A STUDY ON PHYTOREMEDIATING EFFICIENCY OF Pb, Cu AND Zn IN *SANSEVIERIA ROXBURGHIANA* SHULT AND SHULT.F

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ABSTRACT: The phytoremediative effect of medicinal plant (*Sansevieria roxburghiana*) was studied under different concentrations of metals in soil. Physiological parameters and antioxidative enzyme activities are investigated. A pot experiment setup was conducted to investigate the phytoremediative efficiency of plant *Sansevieria roxburghiana* species to accumulate Pb, Cu and Zn. On the basis of growth and enzymatic parameters in our study *Sansevieria roxburghiana* showed adaptive response during metal induced oxidative stress by enhancing the activities of endogenous enzymes. During both 20 and 40 day treatment chlorophyll contents, lipid peroxidation levels, catalase activity (EC 1.11.1.6) superoxide dismutase activity (EC 1.15.1.1) significantly increased. The accumulation in plant with metals Pb, Cu, Zn after treatment was analyzed with Atomic Absorption spectrophotometer. Translocation factor was found to be highest in Zn compared to Pb and Cu. These results suggest that the *Sansevieria roxburghiana* plant has tolerance towards heavy metal toxicity. This finding suggests that *Sansevieria roxburghiana* has the capability for the phytoextraction of Pb, Cu and Zn from polluted soils.

Keywords: *Sansevieria roxburghiana*, Induced oxidative stress, Translocation factor

INTRODUCTION

The recent introduction of anthropogenic toxic chemicals, and the massive relocation of natural materials to different environmental compartments, has resulted in severe pressure on the self-cleansing capacity of recipient ecosystems (Soil, water and atmosphere). Consequently, accumulated pollutants are of concern relative to both human and ecosystem exposure and potential impact. They include toxic heavy metals, metalloids. Heavy metals such as cadmium lead (Pb), copper (Cu), and Zinc (Zn), are released into the environment heavily by mining, industry, and agriculture, threatening environmental and human health. Currently, efforts are underway in many countries to control the release of contaminants (Schnoor *et al.*, 1990) and to accelerate the breakdown of existing contaminants by appropriate remediation techniques. Among all techniques employed Phytoremediation is best Plant based approach, is relatively inexpensive since they are performed in situ and is solar-driven promising new technology that uses plants to remove, transform, degrade, assimilate, metabolize, or detoxify metals, explosives, hydrocarbons, radionuclides, pesticides, and chlorinated solvents and landfill leachates located in soils, sediments, ground water, surface water and even the atmosphere. With a few notable exceptions, the best scenario for the phytoremediation of elemental pollutants involve plants extracting and translocating a toxic cation or oxyanion to above-ground tissues for later harvest; converting the element to a less toxic chemical species (i.e. transformation); or at the very least sequestering the element in roots to prevent leaching from the site. Heavy metals contamination which has become a serious environmental problem affecting all aspects of nature and their uptake by plants depends not only on the total soluble metal concentration in solution, but also on the species present, because different species such as free hydrated metals and metal–ligand complexes can be taken up by plant roots to different extents (Kwon-Rae Kim *et al.,* 2010). Pollutants can be remediated in plants through several natural, biophysical and biochemical processes: adsorption, transport, translocation, hyperaccumulation, transformation and mineralization. Oxygen free radicals are produced when molecular oxygen accepts electrons from other molecules, and many intracellular reactions reduce oxygen to superoxide (O^-) or hydrogen peroxide (H_2O_2) .This Reduced forms of oxygen produced during the course of essential cellular oxidation can be activated by metals such as

Iron, Zinc and copper, generating hydroxyl radicals responsible for alterations of macromolecules, and ultimately contributing to cell death. Other metals such as aluminum, cadmium, nickel and lead are directly toxic for plants and animals. Which are probably responsible for most of the oxidative damage in biological systems. In this study we investigated the influence of *Sansevieria roxburghiana* plant species on different metal absorption in contrasting soils and enzyme activities in order to know to what extent this plant can contribute for phytoremediative studies.

MATERIALS AND METHODS

Experimental design

The pot culture experiment was conducted in the greenhouse at Sri Venkateswara University Botanical Garden, Tirupati, India. All the soils used in the present study were collected from long-term contaminated sites of the Pb, Cu and Zn smelter operation zones. All treatments with five replicates were set up for period of 40 days, which included a control (without heavy metal addition), All pots were watered each day in order to keep soil moisture at 70–85% and kept for daylight.

Enzyme extraction

Tissues were homogenized in ice cold 25 mM Tris–HCl buffer (pH 7.2) containing 5 mM cysteine and 5 mM EDTA in a chilled pestle and mortar. The homogenate was centrifuged at 15000 rpm for 45 min at 4°C. The supernatant obtained after centrifugation was used as enzyme extract.

Measurement of chlorophyll

The chlorophyll content was measured by the method of Harborne (1980). About 0.2 g of leaf tissue was taken from the plants at random locations from the middle part. Leaves are washed with tap water and deionized water and stored at -22°C not longer than 1 day. Tissue samples were cut into small pieces and homogenized with 80% acetone solution until the tissue was fine slurry. The extract was centrifuged twice for 1 min at 16,000 g. The extract was diluted with 80% acetone solution if the initial reading was out of the linear range of instrument detection. The final volume of the diluted sample was recorded. The 80% acetone solution was used as the blank. Measurement of chlorophyll content was done by direct determination of the absorbance at wavelengths at 663 and 646 nm using a Hitachi UV-VIS Spectrophotometer 2001. The concentration of total chlorophyll, chlorophyll a and chlorophyll b was calculated according to (Arnon, 1949).

Total chlorophyll mg $^{-1}$ FW =
(.0127) x (OD 663) - (.00269) x (OD 645)
Chlorophyll a mg $^{-1}$ FW=
(.00229) x (OD 645) - (.00468) x (OD 663)
Chlorophyll b mg $^{-1}$ FW=
(.0202) x (OD 645) + (.00802) x (OD 663)

Lipid peroxidation assay

Lipid peroxidation was determined according to method described by Heath and Packer (1968) by measuring the amount of malondialdehyde (MDA) produced by the thiobarbituric acid. The crude extract was mixed with the same volume of 0.5% (w/v) thiobarbituric acid solution containing 20% (w/v). The mixture was heated at 95°C for 30 min and then quickly cooled on ice. The mixture was centrifuged at 3000 g for 10 min, and the absorbance of the supernatant was monitored at 532 and 600 nm. After subtracting the non-specific absorbance (600 nm), the MDA concentration was determined using its molar extinction coefficient (155 mM^{-1} cm^{-1}).

Catalase assay (EC 1.11.1.6)

Catalase activity was assayed according to the method of Beers and Sizer (1952). About 0.5 g fresh leaf samples were homogenized in 5 ml of cold 200 mM sodium phosphate buffer (pH 7.8) using chilled mortar and pestle. The homogenates were centrifuged at 10,500g for 20 min at 4 °C and the supernatant was used for enzyme assay. Catalase activity was determined by a UV–Vis Spectrophotometry (Hitachi UV-VIS Spectrophotometer 2001.Japan) at 25°C in 2.8 mL reaction mixture containing 1.5 mL 200 mM sodium phosphate buffer (p^H 7.8), 1.0 mL deionized water and 0.3 mL 0.1 M H_2O_2 prepared immediately before use, then 0.5 mL enzyme extract was added. The CAT activity was measured by monitoring the decrease in absorbance at 240 nm as a consequence of H_2O_2 consumption. Activity was expressed as units (one catalase activity unit defined as absorbance at 240 nm changes 0.1 per minute) per g of leaf fresh weight.

Assay of superoxide dismutase activity (EC 1.15.1.1)

The measurement of SOD activity was based on the reduction of cytochrome C at 550 nm (Schooner and Krause, 1990). The reaction mixture consisted of 50 mM phosphate buffer (pH 7.8), 0.1 mM EDTA, 18 mM cytochrome C and 0.1 Mm xanthine and enzyme extract. The reaction was initiated by addition of xanthine oxidase to the reaction mixture. Cytochrome C reduction was monitored by measuring the change in absorbance per min at 550 nm. Enzyme unit for SOD is defined as the amount of enzyme that resulted in 50% inhibition of the rates of cytochrome C reduction at 25±2°C.

Determination of translocation factor (TF)

Translocation factor (TF) = ratio of metal concentration in shoots/ratio of metal concentration in roots (Alina stingu *et al*., 2010)

Heavy metal analysis

After 40 days duration shoot and roots of each plant were cut and washed with distilled water and wet weight was measured. These samples were packed and dried in oven at 70°c. After recording dry weight, samples are milled to mixture and concentration of lead was measured by wet digestion method with nitric acid and perchloric acid (3:1) using ICP-AAS in both shoots and roots.

RESULTS AND DISCUSSION

RESULTS

Soil heavy metal analysis

Precise control over the amounts metal ions available to plants growing in a soil is technically difficult because metal bio-availability depends highly on soil characteristics. These include organic content, phosphorous content, potash, electronic conductivity. It is observed in our experiment heavy metals declined in treated soils when compare to control soils (Fig. 1 and 2).

Heavy metal accumulation by *Sansevieria roxburghiana*

Estimation of Cu, Zn and Pb through atomic absorption spectroscopy (AAS) was done. Plants show absorption of Cu 137.4 mgl^{-1} in roots following leaves (41.4 mgl^{-1}) and stem (32.08 mgl^{-1}).

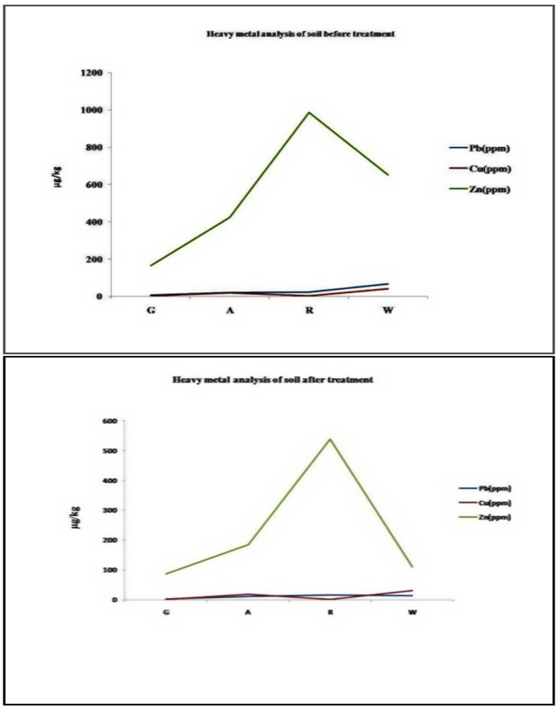

Fig.1and 2

G-Garden soils, **R-**Roadside soils, **A-**Automobile soils, **W-**Welding soils

Zn absorption is observed by (27 mgl^{-1}) in leaves (25.7 mgl^{-1}) in stems and (24 mgl^{-1}) in roots (Fig. 3, 4 and 5). Pb absorption is high in roots (1.9 mgl^{-1}) in leaves by (0.9 mgl^{-1}) and (0.5 mgl^{-1}) in stems in the soils.

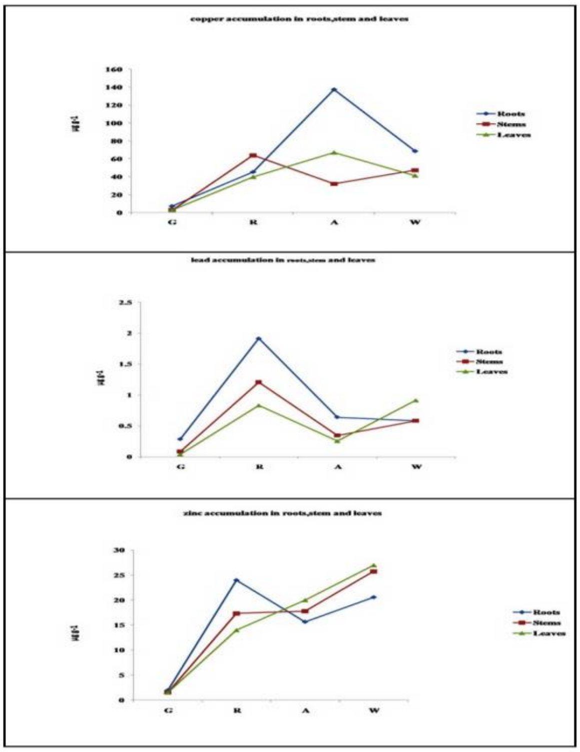

Fig: 3, 4 and 5

Translocation factor (TF)

The translocation factor presented higher values for Cu induced contamination than for others which mean that increasing metal concentration in the soil increased the concentration in the upper parts of the plant. Highest TF is observed with Cu in welding soils contaminated with rich iron following Zn and Pb. Lowest Translocation factor is observed with Pb>Zn>Cu (Fig. 6,7and 8).

Effect of heavy metal ions on chlorophyll content

Chlorophyll concentration decreased in response to heavymetals highest decrease is observed by 21% in garden and welding soils and 23% in roadside and automobile soils in comparison with controls (Fig. 9). Necrotic lesions appeared on the leaves of plants on roadside and automobile soils

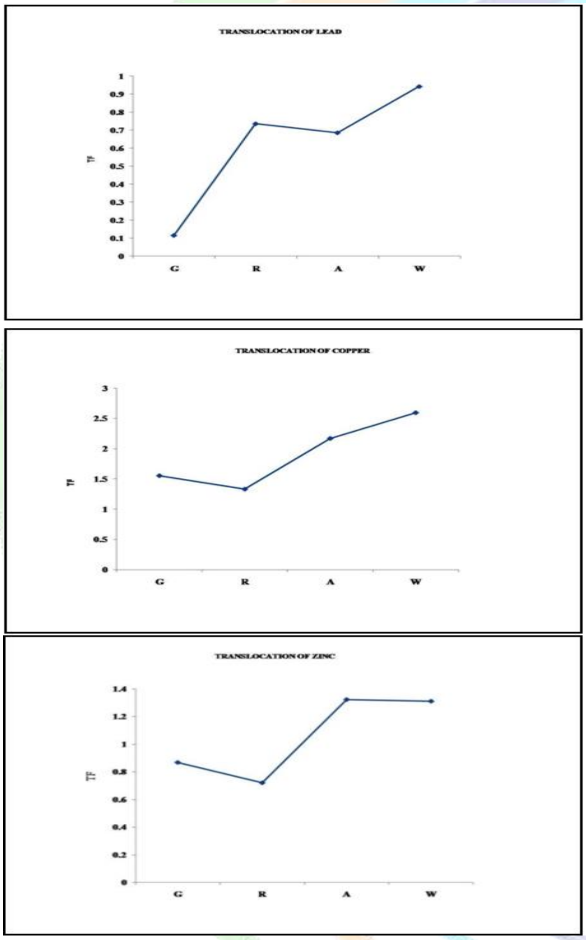

Fig: 6, 7 and 8

Effect of heavy metal ions on mda content

MDA level, which is a measure of membrane lipid peroxidation and lipoxigenase activity was found to increase in the plants treated plants and the increase MDA concentration was observed by 200% in roadside soil 106% in the garden soil when compared to automobile and welding soil by 50% and 66% (Fig. 10). When compared with 20 and 40 days of treatments, MDA content and ion leakage in all six species gradually increased as metal stress intensified as prolonged metal treatments usually increased MDA content. Furthermore, the roots whitish color in the normal plants had evolved to a light brown due to lead in plants.

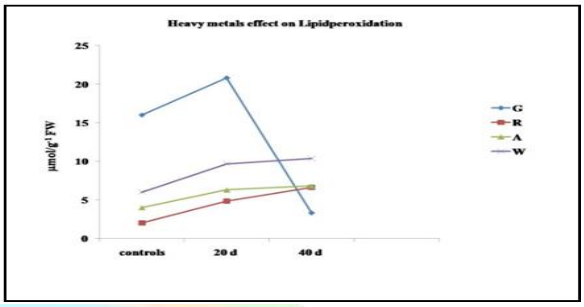

Fig: 9

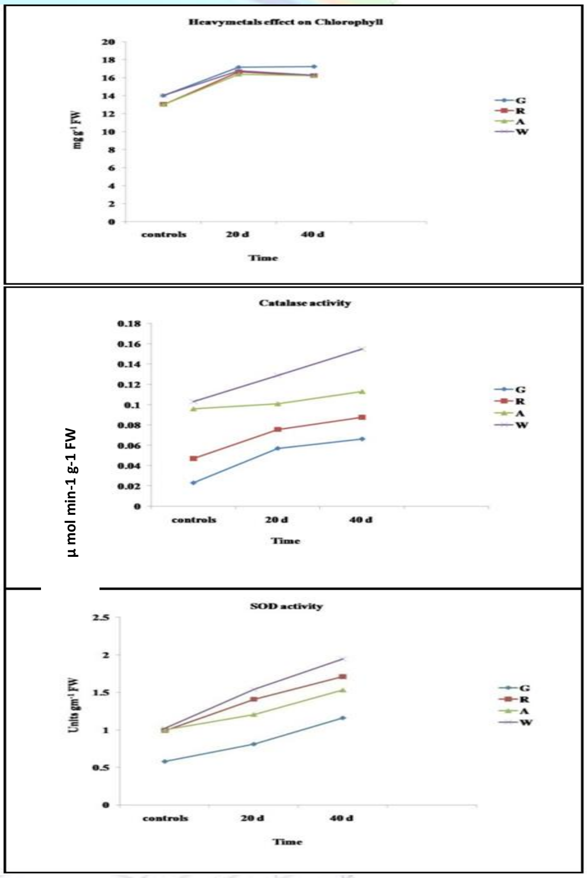

Fig: 10, 11 and 12

Effect of heavy metal ions on catalase activity

Catalase activity increased by increase in the metal ions concentration in treated plants compared with controls. The highest CAT activity was observed high in garden soils at 200% followed by roadside (100%), welding (50%) and Automobile (22%) soils after 40 day duration. In comparison with controls the increase in CAT activity was observed by 50%, 25% in roadside soils 10% 30% in automobile and welding soils between 20 and 40 day treatments in *Sansevieria roxburghiana* (Fig.11).

Effect of heavy metal ions on sod activity

Treated with metal polluted soils in plants for 40 days increased total SOD activity by 59% and 40% respectively in garden and welding soils compared with 20 day treated and 59% and 88% in comparision with controls but Roadside and automobile soils showed decrease SOD activity compared with 20 and 40 day treatments with 40% and 0.9% and 0.2% and 0.9% in comparison with controls (Fig.12).

DISCUSSION

Although some heavy metals are essential as micronutrients uptake of large quantities can be harmful to most plants. Lower presence of Iron, Zinc and copper are essential elements for many cellular processes. Zinc is one of the micronutrients essential for normal growth and development of plants, as it is known to be required in several metabolic processes (Cakmak and Marschner, 1993). Copper (Cu) is important for many structural and functional roles in plants. Copper also serve as constituents of many enzymes which contain copper in the active sites and catalyse the oxidation reactions. It plays a key role in the haemoglobin synthesis. Many oxidative enzymes such as Cu/Zn superoxide dismutase (Cu/Zn SOD), cytochrome oxidase, ascorbate oxidase, polyphenol oxidase and diamine oxidase and electron-transfer proteins (such as plastocyanin in chloroplasts) are Cu dependent (Bell and Dell, 2008).

Pb, is not necessary for plant growth however, induced a higher magnitude of oxidative stress. This may be due to the capability of lead to generate greater amounts of AOS (active oxygen species) which react with the polyunsaturated fatty acids of the chloroplast membranes. Pb is known to be effective in displacing various cationic metals from roots, suggesting that it may play a role in destabilizing the physiological barrier to solute movement in roots and thus limiting the availability of nutrients to the plant (Wierzbicka 1998). In lead-treated plants, lowering of stomatal conductance may be due to several lead-induced alterations in plant metabolism (Romanowska *et al*., 2002).

However, the presence of Lead, Copper and zinc at higher concentrations retarded growth and development of plants, by interfering with certain important metabolic processes (Chaney, 1993; Alia et al., 1995; Ebbs and Kochian, 1997). In view of these considerations, we have carried out a study of the effect of Zn, Cu and Pd on *Sansevieria roxburghiana.* In order to ensure the validity of this hypothesis we measured the activities of superoxide dismutase (SOD), catalase (CAT) and lipid peroxidation levels including Chlorophyll content in plants grown in the presence of toxic levels of zinc, copper and Lead in *Sansevieria roxburghiana*. The oxidative damage caused by metals such as Pb, Cu and Fe can be explained by the involvement of changes in redox state (Fenton reaction), Moreover, Treatments with Pd, Zn and Cu for for 40 days, significantly diminished leaf chlorophyll content with respect to control values. The increased activity of lipoxigenase may also indirectly contribute to the depletion of chlorophyll content (Divya Singh and Tiwari, 2011). Decrease in the concentrations of chlorophyll a and chlorophyll b when plants are subjected to high concentrations of lead in accordance with the observation of Stobart *et al.,* 1985; Phetsombat *et al.,* 2006. As a result, a 70% loss of chlorophyll was measured concomitantly with a comparable percentage reduction in light-saturated photosynthesis.

MDA level is considered as an essential parameter in order to determine membrane damage markedly raised over control values with the three metal ions. The increase in the concentrations of MDA is in accordance with the observation of (Somashekaraiah *et al*., 1992; Piqueras *et al*., 1999) and is due to the increased activity of lipid peroxidation caused by the enzyme lipoxigenase. The increased activity of lipid peroxidation is due to Pd, Cu and Zn induced generation of reactive oxygen species (ROS), which leads to the increased synthesis of catalase, peroxidase etc. (Bhattacharjee *et al.,* 1996). Antioxidant enzymes activity was stimulated by Zn, Pd, and Cu exposure. These enzymes showed a similar behavior when plants were treated with Fe and Cd ions. A growing body of evidence indicates that transition metals act as catalysts in the oxidative deterioration of biological macromolecules, and therefore, toxicity associated with these metals may be due to oxidative tissue damage. The results presented in this study showed that heavy metals increased the activity of catalase and superoxide dismutase with differential inducement among metals. It is possible that heavy metal stress reduce the capacity of the plants to assimilate carbon, this would trigger an increase in photosynthetic electron flux to molecular oxygen, resulting in the increased production of superoxide, hydrogen peroxide, and hydroxyl radical. There are evidences that increased levels of these scavenging enzymes may play a role in limiting the degree of photodamage to plants (Odjegba *et al.,* 2007). Our results confirmed that *Sansevieria roxburghiana* have an exceptional ability to accumulate Pb, Cu and Zn in their tissues under the influence of combined metals.

CONCLUSION

We conclude that exposure of plants to heavy metals provoke pronounced responses of antioxidative systems which protects the plants to some extent against oxidative damage, but the direction of response was dependent on the plant species, the metal used for the treatment and the intensity of the stress.

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