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Evaluation of Antioxidant Defense and Phytoremediation Efficacy of a Submerged Aquatic Macrophyte *Hydrilla verticillata* (L.f.) Royle under Lead Stress

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ABSTRACT

Lead-induced antioxidative response of a submerged aquatic macrophyte *Hydrilla verticillata* was investigated under laboratory condition. The plant was collected from a fresh water pond and bioassayed with varying concentrations of Pb for fifteen days. The activities of enzymatic antioxidants such as Catalase, Superoxide dismutase, Monodehydro ascorbate reductase, Glutathione reductase, Ascorbate peroxidase, Polyphenol oxidase and Phenyl alanine ammonia lyase and non-enzymatic antioxidants (carotenoid, flavonoid, proline, phenol and ascorbate) were analyzed. Phytoremediation efficacy of the macrophyte for lead was evaluated and supported with the quantification of Bioconcentration Factor (BCF). Data were statistically analyzed and levels of significance were recorded. SOD activity, phenol (P-value<0.0001) and proline (P-value=0.0009) accumulation were extremely significant in treated plants as compared to control. APX, GR, PAL, CAT, MDHAR and PPO activity were significant in metal treated plants than control. Very significant amounts of carotenoid and flavonoid were encountered while ascorbate content remained non-significant. Enzymatic and non-enzymatic antioxidants were positively correlated with biosorption of Pb and were significant. Significant lead accumulation suggested that *Hydrilla verticillata* was a promising candidate for phytoremediation of Pb. The present study helped assess the enzymatic and non-enzymatic antioxidant defense in *Hydrilla verticillata* suggesting the enhanced activation of SOD and increased accumulation of phenol and proline as a biomarker of Pb stress and tolerance.

Key Words: Enzymatic Antioxidants; Carotenoid; Flavonoid; Proline; Phenol; Ascorbate; Biomarker, Tolerance

INTRODUCTION

Lead is a transition element acting as a common pollutant in air water and soil. There are variety of industrial processes and commercial products that involve the use of lead such as mining, smelting, manufacture of pesticides and fertilizers, dumping of municipal sewage, burning of lead fuels, paints, ceramic glasses, television glass, batteries, X-rays and electrical equipments. Lead has been listed as a potential carcinogen and it accumulates in the organs including brain and leads to plumbism or lead poisoning and even death. Lead is one of the most abundant ubiquitous toxic elements posing critical concern to human and environmental health. It can cause multiple direct and indirect effects on plant growth and metabolism including stunted growth, membrane disorganization and reduced photosynthesis.(Sharma and Dubey 2005, Ahmad et al. 2008).

Oxidative phytotoxicity is considered to be the most fatal condition in plants under heavy metal stress. Induction and activation of antioxidative defense system is one of the detoxification mechanisms in plants under stressful condition. The tolerance of plants to stress conditions depends on their ability to make balance between the production of toxic oxygen derivatives and antioxidative defense response. Plants have complex ROS scavenging mechanisms at the molecular and

cellular levels. These mechanisms inhibit or slow down the oxidation of biomolecules and oxidative chain reactions, decreasethe cellular oxidative damage and increase resistance to heavy metals (Michalak2006).

The present study was aimed to evaluate anti-oxidant machinery and tolerance in aquatic macrophyte *Hydrilla verticillata* under lead stress. The role of enzymatic oxidants - Catalase (CAT: EC 1.11.1.6), Superoxide dismutase (SOD: EC 1.15.1.1), Mono-dehydro ascorbate reductase (MDHAR: EC 1.6.5.4), Glutathione reductase (GR: EC 1.6.4.2), Ascorbate peroxidase (APX: EC 1.11.1.11), Polyphenol oxidase (PPO: EC 1.14.18.1) and Phenyl alanine ammonia lyase (PAL: EC 4.3.1.5) and nonenzymatic antioxidants - carotenoids, flavonoid, proline, phenol and ascorbate - along with Pb accumulation efficacy and its tolerance were evaluated.

MATERIAL AND METHODS

Hydrilla verticillata, commonly called 'water thyme', is a submersed perennial freshwater macrophyte belonging to the family Hydrocharitaceae. The plant is rooted in the soil and has long stems that branch at the surface where growth becomes horizontal, forming dense mats. *Hydrilla* can be found infesting freshwater lakes, ponds, rivers, impoundments and canals. Plants were collected from a freshwater pond and transferred to the plastic containers and exposed to heavy metal stress in water containing varying concentration of lead nitrate (0.03, 0.09 and 0.180mM; T1,T2, and T3) and the bioassay was performed for fifteen days against a suitable control.Fresh samples were used for the analysis of enzymatic(SOD, CAT, MDHAR, GR, APX, PPO and PAL) and non-enzymatic antioxidants(carotenoids, flavonoid, proline, phenol and ascorbate).

The activity of SOD was assayed following the Nitro Blue Tetrazolium (NBT) method as described by Gong et al. (2005) and CAT activity was determined by decomposition of H_2O_2 spectrophotometrically by assessing the decrease in absorbance at 240 nm (Cakmak et al.1993). MDHAR activity was calculated using the extinction coefficient (6.2 $mM^{-1}{\cdot}cm^{-1}$) according to the method of Miyake and Asada (1992).GR activity was assayed by measuring the decrease in absorbance at 334nm due to the oxidation of NADPH (Foyer and Halliwell 1976) and APX activity was determined according to the method of Nakano and Asada (1981). The activity of PPO was performed according to the method of Esterbauer et al.(1977) and the PAL was spectrophotometrically analysed by the method of Brueske (1980).

Estimation of carotenoid present in the plant sample was carried out by standard method (Arnon1949) and the extraction and estimation of flavonoidcontent was determined according to Meda et al. (2005) with slight modifications.The extraction and estimation of proline was done according to the method of Bates et al. (1973) and the method of Mayr et al. (1995) was used for the estimation of total phenol. Ascorbate present in the samples were analysed by the method of Sadasivam and Balasubramanian(1987). The estimation of Pb in treated macrophyte was carried out following the method of APHA (1992) by aspirating the digested sample into Atomic Absorption Spectrophotometer (AAS) Perkin Elmer model 2380 and values expressed in µg g^{-1}.Bio concentration factor (BCF) was calculated by using standard formula.

BCF= Concentration of metal in dried plant/
Initial concentration of metal in solution

Data were analysed statistically to find out correlation coefficient and P-value using GraphPad InStat DTCG-[DATASET1.ISD] software.

RESULT AND DISCUSSION

The enzymatic and non-enzymatic antioxidants under investigation exhibited profound variation under lead stress. Lead treatment caused concomitant induction in the levels of antioxidative activities of enzymes such as Catalase (CAT),Superoxide dismutase (SOD), Monodehydro ascorbate reductase (MDHAR), Glutathione reductase (GR), Ascorbate Peroxidase (APX), Polyphenol oxidase (PPO) and Phenyl alanine ammonia lyase (PAL) (Figure 1). The enzymatic antioxidants showed increased activity than the control under varying concentration of lead. Gradual increase in the activity of CAT was recorded as concentration of treatments increased whereas more than fourfold increase was evident in the activity of SOD as compared to control.Mishra et al.(2006) reported increased activity of CAT at different concentration of lead in *Ceratophyllum demersum* remained in agreement with the present investigation. CAT accumulation plays an important role in the protection of plant cells against oxidative damage by breaking down hydrogen peroxide (Mittler 2002). A significant increase in SOD activity

was recorded in *Eichhornia crassipes* exposed to lead stress which remained in unison with the present study. The higher activity of SOD indicated that it is an essential component of plant antioxidative system that can be used as biomarker of environmental stress (Dazyet al.2009). Initial increase followed by sudden decline was displayed in the activity of MDHARunder lead treatment. GR and APXactivity increased in initial and final concentrations and a decline was noted in the middle concentration. Lead stressed rice seedlings also show increased activities of APX and GR(Verma and Dubey2003). Increased GR activity under lead toxicity normally helps in recycling oxidized glutathione into reduced glutathione to maintain the ratio of oxidized glutathione to reduced glutathione (GSH/GSSG) and the total glutathione pool(Foyer et al. 1994). Baisaket al.(1994) observed an increase in the activity of GR underPb stress and attributed to de novo synthesis of the enzyme protein. Mohan andHosetti (1997) suggested that the increase of APX activity may be an effect of accelerated senescence, connected with enhanced formation of hydrogen peroxide or secondary metabolites such as phenolic compounds. Both PPO and PAL activity increased steadily with increase in Pb concentrations. PPO was found to be associated to a catalase like activity and could thus have a role in direct hydrogen peroxide removal (Grassmann 2002).Dai et al. (2006) detected a significant increase in PAL activity in fronds of *Azollaimbricata*under cadmium treatment which may be related to its role in response with heavy metal stress.

The non-enzymatic antioxidants like carotenoid, flavonoid, proline, phenol and ascorbate showed evident change under lead stress as compared to control (Figure 2). Initial increase followed by further decline was recorded in the case of carotenoid whereas flavonoid content showed a decreaseunder varying concentration of lead. Carotenoids prevent the formation of singlet oxygen by quenching the triplet of chlorophyll molecule as they arise (Fyfe et al.1995). Carotenoids play an important role in protecting cell against the stress and have ability to quench ROS (Sen and Mukherjee 2009). Aroraet al.(2000)pointed out that flavonoidsare able to alter peroxidation kinetics by modifying the lipid packing order. They stabilize membranes by decreasing membrane fluidity in a concentration-dependent manner and hinder the diffusion of free radicalsand restrict peroxidative reaction.Proline accumulation increased as the concentration of lead increased compared to control. Induction of various molecular anti-oxidants including glutathione, cysteine, ascorbic acid and proline was recorded in an aquatic macrophyte *Najasindica* on application of lead (Singh and Pandey 2011). Costa and Morel (1994) opined that free proline accumulation seems to be widespread among plants and the cumulative capacity is manifestation of self-protective ability of plant exposed to different metals. The important role of proline in response of plants to heavy metal toxicity may also be related to its anti-oxidative properties(Matyasik et al. 2002), its function as metal chelator (Sharma and Dubey 2007) and its ability to protect enzyme from inhibition (Maheswari and Dubey 2007). A slight reduction in phenol content and considerable reduction in ascorbate content were noted in treated *Hydrilla verticillata* than control. Phenolic compounds possess ideal structural chemistry for free radical scavenging activity and shown to be more effective antioxidants. Antioxidative properties arise from their high reactivity as hydrogen or electron donors and from the ability of polyphenol derived radical to stabilize and delocalize the unpaired electron to chelate metal ions (Evans et al. 1997). Both ascorbate and glutathione might play a key role in buffering oxidative stress in most eukaryotic systems (Noctor and Foyer 1998, Smirnoff 1995, Smirnoff et al. 2001).

Bioaccumulation of lead ranged from 2338.0 to 5809.0 μg g^{-1} in *Hydrilla verticillata.* Comparatively higher accumulation of lead was observed under all treatments than control. The BCF values fluctuated between 77.9 and 32.7 (Figure 3) in *Hydrilla verticillata* under Pb stress. Gradual decline in BCF recorded in the present study is in unison with the reports of Khellaf and Zerdaoui (2009) in *Lemna gibba.* Jain et al.`(1990) also reported that the BCF values for *Azollapinnata*treated with Pb observed a gradual decrease with increase in metal concentration in solution.The result revealed that, under the experimental conditions *Hydrilla* accumulated Pb and the biosorption increased as the concentration of metal in the medium increased. The accumulation of Pb in various parts of aquatic macrophytes under laboratory conditions had been reported in several species of aquatic plants such as *Pistia stratiotes* (Main et al. 2001), *Salvinia cucullata* (Phetsombat et al.2006) and *Lemna polyrrhiza* (John et al. 2007). The normal range in the concentration of Pb in plants is 0.1 to10 μg g^{-1} (Leeper1978) and the values obtained in the present study exceed the normal range suggesting the Pb accumulation efficacy of *Hydrilla*. Enzymatic and non-enzymaticantioxidants were positively correlated with biosorption of Pb and found to be significant (Table 1).

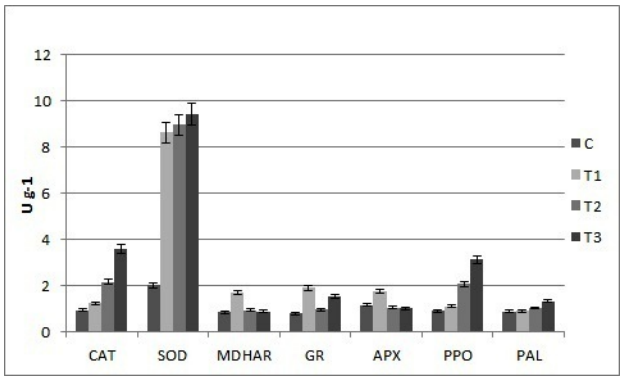

Figure 1. Activity of enzymatic antioxidants in *Hydrilla verticillata* under Pb stress.

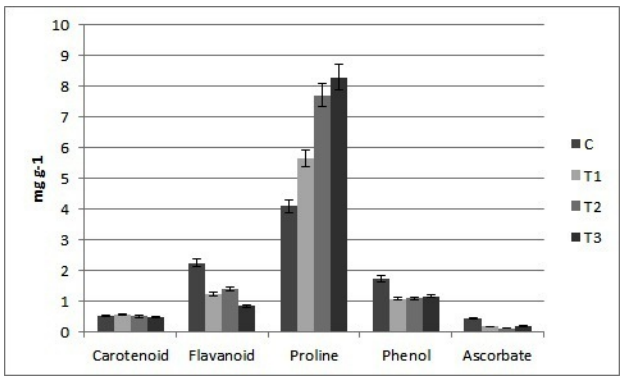

Figure 2. Activity of non-enzymatic antioxidants in *Hydrilla verticillata* under Pb stress.

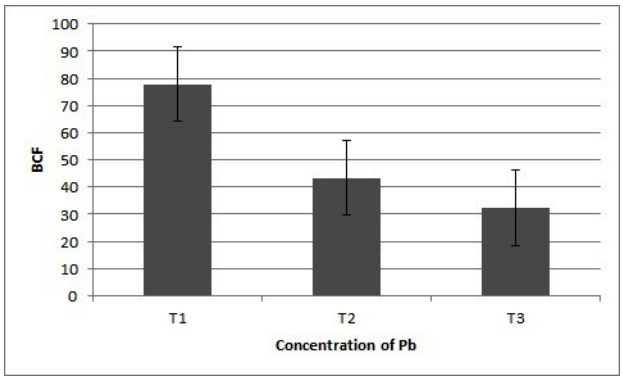

Figure 3. Bioconcentration factor in *Hydrilla verticillata* under Pb stress.

Regression analysis of enzymatic and non-enzymatic antioxidants under varying concentrations of Pb imparted positive correlations. (Figures 4-15, Table 2). Among the enzymatic antioxidants, activity of SOD was extremely significant (P-value<0.0001) and very significant for APX, GR and PAL in treated *Hydrilla*

Table 1. Correlation of antioxidants with biosorption of Pb in *Hydrilla verticillata.*

Antioxidants	Correlation Coefficient (R)	P-value
Enzymatic		
CA	0.8943	0.0162*
SOD	0.8940	0.0163*
MDHAR	0.8930	0.0168*
GR	0.8933	0.0159*
APX	0. 8944	0.0160*
PAL	0. 8938	.0164*
PPO	0.8935	0.0165*
Non Enzymatic		
Carotenoid	0.8940	0.0163*
Flavonoid	0.8944	0.0161*
Proline	0.8941	0.0162*
Phenol	0.8939	0.0165*
Ascorbate	0.8935	0.0168*

Table.2. Regression analysis and level of significance among antioxidants in *H. verticillata* under Pb stress.

Antioxidants	Regression equation	Correlation coefficient (r)	P- value
Enzymatic			
CAT	y = 14.952x + 0.8556	0.8515	0.0315*
SOD	y = 30.817x + 4.9262	0.9986	< 0.0001***
MDHAR	y = -1.6548x + 1.2089	0.8981	0.0150*
GR	y = 1.5976x + 1.1719	0.9244	0.0084**
APX	y = -2.2857x + 1.4114	0.9228	0.0087**
PAL	y = 2.5413x + 0.8434	0.9646	0.0019**
PPO	y = 12.777x + 0.8375	0.8625	0.0271*
Non Enzymatic			
Carotenoid	y = -0.3373x + 0.5525	0.9669	0.0016**
Flavonoid	y = -6.069x + 1.8884	0.9529	0.0033**
Proline	y = 22.508x + 4.7519	0.9754	0.0009***
Phenol	y = -2.0952x + 1.4261	0.9936	< 0.0001***
Ascorbate	y = -1.0278x + 0.3123	0.5354	0.2737^{ns}.

*significant ** very significant ***extremely significant
^{ns}non-significant

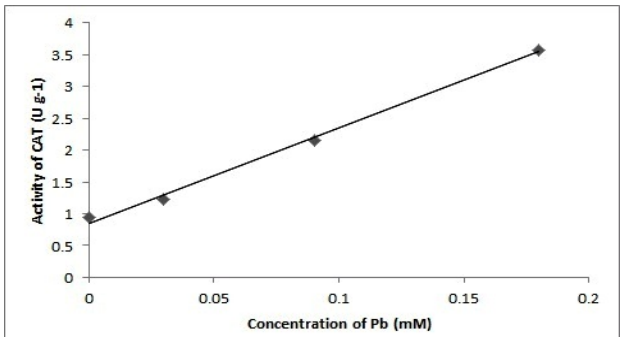

Figure 4. Relationship between CAT and Pb concentrations

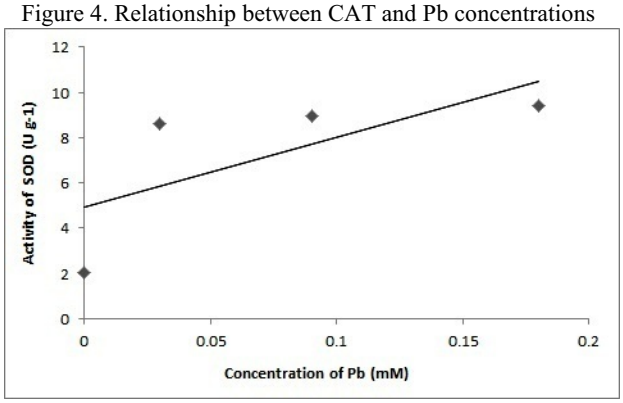

Figure 5. Relationship between SOD and Pb concentrations

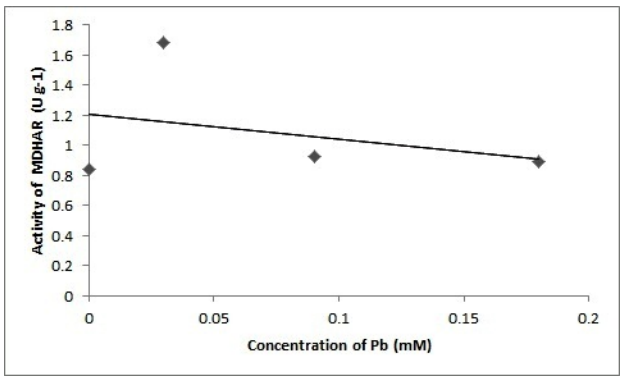

Figure 6. Relationship between MDHAR and Pb concentrations

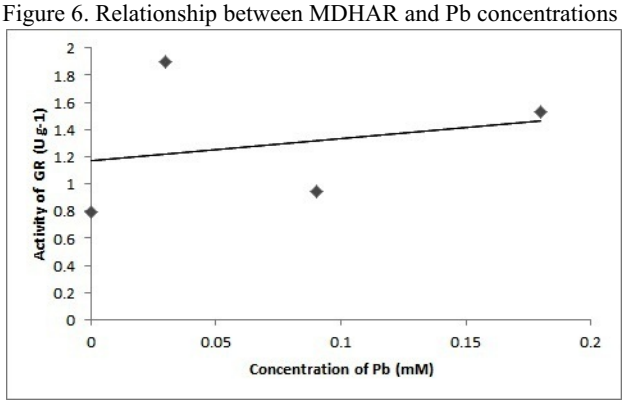

Figure 7. Relationship between GR and Pb concentrations

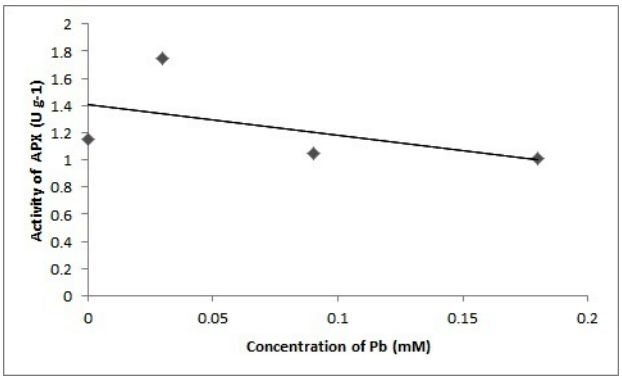

Figure 8. Relationship between APX and Pb concentrations

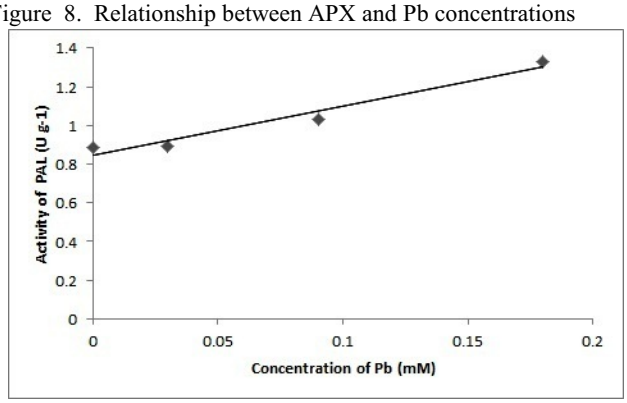

Figure 9. Relationship between PAL and Pb concentrations

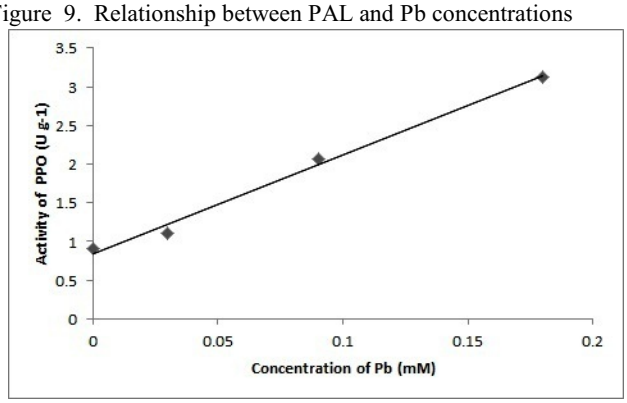

Figure 10. Relationship between PPO and Pb concentrations

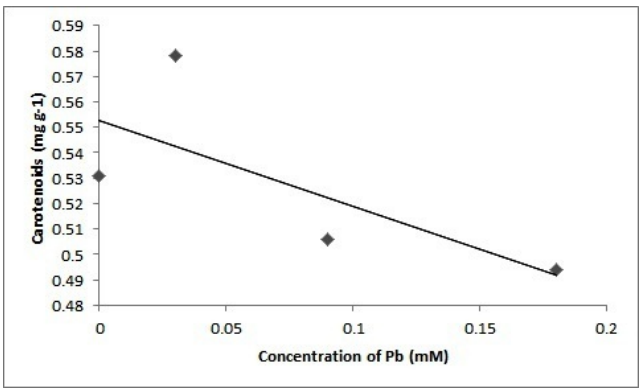

Figure 11. Relationship between carotenoids and Pb concentrations

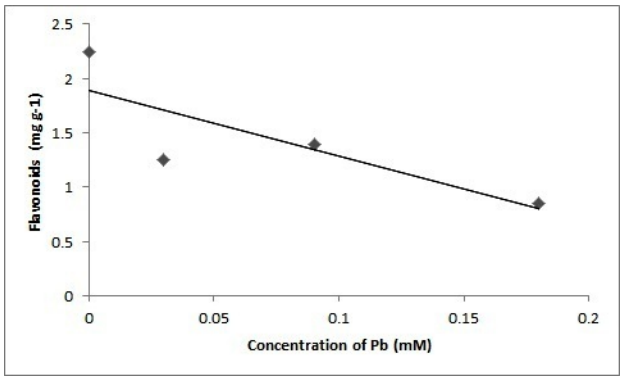

Figure 12. Relationship between flavonoids and Pb concentrations

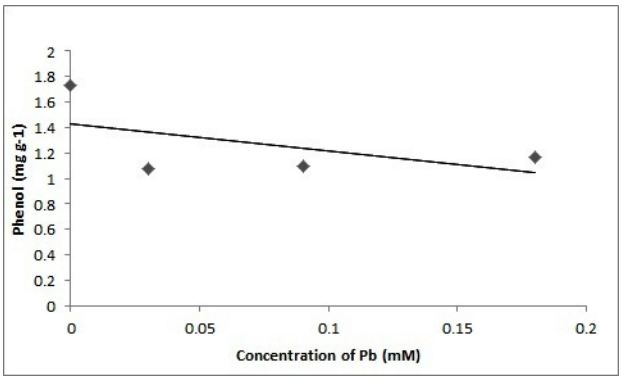

Figure 14. Relationship between phenol and Pb concentrations

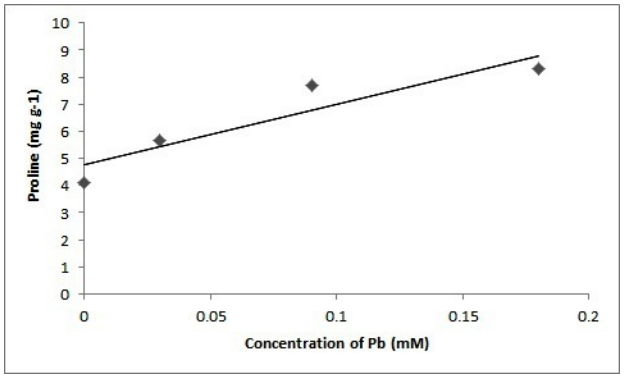

Figure 13. Relationship between proline and Pb concentrations

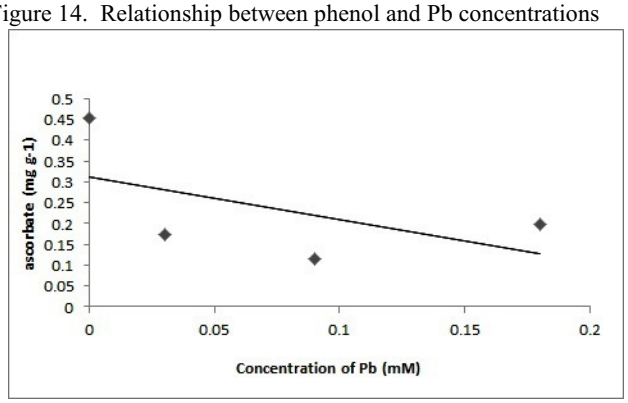

Figure 15. Relationship between ascorbate and Pb concentrations

verticillata than control. Activity of CAT, MDHAR and PPO were found significant than control. Among nonenzymatic antioxidants, proline (P-value=0.0009) and Phenol (P-value<0.0001) accumulation was found extremely significant in treated plant than control. Both carotenoid and flavonoid content found very significant and the value became non-significant in the case of ascorbate. Pb accumulation was extremely significant (p = <0.0001) in treated plants than control.

Profound variation in enzymatic and non-enzymatic antioxidants detected in this study probably occurs in order to buffer the oxidative stress induced by Pb and the enhanced accumulation of Pb in bioparts suggesting the macrophyte as a promising potential tool for phyto-remediation.

REFERENCES

Ahmad, M.S.A.; Hussain, M. Ijaz and Alvi, A.K. 2008. Photo-synthetic performance of two mung bean (*Vigna radiata*) cultivars under lead and copper stress. International Journal of Agriculture and Biology10: 167-172.

APHA 1992. Standard Methods for the Examination of Water and Wastewater. 18th edition. APHA, Washington, DC. 1134 pages.

Arnon, D. 1949. Copper enzymes in isolated chloroplast. Polyphenol oxidase in *Beta vulgaris.*Plant Physiology 24:1-15.

Arora, A.; Byrem, T.M.; Nair, M.G. and Strasburg, G.M. 2000. Modulation of liposomal membrane fluidity by flavonoids and isoflavonoids. Archives of Biochemistry and Biophysics 373: 102-109.

Baisak, R.; Rana, D.; Acharya, P. and Kar, M. 1994. Alterations in the activities of active oxygen scavenging enzymes of wheat leaves subjected to water stress. Plant Cell Physiology 35: 489-495.

Bates, L.S.; Waldren, R.P. and Teare, I. D. 1973. Rapid determination of free proline for water stress studies. Plant and Soil 39: 205-207.

Brueske, C.H. 1980. Phenylalanine ammonia lyase activity in tomato roots infected and resistant to the root-knot nematode, *Meloidogyne incognita*. Physiological Plant Pathology 16: 409-414.

Cakmak, I.; Strbac, D. and Marschner, H. 1993. Activities of hydrogen peroxide scavenging enzymes in germinated wheat seeds. Journal of Experimental Botany44: 127-132.

Costa, G. and Morel, J.L. 1994. Water relations, gas exchange and aminoacid content in Cd- treated lettuce. Plant Physiolology and Biochemistry 32: 561-570.

Dai, L.P.; Xiong, Z. Huang, Y. and Li, M. 2006. Cadmium induced changes in pigments, total phenolics and phenyl alanine

ammonia lyase activity in fronds of *Azolla imbricata*. Environmental Contamination and Toxicology 21(5): 505-512.

Dazy, M.; Masfaraud, J.F. and Ferard, J.F. 2009. Induction of anti-oxidative stress biomarkers associated with heavy metal stress in *Fontinalis antipyretica* Hedw. Chemosphere 75: 297-302.

Esterbauer, H.; Schwarzl, E. and Hayn, M. 1977. A rapid assay for catechol oxidase and laccase using 2, nitro-5-thiobenzoic acid. Analytical Biochemistry 77: 486-494.

Evans, R.C.A.; Miller, N.J. and Paganga, G. 1997. Antioxidant properties of phenolic compounds. Trends in plant sciences 2:152-159.

Foyer, C.H.; Descouvieres, P. andKunert, K. 1994. Protection against oxygen radicals: an important defence mechanism studied in transgenic plants. Plant, Cell and Environment 17: 507-5023.

Foyer, C. and Halliwell, B. 1976. The presence of glutathione and glutathione- reductase in chloroplast. A proposed role in ascorbic acid metabolism. Planta133: 21-25.

Fyfe, P.; Cogdell, R.J.; Hunter, C. N. and Jones, M.R. 1995. Study of carotenoid binding pocket of the photosynthetic reaction center from the purple bacterium *Rhodobacter sphaeroides.* Photosynthesis From Light to Biosphere. Proceedings of 10th International Photosynthesis Congress, Montpellier, France. 4: 47-50

Gong, H.; Zhu, X.; Chen, K.; Wang, S. and Zhang, C. 2005. Silicon alleviates oxidative damage of wheat plants in pots under drought. Plant Science169: 313-321.

Grassmann, J.,Hippeli, S. and Elstner, E. F. 2002. Plant's defence and its benefits for animals and medicine: role of phenolics and terpenoids in avoiding oxygen stress. Plant Physiology and Biochemistry 40:471-478.

Jain, S.K.,Vasudevan, P. and Jha, N.K. 1990. *Azollapinnata* R.Br. and *Lemna minor* L. for removal of lead and zincfrom polluted water. Water Resource 24:177-183.

John, R.; Parvaiz, A.; Kasturi, G. and Sharma, S. 2007. Antioxidative response of *Lemnapolyrrhiza* L. to cadmium stress. Journal of Environmental Biology 28(3):583-589.

Khellaf, N. and Zerdaoui, M. 2009. Growth response of *Lemna gibba* L. (duck weed) to copper and nickel phytoaccumulation. Water Science Technology. Submitted paper.

Leeper, G. W.1978. Managing the heavy metals on the land. Marcel Dekker, Inc. Newyork. 121pages.

Maheswari, R. and Dubey, R.S. 2007. Nickel toxicity inhibits ribo-nuclease and protease activities in rice seedlings: protective effects of proline. Plant Growth Regulators 51: 231-241.

Maine, M.; Durate, M. and Sune, N. 2001. Cadmium uptake by floating macrophytes. Water Resources Research 35: 2629-2634.

Matysik, J. Alia Bhalu, B. and Mohanty, P. 2002. Molecular mechanism of quenching of reactive oxygen species by proline under stress in plants. Current Science 82:525-532.

Mayr, V.; Treutter, D.; Santos Buelga, C.; Bauer, H. and Feucht W. 1995. Journal of Phytochemistry 38:1151-1155.

Meda, A.,Lamien, C. E.,Romito, M.,Millogo, J. and Nacoulma, O. G. 2005. Determination of total phenolic, flavonoid and proline contents in Burkina Fasan honey, as well as their radical scavenging activity. Food Chemistry 91: 571-577.

Michalak, A. 2006. Phenolic compounds and their antioxidant activity in plants growing under heavy metal stress. Polish Journal of Environmental Studies 15: 523-530.

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

Mittler, R. 2002. Oxidative stress, antioxidants and stress tolerance. Trends in Plant Science 7(9): 405-410.

Miyake, C. and Asada, K. 1992. Thylakoid-bound ascorbate peroxidase in spinach chloroplasts and photo reduction of its primary oxidation product monodehydroascorbate radicals in thylakoids. Plant and Cell Physiology 33: 541-553.

Mohan, B.S. and Hosetti, B.B. 1997. Potential phytotoxicity of lead and cadmium to *Lemna minor* L. growth in sewage stabilization ponds. Journal of Environmental Pollution 98: 233-236.

Nakano, Y. and Asada, K. 1981. Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in spinach chloroplasts. Plant and Cell Physiology 22: 867-880.

Noctor, G.andFoyer, C.H.1998.Ascorbate and glutathione: keeping active oxygen under control. Annual Review of Plant Physiology and Plant Molecular Biology 49: 249–279.

Phetsombat, S.; Kuratrachue, M.; Pokethitiyook, P. and Upatham, S. 2006. Toxicity and bioaccumulation of cadmium and lead in *Salvinia cuccullata.*Journal of Environment and Biology27: 671-678.

Sadasivam, S. and Balasubramanian, T. 1987. Practical Manual in Biochemistry Tamil Nadu Agricultural University. Coimbatore. 14 pages.

Sen, S. and Mukherjee, S. 2009. Season controlled changes in biochemical constituents in and oxidase enzyme activities in tomato (*Lycopersicon esculentum* Mill). Journal of Environment and Biology 30: 479-483.

Sharma, P. and Dubey, R.S. 2007. Involvement of oxidative stress and role of antioxidative defense system in growing rice seedlings exposed to toxic concentrations of aluminium. Plant Cell Reports 26(11): 2027-2038.

Sharma, P. and Dubey,R.S. 2005. Lead toxicity in plants. Brazilian Journal of Plant physiology 17:35-52.

Smirnoff, N.1995.Antioxidant systems and plant response to the environment. Pages 217-243, In: Smirnoff, N. (Editor) Environment and Plant Metabolism..Bios Scientific Publishers. Oxford,UK.

Smirnoff, N.; Conklin, P.L.andLoewus, F.A.2001.Biosynthesis of ascorbic acid in plants: a renaissance. Annual Review of Plant Physiology and Plant Molecular Biology 52: 437–467.

Singh, K. and Pandey, S.N. 2011. Effect of nickel stresses on uptake, pigments and antioxidative responses of water lettuce, *Pistia stratiotes* L. Journal of Environment and Biology32(3): 391-394.

Verma, S. and Dubey, R.S. 2003. Lead toxicity induces lipid peroxidation and alters the activities of antioxidant enzymes in growing rice plants. Plant Science164: 645-655.

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