**Cellular & Molecular Biology**
*Cell. Mol. Biol.* 2015; 61 (3): 57-62
Published online June 24, 2015 (http://www.cellmolbiol.com)
Received on June 8, 2015, Accepted on June 20, 2015.
doi : 10.14715/cmb/2015.61.3.12

# Effect of Lead stress on phosphatase activity and reducing power assay of *Triticum aestivum*

U. Gubrelay[1]✉, R. K. Agnihotri[2], S. Shrotriya[1], and R. Sharma[2]

[1] Department of Biochemistry, School of Life Sciences, Khandari Campus, Dr. B.R. Ambedkar University, Agra – 282002, India
[2] Department of Botany, School of Life Sciences, Khandari Campus, Dr. B.R. Ambedkar University, Agra – 282002, India

**Corresponding author:** Dr Udita Gubrelay, Department of Biochemistry,School of Life Sciences, Khandari Campus, Dr. B.R. Ambedkar University, Agra – 282002, India. E-mail: udita_biochem@rediffmail.com

**Abstract**

Lead (Pb) is a highly toxic heavy metal for both plants and animals; the environment is increasingly polluted with heavy metals and reduces crop productivity. Plants possess homeostatic mechanisms that allow them to keep correct concentrations of essential metal ions in cellular compartments and to minimize the damaging effects of an excess of nonessential ones. One of their adverse effects on plants are the generation of harmful active oxygen species, leading to oxidative stress and the antioxidative activity seems to be of fundamental importance for adaptive response of plant against environmental stress. The present study explores the effects of lead (soil treated twice/ week) with (10, 30 and 60 mM) on the specific activities of phosphatases which might lead to reducing power assay in (*Triticum aestivum PBW344*) seedling. A significant decrease in the redox potential of shoot compared to root was observed at the similar concentration of lead. A similar trend on leaves was also noted. Acid and alkaline phosphatase activities were significantly higher in roots than in shoot at all the three concentration of lead i.e. 10, 30 and 60 mM, compared to controls. The above mentioned changes were more pronounced at 60 mM concentration of lead than two other concentrations. These results lead us to suggest that increased lead concentration in soil might lead to adverse effects on plant growth and phosphatase activities.

***Key words:*** Lead induced stress, Reducing power assay, Acid phosphatase, alkaline phosphatase, *Triticum aestivum*.

## Introduction

Wheat is considered good source of protein, minerals, B-group vitamins and dietary fiber i.e. an excellent health-building food (1).Wheat is the most important stable food crop for more than one third of the world population and contributes more calories and proteins to the world diet than any other cereal crops (2,3).It is also rich in pantothenic acid, riboflavin and some minerals, sugars etc. The bran, which consists of pericarp testa and aleurone, is also a dietary source for fiber, potassium, phosphorus, magnesium, calcium and niacin in small quantities. Lutein is the predominant carotenoids present in wheat (4) and the bran/germ fractions of wheat contained greater amounts of carotenoids and antioxidant activity than the endosperm fractions (3). Lutein, and zeaxanthin,are important for the health of skin and eyes in humans (5).

Plants are the target of a wide range of pollutants that vary in concentration, speciation, and toxicity. Such pollutants mainly enter the plant system through the soil (6) or via the atmosphere (7).Among common pollutants that affect plants, lead is one of the most toxic and frequently encountered (8). Once in water it enters the food chain & adversely affects the flora & fauna. Lead also induced reduction in chlorophyll & carotenoid contents significantly (9).

Lead toxicity affects phosphorus nutrition and its metabolism in plants. Activities of the phosphorolytic enzymes acid phosphatase, alkaline phosphatase and ATPase. An acid phosphate (EC- 3.1.3.2) catalyze non-specific hydrolysis of inorganic phosphate from phosphate monoesters in pH ranges from 4 to 6 and plays a major role in the supply and metabolism of phosphate in plants (10). Similarly, alkaline phosphatases (EC 3.1.3.1) have a potential role in utilization of phosphomonoesters as a source of inorganic phosphorus required for maintenance of cellular metabolism (11). Lead inhibits the activity of enzymes at cellular level by reacting with their sulfhydryl groups and further contributing to impairment in oxidative balance. It induces higher activities of antioxidant enzymes and higher contents of non-enzymatic constituents are important for plants to tolerate the stress. These were originally thought to function as osmotic buffers. However, apart from the osmotic adjustment they also seem to play a key role in maintaining the natural state of macromolecules, probably by scavenging ROS (12). The ROS are chemically aggressive species and the attack of free radicals on the polyunsaturated fatty acid components of membrane lipids initiates lipid peroxidation, an autocatalytic process that changes membrane structure and function (13).

Keeping the above in view, the present study was planned to observe the possible change in the physiological parameters as well as behavior of acid and alkaline phosphatase in root and shoot of wheat plants growing under increasing concentration of lead and also to examine reducing power assay and accumulation of lead in root, shoot and leaves.

## Materials and methods

### *Pot and sand culture experiment*

The seeds of (*Triticum aestivum* PBW344) were procured from National Seed Corporation, New Delhi,

India. These were surface sterilized with 0 .1% sodium hypochlorite solution for 10 min and then rinsed with distilled water. The sand was thoroughly washed with water and then treated with 2% sodium hypochlorite solution. Dried surface sterilized seeds of Wheat were sown in Earthen pots containing equal quantities (2 Kg) of washed and acid treated loamy sand. Soil and the potted mixture were treated with Long Ashton nutrient solution (14). Iron was provided as Fe-EDTA. Nitrogen was given as ammonium nitrate along with nutrient solution. Heavy metal lead was provided in the form cadmium chloride of 3 concentrations viz. 10, 30 and 60 mM. Lead treatment was given twice a week followed by irrigation with distilled water. Nutrient solution was also given twice a week. Three identical sets were maintained during the whole experiment and were conducted in green house/glass house to provide controlled experimental conditions. The samples were taken from these pots of 30 days old seedlings for biochemical analysis.

### *Enzyme estimation*

*Acid phosphatase*

Acid phosphatase in shoots and roots were estimated following the method of Fiske and Subbaraow (15). About 1.0g fresh tissues were homogenized in 10ml of ice cold 50 mM citrate buffer (pH 5.3) in a pre chilled pestle and mortar. Filtered through muslin cloth and then centrifuged at 10,000g for 10 min. Supernatant was used as enzyme source and incubated 3ml of substrate solution at 37$^{O}$C for 5min.To this 0.5 ml enzyme extract was added and mixed well again incubated with substrate p-nitro phenyl phosphate for 15 min at 370C.Subsequently 0.5 ml sample was drawn and mixed with 9.5 ml NaOH (0.085N). The absorbance of blank and the incubated tubes was recorded at 405 nm p-Nitro phenol (4 to 20 mM) was used as standard. Enzyme specific activity is expressed as µ mol/min /mg of protein.

*Alkaline phosphatase*

Alkaline phosphatase in shoots and roots was estimated at pH (10.5) and all other conditions were same as (acid phosphatase).

### *Reducing Power Assay*

Reducing power assay associated with antioxidant enzyme system was measured by Ferreira et.al (16) method. According to this method 2.5 ml of sample extracts were mixed with 2.5 ml phosphate buffer (pH 6.6) and 2.5 ml of 1% Potassium Ferricynide. The mixture was incubated at 500C for 20 min. A portion (2.5 ml) of TCA (10%) was added to the mixture, which was then centrifuge at 3000 rpm for 10 min .The Supernatant (upper layer) of the solution 2.5 ml was mixed with distilled water (2.5 ml) and $FeCl_3$ (0.5 ml,0.1%). The absorbance was measured at 700 nm and compared with the Standard 50µg/ml ascorbic acid.

### *Lead concentration*

1.0 g samples (root, shoot, and leaves) were digested with concentrated nitric acid using microwave digestion system (model MDS2100CEM corporation Mathew, NC, USA). Metal analyses were formed using a Perkin Elmer atomic absorption spectrophotometer (model-Analyst 100). For evaluation of accuracy of analytical result, the standard Indian reference material (BND201.02 supplied by National Physical Laboratory, New Delhi, India) was analyzed together with the collected sample. Lead was determined at 283 nm wavelength (17).

### *Statistical analysis*

Data are expressed as mean ± Standard Deviation (SD). Comparisons were carried out by using one way analysis of variance followed by Tukey HSD test (18) to compare means between the different treatment groups.

## Results and Discussion

### *Physiological Parameters*

*Effect of lead on seed germination*

The percentage of germination may reflect the reaction rate of seeds to their living environment. In this investigation, Fig. 1 shows the effect of lead on seed germination of wheat. The maximum % germination (96%) was estimated in the control and lowest germination %was evaluated in the treatment at 60mM lead nitrate which was 63 % ( in respect to control 96%).It has been agreed that decrease in germination may be due to lowering of water potential, osmotic effect or through ionic imbalance of enzymes present in inside seed. Such as β-amylase, and protease degradation which results decrease in germination (19).

*Effect of lead on seedling growth*

Early seedlings mean growth of root and shoot which critically affect plant productivity. It was established that heavy metal viz. Lead reduced early seedling growth by affecting root and shoot length. Higher con-

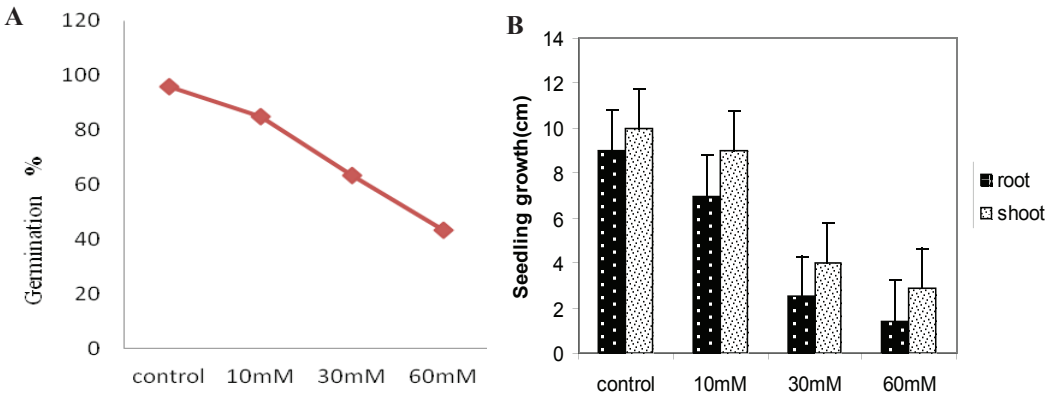


**Figure 1.** Shows **(A)** the effect of lead nitrate on seed germination and **(B)** seedling growth of *Triticum aestivum*.

**Table 1.** Effect of $Pb(NO_3)_2$ on Reducing Power Assay of *Triticum aestivum*.

| Chemical | Conc. | % Inhibition | | |
|---|---|---|---|---|
| | | Root | Shoot | Leaves |
| Lead Nitrate | 10mM | 29±0.30 | 72±0.65 | 20.2±1.24 |
| Lead Nitrate | 30mM | 40±0.45[a] | 90[a].±1.2 | 32.5±2.2[a] |
| Lead Nitrate | 60mM | 101±12.5[a] | 190 [a]±15.6 | 97±18.4[a] |

Values are mean ± SE of 6 observations; [a]P<0.01compared to 10 mM lead nitrate; % inhibition was calculated as $(\frac{a-a_o}{a})$ x 100, where a = control value and the $a_o$ = treated sample value.

centration of Lead affected more the root as compared to shoot. It is depicted in Fig 1 that there was gradual decrease in root and shoot, length dry weight with an increase in lead level from10mM, 30mM, 60mM. Reduction in cell division at higher concentration of Lead are considered a general protoplasmic poison which is slow acting and cause a sharp decrease in crop productivity (20). The higher impact of heavy metal was observed in the root growth as compared to shoot leading to a reduction in its length and fresh weight (21). The reduction in root length due to accumulation of metals within the root reduces the rate of mitosis in the meristematic zones of roots, especially by blocking the metaphase in meristemetic cells. Therefore, root showed reduction in length as demonstrated by (22).

### *Biochemical Parameters*

*Reducing power assay*

Data in Table -1 and Fig - 2 suggests that exposure to lead increased the percent inhibition of reducing power assay. Percent inhibition in roots following exposure to lead (10, 30 and 60 mM) was 29, 40 and 101%, respectively while in shoot the inhibition was more pronounced (i.e. 72, 90, and 190 respectively). The percent inhibition of reducing power assay in leaves also increased following exposure to different lead concentrations (10mM, 30Mm, 60mM) and noted to be 20.2, 32.5 and 97 %. Increased lead concentration in plants significantly increased percent inhibition suggesting oxidative stress in exposed cell compared to normal this result is consistent with investigations done by **o**ther researchers. Lead is not a oxido-reducing metal like iron, therefore the oxidative stress induced by Lead in plants appears to be an indirect effect of Lead toxicity leading to production of ROS, enhancing pro-oxidant status of cell by reducing the pool of reduced glutathione (GSH), activating calcium-dependent systems and affecting iron-mediated processes the sequence of events leading to production of ROS by Lead during membrane-linked electron transport and the role of antioxidative enzymes in scavenging ROS and maintaining the level of antioxidants ascorbate and glutathione (23). In this context, metal activates glutathione-ascorbate cycle. It is a metabolic pathway that detoxifies hydrogen peroxide which is a reactive oxygen species that is produced as a waste product in metabolism (24).To cope with the damages caused by the reactive oxygen species cell possesses their own comprehensive and integrated endogenous antioxidant defence system composed of both enzymatic and non-enzymatic components (12). These non-enzymatic antioxidants prevent the generation of reactive oxygen species by chelation of metals or enzyme catalyzed removal of a potential oxidant (25). These defense systems are composed of metabolites such as ascorbate, glutathione, tocopherol, etc., and enzymatic scavengers of activated oxygen such as superoxide dismutase, peroxidases and catalase therefore control of oxidant levels is achieved by that system, and its response depends on the plant species and tissue analyzed, the metal used for the treatment and the intensity of the stress (26).

### *Acid phosphatase and alkaline phosphatase activities*

Data represented in Table -2 and Fig 2,3 showed that specific activities of acid and alkaline phosphatase were significantly higher in roots and shoot under 10mM 30mM, 60mM compared to control. In roots, acid phosphatase specific activity was 228.94, µmol P/min/mg at 10mM concentration Lead treatment, compared to control. On the other hand, at other two concentrations (30mM and 60mM) 243.23, 592.63 µmol P/min/mg of protein, respectively of lead compared to control (218.51µmol P/min/mg of protein). Specific

**Table 2.** Effect of Pb $(NO_3)$ on specific activity of acid and alkaline phosphatase in different plant parts of *Triticum aestivum*.

| Chemical | Concentration | Acid Phosphatase Specific Activity µmol/P /min /mg of Protein | | Alkaline phosphatase Specific Activity µmo/P /min /mg of Protein | |
|---|---|---|---|---|---|
| | | Root | Shoot | Root | Shoot |
| Control | 0.0mM | 218.51±64.00 | 98.03±1.02 | 175.4±0.37 | 132.66±5.2 |
| Lead Nitrate | 10mM | 228.94±14.2[a] | 183± 3.74[a] | 201.23±3.39[a] | 151.51±8.18[a] |
| Lead Nitrate | 30mM | 243.23±12.59[a] | 269±30.3[a] | 223±14.5[a] | 158.5±14.5[a,] |
| Lead Nitrate | 60mM | 592.63±50.5[a,b] | 361.61±393[a,b] | 241±7.3[a,b] | 173.27±1.66[a,b] |

Values are mean ± SE of 6 observations; [a]p< 0.01 compared to control; [b]p< 0.01 compared to plants exposed to10 mM lead nitrate concentration.

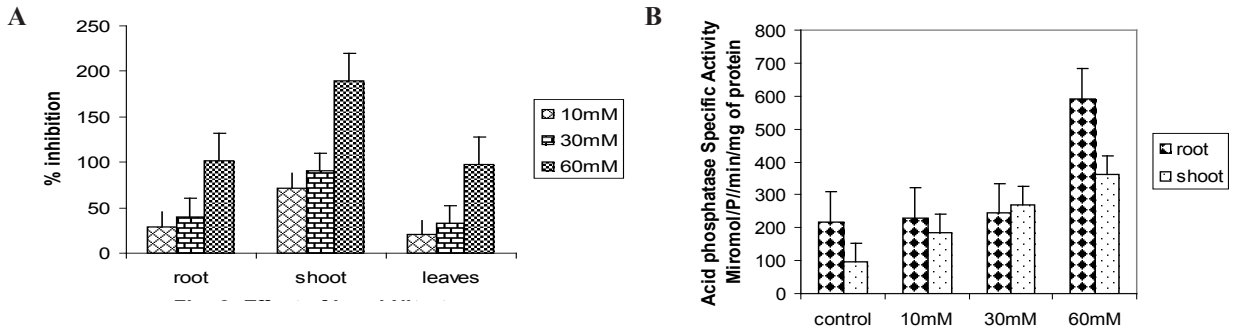


**Figure 2.** Shows **(A)** the effect of $Pb(NO_3)$ on reducing power assay and **(B)** acid phosphatase activity of different plant parts of *Triticum aestivum*.

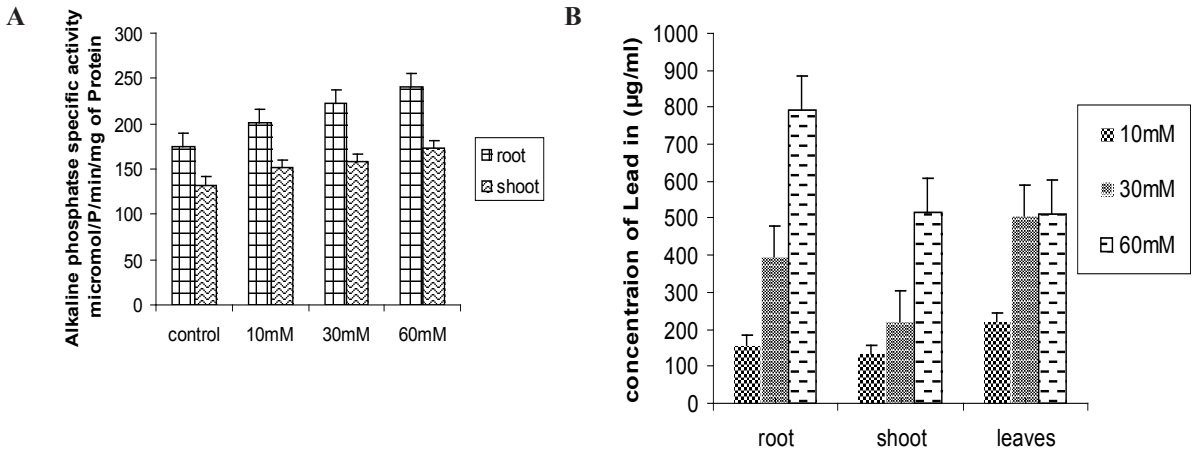


**Figure 3.** Shows **(A)** the effect of $Pb(NO_3)$ on alkaline phosphatase activity and **(B)** accumulation in different plant parts of *Triticum aestivum*.

activity of acid phosphatase was also increased in the shoots of plants treated with lead compared to control. In shoots there were 183, 269.61, 361.61µmol P/min/mg of protein, respectively at 10, 30 and 60 mM of Lead compared to control (98.03, µmol P/min/mg of protein). Similar trend was also obtained in alkaline phosphatase. Alkaline phosphatases specific activities were increased in the roots of plant treated with lead compared to control. In roots, these were 201.23, 223.60, 241, µmol P/min/mg of protein, respectively at 10, 30 and 60 mM of lead compared to control (175.5 µmol P/min/mg of protein of protein). In shoots, these were less increased than roots whose151.51, 158.50, 173.27 µmol P/min/mg of protein, respectively at, 10, 30 and 60mM of lead compared to control (132.66µmol P/min/mg of protein). Alteration in the activity of acid and alkaline phosphatase in plants has been observed under toxic conditions due to various heavy metals (27). Lead showed increased activity of acid phosphatase, α -amylase and peroxidase in leaves (28). Activities of several enzymes are reported to be enhance by after lead treatment this type of changes occur in enzyme synthesis , immobilization of enzyme inhibitors or as a result of effectors molecules, which are synthesized after lead toxicity. Increased activity of hydrolytic enzymes as well as of peroxidase in soybean leaves under Lead treatment parallels with the senescence of leaves. An increase in the activity of the RNA hydrolyzing enzyme ribonuclease and of protease has been observed in submerged aquatic angiospermic plants growing in presence of lead acetate (29). It is the key enzyme involved in transport and recycling of phosphorus (30).

***Accumulation of Lead in different parts of plant:***

Data presented in Table -3 and Fig -3 revealed that the accumulation of lead in different parts of the plants. Result of present study indicates that there was significant increased concentration of lead in root, shoot, then leaves compared to control**.** In roots lead accumulation were 157.1, 395.43, 792.33µg/g of fresh weight at 10, 30 and 60 mM of lead, respectively. In case of shoot Lead accumulation was131.60, 222.66, 513µg/g of fresh weight at 10, 30 and 60 mM of lead, respectively. In leaves lead accumulation were 219.0,507, 799 µg/g of fresh weight at 10, 30 and 60 mM of lead, respectively. Roots are more prone to heavy metal toxicity relative to shoots (31). Plants absorb and accumulate Lead in roots, stems, leaves, root nodules, seeds, etc. Uptake of Lead in plants is regulated by pH, particle size and cation exchange capacity of the soils as well as by exsudation and other physico-chemical parameters (27). The increase depends on the increment of exogenous Lead levels (31). Most of the Lead absorbed by plants accumulates in roots, and only a small fraction is translocated to the aerial part (32). The retention of Lead in the roots is due to binding to ion exchange sites and extracellular precipitation, mainly in the form of Lead carbonates, with both these mechanisms occurring in the cell wall (33). However, Lead does not always penetrate the root endoderm and enter the stele. Here, the endoderm acts as a barrier to Lead absorption and penetration to the interior of the stele and its transport to the aerial plant part (34).

## Conclusion

Based on these result, we may concluded that, the exposure of Triticum *aestivum* to different concentration of lead results in an increase in acid and alkaline phosphatase activity and redox potential. Lead was

**Table 3.** Accumulation Pb ($NO_3$) in Different parts (roots, shoot and leaves) of Triticum *aestivum*.

| Chemical | Lead Nitrate Concentration | Accumulation of Pb in Roots (μg/g fw) | Accumulation of Pb in shoots (μg/g fw) | Accumulation of lead leaves (μg/g fw) |
|---|---|---|---|---|
| Control | 0.0mM | 0.0 | 0.0 | 0.0 |
| Lead nitrate | 10mM | 157.1± 2.2[a] | 131.60±11.89[a] | 219.0± 15.2 [a] |
| Lead nitrate | 30mM | 395.43± 6.8 [a] | 222.66±98.4[a] | 507.0± 12.8 [a] |
| Lead nitrate | 60mM | 792.33± 11.9[a,b] | 516.03±16.12[a,b] | 513.0± 12.0 [a,b] |

Values are mean ± SE of 6 observations ; [a]$p < 0.01$ compared to control; [b]$p < 0.01$ compared to 10 mM lead nitrate exposed group.

accumulated more in the roots of Triticum *aestivum* compared to shoot and leaves. Lead also significantly affected plant growth and development with a lead concentrations in soils. Triticum *aestivum* can be used as a heavy metal accumulator in heavy metal affected soils. The deleterious effects of heavy metals may be alleviated in plants if provided with appropriate concentration and forms.

## References


1. Kumar, P., Yadava, R.K., Gollen, B., Kumar, S., Verma, R.K., Yadav, S. Nutritional Contents and Medicinal Properties of Wheat: A Review Life Sciences and Medicine Research, Volume LSMR-22, (2011)

2. Shewry, P.R., The Health grain programmed opens new opportunities for improving wheat for nutrition and health. *Nutrition Bulletin,* 2009, **34:** 225–231. doi: 10.1111/j.1467-3010.2009.01747.x

3. Alan, J.B., Changrun, L.M.S., Fergus, M., Clydesdale, F.A.C.N., Decker, E.A., Potential of wheat-based breakfast cereals as a source of dietary antioxidants. Journal of the *American College of Nutrition*, 2000, **19:** 308S–311S doi:10.1080/07315724.2000.10718965

4. Abdel-Aal, E.S.M., Sosulski, F.W., Youssef, M.M., Shehata, A.A.Y. Selected nutritional, physical and sensory characteristics of pan and flat breads prepared from composite flours containing fababean. *Plant Foods for Human Nutrition*, 1993, **51:** 409–414.

5. Adams, M.L., Lombi, E., Zhao, F.J., McGrath, S.P., Evidence of low selenium concentrations in UK bread-making wheat grain. *Journal of the Science of Food and Agriculture,* 2002, **82:** 1160–1165. doi: 10.1002/jsfa.1167

6. Arshad, M., Silvestre, J., Pinelli, E., Kallerhoff, J., Kaemmerer, M., Tarigo, A., Shahid, M., Guiresse, M., Pradere, P., Dumat, C. A field study of lead phytoextraction by various scented *elargonium* cultivars. *Chemosphere* 2008; **71**: 2187–2192 doi: 10.1016/j.chemosphere.2008.02.013

7. Uzu, G., Sobanska, S., Sarret, G., Munoz, M., Dumat, C., Foliar lead uptake by lettuce exposed to atmospheric fallouts. *Environ Sci Technol* 2010, **44:** 1036–1042 doi: 10.1021/es902190u

8. Shahid, M., Pinelli, E., Pourrut, B., Silvestre, J., Dumat, C., Lead-induced genotoxicity to *Vicia faba* L. roots in relation with metal cell uptake and initial speciation. *Ecotoxicol Environ Saf* 2011, **74:**78–84.doi: 10.1016/j.ecoenv.2010.08.037.

9. Tiwari, U., Agnihotri, R.K., Shrotriya, S., Sharma, R. Effect of Lead Nitrate Induced Heavy Metal Toxicity on some Biochemical Constituents of Wheat (*Triticum aestivumL*). *Research Journal of Agriculture Sciences* 2013, **4:** 283-285

10. Sano, A., Kiada, R., MakiI, H., Kaneko, T.S., Involvement of an acid phosphatase on cell wall regeneration of tobacco protoplasts. *Physiol. Plant.* 2003, **119:**121-125. doi: 10.1034/j.1399-3054.2003.00160.x

11. Orhanovic´ S, Pavela-Vrancic, M., Alkaline phosphatase activity in sea water: Influence of reaction conditions on the kinetic parameters of ALP. Croat. *Chim. Acta* 2000, **73:** 819-830

12. Miller, G., Shulaev, V. Mitter, R., Reactive oxygen signaling and abiotic stress, *Physiol. Plant.,* 2008; **133:** 481-489. doi:10.1104/pp.109.139287

13. Pinto, E., Sigaud-Kutner, T.C.S., Leitao, A.S., Okamoto, O.K., Morse, D., Coilepicolo, P., Heavy-metal induced oxidative stress in algae. *J. Phycol.* 2003, **39:**1008-1018. doi: 10.1111/j.0022-3646.2003.02-193.x

14. Hewitt, E.J., Sand and water culture methods used in the study of plant nutrition. Second edition. Technical communication no. 22 (Revised). Comm. Wealth Agric. Bureaux, Farnham Royal, Bucks, England, 479-534, 1966.

15. Fiske, C.H., Subbaraow, Y., The Colorimetric determination of phosphorous. *J. Biol. Chem*., 1925, **56**: 375.

16. Ferreira, I.C.F.R., Baptista, P., Vilas-Boas, M., Barros, L., Free-radical scavenging capacity and reducing power of wild edible mushrooms from northeast Portugal: Individual cap and stipe activity. *Food Chem.*, 2007, **100:** 1511-151.

17. Bremner, I., Heavy metal toxicities. *Quart. Rev.Biophy* 1971, **7:** 75-124.

18. Lowry, R., One Way ANOVA – Independent Samples. Vassar. edition. 4th, 2008.

19. Farooqi, Z.R., Zafar Iqbal, M., Kabir, M., Shafiq, M., Toxic effects of lead and cadmium on germination and seedling growth of Albizia lebbeck (L.) Benth. Pak. *J. Bot.,* 2009, **41:** 27-33.

20 Yang, Y-Y, Jung, J-Y., Song, W-Y., Suh, H.S., Lee, Y., Identification of rice varieties with high tolerance or sensitivity to lead and characterization of the mechanism of tolerance. *Plant Physiol.* 2000, **124:**1019-1026. doi: http://dx.doi.org/10.1104/pp.117.3.745

21. Elloumi, N., Ben, F., Rhouma, A., Ben, B., Mezghani, I., Boukhris, M., Cadmium induced growth inhibition and alteration of biochemical parameters of almond seedling grown in solution culture. *Acta. Phytol. Plant,* 2007, **29:** 57. doi: 10.1007/s11738-006-0009-y

22. Pant, P., Tripathi, A.K., Gairola, S., Phytoremediation of Arsenic using Cassia Fistula Lnn.seedling, I*nternational Journal of Research in Chemistry and Environment,* 2011; **1:** 24-28.

23. Noctor, G., Gomez, L., Vanacker, H., Foyer, C.H., Interactions between biosynthesis, compartmentation and transport in the control of glutathione homeostasis and signaling, *J. Exp. Bot.* 2002, **53:** 1283–1304. doi:10.1093/jexbot/53.372.1283

24. Foyer, C.H., Noctor, G., Oxidant and antioxidant signaling in reevaluation of the concept of oxidative stress in Physiol. *Cell and Environ.* 2005, **28: 1**056-1071. doi: 10.1111/j.1365-3040.2005.01327.x

25. Flora, S.J.S., Shrivastava, R., Mittal, M., Chemistry and pharmacological properties of some natural and synthetic antioxidants for heavy metal toxicity. C*urrent Medicinal Chemistry,* 2013, **20**: 4540-4574.

26. Mishra, S., Srivastava S., Tripathi R. D., Kumar R., Seth C. S.,

Gupta D. K., Lead detoxification by coontail (Ceratophyllum demersum L.) involves induction of phytochelatins and antioxidant system in response to its accumulation. Chemosphere 2006, **65**:1027–1039. doi:10.1016/j.chemosphere.2006.03.033

27. Sharma, P., Dubey, R.S., Lead toxicity in plants. *Braz. J. Plant Physiol.* 2005***;*** 17: 35-52. doi: 10.1590/S1677-04202005000100004

28. Lee, K.C., Cunningham, B.A., Paulsen, G.M., Liang, G.H., Moore, R.B., Effects of cadmium on respiration rate and activities of several enzymes in soybean seedlings. *Physiol. Plant,* 1976, **36:** 4-6.

29. Verma, S., Dubey, R.S., Lead toxicity induces lipid peroxidation and alters the activities of antioxidant enzymes in growing rice plants. *Plant Sci.* 2003, **164:** 645-655. doi:10.1016/so168-9452 (03)00022-0.

30. Yan, X., Liao, H., Trull, M.C., Beebe, S.E., Lynch, J.P. Induction of a major leaf acid phosphatase does not confer adaptation to low phosphorus availability in common bean. *Plant Physiol*., 2001**, 125:** 1901-1911

31. Oncel, I., Keles, Y., Ustun, A.S., Interactive effects of temperature and heavy metal stress on the growth and some biochemical compound in wheat seedling, *Environment Pollution,* 2000, **107:** 315-320. doi.org/10.1016/so269-7499(99)00177-3.

32. Patra, M., Bhowmik, N., Bandopadhyay, B., Sharma, A., Comparison of mercury, lead and arsenic with respect to genotoxic effects on plant systems and the development of genetic tolerance. *Environ. Exp. Bot.* 2004, **52:**199-223. doi:10.1016/j.envexpbot.2004.02.009.

33. Jarvis, M.D., Leung, D.W.M., Chelated lead transport in *Pinus radiata*: an ultrastructural study. *Environ. Exp. Bot*. 2002, **48:** 21-32. doi: 10.1016/S0098-8472(02)00005-9

34. Weis, J.S., Weis, P., Metal uptake, transport and release by wetland plants: implications for phytoremediation and restoration. *Environ. Int.* 2004, **30**: 685-700.