© 2012 Triveni Enterprises
Vikas Nagar, Lucknow, INDIA
editor@jeb.co.in
Full paper available on: www.jeb.co.in

J. Environ. Biol.
33, 265-269 (2012)
ISSN: 0254-8704
CODEN: JEBIDP

Growth, photosynthetic activity and oxidative stress in wheat (*Triticum aestivum*) after exposure of lead to soil

Author Details

Gurpreet Kaur	Department of Environment Studies, Panjab University, Chandigarh - 160 014, India
Harminder Pal Singh (*Corresponding author*)	Department of Environment Studies, Panjab University, Chandigarh - 160 014, India e-mail: hpsingh_01@yahoo.com
Daizy R. Batish	Department of Botany, Panjab University, Chandigarh - 160 014, India
Ravinder Kumar Kohli	Department of Botany, Panjab University, Chandigarh - 160 014, India

Publication Data

Paper received:
25 October 2010

Revised received:
25 February 2011

Accepted:
07 March 2011

Abstract

The present study was conducted to assess quantitative information about lead (Pb) contamination in soil on the growth and physiology of wheat. Solutions with three different concentrations of Pb as [$Pb(NO_3)_2$ at 500, 1000 and 2500 μM] were incorporated into the soil to achieve Pb-stressed conditions in comparison to unstressed, water treated, control variant. Wheat growth measured in terms of root length, shoot length and dry weight exhibited a significant decline with increasing Pb concentrations in the soil. Root and shoot length and seedling weight declined in the range of ~23–51, ~17–44, and ~21–44% in response to 500 to 2500 μM Pb. In addition, there was a significant reduction in the levels of photosynthetic pigments-chlorophyll *a* (16-66%) and *b* (10-24%) and total chlorophyll content (by 14-39%) in plants growing in Pb-contaminated soil. It indicated a negative effect on photosynthetic activity in wheat and was confirmed by reduced photochemical efficiency of PSII (Fv/Fm) in the range of ~ 3-37% in response to 500 to 2500 μM Pb. The reduction in wheat growth in Pb-contaminated soil was accompanied by induction of oxidative stress as indicated by enhanced lipid peroxidation in terms of malondialdehyde (MDA) content (by 18-40%) and hydrogen peroxide (H_2O_2) content (by 34-123%) and alterations in the activity of enzymes, superoxide dismutases (SOD) and guaiacol peroxidases (GPX) in wheat roots. The study concludes that Pb in soil inhibits growth and phototsynthetic activity in wheat through induction of oxidative stress.

Key words

Lead toxicity, Seedling growth, Photosynthetic pigments, Photochemical efficiency, Membrane peroxidation

Online Copy

Introduction

In the present scenario, heavy metal pollution of environment is of major ecological concern, since their contamination is threatening agriculture, wild life, and humans (Gratao *et al*., 2005). Rapid industrialization has significantly added lead (Pb) beyond permissible limits into the soil (Nriagu, 1996). Since, Pb is not an essential nutrient for plants, Pb pollution in soil is of priority concern to agriculturalists and environmentalists. It is easily taken up by the plants from the soil, thereby affecting crop yield, and consequently humans (Patra *et al*., 2004). The ability of a plant to take up significant quantities of Pb depends upon its concentration in soil and its bioavailability. Generally, Pb is present in the forms of Pb-nitrate, Pb-acetate, Pb-sulfide and Pb-citrate in the soil, which are readily available and absorbed into the plants (Lopez *et al*., 2009). The physiology of metal toxicity begins with the increased supply of metal to the root, which results in the failure of well defined essential to life plant process. The first visible symptom of metal toxicity includes reduction in growth (Lin *et al*., 2007) followed by impaired plant metabolism (Seregin and Ivanov, 2001). Toxic amounts of heavy metals can exert deleterious effects on photosynthetic pigments and photosynthetic efficiency of growing plants (Maksymiec *et al*., 2007). Previously most of the studies pertaining to Pb have been conducted under laboratory conditions either in hydroponic conditions or in petri dish bioassays (Kopittke *et al.,* 2007; Malecka *et al*., 2009;

Keser and Saygideger, 2010). We, therefore, conducted a pot study to evaluate the affect of Pb-contaminated soil upon the root and shoot length, dry weight, chlorophyll content, quantum efficiency of photosystem II, lipid peroxidation, hydrogen peroxide (H_2O_2) content, and specific activity of superoxide dismutases (SOD) and guaiacol peroxidases (GPX) of wheat (*Triticum aestivum*) under green house conditions.

Materials and Methods

Experimental design and raising of plants: Plants were raised in polypropylene pots of dimensions 8.5 × 8.5 × 7.5 cm filled with 0.5 kg garden soil (soil:sand::3:1, w/w) incorporated with 100 ml of lead nitrate [$Pb(NO_3)_2$; Mol. wt. 331.21; purity 99%; purchased from Merck Ltd., Mumbai, India] solutions at the rate of 0 (control), 500, 1000 and 2500 μM, respectively. The garden soil had pH=7.51, conductivity=132.3 µS, available N=97.5 kg ha^{-1}, available P=86.1 ppm, available K=3.21 ppm and Pb=9.1 µg g^{-1} soil. The experiments were conducted at the experimental dome of the Department of Environment Science, Panjab University, Chandigarh, India during November, 2009. Five independent (pot) replicates were maintained in a randomized block design for each treatment including control. Certified seeds of wheat (*Triticum aestivum* L. var. PBW 302; germination: 95%) used in the present study were purchased from the seed store in the grain market, Chandigarh, India. These were surface-sterilized with 0.1% sodium hypochlorite for 10 min, thoroughly washed with tap water, and then with distilled water twice. Ten seeds were evenly sown in each pot at a depth of 0.5 cm and the pots were watered daily with respective Pb-solution or water to maintain sufficient water content in the soil during the whole test period.

After 7 days, root and shoot length of emerged seedlings were measured with the help of a centimeter ruler. Seedling dry weight was determined by oven drying seedlings at 70°C for 24 hr. Further, leaves were plucked and used for estimation of photosynthetic pigments. The remnant materials were washed in 10 mM $CaCl_2$ to remove Pb accumulated on their surface. Since the growth reduction was greater in roots, these were further used for various biochemical estimations. Apical portion (~1.5-2.0 cm) of roots was removed, stored at -80°C and used for subsequent biochemical analysis. All the chemicals and reagents used in the present study for biochemical estimations and enzymatic assay were of analytical grade and procured from the best available sources.

Estimation of Chlorophyll (Chl): Photosynthetic pigments in leaf tissue (25 mg) of treated and control plants were extracted in dimethyl sulphoxide (4 ml) after incubation at 60°C for 1 hr (Hiscox and Israelstam, 1979). The extinction value of recovered chlorophyll was measured at dual wavelength of 645 and 663 nm on Shimazdu UV-190 double beam spectrophotometer against DMSO, as a blank. Amounts of Chl *a,* Chl *b*, and total Chl were determined using equation of Arnon (1949) and expressed as µg mg^{-1} tissue on dry weight (d.wt.) basis as suggested by Rani and Kohli (1991).

Photochemical efficiency (Fv/Fm): The maximum potential quantum efficiency of photosystem II (PSII) of intact wheat leaves was measured using the OS-30p pulse modulated chlorophyll fluorometer (Opti Sciences, US). Briefly, a leaf was attached on the leaf holder of the plant efficiency analyser equipment and subjected to dark conditions for about 10 min. Thereafter, its fluorescence characteristics (Fv and Fm) were measured. This was repeated ten times (on independent plants) for each treatment concentration. Measurements were made at 12.00 noon.

Estimation of lipid peroxidation and hydrogen peroxide: Lipid peroxidation was measured in terms of malondialdehyde (MDA) content. It is a major thiobarbituric acid reactive species (TBARS) and end product of lipid peroxidation, in root tissue was determined as per Heath and Packer (1968). Roots (100 mg) were homogenized in 5 ml of 0.1% TCA (w/v). After centrifugation at 10,000xg for 10 min at 4°C rotor temperature, 1 ml of supernatant was mixed with 4 ml of 0.5% TBA in 20% TCA. The mixture was heated at 95°C for 30 min, then cooled over ice, and centrifuged at 10,000xg for 10 min. The absorbance of the supernatant was read at 532 nm, and corrected for non-specific absorbance at 600 nm. MDA amount was calculated using an extinction coefficient of 155 $mM^{-1} cm^{-1}$and expressed as nmol g^{-1}f.wt.

Concentration of hydrogen peroxide (H_2O_2) in the root apices was measured as per Singh *et al*. (2007). Briefly, root tissue (100 mg) was extracted with TCA (5 ml, 0.1%, w/v) in a pre-chilled pestle and mortar. The homogenate was centrifuged at 12,000xg for 15 min. An aliquot of 0.5 ml of sample was mixed with 0.5 ml of PO_4^{3-} buffer (pH 7). To this mixture 1 ml of 1 M potassium iodide was added and read the absorbance at 390 nm. The H_2O_2 content was determined using the extinction coefficient 0.28 $\mu M^{-1} cm^{-1}$ and expressed as nmol g^{-1}f.wt.

Estimation of superoxide dismutase (SOD) and guaiacol peroxidase (GPX): Nearly 250 mg wheat roots were homogenized in 10 ml of ice- chilled 100 mM Na-phosphate buffer (pH 7.0) under ice-cold conditions. The homogenate was centrifuged at 15,000×g for 30 min at 4°C rotor temperature. The supernatant was stored at 4°C for determination of enzyme activities. An aliquot of supernatant was used for the estimation of protein content as per Lowry *et al*. (1951) against bovine serum albumin as standard. All enzymatic activities were measured at 25°C on a UV–VIS spectrophotometer (Model UV 190, Shimadzu Corporation, Japan).

The SOD (EC 1.15.1.1) activity was measured in terms of its capacity to inhibit photochemical reduction of nitro blue tetrazolium, NBT (Beauchamp and Fridovich, 1971). One unit of SOD was defined as the amount of enzyme required to inhibit 50% photoreduction of NBT at 25°C. The reaction mixture of 4 ml was composed of 63 µM NBT, 13 mM L-methionine, 0.1 mM EDTA, 13 mM riboflavin, 0.05 M sodium carbonate and 0.5 ml enzyme extract (or distilled water in control). The reaction was initiated by light illumination for 15 min and inhibited by transferring them to dark for 15 min. The absorbance was read at 560 nm.

Online Copy

The GPX (EC 1.11.1.7) activity was determined in terms of oxidation of guaiacol by measuring increase in absorbance at 470 nm (Egley *et al*., 1983). The assay mixture contained 25 mM phosphate buffer (pH 7.0), 0.05% guaiacol (w/v), 1.0 mM H_2O_2 and 0.1 mM EDTA and 0.2 ml enzyme extract. The enzyme activity was calculated using an extinction coefficient of 26.6 mM^{-1} cm^{-1} and expressed as enzyme unit mg^{-1} protein.

Statistical analysis: The data were analyzed by one-way analysis of variance (ANOVA) and means were separated using post hoc Tukey's test at P<0.05.

Results and Discussion

Seedling growth of wheat (measured in terms of length and dry weight) was adversely affected in Pb-amended soil (Table 1). The inhibitory effect was concentration dependent and growth declined with increasing concentration of Pb in the soil. After 7 days, root length decreased in the range of ~23–51% over 500–2500 μM Pb. Likewise, a reduction in shoot length was also noticed. Shoot length was reduced by ~17, 31 and 44% after 7 days upon exposure to 500, 1000 and 2500 μM Pb, respectively. The reduction in shoot length was comparatively lesser and the inhibitory effect was more pronounced on root length. It was also evident from a declining root/shoot ratio upon Pb exposure.

The observations made in the present study are in agreement with earlier reports indicating inhibitory effect of Pb-amended soil upon the seedling growth of plants. Kopittke *et al*. (2007) reported ~29% reduction in root growth of 13 day-old cowpea growing in 0.025–2.5 mM Pb. Similarly, Ghani (2010) reported ~67% reduction in root growth of maize grown in Pb-contaminated soil, whereas shoot length was not affected much. The greater inhibitory effect on root growth than on shoot growth is not surprising. It is a potent inhibitor of root growth and accumulates largely in the roots (Bashmakov *et al*., 2005). Roots, in fact, act as a barrier against heavy metal, and prevent its distribution to the aerial parts (Fernandes and Henriques, 1991).

Parallel to seedling growth, a significant reduction in dry weight of emerged seedlings was also observed. Seedling weight declined in the range of 21-44% when exposed to 500-2500 μM concentration of Pb in soil (Table 1). Such an observation parallels earlier reports that seedling weight accumulation is severely affected in plants growing in metal-contaminated soils (Lolkema *et al*., 1984; Verkleij and Prast, 1989; Ouzounidou *et al*., 1995). Earlier, Kososbrukhov (2004) observed a considerable decrease in dry weights of plant parts specifically under Pb treatment. Previously, it has been opined that Cu-induced reduction in root biomass involves alterations in physiology and metabolism in the root tissue (Ouzounidou *et al*., 1995).

Chlorophyll content is suggested as a very useful *in vivo* indicator of heavy metal toxicity for calculating the upper critical tissue concentrations. Accordingly, we studied the effect of Pb on Chl *a* and *b*, and total Chl content in wheat seedlings growing in

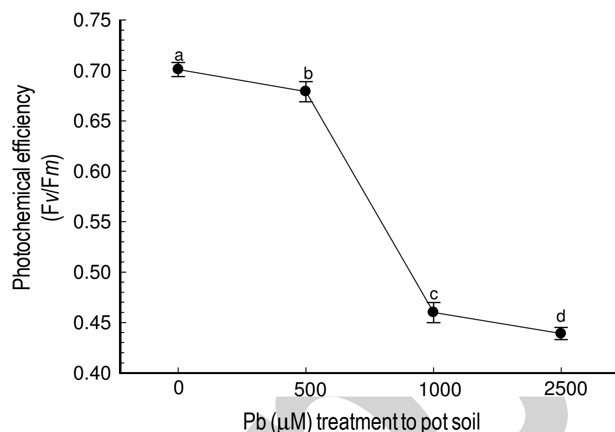

Fig. 1: Effect of lead (Pb) treatment to pot soil on photochemical efficiency (Fv/Fm) in leaves of wheat plant grown for 7 days. Data present as mean ± SE. Different alphabets represent signiucant difference among different concentrations at P<0.05

Pb-contaminated soil (Table 2). Due to Pb toxicity, a significant reduction was noticed in Chl *a*, Chl *b* and total Chl content in wheat seedlings. Chl *a*, Chl *b*, and total Chl content declined by ~16–66%, ~10–24% and 14 to 39%, respectively in 500-2500 µM Pb-contaminated soil. A significant decline was observed in chl *a/b* ratio in wheat leaves.

In order to test if the reduction in chlorophyll content affected photosynthetic efficiency, we measured photochemical efficiency of PSII (Fv/Fm) in dark adapted leaves. Coinciding with the hypothesis, it was found to be severely reduced in wheat seedlings growing in Pb-contaminated soils compared to control plants. Fv/Fm values were decreased by ~34 and 37%, in 1000 and 2500 μM Pb-contaminated soil, over that of control (Fig. 1). A decrease in photosynthetic efficiency with respect to increasing concentration exhibits a reciprocal relation of Fv/Fm with increasing concentrations of Pb in the soil. These results confirm Pb interference with photosynthetic electron transport as evidenced by high fluorescence signal, *i.e.* lower Fv/Fm values (Fig. 1). The current observations are in agreement with Patra *et al*. (2004), who demonstrated that Pb exposure decreases content of photosynthetic pigments, thereby reducing photochemical efficiency of PSII. However, we did not study the exact mechanism of Pb-inhibited photosynthetic activity. Nevertheless, Pb is known to inhibit chlorophyll synthesis either due to impaired uptake of Mg and Fe by plants (Bruzynski, 1987) or because of increased chlorophyllase activity (Drazkiewicz, 1994). Mallick and Mohn (2003) opined that reduced or partial blockage of electron transport from PSII to PSI limits the reoxidation of Q_A (the primary photosystem electron acceptor), thereby resulting in reduction of Fv/Fm. As a result of reduced photosynthesis, the effect of Pb is apparent as decreased plant growth.

The plants growing in Pb-contaminated soil significantly accumulated MDA content (~18–40%) over control. Enhanced MDA content indicated lipid peroxidation in response to Pb-contamination and Pb-induced oxidative stress (Table 3). These observations are in conformity with an earlier observation of Keser and

Online Copy

Table -1: Seedling growth (in terms of length and weight) of 7-day-old wheat (*Triticum aestivum*) plants grown in lead(Pb) treated pot soil

Pb treatment (μM)	Root length (cm)	Shoot length (cm)	Seedling weight (mg/seedling)	Root/Shoot ratio
0	17.3±0.21^{a} (0)	16.7±0.86^{a} (0)	39.1±0.30^{a} (0)	1.04±0.02^{a}
500	13.4±0.27^{b} (–22.5)	13.9±0.40^{b} (–16.8)	31.2±0.20^{b} (–20.5)	0.96±0.02^{b}
1000	9.4±0.19^{c} (–45.7)	11.5±0.36^{c} (–31.1)	28.3±0.21^{c} (–28.2)	0.82±0.02^{c}
2500	8.5±0.11^{d} (–50.9)	9.3±0.46^{cd} (–44.3)	22.1±0.11^{d} (–43.6)	0.91±0.01^{d}

Values are mean of five replicates ± SE. Means with common letters are not significantly different at P<0.05; Figures in parenthesis represent the percent decrease (–) over the control

Table - 2: Level of chlorophyll in leaves of 7-day-old wheat plants grown in lead(Pb) treated pot soil

Pb treatment (μM)	Chl *a* (μg mg^{-1} d.wt.)	Chl *b* (μg mg^{-1} d.wt.)	Chl a/b ratio	Total Chl (μg mg^{-1} d.wt.)
0	6.46±0.39^{a} (0)	4.59±0.27^{a} (0)	1.41±0.28^{a}	11.03±1.2^{a} (0)
500	5.42±0.08^{b} (–16.09)	4.12±0.19^{a} (–10.23)	1.32±0.21^{a}	9.54±1.1^{b} (–13.51)
1000	4.04±0.04^{c} (–37.46)	3.66±0.21^{a} (–20.26)	1.10±0.20^{b}	7.72+0.7^{c} (–30.01)
2500	2.22±0.01^{d} (–65.63)	3.51±0.01^{a} (–23.52)	0.60±0.01^{c}	6.69±0.8^{c} (–39.35)

Values are mean of five replicates ± SE. Means with common letters are not significantly different at P<0.05; Figures in parenthesis represent the percent decrease (–) over the control

Table - 3: Effect of lead (Pb) treatment to pot soil for 7 days on lipid peroxidation (MDA content) and alterations in the activities of superoxide dismutase (SOD) and guaiacol peroxidase (GPX) in the roots of wheat plant

Pb treatment (μM)	MDA (nmol g^{-1} f.wt.)	H_2O_2 (nmol g^{-1} f.wt.)	SOD (EU mg^{-1} protein)	GPX (EU mg^{-1} protein)
0	11.1 ± 0.21^{a} (0)	6.4 ± 0.49^{a} (0)	2.2±0.70^{a} (0)	11.6±1.11^{a} (0)
500	13.1 ± 0.12^{b} (+18.0)	8.6 ± 0.33^{b} (+34.4)	3.3±0.12^{a} (+50.0)	7.6±1.01^{b} (–34.5)
1000	14.2 ± 0.27^{c} (+27.9)	10.7 ± 0.41^{c} (+67.2)	5.0±0.90^{b} (+127.3)	4.2±0.96^{c} (–63.8)
2500	15.5 ± 0.23^{d} (+39.6)	14.3 ± 0.32^{d} (+123.4)	7.1±0.83^{c} (+222.3)	2.5±0.87^{d} (–78.4)

Values are mean of five replicates ± SE. Means with common letters are not significantly different at P<0.05; Figures in parenthesis represent the percent changes (+ = Increase, – = Decrease) increase over the control

Saygideger (2010) who reported MDA accumulation in watercress (*Nasturtium officinale*) in response to Pb-contamination. It further indicated that Pb-induced toxicity is exerted through free radical generation.

The plants growing in Pb-contaminated soil significantly enhanced H_2O_2 content (~34–123%) over control. The H_2O_2 content increased by 34, 67 and 123% in 500, 1000 and 2500 μM Pb-contaminated soils, respectively (Table 3). Such an observation is in agreement with an earlier studies reporting increased H_2O_2 content in plant roots under Pb-stress; for example, in *Hypnum plumaeforme* (Sun *et al*., 2010), and pea (Malecka *et al*., 2009) roots. However, both these studies were conducted in laboratory without using soil as a medium. Nevertheless, H_2O_2 accumulation is another ROS that is implicated in enhanced lipid peroxidation, membrane damage and causing cell death (Stone and Yang, 2006; Singh *et al*., 2009).

Activity of SOD increased linearly with increasing Pb levels in the soil. It enhanced by ~50–222% in Pb-contaminated soil compared to that in control soils (Table 3). A similar increased SOD activity has also been observed in water cress upon Pb exposure under hydroponic conditions (Keser and Saygideger, 2010). In metal-stress studies, SOD activity is of more relevance since it provides first line of defense against oxidative damage (Gratao *et al*., 2005). An up-regulated SOD activity catalyzes dismutation of O_2^- to O_2 and H_2O_2 and this increase can be attributed to *de novo* synthesis of enzyme proteins (Verma and Dubey, 2003).

Unlike SOD, a significant (P<0.05) decline was observed in GPX activity in roots of wheat plants growing in Pb-contaminated soils (Table 3). The GPX activity declined in the range of 35% (at 500 μM Pb) to 78% (at 2500 μM Pb) over that in control (Table 3). Parallel to our observations, a similar decrease in GPX activity has been reported in 7-days-old aquatic plant *Ceratophyllum demersum* in response to 10-100 μM Pb (Mishra *et al*., 2006). It is a stress marker enzyme, located in cytosol, cell wall, vacuole and extra cellular spaces. Broadly, this enzyme is known to catalyze phenolic substrates. It decomposes H_2O_2 to generate phenoxy compounds, which in turn polymerize to produce cell wall components such as lignin (Reddy *et al*., 2005). A decrease in GPX activity results in increased concentrations of H_2O_2 (as observed in present study) to toxic levels, causing oxidative stress to the plants (Sandalio *et al*., 2001).

Thus, based on the results obtained in the present study, it is concluded that Pb-contamination in the soil inhibits plant growth (height as well as dry weight accumulation), impairs photosynthetic activity through induction of ROS-mediated oxidative stress.

Online Copy

Acknowledgments

The Research Fellowship awarded to Gurpreet Kaur by University Grants Commission, New Delhi is highly acknowledged.

References

Arnon, D.I.: Copper enzymes in isolated chloroplasts: Polyphenol oxidase in *Beta vulgaris*. *Plant Physiol.*, **24**, 1-15 (1949).

Bashmakov, D.I., A.S. Lukatkin, V.V Revin, P. Duchovskis, A. Brazaityte, K. Baranauskis: Growth of maize seedlings affected by different concentrations of heavy metals. *Ekologija*, **3**, 22-27 (2005).

Beauchamp, C. and I. Fridovich: Superoxide dismutase: Improved assays and an assay applicable to acrylamide gels. *Anal. Biochem.,* **44**, 276-286 (1971).

Burzynski, M.: The uptake and transpiration of water and the accumulation of lead by plants growing on lead chloride solutions. *Acta Soc. Bot. Poloniae*, **56**, 271-280 (1987).

Drazkiewicz, A.: Chlorophyllase: Occurrence, functions, mechanism of action, effects of external and internal factors. *Photosynthetica*, **30**, 321-331 (1994).

Egley, G.H., R.N. Paul, K.C. Vaughn and S.O. Duke: Role of peroxidase in the development of water impermeable seed coats in *Sida spinosa* L. *Planta*, **157**, 224-232 (1983).

Fernandes, J.C. and F.S. Henriques: Biochemical, physiological and structural effects of excess copper in plants. *Bot. Rev.*, **57**, 246-273 (1991).

Gratäo, P.L., A. Polle, P.J. Lea and R.A. Azevedo: Making the life of heavy metal-stressed plants a little easier. *Funct. Plant Biol.*, **32**, 481-494 (2005).

Ghani, A.: Effect of lead toxicity on growth, chlorophyll and lead (Pb) contents of two varieties of maize (*Zea mays* L.) *Pak. J. Nut.*, **9**, 887-891 (2010).

Heath, R.L. and L. Packer: Photoperoxidation in isolated chloroplast I. Kinetics and stoichiometry of fatty acid peroxidation. *Arch. Biochem. Biophy.*, **125**, 189-198 (1968).

Hiscox, J.D. and G.F. Israelstem: A method for the extraction of chlorophyll from leaf tissue without maceration. *Can. J. Bot.*, **57**, 1332-1334 (1979).

Keser, G. and S. Saygideger: Effects of Pb on the activities of antioxidant enzymes in watercress, *Nasturtium officinale* R Br. *Biol. Trace Elem. Res.,* **137**, 235-243 (2010).

Kopittke, P.M., C.J. Asher, R.A. Kopittke and N.W. Menzies: Toxic effects of Pb^{2+} on growth of cowpea (*Vigna unguiculata*). *Environ. Pollut.,* **150**, 280-287 (2007).

Kososbrukhov, A., I. Knyazeva and V. Mudrik: *Plantago major* plants responses to increase content of lead in soil: Growth and photosynthesis. *Plant Growth Regul.*, **42**, 145-151 (2004).

Lin, R., X. Wang, Y. Luo, W. Du, H. Guo and D. Yin: Effect of soil cadmium on growth, oxidative stress and antioxidant system in wheat seedlings (*Triticum aestivum* L.). *Chemosphere,* **69**, 89-98 (2007).

Lolkema, P.C., M.H. Donker, A.J. Schouten and W.H.O. Ernst: The possible role of metallothioneins in copper tolerance of *Silence Cucubaius*, *Planta,* **162**, 174-179 (1984).

Lopez, M.L., J.R. Peralta-Videa, J.G. Parsons and J.L. Gardea-Torresdey: Effect of indole-3-acetic acid, kinetin and ethylenediaminetetraacetic acid on plant growth and uptake and translocation of lead, micronutrients and macronutrients in alfalfa plants. *Int. J. Phytorem.*, **11**, 131-149 (2009).

Lowry, O.H., N.J. Rosebrough, A.L. Farr and R.J. Rendall: Protein estimation with folin–phenol reagent. *J. Biol. Chem.,* **193**, 265-278 (1951).

Maksymiec, W., Z. Krupa and M. Wójcik: Variation in oxidative stress and photochemical activity in *Arabidopsis thaliana* leaves subjected to cadmium and excess copper in the presence and absence of jasmonate and ascorbate. *Chemosphere,* **66**, 421-427 (2007).

Malecka, A., A. Piechalak and B. Tomaszewska: Reactive oxygen species production and antioxidative defense system in pea root tissues treated with lead ions: The whole roots level. *Acta Physiol. Plant.*, **31**, 1053-1063 (2009).

Mallick, N. and F.H. Mohn: Use of chlorophyll fluorescence in metal stress research: A case study with the green microalga *Scenedesmus*. *Ecotoxicol. Environ. Safety*, **55**, 64-69 (2003).

Mishra, S., S. Srivastava, R.D. Tripathi, R. Kumar, C.S. Seth and D.K. Gupta: Lead detoxification by coontail (*Ceratophyllum demersum* L.) involves induction of phytochelatins and antioxidant system response to its accumulation. *Chemosphere,* **65**, 1027-1039 (2006).

Nriagu, J.O.: A history of global metal pollution. *Science,* **272**, 223 (1996)

Ouzounidou, G., M. Ciamporová, M. Moustakas and S. Karataglis: Responses of maize (*Zea mays* L.) plants to copper stress–I. Growth, mineral content and ultrastructure of roots. *Environ. Exp. Bot.*, **35**, 167-176 (1995).

Patra, M., N. Bhowmik, B. Bandopadhyay and A. Sharma: Comparison of mercury, lead and arsenic with respect to genotoxic effects on plant systems and the development of genetic tolerance. *Environ. Exp. Bot.*, **52**, 199-223 (2004).

Rani, D. and R.K. Kohli: Fresh matter is not an appropriate relation unit for chlorophyll content: Experience from experiments on effects of herbicide and allelopathic substance. *Photosynthetica*, **25**, 655-667 (1991).

Reddy, A.M., S.G. Kumar, G. Jyothsnakumari, S. Thimmanaik and C. Sudhakar: Lead induced changes in antioxidant metabolism of horsegram (*Macrotyloma uniflorum* (Lam.) Verdc.) and Bengalgram (*Cicer arietinum* L.). *Chemosphere*, **60**, 97-104 (2005).

Sandalio, L.M., H.C. Dalurzo, M. Gómez, C. Romero-Puertas and L.A. del Rio: Cadmium induces changes in the growth and oxidative metabolism of pea plants. *J. Exp. Bot.,* **52**, 2115-2126 (2001).

Seregin, T.V. and V.B. Ivanov: Physiological aspects of toxic action of cadmium and lead on higher plants. *Plant Physiol. (Moscow)*, **48**, 606-630 (2001).

Singh, H.P, D.R. Batish, R.K. Kohli and K. Arora: Arsenic-induced root growth inhibition in mung bean (*Phaseolus aureus* Roxb.) is due to oxidative stress resulting from enhanced lipid peroxidation. *Plant Growth Regul.,* **53**, 65-73 (2007).

Singh, H.P., S. Kaur, D.R. Batish, V.P. Sharma, N. Sharma and R.K. Kohli: Nitric oxide alleviates arsenic toxicity by reducing oxidative damage in the roots of *Oryza sativa* (rice). *Nitric Oxide*, **20**, 289-297 (2009).

Stone, J.R. and S. Yang: Hydrogen peroxide: A signaling messenger. *Antioxidant Redox Signal*, **8**, 243-270 (2006).

Sun, S.Q., M. He, T. Cao, Y. Yusufujiang, W. Han and J.L. Li: Antioxidative responses related to H_2O_2 depletion in *Hypnum plumaeforme* under the combined stress induced by Pb and Ni. *Environ. Monit. Assess.*, **163**, 303-312 (2010).

Verkleij, J.A.C. and J.E. Prast: Cadmium tolerance and co-tolerance in *Silene vulagris* (Moench.) Gracke [= *S. Cucubalus* (L) Wibs.] *New Phytol.*, **111**, 637-645 (1989).

Verma, S. and R.S. Dubey: Lead toxicity induces lipid peroxidation and alters the activities of antioxidant enzymes in growing rice plants. *Plant Sci.*, **164**, 645-655 (2003).

Online Copy