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PHYTOREMEDIATION POTENTIAL AND ANTIOXIDANT RESPONSES IN *ALTERNANTHERA SESSILIS* (L) R.BR.

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Abtract: Phytoremediation is a non destructive and cost effective *in situ* technology that can be used for the cleanup of contaminated soils. The present study investigated the potential of weedy plant *Alternanthera sessilis* (Amaranthaceae) for phytoremediation by evaluating its heavy metal phytoaccumulation capacity. The effect of heavy metals generating antioxidant defense system such as SOD, CAT, PPO and POX were also studied in different bioparts of *A.sessilis.* Plants were grown in soil treated with three different concentration of each metal (Cd, Cr and Pb) after conducting range finding test and allowed to grow for one month. Antioxidant enzymes and metal accumulation were recorded after one month. The result showed significant heavy metal absorption and accumulation by *A.sessilis* root and aerial parts. The mean value of heavy metal concentration in the plant was found in descending order Pb>Cr>Cd. Highest accumulation is seen in Pb treated roots which reached 14.83mg kg^{-1}. There is a strong positive correlation between concentration of heavy metals in soil and plant parts. Heavy metal stress enhanced the activity of superoxide dismutase (SOD; E.C. 1.15.1.1) catalase (CAT: E.C 1.11.1.6) polyphenol oxidase (PPO; E.C 1.14.18.1) and peroxidase (POX; E.C. 1.11.1.7). The present study revealed that the plant showed variation in antioxidant response to overcome heavy metal stress and increased antioxidant enzymes may be associated with tolerance capacity of *A.sessilis* to protect the plant from oxidative damage. *A.sessilis* showed moderate tolerance against heavy metal induced stress along with sufficient phytoaccumulation, could make it a suitable candidate for phytoremediation of heavy metal contaminated soil.

Key words: Phytoaccumulation, *A. sessilis*, Heavy metals, Antioxidant enzymes, Reactive oxygen species.

INTRODUCTION

Heavy metals are known to cause irreversible damage to a number of vital metabolic constituents. As the heavy metal is non biodegradable and toxic in nature, the toxicity of metals are threats on ecosystem. Phytoremediation is an integrated multidisciplinary approach to the cleanup of contaminated soils, which combines the disciplines of plant physiology, soil chemistry, and soil microbiology (Cunningham and Ow, 1996). Phytoremediation, also referred as botanical bioremediation (Chaney *et al*., 1997), involves the use of green plants to decontaminate soils, water and air. It is an emerging technology that can be applied to both organic and inorganic pollutants present in the soil, water or air (Salt *et al*., 1998). However, the ability to accumulate heavy metals varies significantly between species as different mechanisms of ion uptake are operative in each species, based on their genetic, morphological, physiological and anatomical characteristics (Garbisu and Alkorta, 2001).

Most of the plant species including crops and weeds cannot survive on polluted sites due to toxic effects of heavy metals (Wong, 2003). Thus, it is urgent to remediate heavy metal contaminated soils (Wei *et al*., 2005). There are several remediation methods such as soil dressing, soil washing and replacement of polluted soils are available, but most of them are too expensive and time consuming and also require huge amount of water and unpolluted soil (Abe *et al*., 2007). Therefore, phytoremediation has attracted great attention as a new and inexpensive technology (Salt *et al*, 1998). In the present study heavy metals Cd, Cr and Pb were selected for evaluation of phytoremediation potential of *Alteranthera sessilis*. Cadmium (Cd) is one of most toxic pollutants found in air, water and soil and is non-essential for plants. Chromium (Cr) in hexavalent state has been recognized as one of the most dangerous environmental pollutant due to its ability to cause mutations. 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. Antioxidative defense mechanism protects the plants cells from oxidative damage caused by Reactive oxygen species (ROS) due to heavy metals. This is based on the fact that activity of one or more of antioxidant enzymes like SOD, CAT, POD etc is generally increased in plants when exposed to heavy metal stress conditions

Present investigation intended to explore possible relationship between heavy metals in soil and their accumulation in bioparts of *Alternanthera sessilis* to evaluate its phytoremediation potential. The study also envisage the antioxidant response of plant due to heavy metal stress.

MATERIALS AND METHODS

Bioassay: Pot culture method was adopted for the bioassay. Healthy plants of *Alternanthera sessilis* were collected from its natural habit. Threshold level of each metal was estimated and three different treatment concentrations (T1, T2, T3) of each metal salt was applied to soil. Garden soil was collected and uniformly saturated with varying concentrations of cadmium sulphate (20, 30 and 50 mg Kg^{-1}), lead nitrate (50, 100 and 150 mg Kg^{-1}), Potassium dichromate (50, 75 and 100 mg Kg^{-1}). 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. After one month, plants were harvested, washed with double distilled water, blotted and separated leaves and roots were used for the study.

Analytical techniques: 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 (Beauchamp and Fridovich, 1971). 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 (Luck, 1974) and was monitored spectrophotometrically at 240 *nm* for 3 min. 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 (Esterbauer *et al*., 1977). 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 (Putter, 1974).

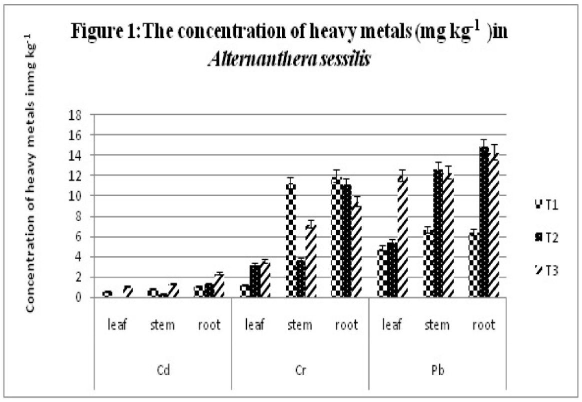
Heavy Metals: Estimation of the heavy metals (cadmium, lead, and chromium) was carried out following the method of APHA, (1992). A known quantity of the sample was subjected to wet digestion using the mixture of concentrated nitric acid and perchloric acid (4:1) for eight hours and made up to a known volume and the solution was aspirated in to Atomic absorption spectrophotometer. The concentration of various heavy metals were computed and expressed as mg Kg^{-1}.

Statistical analysis: The data concerning the antioxidant activity and metal content were analysed by statistical software SPSS (version 7.1)

RESULTS AND DISCUSSION

Heavy metal contents in the control plants were below detectable levels. The result showed significant heavy metal accumulation by *Alternanthera sessilis* roots and aerial parts (Fig 1). The mean value of heavy metal concentration in *Alternanthera sessilis* after treatment followed an order of Pb>Cr>Cd. A similar trend was observed in *Robinia pseudoacacia* and *Philadelphus coronarius* (Celik *et al*. 2005; Kafel *et al*., 2010). There is a clear increase in the concentration of heavy metals in plants with increase in soil metal concentration and a strong positive correlation exist between concentration of heavy metals and plant parts and correlation ranges from 0.93 to 0.97 (Table 1).

Antioxidant enzymes varied drastically with heavy metal treatment. Superoxide dismutase (SOD) is an essential component of plant antioxidation system that can be used as biomarker of environmental stress (Dazy *et al*., 2009). SOD increased with increase in metal concentrations in roots while fluctuated in stem and leaves of *A.sessilis*. The highest accumulation of SOD (Fig 2) was noticed in Cd



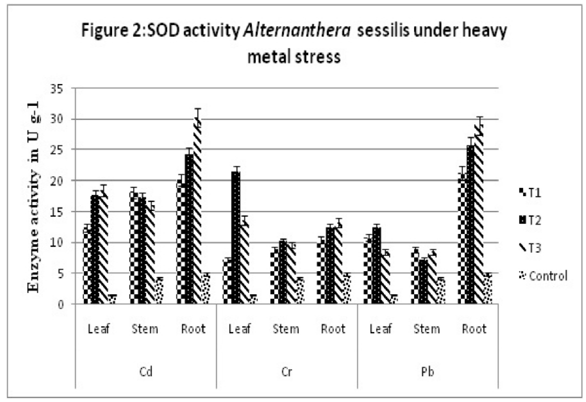


Table 1. Correlation coefficient between the concentration of heavy metal and different plant parts

Plant parts	Cd	Cr	Pb
Leaf	0.9411	0.9786	0.956
Stem	0.9405	0.9771	0.954
Root	0.9375	0.9747	0.9524

T3 roots (30.23 U g^{-1}FW). Cd T3 roots also showed negative correlation between cadmium concentrations (Table 2). Superoxide dismutase is the first enzyme in ROS detoxifying process that with converting O_2^- to H_2O_2 in cytosol, chloroplast and mitochondria plays an axial role in cellular defense mechanisms against the risk of OH formation (Gratao *et al.*, 2005; Salin, 1988). Increase in SOD activity in almost all treatments indicated high accumulation of ROS under heavy metals stress in order to activate antioxidative defense enzymes to inhibit oxygen radical accumulation. Increase in SOD activity appears to be probably attributed to superoxide radical accumulation, de-novo synthesis of the enzymatic proteins (Verma and Dubey, 2003) and induction the expression of genes encoding SOD (Alvarez and Lamb, 1997).

Cells can be protected from reactive oxygen species by the combined action of enzymatic antioxidant systems like SOD, CAT, POD and PPO. In the present study peroxidase and catalase enzyme activity showed an increase under metal stress condition. Peroxidase is an antioxidant enzyme which play crucial role in plant growth and development. POD activity also seen elevated in plant parts as compared to the control. Cd toxicity induced more POD

Table 2. The correlation coefficient between metal concentration and antioxidant measurements in *Alternanthara sessilis*

		SOD	POD	CAT	PPO
	LEAF	0.841	0.8736	0.927	0.9375
Cd	**STEM**	0.8554	-0.8393	0.9246	0.9319
	ROOT	-0.6546	0.7275	0.9174	0.9345
	LEAF	0.9668	0.9786	0.9775	0.9791
Cr	**STEM**	0.975	0.9778	0.9766	0.9749
	ROOT	0.9731	0.9752	0.9768	0.9788
	LEAF	0.9553	0.9483	0.9596	0.9604
Pb	**STEM**	0.9573	0.9534	0.9588	0.9598
	ROOT	0.9401	0.9489	0.988	0.9604

activity compared to Pb and Cr. Highest POD activity was observed in vicinity of Cd T3, which is 25.63 U g^{-1} (Fig. 3). Cd T2 stem showed negative correlation with metal concentration. CAT activity expressed positive correlation with heavy metal concentration and it increased with increase in metal concentration (Fig. 4). Catalase is an important enzyme that protect living system against oxidative stress, being able to scavenge hydrogen peroxide which is a major product produced by SOD (Asada, 1992). Highest PPO activity (Fig. 5) was recorded in Cd T1 stem (4.24 U g^{-1} FW). Cd and Pb treated stem showed elevated PPO comparing to other treatments.

Previous studies recorded a positive relationship between increased POD and CAT enzyme activity with increase in heavy metals such as Cu, Pb and Zn in plant tissue (Girotti, 1985; Mazhoudi *et al.*, 1997; Mocquot *et al*, 1996). These enzymes remove superoxide radicals,

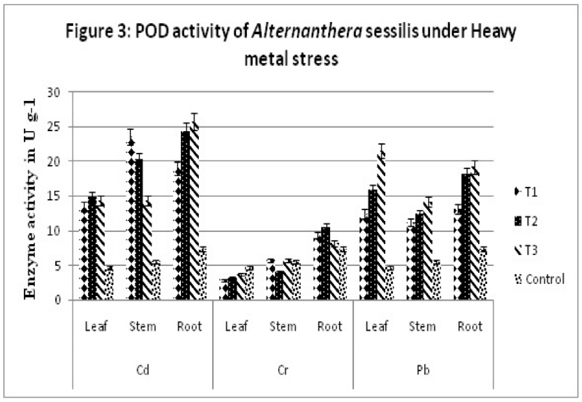


Figure 3: POD activity of *Alternanthera* sessilis under Heavy metal stress

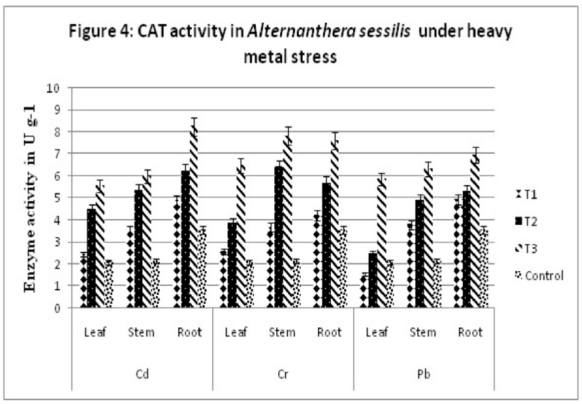


Figure 4: CAT activity in *Alternanthera sessilis* under heavy metal stress

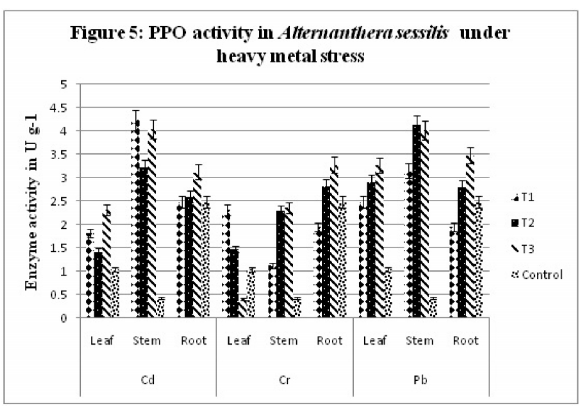


Figure 5: PPO activity in *Alternanthera sessilis* under heavy metal stress

which are harmful to cell membranes. Peroxidase activity sensitive indicators of heavy metal stress and can be used to anticipate events on the organism level (Wu *et al*., 2003; Mac Farlane and Burchett, 2001). The present study revealed that the plant showed variation in antioxidant response to overcome heavy metal stress and increased antioxidant enzymes may be associated with tolerance capacity of *Alternanthara sessilis* to protect the plant from oxidative damage.

CONCLUSIONS

Present study supported the hypothesis that *Alteranthera sessilis* possess the ability to cope with metal stress as a result of oxidative stress defense mechanism. Changes in SOD, CAT, POX and PPO showed a clear correlation with heavy metal concentrations. The data demonstrated a significant increment in the activities of major antioxidant enzymes, which are involved in the detoxification of ROS. However, the magnitude of increase in activity depends on both the enzyme and bioparts (leaves, stem or roots). *Alternanthara sessilis* also showed moderate tolerance against heavy metal induced stress along with good phytoaccumulation of heavy metals which make it a suitable candidate for phytoremediation of heavy metal contaminated soil.

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