organisms, but these ROS can pose a severe threat when produced in larger amounts. (Sharma and Dubey 2005). The aim of this study was to determine the effects of Pb accumulation on contents of chl-a, chl-b, carotenoid, AsA and non-protein SH groups in submerged aquatic macrophyte *Elodea canadensis*.

Materials and Methods

Test material *Elodea canadensis* Michx. (Hydrocharitaceae) was collected from an artificial pond of Gaziantep University Campus, Gaziantep, Turkey. During 2 weeks, the macrophyte was acclimatized in 10% Arnon and Hoagland nutrient solution (Arnon and Hoagland 1940), under 25–27°C, 16 h light (6,000 lux) and 8 h dark periods. Healthy macrophytes (12 g fresh weight) were cultured in plastic vessel containing 1,000 mL solution for Pb treatment during 5-days. The concentrations of Pb in the polluted waters source are in the range of 1–100 mg/L. These concentrations are, however, frequently reduced

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during treatment, prior to discharge to the receiving waters (Harrison and Laxen 1981). Thus, three replicate of macrophytes were exposed to Pb as $Pb(CH_3COO)_2 \cdot 3H_2O$ at 1, 10 and 100 mg/L concentrations which were added to 10% nutrient solution. Plants without added trace elements served as controls. The pH's of solutions were adjusted to 6.5–6.7. All chemicals used in the study were prepared from the analytical grade.

After 5-days duration, fresh leaves of macrophytes were weighted, put separately in 80% acetone and homogenized with mortar and pestle using quartz sand. The supernatant was separated and the absorbances were read using a spectrophotometer (CINTRA 202) at 662, 645 and 470 nm for chl-a, chl-b and carotenoid, respectively. Amounts of these pigments were calculated according to the formulas of Lichtenthaler and Wellburn (1983). Extraction solution was used as blank.

The measurement of total ascorbic acid (AsA) and non-protein SH groups was carried out according to Cakmak and Marschner (1992).

Protein content of *E. canadensis* was determined according to Lowry et al. (1951) using bovine serum albumin as standard.

After harvest, macrophytes were carefully washed three times with distilled water and then weighted. Three replicates of each treatment (4 g fresh weight) dried to a constant weight at 80°C in an electric furnace for dry biomass determination. After that, these samples were pulverized using mortar and pestle. The samples were dissolved in 14 M HNO_3 and residues were dissolved in 1 M HCl. After mineralization, the metal was determined using an atomic absorption spectrometer (Perkin Elmer AAnalyst 400). Control samples were also treated by the same way. Values of uptake were obtained by deducting metal contents of control macrophytes. Bioconcentration factor (BCF) was estimated from ratio of Pb concentrations in *E. canadensis* (μg/g dry weight) to Pb in test medium (mg/L).

All analysis were carried out triplicate. For statistical analyses we chose the analysis of variance (ANOVA) in Statistical Analysis System (SPSS 11.0 for windows). The significance of differences between mean values were determined by a multiple range test (LSD; Least Significant Difference). Correlation analysis (Pearson) was estimated among Pb concentration in macrophyte and analyzed other parameters.

Results and Discussion

Figure 1A shows the concentrations of Pb accumulated in *E. canadensis* shoot tissues. There was an increase in Pb accumulation of the macrophyte with rising external metal concentrations. The increases at 1, 10 and 100 mg/L Pb are about 12.0, 44.6 and 71.1 times greater than control, respectively ($p < 0.05$). Metal concentrations in plant tissue are generally a function of the metal concentration in the growth solution (Kabata-Pendias and Pendias 1984). Lead accumulation by aquatic plants has been reported earlier in aquatic macrophytes (Rai et al. 1995; Gupta and Chandra 1996; Saygideger and Dogan 2004). *E. canadensis* is a submerged aquatic plant that accumulates high amounts of metals (Fritioff and Greger 2003). In the present study, an increase in Pb accumulation in *E. canadensis* by replacing the metal solutions in culture is in agreement with other reports (Fritioff and Greger 2007; Nyquist and Greger 2007).

Bioconcentration factor (BCF), defined as the concentration ratio of Pb in the macrophyte to that in the tested solution, is used to measure the effectiveness of a plant in concentrating Pb into its biomass. There was a decrease in BCF with rising external Pb concentrations (Fig. 1B). In the macrophyte 1 mg/L Pb treated, the maximum BCF, 175.9, was estimated. On the other hand, the minimum BCF, 10.5, was estimated at 100 mg/L Pb treatment. This indicates that *E. canadensis* has high Pb accumulation capability at lower Pb concentrations. Aquatic macrophytes are often used nowadays for phytoremediation of water contaminated with heavy metals.

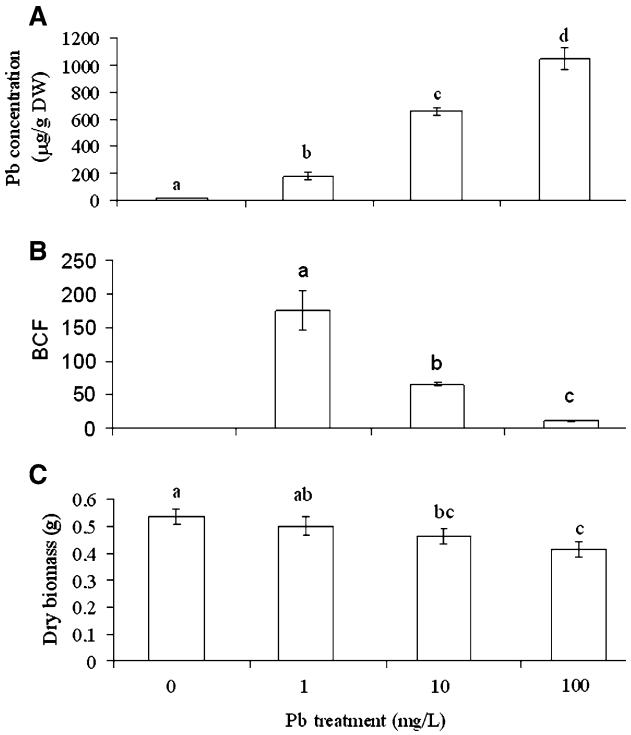

Fig. 1 Pb concentrations, BCF and dry biomass of *E. canadensis* after 5-days Pb treatment. Error bars represent the standard deviation of mean. (n = 3) Means with *different letters* are significantly different from one another according to LSD test ($p < 0.05$)

Visual non-specific symptoms of Pb toxicity are stunted growth, chlorosis and blackening of the root system (Sharma and Dubey 2005). During Pb treatment, any of toxicity symptoms such as necrosis, chlorosis, leaf fall etc., were not observed at 1 mg/L Pb. At 10 mg/L Pb, leaf fall and chlorosis were observed especially in old leaves. During the treatment, firstly chlorosis and leaf fall were observed, then brownish was occured being more marked in old leaves especially at 100 mg/L Pb concentration. Before these macroscopic visible dysfunctions, Pb concentration in macrophyte tissues probably disturbed several biochemical parameters at the cellular level.

Because of Pb toxicity, macrophyte growth was reduced. Dry biomass (g) of macrophyte was not significantly reduced with respect to control for *E. canadensis* at 1 mg/L Pb treatment ($p > 0.05$) (Fig. 1C). In other tested Pb concentrations dry biomasses decreased significantly with respect to control ($p < 0.05$). Correlation analysis showed that there was significant and negative relationship between Pb concentration in *E. canadensis* and dry biomass ($r = -8{,}568$; $p < 0.001$). Adverse effect of Pb on plant growth has been reported by many researchers as well (An 2006; Kopittke et al. 2007).

Chlorophyll content is a parameter that is sensitive to heavy metal toxicity (Gupta and Chandra 1996). Inhibition of chl level by Pb has been observed in aquatic macrophytes (Jana and Chaudhury 1984). According to this study, a dose-dependent reduction was found in total chl-a and chl-b content of *E. canadensis* leaves, as shown in Fig. 2A and 2C, respectively. The minimum content of chl-a and chl-b were determined in the leaves exposed to 100 mg/L Pb and reduction in chl-a and chl-b compared to their respective controls were estimated to be 41.9% and 35.5%, respectively ($p < 0.05$). Our earlier studies revealed that Pb application on aquatic macrophytes *T. latifolia* and *L. minor* decreased total chl content (Saygideger and Dogan 2004; Saygideger et al. 2004). The decreases in chl a content at 0.25, 0.5, 0.75 and 1 mg/L Pb in *L. minor* were 19.8%, 44.8%, 54.3% and 58.6%, respectively, after 4-days treatment. Similar decreases in chl-b content of *L. minor* were found. Reduction ranges of the chl-b at same duration and Pb concentrations were about 21.1%–55.6% (Mohan and Hosetti 1997). Regression analysis showed that there was significant and negative correlation between Pb concentration in *E. canadensis* and contents of chl-a ($r = -0.9045$; $p < 0.001$ (Fig. 2B) and chl-b ($r = -0.7143$; $p < 0.001$) (Fig. 2D). Similarly, carotenoid contents were also reduced by Pb application (Fig. 2E). Carotenoid content of macrophyte leaves decreased by 9.7%, 11.1% ($p > 0.05$) and 38.7% ($p < 0.05$) in 1, 10 and 100 mg/L Pb treatments, respectively, when compared with the control. There was

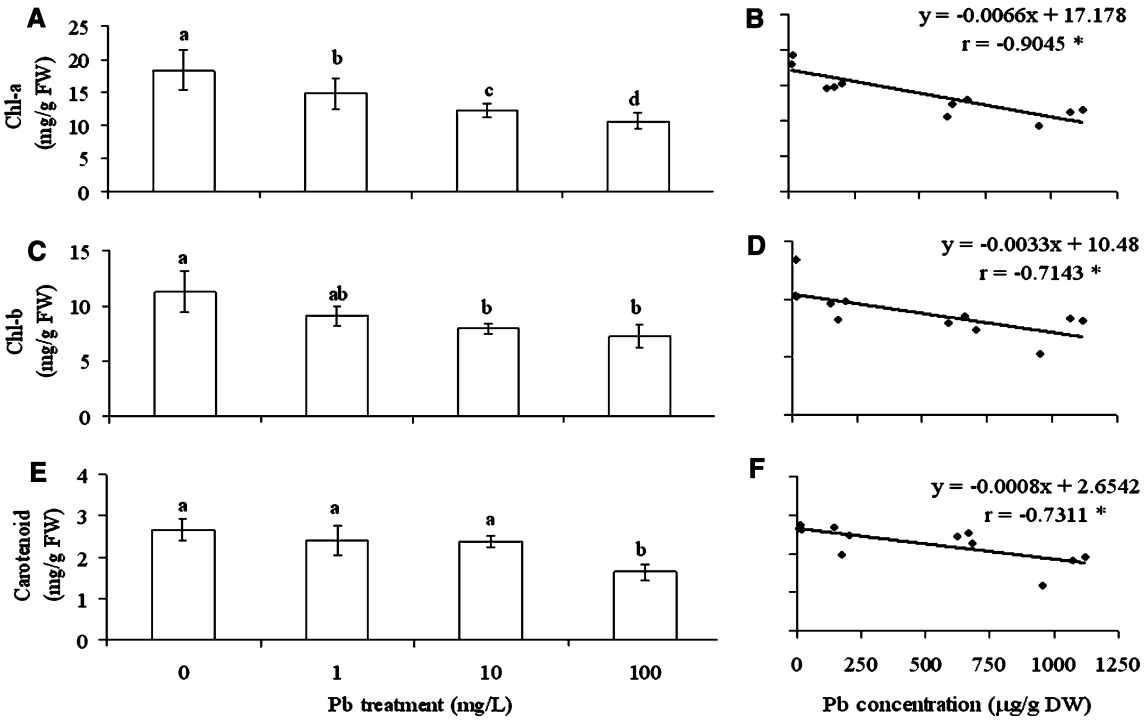

Fig. 2 Effect of Pb treatment on contents of chl-a, chl-b and carotenoid in *E. canadensis* and their statistical evaluations. *Error bars* represent the standard deviation of mean. (n = 3) Means with *different letters* are significantly different from one another according to LSD test ($p < 0.05$). Correlation analysis carried out between Pb concentration in *E. canadensis* and contents of chl-a, chl-b and carotenoid. (* $p < 0.001$)

negative correlation between Pb concentration in *E. canadensis* and carotenoid content ($r = -0.7311$; $p < 0.001$) (Fig. 2F). According to correlation analysis established, negative and significant findings may be due to Pb accumulation. Similar conclusions are indicated by many researchers (Abdel-Basset et al. 1995; Sharma and Gaur 1995). Van Assche and Clijsters (1990) reported that the reduction in chl content in the presence of the Pb may be due to an inhibition of chl biosynthesis. One of the important enzyme of chl biosynthesis is aminolevulinic acid dehydratase (ALAD) which catalyses the formation of porphobilinogen. It is explained that lead inhibits ALAD activity by binding with –SH group of the enzyme (Singh 1995).

Plants usually experience oxidative stress when they are exposed to Pb (Mishra et al. 2006). Pb is not a oxido-reducing metal like iron, therefore the oxidative stress induced by Pb in plants appears to be an indirect effect of Pb toxicity leading to production of ROS (Pinto et al. 2003). Plants possess several protective mechanisms to cope with ROS in which enzymes as well as reducing metabolites, such as AsA, GSH and α-tocopherol. Among these, AsA is a primary as well as a secondary antioxidant (Foyer 1993). As shown in Fig. 3A, AsA content in macrophyte increased with increasing Pb concentrations in the medium. At 5-days after Pb treatment, AsA content in the macrophyte increased by 18.5% ($p > 0.05$), 89.1% and 109.6% ($p < 0.05$) in 1, 10 and 100 mg/L Pb treatments, respectively, when compared with the control. Regression analysis showed that there was significant and positive correlation between Pb concentration in *E. canadensis* and AsA content ($r = 0.8857$; $p < 0.001$) (Fig. 3B). The increased AsA content exhibited that oxidative stress was occured in macrophyte by Pb concentration.

Most of the non-protein SH groups in plants represent glutathione (Grill et al. 1979). Glutathione is involved not only in the detoxification of ROS, but is also essential for the synthesis of metal-binding peptides such as phytochelatins, which inactivate and sequester heavy metals such as Cd, Pb, Hg by forming stable metal-complexes in the vacuole (Cobbett 2000; Hall 2002). Similar as AsA content, non-protein SH groups content in the macrophyte increased by 59.4% ($p > 0.05$), 95.1% and 241.3% ($p < 0.05$) in 1, 10 and 100 mg/L Pb treatments, respectively, when compared with the control (Fig. 3C). Regression analysis showed that there was significant and positive correlation between Pb concentration in *E. canadensis* and SH groups content ($r = 0.8956$; $p < 0.001$) (Fig. 3D). This indicates that non protein SH groups are possibly involved in Pb tolerance of *E. canadensis* either by increasing the antioxidative defense mechanism or enhancing Pb-binding peptides.

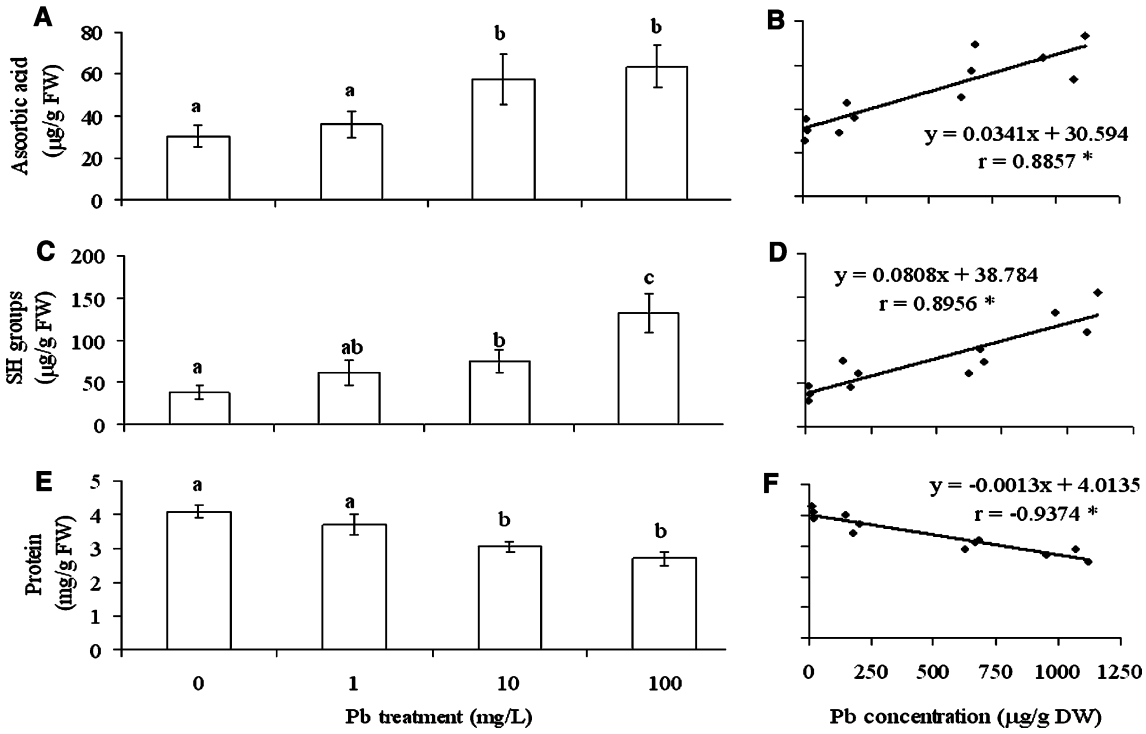

Fig. 3 Effect of Pb treatment on contents of ascorbic acid, SH groups and protein in *E. canadensis* and their statistical evulations. *Error bars* represent the standard deviation of mean. (n = 3) Means with *different letters* are significantly different from one another according to LSD test ($p < 0.05$). Correlation analysis carried out between Pb concentration in *E. canadensis* and contents of ascorbic acid, SH groups and protein. (* $p < 0.001$)

Many studies showed that the protein content of aquatic macrophytes was decreased by Pb accumulation (Gupta and Chandra 1994; Mohan and Hosetti 1997). The reduction in protein content was observed with a progressive increase in Pb concentration (Fig. 3E). Protein content in the macrophyte decreased by 9.8% ($p > 0.05$), 25.2% and 34.1% ($p < 0.05$) in 1, 10 and 100 mg/L Pb treatments, respectively, when compared with the control. Regression analysis showed that there was significant and negative correlation between Pb concentration in *E. canadensis* and protein content ($r = -0.9374$; $p < 0.001$) (Fig. 3F). Nitrogen is one of the primary essential nutrients involved as a constituent of biomolecules such as nucleic acids and proteins. Our earlier studies showed that Pb inhibited nitrogen uptake in aquatic macrophytes (Saygideger et al. 2004; Saygideger and Dogan 2005). During transport of heavy metals into the plants, it can act at different sites to inhibit a large number of enzymes having functional sulphydryl groups. It results in the deleterious effect in the normal protein form (Dua and Sawhney 1991) by disrupting the pathways and protein synthesis (Nagoor 1999). Decrease in protein content of macrophyte may be due to the above reasons.

The present study indicated that accumulation of Pb and its toxicity on chl-a, chl-b, carotenoid and protein contents in the macrophyte *E. canadensis* were dependent upon Pb concentration. Toxicity was also increased with increasing Pb concentration. Induction of non-protein SH groups and AsA showed that Pb accumulation caused oxidative stress. It is also possible that increased non-protein SH groups by Pb accumulation may be due to their role in their Pb detoxification. Results obtained in the present study can be a source for further investigation dealing with similar subjects.

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