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Lead Stress-induced Changes of Antioxidant Enzymes and Biochemical Compounds in Selected Weeds and their Contribution for Phytoremediation

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Abstract

Investigation was carried out to identify tolerant plant species growing in lead contaminated soil. Lead effected Chlorophyll a, chlorophyll b and ascorbicacidoxidase. Induced malondialdehyde and starch contents with up-regulated activities of antioxidative enzymes like catalase and phenylalanineammonialyase were observed. Enzymatic activities have been enhanced in all plants taken indicating that free radical generation was accelerated due to Pb exposure. Concentrations of lead accumulated were *Calotropis procera* (90 mg/kg) and *Bromus tectorum* (77 mg/kg) are higher followed by *Ipomea purpurea* (72 mg/kg), *Parthenium histocarpus* (33 mg/kg), *Anisomilius molabarica* (29 mg/kg) and *Eichornia fasculata* (10 mg/kg). Inspite of accumulation all plants survived successfully. With our findings we can suggest these plants can be used for effective phytoremediation of lead.

***Keywords**:* Free radicals, Lead, Up-regulated, Malondialdehyde, Phytoremediation.

Heavy metal pollution of soils has been increasingly becoming a global problem which poses several risks to human health, decreased soil microbial activity, fertility and yield losses (McGrath, 1995). Inspite of all expensive extraction technologies like chelating and land filling, Phytoremediation is an emerging cheap cleanup technology, which uses green plants to remove, contain or render harmless environmental contaminants.

In heavymetals Lead (Pb) is one of the most abundant heavy metal pollutants in terrestrial and aquatic environments. Lead induces free radicals in various plant species by induction of lipid peroxidation and generation of reactive oxygen species which inhibits metabolic processes such as nitrogen assimilation, photosynthesis, respiration, water uptake and transcription (Boussama *et al.*, 1999). Lead causes two types of unfavorable processes in biological systems: (I) it inactivates several enzymes and (II) it can intensify the processes of reactive oxygen species (ROS) production leading to oxidative stress (Prasad *et al.*, 1999). Reactive oxygen species produced can damage photosystem reaction center proteins by which photosynthesis could decrease and cause viability loss in the cells.

Plants initiate a signal transduction pathway that triggers enzymatic and non-enzymatic antioxidative systems that detoxify the cells (Dalton, 1995). In this mechanisms a wide series of enzymes exist in plants that serve to remove ROS, such as peroxidase, superoxidedismutases, ascorbate peroxidase, catalase, phenylalanine ammonialyase, ascorbicacidoxidase etc. (Verma and Dubey, 2003) thereby preventing the formation of OH radicals. These non-enzymatic and enzymatic systems in plant together keep the reactive oxygen species in low levels and not to be injured by accumulation of ROS. The purpose of this research is to exploit the ability of various plant species to thrive in high lead environments and its affect on antioxidative defense mechanism.

Materials and Methods

Study site

Lead contaminated industrial site (estimated production of about 12 tons per annum) and Pb free site (nearby forest) located at Tirupati, India is selected as a study site which is occupied by luxuriant natural vegetation. Selected weeds with abundant growth (a) *Calotropis procera* (b) *Ipomea purpurea* (c) *Eichornia fasiculata* (d) *Parthenium histocarpus* (e) *Bromus tectorum* (f) *Anisomilus molabarica* were collected from lead contaminated industrial area and nearby lead free site and samples of spoils were taken for laboratory analysis. Pb-contaminated soils were collected from an industrial site. The soil was screened to pass through a 1.0 cm sieve and thoroughly mixed before use. The following procedures were used to characterize the soil. Soil p^H was measured using 1:1 soil/ water ratio; total soil Pb was determined by the atomic absorption spectrophotometer, organic matter content was measured by the Walkley Black method and particle size was measured by the hydrometer method. The selected physical and chemical properties of the Pb-contaminated soils are presented in Table 1.

Total chlorophyll content

Chlorophyll extraction was done according to method described by Wintermans and De Mots (1965) and expressed in mg g^{-1} FW.

TBARS assay

A TBARS (Thiobarbituric acid reactive substances) assay was performed following the method of Heath and Packer (1968).

Catalase activity

The Catalase activity was assayed by the method of Chance and Maehly (1955).

Phenylalanine ammonialyase activity

Phenylalanine ammonialyase activity was done as described by Dickerson *et al*., (1984).

Ascorbic acid oxidase activity

Determination of ascorbic acid oxidase was done according to method of Oberbacher and Vines (1963).

Total starch content

The total starch content was estimated by the anthrone–sulphuric acid method according to Hodge and Hofreiter (1962).

AAS analysis

The properties of soil sample were measured according to Liu (2000). Total metal concentration of Soil and plant metal concentration (digested by 5:1 with concentrated HNO_3 and $HClO_4$ were determined by atomic absorption spectrophotometry.

Statistical analysis

Statistical analysis was performed using spss version 11.5. All data were analyzed using one-way analysis of variance (ANOVA). Different letters in graphs or tables indicate significant differences at $P<0.05$. All values were presented as means ± standard error of the mean (SE), with a minimum of three replicates.

Results and Discussion

Lead uptake

In the present study Pb accumulation by different plants varied significantly (Anova, $P < 0.05$). Among the plants taken *Calotropis procera* showed highest amount of accumulation (90.5 mg/kg). This was about 5 times higher than in *Eichornia fasculata* (10.8 mg/kg) followed by *Bromus tectorum* (77.1 mg/kg), *Ipomea purpurea* (72.1 mg/kg), *Parthenium histocarpus* (33.7 mg/kg) and *Anisomilius molabarica* (29.7 mg/kg).

The Organic carbon content of the soils was determined as moderate which indicated that metals were less likely to be bound to organic matter to form metal-chelate complexes. This will make metals into available form for easy accessibility to the weed plants. The P, K and N help the plants in up taking the metals in bounded forms. Soil particles are sandy and slightly acidic in both control and contaminated soils. Compared to control soils organic carbon (0.23±0.22) and phosphorous content (0.41±3.29) decreased in contaminate soils (0.13±0.35) (0.33±0.15) but nitrogen (55±2.26) and potassium contents (65±1.27) increased (68±1.20) (72±0.03) (Table 1).

Physical and Chemical properties of soil

Table 1: Physical and Chemical properties of soil

Parameters	Control Soil	Contaminated soil
Soil Texture	Sandy loam	Sandy loam
Soil PH	5.7±0.05	5.2±0.08
Organic Carbon (gm/kg)	0.23±0.22	0.13±0.35
Available N (gm/kg)	55±2.26	68±1.20
Available P (gm/kg)	0.41±3.29	0.33±0.15
Available K (gm/kg)	65±1.27	72±0.03
Total Pb (mg/kg)	1.27 mg/kg	164.5 mg/kg

Effect of Pb on chlorophyll content

Chlorophyll content in plants is important factor in determining photosynthetic activity. In our present study, heavy metal Pb decreased chlorophyll content gradually by increased proportionality of metal concentration. Pb significantly effected phototosynthetic pigments (chlorophyll a and chlorophyll b) in weeds. Chlorophyll-a content was decreased from 62% to 34% in all plants when compared to controls with high decline in *Anisomilius molabarica* (62%), *Ipomea purpurea* (52%) and *Eichornia fasculata* (52%) followed by *Parthenium histocarpus* (47%), *Calotropis procera* (34%) and *Bromus tectorum* (40%) in comparision with controls (Fig. 1). Chlorophyll-b decreased from 81% (*Bromus tectorum*) to 36% (*Calotropis procera*) in all plants followed by *Ipomea purpurea* (76%), *Anisomilius molabarica* (75%), *Parthenium histocarpus* (63%) and *Eichornia fasculata* (56%) in comparision with controls (Fig. 2). The content of chlorophylls a, b chlorophyll was significantly decreased corroborates with findings of *Salvinia minima* wild by Jeffrey l. Gardner and Safaa h. al-hamdani (1997) and Zengin and Munzuroglu (2005) in *phaseolus vulgaris L* due to Pb stress. Decreased chlorophyll content associated with heavy metal stress may be the result of inhibition of the enzymes responsible for chlorophyll biosynthesis.

Effect of Pb on MDA content

During present study, a significant increase in malondialdehyde content from 25% to 137% was observed. Here it was observed excess of Pb promoted lipid peroxidation with excessive production of malondialdehyde content in contaminated over controls. The higher activity was observed in *Calotropis procera* (137%) and *Eichornia fasculata* (0.83%) followed by *Anisomilius molabarica* (42%), *Bromus tectorum* (33%), *Parthenium histocarpus* (26%) and *Ipomea purpurea* (25%) (Fig. 3). An enhanced level of lipid peroxidation observed may be due to the generation of toxic oxygen free radicals under metal stress. The ROS attack on free radicals and the polyunsaturated fatty acid components of membrane lipids initiated lipid peroxidation, an autocatalytic process that changes membrane structure and function. Our findings were similar to Qureshi *et al.,* (2005) in *Artemisia annua* and *Withania somnifera* (Khatun *et al.,* 2008) under copper toxicity.

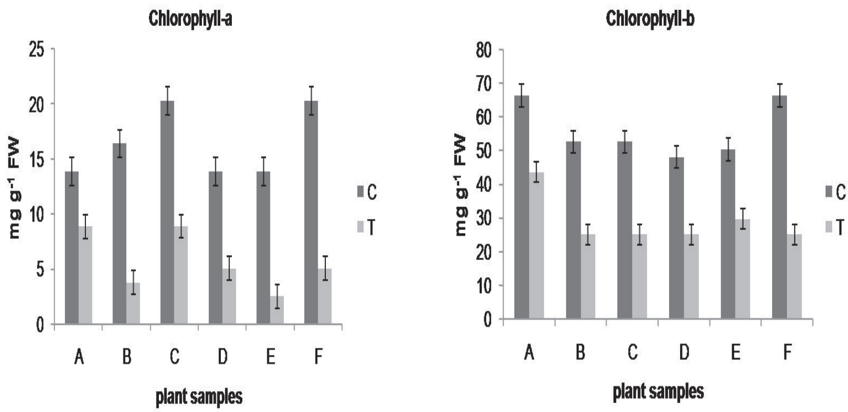
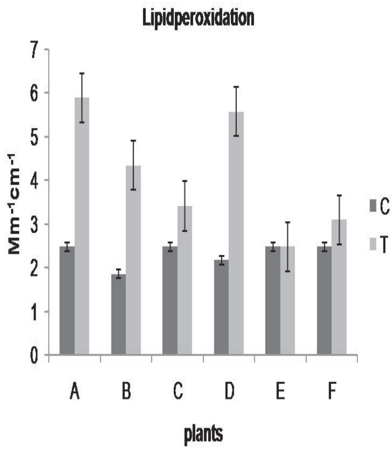


Fig. 1 and 2: Effects of Pb concentration on Chlorophyll a ,Chlorophyll b content

Effect of Pb on Catalase activity

The Catalase increased from 63% to 19% compared to controls in all plants. Highest enhancement was observed in *Calotropis procera* (63%) and lowest in *Bromus tectorum* (19%) followed by *Anisomilius molabarica* (0.47%), *Ipomea purpurea* (0.37%), *Eichornia fasculata* (0.35%) and *Parthenium histocarpus* (0.25%) (Fig. 4). Elevated Catalase would lower H_2O_2 levels, which reduces the lipid peroxidation degree and lessen membrane damage by converting H_2O_2 into water and oxygen. Our results of increasing concentration of catalase activity with the increasing concentration of Pb corroborate with the findings of Verma and Dubey (2003) in rice plants and *Pinus radiate* plants (Jarvis and Leung, 2002).



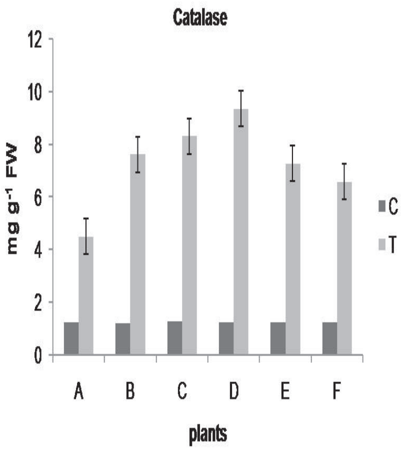


Fig. 3 and 4: Effects of Pb concentration on Catalase activity, and Phenylalanineammonialyase activity

Effect of Pb on Phenylalanineammonialyase activity

Increased Phenylalanineammonialyase activity could be a response to the cellular damage provoked by higher Pb concentrations. Unsurprisingly, phenylalanineammonialyase was more activated by the presence of lead. Significant increase was observed by 20% to 48% in all the plants in compared with controls. *Parthenium histocarpus* showed highest increase in phenylalanineammonialyase activity by 48% and lowest is seen in *Ipomea purpurea* (20%) followed by *Anisomilius molabarica* (46%), *Calotropis procera* (42%), *Eichornia fasculata* (37%) and *Bromus tectorum* (36%) compared with controls (Fig. 5). Overall phenylalanineammonialyase activity was elevated in all plants. Activation of PAL and lignin content increase is considered as common plant responses to various stress factors, including heavy metals (Yang *et al.,* 2007; Kovacik and Klejdus 2008). So it seems that in weed plants the increase of PAL activity is efficient in catalyzing deamination reaction of the amino acid phenyl alanine at the gateway from the primary metabolism into the important secondary phenylpropanoid / phenolic metabolism in plants (Hahlbrock and Scheel, 1989). Overall, Pb had the high inducement on Phenylalanineammonialyase. Similar observations were recorded in previous study under Nacl stress according to Simaei *et al.,* (2012) in Soybean Plants, rice under cadmium toxicity (Ting Hsu and Huei Kao, 2004) and *Luffa cylindrical* under lead toxicity (Jiang *et al.,* 2010).

Effect of Pb on Ascorbic acid oxidase activity

In our experiment, the exposure of plants to lead doses resulted, in decreased ascorbicacidoxidase content from 68% to 6% in all the plants compared with controls. *Calotropis procera* and *Ipomea purpurea* showed highest decrease in ascorbicacidoxidase activity by 68% and lowest is seen in *Parthenium histocarpus* (6%) followed by *Anisomilius molabarica* (67%), *Eichornia fasculata* (63%) and *Bromus tectorum* (59%).compared with controls (Fig. 6). Our results accord with previous researches under Pb stress in ascorbicacidoxidase from findings of

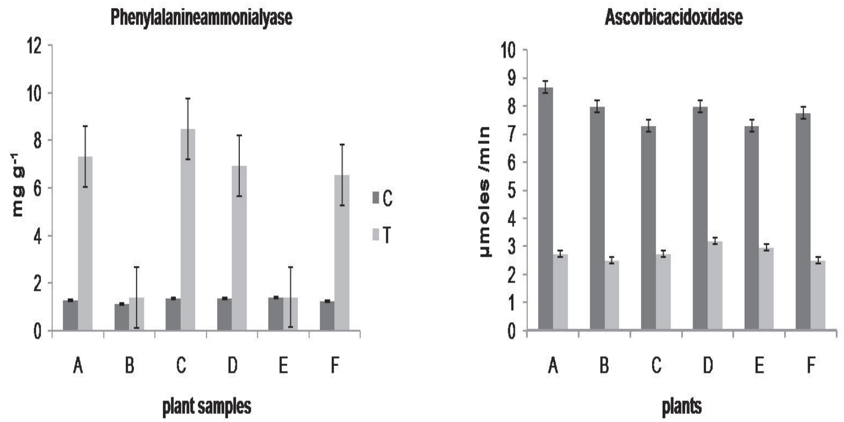


Fig. 5 and 6: Effects of Pb concentration on Ascorbic acid oxidase activity and lipid peroxidation levels

Anuradha *et al.,* (2007) in radish, Ibrahim *et al.,* (2013) in *alfalfa* under cobalt and copper toxicity and cucumber plants due to arsenate toxicity according to Czech *et al.,* (2008). Ascorbicacidoxidase seems more important due to its role in plants not only as an antioxidant, but also as a signal molecule in many developmental and defense responses.

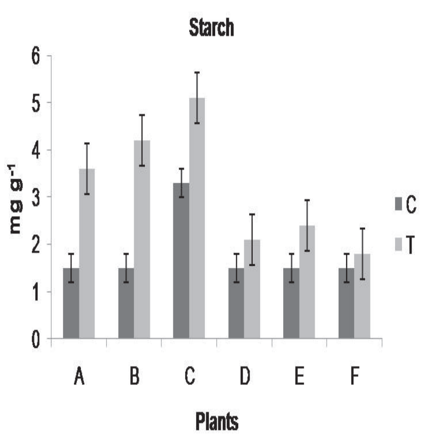


Fig. 7: Effects of Pb concentration on Starch content

Effect of Pb on Starch content

The increase in the starch content might be caused by excessive α-amylosis of the components. In our experiment significant increase of starch accumulation by lead occurred by 14% to 54% in comparision with controls (Fig. 7). Highest increase was observed in *Eichornia fasiculata* (54%) and lowest in *Calotropis procera* (14%) compared to controls followed by *Ipomea purpurea* (18%), *Bromus tectorum* (6%), *Parthenium histocarpus* (4%) and *Anisomilius molabarica* (2%). Increased starch levels were directly proportional with high concentration of heavy metal Pb. Nutrient deficiency and heavy metal toxicities are known to produce starch accumulation within leaves (Vazquez *et al.,* 1987). C_4 photosynthesis when compared to C_3 allows fast biomass accumulation with high nitrogen and water use efficiency (Leegood and Edwards 1996; Sage 2004) which is desired set of trait to increase the productivity of crop plants (Matsuoka *et al.,* 1998) may be the cause for increase in starch and a required character for successful phytoremediation. Similar findings were observed according to verma and dubey (2001) due to cadmium toxicity in rice and in *Tilia argentea* and *Quercus cerris* by Tzvetkova and Kolaro (1996). This may be due to higher resistance of their photosynthetic apparatus and low starch export from the mesophyll.

From the results it has been clear that the concentration of Pb toxicity on the plants posed high oxidative stress on activity of antioxidant enzymes and biochemical compounds in weed plants and both played a vital role in combating oxidative stress in plants for the plant susceptibility and tolerance. Among the plants *calotropis procera* and *Bromus tectorum* are efficient in combating the oxidative stress and accumulating higher amount of Lead than other species. Finally, data which is generated through this study will be very helpful in finding high tolerant plant species to thrive in high lead environments.

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