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Research Article

Effect of lead stress on antioxidative enzymes in leaves, stem and roots of a mangrove *Avicennia marina*

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Abstract

Effect of lead stress on antioxidant enzyme activities in leaf, stem and roots of seedlings of mangrove *Avicennia marina* were assessed using different models like superoxide dismutase, catalase and peroxidase. The seedlings were grown in greenhouse conditions for two months and the soil was amended with various levels of lead (10 ppm, 50 ppm, 100 ppm and 150 ppm) and control were grown without any treatment, for the purpose of comparisons. The seedlings were provided with all conditions necessary for the appropriate growth. The lead stress led to the change of antioxidant enzyme activities at different concentrations. The activities of, superoxide dismutase in leaves, stem and roots, peroxidase in leaves and roots and catalase in roots of *Avicennia marina* seedlings first ascended and then declined at different stress levels compared to control while catalase enzyme activity in leaves and peroxidase activity in stem keeps on ascending upto highest level of concentration provided in the study.

INTRODUCTION

Mangrove ecosystem, possessing great ecological and commercial value, is influenced by a complex interaction of abiotic and biotic factors. Mangrove ecosystem has been widely used as a site where effluents as well as metallic anthropogenic wastes are discharged (Saenger *et al.,* 1990; Peters *et al.,* 1997). Mangrove ecosystem can act as a sink for heavy metals and can become pollution source to plants. Mangrove forests are of special interest because of their metal accumulation potentials. Mangroves can tolerate high contents of heavy metals but toxic levels affect a variety of processes in plants (Maksymeic, 1997).

Lead (Pb) is considered a major pollutant in both terrestrial and aquatic ecosystems. Lead pollution is deleterious to plants at higher concentrations. (Zheljazkov and Nielsen,1996). Lead affected soils contains lead in the range of 400-800 mg/kg soil whereas in industrialized areas it may reach upto 1000 mg/kg (Paivoke, 2002). Different metals such as zinc, copper as well as lead is found in varying quantities in different seasons (Azam and Kulkarni, 2011). Bulk of lead taken up by the plants remains in the roots (Kumar *et al.,* 1995). Zea mays L. plants could translocate and accumulate significant quantities of lead in leaves according to the concentration that it comes in contact (Miller and Koeppe, 1971). In earlier researches lead induced alteration of SOD activity are observed in *Oryza sativa* (Verma and Dubey, 2003), horsegram (Reddy *et al.,* 2005), *Sesbania drummondii* (Thomas *et al.,* 2004) *Sedum alfredii* (Liu *et al.,* 2008), *Cassia angustifolia* Vahl (Qureshi *et al.,* 2007) and *Jatropha curcus* (Gao *et al.,* 2009).

Enhanced production of reactive oxygen species (ROS) damages cell membranes, nucleic acids and chloroplast pigments and the disruption of the balance between their production and the antioxidative enzyme (containing enzymatic antioxidants and non-enzymatic scavengers) causes accumulation of ROS (Tukendorf and Rauser, 1990; Vangronsveld and Clijsters, 1994). Highest SOD, CAT and peroxidase activities are observed in *Avicennia marina* when eight different mangrove leaf extracts are studied (Kirankumar *et al.,* 2014). Increase in antioxidant and antifungal activities are observed with the increase in concentration of aqueous and ethanol extract of mangroves Avicennia marina and Rhizophora mucronata (Somayeh and Mohsen, 2016).

Avicennia marina is a dominant mangrove species but now a day are getting degraded due to industrialization in their vicinity. Hence it becomes necessary to study the correlation between mangrove plants and heavy metals. This study investigated the effects of different concentration of lead on activities of antioxidant enzymes in leaves, stem and roots of *Avicennia marina* seedlings using different models like superoxide dismutase(SOD), peroxidase(POD) and catalase(CAT).

MATERIALS AND METHODS

Seed collection and germination:

The seeds of *Avicennia marina* were collected manually from the field situated in Hazira, Gulf of Khambhat. Healthy, undamaged mature seeds were collected. Seeds were sown in the pots with four seeds in each pot. One pot was kept untreated to be taken as control and three pots were treated with different concentrations (10 ppm, 50 ppm, 100 ppm, 150 ppm) of lead. These pots were provided with all the possible natural conditions for optimum growth of the plant. After two months leaves, stem and roots were harvested for analysis of SOD, POD and CAT activities.

The experiments were triplicated.

Enzyme Assay:

SOD activity was determined by slightly modified procedure (Madamanchi *et al.,* 1994) originally described by Beauchamp and Fridovich (1971) by following the photoreduction of nitroblue tetrazolium (NBT). The 50% inhibition of the reaction between riboflavin and NBT in presence of methionine is taken as 1 unit of SOD activity. The enzyme activity is expressed as units/mg of protein. POD activity was determined according to the procedure given by Chance et al. One unit of POD activity was expressed as POD units per minute and mg of protein. CAT activity was measured according to the Aebi *et al.* (1984). Results were expressed as CAT units per minute and mg of protein.

RESULTS AND DISCUSSION

SOD can eliminate O_2^-, decrease peroxidation of membrane lipids and also plays vital role in maintaining cell membrane stability. The changes in activities of SOD in leaves, stem and roots are shown in Fig 1. SOD activity in leaves increased by 5 %, 21% and 59%under the treatment of 10 ppm, 50 ppm and 100 ppm Pb concentration respectively but there was a sharp decline in enzyme activity under the treatment of high (150 ppm) Pb concentration. For stem SOD activity was at its peak at 100ppm Pb concentration as compared to control and then decreased with further higher concentration. But there was little change in trend of SOD activities in roots as compared to leaves and stem. In roots SOD reached its peak at 50 ppm and then started decreasing with further higher concentrations.

In higher plants, heavy metals induce oxidative stress by generating superoxide, hydrogen peroxide, hydroxyl radical and singlet oxygen collectively known as ROS (reactive oxygen species) (Devi and Prasad, 1998). ROS affects all types of biomolecules such as proteins, lipids, amino acids and nucleic acids (Vos and Schat, 1991; Mehta *et al*., 1992; Luna *et al.,* 1994) that can lead to cell death. Hence, the induction of antioxidant enzymes including SOD, POD and CAT is an important protective mechanism to minimise oxidative damage in polluted environments. In this study SOD activity increased upto certain level with increase in concentration suggesting this increase in SOD has better protection against oxidant damage. Superoxide radical scavenging activity gave similar results in ethanol leaf extract of Eugenia flocosa (Tresina PS *et al*., 2012) Also ethanol, methanol and benzene extract of pneumatophore of Avicennia marina gave maximum superoxide radical scavenging activity which gives similar results with our findings. (M Packia Lincy *et al.,* 2013) Similar results were found by Bowler *et al.,* 1992; Takemura *et al.,* 2000. SOD activity then declined which suggested that oxygen scavenging activity of SOD was impaired. These data were found similar with *Alyssum* species (Schikler and Caspi, 1999)

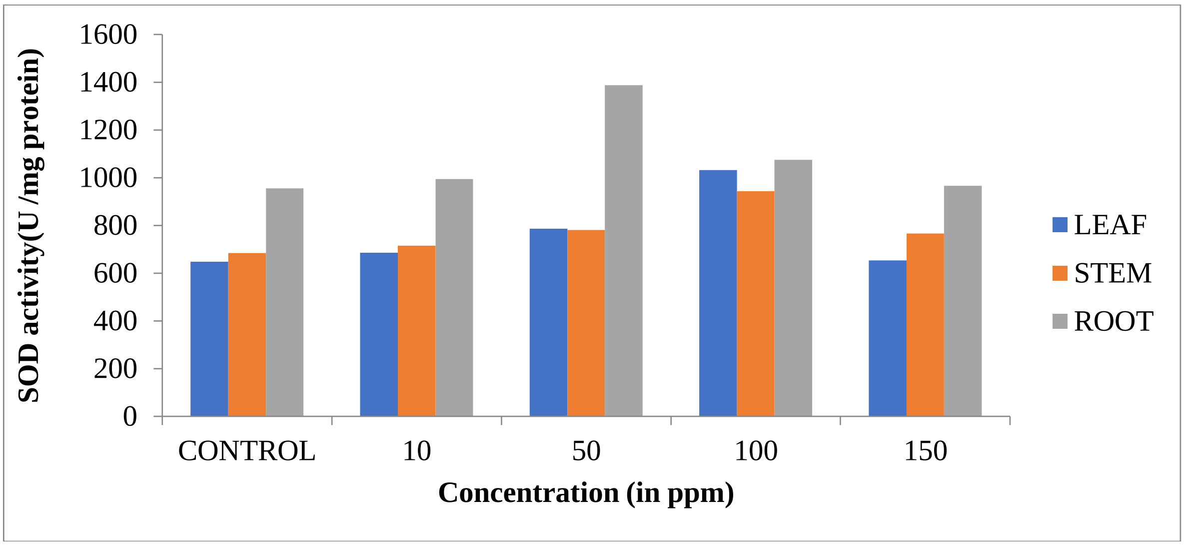

Fig 1: Effect of lead stress on SOD activities in leaves, stem and roots of *Avicennia marina*

The changes in activities of POD in leaves, stem and roots are shown in Fig 2. It was observed that POD activity in leaves and roots peaked at 100 ppm Pb concentration, then there was decline in its activity. In stems POD activity kept on ascending.

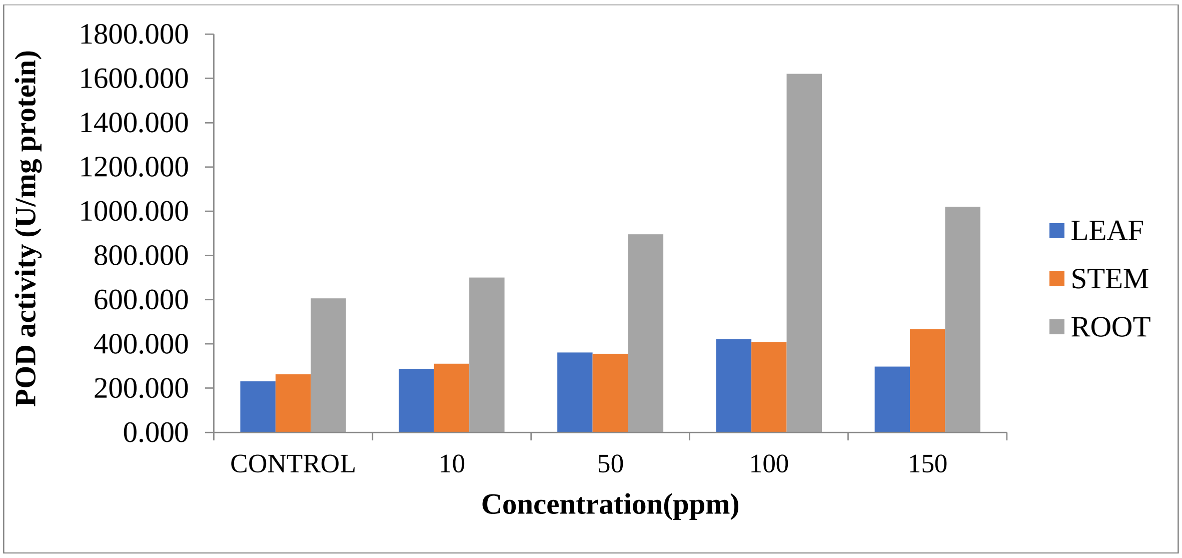

Fig.2: Effect of lead stress on POD activities in leaves, stem and roots of *Avicennia marina*

The changes in activity of CAT activities in leaves, stem and roots are shown in Fig 3.CAT activity in leaves show slight increase with the increase in concentrations. In stem and roots CAT activity show increasing trend and then declined at highest concentration.

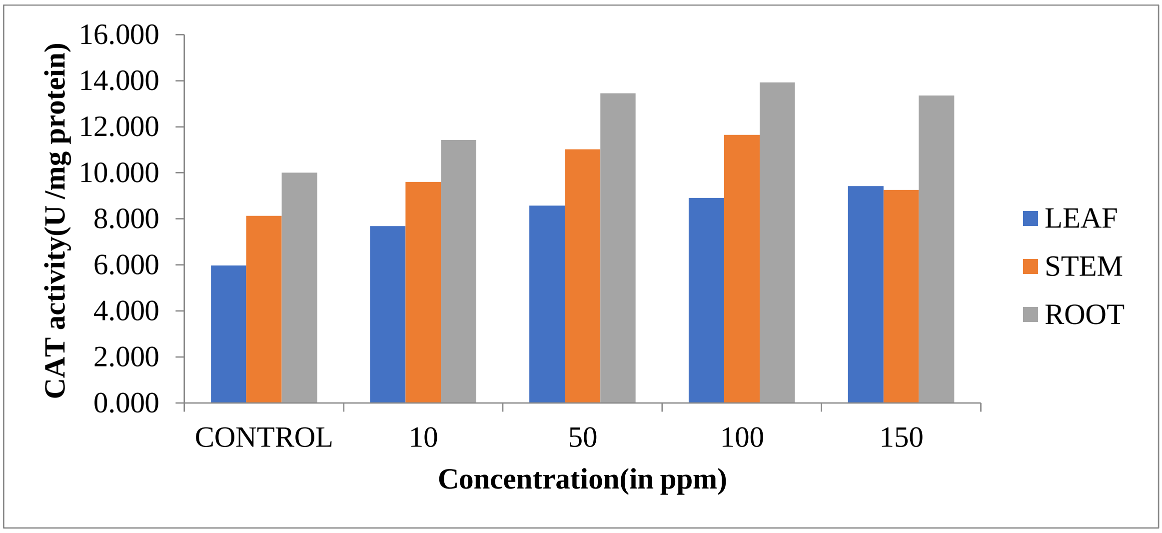

Fig: 3 Effect of lead stress on CAT activities in leaves, stem and roots of *Avicennia marina*

and *Allium sativum* (Zhang *et al.*, 2005). Our observations are similar to the early findings by Gwozdz *et al.*, 1997 and Dixit *et al.*, 2002. POD is considered one of the principal enzymes involved in elimination of active oxygen species. It catalyses hydrogen peroxide dependent oxidation of substrate. POD can also build up physical barrier against toxic heavy metals by participating in lignin biosynthesis (Hegedus *et al.*, 2001). In our study the POD increases at first showing its role in protecting plants against oxidative damage. Similar results were shown by Zhang and Kirkham 1994 that increase in peroxidase activity indicated the formation of large amount of H_2O_2 which could release enzyme from membrane structure. In our study CAT activity keeps on increasing showing its important role in protection of plants. The major function of CAT is to mobilize the peroxide liberated in the peroxisome following the conversion of glycolate during photorespiration (Liu *et al.*, 2008).

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