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RESEARCH ARTICLE

Alleviation of lead-induced physiological, metabolic, and ultramorphological changes in leaves of upland cotton through glutathione

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Abstract Plants face changes in leaves under lead (Pb) toxicity. Reduced glutathione (GSH) has several functions in plant metabolism, but its role in alleviating Pb toxicity in cotton leaves is still unknown. In the present study, cotton seedlings (28 days old) were exposed to 500 μM Pb and 50 μM GSH, both alone and in combination, for a period of 10 days, in the Hoagland solution under controlled growth conditions. Results revealed Pb-induced changes in cotton's leaf morphology, photosynthesis, and oxidative metabolism. However, exogenous application of GSH restored leaf growth. GSH triggered build up of chlorophyll *a*, chlorophyll *b*, and carotenoid contents and boosted fluorescence ratios (F_v/F_m and F_v/F_0). Moreover, GSH reduced the malondialdehyde (MDA), hydrogen peroxide (H_2O_2), and Pb contents in cotton leaves. Results further revealed that total soluble protein contents were decreased under Pb toxicity; however, exogenously applied GSH improved these contents in cotton leaves. Activities of antioxidant enzymes (catalase (CAT), superoxide dismutase (SOD), peroxidase (POD), glutathione reductase (GR), and ascorbate peroxidase (APX)) were also increased by GSH application under Pb toxicity. Microscopic analysis showed that excess Pb shattered thylakoid membranes in chloroplasts. However, GSH stabilized ultrastructure of Pb-stressed cotton leaves. These findings suggested that exogenously applied GSH lessened the adverse effects of Pb and improved cotton's tolerance to oxidative stress.

Keywords Lead · Upland cotton · Reduced glutathione · Metabolic changes · Ultramorphology

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Introduction

In nature, several factors affect the physiology of plants. These include variations in environmental conditions and toxicities of different heavy metals (Niinemets 2007; Islam et al. 2008; Daud et al. 2009; Xu et al. 2014). Lead (Pb) is such a toxic heavy metal, which alters the overall plant growth and inhibits or reduces photosynthesis (Singh et al. 2003; Pourraut et al. 2011; Ali et al. 2014a; Tian et al. 2014). Upward translocation of Pb in plants remains in trace amounts. But, changes appear in leaves, for example, decrease in leaf size, blade thickness, and leaf biomass. Previous research showed that decline occurred in green pigments and essential nutrients of leaves under Pb toxicity (Ali et al. 2014b). Similarly, Pb has also caused disintegration of chloroplasts (Sinha et al. 2006; Gopal and Rizvi 2008; Islam et al. 2008) and changes in biomolecules such as proteins (Pena et al. 2008), lipids (Reddy et al. 2005) and nucleic acids (Gichner et al. 2008).

Basically, Pb causes overproduction of reactive oxygen species (ROS) such as malondialdehyde (MDA) and H_2O_2 which are commonly predicted as stress signs (Sharma and Dubey 2005). To cope with ROS, plants have several antioxidant enzymes such as catalase (CAT), superoxide dismutase (SOD), and ascorbate peroxidase (APX) which convert harmful reactive species into harmless species. Also, there are some low-molecular-weight antioxidants, for example, ascorbic acid (AsA) and glutathione. They act as a shield to protect plants against stress conditions (Mishra et al. 2006; Ahmed et al. 2010; Foyer and Shigeoka 2011). However, variation in antioxidant response and heavy metal tolerance exists among different plant species (Clemens 2006; Daud et al. 2009; Chen et al. 2010; Daud et al. 2013).

Strategies for maintaining plant growth under heavy metal stress are quite diverse in plants. One such is alleviation which by definition means making something less severe or more bearable. Alleviation employs chemical or biological agents for upholding plant growth and productivity under stress conditions. Several chemical agents, such as aminolevulinic acid (Tian et al. 2014), hydrogen sulfide (Ali et al. 2014a), Se and Zn (Cao et al. 2013), etc., have been tested to alleviate Pb toxicity in different plants. However, the role of glutathione in alleviating Pb stress is still unknown at least when it comes to cotton.

Basically, glutathione (γ-Glu–Cys–Gly) is a nonprotein thiol found in almost all eukaryotes. It has multiple roles in photosynthetic organisms (Rouhier et al. 2008), which include cellular defense, sulfur metabolism, xenobiotics removal, and heavy metal detoxification (Foyer and Noctor 2011). Moreover, it detoxifies H_2O_2 in ascorbate-glutathione pathway and serves as a precursor for phytochelatin synthesis. Also, it is a substrate for glutathione-*S*-transferases (Noctor et al. 2002; Foyer and Noctor 2011).

Altered GSH levels can cause changes in leaf physiology. For example, reduced GSH levels, in transgenic *Arabidopsis thaliana*, altered leaf size and biomass and increased plant sensitivity to stress (Xiang et al. 2001). Pb decreases GSH levels in plants, as reported by Gupta et al. (1995) and Kumar et al. (2012), because of its consumption in phytochelatin formation. Therefore, increasing GSH levels by internal manipulation (Freeman et al. 2004) or external addition is important to improving plant tolerance (Chen et al. 2010). Exogenous application of GSH has regulated growth in heavy metal-stressed crops such as rice (Cai et al. 2010; Cao et al. 2013) and barley (Chen et al. 2010). In the light of previous research, plant growth regulation is due to stimulation of GSH-derived mechanisms like increase in photosynthesis (Pietrini et al. 2003), morphogenesis (Shankar et al. 2012), cell signaling (Ball et al. 2004), and stress tolerance (Freeman, et al. 2004). However, plant growth regulation by GSH, at ultrastructural and metabolic levels under Pb toxicity, is yet to be elucidated on some scientific backgrounds.

Today's cotton production is facing several challenges. Especially, the accumulation of heavy metals in agricultural lands has become a major threat (Jiang et al. 2014). It is suggested that roadside fields hold high levels of Pb causing significant uptake in plants and affecting plant growth and crop productivity (Nabul et al. 2006). On the other side, restoring leaf growth can ensure better photosynthesis and greater crop yield (Evans 2013). How leaves contribute to cotton productivity is known. In brief, cotton leaves produce photosynthate, which is transported to the bolls for lint and seed production. Under stressful conditions, photosynthesis remains incompatible with that demand causing falling of bolls (Pettigrew and Meredith 2012). The present research was undertaken to investigate the physiological, metabolic, and ultrastructural changes in cotton leaves due to Pb and GSH application. It is hypothesized that GSH regulates growth, increases photosynthesis, and stabilizes oxidative metabolism and ultrastructural changes in cotton leaves under Pb toxicity. Several studies exist on the alleviation of Pb stress through different plant hormones; however, no study is available in literature specifically focused on alleviating Pb stress by reduced glutathione in cotton leaves. The previously reported studies have mainly either focused on the internal manipulation of glutathione or missing important plant growth parameters when GSH was exogenously applied. In addition, we have focused on alleviation of Pb stress through glutathione at ultrastructural level which provides new insights.

Materials and methods

Plant material and treatments

Seeds of upland cotton variety, TM-1, were surface sterilized in sodium hypochlorite solution (NaClO, 0.1 %) followed by thorough washing. Healthy seedlings (14 days old), grown in peat mass, were transferred to half-strength modified Hoagland solution (Hoagland and Arnon 1950) and allowed to grow for 2 weeks in growth chamber. Growth conditions were set to day/night temperatures of 30/25 °C with a 12-h photoperiod, relative humidity of 60/80 %, irradiance of 40–50 μE m^{-2} s^{-1}, and 350 μM M^{-1} ambient concentration of CO_2 via inbuilt CO_2 control in the growth chamber. Uniform size plants (28 days old) were selected for further study. There were four treatments: (i) control, (ii) 500 μM Pb as $Pb(NO_3)_2$, (iii) 50 μM GSH, and (iv) 50 μM GSH + 500 μM Pb, having the nutrient medium in common. Plants were allowed to grow further for 10 days under respective treatments. The modified Hoagland solution contained (in μM) 500 $(NaH_4)_2SO_4$, 500 $MgSO_4$, 200 K_2SO_4, 1000 KNO_3, 600 $Ca(NO_3)_2{\cdot}4H_2O$, 200 KH_2PO_4, 10 $FeSO_4{\cdot}12H_2O$, 0.5 $MnSO_4{\cdot}H_2O$, 0.25 $ZnSO_4{\cdot}7H_2O$, 0.05 $CuSO_4{\cdot}5H_2O$, 100 H_3BO_3, 0.02 $(NH_4)_6Mo_7O_{24}{\cdot}4H_2O$, and 50 Na-EDTA.

Leaf physiology and photosynthetic features

Different physiological features like leaf length, width, and petiole size were measured by a millimeter scale in 38-day plants. Relative chlorophyll concentration (non-destructive method) was determined as Soil-Plant Analyses Development (SPAD) value by SPAD-502 meter (Zhejiang Top Instrument Co., Ltd, Hangzhou, People's Republic of China). Net photosynthesis (P_n), stomatal conductance (Gs), intercellular CO_2 concentrations (Ci), and transpiration rates (E) were determined by portable photosynthesis system (Li-6400, LI-COR Biosciences, Lincoln, NE, USA).

Chlorophyll fluorescence was measured according to Meyer and Genty (1998), using chlorophyll fluorometer (IMAG-MAXI; Heinz Walz, Effeltrich, Germany). Maximum quantum yield of PSII was calculated by the following formula;

$$F_v/F_m = (F_m - F_0)/F_m$$

$$F_v = Fm - F_0$$

Values were recorded for F_0, F_m, and F_v. Here, F_0 represents minimal fluorescence observed without light, F_m is the maximum fluorescence when high-intensity light was applied, and F_v shows variable fluorescence calculated as below.

Chlorophyll contents were determined according to Porra et al. (1989) and carotenoids by Duxbury and Yentsch (1956). Cotton leaves (0.1 g) were ground in acetone (85 %) and centrifuged at 5000×g for 10 min at 4 °C temperature. Absorbances for Chl a, Chl b, and carotenoids were recorded at 663, 645, and 470 nm, respectively. Blank reading was taken against 85 % acetone.

Determination of stress markers, total soluble proteins, and lead contents

MDA and H_2O_2 concentrations were determined according to Velikova et al. (2000). Fresh cotton leaves (1.0 g) were homogenized in 4 mL trichloroacetic acid (TCA, 0.1 %) and centrifuged at 12,000×g for 20 min at 4 °C temperature. Then, 1 mL TCA (20 %) was mixed with 1 mL supernatant and incubated in boiling water for 30 min followed by immediate cooling on ice bath. Re-centrifugation at 1500×g for 10 min resulted in clear enzyme extract which was used for measuring absorbance at 532 nm. The MDA–TBA content was estimated using extinction coefficient of 155 nM^{-1} cm^{-1} after subtraction of non-specific absorption at 600 nm. For H_2O_2 determination, 0.5-g leaf sample was homogenized with 5 mL 0.1 % TCA solution and centrifuged at 12,000×g for 20 min to get supernatant. Final mixture, for measuring absorbance at 390 nm, involved mixing 0.5 mL potassium phosphate buffer (10 mM, pH 7.0), 1 mL potassium iodide solution (1 M KI), and 0.5 mL enzyme extract. Standard curve was drawn to find H_2O_2 concentration.

Cotton leaves were also analyzed for total soluble proteins (TSP) according to Bradford (1976). Bovine serum albumin (Sigma-Aldrich) was the standard reference. Lead contents were determined by inductively coupled plasma mass spectrometry technique (ICP-MS, 7500a Agilent). Dried leaf samples (0.5 g) were thoroughly powdered and placed in a muffle furnace at a temperature of 250 °C for initial 2 h, followed by heating at 500 °C for 8 h. The samples were then digested with 5 mL of dilute acid (100 mL HNO_3 + 300 mL HCl in 1 L distilled water), and the final volume was raised to 10 mL by adding distilled water. Clear extract was obtained from digested samples following double filtration. Sample uptake time in ICP-MS was 1 mL min^{-1}. Before data acquisition, samples and blanks were aspirated for 2 min. The interval time between the sample and blank was 2 min, and data were expressed as microgram per gram dry weight.

Determination of antioxidant enzymes in cotton leaves

Cotton leaves were ground by a precooled mortar and pestle in liquid nitrogen and chilled phosphate buffer (0.05 M, pH 7.8). Homogenate was centrifuged at 10,000×g for 20 min at 4 °C to get supernatant for testing antioxidant enzymes. CAT (EC 1.11.1.6) was determined according to the method of Aebi (1984). The reaction mixture contained 100 μL enzyme extract, 10 mM H_2O_2, and 50 mM potassium phosphate buffer (pH 7.0). After allowing the mixture to react for 1 min, the absorbance was measured at 240 nm, and CAT activity was calculated using extinction coefficient of 39.4 mM cm^{-1}. SOD (EC 1.15.1.1) activity was done as nitro blue tetrazolium (NBT)-sponsored inhibition of photochemical reduction (Giannopolitis and Ries 1977). One SOD unit is the enzyme's quantity needed for the 50 % inhibition of NBT reduction at 560 nm. The final reaction mixture contained potassium phosphate buffer (50 mM, pH 7.0), methionine (13 mM), NBT (75 μM), riboflavin (2 μM), EDTA (0.1 mM), and supernatant (100 μL). Absorbance was taken at 560 nm. Peroxidase (POD) (EC 1.11.1.7) activity was estimated according to the method of Cakmak et al. (1993) with small changes. The final reaction mixture was composed of potassium phosphate buffer (50 mM, pH 7.0), guaiacol (1 %), H_2O_2 (0.4 %), and supernatant (100 μL). Absorbance was taken at 470 nm. Glutathione reductase (GR) (EC 1.6.4.2) activity was measured according to Jiang and Zhang (2002), by oxidizing NADPH and recording absorbance at 340 nm. Total 1-mL reaction mixture contained 50 mM potassium phosphate buffer (pH 7.0), 2 mM EDTA-Na_2, 0.5 mM GSSG, and 100 μL enzyme extract. APX (EC 1.11.1.11) was assayed according to Nankano and Asada (1981). Reaction mixture, composed of phosphate buffer (100 mM, pH 7.0), sodium EDTA

(0.1 mM), AsA (0.3 mM), H_2O_2 (0.06 mM), and supernatant (100 μL), was subjected to absorbance at 290 nm.

Transmission electron microscopy

Small sections of cotton leaves (2–3 mm) were immersed for more than 4 h in glutaraldehyde (3 %)-containing phosphate buffer (0.1 M, pH 7.2). Then, they were vacuumed several times before fixing in 1 % OsO_4 (osmium(VIII) oxide in phosphate buffer) for 1.5 h. Samples were dehydrated, each for 15–20 min, in graded series of ethanol (50, 70, 80, 90, 95, 100 %) and absolute acetone. Then, they were treated with mixtures of absolute acetone and final spur resin (1:1 and 1:3) for 1 and 3 h, respectively, and kept overnight in final spur resin. Thin leaf sections (80 nm), cut on ultramicrotome, stained with uranyl acetate, and mounted on copper grids, were examined in transmission electron microscope (model H-7650 Hitachi, Japan).

Statistical analysis

Our results are means of three or more replications of independent experiments, expressed as means ± SD. Statistical analysis was performed by SPSS (SPSS, version 16.0, Chicago, IL) using one-way analysis of variance (ANOVA). Significance difference ($P < 0.05$) among different treatment means was determined by LSD test.

Results

Exogenous GSH improves Pb-induced leaf physiological and photosynthetic attributes

Results showed that Pb reduced leaf lengths, widths, and petiole size and SPAD value while GSH application alone and in combination with Pb improved these features as shown in Table 1. Data further showed that GSH alone, in comparison with control, increased leaf length, width, and petiole size by 5, 4, and 15 %, respectively. The SPAD value was also decreased because of Pb; however, no significant difference was observed among rest of the groups. Table 2 shows Pb-induced decline in net photosynthesis, stomatal conductance, and transpiration rates. Data suggested that Pb sponsored non-significant changes in intercellular CO_2 concentrations. Exogenously applied GSH alone caused highest values for photosynthesis parameters while values of GSH + Pb were not different from controls. Photosynthetic pigments were adversely affected by Pb stress; however, GSH improved chlorophyll and carotenoid contents as compared to control and Pb-stressed plants (Table 3). A significant decline under Pb was observed in Chl *a* (−53 %), Chl *b* (−74 %), and carotenoid contents (−13 %) as compared to their respective controls. However, GSH-alone application increased Chl *a* and *b* and carotenoid contents by 19, 26, and 9 %, respectively, as compared to control. Total chlorophyll to carotenoid ratio is a sign of leaf color and stress adaptation. However, a significant decline was noticed in this ratio due to Pb stress alone but exogenously applied GSH increased this ratio significantly (Table 3). Similarly, chlorophyll fluorescence also declined as of Pb stress, but GSH proved a good amendment. The plants in GSH + Pb group displayed highest F_v/F_m (0.82) and F_v/F_0 (4.14) ratios.

GSH reduces the oxidative stress and Pb uptake in cotton leaves under Pb toxicity

Results showed that Pb toxicity alone incited lipid peroxidation (as MDA contents) and excessive H_2O_2 production and declined the content of TSP in cotton leaves (Table 4). Pb-affected plants showed highest MDA values, but there was no significant difference among rest of the treatments. Similarly, Pb and GSH promoted H_2O_2 production by 25 and 24 %, and there was a dramatic decrease of 42 % in combined treatment of GSH + Pb as compared to control. Similarly, Pb stress alone caused 27 % decline in TSP contents in cotton leaves as compared to control, while maximum TSP contents were found with the application of GSH under Pb stress. Moreover, it was also observed that Pb uptake in the cotton leaves was increased under Pb stress as compared to control. Further, exogenous application of GSH reduced Pb uptake in cotton leaves both alone and in combination with Pb (Table 4).

Table 1 Leaf length, width, petiole size, and spade values in cotton seedlings, treated with lead and reduced glutathione

Treatment (μM)	Leaf length (cm)	Leaf width (cm)	Petiole size (cm)	SPAD value
Pb_0	6.49 ± 0.07 b	5.72 ± 0.01 b	2.91 ± 0.28 a	31.57 ± 3.40 a
Pb_{500}	4.85 ± 0.16 d	4.04 ± 0.19 d	2.21 ± 0.13 b	25.37 ± 0.65 b
GSH_{50}	6.80 ± 0.13 a	5.95 ± 0.06 a	3.34 ± 0.40 a	32.20 ± 1.31 a
$Pb_{500} + GSH_{50}$	5.74 ± 0.28 c	5.14 ± 0.13 c	2.45 ± 0.18 b	29.89 ± 0.25 a

Values in columns represent means of three replications ± SD. Variants possessing the same letters are not statistically significant at $P < 0.05$

Table 2 Net photosynthetic rate (P_n), stomatal conductance (Gs), intercellular CO_2 concentration (Ci), and transpiration rate (E) in cotton leaves treated with lead and reduced glutathione

Treatment (μM)	P_n (μmol CO_2 m^{-2} s^{-1})	Gs (mol H_2O m^{-2} s^{-1})	Ci (μmol CO_2 mol^{-1})	E (mmol H_2O m^{-2} s^{-1})
Pb_0	6.48 ± 0.44 b	0.019 ± 0.08 a	423.9 ± 6.9 a	0.96 ± 0.06 b
Pb_{500}	3.04 ± 0.14 d	0.005 ± 0.00 b	404.7 ± 1.44 b	0.29 ± 0.03 c
GSH_{50}	7.70 ± 0.41 a	0.024 ± 0.006 a	409.7 ± 16.77 ab	1.29 ± 0.30 a
Pb_{500} + GSH_{50}	4.58 ± 0.47 c	0.015 ± 0.004 a	417.5 ± 0.18 a	0.77 ± 0.03 b

Data represented here are means of three replications ± SD. Significant difference ($P < 0.05$) among different means is indicated by different alphabets

GSH ameliorates Pb-induced inhibition of antioxidant enzyme activities

The present study stated that higher Pb concentration (500 μM Pb) altered the antioxidant enzyme status of cotton leaves, while exogenously applied GSH modulated these changes under Pb stress (Table 5). Results revealed drastic decrease in CAT (84 %) and APX (63 %) activities under Pb stress as compared to control. In contrast, Pb-treated plants showed an increase in the activities of SOD, POD, and GR as compared to control. However, application of GSH showed synergetic effect and further increased the activities of all aforementioned enzymes under Pb toxicity (Table 5).

Exogenous GSH alleviates the Pb-induced ultrastructural changes

Ultrastructural changes in leaf mesophyll cells of cotton are demonstrated in Fig. 1. Microscopic analysis showed that at control level, there were a large central vacuole, intact thylakoid membranes in chloroplasts, starch grains, and regular-shaped mitochondria (Fig. 1a). In contrast, Pb affected the ultramorphology of cotton leaves by disintegrating chloroplasts. There was an accumulation of debris in the central vacuole and loss of membrane structures (Fig. 1b). On the opposite, exogenous application of GSH alone showed dense chloroplasts, several starch grains, an enlarged vacuole, and integrated mitochondria (Fig. 1c). Further, GSH application stabilized chloroplast ultrastructure under Pb stress that led to observations like expanded but intact thylakoid membranes, visible starch grains, oval-shaped mitochondria, and nucleus integrity (Fig. 1d).

Discussion

Changes in leaf physiology are commonly associated with heavy metal contamination at higher concentrations. The present study reveals the profound physiochemical and morphological changes in cotton leaves treated with Pb and GSH. Leaf physiological traits are the key determinants of plant growth under heavy metal stress (Daud et al. 2009). Pb is known to affect leaf growth and photosynthesis, as discussed earlier. These changes occur due to arrest of chlorophyll biosynthesis, nutrient uptake, and cell division (Pietrini et al. 2003; Sharma and Dubey 2005; Pourraut et al. 2011). Data presented herein showed significant decrease in leaf size and photosynthetic components (Table 1). However, subsequent recovery was observed in GSH-treated plants. Previous research has also suggested GSH-derived growth improvement in white spruce due to increase in DNA and RNA quantities (Belmonte et al. 2005). This increase in nucleotides may be due to an increase in the nuclear GSH levels which create suitable redox environment for DNA synthesis and repair (Garcia-Gimenez et al. 2013). Also, evidences show that GSH stimulates cell proliferation and cell differentiation in the meristematic tissues (Ogawa 2005; Garcia-Gimenez et al. 2013; Pasternak et al. 2014). Another important cause for growth is the nutrient balance. It has been suggested that GSH plays role in sulfur assimilation (Mendoza-Cozatl et al. 2005)

Table 3 Chlorophylls *a* and *b*, carotenoid contents, and chlorophyll fluorescence in cotton leaves treated with lead and reduced glutathione

Treatment (μM)	Chlorophyll *a* (mg g^{-1} FW)	Chlorophyll *b* (mg g^{-1} FW)	Carotenoids (mg g^{-1} FW)	Total chlorophyll (*a* + *b*)/carotenoid	Chlorophyll fluorescence	
					(F_v/F_m)	(F_v/F_0)
Pb_0	6.48 ± 0.44 b	0.019 ± 0.08 a	1.13 ± 0.07 b	5.76 ± 0.35 ab	0.80 ± 0.05 a	3.78 ± 0.78 b
Pb_{500}	3.04 ± 0.14 d	0.005 ± 0.00 b	0.98 ± 0.04 c	3.11 ± 0.12 c	0.48 ± 0.09 b	0.81 ± 0.22 c
GSH_{50}	7.70 ± 0.41 a	0.024 ± 0.006 a	1.23 ± 0.06 a	6.28 ± 0.31 a	0.79 ± 0.04 a	3.85 ± 0.62 ab
Pb_{500} + GSH_{50}	4.58 ± 0.47 c	0.015 ± 0.004 a	1.10 ± 0.11 b	4.18 ± 0.42 b	0.82 ± 0.03 a	4.14 ± 0.50 a

The means represented in table are triplicates ± SD. Significant difference ($P < 0.05$) among means is shown by different letters

Table 4 The effect of lead and glutathione on stress markers, total soluble proteins, and lead uptake in cotton leaves

Treatment (μM)	MDA (μmol mg^{-1} protein)	H_2O_2 (μmol g^{-1} FW)	TSP (mg g^{-1} FW)	Pb contents (μg g^{-1} DW)
Pb_0	16.07 ± 1.24 b	73.09 ± 6.73 b	73.35 ± 1.82 c	8.18 ± 0.62 c
Pb_{500}	28.23 ± 3.62 a	91.49 ± 2.01 a	53.32 ± 1.69 d	378.4 ± 5.84 a
GSH_{50}	13.86 ± 1.48 b	90.76 ± 2.42 a	78.76 ± 3.30 b	6.44 ± 1.07 c
Pb_{500} + GSH_{50}	17.05 ± 0.68 b	42.49 ± 3.27 c	89.70 ± 4.47 a	76.25 ± 8.57 b

The means followed by different letters are significantly different at $P < 0.05$ according to LSD method

and iron availability (Ramirez et al. 2013). Accordingly, we noticed reverted green color and improved leaf size due to GSH application.

Photosynthesis efficiency of plants is dependent on gas exchange parameters, mineral contents, light, and CO_2 concentrations (Evans 2013). Pb brings negative changes in these parameters. On the other hand, GSH plays significant roles in photosynthetic organisms (Rouhier et al. 2008). For example, it protects photosynthetic apparatus from oxidative stress (Pietrini et al. 2003). Previous research has shown that glutamate-cysteine ligase (GCL) and glutathione synthetase (GS) are two basic substrates for glutathione biosynthesis in plants. GCL is located in plastids and GS both in plastids and in cytosol. GCL directly regulates glutathione biosynthesis in chloroplasts which is fortified under stressful conditions (Pietrini et al. 2003). Similarly, both plastid GS via γ-glutamylcysteine synthesis and cytosolic GS contribute to glutathione production and its movement to various cell compartments (Pasternak et al. 2008). Thus, positive changes in photosynthetic parameters (Table 2) may be due to combined effects of GSH on growth and water and nutrient balance (Shankar et al. 2012). Generally, heavy metals slow down or inhibit chlorophyll biosynthesis by oxidizing photochemical apparatus (Pietrini et al. 2003), affecting ALA activity (Cenkci et al. 2010), changing chloroplast morphology (Islam et al. 2008; Daud et al. 2009), and inducing some iron (Fe) imbalance (Gopal and Rizvi 2008). Our study also reports decline in photosynthetic pigments and chlorophyll fluorescence when treated with Pb (Table 3). It is known that under stressful conditions, depletion and inactivation of AsA and APXs lead to photosynthetic inefficiency in plants (Foyer and Shigeoka 2011). We observed subsequent improvement in photosynthesis due to GSH supplementation. Evidences exist for the roles of GSH in protecting key photosynthetic enzymes against stressful conditions. Basically, GSH protects active sites of photosynthetic enzymes where thiols are expected to be binded with heavy metals, thus maintaining photosynthesis efficiency (Pietrini et al. 2003). Similarly, Pasternak et al. (2014) reported stress-defensive role of GSH in *Medicago sativa* L cells. They concluded that cell development by GSH is regulated through several processes like dedifferentiation and cellular activation, stimulation of meristematic cell growth, etc.

Variation in Pb accumulation, among different plants, is dependent on age, concentration, and genetic variation (Sharma and Dubey 2005). We noticed significant Pb levels in young cotton leaves when grown in medium, supplied with higher Pb concentrations (Table 4). Sinha et al. (2006) reported that divalent cations compete with essential nutrients to occupy the same site and their translocation to the roots and other plant parts. Heavy metal translocation pathway is dependent on several sub-mechanisms like presence of metal binding agents in the xylem, radial symplastic passage, and heavy metal load in xylem (Clemens 2006). In the current study, GSH triggered a reduction in Pb contents of cotton leaves. Previous research has shown that Pb activates phytochelatin synthesis (glutathione serves as precursor for phytochelatin synthesis) which captures heavy metals like Pb and reduces its upward translocation to leaves (Clemens 2006). Cai et al. (2010) while working on Cd uptake in rice plants concluded that exogenous GSH application reduces Cd uptake rather than its translocation. Basically, GSH-Pb complex formation

Table 5 The effect of lead and reduced glutathione on the activities of antioxidant enzymes in cotton leaves

Treatment (μM)	CAT (μmol min^{-1} mg^{-1} protein)	SOD (U mg^{-1} protein)	POD (μmol min^{-1} mg^{-1} protein)	GR (μmol min^{-1} mg^{-1} protein)	APX (U min^{-1} mg^{-1} protein)
Pb_0	0.61 ± 0.12 a	463.98 ± 4.20 b	21.26 ± 0.59 c	56.42 ± 3.94 d	3.65 ± 0.23 b
Pb_{500}	0.10 ± 0.01 c	361.22 ± 8.62 d	32.01 ± 0.57 a	78.95 ± 4.90 b	1.34 ± 0.23 c
GSH_{50}	0.40 ± 0.05 b	494.39 ± 12.41 a	25.94 ± 2.03 b	69.82 ± 3.83 c	4.54 ± 0.28 a
Pb_{500} + GSH_{50}	0.57 ± 0.08 a	421.04 ± 11.25 c	34.65 ± 3.14 a	127.79 ± 4.81 a	3.27 ± 0.22 b

Data recorded were the means of three replications. Values followed by same alphabets are not statistically significant at $P < 0.05$, as determined by one-way analysis of variance

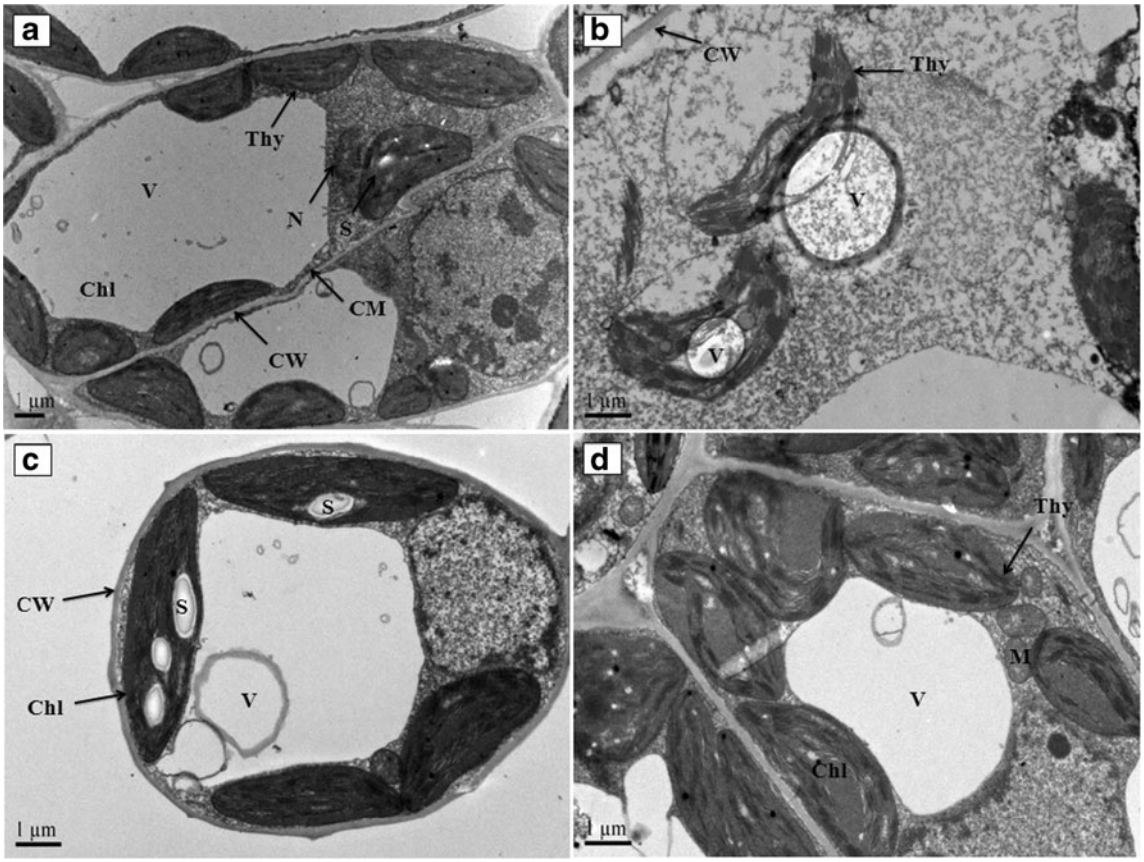

Fig. 1 Transmission electron micrographs of cotton leaf mesophyll cells treated with lead and/or reduced glutathione. Micrograph **a** represents control leaf ultrastructure, **b** indicates variations in cellular morphology under 500 μM Pb, **c** figures out organelle arrangement in plant leaves treated with 50 μM GSH, and **d** shows cellular configuration of leaf mesophyll cells under the effects of GSH addition to the Pb-affected nutrient medium. *Chl* chloroplast, *CW* cell wall, *CM* cell membrane, *N* nucleus, *S* starch grain, *Thy* thylakoid membrane, *V* vacuole, *M* mitochondrion

in the nutrient medium, which may hinder Pb uptake by plant roots, is dependent on GSH/Pb. Vadas and Ahner (2009) observed enhanced Pb uptake when GSH levels were minimal in the nutrient medium, but higher GSH levels reduced Pb uptake. In the current experiment, we have applied 50 μM GSH with 500 μM Pb, expected to have less GSH-Pb complex formation in the nutrient medium. So, it is suggested that, in addition to GSH-Pb complex formation, some other factors may have also been involved in alleviating Pb-induced damage, such as GSH-mediated phytochelatin synthesis, up-regulation of antioxidant enzymes, ROS control, protein synthesis, etc.

ROS are by-products of normal physiological functions; however, under stress conditions, their production is multiplied (Foyer and Shigeoka 2011). It is now well known that glutathione is an intracellular ROS signal transmitter. Moreover, it also acts as reducing agent in metabolic pathways and provides protection against ROS. It detoxifies H_2O_2 by giving electrons to peroxides in ascorbate-glutathione cycle, catalyzed by glutathione peroxidase (Foyer and Noctor 2011). So, it is inferred that decline in H_2O_2 and MDA contents, as reported in the present study, is due to GSH application (Table 4). Our results agree with the previous findings of Chen et al. (2010). Similarly, Chao et al. (2009) also suggested a higher tolerance to heat shock in rice seedlings, due to an early increase in GSH levels.

Antioxidant enzymes play important role in preventing ROS-induced cell injury (Ahmed et al. 2010). Glutathione is a potent antioxidant found extensively in plant cells, mainly as GSH, under normal conditions. It is a cofactor for various enzymes and regulates their activities via glutaredoxin system (Ogawa 2005; Foyer and Shigeoka 2011). In the present study, Pb reduced the activities of CAT and APX and increased those of SOD, POD, and GR in cotton leaves (Table 5). The decline in the activities of CAT and APX may be attributed to the meager quenching capacity of antioxidants to detoxify ROS. On contrary, the increase in the activities of SOD, POD, and GR showed their more involvement in scavenging free radicals. However, the response of antioxidant enzymes depends on stress conditions, plant type/organ, and duration of exposure. Previous studies have shown multiple roles of glutathione in enzyme homeostasis. At first instance, in ascorbate-glutathione pathway, GSH detoxifies H_2O_2 and, secondly, resists denaturation of proteins by inhibiting oxidation of protein

thiol groups (Noctor et al. 2002). Moreover, it modulates protein function by glutathionylation (Ogawa 2005) and maintains redox homeostasis (Foyer and Noctor 2011). We observed GSH-triggered increase in the activities of all abovementioned enzymes. Our results are in line with the findings of Cao et al. (2013) for CAT and APX and Zeng et al. (2012) for CAT, SOD, and GR activities. They have suggested that GSH supplementation increases enzyme activities that led to enhanced plant tolerance to oxidative stress.

Transmission electron microscopy explains leaf ultrastructure and resultant changes under different environmental stresses. This study reports Pb-induced changes in the ultramorphology of cotton leaves and later restoration with GSH treatment (Fig. 1). Earlier research has shown that Pb is mainly deposited in intercellular spaces, vacuoles, and cell walls, and little is found in chloroplasts, endoplasmic reticulum, and nuclei, etc (Sengar et al. 2008). However, even trace amounts of Pb can cause significant changes in leaf ultrastructure (Islam et al. 2008). Generally, ultrastructural modifications in plant cells by Pb are attributed to ROS-induced damage rather than direct effect of Pb. Foyer and Noctor (2009) in their comprehensive review elaborated unique ROS-producing pathways in photosynthetic cells and their distribution. Singlet oxygen, H_2O_2, and peroxides, besides photosynthetic components, are also distributed across plasmalemma and cell walls causing oxidative burst in stress conditions. We, upon treatment with GSH, observed stability in cotton leaf ultrastructure. Wang et al. (2011) have also reported stable chloroplast ultrastructure in barley by exogenous glutathione under cadmium toxicity. Several reasons can be presented here for the improved ultramorphology of cotton leaves treated with GSH such as up-regulation of defensive genes (Wingate et al. 1988; Ball et al. 2004) and stabilization of photosynthetic membranes (Carius et al. 2011). Moreover, as mentioned earlier, GSH is a strong chelating agent and a substrate for S-transferases. It links these molecules with electrophilic substances to form S-conjugates, which are expelled out of the cell (Halliwell and Gutteridge 2007). So, it may be suggested that some restricted entry of Pb into the cells protected subcellular structures.

Conclusions

The findings of the present study highlight the altered growth and disturbed metabolism in cotton leaves treated with Pb. Chlorosis, lamina curling, and smaller number of leaves per plant were some major visible symptoms. Photosynthesis, chlorophyll contents, and chlorophyll fluorescence clearly declined due to Pb treatment. GSH reduced Pb accumulation in leaf cells and increased plant tolerance against toxic levels of Pb. Similarly, Pb exacerbated ROS levels and lipid peroxidation and brought changes in antioxidant enzymes which led to ultrastructural variations. Membrane disturbances caused ultrastructural changes in chloroplasts. However, exogenous GSH improved photosynthesis and overall plant growth by increasing green pigments and fluorescence parameters. Also, GSH heightened plant tolerance against Pb by increasing levels of ROS-scavenging enzymes and protecting chloroplast membranes by stimulating defensive genes, phytochelatin synthesis, and glutathionylation. It can be concluded that GSH successfully alleviated the adverse effects of Pb in cotton leaves, improved photosynthesis, and revived plant growth in cotton. To examine the precise ameliorative role of GSH under Pb stress, a soil environment-based approach is needed.

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References

Aebi H (1984) Catalase in vitro. Methods Enzymol 105:121–126

Ahmed P, Jaleel CA, Saleem MA, Nabi G, Sharma S (2010) Roles of enzymatic and nonenzymatic antioxidants in plants during abiotic stress. Crit Rev Biotechnol 30:161–175

Ali B, Song WJ, Hu WZ, Luo XN, Gill RA, Wang J, Zhou WJ (2014a) Hydrogen sulfide alleviates lead-induced photosynthetic and ultrastructural changes in oilseed rape. Ecotox Environ Saf 102:25–33

Ali B, Mwamba TM, Gill RA, Yang C, Ali S, Daud MK, Wu Y, Zhou WJ (2014b) Improvement of element uptake and antioxidative defense in *Brassica napus* under lead stress by application of hydrogen sulfide. Plant Growth Regul 74:261–273

Ball L, Accotto GP, Bechtold U, Creissen G, Funck D, Jimenez A, Kular B, Leyland N, Mejia-Carranza J, Reynolds H, Karpinski S, Mullineaux PM (2004) Evidence for a direct link between glutathione biosynthesis and stress defense gene expression in Arabidopsis. Plant Cell 16:2448–2462

Belmonte MF, Stasolla C, Katahira R, Loukanina N, Yeung EC, Thorpe TA (2005) Glutathione-induced growth of embryogenic tissue of white spruce correlates with changes in pyrimidine nucleotide metabolism. Plant Sci 168:803–812

Bradford NM (1976) Rapid and sensitive method for quantification of microgram quantities of protein utilizing principle of protein-dye binding. Anal Biochem 72:248–254

Cai Y, Lin L, Cheng W, Zhang G, Wu F (2010) Genotypic dependent effect of exogenous glutathione on Cd-induced changes in cadmium and mineral uptake and accumulation in rice seedlings (*Oryza sativa)*. Plant Soil Environ 56:516–522

Cakmak I, Strbac D, Marschner H (1993) Activities of hydrogen peroxide scavenging enzymes in germinating wheat seeds. J Exp Bot 44:127–132

Carius AB, Henkel M, Grammel H (2011) A glutathione redox effect on photosynthetic membrane expression in *Rhodospirillum rubrum*. J Bacteriol 193:1893–1900

Cao F, Wang N, Zhang M, Dai H, Dawood M, Zhang G, Wu F (2013) Comparative study of alleviating effects of GSH, Se and Zn under combined contamination of cadmium and chromium in rice (*Oryza sativa)*. Biometals 26:297–308

Cenkci S, Cigerci IH, Yildiz M, Ozay C, Bozdag A, Terzi H (2010) Lead contamination reduces chlorophyll biosynthesis and genomic template stability in *Brassica rapa* L. Environ Exp Bot 67:467–473

Chao YY, Hsu YT, Kao CH (2009) Involvement of glutathione in heat shock- and hydrogen peroxide-induced cadmium tolerance of rice (*Oryza sativa* L.) seedlings. Plant Soil 318:37–45

Chen F, Wang F, Wu F, Mao ZG, Zhou M (2010) Modulation of exogenous glutathione in antioxidant defense system against Cd stress in the two barley genotypes differing in Cd tolerance. Plant Physiol Biochem 48:663–672

Clemens S (2006) Toxic metal accumulation, responses to exposure and mechanism of tolerance in plants. Biochimie 88:1707–1719

Daud MK, Variath MT, Shafaqat A, Najeeb U, Muhammad J, Hayat Y, Dawood M, Muhammad IK, Zaffar M, Sardar AC, Tong XH, Zhu SJ (2009) Cadmium-induced ultramorphological and physiological changes in leaves of two transgenic cotton cultivars and their wild relative. J Hazard Mater 168:614–625

Daud MK, Ali S, Variath M, Zhu SJ (2013) Differential physiological, ultramorphological and metabolic responses of cotton cultivars under cadmium stress. Chemosphere 93:2593–2602

Duxbury AC, Yentsch CS (1956) Plankton pigment monograph. J Mar Res 15:93–101

Evans JR (2013) Improving photosynthesis. Plant Physiol 162:1780–1793

Foyer CH, Noctor G (2009) Redox regulation in photosynthetic organisms; signaling, acclimation and practical implications. Antiox Rad Signal 11:861–905

Foyer CH, Noctor G (2011) Ascorbate and glutathione: the heart of the redox hub. Plant Physiol 155:2–18

Foyer CH, Shigeoka S (2011) Understanding oxidative stress and antioxidant system to enhance photosynthesis. Plant Physiol 155:93–100

Freeman JL, Persans MW, Nieman K, Albrecht C, Peer W, Pickering IJ, Salt DE (2004) Increased glutathione biosynthesis plays a role in nickel tolerance in thlaspi nickel accumulators. Plant Cell 16:2176–2191

Garcia-Gimenez JL, Markovic J, Dasi F, Queval G, Schnaubelt D, Foyer CH, Pallardo FV (2013) Nuclear glutathione. Biochim Biophys Acta 1830:3304–3316

Giannopolitis N, Ries SK (1977) Superoxide dismutase 1: occurrence in higher plants. Plant Physiol 59:309–314

Gichner T, Znidar I, Szakova J (2008) Evaluation of DNA damage and mutagenicity by lead in tobacco plants. Mutat Res 652:186–190

Gopal R, Rizvi AH (2008) Excess lead alters growth, metabolism and translocation of certain nutrients in radish. Chemosphere 70:1539–1544

Gupta M, Rai UN, Tripathi RD, Chandra P (1995) Lead induced changes in glutathione and phytochelatin in *Hydrilla verticillata* (I.f.) royale. Chemosphere 30:2011–2020

Halliwell B, Gutteridge JMC (2007) Free radicals in biology and medicine. Clarendon Press, Oxford, (4th edition) UK

Hoagland DR, Arnon DI (1950) The water-culture for growing plants without soil. Calif Agric Exp Stn 337 Circ., p, 347

Islam LD, Li T, Yang JX, Mahmood Q, Tia S, Li J (2008) Effect of Pb toxicity on leaf growth, physiology and ultrastructure in two ecotypes of *Elsholtzia argyi*. J Hazard Mater 154:914–926

Jiang M, Zhang J (2002) Water stress-induced abscisic acid accumulation triggers the increased generation of reactive oxygen species and upregulates the activities of antioxidant enzymes in maize leaves. J Exp Bot 53:2401–2410

Jiang W, Hou Q, Yang Z, Yu T, Zhong C, Yang Y (2014) Annual input fluxes of heavy metals in agricultural soil of Hainan Island, China. Environ Sci Pollut Res 21:876–7885

Kumar A, Prasad MNV, Sytar O (2012) Lead toxicity, defense strategies and associative indicative biomarkers in *Talinum triangulare* grown hydroponically. Chemosphere 89:1056–1065

Mendoza-Cozatl D, Loza-Tavera H, Hernandez-Navarro A, Moreno-Sanchez R (2005) Sulfur assimilation and glutathione metabolism under cadmium stress in yeast, protists and plants. FEMS Microbiol Rev 29:653–671

Meyer S, Genty B (1998) Mapping intercellular CO_2 mole fraction (C_i) *Rosa rubiginosa* leaves fed with abscisic acid by using chlorophyll fluorescence imaging. Plant Physiol 116:947–957

Mishra S, Srivastava S, Tripathi RD, Kumar R, Seth CS, Gupta DK (2006) Lead detoxification by coontail (Ceratophyllum demersum L.) involves induction of phytochelatins and antioxidant system in response to its accumulation. Chemosphere 65:1027–1039

Nabul G, Oryem-Origa H, Diamond M (2006) Assessment of lead, cadmium and zinc contamination of roadside soils, surface films and vegetables in Kampala city. Uganda Environ Res 101:42–52

Nankano Y, Asada K (1981) Hydrogen-peroxide is scavenged by ascorbate-specific peroxidase in spinach chloroplasts. Plant Cell Physiol 22:867–880

Niinemets U (2007) Photosynthesis and resource distribution through plant canopies. Plant Cell Environ 30:1052–1071

Noctor G, Gomez L, Vanacker H, Foyer CH (2002) Interactions between biosynthesis, compartmentation and transport in the control of glutathione homeostasis and signaling. Plant Physiol 53:1283–1304

Ogawa K (2005) Glutathione-associated regulation of plant growth and stress responses. Antiox Rad Signal 7:973–981

Pasternak M, Lim B, Wirtz M, Hell R, Cobbett CS, Meyer AJ (2008) Restricting glutathione biosynthesis the cytosol is sufficient for normal plant development. Plant J 53:999–1012

Pasternak T, Asard H, Potters G, Jansen MAK (2014) The thiol compounds glutathione and homoglutathione differentially affect cell development in alfalfa (*Medicago sativa* L.). Plant Physiol Biochem 74:16–23

Pena LB, Zawoznik MS, Tomaro ML, Gallego SM (2008) Heavy metals effects on proteolytic system in sunflower leaves. Chemosphere 72:741–746

Pettigrew WT, Meredith WR Jr (2012) Genotypic variation in physiological strategies for attaining cotton lint yield production. J Cotton Sci 16:179–189

Pietrini F, Iannelli MA, Pasqualini S, Massacci A (2003) Interactions of cadmium with glutathione and photosynthesis in developing leaves and chloroplasts of *Phragmites australis* (Cav.) Trin. ex Steudel. Plant Physiol 133:829–837

Porra RJ, Thompson WA, Kriedemann PE (1989) Determination of accurate extinction coefficients and simultaneous equations for assaying chlorophyll a and b extracted with four different solvents: verification of the concentration of chlorophyll standards by atomic absorption spectroscopy. Biochim Biophys Acta 975:384–394

Pourraut B, Shahid M, Dumat C, Winterton P, Pinelli E (2011) Lead uptake, toxicity and detoxification in plants. Rev Environ Contamin Toxicol 213:113–136

Ramirez L, Bartoli CG, Lamattina L (2013) Glutathione and ascorbic acid protect Arabidopsis plants against detrimental effects of iron deficiency. J Exp Bot 64:3169–3178

Reddy AM, Kumar SG, Jyothsnakumar G, Thimmanaik S, Sudhakar C (2005) Lead induced changes in antioxidant metabolism of horsegram (*Macrotyloma uniflorum* (Lam.) Verdc) and bengalgram (*Cicer arietinum* L.). Chemosphere 60:97–104

Rouhier N, Lemaire SD, Jacquot JP (2008) The role of glutathione in photosynthetic organisms: emerging functions for glutaredoxins and glutathionylation. Annu Rev Plant Biol 59:143–166

Sengar RS, Goutam M, Sengar RS, Garg SK, Sengar K, Chaoudhary R (2008) Lead stress effects on physiochemical activities of higher plants. Rev Environ Contam Toxicol 196:73–93

Shankar V, Thekkeettil V, Sharma G, Agrawal V (2012) Alleviation of heavy metal stress in *Spilanthes calva* L. (antimalarial herb) by exogenous application of glutathione. In Vitro Cell Dev Biol Plant 48:113–119

Sharma P, Dubey RS (2005) Lead toxicity in plants. Braz J Plant Physiol 17:35–52

Singh RP, Tripathi RD, Dabas S, Rizvi SMH, Ali MB, Sinha SK, Gupta DK, Mishra S, Rai UN (2003) Effect of lead on growth and nitrate

assimilation of *Vigna radiata* (L.) Wilczek seedlings. Chemosphere 52:1245–1250

Sinha P, Dube BK, Srivastava P, Chatterjee C (2006) Alteration in uptake and translocation of essential nutrients in cabbage by excess lead. Chemosphere 65:651–656

Tian T, Ali B, Qin Y, Malik Z, Gill RA, Ali S, Zhou WJ (2014) Alleviation of lead toxicity by 5-aminolevulinic acid is related to elevated growth, photosynthesis and suppressed ultrastructural damages in oilseed rape. BioMed Res Inter Volume 2014, Article ID 530642, 11 pages. doi:10.1155/2014/530642

Vadas TM, Ahner BA (2009) Cysteine- and glutathione-mediated uptake of lead and cadmium into *Zea mays* and *Brassica napus* roots. Environ Pollut 157:2558–2563

Velikova V, Yordanov I, Edreva A (2000) Oxidative stress and some antioxidant system in acid rain-treated bean plants. Plant Sci 151:59–66

Wang F, Chen F, Cai Y, Zhang G, Wu F (2011) Modulation of exogenous glutathione in ultrastructure and photosynthetic performance against Cd stress in the two barley genotypes differing in Cd tolerance. Biol Trace Elem Res 144:1275–1288

Wingate VPM, Lawton MA, Lamb CJ (1988) Glutathione causes a massive and selective induction of plant defense genes. Plant Physiol 87: 206–210

Xiang C, Werner BL, Christensen EM, Oliver DJ (2001) The biological functions of glutathione revisited in Arabidopsis transgenic plants with altered glutathione levels. Plant Physiol 126:564–574

Xu Z, Gao H, Zhao S (2014) Effects of cadmium on the photosynthetic activity in mature and young leaves of soybean plants. Environ Sci Pollut Res 21:4656–4664

Zeng F, Qiu B, Wu X, Niu S, Wu F, Zhang G (2012) Glutathione-mediated alleviation of chromium toxicity in rice plants. Biol Trace Elem Res 148:255–263