J Agri Sci, 2(1): 45-51 (2011)
PRINT: ISSN 0976-6898 ONLINE: ISSN 2456-6535
DOI: 10.31901/24566535.2011/02.01.06

Effect of Heavy Metals on Some Biochemical Parameters of Sal (*Shorea robusta*) Seedling at Nursery Level, Doon Valley, India

Preeti Pandey Pant*, A.K. Tripathi and Vivek Dwivedi#**

Ecology and Environment Division, Forest Research Institute, P.O. New Forest, Dehradun 248 006, Uttarakhand, India
*Contact No. *<+91-9411311838>, **<+91-9412050276>, # <+91-09359936957>*
*E-mail: *<preeti.fri@gmail.com>, **<tripathiak@icfre.org>, # vivek.nature@gmail.com>*

KEYWORDS Heavy Metal. Chlorophyll. Amino Acid. Proline. Ascorbic Acid. Sal (*Shorea robusta*)

ABSTRACT The effects of cadmium, arsenic and lead (1, 5 and 10ppm) on the content of chlorophyll, amino acid, proline, and ascorbic acid were investigated in ten month old sal seedlings (*Shorea robusta*) grown in nursery with various treatment of different concentration of Cadmium, Pb, and As. Control and heavy metal-treated plants were grown for a period of six months in natural condition. Total chlorophyll, amino acid, proline and ascorbic acid content were measured in the leaves of the six month old seedling with the help of UV Spectrophotometer. The result showed that the total chlorophyll and amino acid content decreased with increased concentrations of heavy metals. A significant increase of proline and ascorbic acid content was observed in leaves after a four month exposure to heavy metals. The strongest effect of cadmium, followed by Pb^{++} and As^{++} was observed on total chlorophyll, amino acid, proline and ascorbic acid content of plants.

INTRODUCTION

Heavy metals and metalloids make a significant contribution to environmental pollution as a result of human activities such as emissions from automobile, mining, smelting, electroplating, energy and fuel production, power transmission, intensive agriculture, sludge dumping and military operations (Nedelkoska and Doran 2000). They present a risk for primary and secondary consumers and ultimately humans (Zeller and Feller 1999).

Heavy metals such as Cu and Zn are essential for normal plant growth and development since they are constituents of many enzymes and other proteins. However, elevated concentrations of both essential and non-essential heavy metals in the soil can lead to toxicity symptoms and growth inhibition in most plants (Hall 2002). Toxicity may result from the binding of metals to sulphydryl groups in proteins, leading to inhibition of activity or disruption of structure, or from displacement of an essential element, resulting in deficiency effects (van Assche and Clíjsters 1990). In addition, a heavy metal excess may stimulate the formation of free radicals and reactive oxygen species, perhaps resulting in oxidative stress (Dietz et al. 1999). The lifetime of active oxygen species within the cellular environment is determined by the antioxidant system, which provides crucial protection against oxidative damage. The anti-oxidative system comprises numerous enzymes and compounds of low molecular weight (Noctor and Foyer 1998). The antioxidant properties of plants exposed to various stress factors have been studied (Havaux and Kloppstech 2001), but studies related to the effect of heavy metal-induced stress on vitamin levels in plants are limited. Lead and mercury were reported to cause an increase in ascorbic acid levels in two *Oryza sativa* cultivars (Mishra and Choudhuri 1999).

Detailed studies indicate that heavy metals and metalloids have effects on chlorophyll and amino acid content in plants. Heavy metals are known to interfere with chlorophyll synthesis either through direct inhibition of an enzymatic step or by inducing deficiency of an essential nutrient (van Assche and Clíjsters 1990).) The effect of different concentrations of lead and cadmium on seed germination and seedling growth of *Leucaena leucocephala* was done by Shafiq et al. (2008).

Proline accumulation, accepted as an indicator of environmental stress, is also considered to have important protective roles. Heavy metal stress leads to proline accumulation (Alia and Saradhi 1991). Proline accumulation in plant tissues has been suggested to result from (a) a decrease in proline degradation, (b) an increase

Correspondence Address:
Preeti Pandey Pant
C/O Sameer Pant,
Flat no 664, Om Apartment,
Pocket 2, Sector -14
Dwarka, New Delhi

in proline biosynthesis, (c) a decrease in protein synthesis or proline utilization, and (d) hydrolysis of proteins (Charest and Phan 1990).

This experiment has been carried out for finding the phytoremediation capacities and capabilities of the selected plant species, with the intention of applying these findings to reduce the heavy metal contamination from environment. We have examined the effects of heavy metal cations, namely lead, cadmium and metalloid arsenic, on total chlorophyll, amino acid, proline, and ascorbic acid content in seedling sal (*Shorea robusta*). The results obtained from the experiment have indicated that *S. robusta* is effective and could be used in toxic metal remediation. Therefore, proposed technique may solve the disposal problem of various heavy metals producing industries. This kind of study is beneficial for saving the environment from heavy metals. It is also important that public awareness about this technology is considered and clear and precise information is made available to the general public to enhance its acceptability as a global sustainable technology to be widely used.

MATERIALS AND METHODS

Species were selected on the basis of survey in different sites/locations, which are highly affected by industrial effluents. The species which are commonly available in Dehradun, were taken for the experimental set up. Six month old sal seedlings (*Shorea robusta*) were selected to conduct the study. Heavy metal treatment of CadmiumCl_2 As_2O_3 and Pb $(C_2H_3O_2)_2.3H_2O$ with three different concentrations of 1mg/l, 5mg/l, 10mg/l were prepared in the laboratory. The treatments were given to the species after six months of establishment of seedling. These treatments were given every week for next four months. Three replicates of each plant along with control were treated with different concentrations. Plants were treated with the doses of different concentrations vis-a-vis 1mg/l, 5mg/l, and 10mg/l of each heavy metal to the plant's root.

The chlorophyll contents (mg/g fresh leaf), were analysed by following Arnon (1949) method. Leaf samples were homogenized in 80% acetone and optical densities were measured at 645 and 663 nm with Spectrophotometer (Spectra scan UV 2700).

Free amino acid was measured by the method prescribed by of Moore and Stein (1948) also called as ninhydrin method and is widely used. Absorbance was recorded at 570 nm using Spectrophotometer (Spectrascan UV 2700). Leucine was used as the standard.

Proline was extracted from the leaves and estimated by the methods of Bates et al. (1973). Homogenates of the leaf samples were prepared in 3% sulphosalicylic acid. Pink colour was developed by a reaction with glacial acid and ninhydrin. The colour was separated in toluene layer and intensity of the colour was measured at 529 nm., spectrophotometrically.

Fresh leaf sample (0.5gm) was homogenised in 20ml of extracting media of 0.5gm oxalic acid and 0.075gm EDTA in 100ml distilled water. After centrifuging the homogenate for 15minutes, 1.0 ml of the homogenate was mixed with 5.0 ml of Dichlorophenol indophenols (20 µg/ml). After shaking well, its optical density was measured at 520nm (Sadasivam and Balasubramaniam 1987). Calibration curve for ascorbic acid was prepared using chemically pure ascorbic acid.

Statistical analysis employed SPSS windows version 15.0. The significance of differences between variables at $P<0.01$ or $P<0.05$ was checked with a multiple comparison on (LSD) test. All values are means ±SE. Increases or decreases are expressed as percentages versus the control.

RESULTS

Table 1 and Figure 1 summarize the results for the effects of selected heavy metals (Cadmium, Pb) and metalloid (As) on total chlorophyll, amino acid, ascorbic acid, proline content in leaves of the sal seedlings. Total chlorophyll and amino acid content decreased with increased concentrations of the heavy metal. Significant increases of proline, and ascorbic acid content in the leaf were detected after exposure to heavy metal. The result of Analysis of Variance (ANOVA) shows that, for chlorophyll, the interactions among the metal were found to have significant differences at 0.1% ($P<0.001$) level while between metals and treatment of different concentration at 5% ($P<0.05$) significant level. For proline and ascorbic acid, interactions among metal and treatment were found highly significant at a level of 0.01% ($P<0.001$), and between metal and treatment of different concentration at 5% (P<0.05) significant level. However for amino acids, difference was not significant for the interactions among the metals, and the difference of interactions between metal and treatment were found to have significant at 0.1% (P<0.001) level.

Table 1: Data showing the impact of different treatments of heavy metals on biochemical constituents of sal

Treatment (mg/L)	*Metals*		
Chlorophyll (mg/g) fresh wt.	*Cadmium*	*As*	*Pb*
Control	0.295 ± 0.003	0.295 ± 0.003	0.300 ± 0.006
1	0.220 ± 0.006	0.230 ± 0.006	0.243 ± 0.009
5	0.167 ± 0.017	0.180 ± 0.006	0.170 ± 0.006
10	0.107 ± 0.007	0.140 ± 0.012	0.095 ± 0.003
Proline (mg/g)			
Control	0.088 ± 0.0003	0.078 ± 0.003	0.089 ± 0.004
1	0.072 ± 0.0003	0.011 ± 0.003	0.088 ± 0.003
5	0.087 ± 0.0003	0.099 ± 0.0003	0.114 ± 0.003
10	0.115 ± 0.003	0.180 ± 0.001	0.119 ± 0.003
Ascorbic acid (mg/g)			
Control	2.000 ± 0.104	1.820 ± 0.003	2.000 ± 0.104
1	2.730 ± 0.104	3.185 ± 0.055	4.190 ± 0.104
5	3.915 ± 0.159	2.365 ± 0.107	3.640 ± 0.002
10	3.368 ± 0.579	4.365 ± 0.003	4.360 ± 0.006
Total free amino acid (mg/g)			
Control	4.650 ± 0.673	5.430 ± 0.035	5.271 ± 0.001
1	3.580 ± 0.098	3.315 ± 0.001	3.245 ± 0.020
5	2.140 ± 0.017	2.140 ± 0.017	2.335 ± 0.014
10	1.630 ± 0.006	1.350 ± 0.017	1.525 ± 0.009

Interactions		*Chlorophyll*	*Proline*	*Ascorbic acid*	*Amino acid*
Metals	*Cadmium*	0.0080	0.0029	0.2580	-
	at 5% significance	*	***	***	NS
Treatments	*Cadmium*	0.0092	0.0034	0.2979	0.3146
	at 5% significance	***	***	***	***
Metals* Treatments	*Cadmium*	0.0160	0.0059	0.5160	-
	at 5% significance	*	**	**	NS

Values are mean of three replicates. ± standard error. ***, **, * Significant at the 0.001, 0.01 and 0.05 probability level respectively, NS = non significant

The impact of individual metal on different parameters of sal (*Shorea robusta*) discussed in the following sections.

Cadmium

Figure 1 summarizes the results of percentage variation in total chlorophyll, proline, ascorbic acid and amino acid content of the leaves of seedling after cadmium treatment. The data shows that total chlorophyll content of leaves decreased significantly with increased concentration of doses by 25.4%, 43.5% and 63.8%, respectively (P < 0.05) (Fig. 1a) and amino acid decrease by 23%, 54% and 64.9%. While ascorbic acid increased by 36.5%, and 95.7% and 68.4% in 1, 5 and 10ppm respectively in the leaves of seedlings treated with different concentration of cadmium, Proline content decreased at 1 mg/l (18%) and 5mg/l (1.13%) treatment while increased by treating with 10mg/l at 30.6%.

Lead

It was observed that 1mg/l Pb treatment caused a significant reduction of 19% in total chlorophyll content and 43.3% and 68% reduction at 5 and 10mg/l treatment respectively (Fig. 1a) and amino acid content (34.4 -71.1%) (Fig. 1b). Exposure of seedlings to lead doses resulted increase in ascorbic acid and proline content of the leaves (Fig. 1c and 1d). In leaf treated with concentrations of 1, 5 and 10 mg/l lead doses, exhibit percent enhancement of ascorbic acid 109%, 82% and 118% respectively as compared to control values. It was observed that after Pb treatment exhibit reduction of 1.12% at 1mg/l in proline content while enhancement at 5mg/l and 10mg/l (28.1% and 25.2%) enhancement was observed in proline content as compared with control.

Arsenic

As observed, at 1, 5 and 10mg/l As_2O_3 concentrations, total chlorophyll content in leaves decreased significantly by 22%, 39% and 52%, respectively (P< 0.05) (Fig. 1a). Amino acid content was also decreased by 39%, 60.6% and 75.1% at 1, 5 and 10mg/l as compared to control values. For proline it was observed that, at 1mg/l treatment exhibit reduction (85.8%) while at 5 and 10mg/l treatments, proline content was increased by 26.9% and 130.7% respectively. For 1mg/l treatment ascorbic acid content increased by 75%, at 5mg/l treatment concentration it was 29.9% and 10mg/l ascorbic acid increased by 139.8% as compared to control values.

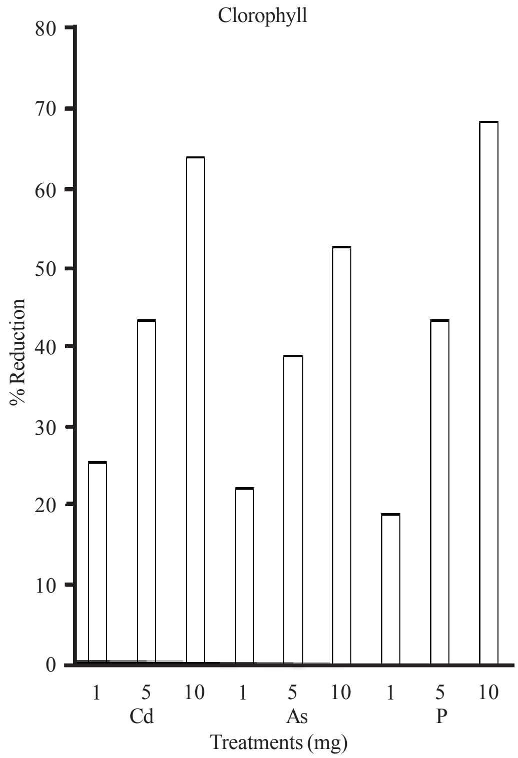

Fig. 1a.

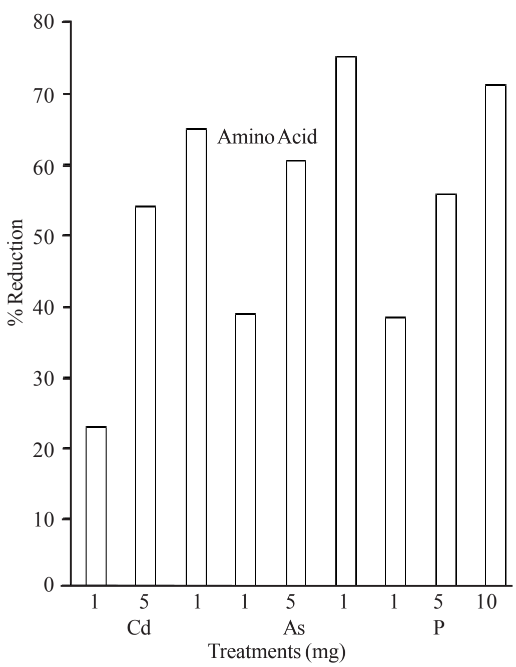

Fig. 1b.

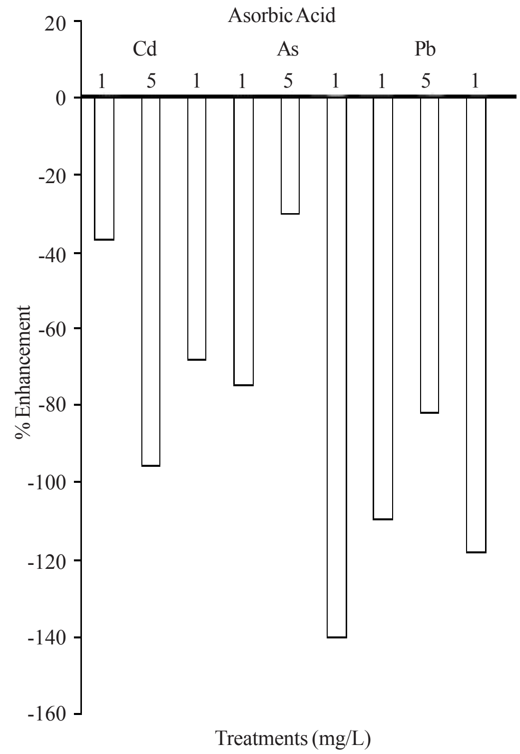

Fig. 1c.

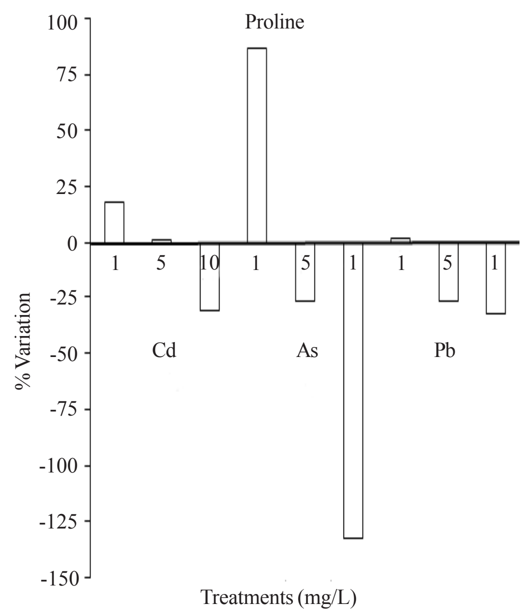

Fig. 1d.

Fig. 1 Percent variation in diferent parameters of *S. robusta*

DISCUSSION

In the present study, exposure to heavy metals affected different parameters of sal viz. total chlorophyll content, proline content, ascorbic acid content and amino acid content. Exposure of six month old sal seedlings to cadmium, arsenic and lead decreased the total chlorophyll, amino acid and increased the proline and ascorbic acid slightly. Increased cadmium, arsenic and lead concentrations significantly enhanced proline accumulation and ascorbic acid in sal seedlings.

Chlorophyll content is often measured in plants in order to assess the impact of environmental stress, as changes in pigment content are linked to visual symptoms of plant illness and photosynthetic activity (Parekh 1990). Researchers have reported decreased chlorophyll in several different plant species under the impact of heavy metals. In two wheat varieties to which cadmium and Pb were applied, total chlorophyll decreased 50% (*Triticum aestivum* cv. Gerek 79) and 70% (Bolal 2973; Oncel et al. 2000). Heavy metals inhibit metabolic processes by inhibiting the action of enzymes, and this may be the most important cause of inhibition. Decreased chlorophyll content associated with heavy metal stress may be the result of inhibition of the enzymes responsible for chlorophyll biosynthesis. Cadmium was reported to affect chlorophyll biosynthesis and inhibit proto-chlorophyll reductase and aminolevulinic acid (ALA) synthesis by Stobart et al. (1985).

Free radical generation is one the initial responses of plants to stress. Generation of free radicals and reactive oxygen species is stimulated in the presence of metals (Halliwell and Gutteridge 1993), and this can seriously disrupt normal metabolism through oxidative damage to cellular components. To mitigate and repair the damage initiated by active oxygen, plants have developed a complex antioxidant system. These antioxidants play an important role in the cellular defence strategy against oxidative stress, inducing resistance to metals by protecting labile macromolecule reported by Zhang and Kirkham (1994) and Galli et al. (1996). It is widely accepted that detoxification of metal ions within plant tissues usually depends on chelation by appropriate ligands. Antioxidants like systeine, proline, ascorbic acid and non-protein thiol (sulfhydryl) play an important role in detoxification of toxic metal ions (Singh and Sinha 2005). Ascorbate is a ubiquitous soluble antioxidant in photosynthetic organisms, and the most important reducing substrate for H_2O_2 detoxification (Singh et al. 2005). It has been suggested that pollutants produce oxy-radicals in plants by Sakaki et al. (1983). These radicals cause widespread damage to membranes and associated molecules, including chlorophyll pigments (Sakaki et al. 1983). Several authors have reported that ascorbic acid can serve as a free radical scavenger against O_3 (van Hove and others 2001; El-Khatib 2003). Ascorbate occurs in the cell wall, where it is a first-line defense against ozone. Cell wall ascorbate and cell wall-localized ascorbate oxidase (AO) have been implicated in control of growth. High AO activity is associated with rapidly expanding cells and a model linking wall ascorbate and ascorbate oxidase to cell wall extensibility has been presented (Smirnoff 1996). Ascorbate has also been implicated in regulation of cell division, through its influence on regulation of cell division, through its influence on progression from the G1 to S phases of the cell cycle

In our study it was determined that all two metals and metalloid (As++, Pb++, Cadmium++) caused a significant increase in proline and ascorbic acid levels. Exposure of *Albizia procera* to various concentrations of nickel, chromium and mercury caused increase in ascorbic acid and proline levels (Tripathi and Tripathi 1998). Our results for decrease in chlorophyll content are corroborated with the findings of Siedlecka and Krupa (1996) who have also found a decrease in chlorophyll content with heavy metal stress in *Zea mays* and *Acer rubrum*. Amino acids play a pivotal role in plant primary metabolism. Being early products of photosynthesis and nitrogen assimilation, they represent an important link between nitrogen and carbon metabolism. Since their biosynthesis is connected to processes that are dependent on environmental factors, the latter influence the variability of the pool of free amino acid (Durzan and Steward 1983). Plants have shown proline accumulation under environmental stress (Ahmad and Jhon 2005; Ahmad et al. 2006; Ahmad et al. 2008).

For over 40 years, plant physiologists have studied the accumulation of free proline in a number of species subjected to hyperosmotic stress. Accumulation of free proline in response to heavy metal accumulation of free proline in response to heavy metal exposure seems widespread among plants (Costa and Morel 1994). Exposure seems widespread among plants (Costa and Morel 1994). Exposure to heavy metals, especially cadmium (Barcelo and others 1986), is known to disturb the plant water balance. Proline accumulation in plants under cadmium stress is induced by a cadmium-imposed decrease of the plant water potential, and the functional significance of this accumulation would lie in its contribution to water balance maintenance; proline-mediated alleviation of water deficit stress could substantially contribute to cadmium tolerance (Costa and Morel 1994). Proline increases the stress tolerance of plants through such mechanisms as osmo-regulation, protection of enzymes against denaturation, and stabilization of protein synthesis (Kuznetsov and Shevyakova 1997). Similar results were observed by Tripathi and Tripathi (1998 and 2006) and John et al. (2008 and 2009).

The present results showed that cadmium, arsenic and lead toxicity decreased the total chlorophyll and amino acid content of the leaves of sal seedlings. In response to heavy metal stress, the plants increased their proline and ascorbic acid content. The highest increases in proline, and ascorbic acid content and greatest reduction in total chlorophyll and amino acid were found in plants exposed to cadmium followed by the sequence > lead > arsenic.

CONCLUSION

The study reveals that biochemical indicators of air pollution which responds to air pollution in predictable way can be used for the bio-monitoring of the air pollution level of that particular region. In this study we used the biochemical parameters of *Shorea robusta* for remediation the contaminated sites. These various heavy metal treatments are used for detecting the lethal level of heavy metals and it is essential for finding hyper accumulating species and also we can say tolerant and sensitive species.

RECOMMENDATIONS

It may be concluded that there is a direct relationship between the concentration of heavy metals and morphological and biochemical responses of plants and chemical characteristics of soil. Physiological and biochemical responses of plants to heavy metal concentration can be viewed as potentially adaptive changes that decline the operation of metabolic regulatory mechanisms which favours the functioning of the plants during or after stress. Thus, these parameters can serve as indicators of heavy metal pollution. Finally, data which is generated through this study will be very helpful in detecting the lethal levels of heavy metals for particular plant species, its tolerance and remediation capacity. Tolerant species with high remediation capacities showing least adverse impact would be proposed for the phytoremediation of industrial waste disposal sites or soil affected by those particular heavy metals. This is recommended that *S. robusta* could be used for phytoremediation of arsenic because of it shows less adverse impact on plant seedlings.

REFERENCES

Ahmad P, Jhon R 2005. Effect of salt stress on growth and biochemical parameters of Pisum sativum L. *Archives of Agronomy and Soil Science,* 51: 665-672.

Ahmad P, Jhon R, Sarwat M, Umar S 2008. Responses of proline, lipid peroxidation and antioxidative enzymes in two varieties of *Pisum sativum* L. under salt stress. *International Journal of Plant Production*, 2: 353-366.

Ahmad P, Sharma S, Srivastava PS 2006. Differential physico-biochemical responses of high yielding varieties of mulberry (*Morus alba*) under alkalinity (Na2CO3) stress *in vitro*. *Physiology and Molecular Biology of* Plants, 12: 59-66.

Alia P, Saradhi PP 1991. Proline accumulation under heavy metal stress. *Journal of Plant Physiology*, 138: 554–558.

Arnon DI 1949. Copper enzymes in isolated chloroplasts. Polyphenol oxidase in *Beta vulgaris*. *Plant Physiology*, 24: 1-15.

Barcelo J, Cabot C, Poschenrieder C 1986. Cadmium induced decrease of water stress resistance in bush bean plants (*Phaseolus vulgaris* L. cv Contender), II effects of cadmium on endogenous abscisic acid levels. *Journal of Plant Physiology*, 125: 27–34.

Bates LS, Waldren RP, Teare ID 1973. Rapid determination of free proline for water stress studies. *Plant Soil,* 39: 205-207.

Charest C, Phan CT 1990. Cold acclimation of wheat (*Triticum aestivum*) properties of enzymes involved in proline metabolism. *Physiologia Plantarum,* 80: 159–168.

Costa G, Morel JL 1994. Water relations, gas exchange and amino acid content in cadmium-treated lettuce. *Plant Physiology and Biochemistry*, 32: 561–570.

Dietz KJ, Baier M, Kramer U 1999. *Free Radicals and Reactive Oxygen Species as Mediators of Heavy Metal Toxicity in Plants: From Molecules to Ecosystems*. Berlin: Springer- Verlag, pp. 73-97.

Durzan DJ, Steward FC 1983. *Nitrogen metabolism.* In: F

C Steward, R G S Bidwell (Eds.): *Plant Physiology*: *A Treatise, l.VIII.* New York: Academic Press, pp. 55-65.

El Khatib AA 2003. The response of some common Egyptian plants ozone and their use a biomonitors. *Environ-mental Pollution*, 124: 419–428.

Galli U, Schiiepp H, Brunold C 1996. Thiols in cadmium and copper-treated maize (*Zea mays* L.). *Planta,* 198: 139–143.

Hall JL 2002. Cellular mechanisms for heavy metal detoxification and tolerance. *Journal of Experimental Botany,* 53: 1–11.

Halliwell B, Gutteridge JMC 1993. *Free Radicals in Biology and Medicine.* Oxford: Clarendon.

Havaux M, Kloppstech K 2001. The protective functions of carotenoid and flavonoid pigments against excess visible radiation at chilling temperature investigated in *Arabidopsis npq* and *tt* mutants. *Planta,* 213: 953–966.

John R, Ahma PK, Gadgil K, Sharma S 2009. Heavy metal toxicity: Effect on plant growth, biochemical parameters and metal accumulation by *Brassica juncea* L. *International Journal of Plant Production,* 3(3): 65-76.

John R, Ahmad P, Gadgil1 K, Sharma S 2008. Effect of cadmium and lead on growth, biochemical parameters and uptake in *Lemna polyrrhiza* L. *Plant Soil Environment,* 54(6): 262–270.

Kuznetsov VV, Shevyakova NI 1997. Stress responses of tobacco cells to high temperature and salinity. Proline accumulation and phosphorylation of polypeptides. *Physiologia Plantarum,* 100: 320–326.

Mishra A, Choudhuri MA 1999. Monitoring or phytotoxicity of lead and mercury from germination and early seedling growth induces two rice cultivars. *Water Air and Soil Pollution,* 114: 339–346.

Moore S, Stein WH 1948. Photometric method for use in the chromatography of amino acid. *Journal of Biological Chemistry*, 176: 367-388.

Nedelkoska TV, Doran PM 2000. Characteristics of heavy metal uptake by plants species with potential for phytoremediation and phytomining. *Minerals Engineering*, 13: 549–561.

Noctor G, Foyer CH 1998. Ascorbate and glutathione: Keeping active oxygen under control. *Annual Review in Plant Physiology and Molecular Biology,* 49: 249–279.

Oncel I, Keles Y, Ustun AS 2000. Interactive effects of temperature and heavy metal stress on the growth and some biochemical compounds in wheat seedlings. *Environmental Pollution*, 107: 315–320.

Parekh D, Puranik RM, Srivastava HS 1990. Inhibition of chlorophyll biosynthesis by cadmium in greening maize leaf segments. *Biochemie Physiologie der Pflanzen*, 186: 239–242.

Sadasivam S, Balasubramanian T 1987. *Practical Manual in Biochemistry*. Tamil Nadu, Agricultural University, Coimbatore, P. 14.

Sakaki T, Kondo N, Sugahara K 1983. Breakdown of photosynthetic pigments and lipids in spinach leaves with ozone fumigation: Role of active oxygen. *Physiology Plant,* 59: 28-34.

Shafiq M, Zafar, I M , Athar M 2008. Effect of lead and cadmium on germination and seedling growth of *Leucaenaleucocephala. J Appl Sci Environ Manage*, 12(2): 61-66.

Siedlecka A, Krupa Z 1996. Interaction between cadmium and iron and its effects on photosynthetic capacity of primary leaves of *Phaseolus vulgaris*. *Plant Physiology and Biochemistry,* 34: 833-841.

Singh A, Agarwal SB, Rarhore D 2005. Amelioration of Indian urban air pollution phytotoxicity in *Beta vulgaris* L. by modifying NPK nutrients. *Environmental Pollution,* 134: 385–395.

Singh S, Sinha S 2005. Accumulation of metals and its effects in *Brassica juncea* (L.) Czern. (cv. Rohini) grown on various amendments of tannery waste. *Ecotoxicology and Environment Safety*, 62: 118–127.

Smirnoff N 1996. Botanical briefing: The function and metabolism of ascorbic acid in plants. *Annals of Botany*, 73: 661– 669.

Stobart AK, Griffiths WT, Ameen BI, Sherwood RP 1985. The effect of cadmium+2 on the biosynthesis of chlorophyll in leaves of barley. *Physiologia Plantarum*, 63: 293–298.

Tripathi AK, Tripathi S 1998. Changes in some physiological and biochemical characters in *Albizia lebbek* as bio-indicators of heavy metal toxicity. *Journal of Environmental Biology*, 20(2): 93-98.

Van Hove L, Bossen M, Son Gabino BG, Sgreva C 2001. The ability of apoplastic ascorbate to protect poplar leaves against ambient ozone content rations: a quantitative approach. *Environmental Pollution*, 114: 371–382.

Van A, Clijsters H 1990. Effect of metals on enzyme activity in plants. *Plant Cell Environment*, 13: 195–206.

Zeller S, Feller U 1999. Long-distance transport of cobalt and nickel in maturing wheat. *European Journal of Agronomy*, 10: 91–98.

Zhang J, Kirkham MB 1994. Drought stress induced changes in activities of superoxide dismutase, catalase and peroxide in wheat species. *Plant Cell Physiology,* 35: 785– 791.