Journal of Hazardous Materials 172 (2009) 479–484

Contents lists available at ScienceDirect

Journal of Hazardous Materials

journal homepage: www.elsevier.com/locate/jhazmat

Short Communication

Antioxidant defense mechanism in hydroponically grown *Zea mays* seedlings under moderate lead stress

D.K. Gupta [a,*,1], F.T. Nicoloso [a,1], M.R.C. Schetinger [b,1], L.V. Rossato [a], L.B. Pereira [b], G.Y. Castro [a], S. Srivastava [c], R.D. Tripathi [c]

[a] Department of Biology, University Federal de Santa Maria, Camobi-97105-900, Santa Maria, RS, Brazil
[b] Department of Chemistry, University Federal de Santa Maria, Camobi-97105-900, Santa Maria, RS, Brazil
[c] Ecotoxicology and Bioremediation Group, National Botanical Research Institute, Lucknow-226001, U.P., India

ARTICLE INFO

Article history:
Received 8 January 2009
Received in revised form 16 June 2009
Accepted 25 June 2009
Available online 3 July 2009

Keywords:
Lead
Antioxidants
Superoxide dismutase
δ-aminolevulinic acid dehydratase
Catalase
Lipid peroxidation

ABSTRACT

The present study was designed to study the process of stress adaptation in roots and shoot of *Zea mays* seedlings grown under hydroponic conditions during exposure to lead (Pb) (0–200 μM) for 1–7 d. The alterations in growth and in the level of various biochemical parameters were accessed vis-à-vis Pb accumulation. The accumulation of Pb increased in a concentration-duration-dependent manner, however its translocation from root to shoot was low. At the same time, the level of malondialdehyde (MDA) increased with increasing Pb concentration. However, growth parameters, such as dry weight and root length did not show a significant decline to any of the Pb concentrations. In addition, the level of photosynthetic pigments decreased only upon exposure to high Pb concentrations. These results suggested an alleviation of the stress that was presumably being achieved by antioxidants viz., superoxide dismutase (SOD) and catalase (CAT) as well as ascorbic acid (AsA), which increased linearly with increasing Pb levels and exposure time. However, the level of non-protein thiols (NP-SH) in roots, in general, showed a decline beyond 4 d that could be attributed to their consumption for the purpose of Pb detoxification. In conclusion, *Zea mays* can be used as an indicator species for Pb, and the various antioxidants might play a key role in the detoxification of Pb induced toxic effects.

© 2009 Elsevier B.V. All rights reserved.

1. Introduction

Heavy metal pollution of the environment is of major ecological concern due to its impact on human health through the food chain and its high persistence in the environment [1]. Lead (Pb) is one of the most abundant, globally distributed toxic elements. Its contamination results from mining and smelting activities, Pb containing paints, paper and pulp, gasoline and explosives as well as from the disposal of municipal sewage sludge enriched with Pb [2]. It exerts adverse effects on morphology, growth and photosynthetic processes of plants and causes inhibition of enzyme activities, water imbalance, and alterations in membrane permeability and disturbs mineral nutrition [2].

In plants, reactive oxygen species (ROS) are produced continuously as byproducts of various metabolic pathways that are localized in different cellular compartments [3,4]. However, under stressful conditions, their formation might increase to excess of antioxidant scavenging capacity, thus creating oxidative stress by reaction and damage to all biomolecules [5]. One of the most damaging effects of ROS and their products is the peroxidation of membrane lipids and ion leakage [2]. To control the level of ROS and to protect the cells, plants possess low molecular weight antioxidants (ascorbic acid (AsA), reduced glutathione (GSH), carotenoids, toco-pherols) and antioxidant enzymes such as superoxide dismutase (SOD), and catalase (CAT) [3]. SOD is the major superoxide radical scavenger and its enzymatic action results in H_2O_2 and O_2 formation. Since H_2O_2 itself is toxic, it is eliminated by conversion to H_2O in subsequent reactions by CAT. Antioxidants such as AsA and glutathione (GSH), which are found at high concentrations in chloroplasts and other cellular compartments (5–20 mM AsA and 1–5 mM GSH), are crucial for plant defense against oxidative stress [6].

Plants possess several other mechanisms that are involved in the detoxification of heavy metals to tolerate the stress, such as reduced uptake of heavy metals or efflux pumping of metals entered in the cytosol [7], binding to ligands viz., organic acids, amino acids, GSH or phytochelatins (PCs) to render them harmless at primary level of metal entry to the cell [8,9]. For Pb, binding to cell wall is one of the major mechanisms of detoxification [10].

Maize (*Zea mays*) is an important food crop plant all around the world and was selected for study because it can be used as an

* Corresponding author. Tel.: +55 55 32208339 213; fax: +55 55 32208022 201.
E-mail address: guptadk1971@gmail.com (D.K. Gupta).
[1] This work was jointly carried out by DKG, FTN and MRCS.

0304-3894/$ – see front matter © 2009 Elsevier B.V. All rights reserved.
doi:10.1016/j.jhazmat.2009.06.141

indicator species to assess ecotoxicity of soils polluted by contaminants and also due to the insufficient information available on Pb toxicity in maize. To get more information on the plant responses to Pb, in our present study, the effects of Pb on MDA concentration (lipid peroxidation), activities of δ-ALA-D, CAT and SOD, and concentration of AsA, NP-SH and total soluble proteins were analyzed under various Pb treatments.

2. Material and methods

2.1. Plant material and growth conditions

Seeds of *Zea mays* Variety BR-3060 were obtained from Feltrin Ltd., Santa Maria, RS, Brazil. Seeds were germinated under dark conditions at 23 ± 2 °C on filter paper humidified with distilled water for 7 d. After sufficient seedling growth, seedlings were transferred on plastic containers (10 L) filled with aerated full nutrient solution of low ionic strength. The nutrient solution had the composition ($mg\,L^{-1}$): 85.31 N; 7.54 P; 11.54 S; 97.64 Ca; 23.68 Mg; 104.75 K; 181.08 Cl; 0.27 B; 0.05 Mo; 0.01 Ni; 0.13 Zn; 0.03 Cu; 0.11 Mn and 2.68 Fe. The solution pH was maintained to 5.8 ± 0.1 by titration with HCl or NaOH solutions (0.1 M) when required. On the 14th d after transplant of seedlings, Lead as $Pb(NO_3)_2$ was added to each container to form five concentrations: 0 (control), 25, 50, 100 and 200 μM. The nutrient solution in the growth containers was renewed once a week. Cultured plants were grown in a growth chamber at 25 ± 1 °C during 16/8 light/dark cycle with 35 $\mu M\,m^{-2}\,s^{-1}$ of irradiance by cold fluorescent lamps. Lead treated seedlings were harvested totally at random on day 1, 4 and 7 as from the plastic containers. In each experiment, second and third leaves of seedlings were selected for analysis. All chemicals used were of analytical grade purchased from Sigma Chemical Company (USA).

2.2. Plant growth parameters

At harvest, the plants were divided into root and shoots. Roots were rinsed twice with distilled water. Subsequently, growth and biochemical parameters were determined. Plant biomass was measured on fresh and dry weight basis. For fresh weight, excess water from the root is dried from tissue paper and weighed on an electronic balance and expressed in $g\,plant^{-1}$. To obtain dry weight, roots and shoots were dried at 65 °C until reaching a constant weight and weighed on an electronic balance and expressed in $g\,plant^{-1}$. Root length was determined according to Tennant [11], and shoot length was measured by Varnier calipers, both expressed in $cm\,plant^{-1}$.

2.3. Metal estimation

Lead content was estimated in both root and shoots. Dried plant tissues, between 0.01–0.25 g, were ground and digested with 5 ml of concentrated HNO_3. Sample decomposition was carried out in an open digestion system, using a heating block Velp Scientific (Milano, Italy). Heating was set at 130 °C for 2 h. Plastic caps were fitted to the vessels to prevent losses by volatilization. The Pb content was determined by Inductively Coupled Plasma Optical Emission Spectrometry, using a PerkinElmer Optima 4300 DV (Shelton, USA) equipped with a cyclonic spray chamber and a concentric nebulizer. The emission line selected was 220.353 nm.

2.4. Total chlorophyll, carotenoids and total soluble protein determination

The content of chlorophyll was estimated by the method of Arnon [12] and carotenoid content was calculated by using the formula given by Duxbury and Yentsch [13]. Protein content was estimated following the method of Lowry et al. [14] using bovine serum albumin as standard.

2.5. Estimation of δ-aminolevulinic acid dehydratase (E.C. 4.2.1.24) activity

Leaves were homogenized in 10 mM Tris–HCl buffer, pH 9.0, at a proportion of 1:1 (w/v). The homogenate was centrifuged at 12,000 g at 4 °C for 10 min to yield a supernatant (S1) that was used for the enzyme assay. The supernatant was pre-treated with 0.1% Triton X-100 and 0.5 mM dithiothreitol. ALA-D activity was assayed as described by Morsch et al. [15] by measuring the rate of porphobilinogen (PBG) formation. The incubation medium for the assays contained 100 mM Tris–HCl buffer, pH 9.0. For the enzyme assay, the final concentration of ALA was 3.6 mM. Incubation was started by adding 100 μl of the tissue preparation to a final volume of 400 μl. The product of the reaction was determined with the Ehrlich reagent at 555 nm using a molar absorption coefficient of $6.1 \times 10^4\,l\,mol^{-1}\,cm^{-1}$ for the Ehrlich-PBG salt. ALA-D activity was expressed as nmol PBG mg^{-1} protein h^{-1}.

2.6. Estimation of lipid peroxidation, catalase (E.C. 1.11.1.6), superoxide dismutase (E.C. 1.15.1.1), ascorbic acid and non-protein thiol concentration

The degree of lipid peroxidation was estimated following the method of El-Moshaty et al. [16]. Catalase (CAT) activity was assayed following the method of Aebi [17]. The activity of superoxide dismutase (SOD) was assayed according to Misra and Fridovich [18]. Ascorbic acid determination was performed as described by Jacques-Silva et al. [19]. Non-protein thiols concentration was measured spectrophotometrically with Ellman's reagent [20].

2.7. Statistical analysis

The experiment was done as randomized block design. Two-way analysis of variance (ANOVA) was done with all the data to confirm the variability of data and validity of results, and Duncan's multiple range test (DMRT) was performed to determine the significant difference between treatments. Graphical work was carried out using Origin v.7.0.

3. Results and discussion

3.1. Absorption of Pb

When *Zea mays* seedlings were raised under increasing concentrations of Pb, plants accumulated significant amounts of Pb in concentration duration-dependent manner (Fig. 1A and B). The Pb levels in roots and shoots showed positive linear relationships with the Pb concentration in the nutrient solution. The maximum Pb concentration was found to be 70,425 $\mu g\,g^{-1}$ DW in roots and 995 $\mu g\,g^{-1}$ DW in shoots at 200 μM after 7 d of Pb treatment. Hence, translocation of Pb from root to shoot was very low. Based on comparative studies of metal content in plant parts, Baker and Walker [21] suggested that uptake, translocation and accumulation mechanisms differed for various heavy metals and for the species. It is known that the root system partially defends the above ground parts from Pb [22,23] as was found in the present study. Mostly, the plants with highest tolerance take-up the smallest proportion of the total soil metal and had the lowest shoot metal contents [24].

3.2. Effect of Pb on seedling growth

In general, Pb treatment reduced the root fresh biomass in comparison to control on 1 and 7 d of treatment (Table 1). In the case of

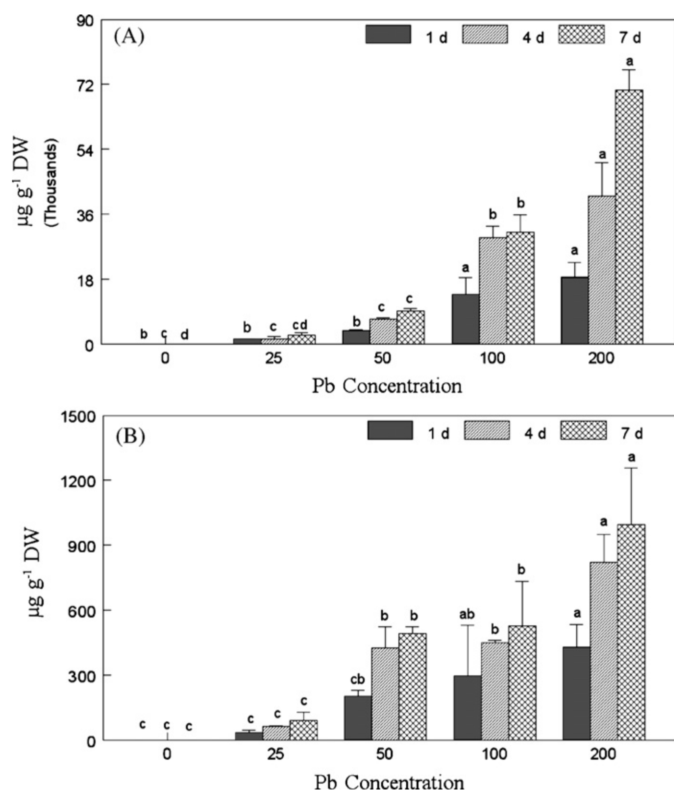

Fig. 1. Pb accumulation by *Zea mays* seedlings ((A) root (B) shoot) treated with different concentrations of Pb for 1, 4 and 7 d. All the values are mean of triplicates ± SD. ANOVA significant at $p < 0.01$. Different letters indicate significantly different values at a particular duration (DMRT, $p < 0.05$).

shoot, fresh weight was not altered in any of treatments as compared to control. Both root and shoot dry weight did not alter upon addition of Pb, with exception after 7 d where shoot dry weight decreased at 200 μM Pb, as compared to control (Table 1). Root length did not show alteration upon addition of any Pb treatment, whereas shoot length was decreased after 7 d at 200 μM Pb (Table 1). Hence, seedlings showed slight reduction of fresh and dry biomass of both root and shoot at higher concentrations after 7 d. Similar types of results were reported by Dey et al. [25] in hydroponically grown wheat seedlings exposed to Cd and Pb. Tanyolac et al. [26] also reported similar type of results in *Zea mays* with Cu stress.

3.3. *Effect of Pb on the level of photosynthetic pigments and total soluble proteins and the activity of δ-ALA-D*

A duration-dependent response of Pb stress was observed on photosynthetic pigments (Table 2). At 1 d after Pb treatment, total chlorophyll increased at 200 μM, on 4 d it remained unchanged, whereas on 7 d it decreased at 200 μM as compared to control. Total carotenoids were decreased only in the presence of 200 μM Pb on 7 d.

Total soluble proteins in roots were increased on 1 d (50, 100 and 200 μM), whereas on 4 d it either increased up to 100 μM or decreased at 200 μM of Pb. On 7 d, proteins declined at all Pb concentrations as compared to control. Shoot total soluble protein concentration increased at 25 μM of Pb on 1 d, whereas it decreased at 100 and 200 μM for 4 d seedlings. Conversely, after prolonged exposure (7 d) it either decreased at 25 and 50 μM of Pb or increased at 100 and 200 μM of Pb.

A duration-dependent response to Pb stress was observed in δ-ALA-D activity (Table 3). On 1 d after Pb treatment, δ-ALA-D activity increased upon addition of 25 and 50 μM Pb, compared to control. Conversely, after 4 d of Pb treatment at 100 and 200 μM, δ-ALA-D activity decreased significantly. On 7 d, δ-ALA-D activity decreased at all Pb levels.

A decline in the level of photosynthetic pigments may be attributed to Pb induced inhibition of chlorophyll and carotenoid biosynthesis [27] that may be caused by the induced nutrient deficiency, such as that of iron [28] as well as due to a reduced δ-ALA-D activity observed in the present study. The decrease in net photosynthesis as a consequence of reduced absorption of essential mineral nutrients is an indirect reason for plant chlorosis [28]. Similar type of result was reported by Tanyolac et al. [26] with *Zea maize* stressed by Cu. Total soluble protein concentration was decreased at higher concentration of Pb in roots as compared to control but was found to increase in shoots after 7 d. It seems that due to high Pb load in roots, there might have greater generation of ROS and hence, more oxidative stress that might have resulted in decline of protein concentration through oxidative damage. The increase observed in shoots might be attributed to induced stress proteins such as antioxidant enzymes and heat shock proteins [29].

Table 1
Effect of Pb concentrations on fresh weight, dry weight and length of *Zea mays* seedlings after addition of different concentrations of Pb for 1, 4 and 7 d.

Pb in μM	Root			Shoot		
	1 d	4 d	7 d	1 d	4 d	7 d
	Fresh weight (g plant^{-1})					
Control	1.27 ± 0.45a	1.37 ± 0.31a	1.58 ± 0.01ab	1.57 ± 0.01a	2.46 ± 0.41ab	3.85 ± 0.41a
25	0.94 ± 0.30ab	1.03 ± 0.17a	1.75 ± 0.74a	2.86 ± 1.26a	3.21 ± 0.55ab	4.28 ± 1.41a
50	0.65 ± 1.04ab	1.56 ± 0.01a	1.59 ± 0.00ab	2.23 ± 0.57a	3.00 ± 0.77a	3.28 ± 0.91a
100	0.63 ± 0.34ab	1.56 ± 0.01a	0.83 ± 0.11b	1.57 ± 0.01a	2.92 ± 0.48a	3.31 ± 0.24a
200	0.28 ± 0.09b	1.24 ± 0.57a	0.80 ± 0.34b	1.56 ± 0.02a	1.57 ± 0.02b	3.08 ± 0.73a
	Dry weight (g plant^{-1})					
Control	0.042 ± 0.02a	0.055 ± 0.01a	0.058 ± 0.02a	0.191 ± 0.04a	0.220 ± 0.04a	0.312 ± 0.09a
25	0.053 ± 0.01a	0.057 ± 0.01a	0.063 ± 0.02a	0.222 ± 0.04a	0.248 ± 0.05a	0.287 ± 0.03ab
50	0.042 ± 0.02a	0.055 ± 0.02a	0.063 ± 0.01a	0.218 ± 0.01a	0.222 ± 0.08a	0.265 ± 0.04ab
100	0.050 ± 0.01a	0.048 ± 0.01a	0.047 ± 0.01a	0.204 ± 0.04a	0.211 ± 0.06a	0.241 ± 0.02ab
200	0.048 ± 0.00a	0.045 ± 0.01a	0.035 ± 0.00a	0.180 ± 0.07a	0.194 ± 0.04a	0.209 ± 0.02b
	Plant length (cm plant^{-1})					
Control	155.9 ± 24.8a	179.5 ± 10.6a	195.7 ± 50.9a	29.7 ± 6.02a	37.1 ± 3.80a	40.6 ± 7.67a
25	115.9 ± 47.5a	162.6 ± 67.2a	185.3 ± 66.7a	31.8 ± 4.26a	32.4 ± 4.75a	39.1 ± 1.07ab
50	114.1 ± 7.6a	152.5 ± 45.6a	172.7 ± 26.9a	30.9 ± 2.05a	34.7 ± 6.66a	37.3 ± 2.13ab
100	104.2 ± 20.4a	170.8 ± 21.9a	156.9 ± 24.3a	30.8 ± 6.91a	32.1 ± 3.31a	34.9 ± 3.78ab
200	137.8 ± 46.2a	132.8 ± 40.2a	129.6 ± 37.1a	30.1 ± 2.85a	30.1 ± 4.03a	31.0 ± 3.27b

All the values are mean of triplicates ± SD. ANOVA significant at $p < 0.01$. Different letters indicate significantly different values at a particular duration (DMRT, $p < 0.05$).

Table 2
Effect of Pb concentrations on total chlorophyll, total carotenoids and on total soluble protein concentrations in *Zea mays* seedlings after addition of different concentrations of Pb for 1, 4 and 7 d.

Pb in μM	Total Chlorophyll (mg g^{-1} FW)			Total Carotenoids (mg g^{-1} FW)		
	1 d	4 d	7 d	1 d	4 d	7 d
Control	1.56 ± 0.24b	1.80 ± 0.07a	1.99 ± 0.08a	0.43 ± 0.08ab	0.47 ± 0.05a	0.54 ± 0.03a
25	1.71 ± 0.17ab	1.80 ± 0.13a	2.00 ± 0.07a	0.46 ± 0.08ab	0.50 ± 0.03a	0.57 ± 0.01a
50	1.71 ± 0.29ab	1.91 ± 0.03a	1.88 ± 0.10ab	0.42 ± 0.16b	0.53 ± 0.01a	0.52 ± 0.03ab
100	1.80 ± 0.09ab	1.96 ± 0.05a	1.86 ± 0.02ab	0.49 ± 0.03ab	0.54 ± 0.02a	0.52 0.01ab
200	1.91 ± 0.08a	1.85 ± 0.16a	1.65 ± 0.14b	0.53 ± 0.03a	0.52 ± 0.04a	0.43 ± 0.04b
Pb in μM	Total soluble protein (mg g^{-1} FW) Root			Shoot		
Control	0.76 ± 0.03c	1.11 ± 0.05c	0.96 ± 0.03a	2.03 ± 0.05b	1.89 ± 0.04a	1.54 ± 0.02b
25	0.73 ± 0.05c	1.43 ± 0.06a	0.75 ± 0.04c	2.13 ± 0.04a	1.96 ± 0.03a	1.24 ± 0.05c
50	1.13 ± 0.01a	1.23 ± 0.02b	0.96 ± 0.03a	2.03 ± 0.05b	1.92 ± 0.04a	1.05 ± 0.04d
100	0.89 ± 0.04b	1.22 ± 0.03b	0.80 ± 0.08cb	2.05 ± 0.04b	1.64 ± 0.04b	1.78 ± 0.05a
200	0.95 ± 0.03b	1.01 ± 0.04d	0.85 ± 0.04b	2.05 ± 0.03b	1.39 ± 0.04c	1.81 ± 0.03a

All the values are mean of triplicates ± SD. ANOVA significant at $p < 0.01$. Different letters indicate significantly different values at a particular duration (DMRT, $p < 0.05$).

Table 3
Effect of Pb concentrations on δ-aminolevulinic acid dehydratase (δ-ALA-D) contents in *Zea maize* seedlings after addition of different concentrations of Pb for 1, 4 and 7 d.

Pb in μM	δ-ALA-D (nmol PBG mg^{-1} protein h^{-1})		
	1 d	4 d	7 d
Control	1.11 ± 0.05cd	1.24 ± 0.02a	1.80 ± 0.23a
25	1.87 ± 0.11a	1.23 ± 0.01a	1.04 ± 0.01b
50	1.49 ± 0.05b	1.12 ± 0.02ab	1.00 ± 0.02b
100	1.19 ± 0.01c	1.05 ± 0.03bc	0.87 ± 0.01c
200	1.00 ± 0.01d	0.94 ± 0.05c	0.65 ± 0.08d

All the values are mean of triplicates ± SD. ANOVA significant at $p < 0.01$. Different letters indicate significantly different values at a particular duration (DMRT, $p < 0.05$).

The decrease observed with prolonged Pb exposure (4 and 7 d) in δ-ALA-D activity may be due to some enzymatic changes and production of ROS in the plants. Noriega [30] also got a similar type of result when soybean plants were treated with Cd, which suggests that accumulation of ALA in the chlorophyll and heme pathways due to ALA-D inhibition, and their consequent generations of ROS are indeed highly responsible for the deleterious action of Cd^{2+} in soybean.

3.4. Effect of Pb on lipid peroxidation

Significant increases in MDA concentration, a measure of oxidative-stress-inducible peroxidation of membrane lipids, in both roots and shoots were observed (Fig. 2). On 1 d, root MDA concentration remained unchanged. Conversely, after prolonged exposure (4 and 7 d), root MDA concentration increased linearly with increased Pb levels in the solution. In case of shoot, MDA concentration was linearly increased with increasing Pb levels independently of duration of exposure. Similar results were found with two cultivars of Horsegram and Bengalgram [4]. Thus, the increased MDA indicates the prevalence of oxidative stress and perhaps this may be one of the possible mechanisms by which toxicity due to Pb could be manifested in the plant tissues. However, the level of lipid peroxidation was lower in roots as compared to that of shoots. This was attributed to low Pb load in shoots that probably resulted in low oxidative stress and membrane damage. This might be also be linked to an inability of the antioxidative enzymes to tolerate such a high Pb load despite the fact that antioxidative enzymes increased significantly [25].

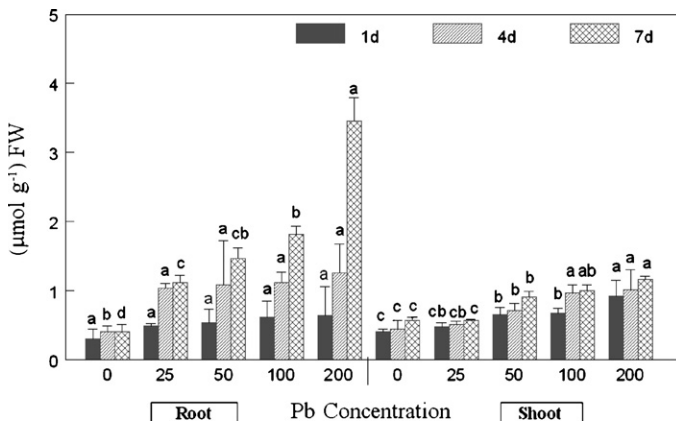

Fig. 2. Effect of Pb concentrations on lipid peroxidation (MDA) in *Zea mays* seedlings treated with different concentrations of Pb for 1, 4 and 7 d. All the values are mean of triplicates ± SD. ANOVA significant at $p < 0.01$. Different letters indicate significantly different values at a particular duration (DMRT, $p < 0.05$).

3.5. Effect of Pb on non-protein thiol and ascorbic acid concentrations

A time and organ-dependent response to Pb stress was observed on NP-SH concentration (Fig. 3A). At the first point of time after Pb exposure (1 d), root NP-SH concentration increased at 100 and 200 μM Pb. Conversely, on 4 and 7 d, it decreased upon addition of Pb levels exceeding 25 μM Pb. Shoot NP-SH concentration increased at 25 μM on 1 and 7 d of Pb treatment, whereas it remained unchanged at most of the exposures.

A time and organ-dependent response to Pb stress was also observed on AsA concentration (Fig. 3B). On 1 d root AsA concentration increased significantly only at 200 μM Pb, compared to control. On 4 d of Pb exposure, root AsA concentration increased only at 100 μM, whereas after prolonged exposure (7 d) it increased at 25, 100 and 200 μM. Shoot AsA concentration increased at 100 and 200 μM Pb at all durations.

An enhancement in NP-SH concentration may be due to an increase of phytochelatins (PCs) and was also consistent with other investigations that have shown increased NP-SH in plants exposed to Pb [31]. The synthesis of PCs could mitigate Pb toxicity by complexing the metal and is an important mechanism of plant adaptation to long periods of metal exposure [31]. This increase in the level of thiols may be due to stimulation of enzymes of the sulfate reduction pathway such as adenosine 5′-phosphosulfate reductase and serine acetyltransferase [32]. The enhancement in NP-SH concentration possibly also reflects a defense reaction to enhanced production of ROS [33] since NP-SH also constitute GSH as one of the major fraction, which also antioxidant properties. In addition, an increase in AsA concentration would have improved

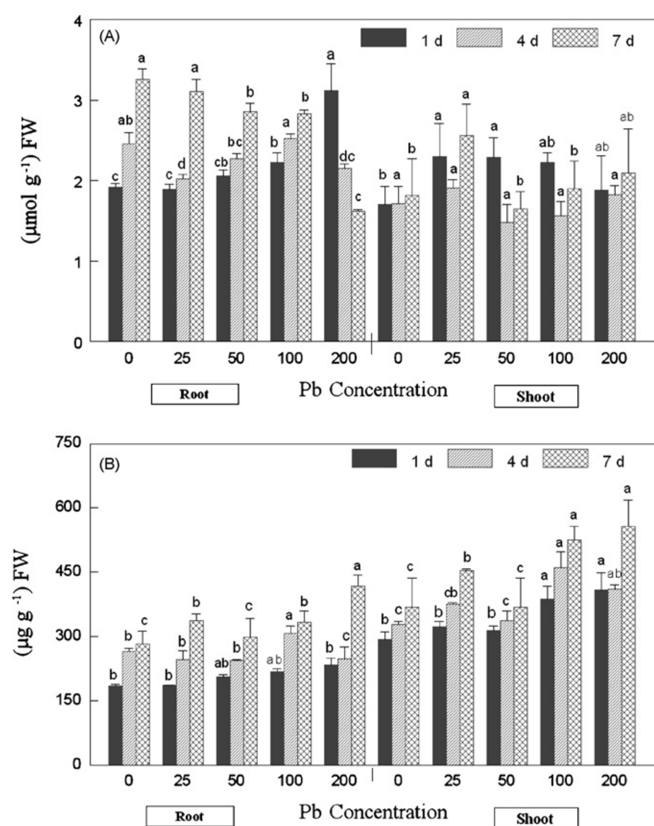

Fig. 3. Effect of Pb concentrations on non protein thiol (NP-SH) and ascorbic acid (AsA) concentrations in *Zea mays* seedlings ((A) NP-SH (B) AsA) treated with different concentrations of Pb for 1, 4 and 7 d. All the values are mean of triplicates ± SD. ANOVA significant at $p < 0.01$. Different letters indicate significantly different values at a particular duration (DMRT, $p < 0.05$).

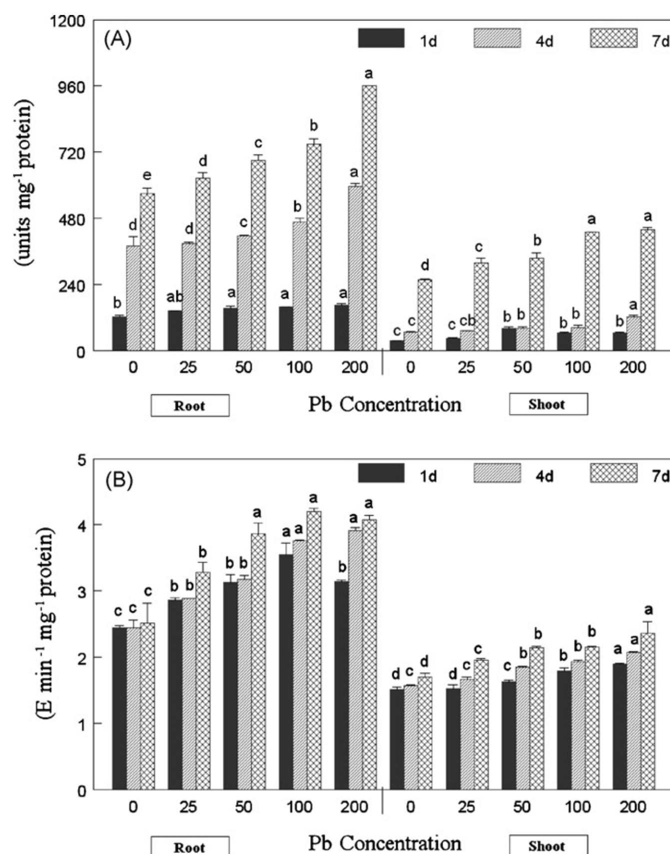

Fig. 4. Effect of Pb concentrations on (A) superoxide dismutase activity (SOD) and on (B) catalase activity (CAT) in *Zea mays* seedlings treated with different concentrations of Pb for 1, 4 and 7 d. All the values are mean of triplicates ± SD. ANOVA significant at $p < 0.01$. Different letters indicate significantly different values at a particular duration (DMRT, $p < 0.05$).

plants antioxidant capacity to Pb induced stress [33]. Liu et al. [34] got similar results with Cd. The enhancement of AsA levels together with the marked increase in NP-SH concentration thus might have effectively taken care of enhanced production of ROS.

3.6. Effect of Pb on superoxide dismutase and on catalase activity

SOD activity was greater in roots than in shoots independently of duration of Pb exposure (Fig. 4A). For both root and shoots, SOD activity increased linearly with increasing Pb levels, but such an increase was greater after prolonged exposure (7 d). CAT activity showed the same trend like SOD activity (Fig. 4B). The maximum CAT activity was recorded after 7 d, i.e. 4.07 and 2.37 $\Delta E\ min^{-1}\ mg^{-1}$ protein, respectively, in roots and shoots of 200 μM Pb treated seedlings.

An increase in SOD activity could possibly be the result of both a direct effect of heavy metal ions and an indirect effect mediated via an increase in levels of superoxide radicals [35]. The effect of Pb stress on SOD expression is likely to be governed by the tissue and sub cellular sites at which oxidative stress is generated as supported by the higher activity of SOD in roots than in leaves of Pb stressed plants. Similar results were also reported for two populations of *Rumex dentatus* in which the resistant varieties had higher SOD activities in the leaves [22,26]. Exposure to higher Cu concentrations also resulted in significant rises in the activities of SOD in *Zea mays* compared with the control [26]. Increase in SOD activity may be linked to an increase in superoxide radical formation as well as to *de novo* synthesis of enzyme protein [36], which in turn may be associated with an induction of genes of SOD by superoxide-mediated signal transduction [37]. An increase in CAT activity was also observed by Vitória et al. [38] for *Raphanus sativus*. CAT is only present in peroxisomes, but it is indispensable for ROS detoxification during stress, when high levels of ROS are produced [39].

4. Conclusion

From our present result we conclude that *Zea mays* can accumulate a high amount of available Pb from the hydroponic medium, however can translocate only a low amount in the upper part of the plant. Pb accumulation resulted in oxidative stress but it was efficiency controlled due to significant increases in antioxidants. Hence, growth of seedlings was affected only to a low extent. So, this plant can be grown in the Pb polluted area as a Pb indicator species, but further study is required to check Pb concentration in the seeds as to whether the translocation of metal is within permissible limits or not for human use.

Acknowledgements

DKG is thankful to Third World Academy of Science (TWAS), Italy and CNPq, Brazil for providing travel and research grant at University Federal de Santa Maria, Brazil. Authors are also thankful to James Macfarlane, 1, Westfield's, Saffron Walden, Cambridge, U.K. for critically checking English language.

References

[1] A. Piechalak, B. Tomaszewska, D. Baralkiewicz, A. Malecka, Accumulation and detoxification of lead ions in legumes, Phytochemistry 60 (2002) 212–218.
[2] P. Sharma, R.S. Dubey, Lead toxicity in plants, Braz. J. Plant Physiol. 17 (2005) 35–52.
[3] P.L. Gratão, A. Polle, P.J. Lea, R.A. Azevedo, Making the life of heavy-metal stressed plants a little easier, Funct. Plant Biol. 32 (2005) 481–494.
[4] A.M. Reddy, S.G. Kumar, G. Jyothsnakumari, S. Thimmanaik, C. Sudhakar, Lead induced changes in antioxidant metabolism of horsegram (*Macrotyloma uniflorum* (Lam.) Verdc.) and bengalgram (*Cicer arietinum* L.), Chemosphere 60 (2005) 97–104.
[5] M.J. Davies, Singlet oxygen-mediated damage to proteins and its consequences, Biochem. Biophys. Res. Comm. 305 (2003) 761–770.
[6] G. Noctor, C. Foyer, Ascorbate and glutathione: keeping active oxygen under control, Annu. Rev. Plant Physiol. Plant Mol. Biol. 49 (1998) 249–279.
[7] J.L. Hall, Cellular mechanism for heavy metal detoxification and tolerance, J. Exp. Bot. 53 (2002) 1–11.
[8] A. Malecka, W. Jarmuszkiewicz, B. Tomaszewska, Antioxidant defense to lead stress in sub cellular compartments of pea root cells, Acta. Biochim. Polon. 48 (2001) 687–698.
[9] R. Kneer, M.H. Zank, Phytochelatins protect plant enzymes from heavy metal poisoning, Photochemistry 31 (1992) 2663–2667.
[10] D. Antosiewicz, M. Wierzbicka, Localization of lead in *Allium cepa* L. cells by electron microscopy, J. Microscopy 195 (1999) 139–146.
[11] D. Tennant, A test of a modified line intersects method of estimating root length, J. Ecol. 63 (1975) 995–1001.
[12] D.I. Arnon, Copper enzymes in isolated chloroplasts: polyphenoloxidases in *Beta vulgaris*, Plant Physiol. 24 (1949) 1–15.
[13] A.C. Duxbury, C.S. Yentsch, Plankton pigment monograph, J. Mar. Res. 15 (1956) 93–101.
[14] O.H. Lowry, N.J. Rosenberg, A.L. Farr, R.J. Randall, Protein measurement with folin phenol reagent, J. Biol. Chem. 193 (1951) 265–275.
[15] V.M. Morsch, M.R.C. Schetinger, A.F. Martins, J.B.T. Rocha, Effects of cadmium, lead, mercury and zinc on δ-aminolevulinic acid dehydratase activity from radish leaves, Biologia Plantarum 45 (2002) 85–89.
[16] F.I.B. El-Moshaty, S.M. Pike, A.J. Novacky, O.P. Sehgal, Lipid peroxidation and superoxide productions in cowpea (*Vigna unguicultata*) leaves infected with tobacco rings virus or southern bean mosaic virus, Physiol. Mol. Plant Pathol. 43 (1993) 109–119.
[17] H. Aebi, Catalase *in vitro*, Methods Enzymol. 105 (1984) 121–126.
[18] H.P. Misra, I. Fridovich, The role of superoxide anion in the autoxidation of epinephrine and simple assay for superoxide dismutase, J. Biol. Chem. 244 (1972) 6049–6055.
[19] M.C. Jacques-Silva, C.W. Nogueira, L.C. Broch, E.M.M. Flores, J.B.T. Rocha, Diphenyl diselenide and ascorbic acid changes deposition of selenium and ascorbic acid in liver and brain of mice, Pharmacol. Toxicol. 88 (2001) 119–125.
[20] G.G. Ellman, Tissue sulphydryl and groups, Arch. Biochem. Biophys. 82 (1959) 70–77.
[21] A.J.M. Baker, P.L Walker, Ecophysiology of metal uptake by tolerant plants, in: A.J. Shaw (Ed.), Heavy Metal Tolerance in Plants: Evolutionary Aspects, CRC Press, Boca Raton, FL, 1990, pp. 155–177.
[22] T.C. Broyer, C.M. Johnson, R.E. Paul, Some aspects of lead in plant nutrition, Plant Soil 36 (1972) 301–313.
[23] S. Mazhoudi, A. Chaoui, M.H. Ghorbal, E. El-Ferjani, Response of antioxidant enzymes to excess copper in tomato (*Lycopercicon esculentum*, Mill), Plant Sci. 127 (1997) 129–137.
[24] J. Liu, K. Li, J. Xu, Z. Zhang, T. Ma, X. Lu, J. Yang, Q. Zhu, Lead toxicity, uptake and translocation in different rice cultivars, Plant Sci. 165 (2004) 793–802.
[25] S. Dey, J. Dey, S. Patra, D. Pothal, Changes in the antioxidative enzyme activities and lipid peroxidation in wheat seedlings exposed to cadmium and lead stress, Braz. J. Plant Physiol. 19 (2007) 53–60.
[26] D. Tanyolac, Y. Ekmekci, S. Unalan, Changes in photochemical and antioxidant enzyme activities in maize (*Zea maize* L.) leaves exposed to excess copper, Chemosphere 67 (2007) 89–98.
[27] C. Boswell, N.C. Sharma, S.V. Sahi, Copper tolerance and accumulation potential of *Chalamydomonas reinhardtii*, Bull. Environ. Contam. Toxicol. 69 (2002) 546–553.
[28] M.D. Vazquez, C. Poschenrieder, J. Barcelo, Chromium (VI) induced structural and ultra structural changes in bush bean plants (*Phaseolus vulgaris*), Ann. Bot. 59 (1987) 427–438.
[29] T.K. Prassad, Mechanisms of chilling-induced oxidative stress injury and tolerance in developing maize seedlings: changes in antioxidant system, oxidation of proteins and lipids, and protease activities, Plant J. 10 (1996) 1017–1026.
[30] G.O. Noriega, K.B. Balestrasse, A. Batlle, M.L. Tomaro, Cadmium induced oxidative stress in soybean plants also by the accumulation of delta-aminolevulinic acid, Biometals 20 (2007) 841–851.
[31] S. Mishra, S. Srivastava, R.D. Tripathi, R. Kumar, C.S. Seth, D.K. Gupta, Lead detoxification by coontail (*Ceratophyllum demersum* L.) involves induction of phytochelatins and antioxidant system in response to its accumulation, Chemosphere 65 (2006) 1027–1039.
[32] G. Noctor, A.C.M. Arisi, L. Jouanin, K.J. Kunert, H. Rennenberg, C.H. Foyer, Glutathione biosynthesis, metabolism and relationship to stress tolerance explored in transformed plants, J. Exp. Bot. 49 (1998) 623–647.
[33] M. Tiryakioglu, S. Eker, F. Ozkutlu, S. Husted, I. Cakmak, Antioxidant defense system and cadmium uptake in barley genotypes differing in cadmium tolerance, J. Trace Elem. Med. Biol. 20 (2006) 181–189.
[34] Y. Liu, X. Wang, G. Zeng, D. Qu, J. Gu, M. Zhou, L. Chai, Cadmium-induced oxidative stress and response of the ascorbate glutathione cycle in *Bechmeria nivea* (L.) Gaud, Chemosphere 69 (2007) 99–107.
[35] P. Chongpraditnum, S. Mori, M. Chino, Excess copper induces a cytosolic Cu, Zn-superoxide dismutase in soybean root, Plant Cell Physiol. 33 (1992) 239–244.
[36] S. Verma, R.S. Dubey, Lead toxicity induces lipid peroxidation and alters the activities of antioxidant enzymes in growing rice plants, Plant Sci. 164 (2003) 645–655.
[37] R.A. Fátima, M. Ahmad, Certain antioxidant enzymes of *Allium cepa* as biomarkers for the detection of toxic heavy metals in wastewater, Sci. Total Environ. 346 (2005) 256–273.
[38] A.P. Vitória, P.J. Lea, R.A. Azevedo, Antioxidant enzymes responses to cadmium in radish tissues, Phytochemistry 57 (2001) 701–710.
[39] H. Willekens, S. Chamnongpol, M. Davey, M. Schraudner, C. Langebartels, M. Van-Montagu, D. Inzé, W. Van-Camp, Catalase is a sink for H_2O_2 and is indispensable for stress defence in C-3 plants, EMBO J. 16 (1997) 4806–4816.