International Journal of Science and Research (IJSR)
ISSN (Online): 2319-7064
Impact Factor (2012): 3.358

Biochemical Mode of Resistance to Environmentally Toxic Heavy Metal Lead in two Species of *Alternanthera*

Devi Chinmayee M[1], Mahesh B[2], Mini I[3], Swapna T S[4]

[1, 2, 3, 4]Department of Botany, University College, Thiruvananthapuram, Kerala, India

Abstract: ***Present study evaluated effect of toxic heavy metal lead in various biochemical parameters in plants. During the exposure of plants to contaminated soils, the antioxidant defense system helps the plant to protect itself from the damage. Greenhouse experiment was conducted and consisted of range finding test and definitive test for various concentrations of heavy metal lead. The bioorganics of the plant such as soluble sugar, protein, lipid, phenol, amino acid photosynthetic pigments and antioxidant enzymes were estimated after 30 days of treatment. The activity of enzymatic antioxidants such as superoxide dismutase (SOD; E.C. 1.15.1.1), catalase (CAT: EC 1.11.1.6), polyphenol oxidase (PPO; EC 1.14.18.1) and peroxidase (POX; E.C. 1.11.1.7) showed profound variations in response to Pb stress from the control plants. It was found that Pb induced oxidative stress in both the species of Alternanthera, enhance antioxidant enzyme activities and antioxidant concentrations. Non enzymatic antioxidants such as proline and phenol increased with Pb concentration. Chlorophyll content seemed to be reduced with Pb concentration. Both species of Alternanthera showed tolerance against Pb induced stress and can be suggested as a suitable candidate for phytoremediation of Pb.***

Keywords: Lead, Antioxidant enzymes, non-enzymatic antioxidants, Heavy metal stress**.**

1. Introduction

Soil pollution by metals differs from air or water pollution, because heavy metals persist in soil much longer than in other compartments of the biosphere [1]. Toxic metal contamination of soil, aqueous waste streams and groundwater causes major environmental and human health problems. Heavy metals can be emitted into the environment by both natural and anthropogenic causes. The major causes of emission are the anthropogenic sources specifically mining operations. Plants are ideal agents for soil and water remediation because of their unique genetic, biochemical and physiological properties. As the heavy metal is non biodegradable and toxic in nature, the toxicity of metals are threats on ecosystem. Lead (Pb) is one of the most abundant, ubiquitous toxic elements posing a critical concern to human and environmental health. It can cause multiple direct and indirect effects on plant growth and metabolism, along with visible symptoms including stunted growth, as well as leading to membrane disorganization and reduced photosynthesis [2], [3]. Elevated levels of heavy metals in plant tissue are known to generate reactive oxygen species (ROS). To eliminate the harmful effects of ROS, plant cells are equipped with a well-developed antioxidant defense system comprising enzymes; namely, catalase (CAT), guaiacol-specific peroxidase (POX) superoxide dismutase (SOD), polyphenol oxidase (PPO) as well as nonenzymatic components such as, proline (PRO), phenols, Carotenoid etc [4].

Present study intended to evaluate the physiological responses of two closely related species of *Alternanthera* (*Alternanthera sessilis* and *Alternanthesa tenella)* belonging to the family Amaranthaceae, to various lead concentrations, with reference to enzymatic and nonenzymatic antioxidants. This will be helpful in stating the toxic effect of lead in *Alternanthera* species and to evaluate the potential of these plants for phytoremediation of lead contaminated soil.

2. Materials and Methods

Two species of *Alternanthera* collected from its natural habit and grown under green house conditions were used as test plants. Lead nitrate solution was used as a source of lead and mixed uniformly with soil. The lead solution was used in three concentrations 50, 100 and 150 mg kg^{-1} (T1, T2 and T3). The test plants were grown in pots containing 2 kg garden soil saturated with corresponding concentrations of metal. Untreated soil was used to raise control plants and the biochemical parameters were recorded after one month.

Bioorganics comprises a wide variety of biologically significant macromolecules such as pigments, primary and secondary metabolites which regulate various biological processes. Bioorganics in plant samples were estimated following standard procedures. The amount of total soluble sugars was estimated colorimetrically at 540 nm, using anthrone reagent according to Roe [5]. Protein content in the extracts was determined according to Lowry *et al* [6]. For lipid estimation, the powdered sample was extracted with chloroform methanol mixture and was estimated using the method of Bligh and Dyer [7]. The amount of amino acids present in the samples was estimated following Moore and Stein [8].

2.1 Non enzymatic antioxidents

Proline was analysed spectrophotometerically at 520 *nm* using toluene for a blank as per Bates *et al* [9]. Chlorophyll Content was measured by Arnon's method [10], and total phenols by Folin-Ciocalteau method [11]

2.2 Enzymatic antioxidents

The activity of Superoxide dismutase (SOD; E.C. 1.15.1.1) was assayed spectrophotometrically by measuring its ability to inhibit the photochemical reduction of Nitro blue Tetrazoilum [12]. One unit of SOD is the amount of extracts that gives 50% inhibition in the rate of NBT reduction. Catalase activity (CAT; EC 1.11.1.6) was determined by consumption of H_2O_2 and was monitored spectrophotometrically at 240 *nm* for 3 min [13]. For Polyphenol oxidase (EC 1.14.18.1) activity, catechol was used and the activity was expressed as changes in absorbance at 495 *nm* min^{-1} g^{-1} fresh weight of tissue [14]. For Peroxidase assay (POX; E.C. 1.11.1.7) the increase in absorbance due to oxidation of guaiacol (extinction coeffi-cient 26.6 mM^{-1} cm^{-1}) was monitored at 470 *nm* [15]. Experiments were repeated thrice and significance of differences between the treatments was statistically evaluated by standard deviation and Student's *t*-test methods.

3. Results and Conclusions

Lead is one of the heavy metal widely used in modern industry that has been recognized as highly toxic and carcinogenic. It can affect growth and metabolism of plant to varying degrees depending on the concentration and tissue types of plant species [2]. Previous studies have suggested that lead exerts its adverse actions by promoting or exacerbating oxidative damage to the cells and it has been shown that antioxidant defense enzymes play a key role in the protection against heavy metal toxicity [16], [17], [18]. These findings are important to understand the behavior of those enzymes in the presence of highly toxic metals.

The concentration of bioorganics such as total soluble sugars, total protein, lipids, phenol, aminoacids, proline and photosynthetic pigments (Figure1 to 7) exhibited profound variation in response to the accumulation of lead. Lead stress enhanced the activity of antioxidant enzymes as shown in fig 8-11. This increase had been reported in other plant species under lead stress, such as *Sesbania drummondii* [16], *Cassia angustifolia* [19] and *Jatropha curcas* [20] Thus increased SOD activity is response to lead stress appears to be due to the need for combating oxidative stress. The results obtained are similar to Zn stress in *Alternanthera philoxeroides* where it changed the activities of superoxide dismutase (SOD), catalase (CAT) and peroxidase (POX) [21]. The present study suggested that treatment with different levels of lead enhances the antioxidant enzymes and changes in biochemical parameters. Both the species if *Alternanthera* showed moderate tolerance against heavy metal induced stress which makes it a suitable candidate for phytoremediation of heavy metal contaminated soil.

4. Future Perspectives

Evaluation, separation and identification of heavy metal stress induced proteins and identification of genes responsible for these will be helpful for getting better knowledge of stress tolerance and resistance in plants.

5. Acknowledgements

Authors are thankful to the Department Of Environment and Climate Change, Govt. of Kerala for providing financial support

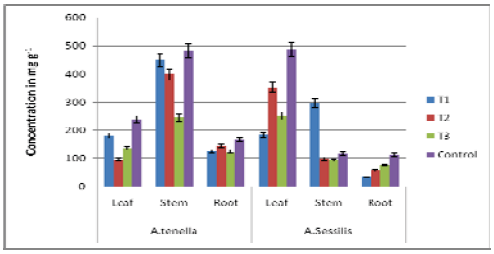

Figure 1: Carbohydrate content in in two species of *Alternanthera* under Lead stress.

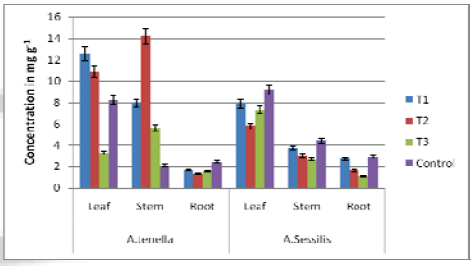

Figure 2: Protein content in in two species of *Alternanthera* under Lead stress

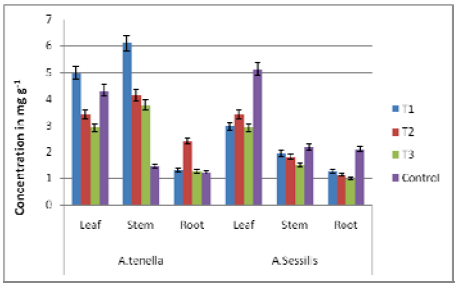

Figure 3: Lipid content in in two species of *Alternanthera* under Lead stress

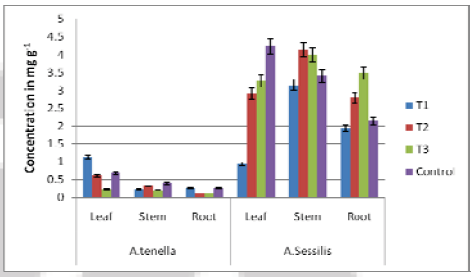

Figure 4: Amino acid content in in two species of *Alternanthera* under Lead stress

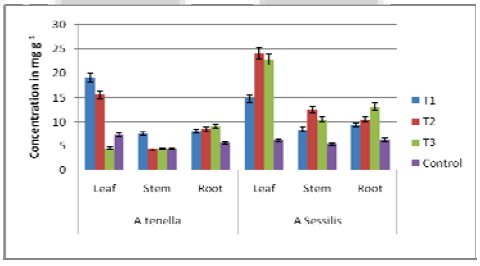

Figure 5: Proline content in in two species of *Alternanthera* under Lead stress

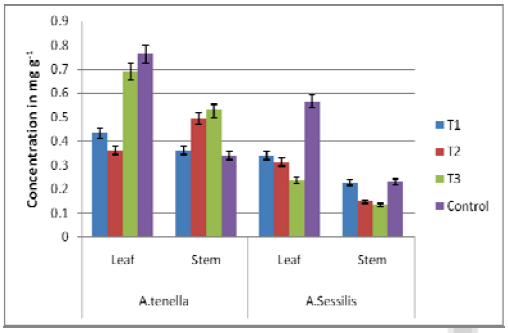

Figure 6: Chlorophyll content in in two species of *Alternanthera* under Lead stress

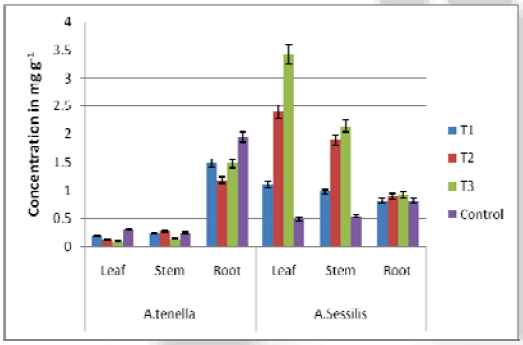

Figure 7: Phenol content in in two species of *Alternanthera* under Lead stress

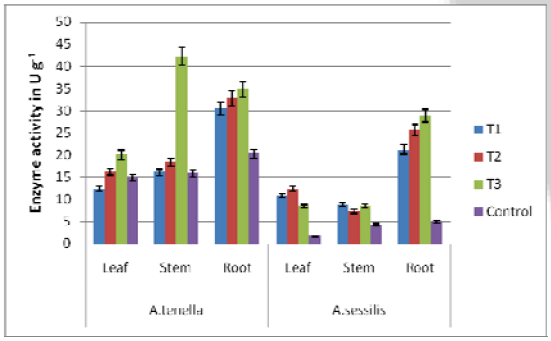

Figure 8: SOD activity in two species of *Alternanthera* under Lead stress

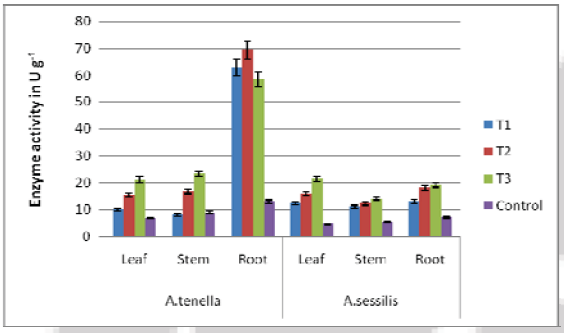

Figure 9: POD activity in two species of Alternanthera under Lead stress

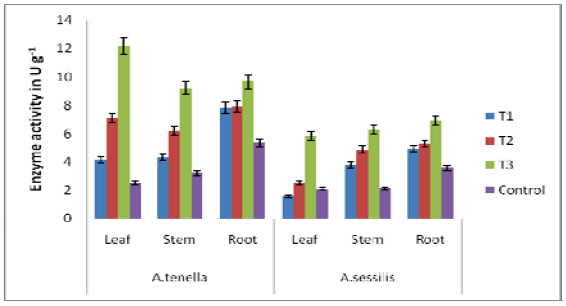

Figure 10: CAT activity in two species of *Alternanthera* under Lead stress

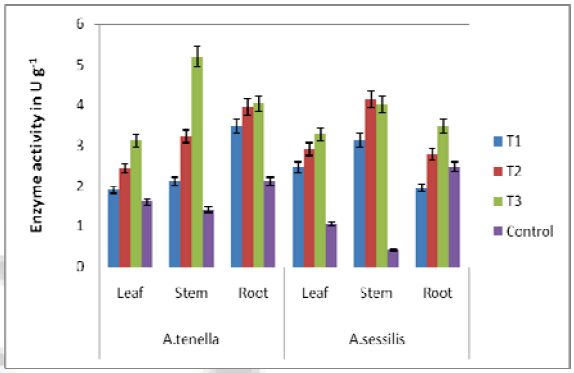

Figure 11: PPO activity in two species of *Alternanthera* under Lead stress

References

[1] M. M. Lasat, Phytoextraction of toxic metals – A review of biological mechanisms.*Journal of Environmental Quality* 31, 2002, pp 109–120.

[2] Sharma, P. and R.S. Dubey, 2005. Lead toxicity in plants. *Brazil J. Plant Physiol.*, 17: 35–52.

[3] Ahmad, M.S.A., M. Hussain, S. Ijaz and A.K. Alvi, 2008. Photosynthetic performance of two mung bean (*Vigna radiata*) cultivars under lead and copper stress. *Int. J. Agric. Biol.*, 10: 167–172.

[4] Briat, J.F. 2002. Metal ion-activated oxidative stress and its control. pp. 171-189. In: Inze, D. and Montagu, M.V. (Eds.). Oxidative Stress in Plants. New York: Taylor and Francis.

[5] Roe J H (1955) The determination of dextran in blood and urine with anthrone reagent. J Biol Chem Jun; 208(2): 889-896.

[6] Lowry, O.H., *et al.* (1951). Protein measurement with phenol reagent. J.Biol. Chem.193, 265-275.

[7] Bligh, E.G. and Dyer, W.J. (1959). Rapid method of total lipid extraction and purification. Can. J. Biochem. Physiol., 37: 911-917.

[8] Moore, S., & Stein, W. H. (1948). In S. P. Colowick & N. D. Kaplan (Eds.), Methods in enzymology (Vol.3). New York: Academic. 468 pp.

[9] Bates L S, Waldren R P and Teare I D, Rapid determination of free proline for water stress studies. *Plant Soil*, *Vol*. **39** (1973), pp. 205-207.

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

[11] Malick C P and Singh M B, ln:plant enzymology and histoenzymology, Kalyani publishers, New Delhi, (1980), P286.

[12] Beauchamp C H and Fridovich I, Superoxide dismutase: improved assays and an assay applicable to

acrylamide gels. *Analytical Biochemistry* **44**, (1971) 276-87.
[13] Luck H, In the Methods in Enzymatic Analysis, 2nd edition, Bergmeyer, Academic Press, New York, 1974, p. 885.
[14] Esterbauer H, Schwarzl E and Hayn M, A rapid assay for catechol oxidase and laccase using 2, nitro-5-thiobenzoic acid, *Anal. Biochem*., **77** (1977), 486-494.
[15] Putter, J. *In: Methods of Enzymatic Analysis*, 2 (*Ed. Bergmeyer*), Academic Press, New York, (1974). pp. 685.
[16] Thomas, R.A., N.C. Sharma and S.V. Sahi, 2004. Antioxidant defense in a lead accumulating plant, *Sesbania drummondii*. *Plant Physiol. Biochem.*, 42: 899–906.
[17] Reddy, A.M., S.G. Kumar, G. Jyothsnakumari, S. Thimmanaik and C. Sudhakar, 2005. Lead induced changes in antioxidant metabolism of horsegram (*Macrotyloma uniflorum* (Lam.) Verdc.) and bengalgram (*Cicer arietinum* L.). *Chemosphere*, 60: 97–104.
[18] Wang, C.R., X.R. Wang, Y. Tian, H.X. Yu, X.Y. Gu, W.C. Du and H. Zhou, 2008. Oxidative stress, defense response and early biomarkers for lead-contaminated soil in *Vicia faba* seedlings. *Environ. Toxicol. Chem.*, 27: 970–977.
[19] Qureshi, M.I., M.Z. Abdin, S. Qadir and M. Iqbal, 2007. Lead-induced oxidative stress and metabolic alterations in *Cassia angustifolia* Vahl. *Biol. Plant.*, 51: 121–128
[20] Gao, S., Q. Li, C. Ou-Yang, L. Chen, S.H. Wang and F. Chen, 2009. Leadctoxicity induced antioxidant enzyme and phenylalaninecammonia-lyase activities in *Jatropha curcas* L. radicles. *FreseniuscEnviron. Bull.*, 5: 811–815
[21] Yuan Q H , Shi G X , Zhao J , Zhang H, and Xu Q S , Physiological and Proteomic Analyses of *Alternanthera philoxeroides* under Zinc Stress *Russian Journal of Plant Physiology,* **56** (2009), 495–502.

Author Profile

Devi chinmayee .M received the B.Sc, M.Sc and M.phil degrees in Botany from University of Kerala in 2007, 2009 and 2011 respectively and worked as a Research assistant in the research project funded by Department of Environment and Climate change, Government of Kerala, Presently doing PhD in Botany in University College, Thiruvananthapuram

Mahesh B studied BSc, MSc and MPhil degrees from Kerala University & currently doing PhD In University College, Thiruvananthapuram

Mini I Associate Professor in Department of Botany, University College and has several years of teaching and research experience in Environmental Science

Swapna T S Assistant Professor of Botany, University College with research interest in Plant and Environmental Biotechnology