

## **Phytoremediation of Lead, Nickel, and Copper by *Salix acmophylla* Boiss.: Role of Antioxidant Enzymes and Antioxidant Substances**

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The metals like  $\text{Cu}^{2+}$  and  $\text{Zn}^{2+}$  are essential micro-nutrients taking part in redox reactions, electron transfers, a multitude of enzyme catalyzed reactions and structural functions in nucleic acid metabolism (Zenk 1996). The concentration of toxic metals (Cd, Ni, Pb, Hg etc.) in terrestrial as well as aquatic ecosystem has been increased manifolds over the past decades. These metals are strongly poisonous, reduce the plant growth and other cellular activities at higher concentrations. The metal contamination of soil, aquatic bodies and ground water poses a major environmental and human health problem due to various industrial processes and hence, need our immediate attention for effective and affordable technological solution. It has been reported that plants could accumulate considerable amount of toxic metals and thus, play significant role in cleaning of metal pollutants (Salt et al 1998). Such plants could tolerate toxic amount of metals by inducing different enzymes, stress proteins and synthesize enzymatically phytochelatins (Van Assche and Clijsters 1990). It does not mean that they remain unaffected with pollutant uptake. The accumulation of phytotoxic amounts of metals by higher plants causes retardation of growth, reduction of phytomass, generation of active oxygen species, loss of membrane integrity and inhibition of sulfhydryl group containing enzymes (Weckx and Clijsters 1996). To protect themselves from oxidative damage, generated by the heavy metal ions, plants have some protective systems composed of antioxidant enzymes and antioxidant substances. Some terrestrial plants accumulate large quantities of heavy metals but the exact mechanism of tolerance exhibited by these plants under heavy metal stress is yet to be investigated.

*Salix acmophylla* Boiss. is an economically important tree which grows generally on the banks of water bodies. Some parts of the stem and roots grow submerged in water. The plant has been reported to accumulate some trace metals (Zn, Pb, Cu, Fe etc.) while growing along a perennial lake (Ali et al 1999). The metal tolerance mechanism of this plant is yet to be investigated. Hence, present study has been designed to investigate the impact of Pb, Ni and Cu (common metal pollutants of lakes) on biomass, total chlorophyll content, lipid peroxidation, cysteine, non protein thiol content, superoxide dismutase and peroxidase activities of plant to ascertain the role of antioxidant enzymes and cellular antioxidants in providing protection to the plant against heavy metal toxicity.

## MATERIALS AND METHODS

The soil used in this experiment was collected from the garden of National Botanical Research Institute, Lucknow, India. The soil was oven dried at 80°C for 4 d and sieved through 6 mm mesh. Physico-chemical properties of the soil were assayed according to the method described by Piper (1966). The garden soil has pH-7.4; electrical conductivity-1.12 dSm<sup>-1</sup>; total nitrogen (%) 0.09; total phosphorus (%) 0.78; organic carbon (%) 0.483; Zn 22.6; Fe 709.45; Mn 44.6; Cu 20.22; B 11.50; Al-115.96; Si-2172; Ni-21.4 mg kg<sup>-1</sup>dw, respectively.

Different metal concentrations (0, 500, 1000, 2000, 5000 and 10000 mg/kg) were prepared by mixing definite amounts of Pb (CH<sub>3</sub>COO)<sub>2</sub>.3H<sub>2</sub>O, NiSO<sub>4</sub>.6H<sub>2</sub>O and CuSO<sub>4</sub>.5H<sub>2</sub>O to 3 kg garden soil. Three earthen pots were filled for each concentration of each metal. Fifteen days old saplings of same height (approx. 10 cm) and fresh weight (approx. 1.5 g) were uprooted from acid washed sand (HNO<sub>3</sub>, 1N) and transplanted in pots containing metal supplemented soil. Plants grown in garden soil served as control. The plants from each treatment were placed under natural conditions. The plants were watered daily and care was taken to avoid leaching of water from the pots. A plastic tub was placed below each pot to collect the leachates. The collected leachates were again returned to the experimental pot. No rainfall was recorded during the period of experiment. The plants were harvested 60 d after transplantation in metal-amended soil. The roots from each plant were detached and washed repeatedly into tap water to remove unwanted debris and blotted dried. Fresh weights of the plants were recorded and biomass was presented on dry weight (DW) basis. Photosynthetic area of 10 leaves of each plant was estimated using Delta-T area meter and mean (n =10) photosynthetic area per plant (cm<sup>2</sup>) has been presented in paper. For metal analysis, dried (1g) plant samples were ground in a grinder and digested in HNO<sub>3</sub>:HClO<sub>4</sub> (3:1, v/v) at 80°C and metals (Cu, Ni and Pb) were estimated by Atomic Absorption Spectrophotometer (Perkin Elmer 2380). The standard reference materials (E - Merck, Germany) of Cu, Pb and Ni were used to provide calibration and quality assurance for each analytical batch. The efficiency of digestion of plant samples was determined by adding standard reference material of metals, samples were digested and metals were estimated as above. Mean recoveries of copper, lead and nickel were 96± 6%, 97 ±5%, 96 ± 4%, respectively. The detection limits of Cu, Pb and Ni were 0.001,0.005 and 0.002 µg ml<sup>-1</sup>, respectively. Replicate (n= 3) analysis was conducted to assess the precision of the analytical techniques. Triplicate analysis for each metal varied by no more than 5%. Photosynthetic pigments were extracted in 80%-chilled acetone as per procedure of Arnon (1949). Protein was estimated by the method of Lowry et al (1951). The lipid peroxidation in the leaf tissue was measured in terms of malondialdehyde (MDA) content following the method of Heath and Packer (1968). Non-protein thiol and cysteine contents were measured following the method of Ellman (1959) and Gaitonde (1967), respectively. Superoxide dismutase (SOD) (EC 1.15.1.1) activity was assayed by measuring its ability to inhibit the photochemical reduction of nitroblue tetrazolium (NBT) using the

method of Dhindsa et al (1981). Peroxidase (EC 1.11.1.7) activity in leaves was estimated according to Hemeda and Klein (1990).

A one way analysis of variance (ANOVA) in randomized complete block design was performed to check the variability of data and validity of the results. Whenever, ANOVA was significant, comparison between means was done by Duncan's multiple range test (DMRT) (Gomez and Gomez 1984).

## RESULTS AND DISCUSSION

The toxicity of Cu, Ni and Pb to test plant was evaluated by measuring plant biomass and photosynthetic area (Table1). The plant biomass and photosynthetic area of *S. acmophylla* was not found affected by 500 mg kg<sup>-1</sup> concentration of these metals in garden soil (DMRT, P>0.05). The toxicity to biomass and photosynthetic area appeared at 1000 mg kg<sup>-1</sup> level of all the three metals which increased progressively with augmenting concentrations of these metals (Cu, Ni and Pb) in soil. Maximum loss of plant biomass and photosynthetic area was observed at the highest concentration (10000 mg kg<sup>-1</sup>) of all the three metals. The deleterious effect was highest in case of Pb, followed by Ni and Cu. *S. acmophylla* accumulated (ANOVA, P<0.05) Cu, Pb and Ni in its different plants parts (leaves, stem and roots) in an order of Pb>Ni>Cu (Table2). However, the uptake of these metals varied widely for the three metals investigated. Further, maximum concentration of these metals was observed in roots followed by stem and leaves.

All the three metals reduced total chlorophyll content (ANOVA, P<0.05) in concentration dependent manner (Table 1). The adverse effect tended to increase from Cu<Ni<Pb. However, the reduction in total chlorophyll level was not significant when exposed to 500 mg kg<sup>-1</sup> Cu, Ni and Pb (DMRT, P>0.05). The contamination of soil by metals affects the protein content significantly (ANOVA P<0.05) (Table 3). All the three metals increased protein level upto 2000 mg kg<sup>-1</sup> (Cu/Ni/Pb). Lead induced maximum protein level followed by Ni and Cu. The toxic effect of these metals on protein level has been observed first on 5000 mg kg<sup>-1</sup> level (DMRT, P<0.05) which increased with increasing levels of these metals. Further, copper treated plants (10000 mg kg<sup>-1</sup>) showed maximum reduction in protein level followed by Ni and Pb at the same concentration. However, all the three metals (Cu, Ni and Pb) enhanced cysteine and non- protein thiol contents in *S. acmophylla* upto 2000 mg kg<sup>-1</sup> (Table 3). The maximum increase in cysteine and NP-SH content was recorded in lead treated plants followed by copper and Ni. The concentrations of these metals >2000 mg kg<sup>-1</sup> in garden soil reduced significantly cysteine and non-protein thiol contents (DMRT; P<0.05). Malondialdehyde content (MDA) is the measure of lipid peroxidation. All the three metals induced lipid peroxidation in *S. acmophylla* in concentration dependent manner (Table 4). However, increase in MDA level (lipid peroxidation) was not significant (DMRT, P>0.05) when exposed to 500 mg kg<sup>-1</sup> level of these metals. The degree of lipid peroxidation was more pronounced in case of Pb treated plants than Cu and Nickel treated plants (Table 4). Cu, Pb and Ni also induced (ANOVA, P<0.05) SOD and POD activities in *S.*

**Table 1.** Effect of copper, nickel and lead on biomass, photosynthetic area, total chlorophyll content of *S. acmophylla*.

Metal (mg kg <sup>-1</sup> )	Biomass (g DW)			Photosynthetic area (cm <sup>2</sup> )			Total chlorophyll (mg g <sup>-1</sup> fw)		
	Copper	Nickel	Lead	Copper	Nickel	Lead	Copper	Nickel	Lead
0.0	0.97 <sup>a</sup> ±0.05	0.97 <sup>a</sup> ±0.04	0.98 <sup>a</sup> ±0.04	53.5 <sup>a</sup> ±2.5	53.5 <sup>a</sup> ±2.3	53.5 <sup>a</sup> ±2.2	2.356 <sup>a</sup> ±0.1	2.356 <sup>a</sup> ±0.1	2.356 <sup>a</sup> ±0.1
500	0.96 <sup>a</sup> ±0.04	0.95 <sup>a</sup> ±0.04	0.97 <sup>a</sup> ±0.04	52.4 <sup>a</sup> ±2.2	51.5 <sup>a</sup> ±2.1	50.4 <sup>a</sup> ±2.3	2.310 <sup>a</sup> ±2.2	2.273 <sup>a</sup> ±1.1	2.25 <sup>a</sup> ±1.1
1000	0.73 <sup>b</sup> ±0.03	0.70 <sup>b</sup> ±0.02	0.68 <sup>b</sup> ±0.03	45.6 <sup>b</sup> ±2.3	42.4 <sup>b</sup> ±1.7	40.7 <sup>b</sup> ±2.0	1.68 <sup>b</sup> ±0.08	1.52 <sup>b</sup> ±0.06	1.34 <sup>b</sup> ±0.07
2000	0.58 <sup>c</sup> ±0.02	0.53 <sup>c</sup> ±0.03	0.43 <sup>c</sup> ±0.02	40.7 <sup>c</sup> ±2.0	35.1 <sup>c</sup> ±1.6	32.0 <sup>c</sup> ±1.5	1.30 <sup>c</sup> ±0.05	1.23 <sup>c</sup> ±0.05	1.02 <sup>c</sup> ±0.05
5000	0.38 <sup>d</sup> ±0.02	0.33 <sup>d</sup> ±0.02	0.24 <sup>d</sup> ±0.01	30.7 <sup>d</sup> ±1.4	28.6 <sup>d</sup> ±1.3	25.2 <sup>d</sup> ±1.3	1.06 <sup>d</sup> ±0.04	0.93 <sup>d</sup> ±0.05	0.86 <sup>d</sup> ±0.04
10000	0.27 <sup>e</sup> ±0.01	0.25 <sup>e</sup> ±0.01	0.18 <sup>e</sup> ±0.001	20.1 <sup>e</sup> ±1.0	18.2 <sup>e</sup> ±0.8	15.4 <sup>e</sup> ±0.7	0.82 <sup>e</sup> ±0.03	0.63 <sup>e</sup> ±0.03	0.51 <sup>e</sup> ±0.02

Mean ± SD; ANOVA, p < 0.05; identical superscripts denote no significant (p < 0.05) difference between means in a column (Cu/ Ni/ Pb) according to DMRT.

**Table 2.** Heavy metal (Cu, Ni, and Pb) accumulation (µg g<sup>-1</sup> DW) by *S. acmophylla*.

Metal (mg kg <sup>-1</sup> )	Copper			Nickel			Lead		
	Leaf	Stem	Roots	Leaf	Stem	Roots	Leaf	Stem	Roots
0.0	2.54 <sup>f</sup> ±0.1	4.00 <sup>f</sup> ±0.2	6.85 <sup>f</sup> ±0.2	2.86 <sup>f</sup> ±0.1	3.54 <sup>f</sup> ±0.1	4.29 <sup>f</sup> ±0.2	1.86 <sup>f</sup> ±0.1	2.50 <sup>f</sup> ±0.1	3.10 <sup>f</sup> ±0.1
500	10.8 <sup>e</sup> ±0.4	25.3 <sup>e</sup> ±1.1	24.8 <sup>e</sup> ±1.1	17.7 <sup>e</sup> ±0.8	26.9 <sup>e</sup> ±1.2	43.9 <sup>e</sup> ±2.1	26.0 <sup>e</sup> ±1.3	40.0 <sup>e</sup> ±1.8	49.2 <sup>e</sup> ±2.3
1000	17.2 <sup>d</sup> ±0.8	73.3 <sup>d</sup> ±2.8	75.8 <sup>d</sup> ±2.8	25.7 <sup>d</sup> ±1.3	107.9 <sup>d</sup> ±4.4	156.1 <sup>d</sup> ±6.8	33.1 <sup>d</sup> ±1.5	150.3 <sup>d</sup> ±6.4	208.3 <sup>d</sup> ±9.4
2000	49.4 <sup>c</sup> ±2.3	103.9 <sup>c</sup> ±4.1	177.5 <sup>c</sup> ±6.1	58.0 <sup>c</sup> ±2.8	172.5 <sup>c</sup> ±7.8	291.1 <sup>c</sup> ±14.5	76.3 <sup>c</sup> ±3.6	192.2 <sup>c</sup> ±8.6	493.9 <sup>c</sup> ±22.9
5000	82.3 <sup>b</sup> ±3.1	179.2 <sup>b</sup> ±6.5	345.3 <sup>b</sup> ±16.3	86.8 <sup>b</sup> ±4.2	204.7 <sup>b</sup> ±9.8	533.3 <sup>b</sup> ±25.7	105.9 <sup>b</sup> ±4.2	231.2 <sup>b</sup> ±10.4	764.1 <sup>b</sup> ±36.2
10000	126.3 <sup>a</sup> ±5.3	203.7 <sup>a</sup> ±8.8	624.4 <sup>a</sup> ±28.2	139.1 <sup>a</sup> ±6.9	264.3 <sup>a</sup> ±12.1	746.3 <sup>a</sup> ±36.5	180.4 <sup>a</sup> ±8.2	284.0 <sup>a</sup> ±13.5	1038.5 <sup>a</sup> ±50.2

Mean ± SD; ANOVA, p < 0.05; different superscripts denote significant difference between means in a column (Cu/ Ni/ Pb) according to DMRT.

**Table 3.** Effect of Cu, Ni and Pb on protein, non protein thiol (NP- SH) and cysteine contents of *S. acmophylla*

Metal (mg kg <sup>-1</sup> )	Protein (mg g <sup>-1</sup> fw)			Non-protein thiol (μ mol g <sup>-1</sup> fw)			Cysteine (μ mol g <sup>-1</sup> fw)		
	Copper	Nickel	Lead	Copper	Nickel	Lead	Copper	Nickel	Lead
0.0	14.05 <sup>d</sup> ±0.7	14.05 <sup>d</sup> ±0.7	14.05 <sup>d</sup> ±0.7	36.82 <sup>d</sup> ±1.7	36.38 <sup>d</sup> ±1.7	36.82 <sup>d</sup> ±1.7	66.12 <sup>d</sup> ±3.1	66.12 <sup>d</sup> ±3.1	66.12 <sup>d</sup> ±3.1
500	16.39 <sup>c</sup> ±0.8	18.23 <sup>c</sup> ±0.9	18.04 <sup>c</sup> ±1.1	49.17 <sup>c</sup> ±2.4	46.06 <sup>c</sup> ±2.2	52.40 <sup>c</sup> ±2.5	76.36 <sup>c</sup> ±3.6	72.12 <sup>c</sup> ±3.5	81.62 <sup>c</sup> ±3.1
1000	20.32 <sup>b</sup> ±0.9	23.12 <sup>b</sup> ±1.1	25.12 <sup>b</sup> ±1.2	58.38 <sup>b</sup> ±2.8	54.79 <sup>b</sup> ±2.6	66.03 <sup>b</sup> ±3.3	88.68 <sup>b</sup> ±4.1	80.32 <sup>b</sup> ±3.9	98.13 <sup>b</sup> ±4.7
2000	27.12 <sup>a</sup> ±1.3	28.32 <sup>a</sup> ±1.3	30.02 <sup>a</sup> ±1.4	68.12 <sup>a</sup> ±3.4	63.12 <sup>a</sup> ±2.9	76.13 <sup>a</sup> ±3.8	103.92 <sup>a</sup> ±4.2	96.32 <sup>a</sup> ±4.6	124.84 <sup>a</sup> ±5.8
5000	6.04 <sup>e</sup> ±0.3	9.02 <sup>e</sup> ±0.4	11.12 <sup>e</sup> ±0.5	30.12 <sup>e</sup> ±1.3	28.13 <sup>e</sup> ±1.4	33.21 <sup>e</sup> ±1.7	50.02 <sup>e</sup> ±2.5	42.12 <sup>e</sup> ±2.1	53.10 <sup>e</sup> ±2.5
10000	3.12 <sup>f</sup> ±0.1	4.01 <sup>f</sup> ±0.1	8.13 <sup>f</sup> ±0.4	17.60 <sup>f</sup> ±0.7	13.15 <sup>f</sup> ±0.6	25.12 <sup>f</sup> ±1.2	32.12 <sup>f</sup> ±1.6	25.21 <sup>f</sup> ±1.2	42.32 <sup>f</sup> ±2.1

Mean ± SD; FW = fresh weight ; ANOVA, p< 0.05; different superscripts denote significant ( p< 0.05) difference between means in a column ( Cu/ Ni/ Pb) according to DMRT.

**Table 4.** Effect of Cu, Ni and Pb on MDA, POD and SOD activities of *S. acmophylla*

Metal (mg kg <sup>-1</sup> )	MDA (μ mol g <sup>-1</sup> FW)			POD (μ mol g <sup>-1</sup> FW)			SOD(unit g <sup>-1</sup> FW)		
	Copper	Nickel	Lead	Copper	Nickel	Lead	Copper	Nickel	Lead
0.0	31.69 <sup>e</sup> ±1.6	31.69 <sup>e</sup> ±1.4	31.69 <sup>e</sup> ±0.7	144.9 <sup>f</sup> ±7.0	144.9 <sup>f</sup> ±6.7	144.9 <sup>f</sup> ±6.5	17.4 <sup>f</sup> ±0.9	17.4 <sup>f</sup> ±0.8	17.4 <sup>f</sup> ±0.6
500	33.98 <sup>e</sup> ±1.4	33.19 <sup>e</sup> ±1.2	33.98 <sup>e</sup> ±1.1	150.6 <sup>e</sup> ±7.4	164.5 <sup>e</sup> ±8.2	170.7 <sup>e</sup> ±7.5	20.5 <sup>e</sup> ±1.0	21.2 <sup>e</sup> ±1.1	23.6 <sup>e</sup> ±1.2
1000	39.70 <sup>d</sup> ±1.9	47.29 <sup>d</sup> ±1.6	37.90 <sup>d</sup> ±1.5	171.7 <sup>d</sup> ±8.2	192.5 <sup>d</sup> ±7.6	201.8 <sup>d</sup> ±9.3	28.5 <sup>d</sup> ±1.4	30.5 <sup>d</sup> ±1.4	36.2 <sup>d</sup> ±1.7
2000	45.51 <sup>c</sup> ±1.6	59.00 <sup>c</sup> ±2.5	54.58 <sup>c</sup> ±2.4	203.6 <sup>c</sup> ±9.4	226.4 <sup>c</sup> ±10.9	241.7 <sup>c</sup> ±12.0	34.1 <sup>c</sup> ±1.7	37.5 <sup>c</sup> ±1.6	45.6 <sup>c</sup> ±2.2
5000	51.83 <sup>b</sup> ±2.3	63.45 <sup>b</sup> ±3.0	64.78 <sup>b</sup> ±3.2	253.1 <sup>b</sup> ±11.3	296.4 <sup>b</sup> ±11.4	310.7 <sup>b</sup> ±15.2	46.4 <sup>b</sup> ±2.0	50.3 <sup>b</sup> ±2.1	55.15 <sup>b</sup> ±2.5
10000	61.13 <sup>a</sup> ±2.9	72.06 <sup>a</sup> ±3.1	84.44 <sup>a</sup> ±3.8	332.8 <sup>a</sup> ±15.7	388.7 <sup>a</sup> ±19.2	398.4 <sup>a</sup> ±19.5	53.1 <sup>a</sup> ±2.4	56.12 <sup>a</sup> ±2.4	60.65 <sup>a</sup> ±2.7

Mean ± SD; FW = fresh weight ;ANOVA, p< 0.05; identical superscripts denote no significant (p< 0.05) difference between means in a column ( Cu/ Ni/ Pb) according to DMRT.

*acmophylla* in concentration dependent manner (Table 4). Lead induced maximum SOD and POD activities, followed by Ni and Cu.

Heavy metal (Cu, Ni and Pb) analyses in plant tissues indicate that *S. acmophylla* is a good accumulator of these metals. Therefore, it could be used as heavy metal filter for metal polluted sites. The maximum concentration of copper, nickel and lead found in roots of *S. acmophylla* might be attributed to the restricted transportation of these metals to the aerial parts.

In the present study, total chlorophyll content of *S. acmophylla* grown in Cu, Ni and Pb contaminated soils was found negatively correlated to the metal concentration in the soil. Besides, reduction in total chlorophyll content was concomitant with increase in MDA level. Phytotoxic effects of lipid peroxidation on chlorophyll contents and reduction in chlorophyll biosynthesis of different plant species due to interaction of toxic metals to -SH group of vital enzymes have been documented under heavy metal stress (Somashekaraiyah et al 1992; Devi and Prasad 1998). It could be inferred from present results that reduction in total chlorophyll content of *S. acmophylla* was probably caused by interaction of these metals to -SH group of enzymes of chlorophyll biosynthesis as well as lipid peroxidation mediated degradation. Further, biomass and photosynthetic area are measure of the plant's productive investment. Reduction in photosynthetic area and biomass of *S. acmophylla* might be attributed to the phytotoxic effects of Cu, Ni and Pb to the total chlorophyll content. Reduced biomass and photosynthetic area of *Cassia surattensis* grown in fly-ash (a mixture of heavy metals) have been reported by Vajpayee et al (2000).. The synthesis of new proteins under metal stress indicates presence of tolerance mechanism in the plant (Toppi and Gabbrielli 1999). Hence, increase in the protein content of *S. acmophylla* treated by Pb, Cu and Ni (up to 2000 mg kg<sup>-1</sup>) might be attributed to the synthesis of stress proteins to combat metal toxicity.

The plant cell membranes are generally considered as primary sites of metal injury. It has been reported that accumulation of heavy metals in plant tissues results in production of free radicals which induces peroxidation of membrane lipids (Devi and Prasad (1998). Further, membrane destabilization is directly correlated with MDA production, which is one of the decomposition products of polyunsaturated fatty acids of membranes. Therefore, high level of MDA observed in *S. acmophylla* under metal stress might be attributed to the peroxidation of membrane lipids caused by reactive oxygen species produced due to metal stress. Cysteine and thiols are regarded as cellular antioxidant substances (Devi and Prasad 1998). Cysteine is the major limiting amino acid for glutathione synthesis. Further, Cysteine and non-protein thiol play a significant role for detoxification of metals by the synthesis of phytochelatin ( Tripathi et al 1996). During present study, cysteine and non-protein thiols contents of *S. acmophylla* exhibited varied response to the heavy metals depending upon the concentrations. The enhancement of cysteine content in Cu, Pb and Ni treated (up to 2000 mg kg<sup>-1</sup>) plants of *S. acmophylla* might be attributed to the active involvement of ATP sulphurylase and ATP sulfotransferase enzyme (Nussabaum et al 1988). The elevated level of non-protein thiol content in Cu, Pb and Ni treated (up to 2000 mg kg<sup>-1</sup>) plants indicated metal tolerance of *S. acmophylla* through phytochelatin

synthesis. It has been reported that ninety percent of non protein thiol contents accumulated in cadmium treated cell suspension culture of *Rauwolfia serpentina* were accounted for phytochelatin synthesis (Grill et al 1987). Therefore, increased levels of cysteine and non -protein thiols up to 2000 mg kg<sup>-1</sup> concentration of Cu, Ni and Pb indicated metal tolerance in *S. acmophylla* through possible synthesis of phytochelatins.

The antioxidant enzymes are considered to be an important defense system of plants against oxidative stress caused by metals (Weckx and Clijsters 1996). The oxidative damage of cells of *S. acmophylla* seems to be controlled by the hyper activity of superoxide dismutase and peroxidase. Superoxide dismutase plays role in dismutating of free hydroxyl radicals by the formation of hydrogen peroxide. The hydrogen peroxide is toxic for plants as it acts both as an oxidant as well as a reductant. The hyper activity of POD under Cu, Ni, and Pb stress indicated the scavenging of H<sub>2</sub>O<sub>2</sub> generated through activity of SOD and photorespiration in plant cells. Therefore, an increase in POD activity prevents plant from toxic effects of H<sub>2</sub>O<sub>2</sub>. During present study, continuous enhancement of POD activity suggests its role in constant detoxification of H<sub>2</sub>O<sub>2</sub> in metal toxicity. The hyper activity of antioxidant enzymes (POD and SOD) in *Salix acmophylla* might be consequences of the strategies adapted by plant for its survival under stress imposed by metals like Cu, Ni, and Pb.

Our results concluded that *S. acmophylla* accumulated considerable amounts of Cu, Ni and Pb in different plant parts and exhibited high tolerance to these metals. The plant showed ability for their detoxification by antioxidants enzymes and cellular antioxidants like cysteine and thiols. The study further indicated that metal induced hyperactivity of antioxidant enzymes in *S. acmophylla* could be used as bio- monitoring tool for identifying metal contamination in soil and water bodies with these chemical traits. Plant is highly suitable for phytoremediation of metal contamination from lakes and soils.

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## REFERENCES

- Ali MB, Tripathi RD, Rai UN, Pal A, Singh SP (1999) Physico-chemical characteristics and pollution level of lake Nainital (U.P. India): Role of macrophytes and phytoplankton in biomonitoring and phytoremediation of toxic metal ions. *Chemosphere* 139: 2171-2182
- Arnon DI (1949) Copper enzyme in isolated chloroplasts. Polyphenol/oxidase in *Beta vulgaris*. *Plant Physiol* 130: 267-272
- Devi SR, Prasad MNV (1998) Copper toxicity in *Ceratophyllum demersum* L. (coontail), a free-floating macrophyte: response of antioxidant enzymes and antioxidants. *Plant Sci* 138: 157-165

- Dhindsa RS, Dhindsa PP, Thorpe TA (1981) Leaf senescence correlated with increased levels of membrane permeability and lipid peroxidation and decreased levels of superoxide dismutase and catalase. *J Exp Bot* 32: 93 - 101
- Ellman GL (1959) Tissue sulfhydryl groups. *Arch Biochem Biophys* 82: 70 -77
- Gaitonde MK (1967) A spectrophotometric method for the direct determination of cysteine in the presence of other naturally occurring amino acid. *Biochem J* 104: 627-633
- Gomez KA, Gomez AA (1984) *Statistical Procedures for Agricultural Research*. John Wiley & Sons, New York
- Grill E, Winnacker EL, Zenk MH (1987) Phytochelatins, a class of heavy metal binding peptides from plants, are functionally analogous to metallothioneins. *Proc Nat Acad Sci USA* 84: 439-43
- Heath RL, Packer L (1968) Photoperoxidation in isolated chloroplast. I. Kinetics and stoichiometry of fatty acid peroxidation. *Arch Biochem Biophys* 125: 189-198
- Hemeda HM, Klein BP (1990) Effects of naturally occurring antioxidants on peroxidase activity of vegetable extracts. *J Food Sci* 55: 184-185
- Lowry OH, Rosenbrough NJ, Farr AL, Randall RJ (1951) Protein measurement with folin phenol reagent. *J Biol Chem* 193: 265-275
- Nussbaum S, Schmutz D, Brunold C (1988) Regulation of assimilatory sulfate reduction by cadmium in *Zea mays* L. *Plant Physiol* 88: 1407-1410
- Piper CS (1966) *Soil and plant analysis*. Inter-Science, New York
- Salt DE, Smith RD, Raskin I (1998) Phytoremediation. *Ann Rev Plant Physiol Plant Mole Biol* 49: 643-668
- Somashekaraiah BV, Padmaja K, Prasad ARK (1992) Phytotoxicity of cadmium ions on germinating seedlings of mung bean (*Phaseolus vulgaris*): involvement of lipid peroxides in chlorophyll degradation. *Physiol Plant* 85: 85-89
- Toppi, LSD, Gabbrielli R (1999) Response to cadmium in higher plants. *Environ Exp Bot* 41:105-130
- Tripathi RD, Rai UN, Gupta M, Chandra P (1996) Induction of phytochelatins in *Hydrilla verticillata* (L.f.) Royle under cadmium stress. *Bull Environ Contam Toxicol* 56: 505-512
- Vajpayee P, Rai UN, Chaudhary SK, Tripathi RD, Singh SN (2000) Management of fly-ash landfills with *Cassia surattensis* Burm: a case study. *Bull Environ Contam Toxicol* 68 : 675-683
- Van Assche F, Clijsters H (1990) Effect of metals on enzyme activity in plants. *PlantCell Environ* 13: 195-206
- Weckx J, Clijsters H (1996) Oxidative damage and defence mechanisms in primary leaves of *Phaseolus vulgaris*. *Physiol Plant* 96:506-512
- Zenk, MH (1996) Heavy metal detoxification in higher plants – a review. *Gene* 179: 21-30