

# Biochemical Adaptations in *Zea mays* Roots to Short-Term Pb<sup>2+</sup> Exposure: ROS Generation and Metabolism

Gurpreet Kaur<sup>1</sup> · Shubhpreet Kaur<sup>2</sup> · Harminder Pal Singh<sup>1</sup> · Daizy Rani Batish<sup>2</sup> · Ravinder Kumar Kohli<sup>2</sup> · Valbha Rishi<sup>3</sup>

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**Abstract** The present study investigated the effect of lead (0, 16, 40 and 80 mg L<sup>-1</sup> Pb<sup>2+</sup>) exposure for 3, 12 and 24 h on root biochemistry in hydroponically grown *Zea mays* (maize). Pb<sup>2+</sup> exposure (80 mg L<sup>-1</sup>) enhanced malondialdehyde content (239 %–427 %), reactive carbonyl groups (425 %–512 %) and H<sub>2</sub>O<sub>2</sub> (129 %–294 %) accumulation during 3–24 h of treatment, thereby indicating cellular peroxidation and oxidative damage. The quantitative estimations were in accordance with in situ detection of ROS generation (using 2',7'-dichlorodihydrofluorescein diacetate dye) and H<sub>2</sub>O<sub>2</sub> accumulation. Pb<sup>2+</sup> treatment significantly reduced ascorbate and glutathione content during 3–24 h of exposure. On the contrary, levels of non-protein thiols were enhanced by 3–11.8 time over control in response to 16–80 mg L<sup>-1</sup> Pb<sup>2+</sup> treatment, after 24 h. A dose-dependent induction in ascorbate peroxidase and lipoxygenase enzyme activity was observed in *Z. mays* roots. The activities of ascorbate-recycling enzymes (dehydroascorbate reductase and monodehydroascorbate reductase) were significantly increased in relation to concentration and duration of Pb<sup>2+</sup> treatment. The study concludes that Pb<sup>2+</sup>-exposure induces ROS-mediated oxidative damage during early period of exposure despite the upregulation of enzymes of ascorbate–glutathione cycle.

**Keywords** Cellular peroxidation · ROS accumulation · Cell death · Defense mechanisms

Lead (Pb), a contaminant of almost every ecosystem (soil, water, atmosphere and living organisms), enters the plant system through soil (Sharma and Dubey 2005) or via foliar uptake (Uzu et al. 2010). Pb is transmitted from soil to the living organisms, via – the food chain, and it has now become necessary to explore the phytotoxic effect of Pb, especially in the context of food quality. It induces a broad range of toxic effects in plants, such as, it impairs plant growth, affects root elongation, seed germination, seedling development, transpiration, photosynthesis and cell division (Sharma and Dubey 2005; Kaur et al. 2014). However, plants respond to different intensities of Pb stress in a different manner, since it depends on the amount and duration of the exposure, plant developmental stage and specific organ under observation. At low Pb levels, sometimes plants show adaptive response (Gupta et al. 2009); whereas, at high Pb levels plants fail to combat Pb-induced oxidative stress (Kaur et al. 2012). To deal with metal toxicity, plants selectively uptake metals, form complexes with specific ligands, follow excretion and do compartmentalization (Krzyszowska et al. 2010; Maestri et al. 2010). Different responses of plants towards Pb exposure serve as bioindicators and help us to understand the mechanisms involved in uptake, transfer and toxicity (Pourrut et al. 2011). Pb primarily targets biochemicals and enzymes involved in the physiological processes, thus, affecting plant growth and causing visible injury (Sengar et al. 2008). Pb toxicity has been investigated in various crop plants like wheat (Kaur et al. 2012); maize (Gupta et al. 2009), pea (Malecka et al. 2009) and onion (Kaur et al. 2014). Most of the studies have been conducted under

✉ Harminder Pal Singh  
hpsingh\_01@yahoo.com

<sup>1</sup> Department of Environment Studies, Panjab University, Chandigarh, India

<sup>2</sup> Department of Botany, Panjab University, Chandigarh, India

<sup>3</sup> Chandigarh College of Engineering and Technology, Chandigarh, India

longer periods of exposure, ranging from 24 h to few weeks. However, not much information is available for cellular peroxidation (lipid and protein), ROS (reactive oxygen species) generation, non-protein thiols (NPTs) and modulation of ascorbate–glutathione cycle in *Zea mays* (maize) during early period of exposure (3–24 h). We chose *Z. mays* as a test plant because it is one of the most important cereal crops of the world, has high biomass and an ability to tolerate heavy metals and remediate both organic and inorganic pollutants from the soil and water; and does not accumulate these in the fruits (Komárek et al. 2007).

## Materials and Methods

Seeds of maize (var. Subeej) purchased locally from the market were surface sterilized with sodium hypochlorite (0.1 %, w/v), washed under running tap water, and then rinsed in distilled water.  $\text{Pb}^{2+}$  was supplied in the form of lead nitrate (MW = 331.21; purity = 99 %; Merck Ltd., India). Imbibed maize seeds (for 6 h) were germinated on a wet filter paper in enamel trays (32 cm × 23 cm × 7 cm) lined with a moist cotton wad. After 24 h, germinating seeds were transferred to nylon mesh floating on distilled water in glass beakers (500 mL capacity) in growth chamber set at 25/20 ( $\pm 2$ )°C, 75 %  $\pm$  3 % RH, and a 12 h photoperiod of  $\sim 240 \mu\text{mol photons m}^{-2} \text{s}^{-1}$  PFD. On third day, seedlings were exposed to 16, 40 and 80 mg  $\text{L}^{-1}$   $\text{Pb}^{2+}$  or distilled water (control) for 3, 12, and 24 h. Since the  $\text{Pb}^{2+}$ -toxicity was more pronounced in roots; these were excised, washed with 10 mM  $\text{CaCl}_2$ , and stored at  $-20^\circ\text{C}$  for further studies. The amount of Pb in root tissues was determined using an Atomic Absorption Spectrophotometer (EC 4139; ECL, Hyderabad, India).

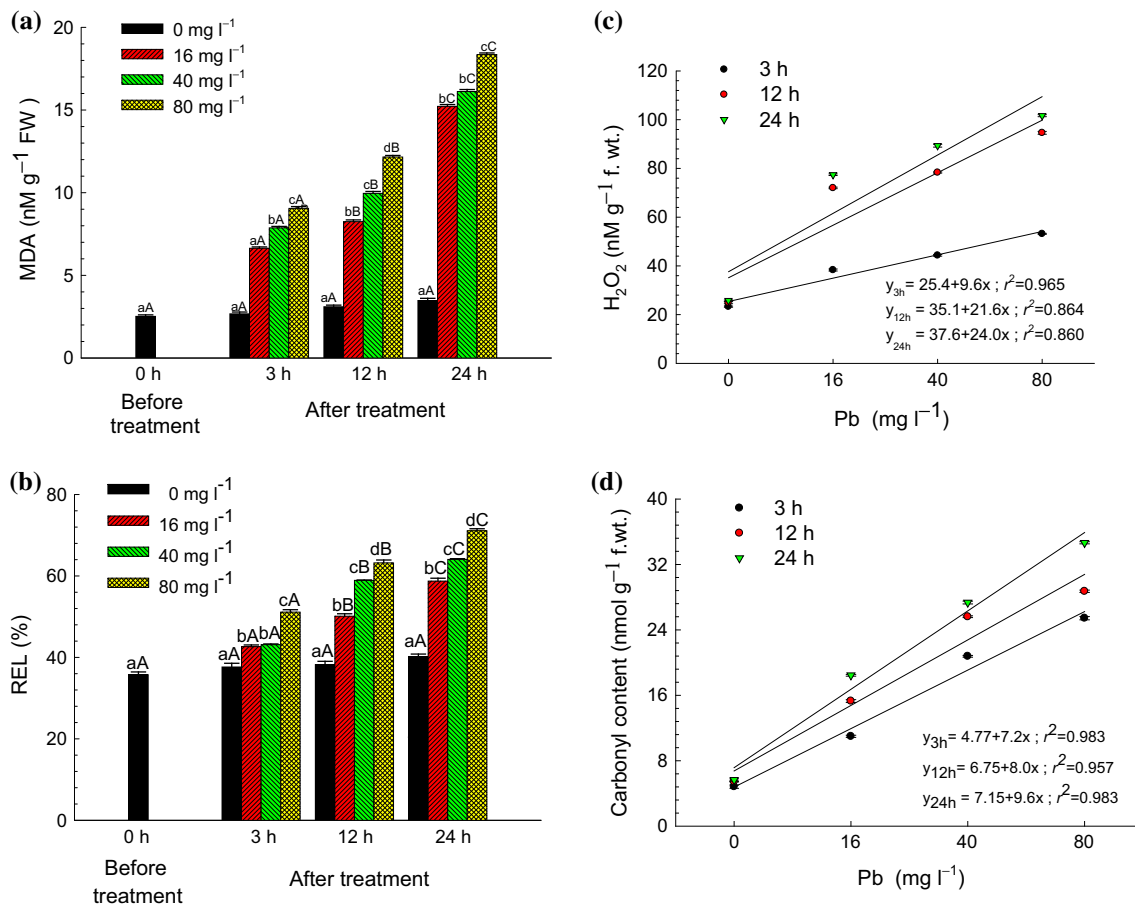
$\text{Pb}^{2+}$ -induced ROS accumulation was determined in terms of hydrogen peroxide ( $\text{H}_2\text{O}_2$ ) generation according to Singh et al. (2008), and malondialdehyde (MDA) accumulation (an indicator of lipid peroxidation) as per Heath and Packer (1968). Effect of  $\text{Pb}^{2+}$  on membrane integrity was determined in terms of electrolyte leakage from root tissue (Singh et al. 2008). Protein oxidation was measured in terms of formation of reactive carbonyl groups (Levine et al. 1994). Histochemical detection of ROS was done using  $\text{H}_2\text{DCFDA}$  (2',7'-dichlorodihydrofluorescein diacetate) (Ezaki et al. 2000). In vivo root plasma membrane integrity was detected using Evans Blue solution (Singh et al. 2008) and  $\text{H}_2\text{O}_2$  was localized by 3,3'-Diaminobenzidine (DAB)– $\text{H}_2\text{O}_2$  complex (Thordal-Christensen et al. 1997). Non-protein thiols (NPTs) content was assayed with Ellman's reagent (Ellman 1959). Changes in low molecular mass antioxidants such as ascorbate (AsA) and glutathione (GSH) were determined as per the method given by Law et al. (1983) and Griffith (1980), respectively. ROS

scavenging enzymes such as ascorbate peroxidases (APX), dehydroascorbate reductase (DHAR), and monodehydroascorbate reductase (MDHAR) were estimated according to Singh et al. (2008), Hossain and Asada (1984), and Hossain et al. (1984), respectively. Lipoygenases (LOX) activity was determined at 234 nm using an extinction coefficient ( $\epsilon$ ) of  $0.25 \text{ mM}^{-1} \text{ cm}^{-1}$  for linoleic acid (Chowhan et al. 2013). For determining the activities of APX and LOX, frozen root tissue was homogenized in 100 mM  $\text{PO}_4^{3-}$  buffer (pH 7.0) in a pre-chilled pestle mortar. For the assay of DHAR and MDHAR, frozen root tissue was ground with 5 mL of ice-cold 25 mM HEPES buffer (pH 7.8) containing 0.2 mM EDTA, 2 mM ascorbate and 2 % PVP. The homogenates were passed through three layers of cheesecloth followed by centrifugation at  $15,000 \times g$  at  $4^\circ\text{C}$  rotor temperature for 30 min. The supernatant was stored at  $-20^\circ\text{C}$  and used further for enzyme assays. Protein content in the homogenate was determined against a standard of bovine serum albumin (Lowry et al. 1951). The experiments were performed in a randomized block design with at least five replicates, each consisting of a beaker with 15 seedlings. The data are presented as mean  $\pm$  SE, and analyzed by one-way ANOVA followed by the comparison of mean values using Tukey's test at  $p \leq 0.01$  and  $p \leq 0.05$ .

## Results and Discussion

Exposure to 16, 40, and 80 mg  $\text{Pb}^{2+} \text{ L}^{-1}$  for 24 h caused accumulation of  $4.9 \pm 0.37$ ,  $6.12 \pm 0.49$ , and  $6.89 \pm 0.47 \mu\text{g Pb}^{2+} \text{ mg}^{-1}$  root tissue (significant at  $p \leq 0.01$ ) compared to the control ( $0.001 \mu\text{g Pb}^{2+} \text{ mg}^{-1}$ ). The difference in  $\text{Pb}^{2+}$  content between 16 and 40 mg  $\text{Pb}^{2+} \text{ L}^{-1}$  was significant at  $p \leq 0.01$ , whereas it was insignificant between 40 and 80 mg  $\text{Pb}^{2+} \text{ L}^{-1}$  treatments. Maize roots absorb/uptake Pb ( $p \leq 0.05$ ) from the medium in a concentration-dependent manner; however, the uptake was limited at higher Pb dose. It suggests that beyond a limit (up to which a plant can withstand Pb exposure) plants activate resistance mechanisms that limit absorption/uptake of Pb, and consequently protect from injury ensued at higher doses. In other words, retarded absorption at high doses acts as defense strategy against metal-induced toxicity. Possibly, it results in a trade-off between metal resistance and plant fitness that enables plant to divert greater energy for survival in stressed environment and thus lesser energy is made available for growth and development (Maestri et al. 2010).

MDA content in *Z. mays* roots was enhanced by 149 %, 166 % and 337 % over their respective controls, after 3, 12 and 24 h of exposure to 16 mg  $\text{L}^{-1}$   $\text{Pb}^{2+}$  (Fig. 1a). It increased further and was 194 %, 221 % and 363 %



**Fig. 1** Effect of short-term Pb<sup>2+</sup> exposure on **a** MDA content, **b** REL, **c** H<sub>2</sub>O<sub>2</sub> accumulation and **d** carbonyl content in roots of 4-day-old *Zea mays* seedlings measured at 3, 12 and 24 h after Pb<sup>2+</sup> treatment (16, 40 and 80 mg L<sup>-1</sup>). Data presented as mean ± SE. In figures **a**, **b**, different alphabets (lower case at a particular time

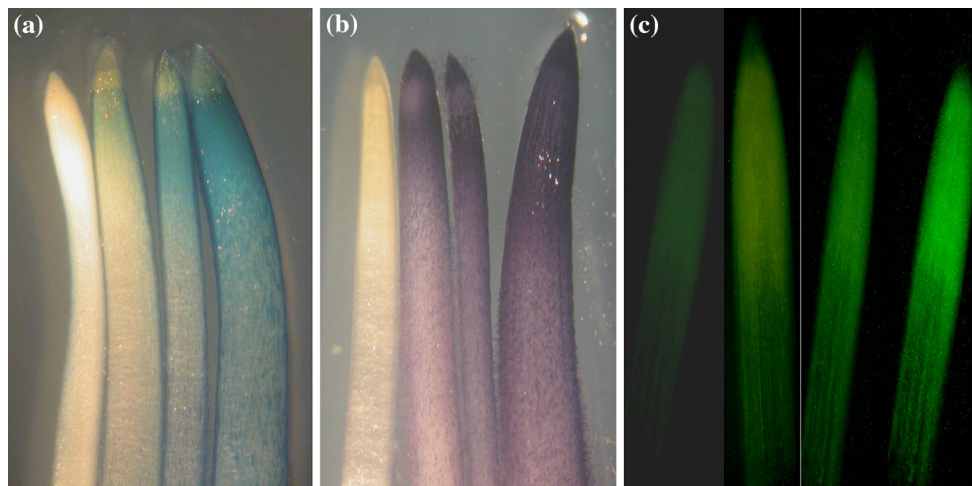
period, upper case within a particular concentration at different time period) represent significant difference among them at  $p \leq 0.05$  applying Tukey's test. In figures **c**, **d**, data were analyzed by linear regression models and  $r^2$  represents the correlation coefficient

greater over the respective control in response to 40 mg L<sup>-1</sup> Pb<sup>2+</sup>, after 3, 12 and 24 h, respectively. In response to 80 mg L<sup>-1</sup> Pb<sup>2+</sup>, MDA content increased by 239 %–427 % over the controls during 3–24 h of exposure (Fig. 1a). MDA accumulation under Pb-treatment suggested lipid peroxidation. In support to present results, stimulation of lipid peroxidation was observed in onion roots exposed to Pb for 24 h (Kaur et al. 2014). Logani and Davies (1980) explained that lipid peroxidation initiates cyclic reactions that lead to the formation of short chain alkane and aldehydes, thereby causing dimerization and polymerization of proteins, thus damaging the membranes. Lipid peroxidation is the most common indicator of oxidative stress resulting in the disturbance in membrane integrity and consequently enhanced permeability (Heath and Packer 1968).

Membrane permeability was estimated on the basis of electrolyte leakage upon exposure to Pb<sup>2+</sup>. After 3 h, not much change in REL was observed in response to

16 mg L<sup>-1</sup> Pb<sup>2+</sup> (Fig. 1b). However, after 12 h of exposure REL increased by 31 %–65 % over the control upon treatment with 16–80 mg L<sup>-1</sup> Pb<sup>2+</sup>. At 80 mg L<sup>-1</sup> Pb<sup>2+</sup>, REL was greater over the control by ~77 % after 24 h of treatment (Fig. 1b). Increased REL in the present study, thus, suggests the toxic effect of Pb<sup>2+</sup> in causing damage to the membrane in *Z. mays* roots. Seregin et al. (2004) explained that 10 mM Pb (lethal dose) penetrates all radicular tissues, and the cell membranes no longer play the role of physical barriers, thus causing disorganization of the membranes. Membrane disruption results in cellular disintegration leading to cell death (Baker and Mock 1994). The quantitative estimations in the present study were in agreement with the in situ histochemical localization where Pb<sup>2+</sup> accumulation resulted in cell death as evidenced by Evans Blue staining (Fig. 2a).

Parallel to MDA accumulation, exposure to Pb<sup>2+</sup> resulted in significant accumulation of H<sub>2</sub>O<sub>2</sub> in root tissue. Accumulation of H<sub>2</sub>O<sub>2</sub> reached up to 200 % after 24 h of



**Fig. 2** In situ histochemical localization showing the effect of  $Pb^{2+}$  ions on **a** membrane integrity, **b**  $H_2O_2$  generation and **c** ROS generation in roots of *Zea mays* after 24 h of treatment. In each figure, roots from *left to right* include: control, 16, 40 and 80  $mg\ L^{-1}\ Pb^{2+}$

exposure to 16  $mg\ L^{-1}\ Pb^{2+}$  as compared to the control (Fig. 1c). At 40  $mg\ L^{-1}\ Pb^{2+}$  exposure, 90%–247% greater accumulation of  $H_2O_2$  over that in the control was observed during 3–24 h of treatment.  $H_2O_2$  levels enhanced further with the increase in dose of the treatment. After 3, 12 and 24 h of treatment to 80  $mg\ L^{-1}\ Pb^{2+}$ ,  $H_2O_2$  content increased by ~129%, 280% and 294% compared to their respective controls (Fig. 1c).  $H_2O_2$  accumulation in *Z. mays* roots upon  $Pb^{2+}$  exposure was confirmed by in situ histochemical localization using DAB (Fig. 2b). Appearance of brown color in  $Pb^{2+}$ -treated roots represented the formation of DAB– $H_2O_2$  complex (Fig. 2b). ROS production in roots was monitored by imaging the ROS-sensitive fluorescent dye,  $H_2DCFDA$  (Fig. 2c).

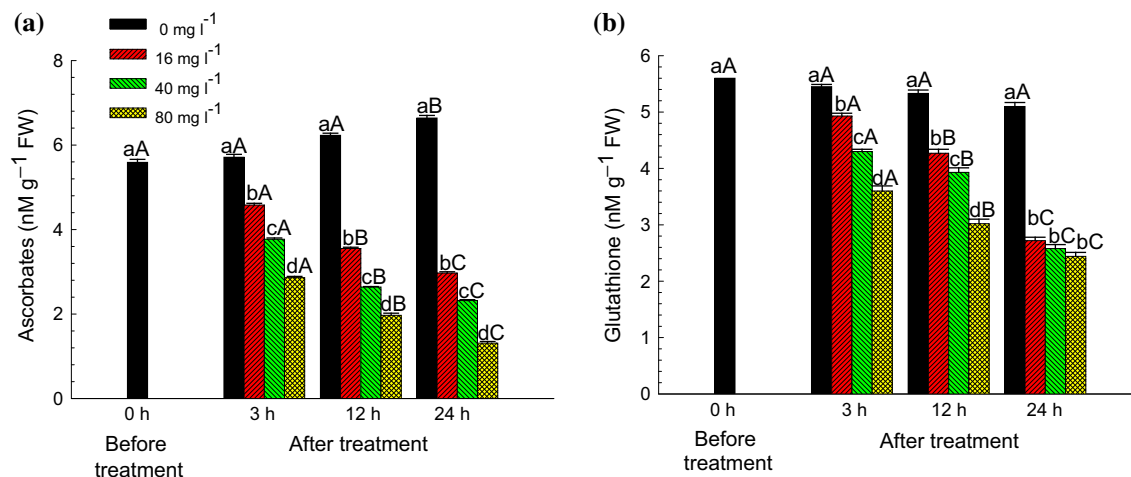
In accordance with the quantitative measurement of ROS, fluorescence intensity was higher at 80  $mg\ L^{-1}\ Pb^{2+}$  as the dye is oxidized by ROS to DCF, indicating increased ROS generation (Fig. 2c).  $H_2O_2$ , one of the ROS, acts as a signalling molecule and serves a dual role in plant defense mechanism (Stone and Yang 2006). Significant accumulation of  $H_2O_2$  content in *Z. mays* roots in the present study suggests the induction of ROS-mediated oxidative stress. It is in agreement with previous findings reporting  $H_2O_2$  accumulation in pea root cells (Malecka et al. 2012), wheat roots (Kaur et al. 2012) and onion roots (Kaur et al. 2014) exposed to  $Pb^{2+}$ . Direct estimation of ROS indicates that  $Pb^{2+}$  exposure increases the production of  $H_2O_2$ , as reported by Pirslova et al. (2011) and Kaur et al. (2012).

$Pb^{2+}$  exposure induced protein oxidation. Amount of carbonyls increased to 127%, 181% and 226% after 3, 12 and 24 h of exposure to 16  $mg\ Pb^{2+}\ L^{-1}$  (Fig. 1d). Further, upon increasing the dose of treatment to 80  $mg\ L^{-1}$ , ~512% increase in carbonyl content was noticed after

24 h, compared to control (Fig. 1d). The results are in concordance with that reported by Biteur et al. (2011) who noticed carbonyl accumulation in radish exposed to 500–1000  $mg\ L^{-1}$  of  $Pb^{2+}$ . Carbonyl content is a sensitive marker of oxidative damage to proteins (Levine et al. 1994). Mitochondria are the main source of ROS in roots and regarded as the main target for oxidative damage to proteins (Møller and Kristensen 2004). Boscolo et al. (2003) and Pena et al. (2008) associated the levels of carbonylated proteins in plants with the oxidative stress induced by heavy metals. The observed formation of carbonyl reactive groups paralleled the ROS generation, oxidative stress and cellular injury. Excessive ROS generated in mitochondria has been related to oxidative carbonylation of proteins such as heat shock proteins and enzymes of Krebs cycle in mitochondria, and results in structural/functional modification that affect its catalytic properties (Møller and Kristensen 2004). Enhanced carbonyl content indicate an imbalance between the quantity of free radicals generated and the capacity of antioxidant defensive system under  $Pb^{2+}$  stress.

Non-enzymatic radical scavengers such as ascorbate and glutathione are known for their antioxidant potential for the removal of  $H_2O_2$ . Both ascorbate and glutathione content showed a progressive decline with increasing concentrations and duration of  $Pb^{2+}$  treatment (Fig. 3). After 3 h, ascorbate content was reduced by ~20%, 34% and 50% over the control on exposure to 16, 40 and 80  $mg\ L^{-1}\ Pb^{2+}$ , respectively (Fig. 3a). A continuous decline was noticed with increasing duration of the treatment. Exposure to 16, 40 and 80  $mg\ L^{-1}$  of  $Pb^{2+}$  caused ~55%, 65% and 80% reduction in the ascorbate content after 24 h. Likewise, we observed a decline in the glutathione content on exposure to  $Pb^{2+}$  (Fig. 3b). After 3 h,



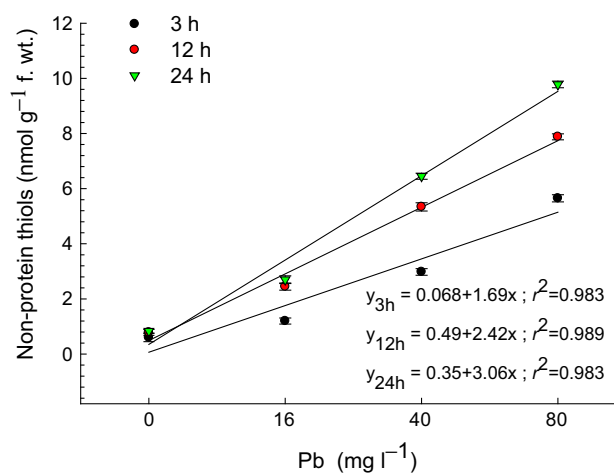


**Fig. 3** Effect of Pb<sup>2+</sup> exposure on the content of ascorbates and glutathione in maize roots measured at 3, 12, 24 h of the treatment. Data represented as mean  $\pm$  SE. Different alphabets (lower case at

particular time period, upper case within a particular concentration at different time period) represent significant difference among them at  $p \leq 0.05$  applying Tukey's test

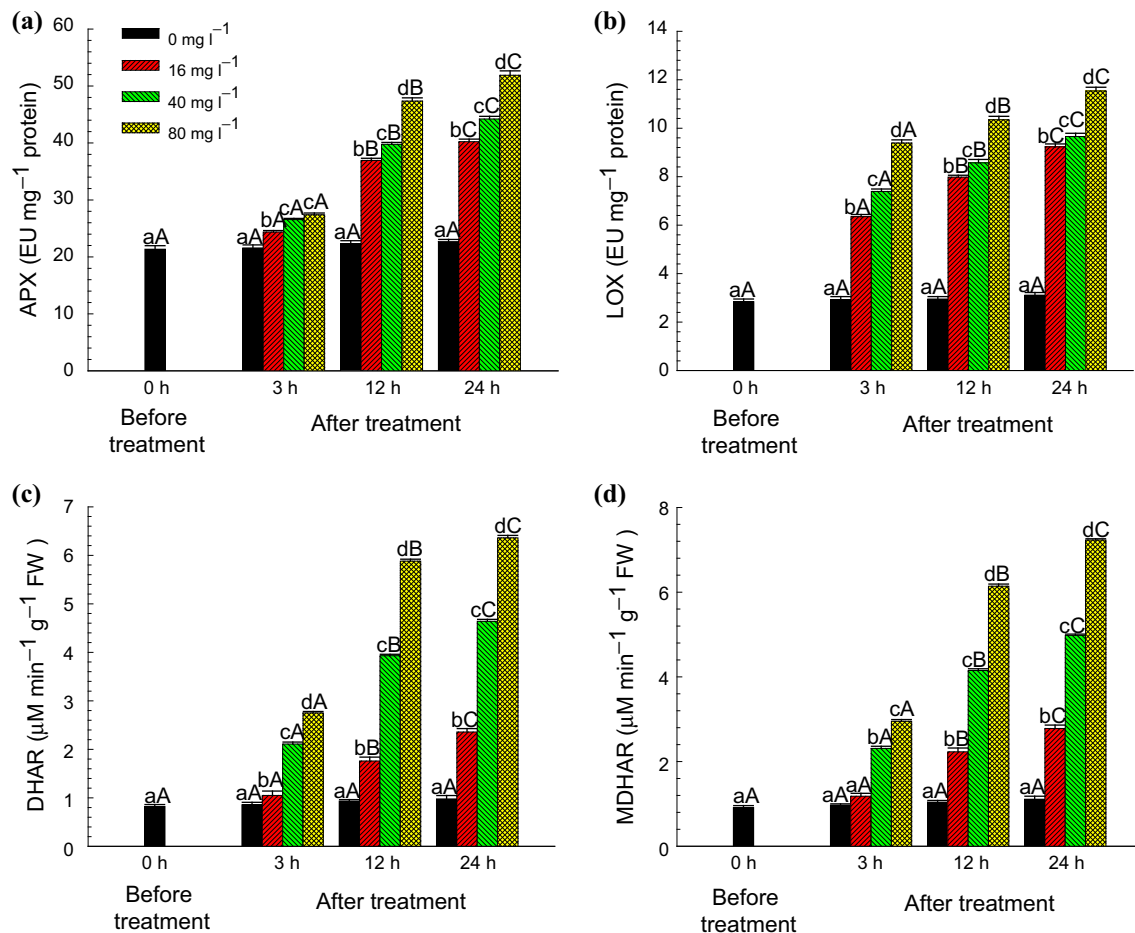
the glutathione content was decreased by 9 %–34 % as compared to control in response to 16–80 mg L<sup>-1</sup> Pb<sup>2+</sup>. At 80 mg L<sup>-1</sup> Pb<sup>2+</sup>, glutathione content decreased by 43 % and 52 % over control after 12 and 24 h, respectively (Fig. 3b). Non-enzymatic radical scavengers, such as ascorbate and glutathione, play an important role in endogenous protective mechanism and help the plant cells to tolerate ROS (Nakano and Asada 1987). Ascorbates affect the redox balances of other metabolites, such as glutathione, since, both are involved in the maintenance of cellular redox balance and a failure to maintain this equilibrium leads to signal amplification (Apel and Hirt 2004). Glutathione is a precursor of low molecular mass peptides such as phytochelatins, produced by the plants to immobilize heavy metals; and correlates with the plant's ability to adapt to metal stress (Seth et al. 2012). In the current investigation, the concentration of ascorbate and glutathione, both, decreased with increasing dose of lead treatment. This indicates the failure of the plant to alleviate oxidative stress induced by Pb<sup>2+</sup>.

In our study, the levels of NPTs were enhanced by  $\sim 2$  to 9 times over the control in response to 16–80 mg L<sup>-1</sup> of Pb<sup>2+</sup> after 3 h ( $R^2 = 0.983$ ) (Fig. 4). Higher concentrations of Pb<sup>2+</sup>–induced greater accumulation of thiol compounds with respect to increasing duration of the treatment. Treatment with 80 mg L<sup>-1</sup> Pb<sup>2+</sup> for 12 h, accumulated ten times higher NPTs with respect to control. After 24 h, the NPT levels were 3, 7.7 and 11.8 times higher than the control at 16, 40 and 80 mg L<sup>-1</sup> Pb<sup>2+</sup>, respectively (Fig. 4). NPTs levels increase under metal stress and these play a significant role in detoxification of metals through the possible synthesis of phytochelatins (Nadgórska-Socha et al. 2011).



**Fig. 4** Effect of short-term Pb<sup>2+</sup> exposure on non-protein thiols (NPTs) in roots of 4-day-old *Zea mays* seedlings at 3, 12 and 24 h after treatment. Data presented as mean  $\pm$  SE, and analyzed by linear regression models and  $r^2$  represents the correlation coefficient

Pb<sup>2+</sup> significantly enhanced the LOX enzyme activity in a concentration- and time-dependent manner (Fig. 5a). LOX activities ranged from 117 % to 222 % in response to 16–80 mg L<sup>-1</sup> Pb<sup>2+</sup>, with respect to control, after 3 h. The highest LOX activity (271 % increase over the control) was observed at 80 mg L<sup>-1</sup> Pb<sup>2+</sup> after 24 h of treatment. Heavy metal stress is known to induce membrane lipid peroxidation by the initiation of enzyme LOX, which produces hydroperoxide and oxy-free radicals by catalyzing the peroxidation of unsaturated fatty acids of biomembranes (Kumar et al. 2013). Increased LOX activity in the present study is well supported by increased H<sub>2</sub>O<sub>2</sub> generation and greater lipid peroxidation. LOX-mediated lipid peroxides accumulation has also been reported in



**Fig. 5** Effect of short-term Pb<sup>2+</sup> exposure on alterations in the activities of **a** APX, **b** LOX, **c** DHAR and **d** MDHAR in roots of 4-day-old *Zea mays* seedlings measured before treatment (0 h stage), and after 3, 12 and 24 h of Pb<sup>2+</sup> treatment (16, 40 and 80 mg L<sup>-1</sup>).

roots of *Talinum triangulare* (Kumar et al. 2013) and *Sedum alfredii* (Huang et al. 2012) upon Pb-exposure. The possible cause of Pb-induced oxidative stress could be the production of hydroperoxide derivatives by the enhanced LOX activity that attacks the cell membranes in *Z. mays* root cells.

We observed alterations in the activities of APX, DHAR and MDHAR under Pb<sup>2+</sup> stress (Fig. 5b–d). APX activity was slightly enhanced after 3 h exposure to 16–80 mg L<sup>-1</sup> of Pb<sup>2+</sup> compared to control. Further, with increasing dose and duration of Pb<sup>2+</sup> treatment, the maximum increase by 112 % and 129 % was noticed at 80 mg L<sup>-1</sup> Pb<sup>2+</sup> after 12 and 24 h, respectively (Fig. 5b). These observations are supported by similar findings made earlier in *Oryza sativa* (Verma and Dubey 2003), onion roots (Kaur et al. 2014) and *Cassia angustifolia* (Qureshi et al. 2007) under Pb treatment. In the ascorbate–glutathione cycle (Foyer–Halliwell–Asada cycle) H<sub>2</sub>O<sub>2</sub> produced by SOD is further detoxified by APX, thereby producing monodehydroascorbate. An increase in APX

Data presented as mean ± SE. Different alphabets (lower case at a particular time period, upper case within a particular concentration at different time period) represent significant difference among them at  $p \leq 0.05$  applying Tukey's test

activity should probably decrease H<sub>2</sub>O<sub>2</sub> accumulation and can help to tolerate metal stress, but an increase in APX activity alone is not sufficient to confer abiotic stress induced by Pb. More likely, a coordinated action of all enzymes of ascorbate–glutathione cycle is required for plant stress tolerance.

The activities of ASC-recycling enzymes (DHAR and MDHAR) also showed significant ( $p \leq 0.05$ ) increase with respect to increasing concentration and duration of the treatment (Fig. 5c, d). A significant increase of 1.2–3.2 fold in DHAR activity was observed with respect to control in the roots of *Z. mays* subjected to 16–80 mg L<sup>-1</sup> of Pb<sup>2+</sup> for 3 h (Fig. 5c). With increase in period of exposure, DHAR activity was almost 1.9–6.3 fold and 2.41–6.50 fold higher than the control in response to 16–80 mg L<sup>-1</sup> Pb<sup>2+</sup> after 12 and 24 h, respectively (Fig. 5c). Parallel to DHAR, a significant rise in MDHAR activity was observed in *Z. mays* roots exposed to Pb<sup>2+</sup> (16–80 mg L<sup>-1</sup>) for different time periods (3, 12 and 24 h) (Fig. 5d). In response to 16, 40 and 80 mg L<sup>-1</sup> Pb<sup>2+</sup>, ~1.22, 2.38, 3.04 fold increase in MDHAR activity,

compared to control, was noted after 3 h of exposure. After 12 and 24 h, MDHAR activity increased by ~5.9 and 6.5 fold over the control, when exposed to 80 mg L<sup>-1</sup> Pb<sup>2+</sup> (Fig. 5d).

The enhanced DHAR activity in response to Pb in the current study is in agreement with previous finding demonstrating stimulation of DHAR by Pb<sup>2+</sup> in *Phaseolus vulgaris* (Geebelen et al. 2002), rice (Verma and Dubey 2003), and lupin roots (Rucińska-Sobkowiak and Pukacki 2006). DHA is reduced to ascorbate by DHAR using glutathione, as a reducing power, which in turn is regenerated by glutathione reductase (Blokhina et al. 2003). MDHAR is responsible for the reduced form of ascorbate, where NAD(P)H or ferredoxin acts as a reductant in the ascorbate–glutathione pathway (Foyer and Halliwell 1976). Enhanced MDHAR activity under metal stress has been linked to the increased resistance of plants to metal stress (Schützendübel et al. 2001).

After analyzing all the observations together, it can be suggested that Pb induces a transitory loss in ‘antioxidative capacity’, perhaps accompanied by stimulation of oxidant producing enzymatic machinery, thus causing H<sub>2</sub>O<sub>2</sub> accumulation and an increase in the oxidative stress markers and alteration in the related enzymes of ascorbate and glutathione cycle. The used Pb concentrations are ecologically relevant and comparable to those found under natural field conditions (Kaur et al. 2014). The obtained findings suggest that maize could be successfully grown in Pb-contaminated soil without any visible injury symptoms, and used for Pb-phytoextraction under natural field conditions.

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**Conflict of interest** The authors declare that they have no conflict of interest.

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