

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

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**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<sup>-1</sup> (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 antioxidants

Proline was analysed spectrophotometrically 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-Ciocalteu method [11]

## 2.2 Enzymatic antioxidants

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 Tetrazolium [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 coefficient  $26.6 mM^{-1} cm^{-1}$ ) 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 (Figure 1 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 in 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 of *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

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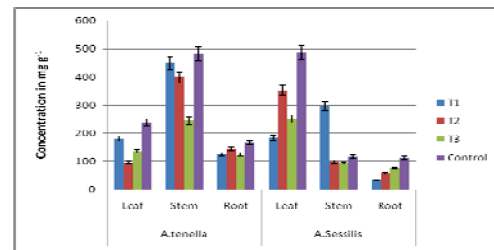


Figure 1: Carbohydrate content in in two species of *Alternanthera* under Lead stress.

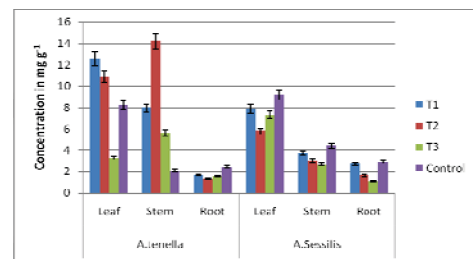


Figure 2: Protein content in in two species of *Alternanthera* under Lead stress

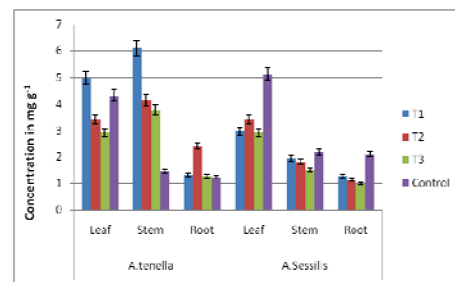


Figure 3: Lipid content in in two species of *Alternanthera* under Lead stress

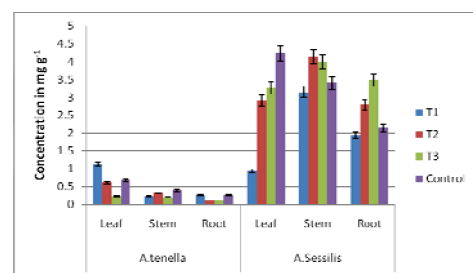


Figure 4: Amino acid content in in two species of *Alternanthera* under Lead stress

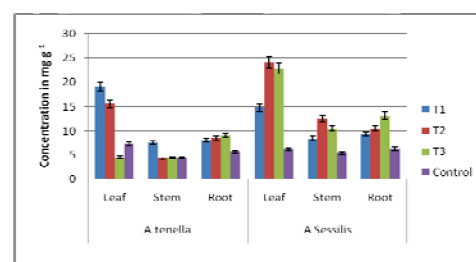


Figure 5: Proline content in in two species of *Alternanthera* under Lead stress

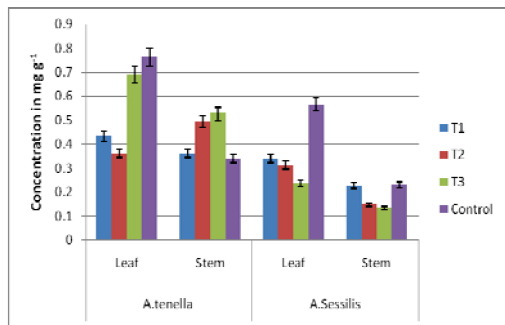


Figure 6: Chlorophyll content in in two species of *Alternanthera* under Lead stress

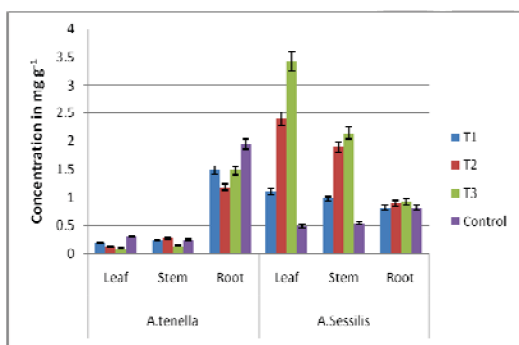


Figure 7: Phenol content in in two species of *Alternanthera* under Lead stress

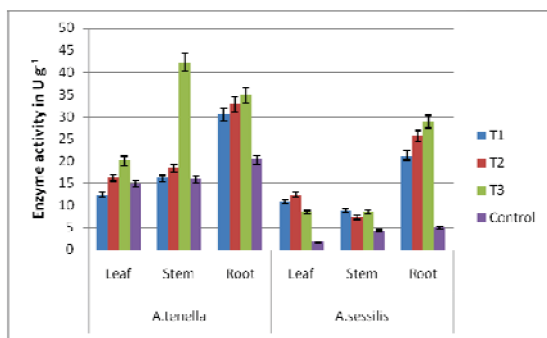


Figure 8: SOD activity in two species of *Alternanthera* under Lead stress

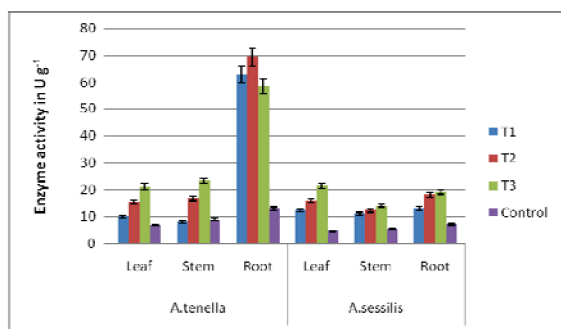


Figure 9: POD activity in two species of *Alternanthera* under Lead stress

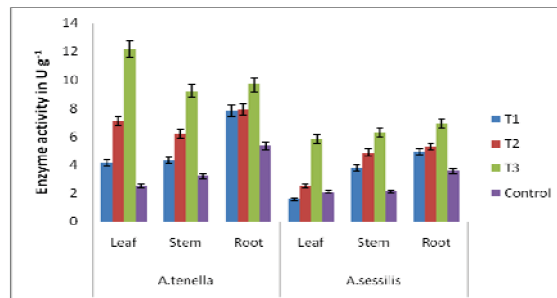


Figure 10: CAT activity in two species of *Alternanthera* under Lead stress

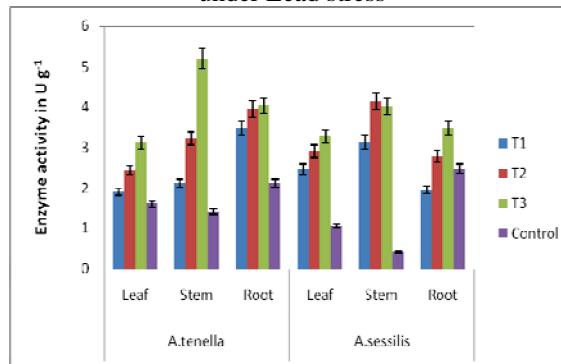


Figure 11: PPO activity in two species of *Alternanthera* under Lead stress

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