Physiological and Biochemical Studies on Heavy Metal Toxicity in Selected Medicinal Plants

Thesis submitted to the
University of Calicut for the partial fulfilment of the
requirements for the Degree of

DOCTOR OF PHILOSOPHY IN BOTANY

By

ABDUSSALAM, A. K.

PLANT PHYSIOLOGY AND BIOCHEMISTRY DIVISION
DEPARTMENT OF BOTANY
UNIVERSITY OF CALICUT
KERALA-673 635
2010

CERTIFICATE

This is to certify that the thesis entitled "**Physiological and Biochemical Studies on Heavy metal Toxicity in Selected Medicinal Plants**" Submitted by **Abdussalam, A. K**. in partial fulfilment of the requirement for the Degree of **Doctor of Philosophy** in Botany, University of Calicut, is a bonafied record of the research work undertaken by him in this Department under my supervision during the period 2006-2010 and that no part thereof has been presented before, for the award of any degree or diploma.

Dr. Nabeesa Salim

DECLARATION

I hereby declare that the thesis entitled "**Physiological and Biochemical Studies on Heavy Metal Toxicity in Selected Medicinal Plants**" submitted by me in partial fulfilment of the requirements for the Degree of **Doctor of Philosophy in Botany**, **University of Calicut** is the bonafied work carried out by me and no part of the work has formed the basis for the award of any other degree or diploma.

C. U. Campus **Abdussalam, A. K**

ACKNOWLEDGEMENT

*I wish to express my deep sense of gratitude to my supervising teacher **Dr. Nabeesa Salim**, Professor and former Head of the Botany Department, University of Calicut, for her valuable guidance and ceaseless motivation throughout my research work that helped me to complete the work in stipulated time. It is she who fired my vision and who catalyzed my ambition. She is the one who drew me to the broad horizon of plant physiological research.*

*I record my deep sense of gratitude to **Prof. K. V. Mohanan**, Head of the department of Botany, University of Calicut, for providing all the necessary facilities to carry out my work. I wish to take this opportunity to acknowledge our beloved former HODs, **Prof. S. Nandakumar**, **Prof. P. V. Madusoodhanan and Prof. M. Sivadasan** in this auspicious juncture for their assistance.*

*No statements would be adequate to articulate my deep sense of gratefulness to **Dr. K. P. Geneshan**, Technical Assistant, Dept. of Botany, University of Calicut, who was instrumental in my coming to the university for research.*

*I specially recall here the names of my beloved teachers, **Dr.K. M. Jayaram, Dr. Jos T. Puthur, Prof. M. Sabu, Dr. John E. Thoppil, Prof. P. Manimohan, Dr. Sunoj Kumar** and **Dr. Harilal** for their timely guidance and advice during the work. I would like to express my sincere thanks to **Dr. A. K. Pradeep**, Herbarium Curator, Department of Botany, University of Calicut for the identification of taxa that I have selected for the research work. I also extend my gratitude to all the non-teaching staff in the Dept. of Botany particularly, **Mr. Zakariya, Mr. Syed Ali, Mr. Ram Bahadhur Tapa, Mrs. Prasanna, Mr. Muhammed Shafi** and **Mr. Vijyan** for their help. **Mrs. Geeth Nair**, **Mrs. Joslyn, Mr. Prakashan**, Librarians, Department of Botany, University of Calicut, who were really helpful in locating and issuing books and reference materials for my thesis. I wish to acknowledge here their sincere support.*

*I would like to express my sincere thanks to **Prof. Ismail Cakmak**, Faculty of Engineering and Natural Sciences, Sabanci University, Istanbul Turkey and **Prof. Fabio N. Moreno**, Soil and Earth Sciences, Massey University, New Zealand for providing literature and protocols.*

*At this point, I specifically would like to mention about **Mr. Ratheesh Chandra, P**., Research scholar, Physiology and Biochemistry division with whom I had studied for B. Sc., M. Phil. and Ph. D. courses, from the bottom*

of my heart I would like to thank for his wholehearted co-operation, motivation, concern and the helps rendered by him. I am also thankful to all my M. Phil. Classmates, ***Mr. Thomas, V. P., Mr. Sreenivas, V. K., Mr. Babu E. A., Mr. Sanoj, E., Mrs. Dolly mol, Mrs. Jisha, Mrs. Jeeja Mathew*** *and my friends* ***Mr. Rajesh Kumar, Mr. Shameer, Mr. Prabu, Miss. Vidya Varma, Miss. Asha, Mrs. Seema, Miss. Shakira and Miss. Viji and Mr. Sudeep*** *in the department especially for their support, help and love and also from other departments and to my well wishers for their direct and indirect support.*

. I record special gratitude to ***Cashew Promotion Council*** *for proving the Heavy Metal Analysis facilities. I express my special thanks to* ***Dr. K. Azees*** *and* ***Dr. Hussain****, Lecturers, Unitiy Womens College, Manjeri,* ***Dr. Venugopalakrishna Kurup****, N. S. S. College, Ottapalam,* ***Dr. S. Anilkumar****, S. N. College, Chembazhanthi, Trivandrum and* ***Mr. Habeeb Rahman****, U., Palakkad for their love, care and support. I would like to express my deep sense of gratitude to all of my friends in Calicut University Campus particularly,* ***Mr. Sajnesh E. V****,* ***Mrs. K. P. Geetha, Mr. Sukul P. B., Mr. K. Ravi, Mr. Shibi C., Mr. Sameer Kavad, Mr. Lineesh K****. and* ***Mr. Rovith*** *for their inspiration and affection.*

I express my sincere gratitude to ***Adv. P. A. Salim*** *for his encouragement and love throughout the period of work. I am indebted to* ***Dr. Sheela Sathyan****, Lecturer, Govt. College, Madappally for her valuable suggestions. I express my special thanks to all the members of* ***Eranjithodiyil family****, Liberty street, Pattambi for their love, concern, support and friendliness. My profound sense of gratitude is also extending to* ***Mrs. Jyothi****, P. V. Lecturer, M. E. S. Ponnani College,* ***Dr. Smitha****,* ***R. B.*** *Trivandrum,* ***Mr, Sai Dev Sidharth****, Pondichery University, Andaman Island and* ***Dr. Vijayalakshmi****, for their support, love and care throughout my study.*

I would like to take this occasion to thank two privileged gentlemen, ***Dr. Mohammed Aslam****, Reader, Department of Earth Science, Pondicherry University, and* ***Mr. Zameel Ahammed****, 'Sreyas', Anangore, Kasaragod for their sheer enthusiastic and moral pumping remarks. It is worth placing the record of sincere thanks to* ***Mr Rafeek,*** *Kuwait University,* ***Mr. Binu Ramachandran****, German University,* ***Mr. Ajeesh****, Germany* ***Mr. Shibin Mohanan****, CFTRI, Mysore,* ***Dr. Ramasree, Mrs. Sareena*** *and* ***Mr. Satheesh,*** *CMPR, Kottakkal for their contributions in the research path.*

I could not have completed this work without the invaluable guidance of ***Adv. Abdul Kadar****, 'Sreyas', Anangore, Kasaragod,* ***Mr. Ashraf, K. H.*** *and*

***Ahammed Rafi B. Fery**, Wisdom Institute, UAE for their genuine caring, concern, and relationship which supported me to finish the work.*

*It would be inappropriate without remembering the constant and regular support and motivations rendered by **Mr. P. T. Assainkutty**, Koduvally, Calicut as he was one of the key-person in my life and career. He will be ever-remembered as my path-finder since my childhood. The affectionately guidance, support, inspiration and love showered by all of my family members, including my **Brothers**, **Sisters**, **Nieces** and **Nephews**. My **Mother** is a constant source of support on my work. A formal word will not reveal my indebtedness to them at all.*

*I will be failing in my duties, if I do not record my sincere thanks to FIP Teachers, especially, **Dr. Abis . V. Cherussery, Dr. Kochuthresiamma Andrews, Dr, Radha, P. G. Dr. Beena Anto** and also to FIP scholars in the Physiology and Biochemistry Lab, especially **Mrs. Viji**, and **Mrs. Swapna** for their various help. I am grateful to **Mr. Ajayakumar**, **Mr. Shaji**, **Mr. Santhosh** and **Mr. Azees**, Art and Photography Department for their assistance for the photographic work. A big applause also goes to Mr. **Rajesh** and his crew, **Bina Photostat** for their fantastic DTP job, which is indeed very appreciable. I express my special thanks to Mr. **Pauli Thomas**, Chemind Chemicals, Calicut for the timely supply of chemicals and glassware.*

*I express my sincere gratitude to the teachers of Botany Department, M. E. S. Asmabi College and also express special thanks to **Dr. N. Anil Kumar**, Director, **Mr. G. Girijan**, **Dr. Ratheesh Naryanan**, **Mrs.Swapna** M.S.S.R. F. Kalpetta and **Dr. Geetha Rani,** Scientist,Gene Bank, M. S.S.R.F. Chennai for their assorted helps during my career.*

*I express my loving acknowledgment and heartfelt gratitude to **Zahira**, my better half. She shared with me all the emotional dynamics of my study patiently and understandingly and tolerated my absence at home. Her constant encouragement and love made my whole work tedious- free and comfortable. Last but certainly not the least, I am really very much thankful to the **Almighty** for His grace which gave me the positive energy and determined will power in completing my work without any problem.*

Abdussalam, A. K.

CONTENTS

INTRODUCTION

The cycling of heavy metals in soil~~s~~ and ecosystem has received a great deal of attention in recent decades due to the increased release of metals that occurs during anthropogenic activities such as ~~metals~~ smelting, refineries and combustion of fossil fuels. The accumulation of heavy metals in soil is becoming a serious problem as a result of industrial and agricultural practices and mining waste. Vegetation is usually the first interceptor of heavy metals deposited to an ecosystem. There has been an increasing concern with regard to the accumulation of toxic heavy metals in the environment and their impact on both public health and the natural environment. ~~The accumulation of heavy metals in soil is becoming a serious problem as a result of industrial and agricultural practices and mining waste.~~

Plants growing in metal enriched soils, take~~s~~ up metals to varying degrees in response to external and internal factors. Considerable interest has been focussed on pattern of metal uptake and accumulation because experimental studies of heavy metal tolerance show subtle differences ~~exists~~ in up take characteristics by tolerant and ~~no~~in tolerant genotypes. There is vast literature of analytical data relating to metal up take, illustrating the scale of differences between species and genotypes and between metals in the field and laboratory studies ranging from trace nutrient elements to toxic heavy metals (Foy *et al*., 1978; Lepp, 1981; Fitter and Hay, 1983; Boro~~u~~vik, 1990; Frie~~n~~dland, 1990; Cseh, 2002).

Heavy metals like cCadmiumd, Crchromium, Hgmercury, Pblead etc. are having no beneficial properties for the plant growth and are highly reactive and consequently are toxic to plants. Cadmium is a natural element and is released to the environment during mining, and weathering, industrial process and agriculture. tThe element is released to the environment. Cadmium is a metal pollutant which enters the environment mainly from industrial process and phosphate fertilizers, as well as from the exhaust gases of automobiles (Foy *et al*, 1978; Wagner, 1993). Chromium occurs naturally in the environment and also produced by industrial process like manufacturing of dyes and paints, chromium plating, leather tannery etc. The impact of chromium contamination in the physiology of plants depends on the metal speciation, which is responsible of its mobilization, subsequent up take and resultant toxicity in the plant system (Shanker *et al*., 2005). Mercury (Hg) is a global environmental pollutant that is present in soil, water, air and biota. The naturally occurring Hg^{2+} can be released into the atmosphere and then exchanged between the soil and water systems by processes such as wind erosion, degassingdegassing from Hg mineralized soil and rock formations, volcanic eruptions and other geothermal activities (Ebinghaus *et al*., 1999). Mercury shows a tendency to combine with other elements get methylated in soil and water by bacteria and highly toxic to plant at very low concentration levels. Lead is a naturally occurring element in the earth crust. Bbut increases ed in the environment by anthropogenic activities. The toxic effects of lead have been known for centuries. Many useful properties give rise to a dramatic escalation of lead use around the time of the industrial revolution, as lead poising was

common amongst workers in the smelting, painting, plumbing, printing and other industries (Landrigan, 1999).

~~Cadmium has been investigated to elucidate phytotoxicity, accumulation, metabolism and phytoremediation (Kim *et al*., 2002, Linger *et al*., 2007). Cadmium contamination in soil has became a global concern as Cd^{2+} is not only absorbed by plants but is easily transferred to food chain (Liu *et al*., 2009).~~

General symptoms of cadmium toxicity in plants are growth inhibition, low biomass production, impaired water relations, respiration, photosynthesis and nitrogen metabolism (Seregin and Ivanov, 2001; Perfus-Barbeoch *et al.*, 2002; Linger *et al.*, 2002). Cadmium induces oxidative stress by generating free radicals which are highly reactive with metabolites (Hendry *et al.*, 1992; Ederli *et al.*, 2004). Absorption and translocation of cadmium by plants occur through transporters and during prolonged growth, Cd^{2+} ions cause growth retardation (Cohen *et al.*, 1998; Pance *et al.*, 2000). Synthesis of cadmium- induced sulfhydryl-rich peptides has been described as effective chelators for detoxification or sequestration (Meuwly *et al.*, 1995; Zenk *et al*., ~~1996~~, 1996). According to Sanita- di-Toppi (2002) cadmium has been the metal of choice for most phytochelatin-metal binding studies and Cd^{2+} ions are probably the most powerful inducers of phytochelatin synthesis. Cadmium exerts inhibitory effects in plant cells by binding to specific groups of proteins thereby inhibiting its normal function especially channel proteins of membranes (Cseh, 2002). Cadmium has been investigated to elucidate phytotoxicity, accumulation, metabolism and

phytoremediation (Kim *et al*., 2002; Liu *et al*., 2003). Cadmium contamination in soil has became a global concern as Cd^{2+} is not only absorbed by plants but is easily transferred to food chain also (Liu *et al*., 2009). ~~Synthesis of cadmium- induced sulfhydryl rich peptides has been described as effective chelators for detoxification or sequestration (Meuwly *et al*., 1995; Zenk *et al*., 1996). According to Sanita di-Toppi, (2002) cadmium has been the metal of choice for most phytochelatin-metal binding studies and Cd^{2+} ions are probably the most powerful inducers of phytochelatin synthesis.~~

~~–~Chromium toxicity in plants is observed at multiple levels such as reduced yield, inhibited growth of leaves and roots, inhibition on metabolism, enzymatic activities and mutagenesis (Clijsters and Van Assche, 1985; Bishnoi *et al*., 1993; Shanker *et al*., 2005), . ~~W~~water relations (Vazques *et al*., 1987) and mineral metabolism (Adriano, 1986). Toxic effects of chromium have been reported in seed germination (Rout *et al*., 2000; Peralta *et al*., 2001) ~~enzymatic activities~~, translocation of sugars (Zeid, 2001), growth and development (Rout *et al*., 1997; Iqbal *et al*., 2001). ~~,~~ Induction and activation of free radical-scavenging enzymes like s~~S~~uperoxide dismutase and ~~antioxidant catalase~~peroxidase have been reported as one of the detoxification mechanisms of chromium toxicity in plants (Prasad, 1998; Shanker *et al*., 2004, 2005; Panda and Choudhury, 2005).

~~Mercury and its compounds are persistent, bioaccumulative and toxic, and there pose a risk to both humans and the ecosystem.~~ Even trace quantity of mercury can have detrimental effects on plant growth and development (Woolhouse, 1983; Lenka *et al*., 1993). The mode of action of this toxic metal~~s~~ includes membrane distortion (Ouar~~r~~iti *et al*., 1997; Nag *et al*., 1980), reaction with thiol groups of metabolites (Meuwly *et al*., 1995), site competition with metabolites (Perfus-Barbeoch *et al*., 2002), interactions with other elements (Orcutt and Nilsen, 2000) and synthesis of reactive free radicals (Stohs and Bagchi, 1995). Eventhough mercury is highly toxic to plants, the physiological effects and toxicity- induced metabolic changes are not well documented. However, the major impact of mercury on plant growth such as uptake and distribution (Beauford *et al*., 1977; Velasco-Alinsug *et al*., 2005), tolerance mechanism (Lenka *et al*., 1993; Ahmed and Tajmir-Riahi, 1993), photosynthesis (Bernier *et al*., 1993), chlorophyll synthesis (Jain and Puranik, 1993; Prasad *et al*., ~~;~~ 1991; Shaw, 1995), sensitivity and accumulation (Orcutt and Nilsen, 2000) have been studied in various plants. Phytoremediation capacity of *Brassica juncea* to remove mercury has~~ve~~ been reported by Moreno *et al*. ~~;(~~2005a, 2008).

~~Lead is a highly toxic element for plants and it has been reported to inhibit photosynthesis and respiration by affecting electron transport mechanism (Orcutt and Nilsen, 2000).~~ Toxicity and contamination of lead that occur in plants may be a function of chemical form of the metal and age/or physiological condition of the plants (Driscoll *et al*., 1994; Mishra and Choudhuri, 1999). Lead is a highly toxic element for plants and it has been reported to inhibit photosynthesis and respiration by

affecting electron transport mechanism (Orcutt and Nilsen, 2000). Effect of lead on the physiological aspects such as photosynthesis ~~(Bazzaz *et al*., 1975; Carlson *et al*., 1975), chlorophyll content (Yordanov and Merakchiisk, 1976;~~ (Stobart *et al*., 1985; Gasic *et al*., 1992; ~~Pandey, 1996~~Mohan and Hosetti, 1997; Fodor, 2002), translocation (Tomsig and Suszkiw, 1991), rRapid root growth inhibition (Gzyl *et al*., 1997; M~~e~~alkowski ~~thoworki,~~ *et al*., 2002), ~~.~~ cytological aspects (Eun *et al*., 2000) and bioaccumulation (Arazi *et al*., 1999; Fodor, 2002; Kim *et al*., 2002; Axtell *et al*., 2003) have been investigated in many plants.

Medicinal plants are the raw materials for many herbal formulations of pharmaceuticals and neutracenticals. The use of medicinal plants in therapeutics goes back to beyond record history, but it has increased during the last decades (Woods, 1999; WHO, 2002). High level of toxic metals occur in medicinal preparation when the plant are growing/cultivated in the fields polluted with heavy metals. A number of medicinal plants are herbaceous and grow wildly and many of them may be hyperaccumulators of toxic heavy metals. If medicinal plants are growing in polluted soil, the contaminants get accumulated in the plant body and reach the final marketable medicines leading to health hazard. Herbal medicinal products have been reported to contain toxic heavy metals such as lead, mercury, cadmium, arsenic etc. (Aslam *et al*., 1979; Saper *et al*., 2004; Ernst, 2005). Studies on the prevalence and concentrations of heavy metals in Ayurvedic medicines have shown that many of the medicines produced from South Asia contains potentially harmful level of lead, mercury and or/ arsenic (Saper *et al*., 2004). According to those authors, users of these

medicines may be at risk for heavy metal toxicity and testing of Ayurvedic medicines for toxic metals should be mandatory.

The aim of the study undertaken was the elucidation of the effect of cadmium, chromium, mercury and lead on the metabolism of medicinal plants. The investigation was planned along two distinct but complimentary lines one: determination of the physiological interference of these heavy metals in the distribution of metabolites in selected medicinal plants and bioaccumulation potential of the same towards Cd, Cr, Hg and Pb. The results of these heavy metal treatments in *Boerhavia diffusa* L. a highly medicinal, profusely growing well adapted herb- delineated the physiological role in considerable detail and provided clues to their metabolic activities. So the second line of investigation - bioaccumulation potential - was carried out with twenty selected medicinal plants inclusive of *B. diffusa*.

B~~oerhaavia~~oerhavia diffusa ~~L.~~ (~~Spreading~~ Common name-Hogweed ~~in English~~) belonging to the family of Nyctaginaceae, is a diffused perennial herbaceous ~~creeping weed.~~medicinal plant growing prostrate or ascending upward in habitats like grasslands, agricultural fields, fallow lands, wastelands and residential compounds. (Known also under its traditional name as 'Punarnava' in sans~~c~~krit and -" Chuvanna thazhuthama" in malayalam). The plant was named in honour of Herman Boerhaave, a famous Dutch Physcian of the 18th Century (Chopra, 1969~~). *B. diffusa* is a medicinal plant species growing prostrate or ascending upward in habitats like grasslands, agricultural fields, fallow lands, wastelands and residential compounds.~~).

Boerhavia diffusa plant has a long history of uses in Ayurvedic or natural herbal medicines (Dhar *et al*., 1968). The major active principle present in the root is alkaloidal and is known as 'punarvavine'. The medicinal value of this plant in the treatment of a large number of human ailments is mentioned in Ayurveda, 'Charaka Samhita', and 'Sushruta Samhita'.

About 45 Ayurvedic preparations inclusive of 'Dhanvantaratistam', 'Chyavanaparasam,' 'Ashokarishtam,', 'Punarnavasavam', 'Rasanadikasayam', 'Narvasimharasayam' etc. contain the roots, leave or entire plant of *B. diffusa* (Sivarajan and Balachandran, 1994). The roots, leaves, or the whole plant of *B. diffusa* have been employed for the treatment of various disorders in the Ayurvedic herbal medicine in India, Nepal, Sri Lanka and China. The root is mainly used to treat gonorrhoea, internal inflammation of all kinds, dyspepsia, odema, jaundice, menstrual disorders, anaemia, liver, gallbladder and kidney disorders, enlargement of spleen, abdominal pain etc. (Kirtikar and Basu, 1956). It was also demonstrated that the drug decreased the albumin urea, increased the serum protein and lowered serum cholesterol level (Ramabhimaiah *et al*., 1984) . Singh and Udupa (1972) reported that the dried root powder showed curative efficiency for the treatment of helminth infection.

The purified glycoproteins from *B. diffusa* exhibited strong antimicrobial activity (Aswasthithi and Menzel, 1986; Aswasthiathi and Rizevi, 1998, 1999) and hHepatoprotective activity (Chakraborti and Handa, 1989; Rawat, 19897; Chandan *et al*., 1991). Further experimental studies also evidenced a beneficial activity of the 'Punarnava' root for the treatment of the jaundice (Singh and Pandey, 1980; Gopal

and Shah, 1985). ~~It~~According to Hiruma –Lima (2000) the ~~was evidenced that the~~ leaves and root possessed antifibrinolitic and anti-inflamatory activities ~~(Hiruma – Lima, 2000)~~. In a study, ~~lead by~~ Mehrotra *et al*. (2002) reported that the ethanolic extract of *B. diffusa* showed a significant immunosuppressive activity on human cells and on murine cells as well. The recent study carried out by Pari and Satheesh ~~*et al*.,~~ ~~(~~(2004) demonstrated that the leaves of *B. diffusa* reduce the levels of glucose in the blood increasing the insulin release from the β cells of pancreas. Antiviral activity of *B. diffusa* have been demonstrated in many vegetable crops (A~~s~~wasa~~a~~thi and Kumar, 2003; A~~s~~wa~~s~~thi *et al*., 2003). Different solvent extract of *B. diffusa* have been proved to contain antidiabetic, antioxidant, anticancer, analgesic, hepatoprotective antiviral and anti fungal activities ~~y~~(Sahu *et al*., 2008).

~~Medicinal plants are the raw materials for many herbal formulations of pharmaceuticals and neutracenticals. The use of medicinal plants in therapeutic goes back to beyond record history. But has increased during the last decades (Woods, 1999, WHO, 2002). High level of toxic metals occur in medicinal preparation when the plant are growing/cultivated in the field polluted with heavy metals.~~

~~A number of medicinal plants are herbaceous and grow wildly and many of these may be hyperaccumulator of toxic heavy metals. If medicinal plants growing in polluted soil, the contaminants reach the final marketable medicines leading to health hazard. Herbal medicinal products have been reported to contain toxic heavy metals such as lead, mercury, cadmium, arsenic etc. Studies on the prevalence and concentrations of heavy metals in Ayurvedic medicines have shown that many of the medicines produced from South Asia contains potentially harmful level of lead,~~

~~mercury and or/ arsenic (Saper *et al*., 2004). According to those authors, users of these medicines may be at risk for heavy metal toxicity and testing of Ayurvedic medicines for toxic metals should be mandatory.~~

As described ~~above~~earlier, *B. diffusa* is a medicinal plant widely used as an important ~~increadiant~~ingredient of many Ayurvedic preparations. These plants grow profusely as wild plants and are well adapted to polluted areas such as road side, railway track, banks of drainage, ~~-~~vicinities of public comfort station etc. By trial and error experiments the present author observed that *B. diffusa* plants grow ~~profusely~~well in Hoagland nutrient medium under hydroponic system. ~~.~~ So simulated experiments were set up to analyse the responses of *B. diffusa* by cultivating ~~the~~ rooted ~~—~~propagules in Hoagland solution artificially contaminated with known quantities of ~~—~~Cadmium chloride ($CdCl_2$), Potassium dichromate ($K_2Cr_2O_7$) Mercuric chloride ($HgCl_2$) and Lead acetate $(CH_3\text{-}COO)_2\ Pb\ 3H_2O$.

Even ~~-~~though effect of Cd, Cr, Hg and Pb have been investigated in a number of plants, effect of these heavy metals on medicinal plants in general and *B. diffusa* in particular have not yet been elucidated. Similarly, investigations on the effect ~~of~~ ~~different~~of ~~heavy~~different heavy metals in varying ~~concentrations in~~concentrations in one and the same plant are very scanty. So the objectives ~~-~~of the present study ~~includes .~~ includes ~~:~~ (1) Treatment of *B. diffusa* plant with four heavy metals ~~is proposed~~ to assess the difference in response and tolerance of the plant towards different concentrations of these metals and a comparison of physiological parameters and bioaccumulation potential. (2) Standardisation of different concentrations of Cd, Cr, Hg and Pb on *B. diffusa* plant to impart more or less

similar visible morphological symptoms. (3) Growth retardation pattern by analysing ~~of~~ growth performances ~~for~~ during a period of 20 days in terms of root and shoot length, ~~and~~ leaf area, biomass distribution and tolerance index. (4) Anatomical study to pin point the cellular damage in root and stem occurred due to heavy metal stress. (5) E~~To e~~lucidation of physiology of growth and distribution of metabolites such as proteins, chlorophylls, free aminoacids and nitrate reductase activity in plant parts ~~such as~~ root, stem and leaves ~~f~~ sampled at comparable intervals of 4 days. (6) Electrophoretic analysis of proteins in order to correlate synthesis/ degradation of proteins due to the impact of heavy metals. (7) Since plants show considerable tolerance towards varying concentrations of these metals, in ~~order to~~order to analyse tolerance mechanism~~, estimation~~, estimation of non enzymic antioxidants such as proline, malondialdehyde (MDA) and phenolics was carried out. (8) Antioxidant~~s~~ enzymes like g~~a~~uaiacol~~icol~~ peroxidase~~,~~ catalase and superoxide dismutase also were included~~.~~ to elucidate the tolerance mechanism. (9~~8~~) As the plants show~~s~~ almost similar morphological performances and the optimal concentrations of the metals are var~~y~~ied each other, ~~the~~ the accumulation of these four metals in the root, stem and ~~leaf~~leaves also was studied as another parameter. (10) In order to compare the accumulation potential and check phytoremediation capacity, distribution pattern of each metal in the plant body, residual content of the growth medium and loss, if any, were analysed.

The experimental results of the effect of Cd, Cr, Hg and Pb on *B. ~~diffusa~~ ~~cultivated~~diffusa* cultivated ~~in nutrient~~in nutrient solution (Hydroponics~~) revealed~~) ~~very~~revealed very high accumulation of ~~these metals~~these metals in root and shoot.

But the results ~~of hydroponic~~of hydroponic studies cannot be extrapolated to field condition as these are carried out in nutrient culture and confined ~~to a~~to a short duration (20 days). ~~The accumulation~~The accumulation and effect of contaminants in the natural ecosystem remain for a long duration with ~~many interaction~~many interactions. So another experimental study was conducted to assess the accumulation potential of *B. diffusa* towards Cd, Cr, Hg and Pb by ~~*B. diffusa* growing in~~growing in soil (Pot culture~~s~~) artificially contaminated with known quantities of these metals. For comparative study~~, twenty~~, twenty common medicinal plants which are growing wildly in Calicut University campus and Calicut U~~u~~niversity Botanical Garden also were used for pot culture experiments. This approach ~~is embarked~~is embarked on the ~~to~~ assessment of ~~the~~ bioaccumulation potential of these medicinal plants and the possibility/ gravity of heavy metal con~~centration~~tamination in the medicinal products prepared by ~~preparations~~ using the plants cultivated in soils polluted with cadmium, chromium, mercury and lead. ~~heavy metals.~~

REVIEW OF LITERATURE

Heavy metals make significant contribution to environmental pollution as a result of anthropogenic activities such as mining, energy- and fuel production, power transmission, intensive agricultural practices, sludge and industrial effluent dumping and military operations (Foy *et al*., 1978; Salt *et al*., 1998; Orcutt and Nilsen, 2000; Cseh, 2002; Pilon- Smits, 2005).

Metals such as aluminium, arsenic, cadmium, cobalt, chromium, copper, lead, manganese, mercury, nickel, selenium and zinc have been considered as the major environmental pollutants and their phytotoxicity has already been established (Ross, 1994; Kochian, 1995; Orcutt and Nilsen, 2000; Cseh, 2002; Fodor, 2002). A common characteristic feature of heavy metals in general regardless of whether they are biologically essential or not is that they may exert toxic effects to plants in low concentrations compared to macro nutrients and these metals are considered to be cytotoxic and mutagenic although a few of them are essential for metabolic processes (Alloway, 1990; Shaw,1995; Hadjiliadis, 1997; Salt *et al*., 1998; Orcutt and Nilsen, 2000; Kabata-Pendias and Pendias, 2001, Fodor, 2002).

Availability and toxicity of heavy metals in the environment, responses and adaptive strategies of plants to metal toxicity and phytoremediation technology etc. have been extensively discussed and excellently reviewed by

several authors (Foy *et al.*,1978; Basberg-Pahlsson, 1989; Rauser, 1990; Steffens, 1990; Mc Neilly, 1994; Prasad, 1997; Salt *et al*., 1998; Bhoomik and Sharma, 1999; Chang *et al*., 2000; Orcutt and Nilsen, 2000; Cseh, 2002; Fodor, 2002; Zimmels *et al*., 2004; Pilon–Smits, 2005; Migocka and Klobus, 2007).

Elevated concentrations of both essential and non-essential heavy metals in the soil and water can lead to toxicity symptoms and growth inhibition in most plants (Grant *et al.*, 1998; Jindal and Kaur, 2000; Hall, 2002). Absorption, translocation and accumulation of heavy metal ions of Hg, Pb, Cr, and Cd by plants, reduce qualitative and quantitative productivity of the species and cause serious health hazards through the food chain to other life forms (Turner, 1994; Barman *et al.*, 1999; Petr *et al.*, 1999; Moreno-Caselles *et al.*, 2000; Axtell *et al*., 2003; Cobbett, 2003; Stolt *et al*., 2006).

According to Wilkins (1978), Lata (1989), Hagemayor (1993), Prasad (1997), Linger *et al*. (2005) and Panda (2007) metal toxicity reduces vigour and growth of plants, causes death in extreme cases interferes with photosynthesis, respiration, water relation, reproduction etc. and causes changes in certain organelles, disruption of membrane structure and functions of different plant species. Levitt (1980) suggested that heavy metals in the plant environment operate as stress factors and they cause physiological

changes and in the process they can reduce vigour, or in the extremes totally inhibits plant growth. Different heavy metals at supra-optimal concentrations, have been shown to inhibit various metabolic process in plants resulting in their reduced growth and development (Bala and Setia, 1990; Davies *et al*., 1991; Bernier *et al.*,1993; Lang *et al.,* 1995; Shaw, 1995; Tomar *et al.,* 2000). At cellular and molecular level, metal toxicity causes denaturing of enzymes and damage to DNA and also forms the increased production of free radicals (Cseh, 2002; Khatun *et al.*, 2008; Posmyk *et al.*, 2009).

In higher plants heavy metals induce oxidative stress by generation of superoxide radical (O_2^-), hydrogen peroxide (H_2O_2), hydroxyl radical (OH) and singlet of oxygen ($^1O_2$) collectively termed as reactive oxygen species (ROS). ROS can rapidly attack all types of bio-molecules such as nucleic acids, proteins, amino acids etc. leading to irrepairable metabolic dysfunction and cell death. Reactive oxygen species produced under stress is detrimental to growth because these molecules cause gradual lipid peroxidation, inactivation of anti oxidant enzymes (Teisseire and Guy, 2000) and oxidative dehydro ascorbic acid damage (Kasprzak, 2002). Therefore, induction of antioxidant enzymes including Superoxide Dismutase (SOD), Catalase (CAT) and Peroxidase (POD) is an important protective mechanism to minimise oxidative damage in plants. SOD is ubiquitous enzyme which play a key role in cellular defence mechanism against ROS (Apel and Hirt, 2004). Its activity modulates the relative amount of O^{2-} and H_2O_2 by Haber-Weiss

reactions and decrease the risk of OH radical formation which is highly reactive and may cause severe damage to membrane proteins and DNA (Bowler *et al*., 1992; Noctor and Foyer 1998; Hall, 2002). According to Zhang *et al.* (2007) and Siedlecka and Krupa (2002) CAT is less efficient in eliminating H_2O_2 due to its low substrate affinity whereas the main response of tolerant plants to heavy metal is increase in SOD and POD activities.

Plants develop a complex mechanism by which they control the uptake and accumulation of heavy metals (Cobbett and Goldsbrough, 2002). These mechanisms involve chelation and sequestering of metal ions by a particular class of metal binding ligands denominated as phytochelatin (PC) and metallothionins (MT) (Cobbett, 2000). Metallothionins have possible role in or detoxification in plants and it has been reported in *Vigna radiata* (Shanker *et al.*, 2004).

Comparing the effects of a series of heavy metals (Cd, Pb, Cu and Ni), the highest growth inhibition was found with cadmium (Burzynski and Buczek, 1994). The devastating effects of cadmium on plants have been described by many authors (Foy *et al*., 1978, Fitter and Hay, 1983; Salt *et al*., 1998; Cseh, 2002; Sun *et al*., 2007). Cadmium is known to be one of the most phytotoxic heavy metals (Prasad, 1995; Sanita-di-Toppi and Gabbrielli, 1999; Fodor, 2002; Ederli *et al*., 2004; Pilon-Smits, 2005).

According t o Vazques *et al*. (1992), low concentration of cadmium is sufficient to cause the formation of cell wall ingrowth in the hypodermis of bean roots. Punz and Sieghardt (1993) reported that in herbaceous plants reduced elongation of roots under cadmium stress may be caused by inhibited mitosis, decreased synthesis of cell wall components, damaged Golgi apparatus or changes in the metabolism of polysaccharides in the root cap.

Ye *et al*. (1997) suggested that when *Typha latifolia* was treated with cadmium, leaves became chloretic before the harvest. According to those authors, leaf and root elongation, shoot and root dry weights were significantly reduced by cadmium and the uptake/ accumulation of cadmium also was observed in seedlings of *Typha latifolia* even in the treatment with 50 µg ml^{-1} cadmium solution. Although cadmium adversely affects all growth parameters, root growth is affected the most and faster reduction in root biomass than shoot resulting in an increased shoot/root biomass ratio (Jalil *et al*., 1994). Ouariti *et al*. (1997) reported that significant decrease in lipid level, fatty acid content and biomass production in tomato seedlings exposed to cadmium in nutrient medium. Cadmium was found to inhibit root growth and decrease fresh weight and water contents in roots and shoots of maize, rye and wheat, the most sensitive of which was maize, although cadmium accumulation was lower in this plant (Wojcik and Tukendorf, 1999). According to Zhang *et al*. (2002) addition of cadmium to the nutrient solution significantly decreased shoot and root weight, shoot height, root length and

tiller per plants in wheat genotypes but growth inhibition was different in root and shoot and among genotypes. In *Triticum aestivum* seedlings, cadmium treatment lead to inhibition of root growth and ion uptake (Abdel-Latif, 2008).

Cadmium is an effective inhibitor of plant metabolism particularly photosynthetic processes and chloroplast development in higher plants (Rasico *et al*., 1993). Effects of Cd^{2+} on chlorophyll and chloroplast development act synergistically and inhibit photosynthesis. (Ferreti *et al*., 1993; Lang *et al*., 1995). This heavy metal inhibits chlorophyll biosynthesis by causing a decrease in the sum of reducing equivalence and ATP synthesis by altering the activity of RuBP carboxylase and indirectly affect the uptake and metabolism of CO_2.

Application of cadmium in *Cajanus cajan*, reduceced net photosynthesis indirectly through reduced chlorophyll content by affecting stomatal conductance and electron transport (Sheoran *et al*., 1990). In *Pisum sativum* seedlings, cadmium caused a sharp decline in chlorophyll content, photosynthetic rates, and activity of the photosystems and photosynthetic enzymes (Chugh and Sawhney, 1999). Cadmium had caused reduction in chlorophyll content and carotenoids, the rate of net CO_2 uptake, transpiration and water use efficiency in the presence of 10 µM cadmium in *Zea mays* seedlings (Prasad, 1995; Baryla *et al*., 2001). Detrimental effects of cadmium

on chlorophyll synthesis, water splitting apparatus reaction complex, antenna and energy distribution of photosystem II have been reported in *Cannabis sativa* (Linger *et al.*, 2002). According to Abdel-Latif (2008), retarded chlorophyll synthesis is a characteristic of cadmium treatment in *Triticum aestivum*. Reduction of carotenoids is found to be a serious consequence of cadmium treatment thereby reducing the protective role of carotenoids against stress induced damage of chlorophyll pigment. Liu *et al.* (2009) proposed that in *Lonicera japonica* decreased chlorophyll content related to higher cadmium concentration could be used to monitor cadmium induced damage.

Cadmium ions are also toxic to nitrate reductase which limits the synthesis of amino acids and proteins (Srivastava, 1974). Cadmium inhibits the synthesis of nitrate reductase in *Zea mays* probably by displacement of an essential metal ion from the central functional part of the enzyme (Shankar *et al*., 2000). Another interference of cadmium ions is with sulfhydral groups which often determine the structural and functional aspects of protein (Kumar *et al*., 2002). According to Liao *et al*. (2002) cadmium causes cellular damage by denaturing proteins due to the binding of cadmium ions to sulfhdryl residues or by displacing cofactors from a variety of proteins and enzymes. Reduction of total protein due to cadmium treatment presumed to occur by the utilization of sulphur containing amino acids for chelation followed by sequestration as reported in *Potamogeton pectinatus* (Rai *et al*., 2003). Several studies on cadmium have been reported to induce

phytochelatin synthesis for sequestration of the cadmium toxicity (Grill *et al*., 1985; Rauser, 1987; Steffens, 1990; Prasad, 1997; Stroinski, 1999; Leopold *et al*., 1999; Kubota *et al*., 2000). Cadmium induced phytochelatin synthesis is reported in *Oryza sativa* (Reddy and Prasad 1992), *Zea mays* (Meuwly *et al*., 1995) horse radish (Kubota *et al*., 2000) and *Phragmitis australis* (Ederli *et al*., 2004). Induction of phytochelatin synthesis due to cadmium toxicity has been reported in *Rubia tinctorium* (Kubota *et al*., 1995), *Bacopa monnieri (*Ali *et al*., 2000), *Arabidopsis halleri* (Weber *et al*., 2006). Phytochelatins are reported to be involved in the translocation of cadmium in *Arabidopsis thaliana* (Cheng *et al.*, 2002). According to Clemens (2006) production of phytochelatin leads to increased accumulation of cadmium in plant cells.

Cakmak *et al*. (2000) suggested that cadmium circulates in the plant and gets transported to the phloem hence it is translocated between leaf and stem. Reid *et al*. (2003) also suggested the mobility of cadmium through phloem in Potato. According to Wu and Zhang (2002) cadmium is one of the most aggressive heavy metals and can be taken up by the roots, translocated readily to above ground tissues and get accumulated in the fruits/seeds and hence become a potential threat to human health as it enters the food chain.

According to Stolt *et al*. (2003, 2006) absorption and translocation of cadmium varies from plant to plant and genetic variation exists in the accumulation rate of cadmium in different parts of the same plant. Those

authors suggested that one genotype may accumulate maximum cadmium in the shoots and in another genotype, maximum cadmium may be accumulated in the grains and these types of observations are clearly correlated with the concentration of cadmium in the soil.

Studies on the elucidation of molecular basis of Cd^{2+} uptake into plant cells revealed that for Cd^{2+} being a non – essential metal ion, there would be no specific uptake mechanism. Ca^{2+} channels have long been studied using Cd^{2+} ions and evidences for Cd^{2+} uptake into plant cells via Ca^{2+} channels have come from the studies by Perfus-Barbeoch *et al*. (2002). According to Clemens (2006) there is clear yet so far mostly indirect evidence, that Cd^{2+} is taken up into plant cells by Fe^{2+} , Ca^{2+} and Zn^{2}+ transporters/channels of low specificity.

Ederli *et al*. (2004) discussed that in *Phragmitis australis* cadmium was accumulated mainly in the vacuoles of parenchyma cells of roots immediately below the endodermis. According to those authors, contradictory results may be occurred due to cadmium treatment and effects are dependent on the plant species and *Phragmitis australis* is reported to be a cadmium accumulator plant and accumulation is abundant in roots. Migocka and Klobus (2007) suggested that in cucumber roots, transport of Cd^{2+} through tonoplast membrane may occur through the involvement of Cd^{2+} and H^{+} coupled antiport.

According to Cheng *et al*. (2002) and Pittman *et al*. (2005), there are specific gene families to encode proteins involved in the transport of metals into vacuoles. Cadmium tolerance and hyper accumulation potential have been reported as a characterisitic of *Bidens pilosa* which is a widely growing weed from tropical and subtropical zones (Sun *et al.*, 2009). Similarly, *Lonicera japonica* plant exposed to cadmium for 21 days showed accumulation of cadmium in the leaves, stem and roots with increased cadmium accumulation in the medium (Liu *et al.*, 2009).

Most widely accepted indicator of oxidative damage due to cadmium stress in plants is accumulation of malondialdehyde (MDA) as a breakdown product of lipid peroxidation in *Phaseolous vulgaris* (Chaoui *et al*., 1997), *Lonicera japonica* (Liu *et al*., 2009), *Bidens pilosa* (Sun *et al.*, 2009). According to Song *et al*. (2004) cellular damage due to cadmium toxicity occurs due to the generation of highly reactive oxygen species, which affects deranged metabolism. MDA level is routinely used as an index of lipid peroxidation under stress condition. In a mangrove plant (*Bruguiera gymnorrhiza*) MDA content was increased in the leaves due to lead, cadmium, and mercury compared to the MDA content of another mangrove (*Kandelia candel*) in which MDA content was decreased. (Zhang *et al.*, 2007). According to those authors the differences in the distribution of MDA between the two mangrove implied that *Kandelia candel* is better protected from oxidative stress damage and can rapidly regulate the antioxidative

system. Similarly accumulation of malondialdehyde was reported in the roots of *Lonicera japonica* subjected to cadmium induced oxidative stress (Liu *et al*., 2009). According to those authors as a defensive mechanism antoxidative enzymes especially superoxide dismutase and catalase are increased in the leaves and roots along with increased cadmium concentration in the medium. Increased glutathione content has been observed as an important effect of Cd treatment in *Bacopa monnier*i (Mishra *et al*., 2006), *Triticum aestivam* (Sun *et al*., 2005), *Phaseolus vulgaris* (Smeets *et al*., 2005) and *Brassica juncea* (Szollosi *et al*., 2009). Increased SOD and CAT activity along with high cadmium accumulation have been observed in *Thlaspi cuerulescecns* (Boominathan and Doran, 2003), *Rorippa globosa* (Sun *et al*., 2007), *Solanum nigrum* (Wang *et al*., 2008), *Lonicera japonica* (Liu *et al*., 2009) and *Bidens pilosa* (Sun *et al*., 2009).

Chromium compounds are highly toxic to plants and are detrimental to growth and development. Chromium effects in relation to phytotoxicity have been investigated by many researchers on important crop plants and also on lower groups plants. (McGrath, 1982; Riedel, 1985; Bishnoi *et al*., 1993; Srivastava *et al*., 1994; Panda and Patra, 1998, 2000; Han *et al*., 2004; Shanker *et al*., 2004, 2005; Panda and Choudhury, 2005).

Various forms of growth retardation and physiological changes have been reported in plants by chromium (Bishnoi *et al*., 1993; Lang *et al*., 1995;

Moral *et al*., 1996; Samantaray and Das, 1997; Prasad, 1997; Samantaray, *et al*., 1998). Chromium phytotoxicity results in inhibition of seed germination, degrade pigment status, nutrient balance and antioxidant enzymes and induce oxidative stress in plants (Poschenrieder *et al*., 1991; Barcelo and Poschenrieder, 1997; Panda and Patra, 1997, 1998, 2000; Panda, 2003; Panda *et al*., 2003).

Corradi *et al*. (1993) recorded suppression of lateral roots in *Salvia sclarea* with a diminishing trend with the increase in the dose of chromium. According to those authors, lateral root production was completely inhibited at 10 ppm of Cr VI and high dose of chromium (60 ppm) added to the plants resulted in the death of plants within three days of treatment in hydroponics as well as pot culture experiments.

Excessive content of chromium in the soil had caused severe leaf necrosis and stunting of roots, which led to the death of *Eucalyptus* seedlings and yellowing of leaves in *Acacia mangium* was also recorded due to excessive chromium and nickel content in the soil (Malajczuk and Dell, 1995). Moral *et al*. (1995) and Samantaray *et al*. (1996) reported that chromium toxicity affected root length and damage the architecture of entire root. Chromium is reported to affect root growth more adversely than any other heavy metals and root length and dry weight also were reduced due to chromium treatment (Iqbal *et al*., 2001). According to Davies *et al*. (2002)

although some crop plants are not affected by low concentration of chromium, this metal is toxic to higher plants at 100 mM Kg^{-1} dry weight. Treatment of cauliflower with chromium resulted in decreased water potential and reduction in trachea vessels diameter (Chatterjee and Chatterjee, 2000). Stem length, leaf area and biomass also were reported to be inhibited by chromium (Tripathi *et al*., 1999). According to Shanker *et al.* (2005) biomass production and yield were generally affected by chromium (Cr IV) in many plants. Biomass production was found to be reduced in *Oryza sativa* plants as a consequences of chromium toxicity (Panda, 2007).

Chlorophyll synthesis was reported to be inhibited by chromium in *Zea mays* (Krupa *et al*., 1982), Rice (Samantaray *et al*., 1996) and *Nymphaea alba* (Vajpayee *et al.,* 2000). Inhibition of chlorophyll b synthesis by the influence of chromium is well known in plants (Barcelo *et al.*, 1985; Panda and Chowdhary, 2005; Shankar *et al*., 2005). Due to chromium application, mung bean plants showed severe stunted growth and leaf chlorosis (Rout *et al*., 1997). According to Samantaray *et al.* (1998) chromium reduces chlorophyll and carotenoid synthesis indirectly by the inhibition of iron and zinc transport to the leaves. Bera *et al*. (1999) studied the effect of chromium on chloroplast pigments content in mung bean and opined that irrespective of the concentration, chlorophyll a, chlorophyll b and total chlorophyll were decreased in six-day-old seedlings. Toxic effect of chromium has been shown by *Ocimum teneflorum* as reduction of

photosynthetic pigments and protein (Rai *et al.*, 2004). Panda and Choudhury (2005) suggested that oxidative stress induced by chromium initiated degradation of photosynthetic pigments causing decline in growth and high concentration of chromium disturbed chlorophyll ultra structure and affect photosynthesis. Chromium stress has been reported as one of the important factors that affect photosynthesis (Shanker *et al*., 2005).

Chromium is found to be accumulated mainly in the roots and poorly transported to shoots (Moral *et al*., 1994, 1996; Samantaray and Das, 1997) possibly due to the spatial localisation in a specific sub cellular compartment in the root cells as suggested earlier by Barcelo *et al*. (1985). Samantaray and Das (1997) reported the accumulation of chromium by mung bean plants upto 70 ppm in their roots when the plants were grown in chromate mine waste. According to Pulford *et al.* (2001) chromium is poorly translocated to aerial parts and held predominating in the roots.

Plant species such as *Scirpus lacustris, Phragmitis karka* and *Bacopa monnieri* exhibit high potential to absorb, translocate and concentrate chromium in their tissues (Yadav *et al*., 2005). Those authors further stated that about 99% of the absorbed chromium is retained in the root tissue because most plants show low chromium concentration in the shoot tissue even when grown in chromium rich soil. So the food chain is well protected against the chromium toxicity.

Uptake and accumulation pattern of chromium varies from crop to crop. In sunflower, root accumulates 212 mg kg^{-1} chromium, while shoot accumulated only 5.8 mg kg^{-1} and in cauliflower accumulation of chromium in root and shoot are 350 mg kg^{-1} and 2 mg kg^{-1} respectively (Zayed *et al*., 1998). In spinach the accumulation limit of chromium varies from 2.8-3.1 (Singh, 2001). In general, more chromium is reported to accumulate in the root compared to the shoot (Shanker *et al*., 2005). Shanker *et al.* (2005) have reported characteristic of *Albizia amara* as a potential chromium accumulator and recommended the plant for phytoremediation. Distribution and bioaccumulation of cadmium and chromium are reported to be species specific in *Vigna* (Ratheesh Chandra *et al*., 2010). According to those authors both metals exert specific influences on the anatomy of root and stem and accumulation of chromium was very high both in root and stem compared to cadmium.

Chromium treatment is known to induce increased production of more biomolecules such as glutathione and phytochelatin which may confer resistance or tolerance to chromium. Chromium has been demonstrated to stimulate formation of free radicals and reactive oxygen species (ROS) such as superoxide radicals (O_2^-), hydrogen peroxide H_2O_2) and hydroxyl radicals (OH) either by direct electron transfer involving metal cations or as a consequence of metal mediated inhibition of metabolic reactions, their presence cause oxidative damage to the biomolecules such as lipids, proteins

and nucleic acids (Stohs and Bagchi, 1995; Kanazawa *et al*., 2000). Shanker *et al.* (2004) studied differential antioxidative response of ascorbate glutathione pathway enzymes and metabolites to chromium speciation stress in green gram roots and suggested that significant increase in lipid peroxidation and H_2O_2 generation was occurred after five hours of chromium (Cr VI) treatment. But similar results were seen only after 12 hours in Cr (III) treated plants. No significant increase in catalase activity and monodihydro – ascorbate- reductase was observed in Cr (III) treated plants. But in Cr (VI) treated plants, superoxide dismutase and ascorbate peroxidase activities were increased. Shanker e*t al.* (2003) reported induction and activation of antioxidant enzymes as one of the detoxification mechanisms of chromium in plants. Pea plants exposed to chromium (Cr VI) resulted in an increased activity of superoxide dismutase in root tissues (Dixit *et al*., 2002). In an excellent review on chromium toxicity in plants Panda and Choudhury (2005) suggested that decreased activity of catalase is an important effect of chromium. Inhibition of catalase activity due to chromium exposure was reported in wheat seedlings (Panda and Patra, 2000; Panda *et al*., 2003). Panda and Choudhury (2005) further opined that chromium affects catalytic activities of antioxidant enzymes like superoxide dismutase, catalase, peroxidase and glutathione reductase.

According to Rai *et al.* (2004), hyperactivity of superoxide dismutase, guaicol peroxidise and catalase have been reported in *Ocimum tenuiiflorum*

treated with chromium salt as a measure of protecting the plant from chromium stress. Increased activity of catalsase and peroxdase due to the exposure of two cultivars of *Brassica napus* to chromium revealed that these enzymes are increased proportional to the chromium concentration. But more activity of both the enzyme was occurred in more resistant cultivars (Hosseini *et al*., 2007).

Panda (2007) opined that chromium induces oxidative stress in the root cells of rice seedlings. According to the author chromium translocation take place via membrane transporters like sulfate carriers. Generation of ROS like H_2O_2, and O_2^- indicative of lipid peroxidation and resultant increase in MDA also occur in plants due to chromium stress (Panda *et al*., 2003). Antioxidant enzymes, guaiacol peroxidise and glutathione reductase showed reduction while superoxide dismutase activity was increased to compensate the oxidative stress induced by chromium.

Mercury belongs to the common group of heavy metals that accumulates in plants and reach animals through food chain. This metal causes serious health hazards like biotransformation, bioaccumulation and toxicity to living systems throughout the world due to its widespread distribution in the environment (Anonymous, 1979).

Toxicity of mercury has been reported in many plants and even in very low concentrations it causes hazards to plant growth (Vallee and Ulmer, 1972;

Sandmann and Boger, 1983; Kagi and Hapke, 1984; Baker *et al*., 1985; De *et al*., 1985). Those authors suggested that mercury is phytotoxic even in small quantities and the toxicity is bearing its strong affinity to acidic and thiol groups of proteins and nucleotides, thus interfering with the function of metabolites /organelles. In addition, mercury competes with other metal such as copper or zinc within the cell (Marschner, 1983). Mercury enters the plants as inorganic form from the soil or water or by the process of methylation that occurs in plants.

Various forms of growth retardation and physiological changes have been reported in plants by mercury toxicity (Nag *et al*., 1980). In *Cyperus rotundus* and *Chloris barbata* root growth inhibition occurred due to mercury treatment and the rate of inhibition was increased with the increase in concentration of mercury (Lenka *et al*., 1993). Maitani *et al*. (1996) observed a reduction in the relative root elongation of *Rubia tinctorium* when treated with 10µM Hg^{2+}.

In *Zea mays* leaves, inhibition of chlorophyll synthesis has been observed due to mercury treatment (Jain and Puranik, 1993). Prasad and Prasad (1987), Prasad (1997) and Parmar *et al*. (2002) have reported the decline of chlorophyll content due to mercury toxicity and according to them the decline is linked to the photosynthetic productivity. According to Prasad

et al. (1991) mercury has got direct effect on the photosynthetic electron transport system.

Interaction of mercuric chloride with primary amino groups of membranes containing phosphatidyl ethanolamine was studied by Delnomdedien *et al*. (1989) and the results revealed that the neutral, positively-and negatively charged-phospholipids are strongly affected by mercury. This mercury-lipid interaction suggested new mechanism for biological effect of mercury at membrane level. Studies on the effect of heavy metals like Zn and Hg on growth and biochemical constituents of *Vigna radiata* seedlings showed a decline in the respiratory rate of seedlings (Pratima *et al*., 1989). Mercury forms stable complexes with a variety of organic ligands and has exponential affinity for sulfhydryl groups of proteins (Falchuk *et al*., 1977; Nath *et al*., 1993). Strongest covalent complexes are formed with sulfur containing ligands such as cysteine, the next strongest with amino acids and hydroxyl carboxylic acids. According to Orcutt and Nilsen (2000) major impact of mercury is that it has a high affinity for sulfhydryl group and thus can inactivate many proteins and enzyme systems in plants.

Mercury is, an enzyme-and protein-inhibitor in biological systems and all mercury compounds are highly toxic to plants in general and aquatic plants in particular (De Fillippis, 1979; Baker and Walker, 1989; Reed and Gadd, 1990). Brzyska *et al.* (1991) reported that mercury treatment resulted in a

decrease of both soluble and immobilized enzyme activity even at low concentrations (0.2 mM) by 20 – 50% and addition of magnesium ions to the soluble forms of endopolygalacturonase counteracted the inhibitory effect of Hg. According to Jeana and Chaudhuri (1982) mercury causes increased permeability of tissues due to membrane damage in some aquatic plants.

Increased synthesis of malondialdehyde in plants treated with mercury was reported due to the inhibition of enzymes of photosynthetic carbon reduction cycle (Sheoran *et al*., 1990; Van Assche and Clijsters, 1990; Shaw, 1995).

According to Maitani *et al*. (1996) phytochelatin (PC) is the most abundant class III metallothionein produced in higher plants due to Hg^{2+} exposure. Those authors suggested that since mercury (II) has a linear configuration in co-ordination compounds, phytochelatin can effectively protect plants against the Hg^{2+} toxicity. Reduced glutathione (GSH) is the predominant free thiol present in plants and the concentration of GSH in plant cell is modified by developmental and environmental factors such as heavy metals and cell culture studies have indicated that GSH is the precursor for synthesis of heavy metal binding phytochelatins (Rauser, 1987; Scheller *et al*., 1987; Obata *et al*., 1994).

Eventhough, extraction and characterisation of phytochelatins induced by mercury in higher plants have not been well studied, higher levels of non-

proteinaceous thiol containing compounds have been detected in algae (Tukendorf and Rauser, 1990; Satoh *et al*., 1999). According to Velasco-Alinsug *et al*. (2005) specific mercury binding peptides are found in *Chromolaena odorata* treated with mercury. Detection by using RP-HPLC studies revealed that this proteins contain a series of five to nine cysteine residues repeatedly attached to the long chain of mercury binding peptides. The ability of the plant to accumulate and sequestrate mercury is primarily attributed to the production of Hg binding proteins.

According to Shaw (1995), mercury significantly inhibited seed germination and seedling growth in *Phaseolus aureus* but had little primary damaging effect on membranes. Lipid peroxidation occurs in *Phaseolus* by mercury treatment and it is indicated that guaiacol- and ascorbate peroxidases and catalases are actively involved in scavenging cellular H_2O_2 and other free radicals. The lipid peroxidation induced by this metal is a consequence rather than the primary cause of toxicity. According to Woolhouse (1983) mercury after being absorbed from the soil remain deposited mostly in root tissues. Lipid peroxidation and IAA oxidative activity are found to be increased in *Phaseolus vulgaris* due to the treatment with mercury. (Parmar and Chanda 2005).

Translocation and accumulation of mercury are very feeble in plants and no plants have been reported as hyperaccumulator of this element (Henry,

2000; Raskin and Ensley, 2000). According to those authors transgenic plants of *Arabidopsis thaliana* are known to accumulate mercury. Velasco-Alinsug *et al*. (2005) reported *Chromolaena odorata* as an accumulator of mercury and hence this plant has been recommended for phyotoremediation technology. Although no angiosperms have been recognised as natural hyper accumulator of mercury (Henry, 2000, Raskin and Ensley, 2000), *Azolla corolimiana* was reported as a hyper accumulator of mercury (Bennicelli *et al*., 2004). An investigation conducted on *Berkheya coddii* and *Atriplex cansescens*, revealed elevated mercury translocation and accumulation (Moreno *et al*., 2004). The results suggested that there is a potential for induced mercury accumulation for phytoremediation in these plants.

Moreno *et al*. (2005a) carried out experiments in plant growth chambers to investigate mercury accumulation and volatalisation in *Brassica juncea* and estimated mercury concentration in shoot and root and volatalisation rate. According to these authors volatalisation is a dominant pathway for mercury removal from the accumulator plant parts. In another study Moreno *et al*. (2005b) investigated the effect of thioligands on mercury volatalisation and accumulation in *Brassica juncea* and found that mercury accumulation was enhanced in the presence of ammonium thiosulphate, but volatalisation was not affected. Based on the experimental studies in *Brassica juncea* Moreno *et al*. (2005b, c) concluded that this plant exhibits phytofiltration potential of mercury from waste water contaminated with

mercury and loss of mercury from the plant-soil system occurs by volatalisation and this plant can also remove mercury from the waste water both by volatalisation and accumulation.

Aquatic plants such as *Eichhornia crassipes*, *Pistia stratiotcs*, *Scirpus tabernaemontani* and *Colocasia esculenta* are capable of removing mercury from water (Skinner *et al.*, 2007). According to those authors, the higher the concentration of mercury in water, the greater the amount of mercury removed by the plants. The roots of *Pistia* exhibited the largest uptake and accumulation capability over all plants followed by *Eichhornia, Colocasia* and *Scirpus*. Roots showed more mercury accumulation because roots are the only plant structure submerged in the test water containing mercury. Mercury storage in the roots may be a strategy of exclusion since roots are usually at the base of the plants (Skinner *et al*., 2007).

In an elaborated study on *Brassica juncea* Moreno *et al*. (2008) suggested that solution of 5 and 10 mg/l mercury detrimentally affected transpiration and roots accumulated 100-270 times mercury (on dry weight basis) above initial concentration. Nevertheless, the plant is found to translocate very little mercury to the shoot accumulating 0.7- 2% of the total mercury in the plant. According to those authors mercury volatalisation is in the form of Hg (o) vapour. Volatalisation is more from the experimental vessels and the rate is increased linearly as a function of mercury

concentration in the solution and it was concluded that, volatalisation of mercury from the solution may be due to the activity of root- associated algae or mercury resistant bacteria. However, phytofiltration effectively removed up to 95% of mercury from the contaminated solution by both volatalisation and accumulation.

Lead is physiologically not essential for plants at the same time it is harmful to growth. Mazen and Maghraby (1997) reported that wide spread use of lead in Roman times and the fall of Roman Empire, was the result of lead poisoning. Although lead is not an essential element, it is absorbed and accumulated in plants (Kabata- Pendias and Pendias, 1999).Various effects of lead on plants have been reviewed extensively (Foy *et al*., 1978; Koeppe, 1981; Kahle, 1993; Wozny *et al*., 1990; Orcutt and Nilsen, 2000; Fodor, 2002).

In a number of studies, the effect of lead on plants revealed that growth inhibition is more prominent in root systems than the shoots (Wozny and Jerczynska, 1991; Kahle, 1993). In sunflower, lead reduces leaf area, dry mass and plant height (Kastori *et al.*, 1998). According to Gzyl *et al*. (1997) and Malkowski *et al*. (2002) major symptoms of lead toxicity in plants are rapid inhibition of root growth. In *Phaseolus vulgaris*, lead in 10 µM concentration inhibits growth of main roots and lateral roots (Wozny and Jerczynska, 1991). In onion roots, the cell wall is remarkably thickened by

the effect of lead which is observed within one hour at a higher lead concentration (Wierzbicka, 1998). According to this author the first step of lead effect is the attachment to the cell wall and the thickening is not uniform. After 24 hours, tubular invaginations of the cell membrane are formed, which bind the majority of lead. According to Jiang *et al*. (2000) even at millimolar concentration of lead, root and shoot growth get inhibited. Cseh *et al.* (2000) suggested that at physiological concentration of 10µM lead does not inhibit shoot growth since lead is captured in the root apoplast by restricting translocation to the shoot. Reduction of photosynthesis associated with reduced stomatal conductance and transpiration have been reported in maize plant treated with lead (Stefanov *et al*., 1993).

In excised leaf segments of pea, Sengar and Pandey (1996) found that lead treatment resulting in lowered photosynthesis specifically by the inhibition of δ- aminolevulinic acid synthesis and the decrease in the 2-oxoglutarate and glutamate pool, which may be caused by the competition between the essential ions required for chlorophyll synthesis and Pb^{2+}. Most common symptoms of lead toxicity is chlorosis of leaves due to decrease of chlorophyll synthesis (Salati *et al*., 1999). In the leaves of *Festuca arundinacea*, the primary target of lead toxicity is the photosynthetic carbon dioxide fixation (Fodor, 2002). Inhibition of growth by lead is interpreted in different ways and most widely accepted conclusion is the direct or indirect effect on photosynthesis (Krupa and Baszynski, 1995). Decreased chlorophyll

content was found in *Lemna minor*, sunflower and cucumber treated with lead (Fodor, 2002).

According to Gregory (1988) the movement of lead from soil solution to root surface is by mass flow or diffusion, and it is controlled by concentration of the metal in the solution and transpiration rate of plant. Lead is taken up from the soil solution into root symplasts (Huang and Cunningham, 1996; Huang *et al*., 1997). In cucumber once lead enters root tissue, it can precipitate or flow into the xylem (the primary transpiration flow) or can exchange with the phloem (secondary bulk flow) structures which balance nutrient concentrations among plant tissues. Once in the xylem the metal can be absorbed to cation exchange sites within the walls of tubular structures (Marschner, 1986; Kochian, 1991; Raskin *et al*., 1994; Salt and Rauser, 1995). In plants treated with lead, transport mechanism of nutrients through the root cell is inhibited up to an extent of 50% (Cseh *et al*., 2000).

Turner and Dickinson (1993) found that concentration of lead in the roots of *Acer pseudoplatanus* was very high which caused stunted growth and lack of lateral roots. According to Kastori *et al*. (1996) bioaccumulation of lead was significantly increased with the increase of lead concentration in the rooting media of *Beta vulgaris* and maximum lead was accumulated in the roots while dry weight of root and shoot was reduced proportional to the

concentration of lead. Ye *et al*. (1997) reported that the lead content in the roots of *Typha latifolia* was greater than in the shoots when the plants were grown in lead containing medium.

According to Brennan and Shelly (1999) the degree of precipitation of lead in *Zea mays*, increases the concentration of lead in the shoot and decreases the concentration in the root. Increasing the precipitation rate had the opposite effect. This behaviour can be explained by the fact that the faster precipitation occurs the more metal builds up at its point of entry in to the root system. As more precipitation occurs in the root, less is available for translocation to the shoots. The lead that is not precipitated only is available for translocation to the shoots.

The accumulation of lead is not uniform among the different zones of corn seedling roots (Michalak and Wierzbicka, 1998). It has been shown that lead absorption by the *Allium cepa* is not uniformly distributed within the root. In this case root tips contained the highest concentrations of lead, intermediate concentrations were found in the proximal parts of the roots while the lowest concentrations of lead were found in the root base. There are plants like cucumber in which despite the retention of lead in the root, a significant amount is transported to the shoot and entering the xylem is evidenced by the significant amount of lead measured in the xylem sap (Varga *et al*., 1999). In plants exposed to lead, greater concentrations were

observed in the root tip, lower in the elongation zone and the least in the basal part of the root (Malkowski *et al*., 2002). Fodor (2002) suggested that in terrestrial plants most of the lead enters the root through the root hair region and accumulates at the endodermis which serve as a barrier to radial movement of lead and hence the translocation to the shoot is restricted.

A comparative study of translocation and accumulation of chromium, cadmium and lead in *Momordica charantia* showed accumulation of all the three elements in shoot, root and fruit. Eventhough this plant is more sensitive to cadmium, maximum quantity of accumulation was observed in the root (Daniel *et al*., 2009). According to Chandra *et al*. (2009) bioaccumulation of copper, cadmium, chromium, zinc, iron, nickel, manganese and lead in *Triticum aestivum* and *Brassica compestris* irrigated with distillary and tannerary effluents revealed that the concentration of all the treated metals was significantly higher than the permissible limit by FAO/WHO (1984).

Gbaruko and Friday (2007) reported that *Hibiscus esculentum* and *Vernonia amygdalina* grown in the soil of polluted coastal area where massive oil exploration takes place, showed the presence of mercury, lead, chromium, copper, nickel and zinc content and the heavy metal content in the roots of both plants were more compared to the leaf/fruit and the authors opined that nutritional implication was that consumers of these food materials may be exposed to heavy metal toxicity. Studies on the effect of waste water

irrigation on the accumulation of lead, copper, zinc and manganese in the vegetable available in one province of Iran revealed that concentration of these metals in the vegetables was very high (Cheraghi *et al*., 2009). Bioaccumulation /phytoextraction potential of *Arachis hypogea* was established by irrigating the plant with effluent water of Biomass Power Plant (Nagajyoti, *et al*., 2008). The effluent contained heavy metals such as chromium, copper, manganese, iron, cobalt, nickel, cadmium, lead and zinc and accumulation of these metals varied from metal to metal and depend on duration and concentration. Maximum accumulation was noticed in 10% effluent. According to those authors, phytoremediation technology can be applied to *Arachis hypogea* and at lower concentration (25%) the effluent can be used for irrigation purpose since it contains some nutrient element in addition to the toxic heavy metals which showed only negligible accumulation.

A new approach termed phytoremediation, using plants to remove heavy metals from contaminated environment has been emerged during 1990's (Cunningham and Berti, 1993; Baker *et al*., 1994; Raskin *et al*., 1994; Salt and Rauser 1995). Some plants, known as heavy metal hyperaccumulators can extract unusually high content of heavy metals from environment via root system and translocate them to the above ground parts (Baker and Brooks, 1989; Brown *et al*., 1994; Kumar *et al*., 1995; Xiong, 1998; Baker *et al*., 2000). Phytoremediation is the process or technology

involving the use of plants and their associated microbes for environmental cleanup (Pilon-Smits, 2005). During the past 10 years, phytoremediation has gained acceptance as a cost effective, non invasive, alternative or complementary technology for engineering- based remediation method against heavy metal pollution.

Thlaspi caerulescens, a herbaceous annual has received much attention because it has been known to hyperaccumulate Zn (Baker *et al* ., 1994; Brown *et al*.. 1995; McGrath *et al*., 1997; Shen *et al*., 1997; Kupper *et al*., 1999; Escarre *et al*., 2000; Pance *et al*., 2000). *Brassica juncea* also is well known due to its hyperaccumulation of Cd and Zn and hence phytoremediation potential of this plant has been established (Kumar *et al*., 1995; Salt and Rauser, 1995; Blaylock *et al*., 1997; Ebbs and Kochian, 1998; Haag-Kerwer *et al* ., 1999; Hale *et al*., 2001; Hamlin *et al*., 2003; Podar *et al*., 2004; Abe *et al*., 2006; Ishikawa *et al*., 2006).

The plant species like sharp dock (*Polygonum amphibium*), duck weed (*Lemna minor*), water hyacinth (*Eichhornia crassipes*), water dropwort (*Oenanthe javanica*) and calamus (*Lepironia articulatae*) are good candidates for phytoremediaion of polluted waters (Wang *et al*., 2002). *Polygonum amphibium* showed accumulation of nitrate and phosphate in its shoots. *Eichhornia crassipes* and *Lemna minor* were hyper accumulators of cadmium.

Water dropwort and calamus were hyperaccumulators of Mercury and Lead respectively (Skinner *et al*., 2007).

Phytoremediation of cadmium has been effectively performed by *Brassica juncea* (David *et al*., 1995), *Thlaspi caerulescens* (Brown *et al*., 1995) and *Polygonum thunberjii* (Shinmachi *et al*., 2003) and these plants are reported to be hyper accumulators of Cd. Similarly, weed species such as *Silene vulgaris* (Joop *et al*., 1994) and *Lepidium campestris* (Wenzel and Jockwer, 1999) have also been reported to grow actively in Cd polluted soil and Cd accumulation was noticed.

Phytoremediation potential of native plants under field condition revealed that severe phytotoxicity may act as a powerful force for evolution of tolerant plant populations (Shu *et al*., 2002; McGrath and Zhao, 2003). Effect of cadmium on weed species growing in the paddy field are found to be accumulators of Cd from the soil and hence can be used for phytoremediation of Cd polluted soil and bioassay for Cd in soil.

Phytoremediation is a promising method for the removal of cadmium from the soil. Plants remove Cd^{2+} from soil very slowly. To increase cadmium uptake by plants, the effect of application of chloride ions (Cl^-) and decrease in soil pH on the quantity of cadmium taken up by plants were investigated in pot experiments using soil collected from paddy fields contaminated with cadmium from mining waste water and planting *Hibiscus*

nannabinus, *Helianthus annuus* and *Sorghum vulgare* (Hattori *et al*., 2006). The application of Cl^- approximately doubled the quantity of Cd uptake by *Helianthus* and *Hibiscus* compared to the control.

Plants used for phytoremediation enhance the removal of pollutants by consuming part of them in the form of plant nutrients (Fitz and Wenzel, 2002). Tong *et al*. (2000) suggested that plants used for phytoremediation of soil polluted by heavy metals should grow fast, develop a large amount biomass quickly and be easy to cultivate and harvest, preferably multiple times/year. According to Ziammels *et al*. (2004); Sersen *et al*. (2005), aquatic plants such as *Eichhornia crassipes*, *Pistia stratiotes*, *Lemna minor* and *Salvinia molesta* which are having high efficiency in removing metals and minerals from contaminated water are used for improving river water quality . So the aquatic plant system offers an eco-friendly and cost effective technology for treatment of urban and agricultural waste water.

Phytoextraction of lead, zinc and cadmium from the soil by selected plants such as *Sinapis alba*, *Raphanus sativus*, *Amaranthus spp*. investigated by cultivating in soil samples contaminated with these heavy metals alone as well as in combination with chelating agents, EDTA (Ethylenediamine-tetraacetic acid) and EDDS (Ethylenediamine-di succinic acid) revealed several fold uptake of these heavy metals (Kos *et al*., 2003). Those authors further stated that EDTA appeared to be more effective chelating agent

while biodegradable EDDS was less effective. Biomass increase was reported as another effect of these chelating agents in most of the plants.

Phytoremediation of chromium - polluted soil and water can be done with harvestable plants (Phytoextraction) and by accumulation in the root tissue of aquatic plants by growing in contaminated water (Rhizofiltration). Yadav *et al*. (2005) reported phytoextraction of chromium from contaminated soil by *Scirpus lacustris*, *Phragmities karka* and *Bacopa monnieri*.

Metal accumulation potential of plants varies from metal to metal and plant to plant. Several plants have been reported as tools for phytoextraction of metals. Chromium accumulation is very high in *Arabidopsis thaliana* (Baker, 1990), *Brassica juncea* (Gleba *et al.*, 1999), *Alternanthera sessilis* and *Pistia stratiotes* (Moodley *et al*., 2007). *Azolla carlilniana* is a hyperaccumulator of mercury (Bernier *et al*., 1993). Accumulation of arsenic is very high in *Pteris vitatta* (Wei *et al.*, 2006), *Pistia*, maize and *Alternanthera* are very good accumulators of lead (Huang and Cunningham, 1996; Moodley *et al.*, 2007). Nickel shows very high potential of bioaccumulation in *Brassica compestris* (Glick, 2003).

Brassica nigra plants have been reported to have high potential to tolerate and accumulate high quantities of toxic trace elements and can be used for phytoremediation of metal –polluted soils (Minglin *et al.*, 1995; Schafer *et al.*, 1997). Bharagava *et al.* (2008) reported that *Brassica nigra*

irrigated with industrial effluents have accumulated toxic trace elements in different parts beyond the permissible limit as suggested by FAO/WHO (1984).

According to Liu *et al*. (2009) the bioaccumulation coefficient (BC) was used to evaluate the metal accumulation efficiency in plants. The elevated translocation factor (TF) shown by *Lonicera japonica* reveals the stronger tolerance to cadmium and translocation from root to shoot indicating high potential of this plant for phytoremediation of soil polluted by cadmium.

Although phytoremediation potential of *Bacopa monnieri* has already been reported by cultivating this plant in nutrient medium artificially contaminated with heavy metals like cadmium chromium, mercury and lead (Sinha, 1999; Hussain, 2007), studies on accumulation of different heavy metal by naturally growing *Bacopa monnieri* collected from different polluted habitat confirmed the phytoremediation potential of this medicinal plant. (Hussain *et al*., 2010).

Medicinal plants growing in natural habitat are exposed to heavy metals and poisoning, associated with these toxic metals have been reported in Asia Europe and United states (Dunbabin *et al.,* 1992; Markowitz *et al.,* 1994; Olujohungbe *et al*., 1994; Kakosy *et al.*, 1996). Plants absorb and accumulate heavy metals from the soil and water polluted with the toxic metals and medicinal preparations using these plants as active ingredients of

many Ayurvedic medicine get contaminated. Lead and mercury have been reported in Chinese and Indian medicines as the plants are grown in polluted area near road ways or metal mining operations (Levitt, 1984). In addition, high level of heavy metals are formed by agricultural expedients including cadmium contaminated fertilizers, organic mercury or lead- based pesticides and contaminated irrigation water. Aneesh *et al.* (2009) reviewed the international market scenario of traditional Indian herbal drugs and stated that overall 20.7% of Ayurvedic medicines contained detectable quantity of lead, mercury and arsenic. Exposure to cadmium cause nephro toxicity in humans and lead and mercury adversely affect neuron system (Tong *et al.*, 2000, WHO, 2003).

Hussain *et al*. (2006) in an elaborated study of bioaccumulation pattern of heavy metals such as lead, copper, zinc, chromium, iron and nickel in medicinal plant parts including root, stem, leaves and seeds revealed that, high amount of lead was accumulated in the roots of *Datura alba* followed by leaves of *Achyranthes aspera* and *Withania somnifera*. Concentration of copper, iron and lead also was high in all samples.

A study on the herbal medicines used in Brazil (Caldas and Machado, 2004) revealed the presence of lead, cadmium and mercury in 138 samples of powdered dry extracts of the plants. Samples contained detectable level of mercury, lead and cadmium and the quantities were 38, 88 and 76% of

samples respectively. This study recommended the need for a systematic control of toxic heavy metals in plants which are used as medicines. According to Zheljazkov *et al*. (2008), medicinal plants such as *Bidens tripartia, Leonurus cardiaca, Marrubium vulgare, Melissa officinalis and Riganum heracleotieum* cultivated in the soils near metal smelter and contaminated with Cd, Mn, Zn, Cu and Pb exhibited slight growth retardation. But the accumulation of these metals was negligible in the shoot particularly compared the roots and the oil content of these plants was metal free since translocation of heavy metals to shoot was very low. Barthwal *et al*. (2008) estimated the heavy metal content of soil and selected medicinal plants such as *Abutilon indicum, Calotropis procera*, *Euphorbia hirta* and *Tinospora cordifolia* collected from three environmentally different sites. The study revealed that the level of heavy metals was higher in the plants of traffic area and lowest in plants collected from industrial and residential area. According to those authors, the level of heavy metal content differed in the same medicinal plant collected from environmentally different sites.

Accumulation of high amount of iron, zinc and manganese have been reported in selected medicinal plants such as *Artemissia vulgaris*, *Asparagus adscendens, Stevia rebaudiana, Withania somnifera* etc. collected from natural habitat (Khan, *et al.,* 2008).

Assessment of heavy metals by different aquatic plants grown along Lana river, Albania revealed that considerable quantity of nickel and cadmium was accumulated in the species, *Typha latifolia*, where as *Arundo donax* plants accumulated more manganese. All the species showed almost same quantity of lead and nickel accumulation (Shehu *et al*., 2010).

MATERIALS AND METHODS

Plant Material

Boerhavia diffusa L. cuttings were collected from Calicut University Botanical Garden. Earthen- ware pots half filled with potting mixture (garden soil: sand: cowdung 1:1:1) were used for cultivation. Five cuttings were planted in each pot, irrigated with tap water and maintained under green house condition. Growth performance were observed and most profusely growing plants were selected for further cultivation. Continuous propagation of plants were done throughout the period of experimentation by re-planting 2-3 times in a year. Healthy cuttings of length 10-15 cm consisting of 3-4 nodes were selected for culture studies.

1. Rooting

The cuttings selected as described above were planted in Hoagland nutrient solution and kept under green house condition for rooting. Rooted propagules were used for heavy metal treatments.

3. Chemicals

Either AR or GR grade chemicals were purchased from MERCK, BDH, SRL and GLAXO companies. PVPP, Riboflavin and BSA were purchased from Sigma Chemicals.

Containers for Culture

Good quality glass bottles of size 8.5 cm height and 5.5 cm diameter were used for the setting of hydroponics system.

5. Composition and Preparation of Nutrient Solution

Modified Hoagland solution (Epstein, 1972) prepared as described by Taiz and Zeiger (2002) was used for hydroponic study (Table 1). The stock solution of each nutrient was prepared separately and appropriate volume of each was mixed together to makeup the final volume and concentration of the nutrient solution. pH of the solution was adjusted to 6.8 using 0.1 N HCl or NaOH.

Table 1. Composition of nutrient solution employed in the present study

	Compounds	Molecular weight	Concentration of Stock solution	Concentration of Stock solution	Volume of stock solution per litre of final solution
		(g mol^{-1})	(m*M*)	(g L^{-1})	(mL)
Macronutrients	KNO3	101.10	1,000	101.10	6.0
	Ca $(NO_3)_2$ 4 H_2O	236.16	1,000	236.16	4.0
	NH_4 H_2 PO_4	115.08	1,000	115.08	2.0
	$MgSO_4$. $7H_2O$	246.48	1,000	246.49	1.0
Micronutrients	KCl	74.55	25	1.864	2.0
	H_3BO_3	61.83	12.5	0.773	
	$MnSO_4$. H_2O	169.01	1.0	0.169	
	$ZnSO_4$. $7H_2O$	287.54	1.0	0.288	
	$CuSO_4$. $5H_2O$	249.68	0.25	0.062	
	H_2MoO_4	161.97	0.25	0.040	
	NaFe EDTA	558.50	53.7	30.0	0.3

6.Treatment with Heavy metals

Screening tests on the effect of treatments of *B. diffusa* cuttings with cadmium chloride ($CdCl_2$), potassium dichromate ($K_2Cr_2O_7$), mercuric chloride ($HgCl_2$) and lead acetate $(CH_3\text{-}COO)_2\ Pb\ 3H_2O$ showed that tolerance of this plants to cadmium, chromium, mercury and lead varied and hence the concentrations in which seedlings survived but exhibited approximately 50% growth retardation were selected for the treatments. Table 2 shows the optimal concentration of each treatment, which brought about 50% growth retardation. Rooted cuttings (3 numbers) were planted in one bottle containing 100 ml of Hoagland solution to which the heavy metal solutions were added to obtain the final concentration as given in Table 2. Minimum 25 bottles were used for each treatment so as to get sufficient tissues for experiments. The hydroponic system was maintained under green house conditions. Plants cultivated in Hoagland solution without any heavy metal salt served as the control.

Table 2. Concentrations of Heavy Metal salts used for treatments of *B. diffusa* seedlings.

Heavy metal salts	**Concentrations** (µM)
Cadmium chloride	30
Potassium dichromate	400
Mercuric chloride	10
Lead acetate	600

7. Sampling

Samples of treatments and control were collected at comparable interval of four days up to 20 days of growth. At each interval, plants were harvested from each treatment, washed thoroughly in distilled water and blotted to dryness. Morphological parameters such as root/shoot length, leaf area, tolerance index and stomatal index were recorded. For biochemical analyses, root, stem and leaves were sampled. A minimum of 5 plants of each treatments were separately cut into pieces, randomized and sampled in duplicates for each analysis.

8. Morphological measurement

Growth of plants were assessed in terms of root length, stem length, and leaf area.

8. 1. Root and stem length

The sampled propagules were washed in distilled water, blotted and length of root, stem and leaf area were measured manually, using a graduated scale. Measurements of not less than five propagules were recorded each time.

8. 2. Tolerance Index percentage

Tolerance Index percentage was calculated according to the method of Turner (1994).

$$TI = \frac{\text{Observed value of root length in solution with metal}}{\text{Observed value of root length in solution without metal}} \times 100$$

8. 3. Stomatal Index

Stomatal density on abaxial and adaxial sides of the leaf was counted under a light microscope, by using nail polish impressions of leaf surface. Stomatal index was calculated according to the method of Meidner and Mansfield (1968).

$$\text{Stomatal Index} = \frac{\text{Number of stomata per unit area}}{\text{Number of stomata + number of epidermal cells per unit area}} \times 100$$

9. Physiological and Biochemical Studies

9.1. Dry weight

Samples of root, stem and leaves collected as described earlier were weighed in pre-weighed containers using electronic balance. Fresh weight obtained was recorded and the weighed samples were then placed in hot air oven at 100°C for one hour followed by at 60°C for overnight. Dry weight of each sample was taken on the next day and drying and weighing were repeated until values become constant. Dry weight percentage was calculated by using the following formula.

$$\text{Dry Weight Percentage} = \frac{\text{Dry weight}}{\text{Fresh weight}} \times 100$$

9. 2. Estimation of Total Protein

Total protein of *B. diffusa* was estimated according to Lowry *et al*. (1951). Bovine Serum Albumin (66 KDa) was used as the standard.

Extraction

Two hundred mg each of root, stem and leaves from the randomized samples of each treatment and control were weighed separately. The weighed tissues were ground using mortar and pestle in chilled distilled water. The homogenate was transferred to centrifuge tubes and equal volume of 10% Trichloroacetic acid was added, mixed well and kept undisturbed in a refrigerator for flocculation.

The precipitated homogenate was centrifuged for 10 minutes, supernatant was decanted off and 2% TCA was added to the residue and again centrifuged, supernatant was decanted off. The precipitate was washed with 80% acetone to remove the pigments. Two washes were carried out in 80% acetone and final washing in anhydrous acetone. Five ml of 0.1 N NaOH was added to the pellet in each centrifuge tube and boiled for 5 minutes in water bath, cooled and centrifuged. The supernatant was then transferred to test tubes and used for protein estimation.

Estimation

Reagents

A – 2% Sodium carbonate in 0.1N Sodium hydroxide

B – 0.5% Copper sulphate in 1% Potassium sodium tartarate

C – Alkaline Copper sulphate solution: Mixed 50 ml A and 1 ml of B prior to use.

D – Folin-Ciocalteau reagent

Suitable aliquots were taken in duplicates from each preparation. Volume was made up to 1 ml with double distilled water. Then 5 ml of reagent C was added to each tube, mixed well and kept at room temperature for 10 minutes, and 0.5 ml 1N Folin-Ciocalteau reagent was added with immediate mixing. The tubes were kept for 30 minutes for color development. Absorbance (Optical Density) was read at 700 nm using a Shimadzu UV-Visible spectrophotometer (Model UV – 1601).

9. 3. Protein Profile

Protein profile of the treatments and control (root, stem and leaves) of *B. diffusa* were analysed by SDS Poly Acrylamide Gel Electrophoresis (Gaal *et al*., 1980).

Extraction of Protein

Five hundred milligrams each of the root, stem, and leaf tissues of the plants of control as well as the treatments were used for PAGE analysis. Tissues were homogenized using a chilled mortar and pestle in 50mM phosphate buffer in the presence of mercaptoethanol as phenolic binder and 10% sodium dodecyl sulphate. The homogenate was centrifuged at 16 000Xg for 20 minutes using a Plastocraft (model ROTA R4 Rv/Fm) refrigerated centrifuge at 4^0C and the supernatant was collected.

Reagents of SDS PAGE

Stock Acrylamide solution (30%)

Acrylamide	- 30 g
Bis-acrylamide	- 0.8 g
Double distilled water	-100 ml

Stored in amber colored bottle.

Stacking Gel Buffer (0.5 M Tris HCl pH 6.8)

Tris Buffer	- 6g
Double distilled water	- 40 ml
1 M HCl	- 48 ml

Made up to 100 ml,filtered with Whatman No. 1 filter paper and stored at 4ºC.

Resolving Gel Buffer (2 M Tris HCl pH 8.8)

Tris Buffer	- 24.2 g
1 M HCl	- 32 ml

Made up to 100 ml, filtered with Whatman No. 1 filter paper and stored at 4ºC.

Reservoir Buffer (pH 8.3)

Tris Buffer	- 3 g
Glycine	- 14.4 g
Sodium Dodecyl Sulphate	- 1 g

Dissolved and made up to 1 Litre, stored at 4ºC.

Staining Solution (Coomassie Brilliant Blue)

Coomassie Brilliant Blue	- 200 mg
Methanol	- 90 ml
Glacial Acetic acid	- 10 ml

Destaining Solution

Methanol	- 40 ml
Glacial Acetic acid	- 10 ml
Double distilled water	- 50 ml

Protein Marker (Genei Narrow Range- 3-43 KDa)

Protein	KDa
Ovalbumin	- 43
Carbonic Anhydrase	- 29
Soybean Trypsin Inhibitor	- 20.1
Lysozyme	- 14.3
Aprotinin	- 6.5
Insulin	- 3

Gel Casting

The glass plates, comb and spacers were washed in absolute alcohol and wiped with tissue paper. Then the plates were cleaned with acetone and spacer was positioned in-between the glass plates one on each side. The glass plates with the spacer were placed in casting tray and clips were tightened, so as to avoid any leakage while casting the gel.

Preparation of Resolving Gel

Resolving gel buffer	- 5 ml
30% Acrylamide stock	- 6.6 ml
Double distilled water	- 8.12 ml

10% Sodium Dodecyl Sulphate	-100 µl
0.1% Ammonium persulphate	-100 µl
TEMED	- 10 µl

The resolving gel was poured into the space between the glass plates placed in the casting tray. Distilled water was added on the top of the resolving gel to form a layer so as to prevent its contact with air and kept it for half an hour. Later distilled water was removed by decanting and the edge of the glass plate was wiped with tissue paper.

Preparation of Stacking Gel

Acrylamide	-0.99 ml
Double distilled water	-1.94 ml
Stacking gel buffer (1 ml diluted to 4 ml)	-3.0 ml
10% Sodium Dodecyl Sulphate	-30 µl
0.1% Ammonium persulphate	-30 µl
TEMED	-10 µl

The stacking gel was poured between the glass plates on the top of the resolving gel layer. Then the comb was placed gradually from one end slanting to avoid bubble formation and the setup was kept undisturbed. The

comb was removed after half an hour and the wells were cleaned with the reservoir buffer.

Sample Preparation for Gel Loading

Test sample - 40 µl

10% Sucrose - 10 µl

Bromophenol blue - 10 µl

Gel loading buffer - 1 ml

Fifty-microlitre sample was taken to load the wells. Protein marker diluted with extraction buffer was also run along with the sample. Then the apparatus (Genei Mini vertical gel casting unit) was assembled for running. The lower and upper tank was filled with 300 ml of reservoir buffer and the electrodes were connected to a power pack. Then power was switched on and voltage was set at 80 V. After about one hour the voltage was raised to 100 V when the loaded protein stored at bottom of the well. Power was switched off when the bands reached at the bottom of the resolving gel. The Gel Unit was taken out and the buffer drained off. The apparatus was unscrewed and the spacers were removed. The gel was taken out and kept in the staining solution for overnight. Then it is transferred to destaining solution. After proper destaining, photographs of the gels were taken using a digital camera

(Canon, EOS 450D, 12MP). Molecular weights of the bands were determined using Quantity One software.

9. 4. Total Free Amino Acids

Total free amino acids of plant parts such as root, stem and leaves of treated and control plants of *B .diffusa* were estimated according to the method of Lee and Takahashi (1966).

Extraction

One gram of fresh tissue was homogenized in 80 % (v/v) alcohol using a clean glass mortar and pestle. The homogenate was transferred to a round –bottomed flask fitted with vertical condensor and refluxed on a boiling water bath for 2 hours. Then the suspension was centrifuged and the supernatant was collected. The residue was re-extracted with 80% alcohol and after each centrifugation the supernatant was combined with the original supernatant. The combined supernatant was then evaporated to dryness over a boiling water bath, eluted using a known quantity of 10% iso-propanol. This extract was used for the determination of total free amino acids using ninhydrin.

Preparation of ninhydrin-citrate –glycerol reagent

One ml of 1% (w/v) ninhydrin solution in 0.5M citrate buffer (pH 5.5) was mixed thoroughly with 2.4 ml of glycerol and 0.4 ml of 0.5M citrate buffer (pH 5.5).

Estimation

To 0.2 ml of the sample, 3.8 ml of ninhydrin-citrate-glycerol mixture was added. After shaking well, the mixture was heated in a boiling water bath for 12 minutes and cooled to room temperature, by keeping under tap water. Within one hour the optical density of the resultant solution was measured at 570 nm using a Spectrophotometer GENESIS 20. The reagent blank was prepared by mixing 0.2 ml water and 3.8 ml ninhydrin-citrate-glycerol. Glycine was used as the standard.

9. 5. Proline

Proline content in the plant parts was estimated according to the method of Bates *et al*. (1973).

Extraction

One gram fresh tissue of plant parts such as root, stem and leaves each of the experimental and control plants was homogenised in 10 ml of 3% (w/v) aqueous sulfocylic acid using a clean glass mortar and pestle. The homogenate was transferred to centrifuge tubes and centrifuged for 10

minutes at 10, 000 rpm and the supernatant was collected and estimation of proline was done using acid ninhydrin.

Preparation of acid ninhydrin

Acid ninhydrin was prepared by dissolving 1.25 gm of ninhydrin in a mixture of 30 ml of glacial acetic acid and 20ml of 6 M ortho phosphoric acid.

Estimation

From the supernatant, 2 ml was taken in test tubes in triplicate and equal volume of glacial acetic acid and acid ninhydrin were added to it. The tubes were then heated in a boiling water bath for one hour and then the reaction was terminated by placing the tubes in an ice bath. For colour development, 4 ml of toluene was added to the reaction mixture and stirred well for 20-30 seconds. Then the chromophoric toluene layer was separated carefully and brought to room temperature. The colour intensity of the solution was measured at a wave length of 520 nm using Spectrophotometer (GENESIS 20). L-proline was used as the standard.

9. 6. Quantitative Estimation of Chlorophyll

Chlorophyll estimation was done according to Arnon (1949). Two hundred mg fresh leaf tissue was homogenized with 80% acetone. The extract was centrifuged for 5 minutes and the supernatant was collected. The residue

was re-extracted with 80% acetone and centrifuged. The process was repeated till the pellet became colorless. The final volume of the combined supernatant was noted. The absorbance of the extract was read at 663 & 645 nm using a UV-Visible spectrophotometer (Shimadzu Model UV –1601). Total chlorophyll, chlorophyll 'a' chlorophyll 'b' and chlorophyll a/b ratio were calculated using the formula suggested by Arnon (1949). The chlorophyll was expressed as mg/g fresh tissue.

Chlorophyll a = 12.7 (A663) – 2.69 (A645) V/1000 x W

Chlorophyll b = 22.9 (A645) – 4.68 (A663) V/1000 x W

Total Chlorophyll = 20.2 (A645) + 8.2 (A663) V/1000 x W

Where, A663 - Absorbance at 663 nm

A645 - Absorbance at 645 nm

V - Volume of extract

W - Weight of tissue

9.7. **Nitrate Reductase assay**

Nitrate reductase activity was measured according to the method suggested by Hageman and Reed (1980) as described by Sadasivam and Manikam (1992). Potassium nitrate was used as substrate. Sodium nitrate was used as standard.

Five hundred mg of fresh samples of root, stem and leaves of *B. diffusa* was homogenized in a known volume of medium containing 1 mM EDTA, 10 mM cysteine and 25 mM potassium phosphate adjusted to a final pH 8.8 and filtered through four layers of cheese cloth and then centrifuged for 20 minutes at 20,000g. The supernatant was decanted through glass wool and used for assay.

The reaction was initiated by adding 0.2 ml enzyme extract to a reaction mixture containing 0.5 ml of 0.1 M phosphate buffer (pH 7.5), 0.2 ml of 0.1 M potassium nitrate solution, 0.4 ml of 2mM NADH solution and 0.7 ml water and the assay system was incubated at 30°C for 15 minutes. The reaction was terminated by the rapid addition of 1 ml of 1% sulphanilamide prepared in 2.4 N HCl followed by 1 ml of 0.02% N-(1- naphtyl) ethylenediamine dihydrochloride reagent. Absorbance was read at 540 nm after 30 minutes. Calculation was made with the help of a standard graph prepared with sodium nitrite and activity was expressed as quantity of nitrite formed per hour per gram dry tissue.

9.8. Phenolics

Total phenolics was estimated using Folin-Denis Reagent (Folin and Denis, 1915). Tannic acid was used as standard.

Extraction

Extraction was done as per the procedure described under free amino acid estimation. The supernatant was collected after centrifugation of refluxed homogenate. The total volume of the supernatant was noted.

Estimation

Aliquots of 2 ml in triplicate were pipetted out and equal volume of Folin-Denis reagent was added. The contents were thoroughly mixed and after 3 minutes, 2 ml of IN sodium carbonate was added. This mixture was kept for one hour after thorough mixing for colour development. The optical density of the resultant solution was measured at 700 nm using a Spectrophotometer (GENESIS-20).

9.9. Lipid Peroxidation

The lipid peroxidation in different tissues of *B.diffusa* was estimated according the procedure of Heath and Packer (1968). Five hundred milligram each of root, stem and leaf was crushed in 5 ml of 5% Trichloroacetic acid. The homogenate was centrifuged at 12, 000 rpm for 15 minutes at 25^{o}C. Two ml of supernatant was mixed with an equal aliquote of 0.5 % thiobarbaturic acid in 20 %TCA.

The solution was heated at 95^{o}C for 25 minutes and then centrifuged at 3000 rpm for 2 minutes.The absorbance of the supernatant was measured at

532nm and 600nm against the reagent blank using Spectrophotometer (GENSIS-20). The absorbance value at 532 nm was correlated for non specific turbidity by subtracting absorbance value at 600nm; the amount of malondialdehyde was calculated by using an extinction coefficient of 155 mM^{-1} Cm^{-1} .

9.10. Guaiacol Peroxidase Assay

Five hundred mg each of root, stem and leaf of control as well as treatments were weighed and homogenised using a pre–chilled glass mortar and pestle. The medium for homoginsation was prepared by mixing 50 mM Tris –HCl of pH 7.5 and 200 mg polyvinylpolypyrolidone (PVPP) as phenolic binder. The extract was filtered through two layers of muslin cloth and the filtrate was made up to 10 ml using the TRIS-HCl buffer. The filtrate was centrifuged at 16000x g for 15 minutes in a Kubota refrigerated centrifuge (Model KR 20000 T) at 4^{o} C. The supernatant was transferred to a clean test tube and kept in an ice bath. A part of the extract was used for enzyme assay and the other part was used for the estimation of soluble protein.

Enzyme Assay

The assay system was followed after Ablees and Biles (1991) with minor modifications.

The reaction mixture consisted of 1.5 ml of 100 mM potassium phosphate buffer at pH 7.0, 0.3 ml of 10mM guaiacol, 0.3 ml of 10 mM hydrogen peroxide, 0.3 ml of enzyme extract and 0.6 ml of distilled water together making up to 3. 0 ml. All the ingredients of the reaction mixture except hydrogen peroxide were added and mixed well in a test tube. The hydrogen peroxide was added at the end to initiate the enzyme activity. The enzyme activity was read at 470 nm by direct spectrophotometry for a period of 3 minutes immediately after the addition of hydrogen peroxide using a UV –Visible Spectrophotometer (Shimadzu). A total of 6 readings were taken at an interval of 30 seconds each. The enzyme activity was measured in terms of the amount of enzyme required to decompose 1.0 µmol of H_2O_2 and is expressed as µmol of H_2O_2 mg^{-1} protein min^{-1}.

9.11. Superoxide Dismutase Assay

For the estimation of superoxide dismutase (SOD) activity the protocol of Giannoplitis and Ries (1977) was adopted. Five hundred milligram of tissue of plant parts were thoroughly homogenised using a chilled glass mortar and pestle in a medium consisting of 50 mM phosphate buffer (pH 7.8) and 100 mg of polyvinyl polypyrolidone as phenolic binder. The homogenate was centrifuged at 16000xg for 15 minutes in a refrigerated centrifuge (Kubota KR 20000 T) at 4^{o} C. Part of the supernatant collected was used for

the assay of SOD activity and the remaining part was used for the estimation of protein.

Enzyme assay

The SOD activity was assayed photochemically. The sets of assay system were prepared separately, one for the assay, one for the dark–control and one for the light-control. The reaction mixture consisted of 0.1 ml of 1.5 M sodium carbonate, 0.3 ml of 0.13 M methionine, 0.3 ml of 10µM. EDTA, 0.3 ml of 13 µM riboflavin, and 0.3 ml of 0.63 mM nitrobluetetrazolium. The nitroblue tetrozolium was withheld in the light –control system. To the reaction system, 0.1 ml of the enzyme extract was added to the blank and to the light-control system. The reaction mixture was made up to 3.0 ml using 50 mM phosphate buffer (pH 7.8). The tubes with dark –control samples were kept in a dark chamber and the tubes of the light-control and the assay systems were kept under a fluorescent lamp. After 30 minutes of incubation the optical density of the solutions of the assay, the dark –control and the light-control systems were measured at 560 nm using Spectrophotometer (GENESIS-20). Results were expressed as units SOD mg^{-1} gram tissue. One unit of SOD was defined as the enzyme activity that inhibited the photoreduction of nitroblue tetrazolium to blue formazan by 50%.

Soluble protein

The amount of protein in the enzyme extract was determined according to the procedure of Lowry *et al*. (1951).

9.12. Catalase Assay

Five hundred mg of the root, stem and leaf tissue of *B. diffusa* were homogenized using a chilled glass mortar and pestle in a medium consisting of 50 mM phosphate buffer (pH 7.0) and 200 mg of polyvinyl polypyrolidone as phenolic binder. The homogenate was filtered through two layers of muslin cloth and was made up to 10 ml using phosphate buffer. The filtrate was then centrifuged (Kubota KR 20000 T) at 16000xg for 15 minutes in refrigerated centrifuged at 4^0C. A part of the extract was used for enzyme assay and the remaining part was used for the estimation of protein.

Catalase assay system Kar and Mishra (1976) consisted of 1.0 ml of 50 mM phosphate buffer pH 7.0, 2.0 ml of the enzyme extract and 1.0 ml of 30mM hydrogen peroxide. The phosphate buffer and the enzyme extract were pipetted out and mixed well in a test tube. To this hydrogen peroxide was added to initiate the enzyme activity. Immediately after the addition of hydrogen peroxide, enzyme activity was measured at 240 nm for 90 seconds at 15 seconds interval using a Shimadzu UV- Visible Spectrophotometer (Model UV-1601).

9.13. Quantitative Estimation of Cadmium, Chromium, Mercury and Lead

Cadmium, chromium, mercury and lead content of the root, stem and leaf tissues were analyzed using Atomic Absorption Spectrophotometer. Samples were prepared according to the method of Allan (1969). Different plant parts- root, stem and leaf tissues of treatments and control were sampled and were dried at 60°C in a hot air oven.

Known weight of the dried sample were digested by refluxing in 10:4 ratio of nitric acid and perchloric acid until the solution become colorless using Kjeldahl's flask heated in a sand bath. Then the digest was transferred to standard flask and volume was made up to 50ml and kept in screw-capped containers. Atomic Absorption Spectrophotometer (PERKIN ELMER Model A, Analyst 300) available at Cashew Export and Promotion Council (CEPC), Kollam, was used for the estimation of heavy metals present in the digested samples.

10. Anatomical Study

For anatomical studies, uniformly cut pieces of root and stem of control and treatments were fixed in FAA, dehydrated through alcohol TBA series and embedded in paraffin wax (Johansen, 1940; Berlyn and Miksche, 1976). Using a Rotary Microtome (Leica Model RM 2125RT). Individual blocks were cut at 10µm and sections were used for anatomical staining.

Deparaffinised sections were stained with Toluidine Blue according to the procedure of Khasim (2002). Photomicrographs were taken by using Nikon Microscope (Model, ECLIPSE E 400) fitted with Nikon Digital Camera and Digital image analyser.

11. Bioaccumulation of Heavy Metals in Medicinal Plants

11. 1. Selection of plants

Twenty medicinal plants namely *Aerva lanata* (L.) Juss. ex Schult., *Alternanthera tenella* Colla, *Amaranthus spinosus* L., *Andrographis paniculata (*Burm.f.) Wall. ex Nees., *Beloperon plumbaginifolia* L., *Blepharis maderaspatensis* (L.) Roth, *Boerhavia diffusa* L., *Catharanthus roseus* (L.) G. Don, *Eclipta prostrata* (L.) L., *Eupatorium triplinerve* Vahl., *Kyllinga nemoralis* (J. R. & G. Forst.) Dandy *ex* Hutch. & Dalz., *Leucas lavandulifolia* Sm., *Oxalis corniculata* L., *Phyllanthus amarus* Schum. &Thonn., *Portulaca oleracea* L., *Rubia cordifolia* L., *Scoparia dulcis* L. *Sida acuta* Burm.f. subsp.*acuta*, *Sida alnifolia* L. and *Vernonia cinerea* (L.) Less. were selected for the study of pattern and potential of these plants to accumulate cadmium, chromium, mercury and lead.

11. 2. Cultivation

Seedlings/ vegetative propagules were collected from different locations near Calicut University campus and Botanical Garden. Pots were

filled with garden soil: sand: dried powdered cow dung in 2:1:1 ratio and seedlings / propagules were planted in each pot. The pots were placed in net House of Calicut University Botany department and maintained under natural conditions and watered regularly.

11. 3. Treatments with Heavy metals

For treatment with heavy metals, healthy propagules cultivated as described above, were planted in plastic pots containing 4 Kg of garden soil and maintained under green house condition. After acclimatization of the seedlings in plastic pots, solutions of $CdCl_2$, $K_2Cr_2O_7$, $HgCl_2$ and Pb $(CH_3COO)_2$ prepared in various concentrations were added. The concentration of each solution to impart visible growth retardation varied among the 20 species and hence specific concentrations were selected by trial and error methods and standardized. List of 20 medicinal plants and quantities of the four metals each added for treatments are given in Table 3. Seedlings cultivated in pots without any heavy metal addition served as control.

Table 3: Selected plants and concentrations of Heavy Metal treatment for bioaccumulation study

Sl. No	Name of the plant	Family	Treatments	Heavy metal applied(mg)
1	*Aerva lanata*	Amaranthaceae	Cadmium	1.979
			Chromium	70.56
			Mercury	0.203
			Lead	182.0
2	*Alternanthera tenella*	Amaranthaceae	Cadmium	0.593
			Chromium	21.16
			Mercury	0.025
			Lead	54.62
3	*Amaranthus spinosus*	Amaranthaceae	Cadmium	2.639
			Chromium	94.08
			Mercury	0.135
			Lead	24.27
4	*Andrographis paniculata*	Acanthaceae	Cadmium	0.650
			Chromium	23.52
			Mercury	0.025
			Lead	60.69
5	*Beloperon plumbaginifolia*	Acanthaceae	Cadmium	2.639
			Chromium	94.08
			Mercury	0.135
			Lead	24.27
6	*Blepharis maderaspatensis*	Acanthaceae	Cadmium	1.978
			Chromium	70.56
			Mercury	0.101
			Lead	182.0
7	*Boerhavia diffusa*	Nyctaginaceae	Cadmium	2.639
			Chromium	94.09
			Mercury	0.135
			Lead	24.27
8	*Catharanthus roseus*	Apocynaceae	Cadmium	0.593
			Chromium	21.16
			Mercury	0.025
			Lead	54.62
9	*Eclipta prostrata*	Asteraceae	Cadmium	0.593
			Chromium	21.16
			Mercury	0.025
			Lead	54.62
10	*Eupatorium triplinerve*	Asteraceae	Cadmium	2.639
			Chromium	94.08
			Mercury	0.135
			Lead	24.28

11	*Kyllinga nemoralis*	Cyperaceae	Cadmium	3.300
			Chromium	117.6
			Mercury	0.169
			Lead	303.4
12	*Leucas lavandulifolia*	Lamiaceae	Cadmium	0.593
			Chromium	21.16
			Mercury	0.025
			Lead	54.62
13	*Oxalis corniculata*	Oxalidaceae	Cadmium	0.650
			Chromium	23.52
			Mercury	0.025
			Lead	60.69
14	*Phyllanthus amarus*	Euphorbiaceae	Cadmium	0.593
			Chromium	21.16
			Mercury	0.025
			Lead	54.62
15	*Portulaca oleracea*	Protulacaceae	Cadmium	0.593
			Chromium	21.16
			Mercury	0.025
			Lead	54.62
16	*Rubia cordifolia*	Rubiaceae	Cadmium	1.979
			Chromium	70.60
			Mercury	0.101
			Lead	182.0
17	*Scoparia dulcis*	Scrophulariaceae	Cadmium	1.979
			Chromium	94.08
			Mercury	0.135
			Lead	24.27
18	*Sida acuta*	Malvaceae	Cadmium	1.979
			Chromium	70.56
			Mercury	0.203
			Lead	182.0
19	*Sida alnifolia*	Malvaceae	Cadmium	1.970
			Chromium	70.56
			Mercury	0.203
			Lead	182.0
20	*Vernonia cinerea*	Asteraceae	Cadmium	0.593
			Chromium	21.16
			Mercury	0.124
			Lead	54.26

11. 4. Sampling

Sampling for estimation of bioaccumulation of metals was done after 30±2 day's growth. Plants were uprooted from the pot without loosing any part of the roots. After washing, seedlings were cut in to root, stem and leaf and the samples were analyzed for biomass and estimation of heavy metal (Cd, Cr, Hg and Pb).

10. 5. Dry weight

Dry weight of root, stem and leaves samples was determined following the method described earlier (9.1).

11.6. Quantitative Estimation of Cadmium, Chromium, Mercury and Lead

Cadmium, chromium, mercury and lead contents of the root, stem and leaf tissues were analyzed using Atomic Absorption Spectrophotometer as per the procedure described earlier (9.13).

12. Statistical Analyses

All experiments were carried out for a minimum of five times and the mean values are given in tables and figures. Standard deviation and standard error were also calculated. The values in tables are mean ± standard error. Test of significance was done following Fisher's 't' test.

DISCUSSION

Plants encounter elevated levels of essential elements and trace or moderate ~~quantit~~quantities~~y~~ of toxic, non - essential elements by exhibiting growth retardation, deranged metabolism and/ or acquiring tolerance mechanism. An emphatic aspect of metal toxicity and tolerance is dissimilar specificity of plant species towards each and every element~~s~~ (Prasad, 1997; Fodor, 2002; Clemens, 2006). A striking feature found in the plant kingdom is the naturally selected hyper accumulation potential of some plants towards metals -(Baker and Brooks,1989). According to Clemens (2001) metal tolerance is found in all plants but the degree of tolerance varies from species to species and varieties. Hence laboratory investigations dealing with heavy metals in plants involve simulation experiments with a wide range of concentration levels and durations.

Several simulated experiments on plants with heavy metals have shown that optimal concentration to impart toxicity varies as 25-50 µM $CdCl_2$ in *Bacopa monnieri* (Ali *et al*., 1998), 10-100µM $CdCl_2$ in *Arabidopsis thaliana*, (Perfus – Barbeoch, *et al*., 2002), 0.05mM $CdCl_2$ in *Triticum aestivum* (Abdeu-l-Latif, 2008), 64 µM chromium in *Bacopa monnieri* (Sinha, 1999), 12-24 mg/l chromium in *Medicago sativa* (Shanker *et al*., 2003), 5-10µM $HgCl_2$ in *Pisum sativum* (Beauford *et al*., 1977), 1-2 µM $Hg(NO)_2$ in *Chromolaena odorata* (Velasco-Alinsug *et al*., 2005), 1-20 µM $PbCl_2$ i~~I~~n *Oryza sativa* (Kim *et al*., 2002) and 100-800 mg/kg lead in *Xanthium strumarium* (Sonmez *et al*., 2008).

By conducting repeated screening experiments under simulated laboratory conditions on *Boerhaavia diffusa* cultivated in Hoagland nutrient solution containing different concentration of $CdCl_2$, $K_2Cr_2O_7$, $HgCl_2$ and $(CH_3COO)_2Pb$ for different periods, the present author selected the concentrations of 30 µM cadmium, 400 µM chromium, 10 µM mercury and 600 µM lead to which the experimental plant showed approximately similar visible symptoms of growth retardation retaining their survival in those concentrations (Figs. 1 & 2).

General growth retardation is an important established symptom of heavy metal toxicity in plants (Foy *et al*., 1978; Lepp, 1981; Fett *et al*., 1994; Shaw, 1995; Prasad, 1997; Salt *et al*., 1998; Orcutt and Nilsen, 2000; Fodor, 2002; Pilon-Smits, 2005; Chen *et al*., 2005). *B. diffusa* exhibits reduced root growth as a result of cadmium, chromium, mercury and lead and more or less similar trend is shown during all intervals of growth (Table 4); Figs. 3 & 4). In nutrient culture, roots are directly in contact with metal ions and hence the immediate effect is expressed as stunted growth of roots. More inhibition of root growth is affected by cadmium followed by mercury compared to other metals in *B. diffusa* .

Tolerance index calculated on the basis ratio of root length ratio of experimental to that of control (Turner, 1994) shows gradual reduction of tolerance index during growth in all treatments and maximum tolerance index is shown by plants treated with lead followed by chromium (Table 5). According to Wilkins (1978) and Wong and Bradshaw (1982), primary toxic effects of

heavy metals is root growth inhibition and this parameter is an ideal index to measure the degree of tolerance. Rooted propagules of *B. diffusa* when exposed to the heavy metal showss rapid response and resultant impact as observed in significant changes in root length and tolerance index. The difference in the tolerance index towards different metals —and the bioaccumulation pattern of the respective heavy metals are found to be integrally related each other (Table 518 & 18; Figs. 5 & 40)). Maximum tolerance index is shown by *B. diffusa* towards lead followed by chromium (Table 5) and these values are positively correlated and the bioaccumulation potential of these metals are high and very high comparatively that accumulate maximum in the root tissue (Table 18); Fig. 40) are positively correlated whereas plants treated with cadmium and mercury are characterised by the least value of tolerance index as well as very low and the accumulation potential of these metals are very low in all parts of the plant.

According to Zou *et al* ., (2006) inhibited mitotic index in the root apex leads to stunted growth in *Amaranthus viridis*. Although root growth retardation is the symptoms of heavy metals, the metabolic role of heavy metals in root growth impairment phenomenon is not fully known. Linger *et al.*, (2005) suggested that cadmium changes the capability of cell division of mersistematic cells and the author suggested that this view is highly plausible after scrutinising many reports on adverse effects of heavy metals on cell division and cell elongation. So According to Zou *et al*. (2006) inhibited mitotic index in the root apex leads to stunted growth in *Amaranthus viridis*. tThe sensitivity/

tolerance exhibited by *B. diffusa* towards different concentrations of cadmium, chromium, mercury and lead are depicted in the pattern of root length also (Table 4~~);~~ ~~Figs. 3 & 4)~~. Contradictory to the above views, Kiran and Sahin (2006) reported that $CdCl_2$ and $HgCl_2$ induce root development by stimulating cell division in the roots of *Lens culinaris* ~~lenticell plant~~. Similarly in *Medicago sativa* low concentration of CrVI (5ppm) and Cd (20 ppm) do not inhibit root growth (Peralta *et al*., 2000). These differences may be due to either to significant differences of concentration or genotypic variations.

Growth inhibition has been reported as an important and established visible effect in plants by heavy metals like cadmium in *Cannabis sativa* (Linger, *et al*., 2005) and *Oryza sativa* (Kim *et al*, 2002), *Vigna* species (Jamal *et al*., 2006; Ratheesh~~-—~~Chandra *et al.*, 2010), lead in *Brassica* species (Hoss~~aa~~einii *et al*., 2007), chromium in *Amaranthus viridis* (Zou *et al*., 2006), mercury in *Triticum aestivum* (Setia and Bala, 1994) and *Bacopa monnieri*, (Hussain, 2007).

Anatomy of root and stem shows cellular damage due to the treatment with all four metals. Since the plants are grown in nutrient culture medium containing cadmium, chromium, mercury and lead, the roots are in direct contact with the toxic metal ions and hence are adversely affected, resulting in cellular damage and general thinning and brittle texture of roots. Among the four metals, accumulation as stained masses are seen in the root tissue of ~~only in the~~ plants treated with cadmium, chromium and lead ~~lead~~ which show

maximum bioaccumulation as shown by estimated data (Table 18); Figs. 40, 42, 43 & 45).

Anatomy of the stem also exhibit direct impact of the toxic metals. Damage of secondary phloem and reduced size and number of vessels, are found to be the reason for comparatively low translocation of these metals to leaf and adversely affect the influx of photosynthates to the stem and root culminating in general growth retardation. Development of trichomes in the stem epidermis of plant treated with chromium and mercury (Figs. 43 & 44)) is also found to be related to the accumulation of these metals in the stem as nd suggested by Dominguez- Solis *et al*. (2004) who reported the involvement of trichomes in the accumulation of cadmium in *Arabidopsis thaliana*.

Reduction of leaf growth is an important visible symptoms of heavy metal stress in many plants (Prasad, 1997; Fodor, 2002). *B. diffusa* exhibits significant reduction of leaf area as an important effect of all the four metals. Due to cadmium treatment, maximum reduction occurs followed by chromium, mercury and lead (Table 4); Figs. 3 & 4). Significant variations are shown in the stomatal index of *B. diffusa* treated with all the metals. Maximum value of stomatal index in both lower and upper epidermis is shown by plants treated with mercury. Other treatments show only slight increase (Table 6); Fig. 8). Although stomatal distribution in relation to heavy metal stress is not well documented, effect of cadmium has been shown to inhibit water stress tolerance in *Phaseolus vulgaris* (Meidner and Mansfield, 1968; Barceleo and Poschenrieder, 1990), reduce cell wall elasticity (Becerril *et al*.,

19869) and reduce transpiration and increase stomatal resistance (Hernandeg *et al*., 1997)Hussain *et al*., 2006). These views are in consonance with the behaviour of *B. diffusa* in which stomatal index shows only negligible changes in all treatments except mercury. Increased stomatal index is essential only if more transpiration is ought to occur. Perterbarbation of plant- water relationship and osmoregulation of stomatal conductance in *Arabidopsis thaliana* treated with $CdCl_2$, revealed the non – essentiality or requirement of increased stomatal index (Perfus- Barbeoch *et al*., 2002). Nevertheless, *B. diffusa* under mercury stress, exhibit significantly increased stomatal index in both upper and lower epidermis. This observation is directly related not only to transpiration but detoxification of mercury also by phytovolatialization process as reported in *Bacopa monnieri* (Hussain, 2007) and in *Brassica juncea* ((Moreno *et al*., (2008). This aspect will be discussed under bioaccumulation chapter section.

Irrespective of the significant differences in the concentration of each heavy metal which are applied to the nutrient solution on the basis of visible growth retardation, only slight increase of biomass is shown by all treatments without significant difference between the treatments. EventhoughEven though the water potential reduction is known to be affected by heavy metal absorption (Costa and Morel, 1994) and resultant stunted growth (Lepp, 19851; Shaw and Rout,- 19968; Orcutt and Nilsen, 2000; Fodor, 2002), biomass is expected to be reduced in plants under heavy metal stress. In accordance with several reports, biomass reduction is a typical impact of heavy metal stress in

plants which are intolerant to the respective metals (Prasad, 1997; Orcutt and Nilsen, 2000; Cseh, 2002). Notwithstanding, in *Arabidopsis thaliana*, biomass remained unaltered and this observation is interpreted as a detoxification mechanisms of cadmium stress. (Perfus- Barbeoch *et al*., 2002). *B. diffusa* plants exhibits no significant changes in biomass or as the other similar responses of growth performances to different concentrations of cadmium, chromium, mercury and lead (Table 7) presumably due to the tolerance towards the selected concentration. Biomass reduction has been reported in Sunflower under lead stress (Kastoori *et al*., 1998). Similarly, Zhang *et al*., (2000) suggested that cadmium induced biomass reduction in wheat.

According to Baker *et al*., (1994), Ebbs *et al*.,and Kochian (1997, 1998) (1997) and Ebbs and Kochian (1999) biomass production is a significant factor contributing to phytoextraction of metals by plants from polluted soil/water. Pilon-Smits (2005) suggested that phytoextraction is a process defined as use of plants to clean up pollutants accumulation in harvestable tissues. Due to the treatment with all the four heavy metals *B. diffusa* showed slight increased in biomass though not much significant, indirectly exhibiting mild phytoextraction potential the accumulation potential of the plant towards cadmium, chromium, mercury and lead.

Distribution of metabolites also is changed as the response of heavy metal stress in *B. diffusa*. Both total and soluble protein fractions of root, stem and

leaves are significantly reduced in plants treated with cadmium, chromium, mercury and lead in comparison with the control plants during all stages of growth. However, ~~—~~between the metals~~,~~ the difference in the distribution ~~i~~was negligible (Table ~~9~~8)~~;~~; Figs. 8 & 9). ~~The rate of reduction was less in the soluble protein compared to the control.~~ Impaired protein synthesis has~~ve~~ been reported in plants under heavy metal stress. (~~Prasad, 1977,~~ Reddy and Prasad, 1992; Prasad, 1997). Reduced protein content may be due to either inhibition of protein synthesis as reported by (Reddy and Prasad, 1992; ~~,~~ Orcutt and Nilsen, 2000) or unavailability of essential component aminoacids (Prasad, 1997) or inhibition of aminoacids mobilization to the site of protein synthesis (Bishnoi *et al.*, 1993). In *B. diffusa* free aminoacid content of plants treated with all heavy metals is ~~exhibited~~ very high ~~accumulation~~(Table 9) plausibly as a consequence of impaired protein synthesis. Hence it is conceivable that in addition to inhibited translocation of aminoacids due to heavy metal stress as suggested by Bishnoi *et al.*~~,~~ (1993), proper function of protein synthesis machinery also is found to be impaired in all tissues where free aminoacid accumulation is very significant (Table~~–~~ 9; Fig. 13)~~)~~. When the distribution of increasing free aminoacid content is compared to total protein reduction during different stages of growth, both are not parallel each other. More accumulation of total aminoacid reveals indirect evidence for impaired protein synthesis and resultant accumulation under heavy metal stress.

Reduction of soluble protein is not much differed between tissues of the same treatment and between treatments. Majority of enzymes come in the

soluble fraction and hence, inhibition of enzyme activity by heavy metals like cadmium (Sheoran *et al*., 1990), chromium (Bishnoi *et al*., 1993) have been reported to occur in plants. Many enzymes get inactivated by cadmium (Ross, ~~*et al*.,~~ 199~~0~~4). Despite the inhibition of enzyme activity by heavy metals as reported in many plants, drastic reduction of soluble protein is not occurred in *B. diffusa* presumably due to the tolerance ~~of *B. diffusa* the plants~~ towards cadmium, chromium, mercury and lead at the concentration levels applied to the plant. Protein metabolism in *Potamogeton pectinatus* under cadmium stress has been studied (Sanit-di Toppi and Gabbrielli, 1999, Rai *et al*., 1995, 2003) and the result revealed that at lower concentration (0.5-50µM) of cadmium, protein content was decreased where as high concentrations more than 50 µM showed toxic effects. Those authors suggested that decreased protein content in the presence of cadmium is due to breakdown of protein by catabolic enzymes which are activated and destroy the proteins. In *B. diffusa* treated with $CdCl_2$, reduction of both soluble and insoluble protein content can be attributed to the cadmium stress that breakdown protein content and/ or inhibit protein synthesis (Table 8; Figs. 8 & 9)~~)~~.

Polyacrylamide Gel electrophoretic profile of root tissue showed two new bands in *B. diffusa* treated with cadmium, chromium and mercury. But these two bands are dissimilar in molecular weight (Fig. ~~7~~10). A number of new bands are appeared in the stem tissue of plants treated with cadmium, chromium and mercury ~~:~~(Fig. 11). Only one feeble band ~~wa~~is shown by lead treatment. The new bands appeared in the plants treated with heavy metals are absent in the

control ~~plant~~ and hence can be presumed as stress -induced proteins ie. phytochelatins, which have already been reported to be synthesised in plants under heavy metals such as cadmium, chromium, mercury, lead etc. (Grill *et al*., 1985; Verkleij *et al*., 1990;~~,~~ Salt *et al*., 19~~89~~98; Choudhury and Panda, 2005)~~, Verkleij *et al*., 1990~~). According to Grill *et al*. (1989) phytochelatins are synthesised due to heavy metals and the role of phytochelatin synthase activity differs from metal to metal. Phytochelatin synthesis for the sequestration of heavy metal toxicity has been reported in plants (Rauser, 1987; Reddy and Prasad, 1990; Kubota *et al*., ~~abata *et al*.,~~ 2000; Cobbet and Goldsbrough, 2002). The protein bands observed under individual metals~~, –~~exhibit difference in number and molecular weight probably either due to the differences in their metal binding property or differences in tolerance potential of *B. diffusa* towards each metal.

~~s. A class of metal binding peptides in plants have been reported to be synthesised due to heavy metals and the role of phytochelatin synthase activity differs from metal to metal. (Grill *et al*., 1989 a, 1989 b).~~ Since the PAGE analyses of protein was done only in the samples of the first interval, ~~(2nd day),~~ the role of the phytochelatin in the sequestration of heavy metals is ambiguous in *B. diffusa* because phytochelatin synthesis has been reported to start during some hours after the heavy metal treatment (Rauser, 1995). Nevertheless, unlike the root and stem, maximum number of new protein bands are observed in the leaf tissue (Fig. 12~~-~~) probably revealing the sequestration and hence the leaf metabolism is not adversely affected. It is also worth mentioning that leaf

tissues exhibit accumulation of all heavy metals in the order Pb>Hg>Cr>Cd (Table 18). So their sequestration is presumed to take place by the formation of phytochelatins which occur in abundance in the leaf. (Fig. 12). Induction of new protein synthesis has been reported in wWheat and mMustard plants irrigated with tannerary effluents containing many heavy metals inclusive of cadmium . (Chandra *et al*., 2009). Zhang *et al*., (19992002) suggested that cadmium tolerance is enhanced by over - expressing the enzyme, —γ-glutamyl cysteine synthetase which is the key enzyme involved in the synthesis of phytochelatins. In *Solanum tuberosum*, phytochelatin synthase activity increases proportional to the concentration of $CdCl_2$ and there by the tissues possess more efficient cadmium detoxification mechanism (De Vos *et al*., 1992; Stroinski and Zielezinska, 2001).

Leaf protein profile shows many bands and significant variation is observed between treatments. Maximum new protein bands are present in the leaves of plants treated with mercury whereas the plants treated with lead exhibit very few protein bands (Fig. 12) revealing different responses of *B. diffusa* towards different metals and / or different concentrations. In *Potomogeton pectinatus*, protein synthesis is induced under cadmium stress up to 50 µM concentration, where as in *B. diffusa* protein content is reduced at the concentration 30µM cadmium. This disparity may be due to genetic variations of plants towards the sensitivity/tolerance to heavy metals as suggested by Angelova *et al*., (2006) and Meers *et al*., (2007). When the distribution of increasing free aminoacid content is compared to total protein reduction during

~~different stages of growth, both are not parallel each other. More accumulation of total aminoacid reveals indirect evidence for impaired protein synthesis and resultant accumulation under heavy metal stress.~~

Proline accumulation is observed in all tissues of *B. diffusa* due to the treatments with cadmium, chromium, mercury and lead. Even ~~-~~though the root system is in contact with the heavy metals, proline accumulation of the root tissue remains unchanged in all treatments (Table 10; Fig. 14). Under stressed conditions proline has multiple functions and the important one is regulation of osmoticum change imposed by drought~~.~~ (Sardhi and Saradhi, 1991; Rout *et al*., 1998~~7, Sardhi *et al*., 1991~~). In consonance with this view, *B. diffusa* roots suffer more osmoticum stress due to the heavy metal ions in the growth medium to which the roots are exposed. However, the effect of variation in the molar concentration is not reflected in the distribution of proline in the roots.

~~Accumulation of prolin has been reported in plants *Ocimum tenuiiflorum* due to chromium treatment (Rai *et al*., 2004), cadmium *Triticum aestivum* (Abdul – Latif, 2008) and *Phaseolus vulgaris* treated with cadmium (Zengin and Munzuroglu, 2005), According to those authors, lead, copper and mercury also induce proline accumulation in *Phaseolous vulgaris*.~~

Proline accumulation increase is observed in the stem tissue mostly after 12 days and maximum accumulation occurs in the samples of 20th day compared to all other tissues (Table 10~~)~~; Fig. 14) and the ~~accumulation~~abundant occurrence of proline in the stem is found to be related to the bioaccumulation of all the

metals in the stem because significant increase of both proline and bioaccumulation of metals occur concurrently. This observation is in conformity with the view put forth by Hopkins, (2000) and Rai *et al*. , (2004) who suggested that high accumulation of proline in the chromium - treated *Ocimum tenuiiflorum* is related to the strategies adapted by the plant to cope up with heavy metal toxicity as proline has multiple functions such as osmoticum maintenance, scavenging of free radicals stabilizing of membranes etc. Accumulation of proline has been reported in *Triticum aestivum* (Abdel – Latif, 2008) and *Phaseolus vulgaris* treated with cadmium (Zengin and Munzuroglu, 2005). –Free radicals scavenging by proline is reported by Matysuik *et al*., (2002). However, in the leaves of *B. diffusa* similar correlation is not feasible because leaves contain comparatively low bioaccumulation of all metals except lead.

According to Costa and Morel, (1994), cadmium stress imposes decrease in water potential which is alleviated by proline accumulation. Significant increase of proline content occurs in *B. diffusa* during all stages of growth under cadmium, chromium, mercury and lead treatments whereas as a constitutive aminoacid, proline content of control plants is very low (Table 10); Fig. 14). Among the four treatments metals, more proline accumulation is observed in plants treated with chromium and lead , and these metals exhibit comparatively more bioaccumulation potential (Table 10); Fig. 14) leading to reduced water potential and the resultant this hyper osmotic stress which is

mitigated by proline as suggested by Zengin and Murzuroglu (2005) in *Phaseolus vulgaris* seedlings treated with cadmium, copper, lead and mercury.

In *Boerhavia diffusa*, cadmium, chromium, mercury and lead treatment resulted in more or less uniform reduction of chlorophyll a, chlorphyll b, and chlorophyll a/b ratio during all developmental stages (Table 11; Fig. 15). Chlorophyll is often measured in order to assess the impacts of environmental stress since the changes in the pigments are linked with visual symptoms of growth disorder and photosynthetic productivity (Parekh *et al*., 1990). But due to lead and cadmium treatment, reduction of chlorophyll a is comparatively loweress than other metals and so chlorophyll a/b ratio is higher. An indirect correlation can be drawn between these values and cadmium accumulation. Similar results have been reported in plants such as Radish and Lettuce due to cadmium stress (Baszynskizyml *et al*., 1980), *Phaseolus aureus* treated with mercury (Shaw and Rout, 1998), Mung bean due to lead (Prasad and Prasad, 1987) and chromium inhibited chlorophyll content in Wheat (Mukhopadhyay and Aery, 2000). Reduction of total chlorophyll content and inhibited photosynthesis as a result of cadmium stress have been reported in *Phaseolus vulgaris* (Krupa *et al*., 1993). Reduced chlorophyll a/b ratio has been reported as a symptoms of cadmium stress in Barley leaves (Stobart *et al.*, 1985). Inhibition of chlorophyll synthesis due to mercury treatment was reported in many plants (Kupper *et al*, (1998);; Mysliwa-Kurdziel and Strzalka, (2002). Kupper *et al*.; (1996, 1998) described the results of many trace elements -copper, cadmium,

mercury, nickel, lead, zinc etc. interfering with chlorophyll synthesis by substituting Mg^{2+} of chlorophyll molecule and resultant inhibition of photosynthesis. Mercury and cadmium interact with light harvesting proteins of chlorophyll in ~~of spinach~~ *Lactuca sativa* leaves (Ahmed and Tajmir-Riachi, 1993). According to Oncel *et al.*, (2000) in wheat varieties treated with cadmium and lead, total chlorophyll content was decreased to 50-70% and this reduction may be the result of inhibition of enzymes involved in chlorophyll biosynthesis.

Striking changes have been reported in the chloroplast fine structure, reduction in grana stacks and amount of stroma etc. by the toxicity of lead (Koeppe, 1981). According to Kupper *et al.*,– (1998) chlorophyll synthesis is inhibited due to the substitution of Mg^{2+} by Pb^{2+} in plants exposed to lead. Stefanory *et al.*, (1993) suggested that reduced rate of photosynthesis is associated with chloroplast damage in maize plants treated with lead. Reduction of chlorophyll pigment due to the presence of heavy metals have been interpreted in different perspectives by various authors. According to Vajpayee *et al.*, (2000) chromium inhibit biosynthesis of chlorophyll by impaired γ aminolevulinic acid dehydratase activity leading to reduced pigment in *Nymphaea alba*.

~~Chromium is reported to reduce total chlorophyll a and chlorophyll b in *Ocimum tenuifloram* (Rai *et al*.,2004).~~

~~–~~Lipid peroxidation, as an important impact of heavy metals, also causes degradation of photosynthetic pigments. Inhibition of photosynthesis due to impaired chlorophyll synthesis by cadmium has been reported by Dubey (1997), Siedleaka and Krupa (1997), Prasad and ~~Strazalka~~Prasad (1997) and ~~;~~ Linger *et al*.~~,~~ (2005). Zengkin and Munzuroglu, (2005) found that cadmium, lead, copper and mercury inhibit the enzyme activity associated with chlorophyll biosynthesis resulting in a drastic reduction of chloroplast content in *Phaseolus vulagaris*. In the present study chlorophyll a/b ratio remained unchanged in plants treated with l~~L~~ead and other ~~metal~~ treatments resulted in a reduction of a/b ratio (Table 11; Fig. 15). Incidentally accumulation of lead also is found to be maximum in the leaves and hence positively correlated to the tolerance of *B. diffusa* towards lead. ~~.–~~Increased chlorophyll a/b ratio is considered as a stress indicator as reported in *E~~mperon~~upatorium nigrum* ~~*nigrum*~~ leaves under copper and nickel stress (M~~u~~onni *et al*., 2001). ~~In *B. diffusa* chlorophyll a/b ratio is maximum in control leaf compared to the treatment. Incidentally accumulation of lead is also maximum in the leaves compared to other treatments.~~

Phenolic content of plants treated with cadmium, chromium, mercury and lead is significantly increased in comparison with control. During growth,

gradual increase of phenolic content was observed in all samples of treatments and control (Table 13; Fig. 19). Enhanced phenolic content is a characteristic of leaf tissue of plants treaded with mercury and lead. Similarly, pPlants treated with lead showed very high phenolic content in the stem and root. Enhancement of phenolic synthesis has been reported by environmental stresses (Osborne, 1998Harborne, 1980)). Lipid peroxidation induced by phenolics in conjunction with aluminium ions have already been reported in (Sakihama and Yamakskai, 2002; Michalka, 2006). As mentioned earlier, root growth inhibition by heavy metal ins general and lLead in particular occur in plants (Kahle, 1993, Goldsbold and Kettererener, 1991). and sStunted and woody nature of roots due to extra lignifications in *Phaseolus vulgaris* treated with 10 µM lead has also been reported by Croznyseh and Jerzeymaska, *et al.* (2000(1991). Since phenolics are the precursor of lignin (Buchanan *et al*., 2000), increased phenolic content due to heavy metal stress is directly related to lignin synthesis resulting in woody texture which is another effect, rather, than a defensive mechanism of heavy metal toxicity stress in plants. Induction of phenolic compounds biosynthesis has been reported in mMaize in response to aluminium (Winkel-Shirley, 2002), in Wheatpepper due to nickelcopper (Diaz *et al*., 2001) and in *Phaseolous vulgaris* exposed to cadmium (Diaetz and Schnoor, 2001).z *et al*, 2001). According to Michalak (2006) *de novo* synthesis of soluble phenolics under heavy metal stress which are acting as intermediates in lignin biosynthesis is reflected in the anatomical changes such as cell wall

thickening as observed in *B. diffusa* (Figs. 42, 43, 44 & 45) due to lignifications induced by heavy metal stress. as suggested by Buchanan *et al*., (2000).

Phenolic compound have been attributed to function also as antioxidants in plants under stressful condition (Michalak, 2006). According to this author, flavenoid group of phenolics can directly scavenge molecular species of ROS due to their ability to bind with phenolic molecules. Significant increase of phenolic compounds (Table 13; Fig. 19) in all tissue may act as antioxidnatantioxidant as a defensive mechanism in *B. diffusa*. and this aspect will be discussed later. Increased phenolics content This observation is in conformity with the views of Posmyk *et al*., (2009) who suggested that in red Cabbage, accumulation of phenolics, anthocyanine and other isoflavoinoides is an effective strategy against reactive oxygen species under copper stress. Antioxidant property of phenolic compound is due to their high tendency to chelate metals, because phenolics possess hydroxyle and carboxyl groups which are able to bind metal ions (Morgen *et al*., 1997). Polyphenols possess ideal structural chemistry for scavenging activity and have been shown to be more effective than ascorbate under *in vitro* condition (Rice –Evans *et al*., 1997). According to Lavid *et al*., (2001) direct chelation of chromium, lead and mercury by binding to phenolics occur in *Nymphaea alba*. In *B. diffusa* co-incidents of phenolics abundance and maximum accumulation of heavy metals in general and chromium and lead in particular in the roots indicate the chelation of these metals by phenolics (Tables 123, 18; Figs. 19 & 40) resulting in the

sequestration and reduced translocation to the aerial parts. More or less similar ~~co- occurrence~~incidents of abundant phenolics and accumulation of all metals except cadmium is evident in the leaves of *B. diffusa*. ~~Flavinoid group of phenolics can directly scavenge molecular species of ROS due to their ability to bind with phenolic molecules (Michalak, 2006).According to Rice –Evans *et al*., (1997) Polyphenols possess ideal structural chemistry for scavenging activity and have been shown to be more effective than ascorbate under *in vitro* condition.~~

Generation of free radicals and reactive oxygen species (ROS) is an established impact of stresses and their synthesis is stimulated in the presence of heavy metals in plants (Halliwell and Gutter~~inds~~idge, 199~~3~~3) and hence normal metabolism is disrupted by lipid peroxidation of membrane system. To mitigate and repair the damage initiated by ROS, plants develop a complex antioxidative system. Maximum lipid peroxidation is observed as accumulation of MDA content in the root tissue of *B. diffusa* compared to other tissues. Manifold increase of lipid peroxidation was observed in the roots of these plants treated with mercury and lead resulting in very high increase of MDA content (Table 14; Figs. 20, 21& 22). Lipid peroxidation in all tissue of *B. diffusa* plants treated with mercury is very high followed by lead treatment compared to other metals. Formation of MDA- a product of lipid peroxidation act as an indicator of peroxidation of membrane lipid in plants. Evidences ~~for~~of the involvement of oxidative stress due to heavy metal stress has been reported

by several authors (Dietz *et al*., 1999; Sinha and Sexana, 2006; Singh *et al*., 2004). MDA is routinely produced as a result of lipid peroxidation under stressed conditions and used as an index of stress status (Zhang *et al*., 2007). If MDA content is considerably reduced due to antioxidative enzymes, reduced H_2O_2 level and low membrane damage occur by lipid peroxidation. Such a situation has been reported in root tissue of *Lycopersicon peruvianum* under salt stress (Shalata *et al*., 2001). Increased MDA content has been noticed in the roots and leaves of *Brassica jucnce* and *Cajanus cajan* treated with zinc (Alia *et al*., 1995). *Helianthus annuus* treated with many heavy metals (Gallego *et al*., 1996), *Triticum aestivum* and *Brassica campestris* due to the toxicity of copper, cadmium, chromium and lead (Chandra *et al*., 2009). Maximum MDA content in all tissues treated with mercury indicates the lack of tolerance since *B. diffusa* is sensitive to very low quantity of $HgCl_2$ ($\geq$10µM) whereas concentration of other metals are comparatively more.

According to Bradly and Munin, (1992) oxidation of unsaturated fatty acids of membrane-lipid by singlet of oxygen produces different products including malonedialdehyde. Hydroxyl radicals are one of the components of reactive oxygen species generated in response to heavy metal stress, also induces lipid peroxidation. Increased MDA content produced from thiobarbituric acid reaction in plants treated with chromium has been reported in *Oryza sativa* (Panda, 2007). Enhanced MDA content in the root tissue of *B. diffusa* treated

with all metals ~~cadmium, chromium, mercury and lead~~ compared to stem and leaf reveals enhanced lipid peroxidation in the root tissue since roots are in direct contact with toxic ions. A correlation can also be drawn between MDA content and bio accumulation of mercury and lead in the roots which is very high compared to other tissues as well as other treatments (Table17a; Fig. 38)~~)~~.

In *B. diffusa* maximum peroxidase activity is shown by root tissue under the stress of all metals whereas only marginal increase of peroxidase activity was exhibited by stem and leaf (Table 15~~)~~; Figs. 23, 24, 25, 26, 27& 28). Peroxidase is one of the principal enzyme~~s~~ involved in the elimination of reactive oxygen species. Zhang *et al*~~–.~~, (2007) opined that in m~~M~~angrove seedlings (*Kand~~a~~e*lia* and *Brugui*era~~ari~~a*) peroxidase activity was found to be very efficient in avoiding damage due to heavy metals such as cadmium, lead and mercury by enhanced activity of this enzyme ~~peroxidase~~ in the roots and leaves. According to Radotic *et al*.~~,~~ (2000) and Sakihamay and Yamasaki, (2002) peroxidase enzyme~~s~~ ~~are~~is known to play a significant role in oxidative stress condition and this enzyme activity can be used as a potential biomarker for sub-lethal metal toxicity in plants. The increase of MDA and peroxidase activity in *B. diffusa* are parallel due to the treatment with all heavy metals irrespective of their wide difference in the concentration~~s~~ (Tables 14& 15; Figs. 20, 21, 22, 23, 24, 25, 26, 27& 28). This results indicate that the plant is not fully protected from the oxidative damage induced by cadmium, chromium, mercury and lead by the mechanism of peroxidase activity. Peroxidase activity

and MDA formation are low in plants treated with chromium compared to the other treatments revealing comparatively reduced lipid peroxidation. So it seems that in this context *B. diffusa* is more tolerant to chromium than other metals.

When a comparison is made between the MDA content and peroxidase activity in *B. diffusa* due to the treatment of cadmium, chromium, mercury and lead and the quantity of former is not proportional to the activity of the latter. A significant increase (about 3-4 times) of MDA content of root, stem and leaves of all treatments (Table 14; Figs. 20, 21 & 22)) compared to the control reveals that lipid peroxiation is very high and attenuation of the same is not takes place by the peroxidasetion activity which exhibit only marginal increase. Nevertheless, the distribution of MDA content and peroxidase activity show similar trends in ameliorating stresses due to these elements irrespective of the significant variation in the available quantity of metals among the treatments as well as bioaccumulation.

The stress enzyme, superoxide_dismutase activity is also dramatically increased in all tissues in general and roots tissue in particular of in *B. diffusa* plants treated with cCadmium, chromium, mercury and lead. Mercury treatment resulted in maximum SOD activity in the root tissue followed by plants treated with lead (Table 16; Figs. 29, 30 & 31)). Superoxide_dismutase is the first enzyme to detoxify highly reactive oxygen species in plants by converting O_2^-

radicals to H_2O_2 (Giannopolitis and Ries, 1977). The product of SOD activity, H_2O_2 is still toxic and must be eliminated by conversion to water in subsequent reactions. In plants, enzyme like catalase, ascorbate peroxidase and guaicol peroxidise are considered as ~~of~~ most important~~ce~~ in scavenging H_2O_2 (Noctor and Foyer, 1998; Zhang *et al*., 2007). Catalase eliminates H_2O_2 by breaking it directly to water and oxygen. This enzyme is less efficient than other peroxidases in scavenging H_2O_2 because of its low substrate affinity. In *B. diffusa*, SOD and peroxidase activities show linear increase during growth under heavy metal stress (Table 16; Figs. 29, 30 & 31)~~)~~.

Catalase activity is maximum in plants treated with chromium compared to other metals and activity of SOD and peroxidase are lower~~ess~~ than the other treatments. MDA content also is very low compared to other metals, revealing reduced lipid peroxidation and more tolerance to chromium as mentioned earlier. Sidlecka and Krupa, (2002) suggested that as long as the heavy metal stress is not too strong for the plants defence capacity, the maximum response to heavy metal is an increase in SOD and peroxidase activities. According to Shanker *et al*.~~,~~ (2004) the combined activity of SOD and catalase~~CAT~~ is critical in mitigating the effect of oxidative stress imposed by chromium in *Vigna radiata*.When a comparison is made between SOD and catalase activity in *B. diffusa* treated with lead, maximum activity of both the enzymes are ~~observe~~coincided~~d~~. So it can be presumed that since the catalase activity is co-ordinated with SOD activity, the protective role is performed in scavenging the O_2^- and H_2O_2 and hence the plant is more tolerant towards lead in consonance

with the views of Liang *et al*.~~,~~ (2003) and Badavi *et al*.~~,~~ (2004) who opined that SOD reduces lipid peroxidation in salt stressed barley and SOD activities induces tolerance in salt stressed tobacco respectively. ~~.~~

During the entire period of growth, peroxidase activity is increased slowly but MDA accumulation is suppressed in *B. diffusa*. Szollosi *et al*.~~,~~ (2009) suggested that in *Brassica juncea* cadmium- induced lipid peroxidation and the MDA formation ~~was~~ are time - dependant and the peroxide formed get scavenged by other antioxidant enzymes or non enzymic antioxidants such as glutathione or ascorbate.

Behaviour of *B. diffusa* towards mercury is found to be unique because maximum MDA content indicate enhanced lipid peroxidation~~,~~ and ~~similarly~~ SOD, peroxidase and catalase are not much active compared to plants treated with the other metals, imposing more toxicity by mercury. It is recalled that among the four metals, *B. diffusa* shows more ~~toxicity~~ sensitivity towards mercury which is given in least molar concentration for the treatments confirming the least tolerance of *B. diffusa* towards mercury. Since both SOD and peroxidase are not much active compared to the other treatments, scavenging of ROS may not be sufficient to protect the plant. But comparatively more catalase activity is found to be sufficient for the scavenging of stress-generated ROS. More or less similar observations have been reported in tobacco under salt stress ((Badawi *et al*.,1984, 2004) and *Kandelia candel* and

Bruguiera gymnorrhiza treated with mercury, lead and cadmium ~~(Zhang *et al.*, 2007)~~which showed increased SOD activity in shoot and root- the highest activity was observed in the root tissue (Zhang *et al.*, 2007). ~~.~~ In the present study, increase of SOD activity is observed during growth up to 20 days presumably due to the 'optimal' concentrations at which the plants show only growth retardation —(It was standardised during the design of experiments) but are not lethal to *B. diffusa.*

Maximum activities of all ~~antioxidnant~~antioxidant enzymes~~,~~ guaicol peroxidase, catalase and superoxide dismutase are observed in the root tissue and linear increase is noticed during growth. Given the differences in the response of *B. diffusa* towards the four metals in the distribution and bioaccumulation potential, the tolerance mechanism is found to be more or less alike but with obvious fluctuations. ~~in *B. diffusa*.~~ Notwithstanding, the marked differences in the molar concentration of c~~C~~admium, chromium, mercury and lead applied on the basis of similar morphological manifestations reveals that, each heavy metal ion, though non- essential, exhibit specificity in the metabolic interference or impact and this data confirm~~revealed~~ that cadmium, chromium, mercury and lead ions are not alike and hence exhibits specificity in their metabolic interference/activity in *B. diffusa*.

The protective mechanism to minimise oxidative stress, adopted by *B. diffusa* towards the four heavy metals vary from one another. As mentioned earlier, plants treated with lead and chromium show more tolerance due to the enhanced activity of antioxidant enzymes with slight differences in the characteristics of enzymes. A correlation can be drawn between the efficiency of antioxidant enzymes to attain tolerance and the bioaccumulation pattern of lead and chromium because maximum accumulation of these metals occurs during the growth period. More or less similar correlation is also seen in the treatment with mercury. But the magnitude is different. Antioxidants production against stress - induced damage in root of *B. diffusa* is much powerful in plants treated with chromium and lead. Bioaccumulation of mercury is comparatively minimum and the plant is not as tolerant as towards the other metals. . Even thoughEven though, plants treated with cadmium do not show a specific mechanism involving antioxidant system, the tolerance of *B. diffusa* towards cadmium is almost similar to that of mercury. The bioaccumulation of cadmium and the molar concentration of the treatment also are agreeable to the above conclusion.

Heavy metal accumulation pattern showed a linear increase of cadmium, chromium, mercury and lead in the tissue such as root, stem and leaf only up to a limited period (12 days). . There after, accumulation rate is very slow (Table18; Fig. 40). Analysis of metal concentration increments show highest (cCumulative) quantity in the samples of 20th day. The quantity and

accumulation pattern differed among the metals and ~~is~~are found to be dependent on type of metals ~~absorbed,~~ thereby exhibiting specificity of individual heavy metals in process of absorption, translocation and accumulation (Table 19).

Cadmium accumulation is maximum in the root tissue of *B. diffusa* and content is 46% of the total available cadmium in the growth medium compared to other metals during 20 days of growth ~~and content is 46% of the total available cadmium in the growth medium and~~ while content accumulated in the entire of the total plant is 92%. These findings confirm that *B. diffusa* can accumulate very high cadmium without much obvious toxic symptoms indicating high tolerance to cadmium toxicity and this view is in accordance with the characteristics of cadmium such as high mobility in the soil-root-system (Sanita-di-Toppi and Gabbrielli, 1999) and ~~,~~ accumulation potential depends on available cadmium content (Cobbett and Golds~~-~~brough, 2002; Guo-Sheng *et al*., 2007). Nevertheless, Sanita-di-Toppi and Gabbrielli (1999) suggested that metal is taken up with the plants more rapidly from the solution than from the soil. In the present study the plants are cultivated in nutrient solution containing known quantities of the heavy metals and hence the accumulation potential observed need not be comparable or equ~~a~~alent to ~~comply~~comply with the accumulation pattern in the soil system. An important ~~Important~~ aspect of mineral nutrition of ~~in~~ plants is antagonism and/~~-~~or~~-~~ synergism between ions as suggested by many authors ~~(~~Orcutt and Nilsen ~~(~~(2000)~~;~~ ~~,~~ Cseh, (2002)~~;~~ ~~,~~ Taiz and Zeiger (200~~2~~3)~~;~~ ~~,~~ Hopkins~~,~~ ~~-~~(200~~3~~4)~~;~~ Singh *et al*. (2004).~~-~~ According to those authors~~,~~ absorption/

accumulation of one element may be antagonistic or synergistic to another element and depend on the availability and /or interaction of other ions as well as the general scenario of nutrition and these two phenomena are ~~also~~ found to be species specific~~.~~ also (Orcutt and Nilsen, 2000).

Cadmium absorption and translocation have been reported in plants like ~~plants like *Zea mays* (Gampus and Gorlach, 1995),~~ *Thal*a*spi caerulescens* (Escarre *et al*., 2000) and *Cucurbita pepo*, *Hord*e~~i~~*um vulgar*~~a~~*e* and *Zea mays* (Ciura *et al*., 2005). As mentioned earlier, Cd^{2+} ions are fast mobile in plants. Many plants such as *Pot*a~~o~~*mogeton pectinatus* (Rai *et al*., 2003), *Arabidopsis thaliana* (Perfus- Barbeoch *et al*., 2002), *Phragmites australis* (Ederli *et al*., 2004), *Cannabis sativa* (Linger *et al*., 2005), *Brassica juncea* (Ishikawa *et al*., 2006; Szollosi *et al*., 2009) and *Helianthus a*~~ur~~*ennus* (Zou *et al*., 2008) are reported as hyperaccumulators of cadmium. High mobility of Cd^{2+} have been established in r~~R~~ice plant by adding $Ca_2(OH)$ to induce more mobility (Kim *et al*., 200~~3~~2). The authors suggested that Cd^{2+} may substitute Ca^{2+} resulting in enhanced cadmium accumulation using Ca^{2+} channels for the passage of Cd^{2+}.

Boerha~~a~~*via diffusa* shows considerable accumulation of chromium in the order root> stem>leaf. Progressive ~~increase~~accumulation of chromium with more content in roots (10-200 times) than the shoots have been reported in ~~Spinach~~ *Lactuca sativa* (Singh, 2001) and ~~,~~ *Nel*e*umbo nucifera* (Vajpayee *et al*., 1999). *Ver*~~n~~*oni*c*a becc*~~e~~*ab*~~h~~*anga* and several hydrophytes showed high

chromium removal from the soil. irrigated with tannerary effluents containing 4-8 mg/ L Cr VI (Zurayk *et al*., 2001). According to Kabata-Pendias and Pendias (2001)Kornik and Havsky, (1994), progressive increase of chromium accumulation occur in the roots and shoots of *Helianthus annuus*sunflower, *maize**Zea mays* and *Vicia faba*. This observation is comparable to the chromium concentration pattern of *B. diffusa*. Accumulation of chromium in the stem tissue of *B. diffusa* is found to be related to the anatomy of stem which show development of multicellular and densely stained trichomes (Fig. 43). The trichomes have been reported to involve in the sequestration of heavy metals like cadmium in *Arabidopsis thaliana* (Dominguez-Solis *et al*., 2004).

Accumulation of mercury in *B. diffusa* is maximum in roots and the increase is linear to the period of growth. and cCompared to the quantity applied in the nutrient media, the accumulation is significant. However, , in the shoot, accumulation is comparatively low. But on the basis of percentage accumulation, stem tissue of *B. diffusa* shows high mercury content. Anatomy of *B. diffusa* plants treated with mercury also shows multicellular denseily stained trichomes in the stem (Fig. 44). This epidermal modification is found be related to mercury accumulation/sequestration as reported in *Arabidopsis thaliana* in which cadmium is sequestered in trichomes (Dominguez-Soils *et al*., (2004). Even though no plants have yet been reported as natural hyper-accumulator of mercury (Henry, 2000; Raskin and Ensly, 2000), transgenic plants such as *Arabidopsis thaliana* and *Nicotiana tabcum* are capable of

accumulating and converting methyl mercury to Hg^{2+}. A study to demonstrate the effectiveness of mercury accumulation and to evaluate the capacity in removing mercury from water containing different concentration of mercury on four aquatic plants. *Eichhornia recipes*, *Pistia stratiotes*, *Scirpus tabernaemontani* and *Colocasia esculenta* ~~and to evaluate the capacity in removing mercury from water containing different concentration of mercury~~ revealed that accumulation i~~wa~~s maximum in plants treated with more amount of mercury salts (Zi~~a~~mmels *et al*., ~~,~~ 2004). Loss of ~~M~~mercury ~~loss~~ has also been reported to occur by volatalisation from plants (Skinner *et al*., 2007). Generally aquatic plants, ~~,~~ *Azolla caroliniana*, *Eichhornia crassipes*, *Scirpus cyperinus*, *Eaberma mostarium*, *Colocasia esculenta* are able to accumulate mercury in their net like root structure (Skinner *et al*., 2007). *Brassica juncea* exhibit phytoremediation potential of mercury from contaminated waste water (Moreno *et al*., 2005 a, 2005 b, 2005c). According to Moreno *et al*.~~,~~ (2008), *Brassica juncea* roots accumulate mercury 100-270 times above initial concentration. But the translocation to the shoot is very little. *B. diffusa* also exhibit significant accumulation of mercury in the root system although the content is dependent on the availability in the growth medium.

Accumulation of lead in *B. diffusa* is very high in the root, stem and leaf and these values are maximum compared to other metals. ~~Lead accumulation is maximum in the root tissue compared to other metals/plant parts.~~ This may provide the plant with better tolerance to heavy metal concentration as

suggested by Weis and Weis ,(2004). Studies on the accumulation of lead in *Typha latifolia* showed maximum lead content in the root while and shoot maintained a low level always (Ye *et al.*, 1997). According to Zhaeljazkove *et al.*, (20006) lead accumulation in *Bidens leonorum*, *Melijsa* and *Ourianum* was maximum in the root and the accumulation pattern varied from plant to plant and dependant on the availability of the metal in the soil. Lead accumulation was reported in the root system of *Bacopa monnieri* (Sinha, 1999). Studies on translocation of lead in rice plants showed that calcium ion ($Ca^{+)}$ ameliorated lead toxicity in rice plant by simulating the translocation of Pb^{2+} through Ca^{2+} channels (Kim *et al*., 2002).

In the present study, since known quantities of each metal are given to the plants cultivated in each container and the data of total amount accumulated in the total plants of each container and quantity retained after twenty days of growth are calculated and considerable loss of each metal is found to occur. The loss is continuously decreasinged during 20 days growth showing the range : - cadmium 13 to 0.5%, chromium 12 to 4%, mercury - 31 to 13% and lead - 19 to 2.5%. When a comparison is made between the accumulation potential of *B. diffusa* with respect to the quantity of each metal available in the growth medium, significant variations are observed in the pattern of accumulation and loss of metals observed during growth. Loss of metals are in the order Pb>Cr>Hg>Cd (Table 19). The loss may be due to the escape

from the leaf through stomata as reported in *Bacopa monnieri* in which mercury loss was noticed after treatment with the 10µM $HgCl_2$ (Hussain, 2007). Loss of mercury through trichomes which are developed in abundance in the stem of *Vigna radiata* seedlings cultivated in nutrient solution containing 15µM $HgCl_2$ has already been reported by Sahadevan, (2001). According to Moreno *et al.*, (2008) *Brassica juncea* cultivated in nutrient culture showed very little translocation to the shoots (0.7-2%) and mercury volatalization in the form of Hg (O) vapour occurred from the plant and from the soil medium due to some mercury tolerant bacteria which reduce Hg^{2+} to metallic mercury.

Loss/ release of mercury by plant from growth medium is one type of phytoremediation technology designated as phytovolatalization as suggested by Pilon-Smits (2005), according to whom phytovolatialization is the release of pollutants from the site as gas without any need for harvesting and disposal of the agent or plant. The author suggested that phytovolatialization can be maximised by promoting transpiration rate through sufficient irrigation. Two plants (*Myriophyllum aquaticum* and *Ludwigia palustris*) haves shown higher capacity in mercury removal contaminated with mercury (Kamal *et al.*, 2004). According to those authors, by aquatic species mercury is removed at higher efficiency than other heavy metals and the removal rate of mercury is dependent on contamination rate.

Leaf tissue of *B. diffusa* exhibit maximum cadmium content compared to the available quantity in the medium (Table 18). Even though leaf anatomy is not done in the present study, the accumulation of cadmium can be correlated to the views of Dominguez- Solis *et al*., (2004) who reported that by employing transgenic approach a plant line of *Arabidopsis thaliana* has been shown to accumulate very high cadmium in the leaves in which trichomes are involved in the accumulation and there by detoxification of cadmium in *Arabidopsis thaliana* (Lee *et al*., 2002). According to those authors, the trichomes are having about 200 fold higher volume than the epidermal cells and the vacuoles of the trichomes occupy above 90% of the volume. The trichomes contain phytochelatin synthase enzyme which is involved in the synthesis of PC-Cd complex (Lee *et al*., 2002). In *Arabidopsis thaliana*, the sequestration of cadmium occurs also by the synthesis of cystine rich glutathione because the GSH biosynthetic pathway is highly active in trichomes.

Bioaccumulation pattern of heavy metal can also be interpreted in terms of Bio cConcentration Factor (BCF) and TTranslocation FFactor (TF) following the method suggested by Yoon *et al*., (2006). According to those authors Bio concentration Factor (BCF) is defined as the ratio of metal concentration in the roots to that of soil and the plants ability to translocates metal form the root to shoot is named as tTranslocation Ffactor (TF) which is defined as the ratio of metal concentration in the shoot to root. In *B. diffusa* the BCF of all the four metals seems to be same where as TF exhibit wide variations such as least value

is shown by chromium and maximum by mercury. Uniformity of BCF value of all the metals indicates that despite the wide difference in the molar concentration of four metals supplied in the growth medium, uniform absorption rate in comparison with the availability ~~is~~is maintained by *B. diffusa* as far as the four heavy metals are concerned (Table 19a; Fig. 41).

Comparatively low TF values of chromium and maximum TF values shown by mercury reveal very low and high translocation of these metals respectively indicating that translocation potential of *B. diffusa* varies from metal to metal. Based on the TF value of the chromium, accumulation is found to be more in the roots compared to the shoot but mercury shows comparatively low ~~less~~ accumulation potential in the root and these ions are more mobile. More or less similar results have been reported in the accumulation pattern of copper and lead in *Bidens tripartita* (Zheljazkov *et al*., 2008). Those authors suggested that accumulation potential of plants towards cadmium, chromium, mercury, lead, copper and zinc depends on the availability of the metals in the soil/ growth media as well as on the plant genotype. According to Kabata- Pendias and Pendias~~,~~ (1991), Angelova, *et al*.~~,~~ (2006) and Meers *et al*.~~,~~ (2007)~~,~~ the concentration of heavy metal in different plant parts reflect genetic differences among the species with respect to heavy metal transport and accumulation. But in the present study, ~~,~~ the differences in the BCF and TF values in *B. diffusa* shows metal specificity on one hand and difference in molar concentration or the availability of the metals ~~,~~ on the other. Molar

concentrations of each metal in the nutrient growth medium has been selected on the basis of tolerance/ sensitivity of *B. diffusa* to impart more or less similar performance of growth retardation.

To explore the metal accumulation in different tissue of *B. diffusa*, association between metal content and oxidative potential expressed by antioxidant enzymes is found to be effective in the scenario of toxicity induced by cadmium, chromium, mercury and lead. Concerning the accumulation of these metals proportionality is observed between accumulation and antioxidant enzyme activity among different tissues in each treatment. Positive correlation can be drawn between the metal content, MDA and proline accumulation (Table 17 b; Fig. 39). When the accumulation is expressed as percentage of available metal content in the growth medium, in spite of significant differences in the molar concentrations of each element, almost similar quantities of all metals are accumulated in the roots; (Table 18; Fig. 40) whereas this pattern is not followed by accumulation pattern of stem and leaf tissue presumably due to significant difference in the TF values as a result of differences in translocation flux. This observation is in conformity with the views of Reid *et al.*; (2003) who suggested that the mode of translocation varies from metal to metal. Thoese authors –also demonstrated that cadmium is highly mobile and get translocated even to the seeds; of *Solanum tuberosum*-. Accumulation of cadmium in all plant parts inclusive of seeds has been reported in *Oryza sativa* (Tanaka *et al.*,

2007) while translocation of lead is very slow in *Bacopa monnieri* (Sinha, 1999), and in *Momordica charantia* fruit (Daniel *et al*., 2009).

Generally the plants having TF factor less than 1 is considered to have in significant phytoremediation potential (Zheljazkov *et al*., 2008). So the present study demonstrates that *B. diffusa* is not suitable for phytoremediation which is a technological process for mitigation of metal pollution of soil by using plants as biological detoxification system. The term hyper-accumulator is used to describe plants that accumulate more than 1000µg Ni/gram dry weight in their shoots in the natural habitat (Brooks *et al*., 1977). Metal-hyper accumulator has been identified in at least 45 plant families (Clemens *et al*., 2002; Pilon – Smits, 2005). Many plants accumulate very high quantities of heavy metals. *Potamogeton pectinatus* and *Lonicera japonica* (Liu *et al*., 2009) are hyper accumulator of cadmium (Zayed *et al*, 1998; Rai *et al*., 2003; Liu *et al*., 2009) *Chromolena odorata* is an accumulator of mercury (Velasco- Alinsug *et al*., 2005). *Brassica juncea* accumulates chromium (Moodley *et al*., 2007) and cadmium (Szollosi *et al*., 2009), lead is accumulated by *Alternanthera sessilis* (Moodley *et al*., 2007; Huang and Cunningham, 1996). All these plants are considered as hyper-accumulators and most of them are recommended for phytoremediation (Pilon-Smits, 2005). According to Yoon *et al*., (2006) and Zheljazkove *et al*. (2008), if the Bioaccumulation Factor (BCF) and and Translocation Factor (TF) values are above 1, the plant is suitable for phytoremediation. All hyper accumulator plants species are known to have BCF

and TF values more than unity (Brown *et al*., 1995). Values of both BCF and TF shown by *B. diffusa* towards cadmium, chromium, mercury and lead come below one and hence this plant is neither a hyperaccumulator nor a phytoremediant. Neverth~~e~~less, considerable quantities of all the four toxic metals are accumulated in the root and shoot when the plants are cultivated under hydroponic system. *B. diffusa* ~~It~~ is an important medic~~i~~nal plant and ~~incrediant~~ingredient of many Ayurvedic products such as ‘Ch~~a~~yavanaprasham’ and ~~*Lonicera japonica* (Liu *et al*., 2009).~~ ‘Dan~~v~~u~~a~~v~~~~n~~tharishtam’, ‘Sukumaragritam’, ‘Dashamoolarishtam’ etc. So the risk factor due to heavy metal contamination in these products is a concern because these medicines are commonly consumed and the health hazard due to cadmium, chromium, mercury and lead includes cognitive diseases, kidney damage, cardiovascular dysfunction etc.

In a~~A~~n important precision experiment designed to explore the bioaccumulation potential of ~~for the treatment of~~ twenty medicinal plants treated with known quantities of four toxic heavy metals Cd, Cr, Hg and Pb to impart visible symptoms of toxicity, significant differences were observed in the sensitivity towards the quantity of each metal given to each plant. By trial and error method, the present author tested the sensitivity of twenty medicinal plants ~~such as~~namely *Aerva lanata*, *Alternanthera tenella*, *Andrographis paniculata*, *Amaranthus spinosus*, *Beloperon plumbaginifolia*, *Blepharis maderaspatensis*, *Boerha~~a~~via diffusa*, *Catharanthus roseus*, ~~*Drymaria cordata*,~~ *Eclipta*

~~alba~~*prostrata*~~—~~, *Eupatorium triplinerve*, *Kyllinga nemoralis*, *Leucas* ~~*indica*~~*lavandulifolia*~~–~~, *Oxalis corniculata*~~–~~, *Phyllanthus amarus*, *P*~~*r*~~*o*r*tulaca oleracea*~~e~~, *Rubia cordifolia*~~–~~, *Scoparia dulcis*~~–~~, *Sida acuta*~~–~~, *Sida alnifolia* and *Vernonia cinerea* towards various concentrations of the four heavy metals and the concentration to which the plant is tolerant but imparting some visible symptoms of toxicity given in table 20.~~is chosen for the study.~~ Most of these medicinal plants are found to be accumulators of Cd, Cr, Hg and Pb.

~~Similar to agricultural crops, medicinal plants also are cultivated in soil contaminated with toxic heavy metals and/ or naturally growing in polluted soil or water and hence get exposed to heavy metal stress. Poisoning associated with the presence of toxic metals in medicinal plants have been reported in Asia, Europe and United states (Dunbalin *et al*., 1992; Olujohungbe *et al*., 1994; Markouitz *et al* ., 1994; Kakosy *et al* ., 1996;). Accumulation of many toxic heavy metals have been detected in root, stem, leaf, fruit and seeds of medicinal plants (Caldas and Machado, 2004; Zheljajkov *et al*., 2008).~~

~~Heavy metal pollution of soil is a major environmental problems that can affect plant productivity, product quality and human health (Alloway, 1990; Kabata- Pendias and Pandias, 2001). Heavy metal contamination of soil occurs in the vicinities of power plants, metal smelters, mines, industrial effluents and other anthropogenic sources such as waste disposal and indiscriminate use of fertilizers and pesticides.~~

Similar to agricultural crops, medicinal plants also are cultivated in soil contaminated with toxic heavy metals and/ or naturally growing in polluted soil or water and hence get exposed to heavy metal stress. Poisoning associated with the presence of toxic metals in medicinal plants have been reported in Asia, Europe and United states (Dunbabin *et al*., 1992; Olujohungbe *et al*., 1994; Markowitz *et al* ., 1994; Kakosy *et al* ., 1996). Accumulation of many toxic heavy metals have been detected in root, stem, leaf, fruit and seeds of medicinal plants (Caldas and Machado, 2004; Zheljazov *et al*., 2008).

Accumulation of heavy metals occurs in medicinal plants cultivated in polluted soil which is used for agricultural purposes. As mentioned earlier, presence of toxic heavy metals in medicinal plants have been reported by Caldas and Machado, (20034), Hussain *et al*., (2006) Khan *et al*., (2008), and Zheljazkov *et al*., (2008). Recently accumulation of cadmium, lead, copper, manganese and zinc was reported in *Leonurus cardiaca*, *Marrubium vulgare*, *Bidens tripartitea*, *Origanum heracleoticum* and *Melissa officinalis* cultivated in polluted soil near a smelter in Bulgaria (Zheljazkov *et al*., 2008). Heavy metal pollution of soil is a major environmental problems that can affect plant productivity, product quality and human health (Alloway, 1990; Kabata- Pendias and Pandias, 2001). The productivity and bioaccumulation potential of the plant may interfere with growth pattern and impose adverse effect of these metals on the metabolites prominently secondary metabolites which are the major sources of medicines.

According to Siedle~~dc~~ka and Krupa (1997)~~-~~, Kim *et al*. (2003) and Sersen *et al*.~~,~~ (2005), cadmium ions are easily translocated to the shoot from the root and absorption by root system also is very fast and accumulation level also is very high.~~-~~ Cadmium is the most studied toxic metal and it is a prevalent pollutant having greater bioavailability than lead or mercury (Clemens, 2006). ~~Cadmium is readily taken up by plants due to high mobility (Kim *et al*., 2003). So accumulation level also is very high.~~ Accumulation of cadmium in medicinal plant is an important concern because cadmium is readily taken up by plants and the subsequent problem is the toxicity of cadmium for animal system comes under 0.5-1mg/kg-^{1} dry plant material. But crop plants are tolerant at least to 10 fold of the concentration in the tissue (Zhe~~lj~~~~kji~~a~~zzsa~~kov~~o~~* et al*., 2008). The toxicity level of cadmium for animal in the range of 0.5- 1 mg/kg dry weight of plant material. Cadmium can cause kidney damage and impair skeletal system and many other health problems (Houston, 2007). Lead can impair cognitive development, reduce intellectual performance and cardiao vascular dysfunctions in human being (Falk *et al*., 2003; Lidsky and Schneider, 2006). Mercury and its compounds cause devastating neurological and kidney damage in animals. Kidney damage and ulcers are caused by chronic toxicity.

~~Another aspect of the present study deals with the accumulation of cadmium, chromium, mercury and lead occurs in all the twenty medicinal~~

~~plants cultivated in soil artificially contaminated with known quantities of these metals . (Table 20).~~ There is a general trend of increasing dry weight in the 20 selected medicinal plants due to heavy metal treatments compared to control plants. But the pattern of dry weight distribution in plant parts such as roots, stem and leaves vary from plant to plant (Table 20; Figs. 46, 47, 48, 49 & 50). Similarly there occurred significant differences in the dry weight distribution of one and the same plant treated ~~independently~~ with four metals- cadmium, chromium, mercury and lead.

Positive correlation is found to exist between the dry weight increase and the bio-accumulation in some species. Increase in root biomass and ~~maiximum~~maximum cadmium accumulation exist in *Aerva lanata*, *Alternanthera tenella* ~~*sessilis*~~ treated with cadmium compared to other treatments ~~.~~ (Table 20; Figs. 46, 47, 48, 49, 50, 51, 52, 53, 54 & 55). Dry weight increase and proportional chromium accumulation are observed in *A. paniculata*, *A. lanata*, *A.* ~~*sessilis*~~*tenella*, *A. spinosus*, *B. maderaspatanesis*, *P. oleracea*~~e~~, *S. dulcis* and *S. alnifolia* . Lead treatment results in the increase in root biomass and maximum accumulation in *A. paniculata*, *A. tenella*~~*sessilis*~~, *B. maderaspatanesis*, *B. diffusa*, *O. corniculata*, *P. oleraceae*, *S. dulcis*, *S. acuta*, and *S. alnifolia*.

The capacity of plants to accumulate metals has been considered a detrimental trait since some plants are directly or indirectly responsible for a proportion of the dietary uptake of toxic heavy metals by human (Chaney *et al*., 1997; Cunninngham *et al*., 1995). The intake of heavy metals through consumption of contaminated crop/~~medicinal~~medicinal plants can have long term effects on human health (Ow, 1996)~~–~~. Based on plant parts ~~of~~where accumulation ~~,~~ take place, ~~-~~ twenty plants are grouped in to two clusters- root accumulators and shoot-accumulators ~~-~~ in order to assess and interpret the rate of metal contamination in the Ayurvedic preparations containing these plant parts. Roots of *Aerva lanata*, *Boerh~~a~~avia diffusa*, *Kyllinga nemoralis*, *Rubia cordifolia*, *Sida acuta* and *S.alnifolia* accumulates cadmium, chromium, mercury and lead (Table 20; Fig. 51, 52, 53, 54 & 55) and roots of these plants are important ingredients of 'Punarnavasavam', 'Sukumaraghrita', 'Chyavanaprasyam', 'Valiyamarmagulika', 'Pathadigulika', 'Dhanvantht~~t~~arishtam'~~.~~ (Sivarajan and Balachandran, 1994).

Present study reveals that medicinal plants like *B. diffusa, E. triplinerve*, *R. cordifolia*, *S. dulcis, S. acuta , S. alnifolia and V. cinereae*, the quantities of cadmium accumulated in the root are 484, 589, 1273, 661, 789, 517 and 430 µg/g dry weight respectively. Since these plants are highly tolerant to cadmium, the chance of accumulation of cadmium in these medicinal plants growing in soil contaminated with even mild quantities of cadmium is very high. Since most of these plants serve as important ingredients of many Ayurvedic

preparations like Brhatidivayam, 'Punarnavasavam', 'Rasanadikasayam', 'Dhanwvantdharishtam' (Sivanrajan and Blachandran, 1994), consumption of the medicines containing the root of the above mentioned plants collected from the field polluted with cadmium may lead to health hazard. Accumulation of cadmium is more in the shoot of *A. paniculata*, *A. lanata*, *P. amarus*, *V. cinereae*, *B. plumbaginifolia*, *B. diffusa*, *C. rosues*, *E.prostrata*, *E. triplinerve*, *K. nemoralis*, *R. cordifolia*, *S. dulcis*, *S. acuta* and *S. alnifolia* presumably due to the high mobility of Cd^{2+} ions (Kim *et al*., 2002; Tanaka *et al*., 2007). Generally the shoot part of these plants are important ingredients of 'Punanrnavasavam', 'Chyavanaprasyam', 'Ashokarishtam', 'Rasanadikashayam', 'Pathadigulika', 'Dhanwvantharishtam'.

Similarly chromium accumulation is very high in the roots of *A. paniculata*, *A. lanata*, *A. tenellasessilis*, *A. spinousous*, *B. diffusa*, *C. rosesus*, *E. prostratra*, *E. triplinerve*, *K. nemoralis*, *L. indica*, *O. corniculata*, *P. oleraceae*, *R. cordifolia*, *S.acuta*, *S. rhombialnifolia and V. cinereae* (Table 20; Figs. 51, 52, 53, 54 & 55). Ayurvedic preparations such as 'Triktakaghritam', 'Goroganaadi gulika', 'Narasimharasayanam', 'Kombangadi gulika', 'Balarishtam' etc.Shoots of *A. spinosous*, *B. diffusa*, *E. prostrata*, *B. maderaspataenesis*, *C. roseus*, *O. corniculata*, *S. acuta* and *P. oleraceae*. contain high amount of chromium content. Shoots of these plants are used in the preparation of 'Tiktaghritam', 'Gorochanadi gulika', 'Candanasavam', 'Narvasimharasayam', 'Mahatraiiphalaghrittam', 'Suranadighritam',

‘Chyavanaprasyam’, and ‘Thipalliyathighrita’. Medicinal plants such as *Artemsilic vimliarium*, *Mucoura prurieus*, *Asparagus adselndeus*, *Wishania sominfera* etc, are reported to accumulate chromium, lead and cadmium (Khan *et al*., 2008).

Generally mercury accumulation is very low in the root tissue. Nevertheless, on the basis of percentage of the quantity given in each treatments, the accumulation values are very high. About 50% of mercury accumulation is observed in root tissue of *E. triplinerve*, *S. dulcis*, *S. acuta*, and *S. rombalnifolia*. This observations reveals sensitivity as well as comparable accumulation potential of almost all plants included in the present study towards the mercury. Plants such as *A. lanata, B. plumbaginifolia, B. maderaspatanensis, B. diffusa, E. triplinerve, R. cordifolia, S. dulcis, S. acuta, S. alnifolia*, accumulate moderately high amount of mercury in the roots. Comparatively low mercury is accumulated in the shoots of *B. maderaspatnensis, P. oleraceae, B. plumbaginifolia, B. diffusa* and *R. cordifolia.* Even though the availability of mercury is comparatively low in soil medium, the shoots of *R. cordifolia* which is highly medicinal, accumulate high amount of mercury. Ayurvedic medicines such as ‘Cancaradigritam’, ‘Pathadigulika’, ‘Narasimharasayanam’, ‘Mahathriphala ghritham’ etc. contain the roots of *R. cordifolia*, as an important ingredients.

Significant accumulation of lead in the roots of *A. paniculata*, *A. ~~sessilis~~tenella*, *A. spinos~~o~~us*, *B. maderaspatensis*, *B. diffusa*, *K. nemoralis*, *P. amarus* and *R. cordifolia* shows the relative immobility of this metal as suggested by Kabata –Pendias and Pendias (1991), Zheljazkov and Warman (2003) and Zhel~~j~~gazkov *et al*.~~,~~ (2006). Similar observations have been reported in *Salix* species (Meers *et al*., 2007). Roots of *A. paniculata*, *A. tenella~~sessilis~~*, *A. spinos~~o~~us*, *B. maderaspat~~a~~nensis*, *B. diffusa*, *K. nemoralis*, *P. amarus and R. cordifolia* show very high amount of lead whereas ~~while increased amount of lead is present in the~~ shoots of *A. paniculata*, *A. spinosous*, *B. plumbaginifolia*, *B. maderaspata~~n~~ensis*, *B. diffusa*, *C. roseus*, *P. oleracea~~e~~*, *V. cinerea~~e~~* ~~-~~, *S. acuta* and *Sida alnifolia* contain enhanced quantities of lead~~-~~. According to Sivarajan and Balachandran (1994) 'Valiyamarmagulika', 'Rasanadikasayam', 'Aswagandha~~diri~~leham', ~~—~~'Sadavarikasayam', 'Balajeerakadi leham', ~~Sadavari kasayam~~ etc. contain the above mentioned plants.

The distribution of heavy metals in the root and shoot can be evaluated using BCF (Bioconcentration Factor) and TF (Transolocation Factor), according to Yoon *et al*.~~,~~ (2006)~~.~~ ~~E~~enrichment or hyperaccumulation occurs when a contaminant taken up by a plant is not degraded rapidly resulting in hyper accumulation. The process of phytoremediation generally requires the translocation of heavy metals to the easily harvestable plant parts i.e. shoots (Pilon –Smits, 2005). By comparing BCF and TF the ability of different plants to take up metals from soil and translocating them to shoots can be compared.

Tolerant plants tend to restrict soil- root and root-shoot transfer and therefore have more or less similar metal accumulation in their biomass, while hyperaccumulators plants absorb and translocate metals into their above ground biomass. Plants exhibiting TF and BCF values less than one (<1) are unsuitable for phytoremediation (Fits and Wenzel, 2002). BCF indicate the bioavailability of the metal from the soil and this value is also related to the absorption potential of each element (Zheljazkov, *et al*., 2008).

Elevated values of BCF pertaining the cadmium absorption is shown by *A. sessilistenella*, *C. roseus*, *L. lavandulifolia*, *R. cordifolia*, *Sida* species and *V. cinereae* (Table 20a) indicating very high accumulation potential in the root system in relation to the availability of the metal in the soil. Correlation between quantity applied, accumulation percentage and BCF is not feasible in the case of cadmium and the sensitivity of each plant towards cadmium varies widely and the quantities are much more. Comparatively more TF values are shown by *A. paniculata*, *B. plumbaginifolia*, *C. rosues*, *E. prostrate*, *K. nemoralis*, *P. amarus*, *S. dulcis*, *S. acuta*, *S. alnifolia and V. cinerae* in the mode of cadmium accumulation.

In spite of very high chromium content accumulated in the root system of many plants (Table 20; Figs. 51, 52, 53, 54 & 55) the BCF values are very low (<0.02) and hence the root system of these plants accumulate very

low quantity of chromium compared to the availability in the soil. Cultivation of medicinal plants in soil polluted with comparatively low amount of chromium is feasible since it never causes any contamination of medicines using the roots of these plants which are found to be tolerant to chromium. It seems that tolerant plants adapt the strategy of avoidance towards absorption of chromium in spite of surplus availability in the soil. Accumulation of chromium in the root system on percentage basis shows very low values (<3%). Similarly BCF values also are meagre (<0.01). But the quantity of chromium applied is very high and wide variation exists (21-94mg) between the plants. Th~~i~~eses~~s~~ observations infer that almost all plants are considerably tolerant to chromium and accumulated quantities in the root system may not cause poisoning of medicines.

~~Even though~~Even though almost all plants out of the twenty investigated in the present study are sensitive to low quantities of mercury due to high toxicity of the metal, the BCF value also are very high indicating more accumulation potential in the root system. Compared to the other metals mercury exhibit maximum BCF values in a number of plants (Table 20 a). Root system of these plants (*B. maderaspatanensis, R. cordifolia, E. prostrata, S. dulcis, S.acuta, and S.alnifolia*) accumulate very high quantity of mercury (more than 50%) compared to the amount available in the soil.

When the quantity of mercury accumulated in the root system is expressed as percentage of the metal available in the medium, the values are

very high so also BCF values (Table 20a). As mentioned earlier tolerance of all plants towards mercury is very low and hence the quantities ~~y~~ applied to the soil are least compared to other metals (Table 20). Another characteristics of mercury accumulation is the difference in the sensitivity, accumulation potential and BCF between different plants such as *A. paniculata, A. tenella~~sessilis~~, C. roseues, E. prostrata, L. lavandulifolia* which are exposed to the same quantity of mercury (0.025mg) and the percentage of accumulation in the root is 21, 25, 13, 36 and 29 respectively and the respective BCF values are 0.21, 0.25, 0.13, 0.3 and 0.29. More or less similar correlation are observed in the accumulation pattern of mercury in other plants also which are exposed to more mercury content and hence are found to be more tolerant. This observations infer that maximum accumulation (percentage) and elevated BCF values are directly related to the sensitivity of these plants towards mercury since the quantities of mercury applied to the soil is minimum compared to cadmium, chromium and lead.

Bioaccumulation of lead in the root system shows wide variations (0.2 to 35%) and BCF values also differ between plants (0.006 to 0.03). But the applied quantity varies in the range of (24 to 303) milligrams. In this context accumulation potential of each plants vary significantly and no correlation exist between accumulation, BCF and the quantity applied. Nevertheless, the quantities of lead applied are comparatively very high and the plants are not much sensitive to this metal. So roots contain comparatively low amount of lead

in *A. lanata, E. triplinerve, P. oleracea~~e~~ and E. prostrata* the root system of which are important ~~incredients~~ingredients of ~~the~~ many Ay~~r~~uri~~i~~vedic medicines.

The Translocation Factor values of lead varies widely between *B. plumbaginifolia* (0.93), *E. triplinerv*e~~e~~ (2.04), *K. nemoralis* (1.0) *P. amarus* (1.54) and *P. oleracea~~e~~* (3.04) and these plants show very high value of T. F (Table 20 a) revealing more translocation to the shoot which are the ~~incredients~~ingredients of 'Mustaristam', 'Cheriya Rasanadi Kasayam', 'Cyavanaprasam', 'Thipalligathighritha', 'Madhyasthyathithyla', 'Satavariude', 'Carngeryadighrtam', 'Vyaghryadi leham', 'Amrtapraghrtam' and 'Chyavanaprasaleham'.

On the basis of percentage accumulation of lead, values are very low (<1) in many plants such as *A. lanata*, *A. tenella~~sessilis~~*, *B. madraspatensis*, *C. roseus* because the percentage values are calculated as percentage of the quantity applied to the soil. So these plants are found to be highly tolerant to lead because the plants survive in 600 µm lead and accumulation potential also is very low.

Levitt~~,~~ (1984) and Chan~~n~~ *et al*.~~,~~ (1993) opined that high level of toxic metals occurs in the medicinal preparations using plants collected from polluted

fields. According to Caldas and Machado, (2004), cadmium and mercury are present in 130 Brazilian herbal medicines prepared by using 105 medicinal plants of various families, eleven samples of *Centella asiatica* had cadmium exceeding limit of 0.3 µg/g recommended for medicinal plants (WHO, 1999). Population growth in the developing countries and increasing interest in the industrialised nation have greatly expanded the demand for medicinal plants and their products. According to Woods, (1999), approximately 80% of the world population use medicinal plants. Since considerable amount of heavy metal accumulation is reported in medicinal plants standard quality control of these products should be enforced. CKaldas and Machaao, (2004) suggested the need for a systematic control of toxic heavy metals in the plant used as medicines.

Vernonia amygdalina plants cultivated in coastal soil polluted with heavy metals such as mercury, lead, chromium, copper, nickel and zinc showed maximum accumulation of these metals in the root, leaf and fruit. (Gbaruko and Friday, 2007). Those authors opined that continuous consumption of these plant materials (medicinal) may lead to toxicity in human being. *Vernonia cinereae* included in the present study is a medicinal plant and accumulation of heavy metals in the root is in the order lead>cadmium>chromium>mercury and cadmium is present in the leaf also.

Generally it is seen that concentration of cadmium, chromium, mercury and lead in the root tissue of each plant vary significantly and accumulation is

proportional to the quantity of each metal present in the soil. But the percentage of accumulation is not at all directly proportional to the quantity given whereas the percentage value is clearly an index of accumulation of the metal. This behaviour is found to be a species specific trait. So the response of one plant to different metals and vice-versa exhibit no direct correlation.

The larger BCF values of cadmium and mercury ions indicate an increased accumulation in the roots of *A. lanata*, *A. sessilistenella*, *A. spinosous and B. maderaspatenesis*, but the TF values of cadmium in the plants mentioned above never go hand in hand. The low TF values of chromium and lead in almost all plants indicate more accumulation potential in the root and slow translocation to the shoot. This observation is corroborated with the views of Basta *et al*., (2005), who suggested that accumulation and bioavailability need not be proportional always. More or less comparable data and interpretation in the pattern of cadmium, lead, zinc and manganese accumulation in four medicinal plants, *Bridens tripartite, Leonarum cardiare*, *Marrubium vulgare* and *Melissa officinalis* cultivated in polluted soil are reported by Zheljazkov *et al*., (2008).

—According to Brooks *et al*., (1977), Brown *et al*. (1995) and Yoon et *al*., (2006) TF value more than 1 of plant species indicates their hyperacumulation potential and are known as hyperaccumulator plants. The TF values more than one (>1) shown by *E. triplinerve, K. nemoralis*,

P. amarus, and P. oleraceae species for cadmium and chromium indicate their hyper-accumulator behaviour. Plants having BCF and TF values more than one are suitable for phytoremediation. Phytoremediation potential of these species on one hand and the medicinal property on other, — are paradoxical because hyper accumulation of any toxic metal contributes more toxicity to the medicinal products. *Bacopa monnieri* is a medicinal plant (Sivarajan and Balachandran, 1994) but the phytoremediation potential of this plant has been reported by cultivating in a medium of mixed metals (cadmium, lead, mercury, and chromium) under simulated laboratory condition (Huang *et al*., 1997; Sinha *et al*., 1996; Sinha 1999).

In the present study T.F. values for chromium and lead shown by *E. triplinerve*, cadmium and lead by *K. nemoralis*, lead by *P. amarus*, and *P. oleraceae* are above one so these plants can be recommended for phytoremediation. All these plants are herbs and are profusely growing in polluted and marshy areas which are commonly used for regular flushing of industrial effluents and sewage waste water which may facilitate more accumulation of toxic heavy metals. In addition, all the four species are profusely growing weeds. Similar to aquatic plants, weeds are considered as hyper accumulators of heavy metal (Moodley *et al*., 2007). According to Sonmez *et al*., (2008) three weeds *Avena sterilis*, *Isatis tinctoria* and *Xanthium strumarium* are hyper accumulator of cadmium, lead and zinc and accumulated different levels of phytoavailable concentration of these metals.

The concentration of heavy metals in different plant parts reflect genetic differences among species with respect to heavy metal absorption, translocation and accumulation (Macnair, 1990; Angelova *et al*., 2006; Meers, *et al*., 2005~~7). ; Macurier, 1990).~~ While testing the sensitivity of all the twenty medicinal plants towards the cadmium, chromium, mercury and lead, a number of plants exhibits similar growth rate and biomass distribution towards the same concentration. But the accumulation pattern differed widely. Accumulation potential of cadmium shows that *A. tenella*~~*sessilis*~~, *C. roseus*, *E prostrata*, *L. lavandulifolia* and *P. amarus* are having similar sensitivity towards 0.593 mg of cadmium. But the quantities~~amounts of~~ accumulated ~~ion~~ in the root, stem and leaf vary significantly and not comparable among plants as well as heavy metals~~are 227, 37, 48; 208, 115, 7.8; 192, 110, 36; 248, 8, 14 and 97, 36, 47 µg cadmium respectively in these four plants.~~

.

RESULTS

Root growth

Growth retardation expressed in terms of reduced root and stem length and leaf area was observed in *Boerhavia diffusa* as a result of cadmium, chromium, mercury and lead treatments. Due to cadmium treatment, root growth was reduced fourth day onwards gradually and the same trend was continued and on 20th day 50% growth reduction was observed. But in plants treated with chromium, root growth retardation was comparatively lower than other metals and more or less similar trend in root length was shown by the plants treated with mercury and lead (Table 4; Fig. 3).

Stem growth

Stem growth of *B. diffusa* was adversely affected by cadmium treatment resulting in significant reduction of stem length in all stages of growth compared to the control. Chromium treatment resulted in maximum growth reduction of stem compared to other metals and on 20th day only 50% growth was observed compared to the control. Stem growth retardation due to mercury and lead treatments was significant compared to the control throughout the experimental period (Table 4; Fig. 3).

Leaf area

Leaf growth was found to be significantly reduced due to the treatment with all metals. About 50% leaf growth retardation was shown by the treatment with cadmium and chromium during all intervals of growth. Plants treated with mercury and lead also showed reduced leaf area but comparatively less retardation in leaf growth was observed compared to that of cadmium and chromium treatments (Table 4; Fig. 4).

Table 4: **Effect of Heavy Metals on Root and Stem length (cm) and Leaf area (mm^2) in *Boerhavia diffusa***

Treatments	Tissues	Interval (Days)					
		0	**4**	**8**	**12**	**16**	**20**
Control	Root length	3.41±0.17	4.57±0.56	6.84±0.79	8.12±0.96	11.30±0.87	13.72±0.84
	Stem Length	7.53±0.39	9.62±0.74	19.5±0.32	22.6±0.53	28.30±0.63	31.62±0.80
	Leaf area	288.6±11.6	490.7±21.6	641.4±10.0	784.2±16.3	967.5±9.50	1158.7±17.3
Cadmium	Root length	3.41±0.97	3.96±0.97	4.28±0.13	5.89±0.31	6.13±0.11	6.89±0.93
	Stem Length	7.53±0.39	7.89±0.72	11.33±1.43	14.84±0.92	17.62±0.81	21.52±0.76
	Leaf area	288.6±11.6	323.4±14.2	386.7±11.3	423.2±8.20	567.5±11.4	593.2±7.20
Chromium	Root length	3.41±0.17	4.16±0.82	5.92±0.98	7.16±0.35	9.28±0.81	10.67±1.13
	Stem Length	7.53±0.39	8.39±0.13	9.67±0.70	13.23±0.93	14.77±0.78	16.39±0.75
	Leaf area	288.6±11.6	319.3±9.30	428.6±9.3	499.3±7.82	584.2±9.31	612.5±12.4
Mercury	Root length	3.41±0.17	4.12±0.35	4.86±0.56	5.28±0.69	6.95±0.52	8.54±0.25
	Stem Length	7.53±0.39	8.95±0.81	17.39±0.39	19.34±0.23	23.21±0.85	24.59±0.31
	Leaf area	288.6±11.6	334.7±11.3	489.8±14.6	596.2±10.2	633.4±12.5	824.2±15.2
Lead	Root length	3.41±0.17	4.39±0.39	5.86±0.67	6.64±0.92	9.16±0.50	10.93±1.25
	Stem Length	7.53±0.39	8.34±1.04	12.39±0.73	17.38±0.52	23.28±0.58	25.34±0.82
	Leaf area	288.6±11.6	413.5±8.95	487.4±12.2	639.3±4.43	832.2±6.30	884.4±7.43

Values are mean of 5 replicates ±standard error

Tolerance Index

Values of tolerance index evaluated on the basis of relative root length of experimental plants and control showed that maximum values were shown in the first interval in all four metals and the values showed slight reduction after 8th day of growth. During further growth, the values showed continuous reduction in Hg and Pb treated plants without any significant difference between the intervals. Cadmium and chromium showed slight increase in tolerance index values in sample of 8th day and significant reduction during further growth in cadmium treated plants, where as in chromium treated plants, the reduction was insignificant (Table 5; Fig. 5).

Table: 5 Effect of Heavy Metals on Tolerance Index percentage pertaining to Root length in *Boerhavia diffusa* during growth

Treatment	Interval (Days)				
	4	**8**	**12**	**16**	**20**
Control	100	100	100	100	100
Cadmium	86.65±2.18	72.57±2.51	62.53±2.31	54.20±2.65	50.20±2.19
Chromium	91.02±3.09	86.54±3.42	88.17±3.91	82.12±3.61	77.76±3.41
Mercury	90.15±2.91	71.05±3.14	65.02±2.40	61.50±2.93	62.20±3.10
Lead	96.06±3.61	85.67±3.58	81.77±2.64	81.06±2.98	79.60±3.41

Stomatal index

Stomatal index of *B. diffusa* plants treated with all heavy metals showed significant changes. Cadmium treatment resulted in significant increase of stomatal index in the lower epidermis in comparison with that of control, whereas stomatal index of upper epidermis remained almost unchanged. Changes due to chromium treatment in the stomatal index of both upper and lower epidermis was negligible compared to control. Maximum value of stomatal index of both lower and upper epidermis was shown by the plants treated with mercury compared to the control as well as other treatments. Plants treated with lead showed only slight increase in the stomatal index values of upper and lower epidermis compared to the control (Table 6; Fig. 6).

Table 6: Effect of Heavy Metals on the stomatal index in *Boerhavia diffusa*

Treatments	Tissues	Interval (Days)					
		0	**4**	**8**	**12**	**16**	**20**
Control	Lower epidermis	17.34±1.24	19.50±1.22	23.68±1.08	25.26±1.50	27.53±1.22	29.64±1.25
	Upper epidermis	15.50±1.30	17.65±1.36	19.31±1.24	22.27±1.54	23.84±1.10	24.39±1.45
Cadmium	Lower epidermis	17.34±1.24	22.34±0.98	25.62±0.95	29.84±1.23	37.61±1.25	41.23±1.50
	Upper epidermis	15.50±1.30	17.39±0.87	19.75±1.26	21.06±0.97	22.93±1.50	23.75±1.24
Chromium	Lower epidermis	17.34±1.24	19.36±1.24	21.39±1.33	24.74±1.25	27.59±1.23	32.36±1.65
	Upper epidermis	15.50±1.30	16.45±1.64	23.23±1.42	24.42±1.45	26.17±1.06	28.10±1.06
Mercury	Lower epidermis	17.34±1.24	28.35±1.75	31.74±1.22	34.52±1.47	36.73±1.24	49.65±0.84
	Upper epidermis	15.50±1.30	24.28±0.88	27.74±1.87	31.63±1.24	36.28±1.53	36.97±1.23
Lead	Lower epidermis	17.34±1.24	23.71±1.37	25.28±0.95	28.61±0.94	33.25±1.06	36.26±1.24
	Upper epidermis	15.50±1.30	19.28±1.31	24.40±1.10	25.64±1.32	28.10±0.98	32.75±1.46

Values are mean of 5 replicates ±standard error

Dry weight distribution

Dry weight distribution of root tissue of *B. diffusa* plants treated with all four heavy metals showed increase compared to the respective controls during all intervals of growth. Though the differences were not significant, the increase in the dry weight of roots of plants were more compared to the control plants during all stages of growth treated with cadmium, chromium, mercury and lead. Stem tissue also exhibited more or less same trend in the distribution of dry weight content. Only negligible fluctuations were observed in the distribution of dry matter content of leaves of the plants treated with cadmium, chromium, mercury and lead (Table 7; Fig.7).

Table 7: Effect of Heavy Metals on the Dry weight percentage in *Boerhavia diffusa*

Treatments	Tissues	Interval (Days)					
		0	4	8	12	16	20
Control	Root	8.900±0.13	10.50±0.29	11.74±0.15	12.35±0.11	13.05±0.16	13.56±0.11
	Stem	18.84±0.06	19.57±0.06	19.61±0.02	20.43±0.08	22.21±0.03	22.89±0.01
	Leaf	11.71±0.07	12.37±0.05	13.43±0.11	13.97±0.07	15.38±0.03	16.76±0.10
	Shoot*	30.59	31.94	33.04	34.40	37.59	39.65
Cadmium	Root	8.900±0.13	10.91±0.23	12.26±0.13	13.74±0.06	14.63±0.02	14.98±0.01
	Stem	18.84±0.06	19.32±0.12	19.97±0.21	21.26±0.44	23.39±0.12	24.67±0.41
	Leaf	11.71±0.07	11.91±0.06	12.64±0.03	13.92±0.16	16.72±0.12	17.77±0.04
	Shoot	30.55	31.23	32.61	35.18	40.11	42.44
Chromium	Root	8.900±0.13	11.17±0.21	12.76±0.20	13.93±0.16	15.27±0.11	15.87±0.15
	Stem	18.84±0.06	20.31±0.23	21.93±0.17	23.34±0.13	24.62±0.28	25.33±0.13
	Leaf	11.71±0.07	12.23±0.07	12.94±0.04	14.62±0.03	15.98±0.06	18.31±0.43
	Shoot	30.55	32.54	34.87	37.96	40.60	43.64
Mercury	Root	8.900±0.13	9.320±0.12	9.960±0.11	12.73±0.29	13.67±0.16	15.14±0.23
	Stem	18.84±0.06	19.27±0.17	19.94±0.06	20.76±0.03	21.17±0.11	23.15±0.15
	Leaf	11.71±0.07	12.07±0.11	12.62±0.17	13.33±0.16	15.72±0.13	15.93±0.07
	Shoot	30.55	31.34	32.56	34.09	36.89	39.08
Lead	Root	8.900±0.13	9.730±0.21	10.91±0.11	12.62±0.17	13.55±0.10	14.72±0.24
	Stem	18.84±0.06	18.96±0.06	19.73±0.02	21.68±0.07	23.12±0.21	24.74±0.17
	Leaf	11.71±0.07	11.88±0.03	12.96±0.07	13.88±0.21	15.37±0.01	17.56±0.03
	Shoot	30.55	30.84	32.69	35.56	36.67	42.30

Values are mean of 5 replicates ±standard error, * Sum of stem and leaf

Protein content

In comparison with the control, total protein content of root tissue exhibited significant reduction in plants treated with all heavy metals. Maximum reduction of protein content was shown by plants treated with chromium during all stages of growth. Protein content of the stem tissue also was significantly reduced in all metal treated plants compared to the control. Maximum reduction was shown by plants treated with cadmium. Protein content of the leaf tissues also were reduced due to the treatment with all heavy metals more or less uniformly (Table 8; Figs. 8 & 9).

Soluble protein content of root tissue was reduced significantly due to the treatment with cadmium, mercury and lead during all developmental stages. Chromium treatment showed more reduction in the soluble protein content compared to other metals. However treatment with all the metals resulted in only negligible changes in the distribution of soluble protein content in the stem tissue. Leaf tissue exhibited significant reduction of soluble protein content in the samples of all intervals ($P<0.01$) respectively of growth on 20^{th} day. Maximum reduction was shown by the plants treated with chromium(Table 8; Figs. 8 & 9).

Table 8: Effect of Heavy Metals on Protein content in *Boerhavia diffusa* (mg g^{-1} dry weight)

Treatments	Tissues	Interval (Days)					
		0	4	8	12	16	20
Control	**Root**	87.12±2.01 (21.34±1.30)	97.65±2.71 (28.63±0.9)	116.5±3.21 (33.67±1.2)	128.0±4.12 (42.35±1.2)	141.21±1.12 (47.5±1.11)	156.2±2.19 (52.64±1.1)
	Stem	19.39±1.10 (8.25±0.93)	28.32±1.18 (14.48±1.3)	39.16±2.10 (21.39±1.2)	47.58±3.10 (26.62±0.9)	53.62±2.37 (28.50±0.8)	62.56±1.17 (29.98±1.1)
	Leaf	116.7±2.31 (28.50±2.10)	128.7±1.17 (36.63±1.3)	144.2±2.21 (47.52±1.7)	163.3±3.12 (54.30±2.0)	173.5±1.18 (66.23±1.2)	189.3±2.45 (73.58±2.0)
Cadmium	**Root**	87.12±2.01 (21.34±1.30)	89.52±2.21 (22.39±0.9)	97.32±1.17 (28.57±1.1)	115.1±2.20 (32.69±2.2)	119.3±1.15 (38.36±0.9)	128.5±1.03 (44.51±0.6)
	Stem	19.39±1.10 (8.25±0.93)	22.35±1.18 (11.39±2.1)	28.39±1.32 (17.64±1.7)	34.63±1.17 (24.38±2.1)	37.58±2.21 (26.41±0.9)	41.84±0.91 (27.54±0.9)
	Leaf	116.7±2.31 (28.50±2.10)	119.5±1.12 (29.39±2.0)	125.3±1.23 (37.30±1.2)	132.3±2.12 (47.21±1.8)	137.6±1.19 (55.39±1.3)	143.6±2.10 (63.23±1.1)
Chromium	**Root**	87.12±2.01 (21.34±1.30)	88.62±2.10 (22.40±1.1)	92.56±1.11 (25.69±2.0)	97.39±1.21 (29.33±1.2)	107.9±0.91 (34.21±0.9)	116.5±1.10 (39.62±0.7)
	Stem	19.39±1.10 (8.25±0.93)	23.78±2.11 (12.61±1.0)	28.39±1.12 (14.39±0.6)	33.59±2.11 (17.63±1.4)	39.62±1.10 (21.56±1.2)	44.21±1.70 (27.30±1.0)
	Leaf	116.7±2.31 (28.50±2.10)	119.6±0.12 (28.39±0.1)	124.6±1.20 (34.21±0.7)	129.4±2.13 (39.54±1.1)	134.5±1.76 (44.91±2.1)	141.6±2.12 (47.23±1.3)
Mercury	**Root**	87.12±2.01 (21.34±1.30)	92.50±0.98 (23.92±1.7)	97.39±1.13 (28.40±0.1)	104.9±1.97 (35.63±1.7)	118.2±2.01 (39.21±0.9)	129.0±1.11 (44.53±1.6)
	Stem	19.39±1.10 (8.25±0.93)	24.46±2.12 (11.56±1.2)	29.53±1.70 (15.39±0.8)	33.80±1.41 (19.62±0.7)	41.62±1.18 (23.37±1.1)	48.31±1.10 (30.34±1.0)
	Leaf	116.7±2.31 (28.50±2.10)	121.52±1.1 (32.27±0.5)	128.9±1.12 (37.65±1.1)	137.6±2.21 (41.39±1.0)	141.2±1.10 (45.32±1.0)	146.5±0.97 (49.65±0.7)
Lead	**Root**	87.12±2.01 (21.34±1.30)	94.39±0.98 (24.39±0.9)	103.6±1.11 (27.30±0.5)	113.2±1.21 (35.61±0.8)	128.5±0.98 (39.33±0.1)	133.0±1.15 (46.43±0.2)
	Stem	19.39±1.10 (8.25±0.93)	25.83±1.91 (13.52±0.8)	29.39±0.98 (17.69±0.1)	36.57±1.05 (20.39±0.2)	41.39±1.15 (24.68±0.2)	57.21±1.12 (27.51±0.9)
	Leaf	116.7±2.31 (28.50±2.10)	123.6±1.23 (34.5±0.6)	128.3±2.11 (39.58±0.1)	133.5±1.09 (43.62±0.4)	142.4±1.87 (47.39±0.2)	151.0±0.98 (56.31±0.2)

(Values in parenthesis are soluble protein); Values are mean of 5 replicates ±standard error

Protein profile

SDS – PAGE profile analysis showed only two distinct bands with molecular weight 14.8 KDa and 18.5 KDa in the root of control plants where as in root tissue of plants treated with cadmium, two new bands (8.85 KD and 18.71KDa) were present (Fig.10). Similarly in chromium treatment also two new bands with different molecular weight were present. Due to mercury treatment, two bands more or less similar to that of chromium treatment were observed. Lead treated root tissue showed only a single additional band that was absent in the control.

Stem tissue of plants treated with cadmium, the range of molecular weight was 5.18KDa to 151.23 KDa compared to the control (Fig. 11). Six new bands which were absent in the control. In chromium treated plants only five new bands were present with different molecular weight which were not present in the control. More or less similar bands were present in mercury treatment but molecular weight range was 4.26 KDa to 80.85KDa. The protein profile of treated tissue showed very thick band with molecular weight 26.78 KDa in addition to 26.78 molecular weight band. Six more bands ranges from 3.63KDa to 80.34KDa.

Protein profile of leaf of plants treated with cadmium showed number of protein bands having molecular weight ranges from 2.81KDa to 62.59KDa compared to the control (Fig.12). Many bands were newly appeared

particularly proteins with high molecular weight range. Plants treated with chromium exhibited lesser number of protein bands which were stained feebly compared to the control as well as the cadmium treated plants. Maximum number of protein bands 1.188 KDa to 65.15 KDa shown by leaf tissue of plants treated with mercury in which many protein were new which were absent both in the control and other treatments. Comparatively lesser number of bands were present in the leaf protein profile of plants treated with lead and the staining intensity of bands also was very high (Fig. 12).

Total Free Aminoacids

Compared to the control plants, total free aminoacid content of *B. diffusa* root tissue treated with heavy metals showed significant increase (Table 9). Among the treatments the increase appeared in the order Pb>Cd>Hg>Cr. The free aminoacid content of stem tissue treated with cadmium and lead was very high compared to the control and other treatments. Distribution of free aminoacid content of leaf tissue showed significant increase in plants treated with cadmium, mercury and lead compared to the control. The distribution pattern was in the order Pb>Cd>Hg>Cr (Fig. 13).

Table 9: Effect of Heavy Metals on Total free Amino acid content in *Boerhavia diffusa* (mg g^{-1} dry weight)

Treatments	Tissues	Interval (Days)					
		0	4	8	12	16	20
Control	Root	0.93±0.070	1.52±0.06	1.75±0.12	1.93±0.11	2.25±0.12	2.86±0.12
	Stem	1.23±0.12	1.45±0.02	1.87±0.01	2.34±0.09	2.87±0.13	3.23±0.19
	Leaf	2.24±0.11	2.85±0.03	3.35±0.04	3.87±0.18	4.52±0.11	4.85±0.10
Cadmium	Root	0.93±0.08	1.95±0.07	2.73±0.21	3.35±0.47	4.24±0.41	4.76±0.31
	Stem	1.23±0.11	2.15±0.09	2.79±0.32	3.80±0.63	4.51±0.11	5.18±0.40
	Leaf	2.24±0.02	3.40±0.39	4.69±0.39	5.67±0.21	6.13±0.23	6.85±0.31
Chromium	Root	0.93±0.02	1.50±0.11	1.85±0.22	2.35±0.07	2.85±0.40	3.17±0.11
	Stem	1.23±0.09	1.87±0.21	2.25±0.07	2.76±0.09	2.97±0.11	3.02±0.12
	Leaf	2.24±0.12	2.93±0.30	3.36±0.04	3.94±0.15	4.28±0.31	4.86±0.16
Mercury	Root	0.93±0.02	1.62±0.08	1.83±0.41	2.67±0.09	3.21±0.20	4.36±0.32
	Stem	1.23±0.21	2.03±0.09	2.68±0.41	2.98±0.11	3.54±0.18	4.19±0.43
	Leaf	2.24±0.14	3.06±0.04	4.18±0.21	4.91±0.23	5.93±0.30	6.12±0.35
Lead	Root	0.93±0.09	2.57±0.21	3.12±0.12	3.88±0.11	4.63±0.39	5.18±0.42
	Stem	1.23±0.11	3.09±0.51	3.93±0.43	4.63±0.33	5.16±0.12	5.88±0.12
	Leaf	2.24±0.18	3.88±0.13	4.59±0.29	5.61±0.21	6.92±0.41	7.19±0.34

Values are mean of 5 replicates ±standard error

Proline

Proline content of roots exhibited gradual increase due to the treatment of heavy metals during all stages of development in comparison with the respective controls, and on 20th day, the increase was more than twice in plants treated with cadmium (Table 10). Almost the same trend was shown by the chromium and mercury treatment where as the increase was comparatively lower in the plants treated with lead (Table 10). Proline content of the stem tissue also showed significant hike in all treatments showing maximum quantity in the plants treated with chromium and lead. Leaf tissue of plants treated with lead exhibited increased proline which is more than eight times compared to the control. Proline content of leaf tissue of plants treated with mercury and cadmium also was very high compared to the control. However proline content of leaf treated with chromium was comparatively lower than the other treatments (Table 10). The increase in the proline content during developmental stages of leaves was highly significant in all treatments compared to the control plants (Fig. 14).

Table 10: Effect of Heavy Metals on Proline content in *Boerhavia diffusa* (mg g^{-1} dry weight)

Treatments	Tissues	Interval (Days)					
		0	4	8	12	16	20
Control	Root	0.351±0.07	0.476±0.01	0.517±0.02	0.620±0.05	0.673±0.03	0.714±0.01
	Stem	0.247±0.01	0.280±0.01	0.299±0.01	0.325±0.04	0.351±0.01	0.373±0.02
	Leaf	0.106±0.06	0.127±0.01	0.139±0.01	0.144±0.01	0.159±0.02	0.166±0.01
Cadmium	Root	0.351±0.02	0.561±0.01	0.770±0.02	0.858±0.01	1.521±0.12	1.891±0.10
	Stem	0.247±0.01	0.297±0.01	0.341±0.03	0.395±0.02	1.324±0.18	1.643±0.10
	Leaf	0.106±0.05	0.144±0.01	0.185±0.01	0.213±0.03	0.263±0.12	0.296±0.02
Chromium	Root	0.351±0.03	0.570±0.01	0.785±0.03	0.930±0.01	1.450±0.031	1.860±0.08
	Stem	0.247±0.02	0.355±0.02	0.452±0.01	1.520±0.01	1.850±0.031	2.530±0.20
	Leaf	0.106±0.01	0.251±0.03	0.363±0.02	0.427±0.04	0.591±0.02	0.914±0.04
Mercury	Root	0.351±0.03	0.623±0.02	0.959±0.01	1.259±0.01	1.537±0.20	1.875±0.02
	Stem	0.247±.0.10	0.452±0.07	0.732±0.02	0.917±0.01	1.253±0.10	1.289±0.02
	Leaf	0.106±0.02	0.315±0.01	0.406±0.01	0.538±0.07	0.686±0.02	0.905±0.01
Lead	Root	0.351±0.01	0.734±0.05	0.858±0.01	0.921±0.02	1.280±0.11	1.530±0.31
	Stem	0.247±0.04	0.625±0.01	0.936±0.01	1.531±0.02	2.425±0.02	2.870±0.82
	Leaf	0.106±0.01	0.381±0.01	0.562±0.01	0.685±0.01	0.934±0.02	1.216±0.08

Values are mean of 5 replicates ±standard error

Chlorophyll

Compared to the control plants, the chlorophyll a and b contents were reduced in the plants treated with cadmium and the a/b ratio remained unchanged (Table 11). Significant reduction of chlorophyll a and b was observed in the plants under chromium toxicity ($P<0.01$). More or less similar results were obtained in the plants treated with mercury. Plants treated with lead exhibited only negligible changes in the distribution of chlorophyll a and b compared to the control retaining the same chlorophyll a/b ratio. Total chlorophyll content was reduced due to the treatments with all the metals (Table 11; Fig. 15).

Nitrate reductase

Nitrate reductase activity of control plants showed very high rate in the leaf tissue, only less than half of this activity was shown by root and stem tissue (Table 12). Cadmium treatment resulted in a significant reduction of nitrate reductase activity in all tissues in general and leaf in particular. Plants with all the other three heavy metals also exhibited significant reduction in the nitrate reductase activity compared to the plants treated with cadmium as well as control plants (Figs. 16, 17 & 18).

Table 11: Effect of Heavy Metals on Chlorophyll content in *Boerhavia diffusa* (mg g^{-1} dry weight)

Treatments	Tissues	Interval (Days)					
		0	4	8	12	16	20
Control	Chl.a	0.98±0.02	1.13±0.06	1.37±0.06	1.57±0.05	1.78±0.08	2.17±0.08
	Chl.b	0.57±0.01	0.63±0.04	0.78±0.03	0.86±0.08	0.92±0.04	1.07±0.05
	a/b	1.71±0.03	1.79±0.10	1.75±0.05	1.82±0.04	1.93±0.08	2.02±0.11
	Total	1.55±0.04	1.76±0.05	2.15±0.12	2.43±0.12	2.70±0.05	3.24±0.16
Cadmium	Chl.a	0.98±0.03	0.91±0.02	1.23±0.07	1.42±0.08	1.55±0.08	1.84±0.10
	Chl.b	0.57±0.01	0.42±0.02	0.53±0.02	0.66±0.03	0.78±0.01	0.93±0.05
	a/b	1.71±0.02	2.16±0.09	2.32±0.12	2.15±0.11	1.98±0.06	1.97±0.05
	Total	1.55±0.04	1.33±0.08	1.76±0.07	2.08±0.02	2.33±0.05	2.77±0.16
Chromium	Chl.a	0.98±0.02	0.63±0.02	0.75±0.03	0.93±0.04	1.23±0.09	1.34±0.08
	Chl.b	0.57±0.06	0.39±0.01	0.41±0.02	0.58±0.02	0.68±0.02	0.84±0.05
	a/b	1.71±0.03	1.61±0.06	1.82±0.08	1.60±0.09	1.80±0.08	1.65±0.06
	Total	1.55±0.06	1.02±0.05	1.16±0.04	1.51±0.06	1.91±0.05	2.23±0.08
Mercury	Chl.a	0.98±0.02	0.72±0.02	0.89±0.03	1.13±0.07	1.37±0.08	1.49±0.07
	Chl.b	0.57±0.02	0.43±0.01	0.59±0.01	0.67±0.01	0.73±0.03	0.86±0.02
	a/b	1.71±0.07	1.67±0.04	1.50±0.04	1.68±0.04	1.87±0.05	1.63±0.07
	Total	1.55±0.03	1.15±0.07	1.48±0.05	1.80±0.04	2.10±0.09	2.27±0.13
Lead	Chl.a	0.98±0.02	0.83±0.03	1.12±0.07	1.37±0.05	1.48±0.04	1.93±0.10
	Chl.b	0.57±0.01	0.45±0.02	0.63±0.01	0.73±0.02	0.83±0.03	0.94±0.13
	a/b	1.71±0.08	1.84±0.08	1.77±0.04	1.87±0.08	1.78±0.09	2.05±0.10
	Total	1.55±0.08	1.28±0.07	1.75±0.05	2.10±0.02	2.31±0.11	2.87±0.10

Values are mean of 5 replicates ±standard error

Table 12: Effect of Heavy Metals on Nitrate reductase activity in *Boerhavia diffusa* (µ moles NO_2 g^{-1} dry weight)

Treatments	Tissues	Interval (Days)					
		0	4	8	12	16	20
Control	Root	9.55±0.16	11.3±0.90	13.2±0.12	13.9±0.22	14.6±0.11	15.2±0.24
	Stem	16.4±0.13	17.2±0.86	17.9±0.15	18.1±0.62	19.6±0.11	21.7±0.32
	Leaf	33.5±1.01	39.2±1.11	46.8±1.24	53.1±0.45	52.6±0.47	53.8±0.21
Cadmium	Root	9.55±0.26	6.78±0.14	7.13±0.21	8.98±0.19	9.96±0.11	10.2±0.14
	Stem	16.4±0.13	12.7±0.12	14.2±0.26	16.1±0.23	17.5±0.15	17.9±0.11
	Leaf	33.5±1.01	30.4±0.05	32.1±1.02	35.6±1.20	37.6±0.41	37.8±1.31
Chromium	Root	9.55±0.36	8.42±0.45	9.21±0.17	10.5±0.74	10.9±0.67	11.4±0.26
	Stem	16.4±0.23	10.5±0.11	11.4±0.88	12.6±0.21	13.3±0.19	15.7±0.01
	Leaf	33.5±1.01	25.7±0.45	26.8±0.56	28.6±0.43	29.6±0.44	31.8±0.41
Mercury	Root	9.55±0.16	5.62±0.14	6.98±0.32	7.54±0.14	9.70±0.12	10.6±0.19
	Stem	16.4±0.23	13.1±0.13	14.7±0.98	15.8±0.51	17.9±0.32	18.0±0.55
	Leaf	33.5±1.01	26.0±0.45	27.8±0.82	28.5±0.56	29.4±0.49	31.7±0.41
Lead	Root	9.55±0.16	9.84±0.21	10.8±0.42	11.6±0.13	12.8±0.52	14.6±0.21
	Stem	16.4±0.23	12.6±0.32	13.7±0.11	13.9±0.31	12.0±0.41	11.9±0.30
	Leaf	33.5±0.31	25.7±0.79	27.7±0.41	28.9±0.43	29.3±0.28	29.7±1.43

Values are mean of 5 replicates ±standard error

Phenolics

Phenolic content of root tissue was increased insignificantly in the plants treated with cadmium whereas the increase was significant in the roots treated with chromium and mercury (Table 13). Phenolics of root tissue treated with lead showed a doubling compared to the control. The stem tissue also followed more or similar trend in the distribution of phenolics as that of root tissue. Phenolic content of leaf tissue of *B. diffusa* plant treated with cadmium did not show any change in comparison with the control, whereas significant increase was observed in the leaf tissue of plants treated with chromium, mercury and lead ($P<0.01$). Among the treatments, the increase in the phenolics of leaf tissue was in the order Cr<Hg<Pb (Fig. 19).

Lipid peroxidation (MDA content)

The malondialdehyde content of root tissue was more than that of the stem and leaf tissue of control plants (Table 14). Plants treated with cadmium showed significant increase in the MDA content and four times increase was observed in root tissue. Significant increase was occurred in the other tissues compared to the control. MDA content of root tissue of plants treated with chromium also was increased during all stages of growth but values were lower than that of cadmium treatment whereas stem and leaf showed similar amount of MDA in the plants treated with cadmium and chromium. Plants treated with mercury and lead exhibited more or less similar values of MDA content as that of cadmium treatment in all tissue at all intervals (Figs. 20, 21 & 22).

Table 13: Effect of Heavy Metals on Phenolics in *Boerhavia diffusa* (mg g^{-1} dry weight)

Treatments	Tissues	Interval (Days)					
		0	4	8	12	16	20
Control	Root	0.45±0.03	0.68±0.02	0.79±0.01	0.93±0.03	1.21±0.03	1.38±0.02
	Stem	0.68±0.01	0.98±0.05	1.54±0.10	1.68±0.01	1.73±0.04	1.89±0.10
	Leaf	0.79±0.04	0.92±0.02	1.24±0.10	1.58±0.09	1.67±0.06	1.81±0.12
Cadmium	Root	0.45±0.02	0.73±0.05	0.95±0.05	1.24±0.10	1.59±0.04	1.67±0.09
	Stem	0.68±0.03	1.12±0.09	1.47±0.06	1.68±0.09	1.98±0.03	2.10±0.08
	Leaf	0.79±0.02	1.14±0.10	1.27±0.08	1.38±0.08	1.59±0.08	1.78±0.10
Chromium	Root	0.45±0.04	0.84±0.03	1.26±0.02	1.58±0.10	1.94±0.09	2.10±0.08
	Stem	0.68±0.04	0.93±0.02	1.45±0.10	1.73±0.02	1.98±0.08	2.51±0.13
	Leaf	0.79±0.02	1.27±0.04	1.59±0.08	1.81±0.08	2.33±0.10	2.85±0.10
Mercury	Root	0.45±0.01	0.95±0.02	1.59±0.10	1.74±0.09	2.36±0.10	2.59±0.12
	Stem	0.68±0.02	0.95±0.02	1.62±0.09	1.84±0.12	2.40±0.11	2.83±0.23
	Leaf	0.79±0.02	1.03±0.04	1.56±0.10	1.89±0.14	2.62±0.11	3.54±0.14
Lead	Root	0.45±0.01	0.54±0.05	1.52±0.10	2.04±0.18	2.85±0.12	3.94±0.10
	Stem	0.68±0.04	0.69±0.03	1.26±0.20	1.89±0.09	2.64±0.12	3.87±0.12
	Leaf	0.79±0.07	1.12±0.01	1.86±0.02	2.58±0.09	3.62±0.12	3.86±0.13

Values are mean of 5 replicates ±standard error

Table 14: Effect of Heavy Metals on Lipid Peroxidation in *Boerhavia diffusa* (MDA content µ moles g^{-1} dry weight)

Treatments	Tissues	Interval (Days)					
		0	**4**	**8**	**12**	**16**	**20**
Control	Root	4.16±0.12	4.60±0.11	4.89±0.13	7.24±0.14	8.94±0.15	9.11±0.43
	Stem	1.22±0.07	1.38±0.08	2.09±0.12	3.67±0.13	4.24±0.07	5.67±0.15
	Leaf	0.95±0.07	1.07±0.04	1.98±0.06	2.33±0.16	2.87±0.08	3.46±0.18
Cadmium	Root	4.16±0.12	19.8±0.32	23.4±0.01	25.4±0.10	28.3±0.46	36.8±1.31
	Stem	1.22±0.04	5.07±0.11	7.30±0.32	12.2±0.22	13.6±0.56	16.1±0.21
	Leaf	0.95±0.04	3.98±0.18	4.13±0.12	5.26±0.31	5.89±0.23	6.30±0.11
Chromium	Root	4.16±0.12	15.7±0.50	18.8±0.19	24.2±1.09	27.9±0.82	29.5±1.45
	Stem	1.22±0.08	4.68±0.05	5.13±0.81	9.30±0.54	12.2±0.47	13.6±0.82
	Leaf	0.95±0.04	2.98±0.03	3.25±0.54	5.47±0.21	6.22±0.13	6.88±0.11
Mercury	Root	4.16±0.12	17.0±0.18	26.0±0.08	29.3±0.94	35.4±0.92	39.4±1.08
	Stem	1.22±0.14	10.6±0.35	12.1±0.82	14.2±0.21	15.0±0.33	17.8±0.91
	Leaf	0.95±0.07	4.24±0.06	4.67±0.13	6.54±0.11	8.23±0.24	11.8±0.81
Lead	Root	4.16±0.12	18.6±0.32	21.9±0.98	25.3±0.41	35.6±1.31	38.4±1.11
	Stem	1.22±0.06	7.06±0.58	9.39±0.21	11.4±0.17	13.9±0.13	15.2±0.84
	Leaf	0.95±0.03	3.53±0.12	5.60±0.06	7.54±0.55	9.27±0.18	9.91±0.23

Values are mean of 5 replicates ±standard error

Peroxidase activity

Guaiacol peroxidase was very active in the root tissue of the control plants whereas in the stem and leaf the activity of the enzyme was feeble (Table 15). Plants treated with cadmium exhibited significant hike in the peroxidise activity during all developmental stages in the root tissue. The changes in activity was significant in the stem but not significant in the leaf compared to the control (Table 15). Chromium treatment resulted in a significant increase of peroxidise activity in the root, stem and leaf tissue. Peroxidase activity of root tissue due to mercury treatment was more or less similar to that of cadmium. Maximum peroxidise activity was observed in the root tissue of plants treated with lead but considerable increase was observed in the peroxidise activity of stem and leaf tissues compared to the respective control and tissue of other treatments. Predominant increase of peroxidise activity of all the treatments was found to be the characteristic of all root tissue. Changes in the other tissues were not much significant. The specific activity of root tissue showed reduced values of peroxidise activity in the treatments. Plants treated with cadmium, chromium, mercury and lead compared to the control, the specific activity of peroxidise enzyme in the stem tissue did not vary significantly among the treatments. In the leaf tissue also the fluctuation were negligible between the control and treatments (Figs. 23, 24, 25, 26, 27 & 28).

Table 15: Effect of Heavy Metals on Peroxidase activity in *Boerhavia diffusa* (Unit g^{-1} dry weight)

Treatments	Tissues	Interval (Days)					
		0	4	8	12	16	20
Control	Root	106.4±3.17 (6.07±0.32)	109.9±2.20 (6.28±0.04)	116.03±3.3 (6.47±0.13)	117.29±8.62 (7.12±0.09)	119.24±3.35 (8.30±0.07)	121.2±4.42 (9.02±0.13)
	Stem	18.04±1.42 (4.94±0.74)	17.34±0.92 (5.09±0.02)	20.24±0.82 (5.11±0.03)	23.12±1.02 (5.23±0.11)	24.70±1.14 (5.67±0.09)	26.71±1.27 (5.89±0.08)
	Leaf	14.94±0.83 (3.42±0.09)	15.38±0.34 (3.97±0.03)	18.42±1.21 (4.23±0.37)	19.67±0.93 (4.20±0.13)	22.63±1.10 (4.33±0.05)	23.97±1.03 (4.67±0.16)
Cadmium	Root	106.4±3.17 (6.07±0.32)	149.5±3.20 (6.54±0.05)	158.2±7.46 (7.43±0.21)	163.3±.6.65 (7.93±0.70)	167.2±2.31 (7.14±0.12)	176.5±4.93 (7.21±0.23)
	Stem	18.04±1.42 (4.94±0.74)	23.36±0.54. (5.02±0.04)	28.76±0.92 (5.43±0.12)	29.87±1.21 (6.02±0.14)	31.33±1.05 (5.85±0.14)	33.56±1.21 (5.92±0.91)
	Leaf	14.94±0.83 (3.42±0.09)	18.34±0.96 (3.89±0.05)	19.20±1.03 (4.36±0.23)	21.21±0.82 (5.03±0.13)	23.46±1.04 (5.02±0.19)	26.82±1.21 (4.97±0.11)
Chromium	Root	106.4±3.17 (6.07±0.32)	106.7±2.10 (6.10±0.05)	116.3±4.78 (6.46±0.19)	128.5±8.65 (6.89±0.13)	135.7±8.20 (6.54±0.12)	143.0±2.98 (6.38±0.27)
	Stem	18.04±1.42 (4.94±0.74)	19.50±0.91 (5.08±0.05)	21.23±0.80 (5.89±0.13)	25.45±1.37 (6.13±0.41)	27.80±1.07 (6.10±0.13)	35.62±1.74 (5.94±0.24)
	Leaf	14.94±0.83 (3.42±0.09)	16.34±0.67 (4.02±0.05)	19.39±0.82 (4.35±0.06)	23.20±0.74 (4.67±0.16)	27.24±0.93 (4.39±0.12)	29.07±1.31 (4.21±0.17)
Mercury	Root	106.4±3.17 (6.07±0.32)	151.1±1.98 (6.6±0.12)	168.7±8.02 6.37±0.10)	173.5±5.57 (7.01±0.12)	177.6±5.53 (7.04±0.14)	183.2±4.12 (7.13±0.26)
	Stem	18.04±1.42 (4.94±0.74)	18.16±0.71 (5.12±0.07)	21.39±1.05 (5.64±0.17)	23.45±0.90 (6.02±0.18)	29.87±0.94 (5.98±0.12)	33.06±1.56 (5.72±0.41)
	Leaf	14.94±0.83 (3.42±0.09)	13.35±0.81 (3.67±0.09)	16.45±1.20 (3.98±0.20)	19.07±0.78 (4.01±0.12)	25.62±1.09 (3.87±0.31)	29.28±1.05 (3.56±0.18)
Lead	Root	106.4±3.17 (6.07±0.32)	177.8±3.98 (6.32±0.10)	189.0±8.32 (6.68±0.21)	197.1±5.58 (7.03±0.16)	212.4±8.70 (7.00±0.12)	219.6±5.34 (7.07±0.09)
	Stem	18.04±1.42 (4.94±0.74)	20.38±0.98 (5.21±0.15)	29.34±1.60 (5.54±0.09)	32.46±1.04 (5.97±0.13)	35.76±1.02 (6.32±0.08)	38.21±1.54 (6.21±0.08)
	Leaf	14.94±0.83 (3.42±0.09)	17.92±0.93 (3.01±0.07)	21.43±0.96 (3.45±0.12)	24.50±0.68 (3.97±0.10)	28.24±1.09 (3.62±0.14)	31.36±1.02 (3.06±0.20)

(Values in parenthesis are specific activity) Values are mean of 5 replicates ±standard error

Superoxide dismutase

Superoxide dismutase activity was maximum in the root tissue of the control and treatments compared to the stem and leaf (Table 16). Due to cadmium treatment, superoxide dismutase activity was doubled and significant increase was observed in the stem and leaf tissues ($P < 0.01$) during all stages of growth. Superoxide dismutase activity of plants treated with chromium was significantly higher than that of the control but there occurred slight reduction in the SOD activity of the chromium treated tissue compared to the cadmium treatment in all tissue samples. Tissue of plants treated with mercury also showed significantly higher superoxide dismutase activity compared to the control. Maximum activity was shown by roots of the plants treated with lead compared to other treatments and control in the samples of all intervals. The increase occurred in the superoxide dismutase activity of the stem and leaves also was highly significant during growth, the activity was continuously increased (Figs. 29, 30 & 31).

Table 16: Effect of Heavy Metals on Superoxide dismutase activity in *Boerhavia diffusa* (unit g^{-1} dry weight)

Treatments	Tissues	Interval (Days)					
		0	4	8	12	16	20
Control	Root	63.93±1.87	72.29±1.43	78.91±1.91	81.82±2.57	83.46±1.36	87.07±1.20
	Stem	11.30±0.76	15.40±0.08	16.60±0.92	18.39±1.10	18.92±0.56	19.35±0.49
	Leaf	9.131±0.90	9.983±0.32	10.50±0.81	11.75±0.56	13.01±0.87	13.48±0.23
Cadmium	Root	63.93±1.87	114.5±2.10	123.1±2.08	137.0±2.01	143.5±3.21	151.6±2.61
	Stem	11.30±0.76	27.12±1.08	33.90±1.12	41.22±1.43	47.86±0.54	51.58±1.08
	Leaf	9.131±0.90	23.17±1.02	27.20±1.20	31.53±0.81	36.82±0.41	39.31±1.51
Chromium	Root	63.93±1.87	83.61±1.23	93.21±2.23	127.6±2.14	132.9±1.52	139.6±1.79
	Stem	11.30±0.76	23.09±1.06	29.31±2.09	38.35±1.17	42.10±4.20	44.29±1.46
	Leaf	9.131±0.90	19.70±1.07	23.21±1.31	32.45±1.06	33.17±0.97	35.87±0.97
Mercury	Root	63.93±1.87	145.9±2.34	167.3±2.12	178.6±2.97	188.2±4.60	189.5±2.23
	Stem	11.30±0.76	29.78±1.07	36.57±1.83	39.41±1.65	42.31±1.50	44.67±1.51
	Leaf	9.131±0.90	21.45±0.06	23.67±1.02	27.87±0.71	34.67±1.52	36.87±0.98
Lead	Root	63.93±1.87	239.4±1.12	246.7±7.82	252.1±8.51	266.7±7.20	269.5±1.52
	Stem	11.30±0.76	49.26±0.32	54.50±1.97	57.21±1.13	57.76±1.02	61.30±1.01
	Leaf	9.131±0.90	31.40±0.32	36.54±1.05	41.25±1.87	43.23±1.12	43.87±1.48

Values are mean of 5 replicates ±standard error

Catalasae

Catalase activity was maximum of all the root tissue of all treatments and control compared to the stem and leaf tissues. Due to cadmium treatment there occurred a significant increase in catalase activity at all intervals (Table 17). Catalase activity was doubled in the root tissues and a significant increase was shown by stem and leaf tissue at all intervals. Maximum catalase activity was shown by root tissue of plants treated with chromium (about 4 times increase) compared to the control. Leaf and stem also showed significant increase than that of control. Plants treated with mercury and lead also showed increased catalase activity. Stem and leaf also showed more catalase activity in the tissue of mercury and lead treated plants compared to the control (Figs. 32, 33 & 34).

Specefic activity of catalase in the root tissues was doubled in the plants treated with cadmium compared to the control. Stem and leaf tissues also showed significantly higher catalase activity in the plants due to cadmium treatment. Plants treated with chromium showed maximum specific activity of catalase in the root tissue compared to other treatments and the control, where as leaf and stem showed slight increase in the specific activity of catalase compared to the control. Specific activity of roots of plants treated with mercury and lead also was very high. Leaf and stem showed more or less similar value as that of chromium treatment. Specific activity of catalase of root tissues treated with lead was less than that of mercury and chromium treatments, but the activity of the leaf and stem tissues remained unchanged compared to the control (Table 17; Figs. 35, 36 & 37).

Table 17: Effect of Heavy Metals on Catalase activity in *Boerhavia diffusa* (Unit g^{-1} dry weight)

Treatments	Tissues	Interval (Days)					
		0	**4**	**8**	**12**	**16**	**20**
Control	Root	194.9±3.12 (32.47±0.56)	203.3±6.29 (34.21±0.23)	207.8±2.23 (35.32±0.12)	213.6±3.87 (36.1±0.34)	215.3±2.56 (38.7±0.46)	217.9±2.21 (39.2±0.23)
	Stem	65.87±1.21 (7.16±0.34)	68.90±2.65 (7.98±0.12)	75.31±2.22 (8.32±0.23)	79.09±3.09 (9.43±0.63)	86.02±1.56 (11.2±0.14)	91.65±1.34 (11.9±0.23)
	Leaf	36.37±0.98 (4.01±0.21)	39.76±0.98 (4.65±0.25)	42.87±1.45 (5.98±0.12)	46.74±1.23 (6.21±0.45)	49.92±1.12 (7.98±0.12)	53.85±0.24 8.23±0.04)
Cadmium	Root	194.9±7.12 (32.47±0.56)	360.9±8.24 (39.98±0.76)	376.9±4.76 (41.34±0.75)	388.9±6.78 (53.21±1.45)	394.2±4.13 (64.22±0.98)	403.7±3.08 (73.21±0.34)
	Stem	65.87±1.21 (7.16±0.34)	96.53±3.06 (26.00±0.31)	98.65±2.56 (29.43±0.67)	113.9±2.45 (33.24±0.04)	116.9±2.07 (34.51±0.85)	125.8±1.06 (36.24±0.44)
	Leaf	36.37±1.98 (4.01±0.21)	67.50±4.21 (13.44±0.34)	76.80±1.09 (17.45±0.51)	84.76±2.23 (19.24±0.50)	89.06±1.34 (21.87±0.64)	92.76±1.54 (21.98±0.32)
Chromium	Root	194.9±2.12 (32.47±0.56)	917.1± 3.27 (114.4±3.11)	976.3±3.08 (123.21±1.95)	987.4±6.54 (134.13±1.09)	996.5±8.27 (165.2±1.56)	998.3±3.65 (168.2±0.32)
	Stem	65.87±1.21 (7.160±0.34)	119.4±4.07 (9.15±0.76)	135.8±3.98 (13.24±0.29)	142.9±2.43 (17.21±0.63)	148.8±4.67 (17.95±0.97)	151.6±2.02 (18.21±0.34)
	Leaf	36.37±0.98 (4.010±0.21)	62.71±0.76 (5.94±0.35)	67.96±1.23 (6.290±0.15)	71.86±2.56 (8.65±0.52)	76.98±1.21 (9.11±0.32)	81.43±2.35 (9.89±0.42)
Mercury	Root	194.9±7.12 (32.47±0.95)	681.2±8.21 (85.08±2.26)	754.7±8.56 (98.21±2.13)	785.3±6.25 (124.09±2.67)	786.9±3.68 (127.6±1.36)	789.5±3.89 (129.8±1.11)
	Stem	65.87±4.21 (7.160±0.34)	143.1±5.45 (11.01±0.24)	176.5±4.98 (14.67±0.54)	185.3±2.26 (17.89±0.55)	197.3±8.09 (18.65±0.91)	213.2±1.12 (19.10±0.12)
	Leaf	36.37±0.98 (4.01±0.21)	69.09±2.96 (4.23±0.56)	73.51±4.98 (5.010±0.3)	78.98±1.35 (9.110±0.12)	81.32±1.67 (10.08±0.11)	89.65±1.28 (10.91±0.14)
Lead	Root	194.9±2.12 (32.47±0.56)	664.9±9.47 (73.74±1.69)	723.7±4.36 (89.09±1.06)	787.4±4.78 (94.76±0.61)	814.6±4.35 (97.87±1.05)	819.4±5.21 (99.14±0.71)
	Stem	65.87±1.21 (7.16±0.34)	87.07±2.10 (5.43±0.36)	98.65±1.36 (7.980±0.94)	112.7±3.98 (9.270±0.61)	118.8±2.25 (11.07±0.13)	122.8±2.21 (11.86±0.23)
	Leaf	36.37±0.98 (4.010±0.21)	54.99±1.87 (5.88±0.14)	67.94±1.56 (6.76±0.88)	72.62±2.21 (8.980±0.33)	83.65±2.28 (9.11±0.22)	89.43±2.56 (9.93±0.28)

(Values in the parenthesis are specific activity)
Values are mean of 5 replicates ±standard error

Table 17 a: Comparative table showing Malonedialdehyde and Stress Enzyme activity ratio between experimental and control

Treatments	Tissues	MDA	POX	SOD	CAT
Cadmium	Root	4.08	1.50	2.00	2.00
	Stem	3.00	1.00	2.50	1.00
	Leaf	2.00	1.00	3.00	1.00
Chromium	Root	3.00	1.00	1.50	4.50
	Stem	2.00	1.00	2.00	1.50
	Leaf	2.00	1.00	2.50	1.50
Mercury	Root	4.50	1.50	2.00	3.50
	Stem	3.00	1.00	2.00	2.00
	Leaf	3.50	1.00	2.50	1.50
Lead	Root	4.00	2.00	3.00	3.50
	Stem	3.00	1.50	3.00	1.00
	Leaf	3.00	1.00	3.00	1.50

Table 17 b: Comparison between bioaccumulation potential of Proline and MDA content and activities of Peroxidase, Superoxide Dismutase, Catalase on 20th day of growth.

Treatments	Tissues	Bioaccumulation µg/g.dry weight	Proline	MDA	POX	SOD	CAT
Control	Root	NDR*	0.714	9.11	121.2	87.07	217.9
	Stem	NDR	0.373	5.67	26.71	19.39	91.65
	Leaf	NDR	0.166	3.46	23.97	13.48	53.85
Cadmium	Root	2.550	1.89	36.8	176.5	151.6	403.7
	Stem	1.300	1.64	16.1	33.56	51.58	125.8
	Leaf	0.104	0.29	6.3	26.82	39.31	92.76
Chromium	Root	26.02	1.86	29.5	143.0	139.6	998.3
	Stem	3.410	2.53	13.6	35.62	44.29	151.6
	Leaf	0.220	0.91	6.8	29.07	35.87	81.43
Mercury	Root	1.110	1.87	39.4	183.2	189.5	789.5
	Stem	0.714	1.28	17.8	33.06	44.67	213.2
	Leaf	0.220	0.90	11.8	29.28	36.87	89.65
Lead	Root	107.1	1.53	38.4	219.6	269.5	819.4
	Stem	26.90	2.87	15.2	38.21	61.30	122.8
	Leaf	0.377	1.21	9.9	31.36	43.87	89.43

* Non-Detectable Range

Bioaccumulation of cadmium, chromium, mercury and lead in *Boerhavia diffusa*

There occurred a significant variation in the pattern of bioaccumulation of cadmium, chromium, mercury and lead in *B. diffusa* grown in Hoagland solution containing known quantities of these heavy metals (Table 18; Fig. 40). The accumulation was found to be mainly depending upon the duration of growth and related to plant parts like root, stem and leaf. After 24 hours, all the metals were present in root tissue only where as occurrence of cadmium, chromium and lead was observed in root and stem on 4th day. As the growth advanced up to 8th day, all metals were present in the root and stem but leaves were devoid of heavy metals. Plants collected on 12th day exhibited cadmium and mercury only in root and stem but chromium and lead were present in the leaf also. Sixteenth day samples showed all elements except cadmium in root, stem and leaves. The 20th day samples, all heavy metals were accumulated in all plant parts.

Accumulation of cadmium in the root tissue was continuously increased proportional to the duration of treatment. Comparatively very low amount of cadmium was present in the stem tissue which started accumulating 4th day onwards and gradually increased. The presence of cadmium in leaves was observed only in the final stage of growth (20th day). First day onwards, chromium started accumulating in the root tissue and

considerable quantities of chromium was accumulated and the increase was linear during the growth. Accumulation of mercury in the root tissue was started 4th day onwards and the rate of increase was linear and proportional to the concentration. Occurrence of mercury in the stem tissue was observed only on 8th day and the increase was proportional to the growth period. Mercury was detected in the leaf tissue only in the samples of 16th day which was doubled on 20th day. After first day of treatment lead was present only on the root tissue. Root and stem showed the accumulation on 4th day. The accumulation of lead in the root tissue was increased several fold on 8th day onwards and the same trend was followed subsequently. The stem also showed significant rate of accumulation on 12th day onwards. All metals were absent in the leaf tissue after 8th day samples. Very low quantities of lead was accumulated in the leaves on 16th day and increase was very meagre thereafter.

Table 18: Bioaccumulation pattern of Heavy Metals in *Boerhavia diffusa* cultivated in Hoagland solution containing known quantities of Cd, Cr, Hg and Pb.

Interval of sample collection (Days)	Tissues	Bioaccumulation of Heavy metals(µg/g) Dry weight			
		Cadmium (5.5µg) *	Chromium (58.8 µg) *	Mercury (2.715µg) *	Lead (227.6 µg) *
1	Root	0.454 (8.2)	3.48 (5.9)	0.116 (4.27)	0.230 (0.09)
	Stem	NDR	NDR	NDR	NDR
	Leaf	NDR	NDR	NDR	NDR
4	Root	0.970 (17.6)	3.850 (6.5)	0.310 (11.4)	0.830 (0.35)
	Stem	0.220 (4.0)	0.192 (0.32)	NDR	0.100 (0.04)
	Leaf	NDR	NDR	NDR	NDR
8	Root	1.670 (33.4)	5.000 (8.5)	0.410 (15.1)	6.080 (2.61)
	Stem	0.280 (5.0)	2.790 (4.7)	0.110 (4.1)	0.230 (0.09)
	Leaf	NDR	NDR	NDR	NDR
12	Root	2.179 (39.5)	20.79 (35.3)	0.750 (27.6)	8.870 (3.8)
	Stem	0.937 (17.6)	6.070 (10.3)	0.340 (12.5)	0.380 (0.16)
	Leaf	NDR	0.110 (0.18)	NDR	0.087 (0.03)
16	Root	2.230 (40.5)	23.26 (39.5)	0.970 (35.7)	87.85 (37.7)
	Stem	1.190 (21.6)	6.140 (10.4)	0.410 (15.1)	11.08 (4.7)
	Leaf	NDR	0.142 (0.24)	0.110 (4.1)	0.230 (0.09)
20	Root	2.550 (46.3)	26.02 (44.2)	1.110 (40.0)	107.1 (46.0)
	Stem	1.300 (23.6)	3.410 (5.8)	0.714 (26.2)	26.90 (11.5)
	Leaf	0.104 (18.9)	0.200 (3.4)	0.220 (8.10)	0.377 (0.16)

***Note:** 5.5 µg cadmium (250 ml of 30 µm $CdCl_2$), 58.8 µg chromium (250 ml of 400 µm $K_2Cr_2O_7$), 2.715 µg mercury (250 ml of 10 µm $HgCl_2$) and 227.6 µg lead (250 ml 0f 600 µm CH_3-$COO)_2$ Pb $3H_2O$).

Values in the parenthesis are percentage of accumulation of each metal

Distribution pattern of cadmium, chromium, mercury and lead in *Boerhavia diffusa*

Distribution pattern of cadmium in *B. diffusa* plants cultivated in Hoagland solution containing known quantities of the metals showed that the increase in the quantity of the accumulation was proportional to growth period and the content retained in the residual solutions was decreasing inversely proportional to the accumulation (Table 19). There occurred significant loss of cadmium during growth in such a way that the quantity of loss was decreasing (13-0.5%) linearly as the growth advanced.

Chromium accumulation also was gradually increasing during growth and sixty three percentage of chromium was found to be accumulated on 20^{th} day. The quantity of chromium present in the residual medium was decreasing during the growth and the rate of chromium loss was 21% - 7% during 1-20 days of growth. Considerable amount of chromium was found to be lost and loss was gradually decreasing during the 20^{th} day growth.

Mercury accumulation in the plant tissue was very feeble during early stages of growth but increased proportional to the growth period. Similarly the quantity of mercury present in the residual solution was decreasing gradually up to 20^{th} day of growth. In the case of mercury, significant quantity was found to be lost during growth starting from 1 to 20^{th} day showing the range of 31 to 13 %.

Accumulation pattern of lead was very interesting, from 1 to 20 days of growth showing the proportion 0.1- 74% on 1^{st} and 20^{th} day respectively.

Similarly very high content of lead was present in the residue during the initial days, which was decreased linearly as the growth advanced. Loss of lead during growth also was vary significant from first day to 20th day showing the range of 19- 2.2%.

Table 19: **Accumulation pattern of cadmium, chromium, mercury and lead in *Boerhavia diffusa* plant tissue and availability of metals in the growth medium**

Heavy metal applied (µg)	Interval (Days)	Accumulation per total plant cultivated(µg)	Residual content (µg)	Loss (µg)
Cadmium (5.5)	1	0.443(8.05)	4.321(78.56)	0.736(13.38)
	4	1.268(23.0)	3.831(69.65)	0.401(7.29)
	8	2.175(39.54)	2.892(52.58)	0.433(7.87)
	12	3.626(65.92)	1.264(22.98)	0.610(11.1)
	16	4.252(77.30)	1.085(19.72)	0.163(2.96)
	20	5.061(92.01)	0.413(7.509)	0.026(0.47)
Chromium (58.8)	1	3.430(5.833)	43.21(73.48)	12.16(20.68)
	4	4.321(7.348)	43.01(73.14)	11.47(19.50)
	8	8.690(14.77)	39.09(66.47)	11.02(18.74)
	12	29.75(50.59)	19.23(32.70)	9.820(16.70)
	16	34.43(58.55)	19.04(32.38)	5.330(9.06)
	20	36.99(62.90)	17.62(29.96)	4.190(7.13)
Mercury (2.715)	1	0.084(3.093)	1.786(65.78)	0.845(31.12)
	4	0.328(12.08)	1.571(57.86)	0.816(30.05)
	8	0.593(21.84)	1.124(41.39)	0.998(36.75)
	12	1.094(40.29)	0.934(34.40)	0.687(25.30)
	16	1.567(57.71)	0.796(29.31)	0.352(12.96)
	20	2.090(76.97)	0.273(10.05)	0.352(12.96)
Lead (227.6)	1	0.240(0.105)	183.3(80.53)	44.10(19.37)
	4	1.057(0.464)	176.7(77.63)	49.90(21.92)
	8	7.210(3.167)	153.2(67.31)	67.19(29.52)
	12	12.10(5.316)	148.6(65.28)	66.90(29.39)
	16	121.2(53.25)	96.30(42.31)	10.10(4.44)
	20	168.4(73.98)	53.62(23.55)	5.580(2.45)

(Values in the parenthesis are percentage distribution)

Table19a: Bioaccumulation pattern of Heavy metals in terms of BCF* and TF*

Period of sample collection (Days)	Cadmium		Chromium		Mercury		Lead	
	BCF	TF	BCF	TF	BCF	TF	BCF	TF
1	0.082	------	0.059	------	0.042	------	0.001	-----
4	0.176	0.226	0.065	0.049	0.114	------	0.003	0.120
8	0.303	0.167	0.085	0.558	0.151	0.268	0.026	0.037
12	0.396	0.430	0.353	0.297	0.276	0.453	0.038	0.052
16	0.405	0.533	0.395	0.270	0.357	0.536	0.384	0.128
20	0.463	0.550	0.442	0.138	0.408	0.841	0.470	0.254

*BCF: Bioaccumulation Factor

*TF : Translocation Factor

Anatomical Studies

Cadmium

Anatomy of root showed cellular damage due to Cd, Cr, Hg and Pb treatment in *B. diffusa*. In the roots of plants treated with cadmium, piliferous layer and cortex cells completely torn compared to control plants. Another effect of cadmium treatment was complete breakage of phloem tissue. Protoxylem cells are underdeveloped in treated plants. In the stem, epidermis and hypodermal layers are not damaged due to cadmium treatment. But secondary phloem and cambium cells are completely damaged and disappeared. Partial damage was observed in sclerenchyma and conjunctive tissue. More cell wall thickening was shown in the outer regions of paranchymatous pith. The meduallary vascular bundles were intact. Some pith cells and vessels showed stained masses accumulated in the lumen presumably due to the accumulation of cadmium (Fig. 42).

Chromium

Roots of *B. diffusa* plants treated with chromium showed general thinning of roots and complete damage of piliferous layer and cortex. Cell wall thickening of parenchyma and xylem vessels was another effect. The number of xylem vessels was reduced compared to the control. Stem of *B. diffusa* treated with chromium showed complete damage of epidermis, hypodermis, secondary phloem and outer part of sclerenchymatous conjunctive tissue. More cell wall thickening was seen in schlerenchymatous conjunctive cells and paranchymatous pith. General reduction in size of medullary bundles (Primary) was observed. Secondary xylem vessels showed reduction in size and number. Medullary bundles were not fully differentiated. Accumulation of stained masses were seen in some cells of pith and vessels. Eventhough epidermal and cortical cells were almost damaged, some regions showed intact epidermal cells, some of which were modified to trichome like appendages which were multicellular filled with stained patches (Fig. 43).

Mercury

Treatment with mercury resulted in a general reduction of cells size in the roots compared to the control and other treatments. Piliferous layer and cortex were fully damaged. Vessel number also was reduced. Stained masses were present in some pith cells. Stem anatomy of *B. diffusa* treated with mercury showed general reduction of epidermal, hypodermal cell size and epidermal cells were modified to multicellular trichomes (Fig. 44). Secondary phloem was not damaged. But medullary bundles showed under developed phloem. Sclerenchymatous layer was highly reduced but cells were slightly thick walled. Paranchymatous pith region was damaged showing some breakage or lysis of cell wall resulting in the formation of many cavities. Accumulation of stained masses was present in the sclerenchymatous conjunctive tissue and secondary phloem. General shrinkage of cells was observed in all cells including vessels (Fig. 44).

Lead

Lead treatment showed minimum adverse effect in the root structure. Slight cell wall thickening was observed. Vessels were larger in size compared to the control. Protoxylem was intact. Partial damage of the piliferous layer was observed and all other cells remained unchanged. In stem, epidermal and hypodermal cells showed partial cell wall breakage. Secondary phloem and outer layer of sclernchymatous conjunctive tissue were damaged. Cell wall thickening and reduction of cell size were seen in the conjunctive tissues. Pith cells were slightly distorted. The number of secondary xylem vessel was reduced. Medullary bundles were almost intact, but some vessels contained stained masses. Stained masses were observed in the pith cells also (Fig. 45).

Bioaccumulation of Heavy Metals in Twenty Medicinal Plants

Dry weight

Twenty medicinal plants cultivated in soil artificially contaminated with known quantities of cadmium, chromium, mercury and lead showed significant morphological and physiological changes during the growth of plants. Generally biomass of the roots was significantly increased due to the treatment of all metals whereas, stem and leaf showed significantly reduced dry weight. In *Aerva lanata*, cadmium induced more biomass production but other elements did not affect the dry weight. Significant increase in dry weight of root was observed in cadmium treatment and only slight fluctuations were shown by stem and leaf. More or less similar result was shown by *Alternanthera tenella* where increased dry weight in the cadmium treatment and the other treatments remained unchanged. Biomass of root tissue in *A. tenella* was significantly increased in the treatments with cadmium, chromium and lead but the increase was negligible in mercury treatment. Biomass production was induced by all heavy metals in *Amaranthus spinosus*. But the increase was not significant. However, the biomass of root tissue was significantly increased in all treatments except lead. *Andrographis paniculata* showed growth retardation and reduction in dry weight by the treatment of cadmium. Biomass was remained unchanged

in plants treated with mercury and lead whereas chromium influenced increased biomass production (Table 20; Fig. 46).

Beloperon plumbaginifolia showed negligible fluctuations in the biomass due to the treatments with all heavy metals. There was very feeble change in the biomass of *Blepharis maderaspatensis* due to cadmium treatment, whereas chromium induced maximum biomass production and lead and mercury treatment resulted in only slight increase in biomass. Roots showed very high dry weight due to chromium and lead treatments and stem dry weight also was increased by chromium whereas mercury treatment induced leaf dry weight. Cadmium, mercury and lead showed reduction in biomass in *B. diffusa* . In *B. diffusa* increase in biomass was significant due to chromium treatment. In *Catharanthus roseus* cadmium, chromium and mercury did not influence biomass production while lead treatment resulted in significant reduction of dry weight (Table 20; Fig. 47).

All metal treatments showed increased biomass in general while significantly increased dry weight was shown by chromium treatment in *Eclipta prostrata*. Root tissue exhibited only reduced dry weight in all treatments compared to the control. But chromium and mercury resulted in more dry weight in stem and leaf. In *Eupatorium triplinerve,* cadmium induced biomass production while mercury showed significant reduction. Dry weight in the plant treated with chromium and lead remained unchanged.

Root showed reduced dry weight in treatments with chromium and mercury. Stem dry weight was more in cadmium treatment in *Kyllinga nemoralis*. Biomass remained unchanged in plants treated with all heavy metals except chromium which showed enhanced biomass production. Dry weight of root and stem was reduced in all treatments. Chromium treatment resulted in significant increase of leaf dry weight. In *Leucas lavandulifolia* treatment with cadmium and lead resulted in a reduction of biomass while chromium and mercury showed increased dry weight. The reduction of root dry weight due to all metals was significant compared to the control (Table 20; Fig. 48).

Biomass of entire plant root and leaves was increased due to mercury and lead treatment in *Oxalis corniculata* while no difference observed in dry weight of plants treated with cadmium and chromium. *Phyllanthus amarus* showed increased dry weight in plants treated with cadmium. Increase in biomass was observed in *Portulaca oleracea* treated with cadmium, chromium mercury and lead. In *Rubia cordifolia* dry weight change due to all treatments were negligible. But the root showed significant reduction of biomass in all treatment compared to the control (Table 20; 49).

In *Scoparia dulcis* effect of cadmium, chromium and mercury showed only very negligible changes in the biomass while significant increase was observed in plants treated with lead. Dry weight of root tissue was significantly increased in chromium and lead treatments. Lead treatment

induced more dry weight in stem and leaf. *Sida acuta* showed significant increase in biomass production due to the treatment with chromium, ($P < 0.1$) mercury and lead compared to the control. The same trend was observed in the root and stem tissue also. Dry weight of leaf was more in mercury and lead treatments. Insignificant increase of biomass was observed in *Sida alnifolia* due to the treatment with chromium, mercury and lead. The increase of dry weight in the root tissue was very high in chromium, mercury and lead. Cadmium and chromium treatment resulted in increased dry weight of stem. Leaf dry weight was maximum in mercury treatment. Dry weight of *Vernonia cinerea* showed only slight changes in all treatments except lead where the increase was significant. Cadmium treatment resulted in reduced dry weight in root whereas stem and leaf tissue was more in lead treated plants (Table 20; Fig. 50).

Bioaccumulation

Aerva lanata showed maximum accumulation of chromium in root tissue followed by cadmium. The order of accumulation pattern is root>Stem>Leaf. Accumulation of mercury was maximum in the root and leaf tissues in relation to the availability in the medium and compared to the other metal accumulation. *Alternanthera tenella* also exhibited maximum accumulation of all metals in the root followed by stem and leaf except lead which was more abundant in the root followed by leaf and stem. In

Amaranthus spinosus maximum accumulation of all metals was observed in root tissue followed by stem and leaf. Maximum amount of chromium and lead was present in the leaf and stem respectively compared to other metals. In *Andrographis paniculata* the quantity of heavy metal accumulation was varied widely between the metals and tissues. But values were not proportional to the amount applied. Accumulation of all metals was maximum in the root tissue. Maximum accumulation occurred in all tissues was noted in the case of lead followed by chromium. Very minute quantities of metals were found to accumulate in the stem and leaf tissue compared to the root tissue as well as other heavy metals (Table 20; Fig. 51).

In *Beloperon plumbaginifolia* root showed maximum accumulation of metals compared to the tissues of other plant parts. Maximum quantity accumulated in the stem and leaf is found to be lead. In *Blepharis maderaspatensis* root accumulated maximum quantity of all metals compared to other parts and lead accumulation was very high in the roots. Accumulation pattern was in the order root> Stem>Leaf except chromium which showed more accumulation in the leaf than other tissues (Table 20). In *B. diffusa* maximum accumulation was observed in roots. Chromium is the most abundant metal accumulated in the root, stem and leaf. Accumulation of metals was in the order Root>Stem>Leaf. In *Catharanthus roseus*, eventhough all metals accumulated was found maximum in the root tissue, stem also showed very high accumulation. In this plants also very high

amount of chromium was present in the root tissue compared to the other elements (Table 20; Fig. 52).

In *Eclipta prostrata* maximum quantity was accumulated in the root tissue, accumulation of chromium was maximum in the root followed by stem and leaf tissue. In *Eupatorium triplinerve* maximum accumulate cadmium and chromium was found in the root, whereas stem tissue showed more chromium and lead, leaf contained maximum amount of cadmium compared to the other metals. The order of accumulation of metals was Root>Stem>Leaf. In *Kyllinga nemoralis* maximum accumulation was found in root, abundant accumulation of metals was found in the root, stem and leaf was lead. Mercury accumulation in the root was very high (47%) compared to the availability in the medium. In *Leucas lavandulifolia* maximum accumulation of metals was present in root system. Stem and leaf shows more amount of lead compared to other elements. Chromium accumulation was maximum in the root tissue followed by stem and leaf (Table 20; Fig. 53).

In *Oxalis corniculata* maximum accumulation was present in the root tissue, very high amount of chromium was seen in leaf tissues compared to other heavy metals. Maximum amount of all elements was found to be in the root system of *Phyllanthus amarus*, lead and chromium were the most abundant metals. Equal amount of lead was accumulated in the stem as that

of root. Very high amount of lead was accumulated in the leaf also compared to other metals. Mercury content also was very high in the root and stem as well as other elements.In *Portulaca oleraceae* maximum accumulation of all metals was seen in root system. Chromium and lead accumulated in stem also was very high. Higher amount of chromium and lead was also present in leaf tissues. Very high amount of all metals was accumulated in the root tissues of *Rubia cordifolia*. Maximum quantity of lead and chromium was present in root tissue. Cadmium is very high in stem and leaf. *Scoparia dulcis* showed maximum cadmium and lead accumulation in the leaf tissue compared to the other elements. Accumulation order was Root>Stem>Leaf. In *Sida acuta* cadmium accumulation was maximum in root tissue compared to other elements. Chromium was very high in the stem and leaf contained maximum amount of cadmium followed by lead. Mercury also was very high in the root. Maximum accumulation of lead was seen in root tissue of *Sida alnifolia*. Very high amount of cadmium and lead were present in the stem tissue whereas leaf tissue showed maximum amount of lead compared to the other elements. In *Vernonia. cinerea* maximum accumulation of all metals was seen in root, maximum quantity accumulated metal was lead in the stem and leaf but cadmium in root. Leaf contain higher quantity of cadmium followed by stem. Lead content was very high in the leaf tissue (Table 20; Fig. 55).

Table: 20 Bio- accumulation pattern of Cadmium, Chromium, Mercury and Lead in Medicinal Plants cultivated in soil containing known quantities of these Heavy Metals

Sl.N0	Name of the Plant	Treatment	Dry weight%			Heavy metal applied(mg)	Accumulation (µg/g)Dry weight		
			Root	Stem	Leaf		Root	Stem	Leaf
1	*Aerva lanata*	Control	36.30 ±0.3	25.33 ±0.3	18.30 ±0.8	0.000	0.000	0.000	0.000
		Cadmium	47.20 ±0.2	31.87 ±0.6	24.65 ±0.9	1.979	298.0 (15.0)	34.50 (1.74)	5.010 (0.25)
		Chromium	39.20 ±0.5	23.69 ±0.6	21.90 ±0.3	70.56	585.0 (0.82)	43.40 (0.06)	19.90 (0.02)
		Mercury	37.90 ±0.6	22.80 ±0.2	22.07 ±0.1	0.203	54.16 (26.6)	3.800 (1.87)	0.830 (0.40)
		Lead	34.37 ±0.8	23.73 ±0.2	21.50 ±0.6	182.0	61.80 (0.03)	17.76 (0.009)	1.630 (0.0008)
2	*Alternanthera tenella*	Control	35.22 ±0.3	25.00 ±0.7	34.48 ±0.7	0.000	0.000	0.000	0.000
		Cadmium	49.36 ±0.5	31.96 ±0.8	24.05 ±0.6	0.593	227.0 (38.2)	37.30 (6.29)	4.800 (0.80)
		Chromium	47.88 ±0.1	26.17 ±0.9	21.83 ±0.7	21.16	567.3 (2.68)	31.70 (0.14)	21.20 (0.10)
		Mercury	39.02 ±0.3	23.42 ±0.7	23.68 ±0.8	0.025	6.300 (25.2)	2.440 (9.76)	0.212 (0.84)
		Lead	43.83 ±0.5	25.41 ±0.8	29.35 ±0.2	54.62	723.4 (1.32)	56.70 (1.03)	93.20 (1.70)
3	*Amaranthus spinosus*	Control	34.52 ±0.3	26.29 ±0.7	19.29 ±0.1	0.000	0.000	0.000	0.000
		Cadmium	38.80 ±0.6	28.32 ±0.8	21.71 ±0.7	2.639	371.0 (14.0)	37.30 (1.41)	8.100 (0.30)
		Chromium	41.03 ±0.7	25.00 ±0.7	18.05 ±0.7	94.08	815.3 (0.86)	413.1 (0.43)	141.4 (0.15)
		Mercury	39.72 ±0.9	27.36 ±0.7	21.28 ±0.3	0.135	33.75 (25.0)	3.180 (2.35)	0.360 (0.27)
		Lead	34.93 ±0.4	28.55 ±0.9	21.33 ±0.5	24.27	763.2 (3.14)	420.0 (1.73)	21.00 (0.08)

(Values in the parenthesis are percentage distribution among tissues)

Contd…

4	*Andrographis paniculata*	Control	20.20 ±0.7	27.86 ±0.3	20.20 ±0.7	0.000	0.000	0.000	0.000
		Cadmium	23.97 ±0.4	20.22 ±0.4	13.13 ±0.4	0.650	133.5 (20.5)	26.09 (4.01)	35.50 (5.46)
		Chromium	31.19 ±0.3	21.15 ±0.5	13.30 ±0.3	23.52	254.1 (1.08)	45.40 (0.19)	20.90 (0.08)
		Mercury	25.26 ±0.7	24.57 ±0.8	14.18 ±0.2	0.025	5.720 (21.1)	2.282 (8.45)	0.465 (1.72)
		Lead	28.21 ±0.7	25.30 ±0.9	13.90 ±0.6	60.69	924.0 (1.52)	41.70 (0.06)	177.8 (0.29)
5	*Beloperon plumbaginifolia*	Control	42.38 ±0.3	33.26 ±0.4	24.39 ±0.9	0.000	0.000	0.000	0.000
		Cadmium	44.16 ±0.3	32.09 ±0.6	26.09 ±0.6	2.639	217.8 (8.25)	91.40 (3.46)	32.60 (1.23)
		Chromium	46.00 ±0.6	32.82 ±0.9	27.16 ±0.8	94.08	585.9 (0.62)	37.30 (0.03)	20.90 (0.02)
		Mercury	42.06 ±0.7	34.09 ±0.8	27.46 ±0.8	0.135	44.78 (33.1)	6.190 (4.58)	0.900 (0.66)
		Lead	45.50 ±0.8	30.90 ±0.7	25.5 ±0.7	24.27	286.2 (1.17)	206.1 (0.84)	61.87 (0.25)
6	*Blepharis maderaspatensis*	Control	38.65 ±0.5	26.09 ±0.1	19.11 ±0.8	0.000	0.000	0.000	0.000
		Cadmium	40.55 ±0.3	26.08 ±0.2	18.78 ±0.3	1.978	362.9 (18.3)	104.2 (5.26)	34.69 (1.75)
		Chromium	57.69 ±0.7	27.54 ±0.9	18.98 ±0.9	70.56	50.00 (0.07)	102.2 (0.14)	169.5 (0.24)
		Mercury	46.83 ±0.8	29.77 ±0.1	24.85 ±0.1	0.101	54.28 (53.7)	5.000 (4.95)	1.040 (1.02)
		Lead	50.00 ±0.1	33.30 ±0.2	22.44 ±0.6	182.0	842.7 (0.46)	694.7 (0.38)	71.92 (0.03)

Contd…

7	*Boerhavia diffusa*	Control	21.00 ±0.7	27.30 ±0.7	21.00 ±0.3	0.000	0.000	0.000	0.000
		Cadmium	24.20 ±0.9	20.43 ±0.2	12.55 ±0.6	2.639	484.3 (18.3)	217.8 (8.25)	124.9 (4.73)
		Chromium	22.40 ±0.4	21.16 ±0.2	16.23 ±0.5	94.09	906.3 (0.96)	331.5 (0.35)	171.4 (0.18)
		Mercury	23.70 ±0.9	22.70 ±0.2	14.56 ±0.9	0.135	77.26 (57.2)	7.820 (5.79)	0.450 (0.33)
		Lead	28.36 ±0.8	24.76 ±0.9	13.90 ±0.2	24.27	854.9 (35.2)	318.6 (1.31)	140.9 (0.56)
8	*Catharanthus roseus*	Control	26.25 ±0.7	15.23 ±0.2	11.08 ±0.1	0.000	0.000	0.000	0.000
		Cadmium	28.18 ±0.2	14.50 ±0.4	12.90 ±0.3	0.593	208.6 (35.1)	115.6 (19.4)	7.870 (1.32)
		Chromium	27.88 ±0.2	15.92 ±0.6	12.19 ±0.4	21.16	383.9 (1.81)	223.7 (1.05)	118.7 (0.56)
		Mercury	27.20 ±0.1	14.64 ±0.9	12.76 ±0.9	0.025	3.260 (13.0)	0.470 (1.88)	0.106 (0.42)
		Lead	28.01 ±0.8	15.47 ±0.5	11.40 ±0.1	54.62	428.6 (0.78)	306.7 (0.56)	106.9 (0.19)
9	*Eclipta prostrata*	Control	38.80 ±0.1	19.35 ±0.6	22.69 ±0.7	0.000	0.000	0.000	0.000
		Cadmium	33.96 ±0.2	25.10 ±0.3	25.83 ±0.2	0.593	192.1 (32.3)	110.4 (18.6)	36.70 (6.19)
		Chromium	35.13 ±0.5	40.14 ±0.8	27.38 ±0.8	21.16	414.2 (1.95)	197.1 (0.93)	103.4 (0.48)
		Mercury	31.48 ±0.8	36.79 ±0.3	28.20 ±0.9	0.025	9.160 (36.6)	1.380 (5.52)	0.900 (3.60)
		Lead	22.50 ±0.9	20.66 ±0.1	40.90 ±0.2	54.62	260.2 (0.47)	35.58 (0.06)	5.880 (0.01)

Contd…

10	*Eupatorium triplinerve*	Control	37.14 ±0.7	22.68 ±0.8	15.76 ±0.6	0.000	0.000	0.000	0.000
		Cadmium	38.00 ±0.4	29.13 ±0.8	21.69 ±0.9	2.639	589.8 (22.3)	108.2 (4.10)	86.88 (3.29)
		Chromium	34.00 ±0.5	24.40 ±0.8	18.06 ±0.3	94.08	114.2 (0.12)	116.5 (0.12)	5.312 (0.005)
		Mercury	26.88 ±0.3	15.84 ±0.9	13.47 ±0.1	0.135	70.35 (52.1)	7.390 (5.47)	1.530 (1.13)
		Lead	35.45 ±0.2	16.60 ±0.1	16.20 ±0.6	24.28	54.73 (0.22)	105.8 (0.43)	5.930 (0.02)
11	*Kyllinga nemoralis**	Control	30.63 ±0.1	14.55 ±0.6	13.89 ±0.2	0.000	0.000	0.000	0.000
		Cadmium	28.40 ±0.5	16.00 ±0.3	11.56 ±0.4	3.300	334.37 (10.1)	275.0 (8.34)	94.40 (2.86)
		Chromium	29.30 ±0.5	15.17 ±0.3	22.00 ±0.8	117.6	414.8 (0.35)	97.25 (0.08)	94.79 (0.08)
		Mercury	29.80 ±0.2	14.47 ±0.1	13.21 ±0.5	0.169	79.23 (46.8)	10.32 (6.10)	2.560 (1.51)
		Lead	29.40 ±0.6	14.96 ±0.7	14.70 ±0.3	303.4	653.1 (0.21)	477.6 (0.15)	184.5 (0.06)
12	*Leucas lavandulifolia*	Control	38.46 ±0.3	24.63 ±0.1	18.00 ±0.7	0.000	0.000	0.000	0.000
		Cadmium	32.07 ±0.2	17.37 ±0.3	20.79 ±0.3	0.593	248.8 (41.9)	8.920 (1.50)	14.11 (2.37)
		Chromium	29.72 ±0.1	37.68 ±0.1	25.00 ±0.1	21.16	406.9 (1.92)	26.67 (0.12)	19.33 (0.09)
		Mercury	33.30 ±0.6	26.06 ±0.4	21.71 ±0.5	0.025	7.340 (29.3)	4.520 (18.0)	0.816 (3.26)
		Lead	23.80 ±0.9	20.83 ±0.6	23.25 ±0.3	54.62	273.8 (0.50)	50.75 (0.09)	38.75 (0.07)

*In *Kyllinga nemoralis the tissue analysed was petiole instead of stem*

Contd…

13	*Oxalis corniculata*	Control	30.37 ±1.2	14.65 ±0.6	15.43 ±0.7	0.000	0.000	0.000	0.000
		Cadmium	31.40 ±1.7	13.29 ±0.8	14.40 ±0.8	0.650	124.6 (18.9)	12.01 (1.83)	8.173 (1.24)
		Chromium	32.24 ±0.9	13.79 ±0.9	16.38 ±0.9	23.52	462.7 (1.96)	207.5 (0.88)	87.39 (0.37)
		Mercury	40.80 ±1.3	18.06 ±0.4	23.05 ±0.3	0.025	7.760 (28.7)	4.880 (18.0)	1.300 (4.81)
		Lead	37.03 ±1.7	18.20 ±0.1	19.05 ±0.1	60.69	424.8 (0.69)	91.27 (0.15)	5.784 (0.009)
14	*Phyllanthus amarus*	Control	34.20 ±1.9	17.46 ±0.2	22.14 ±0.3	0.000	0.000	0.000	0.000
		Cadmium	31.37 ±1.1	16.87 ±0.1	19.49 ±0.5	0.593	97.50 (16.4)	36.20 (6.10)	47.50 (8.01)
		Chromium	27.70 ±1.2	17.20 ±0.1	23.00 ±0.1	21.16	100.2 (0.47)	36.20 (0.17)	47.50 (0.22)
		Mercury	28.00 ±1.1	17.16 ±0.4	25.70 ±0.6	0.025	5.930 (24.7)	3.400 (14.1)	0.510 (2.12)
		Lead	21.90 ±1.1	21.30 ±0.2	25.20 ±0.8	54.62	705.0 (1.29)	710.0 (1.29)	377.0 (0.69)
15	*Portulaca oleracea*	Control	13.04 ±1.2	7.20 ±0.6	10.62 ±0.8	0.000	0.000	0.000	0.000
		Cadmium	14.81 ±1.1	7.61 ±0.3	10.79 ±0.4	0.593	151.3 (25.6)	11.74 (1.98)	28.71 (4.87)
		Chromium	21.42 ±1.2	7.38 ±0.6	11.16 ±0.9	21.16	341.5 (1.61)	223.2 (1.05)	76.60 (0.36)
		Mercury	19.00 ±1.6	6.45 ±0.8	6.230 ±0.4	0.025	6.220 (25.9)	4.040 (16.6)	1.590 (6.62)
		Lead	23.30 ±1.4	6.19 ±0.9	5.990 ±0.3	54.62	110.92 (0.20)	283.8 (0.51)	51.29 (0.09)

Contd…

16	*Rubia cordifolia*	Control	27.50 ±1.8	15.18 ±0.3	16.55 ±0.5	0.000	0.000	0.000	0.000
		Cadmium	24.20 ±1.5	17.85 ±0.5	17.02 ±0.8	1.979	1273 (64.3)	210.0 (10.6)	60.00 (3.03)
		Chromium	19.04 ±1.8	17.18 ±0.2	19.58 ±0.3	70.60	714.5 (1.01)	36.15 (0.05)	7.580 (0.01)
		Mercury	17.85 ±1.9	14.76 ±0.2	20.00 ±0.2	0.101	32.25 (31.9)	6.300 (6.23)	1.530 (1.51)
		Lead	20.00 ±0.5	13.61 ±0.8	19.04 ±0.1	182.0	746.0 (0.40)	33.46 (0.01)	10.00 (0.005)
17	*Scoparia dulcis*	Control	38.39 ±1.4	28.62 ±0.6	27.60 ±0.4	0.000	0.000	0.000	0.000
		Cadmium	34.09 ±1.7	26.74 ±0.8	29.89 ±0.1	1.979	661.0 (33.4)	193.7 (9.78)	101.7 (5.13)
		Chromium	42.39 ±1.2	24.18 ±0.9	33.87 ±0.1	94.08	40.51 (0.04)	2.371 (0.002)	0.416 (0.0004)
		Mercury	39.60 ±1.1	30.43 ±0.5	19.58 ±0.2	0.135	75.32 (55.7)	7.830 (5.8)	2.500 (1.85)
		Lead	42.00 ±1.6	35.07 ±0.8	34.78 ±0.1	24.27	407.2 (1.67)	193.9 (0.79)	94.10 (0.38)
18	*Sida acuta*	Control	38.00 ±1.2	25.31 ±0.2	18.66 ±0.2	0.000	0.000	0.000	0.000
		Cadmium	35.37 ±1.1	30.20 ±0.8	19.24 ±0.1	1.979	789.1 (39.8)	250.2 (12.6)	116.3 (5.87)
		Chromium	44.40 ±1.3	41.73 ±0.6	17.94 ±0.5	70.56	372.1 (0.52)	256.9 (0.36)	10.74 (0.01)
		Mercury	46.75 ±1.6	29.52 ±0.1	23.03 ±0.6	0.203	127.0 (62.5)	2.550 (1.25)	0.760 (0.31)
		Lead	47.32 ±1.2	33.50 ±0.1	23.47 ±0.3	182.0	497.6 (0.27)	231.8 (0.12)	80.94 (0.04)

Contd…

19	*Sida alnifolia*	Control	37.82 ±1.7	25.38 ±0.3	19.47 ±0.1	0.000	0.000	0.000	0.000
		Cadmium	37.08 ±1.5	31.18 ±0.5	17.28 ±0.4	1.970	517.3 (26.2)	230.9 (11.7)	8.740 (0.44)
		Chromium	54.80 ±1.2	42.63 ±0.5	16.66 ±0.1	70.56	553.8 (0.78)	120.4 (0.17)	6.070 (0.008)
		Mercury	38.15 ±1.1	29.60 ±0.6	22.94 ±0.2	0.203	99.13 (48.8)	0.178 (0.08)	0.937 (0.46)
		Lead	47.32 ±1.2	25.95 ±0.6	23.32 ±0.8	182.0	673.4 (0.37)	284.5 (0.15)	16.82 (0.09)
20	*Vernonia cinerea*	Control	42.34 ±1.4	28.34 ±0.8	27.41 ±0.2	0.000	0.000	0.000	0.000
		Cadmium	35.63 ±1.2	26.45 ±0.2	29.00 ±0.6	0.593	430.4 (72.5)	189.1 (31.8)	112.3 (18.93)
		Chromium	41.93 ±1.1	25.80 ±0.3	34.29 ±0.7	21.16	410.6 (1.94)	26.47 (0.12)	7.800 (0.03)
		Mercury	40.84 ±1.5	27.22 ±0.1	21.74 ±0.8	0.124	4.640 (3.74)	3.610 (2.91)	0.780 (0.62)
		Lead	41.00 ±1.2	34.80 ±0.6	37.33 ±0.5	54.26	633.9 (1.16)	367.3 (0.67)	23.28 (0.04)

SUMMARY AND CONCLUSIONS

Non essential heavy metals such as cadmium, chromium, mercury and lead are highly reactive and interfere the normal metabolism and become toxic to plants generating morphological and physiological alterations and modifications. Despite the toxicity symptoms as the consequence of oxidative stress, many plants are hyperaccumulators of heavy metals. Medicinal plants form the raw materials for Ayurvedic medicines. Most of the medicinal plants are herbs which are cultivated or naturally growing in soil, contaminated with heavy metals by natural and anthropogenic activities and the plants accumulate considerable quantities of toxic heavy metals. The metals confined in medicinal plants finally reach food chain leading to health hazard in human and animals and get recycled.

Objective of the present study is the elucidation of metabolic interference of cadmium, chromium, mercury and lead in medicinal plants. The investigation was planned along two complementary lines – one to elucidate the effect of these heavy metals in the metabolism and two to assess the bioaccumulation potential of the same. Data obtained by analysing the distribution of metabolites in *Boerhavia diffusa* L. treated with known quantities of Cd, Cr, Hg and Pb permitted conclusion to be drawn about the physiological events occurring in medicinal plants and hence bioaccumulation

potential was analysed in twenty medicinal plants inclusive of *B. diffusa*. *B. diffusa* belonging to the family Nyctaginaceae is a medicinal plant wildly growing and widely used as an ingredient of many Ayurvedic medicines. Effect of Cd, Cr, Hg and Pb in the metabolism and the potential of *B. diffusa* to accumulate these heavy metals was elucidated by cultivating the plants in Hoagland nutrient solution artificially contaminated with known quantities of these metals.

Responses of *B. diffusa* plants exposed to heavy metals were investigated with respect to time and concentration dependence. The parameters taken to evaluate the effect of heavy metals include growth retardation, tolerance index, stomatal index, root and stem anatomy, distribution of biomass, metabolites such as free amino acids, proteins, chlorophylls, phenolics, and nitrate reductase assay. PAGE analysis of protein also was another parameter. Oxidative stresses were analysed by estimating proline and malondialdyhide accumulation. Assaying antioxidant enzymes such as guaiacol peroxidase, superoxidedismutase and catalase was another parameter. Bioaccumulation of Cd, Cr, Hg and Pb in the plant parts – root, stem and leaves also was analysed. Another separate study was carried out to assess the accumulation potential of 20 medicinal plants inclusive of *B. diffusa* towards Cd, Cr, Hg and Pb by cultivating them in soil artificially contaminated with known quantities of those metals under pot culture method.

Growth retardation in terms of root - and shoot length and leaf area was shown by plants treated with all metals. Toxicity of metals was also shown by anatomical variations in the root and stem. Piliferous layer and cortical cells were almost damaged in the roots. Secondary phloem and cortex were completely damaged in the stem. General cell wall thickening was another important visible effect by treatment with all heavy metals. Darkly stained patches representing the accumulation of heavy metals were present in the stem tissues of plants treated with chromium, mercury and lead. Development of trichomes in the stem tissues was an important observation shown by plants treated with chromium and mercury. Tolerance index values were minimum in treatments with Cd and Hg and maximum in Pb. Stomatal index was maximum in plants treated with mercury.

Total proteins and soluble protein fractions of root, stem and leaves of plants treated with all metals exhibited reduction. PAGE studies showed new protein bands in experimental plants compared to control. Appearance of new protein bands varied among the four metals. Proline accumulation was remarkable in plants treated with all metals. Chlorphyll a, chlorphyll b and total chlorophyll contents affected slightly by all metals. Considerable reduction of nitrate reductase activity was another important impact of heavy metal treatment. Phenolic content of plants treated with all the four metals was significantly increased maximum being in the leaf tissue. Lipid peroxidation was very high due to toxicity of all metals estimated as

accumulation of malondialdehyde content in the root, stem and leaf tissue. One of the antioxidant enzymes, peroxidase activity was maximum in root tissue followed by stem and leaf. Superoxidedismutase activity also was dramatically increased generally in all tissues and particularly in roots. Catalase activity was also very high in plants treated with all metals maximum being in root tissue. Increase in accumulation of all metal was linear during the period of growth. Bioaccumulation quantity of metals in the tissue was in the order Pb>Cr>Hg>Cd.

Out of the twenty medicinal plants, majority of them inclusive of *B. diffusa* accumulated moderate quantities of Cd, Cr, Hg and Pb in root and shoot tissues whereas some plants are found to be hyperaccumulators of these heavy metals exhibiting the potential as phytoremediants. Based on these observations, the data are discussed in the light of current literature and the following conclusions are drawn.

1. Significant variations in the concentration of cadmium, chromium. mercury and lead have been identified to impart more or less uniform growth retardation resulting in similar morphology and tolerance by *B. diffusa* cultivated in nutrient medium artificially contaminated with known quantities of these metals.

2. In spite of the differences in the concentration of Cd, Cr, Hg and Pb, the morphological and physiological performance are almost similar

and sustainable but considerable fluctuations are observed in many parameters among individual metals.

3. Although growth retardation is expressed as more or less similar morphology and anatomical variations due to the differences in the strategies like development of trichomes for metal sequestration are shown by plants treated with chromium and mercury.

4. Distribution of dry weight remains unaltered due to the treatments highlighting similar responses of *B. diffusa* towards widely differed concentrations of the four heavy metals.

5. Maximum tolerance index values are shown by plants exposed to chromium and lead and the quantities of these metals which are supplied to nutrient medium are also maximum revealing significant differences in the tolerance potential of *B. diffusa* towards individual metals.

6. Increased stomatal index exhibited by plants treated with mercury is indirectly linked with escape of mercury by the process of phytovolatalisation which is substantiated by the least values of mercury bioaccumulation in the leaves.

7. Physiological aspects such as distribution of proteins, amino acids, chlorophylls, nitrate reductase etc. registered only negligible

variations in general among treatments but some distinctly differed responses such as enhanced ratio of chlorophyll a/b, low amount of chlorophyll a and b due to the treatment with lead and chromium respectively.

8. Maximum bands of protein profile analysed by PAGE shown by leaf tissue are presumably phytochelatin which are involved in the sequestration of metals accumulated in the leaf tissue.

9. Since the concentration of all metals induce moderate toxicity stress / tolerance, mechanism adopted by *B. diffusa* is synthesis/ accumulation of antioxidant metabolite-proline content, the concentration of which is proportional to the bio accumulation potential specific to tissues such as root/stem/leaf.

10. Antioxidant property of phenolics is also expressed as another strategy of *B. diffusa* to cope up with heavy metal stress exhibited by several fold increase of phenolics in the experimental plants compared to the control.

11. Accumulation of malondialdehyde as a result of lipid peroxidation is an important response towards all metals and the MDA content is proportional to the concentration/availability as well as specificity of metal ions.

12. Lipid peroxidation due to highly reactive oxygen species induced by heavy metals resulting in MDA content is an important strategy of *B, diffusa* and the difference in magnitude of this process confirms the specificity and strategy of tolerance towards different metals.

13. Dramatic increase of superoxide dismutase and catalase activities are important defence mechanisms against ROS induced by all the four metals which exhibit different concentrations in such a way that reduced activity of SOD and catalase shown by plants treated with mercury indicate more toxicity although the molar concentration of this metal is minimum.

14. The protective mechanisms shown by *B. diffusa* in terms of enzymic and non enzymic antioxidnants to ameliorate oxidative stress towards the four heavy metals vary among metals.

15. Bioaccumulation quantity/pattern differed in all the four metals and is found to be dependent on the type and the specificity of all metals in the process of absorption, translocation and accumulation. Accumulation in terms of percentage of availability in the growth medium show similar quantities of all metals in the root in spite of significant variation in the molar concentrations of treatments, indicating the integration between sensitivity/tolerance on one hand

and accumulation as percentage of the availability of metals on the other.

16. Given the differences in the response of *B. diffusa* towards the four metals which are required to impart similar visual inhibitory responses, the distribution of metabolites, bioaccumulation potential and tolerance mechanism are found to be more or less alike in *B. diffusa*. Notwithstanding, the marked differences in the molar concentration of cadmium, chromium, mercury and lead applied on the basis of similar morphological manifestations reveals that, these heavy metal ions though non -essential, exhibit specificity in their metabolic interference or impact and are not alike hence exhibits specificity in their concentration dependent metabolic interference/activity in *B. diffusa*.

17. Twenty medicinal plants cultivated in soil, containing known quantities of Cd, Cr, Hg and Pb facilitated to classify these plants as hyperaccumulators and phytoremediants because many of the medicinal plants included in the study are herbs which are well adapted to the contaminated soil and their hyperaccumulation potential lead to health hazard since most of these plants are important ingredients of many Ayurvedic preparations.

REFERENCES

Abdel-Latif, A. (2008) Cadmium induced changes in pigment content, ion uptake, proline content and phosphoenolpyruvate carboxylase activity in *Triticum aestivuaum* seedlings. *Aust.ralian J.ournal of Basic and Appl.ied Sci.ences* **2**: 57- 62.

Abe, T., Fukami, M., Ichizen, N. and Ogasawara, M. (2006) SusceptibilitySusceptibility of weed species to cadmium evaluated in a sand culture. *Weed Biol. ManageManage*. **6**: 107-114.

Abeles, F. B. and Biles, C. L. (1991) Characterization of peroxidaises in lignifying peach fruit endocarp. *Plant Physiol*. **95**: 269- 273.

Adriano, D. C. (1986) *Trace Elements in the Terrestrial Environment*. Springer-Verlag,, Berlin.

Ahmed, A. and Tajmir-Riahi, H. A. (1993) Interaction of toxic metal ions Cd^{2+}, Hg^{2+} ,and Pb^{2+} with light harvesting proteins of chloroplast thylakoid membranes: An FTIR spectroscopic study. *J. Inorg. Biochem*. **50:** 235-243.

Ali, G., Srivastava, P. S. and Iqbal, M. (1998) Morphogenic response and proline content in *Bacopa monnieri* cultures grown under copper stress, *Plant Sci*. **138**: 191- 195.

Ali, G., Srivastava, P. S. and Iqbal, M. (2000) Influence of cadmium and zinc on growth and photosynthesis of *Bacopa monnieri* cultivated *in vitro. Biol. Plant.* **43:** 599 - 601.

Alia, K. V., Prasad, S. K. and Saradhi, P. P., (1995) Effect of zinc on free radicals and proline in *Brassica* and *Cajanus*. *Phytochem*. **39**: 45- 47.

Allan, J. E. (1969) The Preparation of agricultural samples for analysis by Atomic Absorption Spectrometry. *Varian Techtron Bulletin* 12-69 (S.I.S. Edn.). 12-69 .

Alloway, B. J. (1990) *Heavy Metals in Soils*. John Wiley & Sons, New York.

and Tomaro, M. L. (1996) Heavy metal phytotoxicity induces oxidative stress in a moss, *Taxithellium* sp. *Plant Sci*. **121**: 151-159.

Aneesh, T. P., Hisham, M., Sekhar, S. M., Madhu, M. and Deepa, T. V. (2009) International market scenario of traditional Indian herbal drugs- India declining. *Inter. J. Green Pharma*.~~cy~~. **10**: 184-190.

Angelova, V., Ivanov, K. and Ivanova, R. (2006) Heavy metal content in plants form family Lamiaceae cultivated in industrially polluted region. *J. Herb. Species Med. Plants* **11**: 37-46.

Anonymous, (1979) Effects of mercury in the Canadian environment. Associate Committee on Scientific Criteria for Environmental Quality, National Research Council of Canada, Canada. NRC Publication No. 16739.

Apel, K. and Hirt, H. (2004) Reactive oxygen species: metabolism, oxidative stress and signal transduction. *Annu. Rev. Plant. Biol.* **55**: 373- 399.

Arazi, T., Sunkar, R., Kaplan, B. and Formm, H. (1999) A tobacco plasma membrane calmodulin- binding transporter confers Ni^{2+} tolerance and Pb^{2+} hypersensitivity in transgenic plants. *Plant J.* **20**: 171-182.

Arnon, D. I. (1949) Copper enzymes in isolated chloroplasts. pPolyphenoloxidase in *Beta vulgaris. Plant Physiol.* **24:** 1-5.

Aslam, M., Davis, S. S. Healy, M. A. (19779) Heavy metals in some Asian medicines and cosmetics. *Public Health*. **93**: 274-284.

Awasathi, L. P. and Menzel, G. (1986) Effect of root extract from *Boerhavia diffusa* containing an antiviral principle upon plaque formation of RNA bacteriophages. *Zentralblatt. fur Bakteriol. ogie* **141**: 415-419.

Awasthi, L. P. and Rizvi, S. M. A. (1998) Prevention of infection of a vector borne virus of tomato by *Boerhavia diffusa* glycoprotein. In: Abstracts, XIII Annual convention of the Indian Virological Society and National Symposium on Characterization and Management of Viruses. NBRI and Lucknow University, Lucknow, Uttar Pradesh, India. Abstract no. 27.

Awasthi, L. P. and Rizvi, S. M. A. (1999) Effect of *Boerhavia diffusa* glycoprotein on the transmition of tomato yellow leaf curl virus by *Bemisia tabaci* Gen.: Abstracts, National Symposium on Vectors of Plant diseases, Narendra Dev University of Agriculture and Technology,

Kumarganj, Faizabad, Uttar Pradesh, India **56**.

Awasthi, L. P. and Kumar, P. (2003) Protection of some cucurbitaceous crops against natural infection of virusese through *Boerhavia diffusa* plants. *Indian Phytopathol.* **56**: 318- 324.

Awasthi, L. P., Kumar, P., and Singh, R. V., (2003) Effect of *Boerhavia diffusa* inhibitor on the infection and multiplication of cucumber green mottle mosaic virus in musk melon plants *Inidan Phytopathol.ogy* **56**: 362. 367.

Awasthi, L. P., and Kumar, P. (2003) Protection of some cucurbitaceous crops against natural infection of virusese through *Boerhavia diffusa* plants. *Indian Phytopathology* **56**: 318.

Awsathi, L. P. and Rizvi, S. M. A. (1998) Prevention of infection of a vector borne virus of tomato by *Boerhavia diffusa* glycoprotein. In: Abstracts, XIII Annual convention of the Indian Virological Society and National Symposium on Characterization on and Management of Viruses. NBRI and Lucknow University, Lucknow, Uttar Pradesh, India. Abstract no. 27.

Awsathi, L. P. and Rizvi, S. M. A. (1999) Effect of *Boerhavia diffusa* glycoprotein on the transmition of tomato yellow leaf curl virus by *Bemisia tabaci* Gen. In: Abstracts, National Symposium on Vectors of Plant diseases, Narendra Dev University of Agriculture and Technology, Kumarganj, Faizabad, Uttar Pradesh, India **56**.

Axtell, N. R., Sternberg, S. P. K. , and Claussen, K. (2003) Lead and nickel removal using *Microspora* and *Lemna minor*. *Bioresource Technol*. **89:** 41- 48.

Badawi, G. H., Yamauchi, Y., Shimada, E., Sasaki, R., Kawano, N. and Tanaka, K. (1984) Enhanced tolerance to salt stress and water deficit by over expressing superoxide dismutase in tobacco (*Nicotiana tabacum*) chloroplasts. *Plant Sci*. **166**: 919-928.

Badawi, G. H., Yamauchi, Y., Shimada, F., Sasaki, R., Kawano, N., Tanaka, K. and Tanaka, K. (2004) Enhanced tolerance to salt stress and water deficit by over expressing superoxide dismutase in tobacco (*Nicotiana tabacum*) chloroplasts. *Plant Sci*. **166**: 919- 928.

~~Badawi, G. H., Yamauchi, Y., Shimada, E., Sasaki, R., Kawano, N.~~, Tanaka, K. ~~and Tanaka, K. (1984) Enhanced tolerance to salt stress and water deficit by over expressing superoxide dismutase in tobacco (*Nicotiana tabacum*)~~ C~~hloroplasts. *Plant Sci*. **166**: 919- 928.~~Baker, D. E. (1990) Copper In: B. J. Alloway (Ed.) *Heavy Metals in Soils*. Blackie and Son Ltd. 151- 176.

Baker, A. J. M. and Brooks, R. R. (1989) Terrestrial higher plants which hyperaccumulate metallic elements-a review of their distribution, ecology and phytochemistry. *Biorecovery* **1**: 81- 126.

Baker, A. J. M. and Walker, P. L. (1989) Physiological responses of plants to heavy metals and the quantification of tolerance and toxicity. *Chem. Specia. Bioavai.* **1**: 7-17.

Baker, A. J. M., Reeves, R. D., and Hajar, A. S. M. (1994) Heavy metal accumulation and tolerance in British populations of the metallophyte *Thlaspi caerulescens* J. & C. Presl (Brassicaceae). *New Phytol.* **127**: 61-68.

Baker, A. J. M., McGrath, S. P., Reeves, R. D. Smith, J. A. C. (2000) Metal hyper accumulator of plants. A review of the ecology and physiology of a biological resource for phytoremediation of metal-polluted soils. In: N. Terry and, G. N. Banuelos, G. (Eds.), *Phytoremediation of Contaminated Soil and Water*. Lewis publishers, Boca Raton, F. L. 85- 107.

Baker, A.J.M., Reeves, R. D., and Hajar, A.S.M (1994) Heavy metal accumulation and tolerance in British populations of the metallophyte *Thlaspi caerulescens* . J. & C. Presl (Brassicaceae) . *New Phytol.* **127**: 61-68.

Baker, A. J. M. and Brooks, R. R. (1989) Terrestrial higher plants which hyperaccumulate metallic elements- a review of their distribution, ecology and phytochemistry. *Biorecovery* **1**: 81- 126.

Baker, A. J. M. and Walker, P. L. (1989) Physiological responses of plants to heavy metals and the quantification of tolerance and toxicity. *Chem. Specia. Bioavai.* **1**: 7-17.

Baker, D. E. (1990) Copper. In: Alloway, B. J. (Ed.) *Heavy Metals in Soils*. Blackie and Son Ltd. 151- 176.

Baker, R., Lavie, B. and Novo, E. (1985) Natural selection for resistance to the pollution. *Experientia* **41**: 697-699.

Bala, R. and Setia, R. C. (1990) Some aspects of cadmium and lead toxicity in plants. In: C. P. Malik, D. S. Bhatta, R. C. Setu and P. Singh (Eds.). *Advances in Frontier Areas of Plant Science*. Narendra Publishing House, Delhi. 167- 180.

Barcelo, J. and Poschenrieder, (1990) Plant water relations as affected by heavy metal stress: A review . *J. Plant Nutr*. **13**: 1-37.

Barcelo, J. and Poschenrieder, C. (1997) Chromium in plants. In: S. Carati, F. Tottarelli and P. Seqmi, (Eds.). *Chromium Environmental Issue*. Francotangata Press, Milan. 101-129.

Barcelo, J., Poschenriender, C., Ruano, A. and Gunse, B. (1985) Leaf water potential in Cr (VI) treated bean plants (*Phaseolus vulgaris* L.). *Plant Physiol*. *Suppl*. **77**: 163-164.

Barman, S. C., Kisku, G. C. and Bhargava, S. K. (1999) Accumulation of heavy metals in vegetables, pulse, and wheat grown in fly ash amended soil. *Environ. Biol*. **20:** 15-18.

Barthwal, J. Nair, S. and Kakkar, P. (2008) Heavy metal accumulation in medicinal plants collected form environmentally different sites. *Biomed. Environ. Scie*. . **21**: 319- 324.

Baryla, A., Carrier, P., Franck, F., Coulomb, C., Sahut, C. and Havaux, M. (2001) Leaf chlorosis in oil seed rape plants (*Brassica napus*) grown on cadmium -– polluted soil: causes and consequences for photosynthesis and growth. *Planta* **212**: 696- 709.

Basberg-Pahlsson, A. M. (1989) Toxicity of heavy metals (Zn, Cu, Cd, Pb) to vascular plants . *Water Air Soil Pollut*. **47**: 287-319.

Basta, N. T., Ryan, J. A. and Chaney, R. L. (2005) Trace element chemistry in rResidual- – treated soil: kKey cConcepts and mMetal bBioavailability *J. Environ. Qual*. **34**: 49- 63.

Baszynski, T., Wajda, L., Korl, M., Wolinska, D., Krupa, Z. and Tukendorf, A. (1980) Photosynthetic activities of cadmium - treated tomato plants. *Physiol. Plant*. **48**: 365- 370.

Bates, L. S., Waldren, R. P. and Teare, I. D., (1973) Rapid determination of free proline for water stress studies. *Plant Soil*. **39**: 205- 208.

Beauford, W., Barber, J. and Barringer, A. R. (1977) Uptake and distribution of mercury within higher plants. *Physiol. Plant.* **39:** 261-265.

Becerril, J. M., Gonzalez-Murua, C., Munoz-Rueda, R. and De Felipe, M. R. (1989) Changes induced by cadmium and lead in gas exchange and water relations of clover and lucerne. *Plant Physiol. Biochem*. **27**: 913- 918.

Bennicelli, R., Stezpniewska, Z., Banach, A., Szahnocha, K. and Ostrowski, J. (2004) The ability of Azolla caroliniana to remove heavy metals [Hg (II), Cr (III), Cr (VI)] from municipal waste water. *Chemospere* **55**: 141- 146.

Bennicelli, R., Z., Stepniewska, A. Banach, K. Sszajnocha and Ostrowski, J. (2004) The ability of *Azolla caroliniana* to remove heavy metals (Hg (II), Cr (III), Cr (VI)) from municipal waste water. *Chemosphere*, **55**: 141-146.

Bera, A. K. , Kanta-Bokaria, A. K. and Bokaria, K. (1999) Effect of tannery effluent on seed germination, seedling growth and chloroplast pigment content in mung bean (*Vigna radiatea* L. *Wilczek*). *Environ. Ecol.* **17**: 958-– 961.

Berlyn, G. P. and Miksche, J. P. (1976) *Botanical Microtechnique and Cytochemistry*. The lowa State University Press. Ames, Iowa. 326.

Bernier, M., Popovic, R. and Carpentier, R. (1993) Mercury inhibition at the donor side of photosystem II is reversed by chloride. *FEBS Lett.* **321:** 19-23.

Bharagava, R. N., Chandra, R. and Rai, V. (2008) Phytoextraction of trace elements and physiological changes in Indian mustard plants (*Brassica juncea* L.) grown in post methanated distillery effluent (PMDE) irrigated soil. *Biores. Technol.* **99**: 8316-8324.

Bhoomik, N. and Sharma, A. (1999) Lead toxicity in plant systems: Some aspects. *The Nucleus* **42**: 131- 181.

Bishnoi, N. R., Sheoran, I. S. and Singh, R. (1993) Effects of chromium on photosynthesis, respiration and nitrogen fixation in Pea (*Pisum sativum* L.) seedlings. *J. Plant Physiol*. **142**: 25-30.

Blaylock, M. J., Salt, D. E., Dushenkov, S., Zakharov, O., Gussman, C., Kapulink, Y., Ensley, B. D. and Raskin, I. (1997) Enhanced accumulation of Pb in Indian mustard by soil-applied chelating agents. *Environ. Sci. Technol.* **31**: 860-8685.

Boominathan, R. and Doran, P. M. (2003) Cadmium tolerance and antioxidative defenses in hairy roots of the cadmium hyperaccumulator, *Thlaspi caerulescens*. , *Biotehnol. Bioeng*. **83**: 158- 167.

Borovik, A. J. (1990) Characteristics of Metals in Biological System. In: A. J. Shaw (Ed.). *Heavy Metal Tolerance in Plants: Evolutionary Aspects*. .CRC Press, Florida. 3-5.

Bowler, C., Montagu, M. V. and Inze, D. (1992) Superoxide dismutase and stress tolerance. *Annu. Rev. Physiol. Plant. Mol. Biol.* **43**: 83- 116.

Bradley, D. E. and Min, D. B. (1992) Singlet oxygen oxidation of foods. *Cat. Rev. Food Sci. Nutr*. **31**: 211- 236.

Brennan, N. A. and Shelly, M. L. (1999) A model of the uptake, translocation and accumulation of lead (Pb) by maize for the purpose of

phytoextraction. *Ecol. Eng*. **12**: 271-297.

Brooks, R. R., Lee, J., Reeves, R. D. and Jaffre, T., (1977) Detection of nickeliferous rocks by analysis of herbarium specimens of indicator plants. *J. Geochem. Explor*. **7:** 49-58.

Brown, S. L., Chaney R. L., Angel, J. S. and Baker, A. J. M. (1994) Phytoremediation potential of *Thlaspi caerulescens* and bladder campion for zinc and cadmium – contaminated soil. *J. Environ. Qual*. **23**: 1151-1157.

Brown, S. L., Chaney, R. L., Angle, J. S. and Baker, A. J. M. (1995) Zinc and cadmium uptake by hyper accumulator *Thalaspi caerulescens* grown in nutrient solution. *Soil . Sci. Soc. Am. J*. **59**: 125-133.

Brzyska, M., Ginalska, G. and Lobarzewski, J. (1991) Combined influence of lead, mercury and cadmium ions in the presence of magnesium on endopolygalacturonase activity. *Biotechnol. Appl. Biochem.* **13:** 83-89.

Buchanan, B. B., Gruissem, W. and Jones, R. L. (2000) *Biochemistry and Molecular Biology of Plants*. American Society of Plant Physiologists. Rockville, Maryland.

Burzynski, M. and Buczek, J. (1994) The influence of Cd, Pb, Cu and Ni on NO_3 uptake by cucumber seedlings. II. *in vitro* and *in vivo* effects of Cd, Cu, Pb and Ni on the plasmalemma ATPase and oxidoreductase from cucumber seedling roots. *Acta Physiol. Plant*. **16**: 297-302.

Cakmak, I., Welch, R. , M., Hart, J., Norvell, W. A., Ozturk, L. and Kochian, L. V. (2000) Uptake and retranslocation of leaf- applied cadmium (Cd- 109- Cd) in diploid, tetraloid and hexaploid wheats. *Exp. Bot.* **51**: 221- 226.

Caldas, E. D. and Machado, L. L. (2004) Cadmium, mercury and lead in medicinal herbs in Brazil Food. *Chem. Technol.* **42**: 599- 603.

Chakraborti, K. K. and Handa, S. S. (1989) Antihepatotoxic investigations of *Boerhavia diffusa* L. *Indian Drugs* **27**: 161- 166.

Chan, T. Y. K. , Tomlinson, B. and , Critichley, A. J. H. (1993) Chinese herbal medicines revisited: a Hong Kong perspective. *The Lancet* **342**: 1532- 1534.

Chandan, B. K., Sharma, A. K., and Anand, K. K. (1991) *Boerhavia diffusa*: A study of its hepatoprotective activity. *J. Ethnopharmacol.* **31**: 299- 307.

Chandra, R., Bharagava, R. N., Yadav, S. and Mohan, D. (2009) Accumulation and distribution of toxic metals in wheat (*Triticum aestivum* L.) and Indian mustard (*Brassica campestris* L.) irrigated with distillery and tannery effluents. *J. Hazard. Mater.* **162**: 1514-1521.

Chaney, R., M., Malik, Y. M., Li, S. L., Brown, E. P., Brewer, J. S. Angle, A. J. M. and Baker, E. (1997) Phytoremediation of soil metals *Curr. Opin. Biotechnol.* **8**: 279-284.

Chang, M. Y., Chen, S. L., Lee, C. F. and Chen, Y. M. (2000) Cold-acclimation and root temperature protection from chilling injury in chilling –sensitive mungbean (*Vigna radiata* L.) seedlings. *Bot. Bull. Acad. Sin.* **42**: 53- 60.

Chaoui, A., Mazhoudi, S., Chorbl, M. H. and El- Ferjani, E. (1997) Cadmium and zinc induction of lipid peroxidation and effects on antioxidant enzyme activities in bean (*Phaseolus vulgaris* L.) *Plant. Sci.* **127**: 139-147.

Chatterjee, J. and Chatterjee, C. (2000) ~~Zinc stress changes in the metabolism of blackgram (*Phaseolus mungo*) *Indian J. Agri. Sci.* **70**: 250-252.~~Phytotoxicity of cobalt, chromium and copper in cauliflower. *Environ. Pollut.* **109**: 169-174.

Chen, Z., Zhu, Y. G., Liu, W. J. and Meharg, A. A (2005) Direct evidence showing the effect of root surface iron plaque on arsenite and arsenate uptake into rice (*Oryza sativa*) roots. *New Phytol.* **165**: 91-97.

Cheng, N. H., Pittman, J. K., Shigaki, T. and Hirschi, K. D. (2002) Characterisation of CAX4, an Arabidopsis H+/ cation antiporter. *Plant. Phsyiol.* **120**: 1245- 1254.

Cheraghi, M., Lorestani, B. and Yousefi, N. (2009) Effect of waste water on heavy metal accumulation in Hamedan province vegetables. *Int. J. Bot.* **5**: 190-193.

Chopra, G. L. (1969) *Angiosperms. Systematics and Life Cycles*. S. Nagin & Co.

Jalandhar, Punjab, India. 361- 365.

Choudhury, S. and , Panda, S. K., (2005) Toxic effect, oxidative stress and ultra structural changes in moss *Taxitheelium nepalense* (Schwaegr.) Broth. uUnder lead and chromium toxicity. *Water Air Soil Pollut*. **167**: 73- 90.

Chugh, L. K. and Sawhney, S. K. (1999) Photosynthetic activities of *Pisum sativum* seedlings grown in presence of cadmium. *Plant Physiol. Biochem*. **37**: 297- 303.

Ciura, J., Poniedzialek, M., Sekara, A. and Jedrszczyk, E. (2005) The possibility of using crops as metal phytoremediants. *Polish J. Environ. Stud.* **14**: 17-22.

Clemens, S. (2001) Molecular mechanisms of plant metal tolerance and homeostasis. *Planta* **212**: 475- 486.

Clemens, S. (2006) Toxic metal accumulation, responses to exposure and mechanisms of tolerance in plants ~~(2006)~~. *Biochiemie*. **88**: 1707-1719.

Clemens, S., Palmagren, M. G. and Kraemer, U. (2002) A long way ahead: understanding the engineering plant metal accumulation. *Trends Plant Sci*. **7**: 309-315.

Clijsters, H. and Van Assche, F. (1985) Inhibition of photosynthesis by heavy metals. *Photosynth. Res*. **7**: 31-40.

Cobbett, C. S. (2000) Phytochelatins and their roles in heavy metal detoxification. *Plant Physiol.* **123:** 825-832.

Cobbett, C. S. (2003) Heavy metals and plants- model systems and hyper accumulators. *New Phytol*. **159**: 289- 293.

Cobbett, C.S. (2000) Phytochelatins and their roles in heavy metal detoxification. *Plant Physiol.* **123:** 825-832.

Cobbett, C. S. and Goldsbrough, P. (2002) Phytochelatins and metallothioneins: Roles in heavy metal detoxification and homeostasis. *Annu. Rev. Plant Biol.*. **53**: 159-182.

Cohen, C. K., Fox, T. C., Garvin, D. F. and Kochian, L.V. (1998) The role of iron-deficiency stress responses stimulating heavy-metal transport in plants. *Plant Physiol.* **116:** 1063-1072.

Corradi, M. G., Bianchi, A. K. and Albasini, A. (1993) Chromium toxicity in *Salvia sclarea*: 1. Effect of hexavalent chromium on seed germination and seedling development. *Environ. Expt. Bot*. **33**: 405-413.

Costa, G. and Morel, J. L. (1994) Water relations, gas exchange and amino acid content in Cd- treated lettuce. *Plant Physiol. Biochem*. **32**- 561-570.

Cseh, E. (2002) Metal permeability, transport and efflux in plants. In: M.N.V. Prasad and K. Strzalka (Eds.). *Physiology and Biochemistry of Metal Toxicity and Tolerance in Plants*. Kluwer Academic, *Dordrecht* 1-36.

Cseh, E., Fodor, F., Varga, A. and Zaray, G. (2000) Effect of lead treatment on the distribution of essential elements in cucumber, *J. Plant Nutr*. **23**: 1095-1105.

Cunningham, SC. D. and Berti, W. R. (1993) Remediation of contaminated soils with green plants: An overview. *In vitro Cell Dev. Biol*. **29**: 207- 212.

Cunningham, S. D., Berti, W. R. and Huang, J. W. (1995) Phytoremediation of contaminated soils. *Trends Biotechnol*. **13**: 398- 403.

Daniel, G., Hussain, K. and Nabeesa- Salim, (2009) Bioaccumulation and effect of lead, chromium , and cadmium on peroxidase activity in bitter gourd (*Momordica charantia* L. Walp) cv. PRIYA *Inter. J. Plant . Sci*. **4**: 239-244

David, E. S., Roger, C. P., Ingrid, J. P. and Ilya, R. (1995) Mechanisms of cadmium mobility and accumulation in Indian Mustard. *Plant . Physiol*. **109**: 1427- 1433.

Davies, K. L., Davies, M. S. and Francis, D. (1991) The influence of an inhibitor of phytochelatin synthesis on root growth and root meristematic activity in *Festuca rubra* L. in response to zinc. *New Phytol*. **118**: 565-570.

Davies, F. T., Puryear, J. D., Newton, R. J., Egilla, J. N. and Grossi, J. A. S. (2002) Mycorrhizal fungi increase chromium uptake by sunflower plants: influence on tissue mineral concentration, growth and gas exchange. *J. Plant Nutr*. **25**: 2389-2407.

Davies, K. L., Davies, M. S. and Francis, D. (1991) The influence of an inhibitor of phytochelatin synthesis on root growth and root meristematic

activity in *Festuca rubra* L. in response to zinc. *New Phytol*. **118**: 565-570.

De Fillippis, L. F. (1979) The effect of heavy metal compound on the permeability of *Chlorella* cells. *Z. Pflanzenphysiol*. **92**: 39-43.

De Vos, C. H. R., Vonk, M. J. and Schat, H. (1992) Glutathionine depletion due to copper induced phytochelatin synthesis causes oxidative stress in *Silene cucubalus*. *Plant Physiol*., **98**: 853-858.

De, A. K., Sen, A. K., Modak, D. P. and Jana, S. (1985) Studies of toxic effect of Hg (II) on *Pistia stratiotes*. *Water Air Soil Pollut*. **24:** 351-360.

Delnomdedien, M., Boudou, A., Desmager, J. P. and Georgescauld, D. (1989) Interaction of $HgCl_2$ with the primary amino group of model membranes containing phosphatidyl serine and phosphatidyl ethanolamine. *Biochem. Biophys. Acta*. **986**: 191-199.

Dhar, M. L., Dha, M. M., Dhawan, B. N., Mehrotra, B. N. and Ray, C. (1968) Screening of Indian plants for biological activity: Part I *Indian J. Exp. Biol*. **6**: 232- 247.

Diaz, J., Bernal, A., Pomar, F. and Merino, F. (2001) Inductin of shikimate dehydrogenase and peroxidase in pepper (*Capsicum annum* L.) seedlings in response to copper stress and its relation to lignifications. *Plant Sci*.

161-179.

Dietz, A. C. and Schnoor, J. L. (2001) Advances in phytoremediation. *Environ. Health Perspect*. **109**: 163- 168.

Dietz, K. J., Baier, M. and Kramer, M. (1999) Free radicals and reactive oxygen species as mediator of heavy metal toxicity in plants. In: M. N. V. Prasad M.N.V., and J. Hagemeyer J. (Editorss.). *Heavy Metal Stress in Plants: from Molecules to Ecosystem*. Berlin: *Springer* Berlin 73- 79.

Dixit, V., Pandey, V. and Shyam, R. (2002) Chromium ions inactivate electron transport and enhance superoxide generation *in vivo* in pea (*Pisum sativum* L. cv. Azad) root mitochondria. *Plant Cell Environ.* **25**: 687-690.

Dominguez- Solis, J. R., Lopez-Martin, M., Ager, F. J., Ynsa, M. D., Romero, L. C. and Gotor, C. (2004) Increased cysteine availability is essential for cadmium tolerance and accumulation in *Arabidopsis thaliana*. *Plant Biotechnol. J.* **2**: 469-476.

Driscoll, C. T., Yan, C., Schofield, C. L., Munson, R. and Holsapple, H. (1994) The chemistry and bioavailability of mercury in Adirandack lakes. *Environ*. Sci. *Technol*. **28**: 136-143.

Dubey, R. S. (1997) Photosynthesis in plants under stressful conditions In: M. Pessarakli , M. (Ed.): *Handbook of Photosynthesis* 859- 876 Marcel Dekker, New York. 859- 876.

Dunbabin, J. S. and Bowner, K. H. (1992) Potential use of constructed wetlands for treatment of industrial waste waters containing metals. *Sci. Total Environ.* **111**: 151-168.

Ebbs, S. D. and Kochian, V. K. (1997) Toxicity of zinc and copper to *Brassica* species: implications for phytoremediation. *Environ. Qual.* **26**: 776-778.

Ebbs, S. D. and Kochian, ~~I.~~ V. K. (1998) Phytoextraction of zinc by oat (*Avena sativa*) barley (*Hordeum vulgare*) and Indian mustard (*Brassica juncea*) *Environ. Sci. Technol.* **32**: 802- 806.

Ebbs, S. D. and Kochian, I. V. (1998) Phytoextraction of zinc by oat (*Avena sativa*), barley (*Hordeum vulgare*) and Indian mustard (*Brassica juncea*) *Environ. Sci. Technol.* **32**: 802-806.

Ebbs, S. D. and Kochian, L. V. (1997) Toxicity of zinc and copper to *Brassica* species: implications for phytoremediation. *Environ. Qual.* **26**: 776- 778.

~~Ebbs, S. D. and Kochian, V. K. (1997) Toxicity of zinc and copper to *Brassica* species: implications for phytoremediation *Environ. Qual.* **26**: 776-778.~~

Ebinghaus, R., Tripathi, R. M. and Wallschlager, L. S. E (1999) Natural and anthropogenic mercury sources and their impact on the air- surface exchange of mercury on regional and global scales. In: R., Ebinghaus, R. R., Turner, L. D. Lacerda,, O., Vasilev and W. Salomons, (Eds.) *Mercury Contaminated Sites*: *Characterisation, Risk Assessment and Remediation*. New York, *Springer*- Verlag, Berlin Heidelberg 76-89..

Ederli, L., Reale, L., Ferrauti, F. and Pasqualini, S. (2004) Responses induced by high concentration of cadmium in *Phragmites australis* roots. *Physiol. Plant.* **121:** 66-74.

Epstein, E. (1972) *Mineral Nutrition of Plants*: *Principles and Perspectives*. John Wiley and Sons, New York.

Ernst, E. (2005) Contamination of herbal medicines. *Pharmaceut.* **275**: 167.

Escarre, J., Lefebure, C., Gruber, W., Leblanc, M., Lepart, J., Riviere, Y. and Delay, B. (2000) Zinc and cadmium hyperaccumulation by *Thlaspi caerulescens* from metalliferous and non - metalliferous sites in the Mediterranean area: Implications for phytoremediation. *New Phytol*. **145**: 429- 437.

Eun, S. O., Youn, H. S. and Lee, Y. (2000) Lead disturbs microtubule organization in the root meristem of *Zea mays*. *Physiol. Plant*. **110**: 357-365.

Falchuk, K.H., Goldwater, L. J. and Vallee, B. L. (1977) The biochemistry and toxicology of mercury. In: C. A. Mc Aulifee (Ed.). *The Chemistry of Mercury*. Mac Millan, London. , 259-283.

Falk, H., Walling, R. and Au, W. (2003) A global approach to childhood lead poisoning prevention. *Int. J. Hyg. Environ. Health* **206**: 363-369.

FAO/WHO, (1984) Contaminants. In Codex Alimentarius XVII, Edn.1. FAO/WHO. Codex Alimentarius Commission. Rome.

Ferreti, M., Ghisi, R., Merlo, L., Vecchia, F. D. and Passera, C. (1993) Effect of cadmium on photosynthesis and enzymes of photosynthetic sulphate and nitrate assimilation pathways in maize (*Zea mays* L.) *Photosynthetica* **29**: 49- 54.

Fett, J. P., Cambraia, J., Oliva, M. A. and Jorado, C. P. (1994) Cadmium uptake and growth inhibition in water hyacinth: Effect of nutrient solution factors. *J. Plant. Nutr*. **17**: 1205-1217.

Fitter, A. H. and Hay, R. K. M. (1983) *Environmental Physiology of Plants*. Academic Press, London.

Fitz, W. J. and Wenzel, W. W. (2002) Arsenic transformation in the soil rhizosphere plant system, fundamentals and potential application of Phytoremediation. *Biotechnol*. **99**: 259- 278.

Fodor, F. (2002) Physiological responses of vascular plants to heavy metals. In: M.N.V. Prasad and K. Strzalka (Eds.). *Physiology and Biochemistry of Metal Toxicity and Tolerance in Plants.* Kluwer Academic. Dordrecht. 149-177.

Folin, O. and Denis, W. (1915) A Calorimetric method for the determination of phenols (and phenol derivatives) in urine. *J. Biol. Chem.* **22**: 305- 308.

Foy, C. D. Chaney, R. L. and White, M. C. (1978) The Physiology of metal toxicity in plants. *Annu. Rev. Plant Physiol*. **29:** 511-566.

Foyer, C. H., Lopez- Delgado, H., Dat, J. F. and Scot, I. M. (1997) Hydrogen peroxide and glutathione associated mechanisms of acclamatory stress tolerance and signalling. *Physiol. Plant*. **100**: 241- 245.

Friedland, A. J. (1990) The movement of metals through soils and ecosystems. In: A. J. Shaw (Ed.). *Heavy Metal Tolerance in Plants: Evolutionary Aspects*. .CRC Press, Florida. 7-19.

Gaal, O., Medgyesi, G. A. and Vereczkey, L. (1980) *Electrophoresis in the Separation of Biological Macromolecules*. John Wiley and Sons, Chichester.

Gallego, S. M., Benavioas, M. P. and Tomaro, M. L. (1996) Effect of heavy metal ion excess on sunflower leaves: evidence for involvement of oxidative stress. *Plant Sci*. **121**: 151-159.

Gasic, O., Kastori, R. Petrovic, N. and Stagner, D. (1992) Effect of lead on some biochemistry and physiological parameters in corn (*Zea mays* L.) *Agric. Meditarania*. **122**: 257-261.

Gbaruko, B. C., and Friday, O. U. , (2007) Bioaccumulation of heavy metals in some fauna and flora. *Int. J. Environ. Sci. Tech*. **4**: 197-202.

Giannopolitis, C. N. and Ries, S. K. (1977) Superoxide Dismutase I. Occurance in higher plants. *Plant Physiol*. **59**: 309- 314.

Gleba, D., Borisjuk, N., Borisjuk, L., Kneer, R., Poulev, A., Skarzhinskaya, M., Dushenkov, S., Logendra, S., Gliba, Y. and Raskin, I. (1999) Use of

plant roots for phytoremediation and molecular farming. *Proc. Natl. Acad. Sci.* USA. **96**: 5973- 5977.

Glick, B. R. (2003) Phytoremediation: synergistic use of plants and bacteria to clean up the environment. *Biotechnol. Adv.* **21**: 383- 393.

Goldbold D. L., Kettner, C. (1991) Use of root elongation studies to determine aluminium and lead toxicity in *Picea abides* seedlings J. *Plant Physiol.* **5**: 138-231.

Gopal, G. V. and Shah, G. L. (1985) Some folk medicinal plants used for jaundice in Gujarat, India. *J. Res. Edu. Indian Med.* **4**: 44-49.

Grant, C. A., Buckley, W. T., Bailey, L. D. and Selles, F. (1998) Cadmium accumulation in crops *Can. J. Plant Sci.* **78**: 1- 17.

Gregory, P. J. (1988) Growth and functioning of plant roots. In: A. Wild (Ed.) *Russells Soil Conditions and Plant Growth*. John Wiley, New York. 113-167.

Grill, E., Winnacker, E.L. and Zenk, M.H. (1985) Phytochelatins, the principal heavy-metal complexing peptides of higher plants. *Sci.* **230**: 674-676.

Grill, E., Coffler, S., Winnacker, E. L. and Zenk, M. A. (1989) Phyotochelatins, the heavy metal binding peptides of plants are synthesized from glutathione by a specific γ- glutamyl cysteine dipeptidyl transpeptidase (Phytochelatin synthase). *Proc. Natl. Acad. Sci.* USA. **86**: 6838-6842.

Grill, E., Loffler, S., Winnacker, E. L., Zenk, M. H. (1989) Phytochelatins, the heavy – metal – binding peptides of plants, are synthesised from glutathione by a specific γ- glutamylcystine dipeptidyl transpeptidase (Phytochelatin synthase). *Proc. Natl Acad Sci*. USA 86: 6838- 6842.

Grill, E., Winnacker, E.L. and Zenk, M.H. (1985) Phytochelatins, the principal heavy-metal complexing peptides of higher plants. *Science*. **230**: 674-676.

Guo-sheng, S. Ming-xue, C., Xiu-fu, Z., Chun-mei, X. U., Dan-ying, W. Qian, Q. and Guo-ping, Z. (2007) Cadmium accumulation and its toxicity in Brittle culm 1(bc1), a fragile Rice mutant. *Rice Sci*. **14**: 217-222.

Gzyl, J., Przymusinski, R. and Wozny, A. (1997) Organospecific reaction of yellow lupin seedlings to lead. *Acta Soc. Bot. Pol*. **66**: 61-66.

Haag-Kerwer, A., Schafer, H. J., Heiss, S., Walter, C. and Rausch, T. (1999) Cadmium exposure in *Brassica juncea* causes decline in transpiration rate and leaf expansion without effect on photosynthesis. *J. Exp. Bot*. **50**: 1827-1835.

Hadjiliadis, N. D. (1997) Cytotoxicity, mutagen and carcinogenic potential of heavy metals related to human environment. In: *Environment* NATO-ASI Series, Kluwer, Dordrecht.

Hageman, R. H. and Reed, A. J. (1980) Nitrate reductase from higher plants. In: A. Sanpietra (Ed.) *Methods in Enzymology*. **XX111**: 491-503 Academic Press. New York. 270- 280.

Hagemayor, J. (1993) Indicators for heavy metals in the terrestrial environment. In: B. Market (Ed.) *Plants as Biomonitors*. V. C. H. Weinheim, New York, 541-556.

Hale, K. L., McGrath, S. P., Lombi, E., Stack, S. M., Terry, N., Pickering, I. J., George, G. M. and Pilon-Smits, E. A. H. (2001) Molybdenum sequestration in *Brassica* species. aA rRole for aAnthocyanins? *Plant Physiol.* **126:** 1391-1402.

Hall, J. L. (2002) Cellular mechanisms for heavy metal detoxification and tolerance. *J. Exp. Bot.* **53:** 1-11.

Halliwell, B. and Gutteriodge, J. M. C. (1999) *Free Radicals in Biology and Medicine*,. Oxford, U K, Clarendon Press.

Hamlin, R. L., Schatz, C. and Baker, A. V. (2003) Zinc accumulation in Indian mustard as influenced by nitrogen and phosphorus nutrition. *J. Plant Nutr*. **26**: 177-190.

Han, F.X., Sridhar, B. B. M., Monts, D. L. and Su, Y. (2004) Phytoavailability and toxicity of trivalent and hexavalent chromium to *Brassica juncea*. - *New Phytol*. **162**: 489-499.

Han, F.X., Sridhar, B.B.M., Monts, D.L. and Su, Y. (2004) Phytoavailiability and toxicity of trivalent and hexavalent chromium to *Brassica juncea*, *New Phytol*. **162**: 489.

Harborne, J. B. (1980) Plant Phenolics. In: E. A. Bell and B. V. Charlwood (Eds.). Vol. 8. *Secondary Plant Products*. 329-395. *Encyclopedia of*

Plant Physiology, (New Series) Vol. 8. Springer- Verlag Berlin. Heidelberg New York. 329-395.

Hattori, H., Kuniyasu, K., Chiba, K. and Chino, M. (2006) Effect of chloride application and low soil pH on cadmium uptake from soil by plants. *Soil Sci. Plant Nutr*. **52**: 89-94.

Heath, R. L. and Packer, L., (1968) Photo-peroxidation in isolated chloroplast. I. Kinetics and stoichiometry of fatty acid peroxidation. *Arc. Biochem. Biophys*. **125**: 189- 198.

Hendry, G. A. F., Baker, A. J. M. and Ewart, C. F. (1992) Cadmium tolerance and toxicity, oxygen radical processes and molecular damage in cadmium- tolerant and cadmium sensitive- clones of *Holceus lanatus* L. *Acta Bot. Neerl*. **41**: 271-281.

Henry, J. R. (2000) Phytoremediation of mercury In: *An overview of the Phytoremediation of Lead and Mercury. A report for the U. S. Environmental Protection Agency. Washington*, D. C., USA. 44 - 46.

Hiruma –-Lima, C. A., Grazioso, J. S., Bighetti, E.J., Germonsen Robineou, L. and Souza- Brito, A. R. (2000) The juice of fresh leaves of *Boerhavia diffusa* L. (Nyctaginaceae) markedly reduces pain in mice. *J. Ethnopharmacol*. **71**: 267-274.

Hopkins, W. G., , Norman P. A. and Huner (2004) *Introduction to plant physiology*, III rd Edn. John -Wiley & Sons, Inc. New York

Hopkins L., Malcolm, J., Hawkesford, J. (2000) J. S- supply, free sulphur and free aminoacids pools in Potato (*Solanum tuberosum* I. Cv Desiree) In: C. Brunold C,(Ed.) *editor. Sulphur Nutrition and Sulfur Assimilination in Higher Plants* 259- 261.

Hosseini, R. H., Khanlarian, M. , and Ghorbanli, M. (2007) Effect of lead on germination, growth and activity of catalase and peroxidase enzyme in root and shoot of two cultivars of *Brassica napus* L. *J. Biol. Sci*. **4**: 592-598.

Houston, M. C. (2007) The role of mercury and cadmium heavy metals in vascular disease, hypertension, coronary heart disease and myocardial infarction. *Altern. Ther. Health Med*. **13**: 128-133.

Huang, J.W. and Cunningham, S. D. (1996) Lead phytoextraction: species variation in lead uptake and translocation. *New Phytol*. **134**: 75-84.

Huang, J. W., Chen, J., Berti, W.R. and Cunningham, S. D. (1997) Phytoremediation of lead–contaminated soils: role of synthetic chelators in lead phytoextraction. *Environ. Sci. Technol*. **31**: 800-805.

Hussain, K. (2007) *Ecophysiological Aspects of Bacopa monnieri* (L.) Pennell. Ph.D. Thesis submitted to the University of Calicut.

Hussain, I., Khan, F., Khan, I., Khan, L. and Wali-Ullah, J. (2006) Determination of heavy metals in medicinal plants. *Chem. Soc. Pak.* **28**: 347-351.

Hussain, K. (2007) *Ecophysiological Aspects of Bacopa monnieri* (L.) Pennell. Ph.D. Thesis submitted to the University of Calicut.

Hussain, K., Abdussalam, A. K., Ratheesh- Chandra, P. and Nabeesa-Salim (2010) Bioaccumulation of heavy metals in *Bacopa monnieri* (L.) Pennell growing under different habitat. *Int. J. Ecol. Devel.* **10**:66-73.

Iqbal, M. Z., Saeeda, S. and Muhammed, S. (2001) Effects of chromium on an important arid tree (*Caesalpinia pulcherima*) of Karachi city, Pakistan. *Ekol. Bratislava*. **20**: 414-22.

Ishikawa, S., Ae, N., Murakami, M. and Wagatsuma, T. (2006) Is *Brassica juncea* a suitable plant for phytoremediation of cadmium in soils with moderately low cadmium contamination?- Possibility of using other plant species for Cd- phytoextraction. *Soil Sci. Plant Nutr*. **52**: 32-42.

Jain, M. and Puranik, M. (1993) Protective effect of reduced glutathione on inhibition of chlorophyll biosynthesis by mercury in excised greening maize leaf segments. *Indian J. Exp. Biol.* **31**: 708-710.

Jalil, A., Selles, F. and Clarke, J. M. (1994) Effect of cadmium on growth and uptake of cadmium and other elements by Durum Wheat. *J. Plant Nutr.* **17**: 1839-1858.

Jamal, S. N., Iqbal, M. Z. and Athar, M. (2006) Effect of aluminum and chromium on the germination and growth of two *Vigna species*. *Int. J. Environ. Sci. Techol*. **3**: 53-58.

Jeana, S. and Chaudhuri, M. A. (1982) Senescence in submerged aquatic angiosperms: Effect of heavy metals. *New phytol*. **90**: 477-484.

Jiang, W., Liu, D. and Hou, W. (2000) Hyperaccumulation of lead by roots, hypocotyls and shoots of *Brassica juncea*. *Biol. Plant.* **43:** 603-606.

Jindal, R. and Kaur, B. (2000) Effect of heavy metal toxicity on the productivity of a fresh water ecosystem. *Indian. J. Ecol*. **27**: 27- 32.

Johansen, O. A. (1940) *Plant Microtechnique*. Mc Graw Hill Book Company, Inc. New York.

Joop, A. K., Marjolein, D., Paul, L. M. K., Henk, S., Jos, A. C. V. and Wilfried, H. O. E. (1994) Phytochelatin in cadmium sensitive and cadmium tolerant *Silene vulgaris*. *Plant Physiol*. **104**: 255-261.

Kabata- Pendias, A. and Pendias, H. (1991) *Trace Elements in Soil and Plants* (3rd Edn). CRC Press. LLC. Boka Raton., FL.

~~Kabata- Pendias, A. and Pendias, H. (2001) Trace Elements in Soils and Plants., 3rd ed. CRC. Press.~~ 365.

Kabata-Pendias, A. and Pendias, H. (1999) *Biogeochemistry of Trace Elements* in (Polish) PWN. Warszawa. Poland.

Kabata- Pendias, A. and Pendias, H. (2001) Trace Elements in Soils and Plants. (3rd Edn.) CRC. Press.

Kagi, J.H.R. and Hapke, H.J. (1984) Biochemical interactions of mercury,

cadmium and lead. In: J. O. Nriagu (Ed.). *Changing Metal Cycles and Human Health.* Springer – Verlag, New York. 237-250.

Kahle, H. (1993) Response of roots of trees to heavy metals. *Environ. Exp. Bot*. **33**: 99-119.

Kakosy, T., Hudak, A. and , Naray, M. (1996) Lead intoxication epidemic caused by ingestion of contaminated ground paprika. *J. Toxicol*ogy-*Clinical Toxicol*.ogy **34**: 507-511.

Kamal, M., Ghaly, A. E., Mahmoud, N. and Cote, R. (2004) Phytoaccumulation of heavy metals by aquatic plants. *Environ*. *Int*. **29**: 1029- 1039.

Kanazawa, S., Sano, S., Koshiba, T. and Ushimaru, T. (2000). Changes in antioxidants in cucumber cotyledons during natural senescence: comparison with those during dark induced senescence. *Physiol*. *Plant*.. **109**: 211–216.

Kar, M. and Mishra, D. (1976) Catalse, peroxidase and polyphenol oxidase activities during rice leaf senescence. *Plant Physiol*. **57**: 315-319.

Kasprzak, K. S. (2002) Oxidative DNA and protein damage in metal- induced toxicity and carcinogenesis. *Free radical Biol*. *Med*. **32**: 958- 967.

Kastori, R., Petrovic, N. and Arsenijevic, M. I. (19956) Effect of lead on thewater relations, proline concentration and nitrate reductase activity in Sunflower plants. *Acta Agron*. *Hungarica* **44**: 21- 28. activities of some enzymes of nitrogen metabolism in sugar beet (*Beta vulgaris* L.). *Glasnik*

Instituta Za Botanika I Botanicke Baste Univerziteta u Beograda. **29**: 77-84.

Kastori, R., Plenicar, M., Sakai, Z., Pankovic, D. and Maksimovic, A. I. (1998) Effect of excess lead on sunflower growth and photosynthesis. *J. Plant Nutr*. **21**: 75-85.

Khan, S. A., Khan, L. and Hussain, I. (2008) Profile of heavy metals in selected medicinal plants *Pak. J. Weed Sci. Res*. **14**: 101- 110.

Khasim, S. M. (2002) *Botanical Microtechnique: Principles and Practice*. Capital Publishing Company, New Delhi.

Khatun, S., Ali, M. B., Hahn, E. and Paek, K. (2008) Copper toxicity in *Withania somnifera*: Growth and antioxidant enzymes responses of *in vitro* grown plants. *Environ. Exp. Bot*. **64**: 279- 285.

Kim, Y. Y., Yang, Y. Y. and Lee, Y. (2002) Pb and Cd uptake in rice roots. *Physiol. Plant*. **116**: 368-372.

Kim, I. S., Kang, K. H., Jhnson-Green, P. and Lee, E. J. (2003) Investigation of heavy metal accumulation in *Polygonum thunbergii* for phytoextraction. *Environ. Pollut*. **126**: 235-243.

~~Kim, Y. Y., Yang, Y.Y. and Lee, Y. (2002) Pb and Cd uptake in rice roots. *Physiol. Plant*. **116**: 368-372.~~

Kiran, Y., and Sahin, A., (2006) The effects of cadmium on seed germination, root development and mitosis of root tip cells of lentil (*Lens culinaris* Medik). *World J. Agri. Sci.* **2:** 196-200.

Kirtikar, K. R. and Basu, B. D. (1956) *Indian Medicinal . Plants.:* Lalit Mohan Basu Allhabad, India.

Kochian, L. V. (1991) Mechanisms of micronutrient uptake and translocation in plants. In: J. J. Mordtnet, F. R. Cox, L. M. Shuman and R. M. Welch (Eds.) *Micronutrients in Agriculture*. Soil Science Society of America, Madison, 229-296.

Kochian, L. V. (1995) Cellular mechanisms of aluminium toxicity and resistance in plants. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **46**: 237-260.

Koeppe, D. E. (1981) Lead: understanding the minimal toxicity of lead in plants. In: N.W. Lepp (Ed.) *Effects of Heavy Metal Pollution on Plants*. Vol. 1. Applied Science Publishers, London and New Jersey. 55-76.

Kos, B., Greman, H. and Lestan, D. (2003) Phytoextraction of lead, zinc and cadmium from soil by selected plants. *Plant. Soil. Environ.* **49**: 548-553.

~~Krupa Z., Iquist G. and Huner, N.P.A. (1993). The effects of cadmium on photosynthesis of *Phaseolus vulgaris L.* - a fluorescence analysis. *Physiol. Plant*,, **88**: 626-630.~~

Krupa, Z. and Baszynski, T. (1995) Some aspects of heavy metals toxicity towards photosynthetic apparatus: Direct and indirect effects on light and dark reactions. *Acta . Physiol. Plant*. **17**: 177-190.

Krupa, Z., Ruszkowski, M. and Gilowska-Juny, E. (1982) The effect of chromate on the synthesis of plastid pigments and lipo quinones in *Zea mays* seedlings. *Acta Soc. Bot. Pol*. **51**: 275-281.

Krupa Z., Iquist, G. and Huner, N. P. A. (1993). The effects of cadmium on photosynthesis of *Phaseolus vulgaris L.*- a fluorescence analysis. *Physiol. Plant.* **88**: 626-630.

Kubota, H., Satoh, K., Yamada, T. and Maitani, T. (1995) Phytochelatins (Class III metallothioneins) and their desglycyl peptides induced by Cd in normal roots of *Rubia tinctorium* L. *Plant Sci* **106**: 157- 166.

Kubota, H., Satoh, K., Yamada, T. and Maitani, T. (2000) Phytochelatin homologues induced in hairy roots of horse radish. *Photochem.istry* **53**: 239- 245.

Kubota, H., Satoh, K., Yamada, T. and Maitani, T. (2000) Phytochelatin homologos induced in hairy roots of horse radish. *Phytochem* **53**: 239-245.

Kubota, H., Satoh, K., Yamada, T. and Maitani, T., (1995) Phytochelatins (Class III metallothioneins) and their desglycyl peptides induced by Cd in normal roots of *Rubia tinctorium* L. *Plant Sci* **106**: 157- 166.

Kumar, A., Vajpayee, P., Ali, M. B., Tripathi, R. D., Singh, N., Rai, U. N.and Singh, S. N. (2002) Biochemical responses of *Cassia siamea* Lamk grown on coal combustion residue (fly- ash). *Bull. Environ. Contam. Toxicol* **68**: 675- 683.

Kumar, N., Dushenkov, V. and Motto, H. (1995) Phytoextraction: The Use of Plants to Remove Heavy Metals from Soils. *Environ. Sci. Technol.* **29**: 1232- 1238.

Kumar, A., Vajpayee, P., Ali, M. B., Tripathi, R. D., Singh, N., Rai, U. N.and Singh, S. N. (2002) Biochemical responses of *Cassia siamea* Lamk grown on coal combustion residue (fly- ash). *Bull. Environ. Contam. Toxicol.* **68**: 675- 683.

Kumar, N.P.B.A., Dushenkov, V., Motto, H. and Raskin, I. (1995) Phytoextraction: The use of plants to remove heavy metals from soil. *Environ. Sci. Technol.* **29:** 1232-1238.

Kupper, H., Kupper, F. and Spiller, M. (1996) Environmental relevance of heavy metal substituted chlorophylls using the example of water plants. *Experi. Bot.any.* **47**: 259- 266.

Kupper, H., Kupper, F. and Spiller, M. (1998) *In Situ* detection of heavy metal substituted chlorophylls in water plants. *Photosynth. Res.* **58:** 123-133.

Kupper, H., Zhao, F. J. and McGrath, S. P. (1999) Cellular compartmentation of zinc in leaves of the hyper accumulator, *Thlaspi caerulescens. Plant Physiol.* **119:** 305-311.

Landrigan, P. J. (1999) Risk assessment for children and other sensitive populations. *Ann. N.Y. Acad. Sci.* , **895**: 1-9.

Lang, F., Sarvari, E., Szigeti, Z., Fodor, F. and Cseh, E. (1995) Effect of heavy metals on the photosynthetic apparatus in cucumber. In: P. Mathis (Ed.). *Photosynthesis: From Light to Biosphere.* Vol. IV. Kluwer Academic, Netherlands. 533-536.

Lata, S. (1989) Effect of cadmium on seedlings growth, mobilization of food reserves and activity of hydrolytic enzymes in *Phaseolus aureus* L. seeds. *Acta Bot.* **17**: 290-293.

Lavid, N., Schwartza, A., Yarden, O. and Tel-Or, E. (2001) The involvement of polyphenols and peroxidase activities in heavy metal accumulation by epidermal glands of water lilly (Nymphaeceaea) *Planta* **212**: 323-328..

Lee, J. H., Hossner, L. R., Attrep, Jr. M. and , Kung, K. S., (2002) Uptake and translocation of plutonium, in two plant species using hydroponics. *Environ. Pollut.* **117**: 61- 68.

Lee, Y. P. and Takahashi, T. (1966) An improved colorimetric determination of amino acids with the use of ninhydrin. *Anal. Biochem.* **14**: 71-77.

Lenka, M., Das, B. L., Panda, K. K. and Panda, B. B. (1993) Mecury-tolerance

of *Chloris barbata* Sw. and *Cyperus rotundus* L. isolated from contaminated sites. *Biol. Plant.* **35:** 443-446.

Leopold, I., Gunther, D., Schmidt, J. and Neumann, D. (1999) Phytochelatins and heavy metal tolerance. *Phytochem*. **50**: 1323- 1328.

Lepp, N. W. (1981) *Effect of Heavy Metal Pollution on Plants* Vol. 2. Applied Science Publishers, London.

~~Levitt, C. M. D., (1984) Sources of lead poisoning. *J. Am*erican *Med*ical *Assoc*iation **252**: 3127- 3128.~~

Levitt, J. (1980) *Responses of Plants to Environmental Stress*. (2nd Edn.. Vol. 2) *Academic Press*, New York.

Levitt, C. M. D. (1984) Sources of lead poisoning. *J. Am. Med. Assoc*. **252**: 3127- 3128.

Liang, Y., Chen, Q., Liu, Q. and Zhang, W., R., (2003) Exogenous silicon (Si) increases antioxidant enzyme activity and reduces lipid peroxidation in roots of salt-stressed barley (*Hordeum vulgarae* L.) *Plant Physiol*. **160**: 1157-1164.

Liao, V. H., Dong, L. and Freedman, J. H. (2002) Molecular characterization of a novel, cadmium -– inducible gene from the nematode *Caenorabolitis elegans*. A new gene that contributes to the resistance to cadmium toxicity. *J. Bio. Chem*. **277**: 42049- 42059.

Lidsky, T. I., and Schneider, J. S. (2006) Adverse effects of childhood lead poisoning: the clinical neuropsychological perspective. *Environ. Res.* **100**: 284-293.

Linger, P., Mossig, J., Fischer, H. and Kobert, J. (2002) Industrial hemp (*Cannabis sativa* L.) growing on heavy metal contaminated soil: fibre quality and phytoremediation potential. *Indian . Crops Prod.* **16**: 33- 42.

Linger, P., Ostwald, A. and Haensler, J. (2005) *Cannabis sativusa* L., growing on heavy metal contaminated soil: Growth, cadmium uptake and photosynthesis. *Biol. Plant.* **49**: 567-576.

Liu, D., Jiang, W. and Gao, X. (2003) Effects of cadmium on root growth, cell division and nucleoli in root tip cells of garlic. *Biol. Plant.* **47**: 79-83.

Liu, Z., He, X., Chen, W., Yuan, F., Yan, K. and Tao, D. (2009) Accumulation and tolerance characteristics of cadmium in a potential hyperaccumulator- *Lonicera japonica* Thumb. *Hazard.ous Materials..* **10**: 81-97.

Lowry, O. H., Rosenbrough, N. J., Farr, A. L. and Randall, R. J. (1951) Protein measurement with Folin-Phenol reagent. *J. Biol. Chem.* **193:** 265-275.

Macnair, M. R. (1990) The genetics of metal tolerance in natural populations In: A. J. Shaw. A. J. (Ed.) *Heavy Metal Tolerance in Plants: Evolutionary Aspects*. CRC Press. Boca Raton, F. L. 235-253.

Maitani, T., Kubota, H., Satoh, K. and Yamada, T. (1996) The composition of metals bound to Class III metallothionein (Phytochelatin and its desglycyl peptide) induced by various metals in root cultures of *Rubia tinctorium*. *Plant Physiol*. **110**: 1145-1150.

Malajczuk, N. and Dell, B. (1995) Heavy metals in Philipines soils affect growth and establishment of trees. *ACIR-For. News Letter*. **18**: 2-3.

Malkowski, E., Kitta, A., Galas, W., Karcz, W. and Kuperberg, M. J. (2002) Lead distribution in corn seedlings (*Zea mays* L.) and its effect on growth and the concentrations of potassium and calcium. *Plant Growth Regul*. **37**: 69-76.

Markowitz, S. B., Nenez, C. M., Klitzman, S. (1994) Lead poisoning due to hai ge fen: the porphyryn content of individual erythrocytes. *J. Am..*erican *Med.*ical *Assoc*iation. **271**: 932- 934.

Marschner, H. (1983). General introduction to the mineral nutrition of plants. In: A. Lauchli and R.L. Bieleski (Eds.). *Encyclopedia of Plant Physiology (New Series*). ***Vol. 15 A*** *Inorganic Plant Nutrition vol. 15 A.*. Springer Verlag, New York. 5-60.

Marschner, H. (1986) *Mineral Nutrition of Higher Plants*. Academic Press. England.

Matysik, J., B. Bhalu, and P. Mohanty, P. (–2002): Molecular mechanisms of quenching of reactive oxygen species by proline under stress in plants. *Curr. Sci.* **82**:, 525–532.

Mazen, A. M. A., and Maghraby, O. M. O. (1997) Accumulation of cadmium, lead, strontium and role of calcium oxalate in water hycianth tolerance. *Biol. Plant*. **40**: 411-417.

McGrath, S. P. (1982) The uptake and translocation of tri- and hexavalent chromium and effects on the growth of oat in flowing nutrient solution and in soil. *New Phytol*. **92**: 381-390.

McGrath, S. P. and Zhao, F. J. (2003) Phytoextraction of metals and metalloids from contaminated soils. *Curr. Opin. Biotechnol*. **14**: 1-6.

McGrath, S. P., Shen, Z. G. and Zhao, F. J. (1997) Heavy metal uptake and chemical changes in the rhizosphere of *Thlaspi caerulescens* and *Thlaspi ochroleucum* grown in contaminated soils. *Plant Soil* **188**: 153- 159.

McGrath, S.P. (1982) The uptake and translocation of tri- and hexavalent chromium and effects on the growth of oat in flowing nutrient solution and in soil. *New Phytol*. **92**: 381-390.

McNeilly, T. (1994) Metal toxicity. In: A. R. Yeo and T. J. Flowers (Eds.). *Soil Mineral Stresses*: *Approaches to Crop Improvement*. Springer- Verlag, New York, 145-174.

Meers, E., Vaudecasteele, B., Ruttens, A., Vangrousveld, J. and Tack, F. M. G., (2007). Potential of five Willo species (*Salix* spp.) for phytoextraction of heavy metals. *Environ. Exp. Bot.* **60**: 57-68.

Mehrotra, S., Mishra, K. P., Maurya, R., Srimal, G. and Singh, V. K. (2002) Immunomodulation by ethanolic extract of *Boerhavia diffusa* roots. *Int. Immunopharmacol.* **2**: 987- 996.

Meidner, H. and Mansfield, T. A. (1968) *Physiology of Stomata*. McGraw Hill, Loindon.

Meuwly, P., Thibault, P., Schwan, A. L. and Rauser, W. R. (1995) Three families of thiol peptides are induced by Cd in maize. *Plant J.* **7:** 391-400.

Michalak, A. (2006) Phenolic compounds and their antioxidant activity in plants growing under heavy metal stress. (2006) *Polish J. Environ. Stud.* **15**: 523-530.

Michalak, AE. and Wierzbicka, M. (1998) Differences in lead tolerance between *Allium cepa* plants developing from seeds and bulbs. *Plant Soil*. **199**: 251-260.

Migocka, M. and Klobus, G. (2007) The properties of the Mn, Ni and Pb transport operating at plasma membranes of cucumber roots. *Physiol. Plant.* **129:** 578-587.

Minglin, Y., Yuxiu, Z. and Tuanyao, C. (1995) Identification of genes up-regulated in response to Cd exposure in *Brassica juncea*, L. *Gen*. **363**: 151- 158.

Mishra, A. and Choudhuri, M. A. (1999) Monitoring of phytotoxicity of lead and mercury from germination and early seedling growth induces two rice cultivars. *Water Air Soil Pollut.* **114**: 339-346.

Mishra, S., Srivastava, S., Tripathi, R. D., Govinindarajan, R., Kuriakose, S. V. and Prasad, M. N. V. (2006) Phytochelatin synthesis and response of antioxidants during cadmium stress in *Bacopa monnieri* L. *Plant. Physiol. Biochem*. **44**: 25- 37.

Mohan, B. S. and Hosetti, B. B. (1997) Potential phytoxicity of lead and cadmium to *Lemna minor* grown in sewage stabilization ponds. *Environ. Res*. **98**: 233- 238.

Monni, S., Uhlig, C., Hunttila, O., Hausen, E. and Hynynen, J. (2001) Chemical composition and ecophysiological responses of *Eupatorium nigrum* to above ground element application. *Environ. Pollut.* **112:** 417-426.

Moodley, K. G., Baijnath, H., Southway- Ajulu, F. A., Maharaj, S. and Chetty, S. R. (2007) Determination of Cr, Pb and Ni in water, sludge and plants from settling ponds of a sewage treatment works. *Water* SA **33**: 723-728.

Moral, R., Gomes, I., Pedreno, J.N. and Mataix, J. (1996) Absorption of chromium and effects on micronutrient content in tomato plants

(*Lycopersicon esculentum*). *Agrochimica* **11**: 138-145.

Moral, R., Palacios, G., Gomes, I., Pedreo, J. N. and Mataix, J. (1994) Distribution and accumulation of heavy metals (Cd, Ni and Cr) in tomato plant. *Fresnius Environ. Bull*. **3**: 395-399.

Moral, R., Pedreno, J. N., Gomes, I. and Mataix, J. (1995) Effects of chromium on the nutrient element content and morphology of tomato. *J. Plant Nutr*. **18**: 815-822.

Moral, R., Gomes, I., Pedreno, J. N. and Mataix, J. (1996) Absorption of chromium and effects on micronutrient content in tomato plants (*Lycopersicon esculentum*). *Agrochimica.* **11**: 138-145.

Moreno, F. N., Anderson, C.W. N., Stewart, R. B. and Robinson, B. H. (2004) Phytoremediation of mercury- contaminated mine tailings by induced plant- mercury accumulation . *Environ. Practice* **6**: 165- 175.

Moreno, F. N., Anderson, C. W. N., Stewart, R. B. and Robinson, B. H. (2005 a) Mercury volatilisation and phytoextraction from base- metal mine tailings. *Environ. Pollut*. **136**: 341- 352.

Moreno, F. N., Anderson, C. W. N., Stewart, R. B., Robinson, B. H. Ghomshei, M. and Meech, J. A. (2005 b) Induced plant uptake and transport of mercury in the presence of sulphur containing ligands and humic acid. *New Phytol*. **166**: 445-454.

Moreno, F. N., Anderson, C. W. N., Stewart, R. B., Robinson, B. H., Nomura, R., Ghomshei, M. and Meech, J. A. (2005 c) Effect of thio ligłands on plant-Hg accumulation and volatilisation from mercury– contaminated mine tailings. *Plant soil* **275**: 233-246.

Moreno, F. N., Anderson, C.W. N., Stewart, R. B. and Robinson, B. H. (2004) Phytoremediation of mercury- contaminated mine tailings by induced plant- mercury accumulation . *Environ. Practice* **6**: 165- 175.

Moreno, F. N., Anderson, C. W. N., Stewart, R. B. and Robinson, B. H. (2008) Phytofiltration of mercury – contaminated water: Volatilisation and plant – accumulation aspects. *Environ. Exp. Bot.* **62**: 78-85.

Moreno-Caselles, J., Moral, R., Perez- Espinosa, A. and Perez-Murcia, M. D. (2000) Cadmium accumulation and distribution in cucumber plant. *J. Plant Nutri.* , **2**: 29-34.

Morgan, J. F., Klucas, R. V., Grayer, R. J., Abian, J. and Becana, M. (1997) Complexes of iron with phenolic compounds from soybean nodules and other legume tissues: prooxidant and antioxidant properties. *Free Radic. Biol. Med.* **22**: 861.

Mukhopadhyay, N. and Aery, N. C. (2000) Effect of Cr (III) and Cr (VI) on the growth and physiology of *Triticum aestivum* plants during early seedlings growth . *Biologia*. **55**: 403- 408.

Mysliwa-kurdziel, B. and Strzalka, K. (2002) Influence of metals on biosynthesis of photosynthetic pigments. In: M. N. V. Prasad and K.

Strzalka (Eds.). *Physiology* and *Biochemistry of Metal Toxicity* and *Tolerance in Plants.* Kluwer Academic. The Netherlands. 201-227.

Nag, P., Paul, A. K and Mukherji, S. (1980) Effects of mercury, copper and zinc on the growth, cell division, GA. induced amylase synthesis and membrane permeability of plant tissues. *Indian J. Exp. Biol*. **18**: 822-827.

Nagajyoti, P. C., Dinakar, N., Prasad, T. N., V. K. V., Suresh, C. and Damodharam, T. (2008) Heavy metal toxicity: Industrial effluent effect on groundnut (*Arachis hypogaea* L.) seedlings. *J. Appl. Sci.ences Res.earch.* **4**: 110-121.

Nath, S., Chaudhury, J., Pahan, K. and Mandal, A. (1993) Mercury and organo mercurial resistance in rhizobial strains. *Indian J. Exp. Biol*. **31:** 50-53.

Noctor, G. and , Foyer, C. H., (1998) Ascorbate and glutathione: Keeping active oxygen under control. *Annu. Rev. Plant. Physiol. Plant. Mol. Biol.* **49**: 249-279.

Obata, H., Inoue, N., Imai, K. and Umebayashi, M. (1994) Cadmium tolerance of calli induced from roots of plants with difference in cadmium tolerance. *Soil Sci. Plant Nutr*. **40**: 351-354.

Olujohungbe, A., Fields, P. A. and , Sandford, A. F. , (1994) Heavy metal intoxication from homeopathic and herbal remedies. *Postgrad.*uate *Med*ical. Journal . **70**: 764- 769.

Oncel, I. Keles, Y. and Ustun, A. S. (2000) Interactive effects of temperature and heavy metal stress on the growth and some biochemical compounds in wheat seedlings. *Environ. Pollut.* **107**: 315- 320.

Orcutt, D. M. and Nilsen, E. T. (2000) *Physiology of Plants Under Stress: Soil and Biotic Factors*. John Wiley & Sons, Inc. New York.

Ouariti, O., Boussama, N., Zarrouk, M., Cherif, A. and Ghorbal, M. H. (1997) Cadmium and copper induced changes in tomato membrane lipids. *Phytochem.istry* **45**: 1343-1350.

Ow, D. W. (1996) Heavy metal tolerance genes: prospective tools for bioremediation. *Resour.*ces, *Conserv.*ation *Recycl*ing**. 18**: 135- 149.

Pance, N. S., Larsen, P. B., Ebbs, S. D., Letham, D. L., Lasat, M. M., Garvin, D. F., Eide, D. and Kochian, L. V. (2000) The molecular physiology of heavy metal transport in the Zn/Cd hyperaccumulator *Thlaspi caerulescens*. *Proc. Natl. Acad. Sci.* U.S.A. **97:** 4956-4960.

Panda, S. K. (2003) Heavy metal phytotoxicity induces oxidative stress in *Taxithelium* sp. *Curr. Sci.* **84**: 631-633.

Panda, S. K., (2007) Chromium – mediated oxidative stress and ultra structural changes in root cells of developing rice seedlings. *Plant. Physiol.* **164**: 1419-1428.

Panda, S. K. and Patra, H. K. (1997) Physiology of chromium toxicity in plants. A r~~R~~eview. *Plant Physiol. Biochem.* **24**: 10-17.

Panda, S. K. and Patra, H. K. (1998) Alteration of nitrate reductase activity by chromium ions in excised wheat leaves. *Ind. J. Angio. Biochem*. **2**: 56-57.

Panda, S. K. and Patra, H. K. (2000) Does Cr. (III) produce oxidative damage in excised wheat leaves. *J. Plant. Biol*. **27**: 105-110.

Panda, S.K. (2003) Heavy metal phytotoxicity induces oxidative stress in *Taxithelium* sp. *Curr. Sci.* **84**: 631-633.

Panda, S. K. and Choudhury, S. (2005) Chromium stress in plants. *Braz. J. Plant Physiol*. **17**: 95-102.

Panda, S.K. and Patra, H.K. (1997) Physiology of chromium toxicity in plants. A Review. *Plant Physiol. Biochem*. **24**: 10-17.

Panda, S.K. and Patra, H.K. (1998) Alteration of nitrate reductase activity by chromium ions in excised wheat leaves. *Ind. J. Angio. Biochem*. **2**: 56-57.

Panda, S.K. and Patra, H.K. (2000) Does Cr. (III) produce oxidative damage in excised wheat leaves. *J. Plant. Biol*. **27**: 105-110.

Panda, S. K., Chaudhury, L. and Khan, M. H. (2003) Heavy metals induce lipid peroxidation and affects antioxidants in wheat leaves. *Biol. Plant*. **46**: 289-294.

Parekh, D., Puranik, R. M. and Srivastva, H. S. (1990) Inhibition of chlorophyll biosynthesis by cadmium in greening maize leaf segments. *Biochem.*

Physiol. Pflanzen. **186**: 239- 242.

Pari, L. and Satheesh, A. M. (2004) Antidiabetic activity of *Boerhavia diffusa* L. effect on hepatic key enzymes in experimental diabetes *J. Ethnopharmacol*. **9**: 109- 113.

Parmar, N. G. and Chanda, S. V. (2005) Effects of mercury and chromium on peroxidase and IAA oxidase enzymes in the seedlings of *Phaseolus vulgaris*. *Tur. J . Biol.* **29***:* 15-21.

Parmer, N. G. Vithalani, S. D. and Chanda, S. V. (2002) Alteration in growth and peroxidase activity by heavy metals in *Phaseolus* seedlings. *Acta Physiol. Plant*. **24**: 89-95.

Peralta, J. R., Gardea-Torresdey, J. L., Tiemann, E., Gomez, S., Arteaga, E., Rascon, and Parsons, J. G. (2000) Study of the effects of heavy metals on seed germination and plant growth on alfalfa plant (*Medicago sativa*) grown in solid media. *Proceedings of the conference on hazardous waste research*. 135- 140.

Peralta, J. R., Gardea Torresdey, J. L., Tiemann, K. J., Arteaga, S. and Rascon, E. (2001) Uptake and effects of five heavy metals on seed germination and plant growth in alfalfa (*Medicago sativa* L.). *Bull. Environ. Contam. Toxicol*. **66**: 727-734.

Perfus–Barbeoch, L., Leonhardt, N., Vavasseur, A. and Forestier, C. (2002) Heavy metal toxicity: Cadmium permeates through calcium channels and

disturbs the plant water status. *The Plant J.* **32:** 539-548.

Petr, J., Skerik, J. and Dlouby, J. (1999) Cadmium, lead and mercury contents in ecologically grown crops. *Sci. Agri. Bohemica* **30:** 285-299.

Pilon-Smits, E. (2005) Phytoremediation. *Annu. Rev. Plant Biol.* **56:** 15-39.

Pittman, J. K., Shigaki, T. and Hirschi, K. D. (2005) Evidence of differential pH regulation of the *Arabidopsis* vacuolar Ca^{2+}/H^{+} antiporters CAX1 and CAX 2. *FEBS Lett*. **579**: 2648- 2656.

Podar, D., Ramsey, M. H. and Hutchings, M. J. (2004) Effect of cadmium, zinc and substrate heterogeneity on yield, shoot metal concentration and metal uptake by *Brassica juncea*: Implications for human health risk assessment and phytoremediation. *New Phytol*. **163**: 313-324.

Poschenrieder, C., Vazquez, M. D., Bonet, A. and Barcelo, J. (1991) Chromium III iron interaction in iron sufficient and iron deficient bean plants. II. Ultra structural aspects. *J. Plant Nutr*. **14**: 415-428.

Posmyk, M. M., Kontek, R. and Janas, K. M. (2009) Antioxidant enzymes activity and phenolic compounds content in red cabbage seedlings exposed to copper stress. *Ecotoxicol. Environ. Saf.* **72**: 596-602.

Prasad, M. N. V. (1995) Inhibition of maize leaf chlorophylls, carotenoides and gas exchange functions by cadmium. *Photosynthetica* **31**: 635-640.

Prasad, M. N. V. (1997) Trace metals. In: M. N. V. Prasad (Ed.). *Plant Ecophysiology*. John Wiley & Sons, Inc. 207-249.

Prasad, M. N. V. (1998) Metal-biomolecule complexes in plants: occurrence, functions and applications. *Analysis*. 26-28.

Prasad, D. D. K. and Prasad, A. R. K. (1987) Effect of lead and mercury on chlorophyll synthesis in mung bean seedlings. *Phytochem.* **26:** 881-883.

Prasad, M.N.V. (1995) Inhibition of maize leaf chlorophylls, carotenoides and gas exchange functions by cadmium. *Photosynthetica* **31**: 635-640.

Prasad, M.N.V. (1997) Trace metals. In: M.N.V. Prasad (Ed.). *Plant Ecophysiology*. John Wiley & Sons, Inc. 207-249.

Prasad, M.N.V. (1998) Metal-biomolecule complexes in plants: occurrence, functions and applications. *Analysis*. 26-28.

Prasad, S. M., Singh, J. B., Rai, L. C. and Kumar, H. D. (1991) Metal induced inhibition of photosynthetic electron transport chain of cyanobacterium, *Nostoc muscarum*. *FEMS Microbiol. Lett*. **82:** 95-100.

Pratima, N. P., Paul, A. K. and Mukherji, S. (1989) The effects of heavy metals, Zn and Hg on the growth and biochemical constituents of mung bean (*Vigna radiata*) seedlings. *Bot. Bull. Acad. Sin.* (Taipei) **30**: 241-250.

Pulford, I. D., Watson, C. and McGregor, S. D. (2001) Uptake of chromium by trees: prospects for phytoremediation. *Environ. Geochem. Health* **23**: 307-311.

Punz, W. F. and Sieghardt, H. (1993) The response of roots of herbaceous plant species to heavy metals. *Environ. Exp. Bot.* **33**: 85-98.

Radiotic, K., Ducic, T. and Mutavdzic, D. (2000) Changes in peroxidase activity and isoenzymes in spruce needles after exposure to different concentration of cadmium. *Environ. Exp*. **44**: 105- 113.

Rai, U. N., Tripathi, R. D., Sinha, S. and Chandra, P. (1995) Chromium and cadmium bioaccumulation and toxicity in *Hydrilla verticillata* (L. f.) Royle and *Chara coralline* Wildenow. *J. Environ. Sci. Health* Part- A **30**: 537- 551.

Rai, U. N., Tripathi, R. D., Vajpayee, P., Pandey, N., Ali, M. B. and Gupta, D. K. (2003) Cadmium accumulation and its phytotoxicity in *Potamogeton pectinatus* L. (Potamogetonaceae). *Bull. Environ. Contam. Toxicol*. **70:** 566-575.

Rai, V., Vajpayee, P., Singh, S. N. and Mehrotra, S. (2004) Effect of chromium accumulation on photosynthetic pigments, oxidative stress defense system, nitrate reduction, proline level and eugenol content of *Ocimum tenuiiflorum* L. *Plant Sci.* **167**: 1159- 1169.

Ramabhimaiah, S., Stalin, D. and Kalaskar, N. J (1984) Pharmacological investigations of the water soluble fraction of the methanol extracts of *Boerhavia diffusa* roots. *Indian Drugs* **21**: 343- 344.

Rasico, N., Veechia, F.T., Ferretti, M., Merlow, L. and Ghisi, R. (1993) Some effects of cadmium on maize. *Plant Arch. Environ. Contam. Toxicol*. **25**: 244-249.

Raskin, I. and Ensley, B. D. (2000) *Phytoremediation of Toxic Metals Using Plants to Clean up the Environment.* John Wiley & Sons. Inc. New York.

Raskin, I., Kumar, N. P., ~~B. A.,~~ Dushenkov, S. and Salt, D. E. (1994) Bioconcentration of heavy metals by plants. *Curr. Opin. Biotechnol.* **5**: 285-290.

Ratheesh--Chandra, P., Abdussalam, A. K., Nabeesa- Salim, N. and Puthur, J. T. (2010) Distribution of bio-accumulated Cd and Cr in two *Vigna* species and the associated histological variations. *Stress physiol.ogy & Biochem.istry* **6**: 4-12.

Rauser, W. E. (1987) Changes in glutathione content of maize seedlings exposed to cadmium. *Plant Sci.* **51:** 171-175.

Rauser, W. E. (1990) Phytochelatins. *Annu Rev. Biochem*. **59**: 61-86.

Rauser, W. E. (1995) Phytochelatins and related peptides: Structure, biosynthesis and function. *Plant Physiol*. **109**: 1141-1149.

Rauser, W.E. (1987) Changes in glutathione content of maize seedlings exposed to cadmium. *Plant Sci.* **51:** 171-175.

Rawat, A. K. S., Mehrotra, S., Tripathi, S. K., and Sharma, U. (1997) Hepatoprotective activity in Punarnava - a popular Indian ethnomedicine. *J. Ethnopharmacol.ogy*. **56**: 61- 68.

Reddy, G. N. and Prasad, M. N. V. (1990) Characterization of cadmium binding protein from *Scenedesmus quadricauda* and Cd toxicity reversal by phytochelatin consisting amino acids and citrates *J. Plant Physiol.* **140**: 156- 162.

Reddy, G. N. and Prasad, M. N. V. (1990) Heavy metal binding protein/ peptides: Occurrence, structure,synthesis and functions- A review. *Environ. Exp. Bot.* **30**: 251- 264.

Reddy, G. N. and Prasad, M. N. V. (1992) Cadmium induced peroxidase activity and isozymes in *Oryza sativa*. *Biochem. Archives **8**:* 101- 106.

Reed, R. H. and Gadd, G. M. (1990) Metal tolerance in eukaryotic and prokaryotic algae. In: A. J. Shaw (Ed.). *Heavy Metal Tolerance in Plants*: *Evolutionary Aspects*. CRC Press, Florida. 105-118.

Reid, R. J., Dunbar, K. R. and McLaughlin, M. J. (2003) Cadmium loading into potato tubers: the roles of the periderm, xylem and phloem. *Plant Cell Environ.* **26:** 201-206.

Rice-Evans, C. A., Miller, N. J. and Paganga, G. (1997) Antioxidant properties of phenolic compounds. *Trends Plant Sci*. **2**: 152.

Riedel, G. F. (1985) The relationship between chromium (IV) uptakes, sulphate uptake and chromium (IV) toxicity in the estuarine diatom *Thalassionira pseudonama*. *Aquatic Toxicol*. **7**: 191-204.

Ross, H. B. (1994) On the use of mosses (*Hylocomium splendens* and *Pleurozium schreberi*) for estimating atmospheric trace metal deposition. *Water Air Soil Pollut*. **50**: 63-76.

Rout, G. R., Samantaray S. and Das, P. (1997) Different chromium tolerance among eight mung bean cultivars grown in nutrient culture. *J. Plant Nutr*. **20**: 341-347.

Rout, G. R., Sanghamitra, S. and Das, P. (2000) Effect of chromium and nickel on germination and growth in tolerant and non-tolerant populations of *Echinochola colona* (Link.) *Chemosphere* **40**: 855-859.

Sadasivam, S. and Manikam, A. (1992) *Biochemical Methods for Agricultural Sciences*. Wiley Eastern Ltd., New Delhi. and Tamilnadu Agricultural University, Coimbatore.

Sahadevan, K. K. (2001) *Physiological and Bbiochemical Sstudies on mMercury Toxicity in Vigna mungo* (L.) *Hepper seedlings*. Ph. D. Thesis submitted to the University of Calicut.

Sahu, A. N., Damiki, L., Nilanjan, G. and Dubey, S. (2008) Phytopharmacological review of *Boerhavia diffusa* Linn. (Punarnava). *Phcog. Rev*. **2**: 14-22.

Sakihamay, Y. and Yamasaki, H. (2002) Lipid peroxidation induces by phenolics in conjunction with aluminium ions. *Biol. Plant.arum* **45**: 249.

Salati, E., Eneida, S. and Salati, E. (1999) Wetlands projects developed in Brazil. *Water Sci. Technol*. **40**: 19.

Salt, D. E. and Rauser, W. E. (1995) Mg ATP-dependent transport of phytochelatins across the tonoplast of oat roots. *Plant Physiol.* **107:** 1293-1301.

Salt, D. E., Smith, R. D. and Raskin, I. (1998) Phytoremediation. *Annu. Rev. Plant Physiol. Plant Mol. Biol*. **49**: 643-668.

Salt, D.E. and Rauser, W.E. (1995) Mg ATP-dependent transport of phytochelatins across the tonoplast of oat roots. *Plant Physiol.* **107:** 1293-1301.

Samantary, S. and Das, P. (1997) Accumulation and distribution of chromium, nickel and iron in the mung bean plant. *Fresnius Envir. Bull*. **6**: 633-641.

Samantary, S., Rout, G. R. and Das, P. (1996) A study of soil plant and root-shoot relationship in rice (*Oryza sativa* L. cv. Pathan) grown on chromiferous mine soil. *Proc. Natl. Acad. Sci*. USA. **66**: 349-357.

Samantary, S., Rout, G. R. and Das, P. (1998) Role of chromium on plant growth and metabolism. *Acta Physiol. Plant.* **20**: 201-212.

Sandmann, G. and Boger, P. (1983). The enzymological function of heavy metals and their role in electron transfer process of plants. In: A. Lauchli and R. L. Bieleski (Eds.). *Encyclopedia of Plant Physiology*, (New Series) Vol. **15B**. *Inorganic Plant Nutrition*. 563-596.

Sanita-di-Toppi, L. and Gabbrielli, R. (1999) Response to cadmium in higher

plants. *Environ. Exp. Bot.* **41:** 105-130.

Sanità di Toppi, L., Prasad, M. N. V. and Ottonello, S. (2002) Metal chelating peptides and proteins in plants. In: M. N. V. Prasad and K. Strzalka (Eds.) *Physiology and Biochemistry of Heavy Metal Toxicity and Detoxification and Tolerance in Plants*. Kluwer Academic, Publishers, Dordrecht. 59–93.

Sanita-di-Toppi, L. and Gabbrielli, R. (1999) Response to cadmium in higher plants. *Environ. Exp. Bot.* **41:** 105-130.

Saper, R. B., Kales, S. N., Paquin, J. Burns, M. J., Eisenberg, D. M. and Davis, R. B. (2004) Heavy metal content of ayurvedic herbal medicine products. *Jama*, **292**: 2868-2873.

Saradhi, A. and Saradhi, P. P. (1991) Proline accumulation under heavy metal stress. *Plant. Physiol*. **138**: 554- 558.

Satoh, M., Karaki, E., Kakehashi, M., Okazaki, E., Gotoh, T. and Oyama, Y. (1999) Heavy-metal induced changes in non proteinaceous thiol levels and heavy metal binding peptides in *Tetraselmis tetrathele* (Prasinophyceae). *J. Phycol*. **35**: 989-944.

Schafer, H. J., Greiner, S., Rausch, T. and Haag- Kerwer, A. (1997) In seedlings of the heavy metal accumulator *Brassica juncea* Cu^{2+} differentially affects transcript amounts for γ-glutamylcysteine synthetase γ-glutamylcysteine

synthethtase (ɣ-ECS) and metallothionein (MT2) *FEBS. Lett*. **404**: 216-220.

Scheller, H. V., Huang, B., Hatch. E. and Goldsbrough, P. B. (1987) Phytochelatin synthesis and glutathione levels in response to heavy metals in tomato cells. *Plant Physiol*. **85**: 1031-1035.

Sengar, R. S. and M. Pandey, (1996). Inhibition of chlorophyll biosynthesis by lead in greening *Pisum sativum* leaf segments. *Biol. Plant*., **38**: 459- 462

Sergin, I. V. and Ivanov, V. B. (2001) Physiological aspects of cadmium and lead toxic effects on higher plants. *Russ. J. Plant Physiol*. **48**: 523- 544.

Sersen, F., Clik, G., Havranek, E. and Sykorova, M. (2005) Bioremediation by natural zeolite in plants cultivated in a heavy metal contaminated medium. *Fresenius Environ. Bull*. **14**: 13-17.

Setia, R. C. and Bala, R. (1994) Anatomical changes in root and stem of wheat (*Triticum aestivum* L.) in response to different heavy metals. *Phytomorph*. **44**: 95-104.

Shalata, A., Mittova, V., Volokita, M., Guy, M. and Tal, M. (2001) Response of the cultivated tomato and its wild salt-tolerant relative *Lycopersicon pennellii* to salt dependant oxidative stress: the root antioxidative system. *Physiol. Plant*. **112**: 487- 494.

Shankar, N., Garg, S. K. and Srivastava, H. S. (2000) The influence of low nutrient pH on nitrate assimilation and nitrate reductase activity in maize seedlings *Plant. Physiol.* **156**: 678- 683.

Shanker, A. K., Sudhagar, R., Pathmanabhan, G. (2003) Growth, Phytochelatin SH and antioxdative response of Sunflower as affected by chromium speciation. 2nd International Congress of Plant Physiology on Sustainable Plant Productivity under Changing Environment. New Delhi. India.

Shanker, A. K., Djnaguiraman, M., Sudhagar, R., Chandrashekar, C. N. and Pathmanabhan, G. (2004) Differential antioxidative response of ascorbate glutathione pathway enzymes and metabolites to chromium speciation stress in green gram (*Vigna radiata* (L.) R. Wilczek. cv CO roots. *Plant Sci.* **166**: 1035-1043.

Shanker, A.K., Cervantes, C., Loza-Tavera, H. and Avudainayagam, S. (2005) Chromium toxicity in plants. *Environ. Intl*. **31**: 739-753.

Shanker, A.K., Djnaguiraman, M., Sudhagar, R., Chandrashekar, C.N. and Pathmanabhan, G. (2004) Differential antioxidative response of ascorbate glutathione pathway enzymes and metabolites to chromium speciation stress in green gram (*Vigna radiata* (L.) R. Wilczek. cv CO 4) roots. *Plant Sci.* **166**: 1035-1043.Shaw, B. P. (1995) Effect of mercury and cadmium on the activities of antioxidative enzymes in the seedlings of *Phaseolus aureus*. *Biol. Plant*. **37**: 587-596.

Shaw, B. P. and Rout, N. P. (1998) Age-dependent responses of *Phaseolus aureus* Roxb. to inorganic salts of mercury and cadmium. *Acta Physiol. Plant*. **20**: 85-90.

Shaw, B.P. (1995) Effect of mercury and cadmium on the activities of antioxidative enzymes in the seedlings *of Phaseolus aureus*. *Biol. Plant*. **37**: 587-596.

Shehu, A., Mullaj, A., Harizaj, F. and Shehu, J. (2010) Assessment of hHeavy metals accumulation by different spontaneous plant species- Grown along Lana River, Albinia. *Balwois Orchid*, *Republic* of *Macedoina* 25- 29.

Shen, Z. G., Zhao, F. J. and McGrath, S. P. (1997) Uptake and transport of zinc in the hyperaccumulator *Thlaspi caerulescens* and the nonhyperaccumulator of *Thlaspi ochroleucum*. *Plant Cell Environ*. **20**: 898- 906.

Sheoran, I. S., Aggarwal, H. and Singh, R. (1990) Effect of cadmium and nickel on *in vivo* carbon dioxide exchange rate of pigeon pea. *Plant Soil* **129:** 243-249.

Shinmachi, F., Kumanda, Y., Noguchi, A. and Hasegawa, I. (2003) Translocation and accumulation of cadmium in cadmium tolerant *Polygonum thunberjgii*. *Soil Sci. Plant Nutr*. **49**: 355- 361.

Shu, W. S., Ye, Z. H., Lan, C. Y., Zhang, Z. Q. and Wong, M. H. (2002) Lead, zinc and copper accumulation and tolerance in populations of *Paspalum distichum* and *Cynodesmon dactylon. Environ. Pollut*. **120:** 445-453.

Siedlecka, A. and Krupa, Z. (1997) Cd/Fe interaction in higher plants- its consequences for the photosynthetic apparatus. *Photosynthetica* **36**: 321-331.

Siedlecka, A. and Krupa, Z. (2002) Functions of enzymes in heavy metal treated plants. In: M.N.V. Prasad and M. N.V., Kazimierz, S.K. Strzalka (Eds.) *Physiology and Biochemistry of Metal Toxicity and Tolerance in Plants*. Kluwer, Academic, Dordrecht.*Netherlands*, 314- 317.

~~Siedlecka, A. and Krupa, Z. (1997) Cd/Fe interaction in higher plants- its consequences for the photosynthetic apparatus. *Photosynthetica* **36**: 321-331.~~

Singh, A. K. (2001) Effect of trivalent and hexavalent chromium on spinach (*Spinacei*a *oleracea* L.) *Environ. Ecol*. **19**: 807-810.

Singh, R. H. and Udupa, K. N. (1972) Studies on the Indian indigenous drug punarnava (*Boerhavia diffusa* Linn.) Part IV: Preliminary controlled clinical trial in nephritic syndrome. *Res. Indian Med.* **7**: 28- 33.

Singh, V. and Pandey, R. P. (1980) Medicinal plantlore of the tribals of eastern Rajasthan. *J. Econ. Taxon. Bot.* 137- 147.

Singh, K. P., Mohan, D., Sinha, S. and Dalwani, R. (2004) Impact assessment of treated/ untreated wastewater toxicants discharged by sewage treatment plants on health, agricultural and environmental quality in the wastewater disposal area. *Chemosphere* **55**: 227-255.

Singh, R. H. and Udupa, K. N. (1972) Studies on the Indian indigenous drug

punarnava (*Boerhavia diffusa* Linn.) Part IV: Preliminary controlled clinical trial in nephritic syndrome. *Res. Indian Med.* **7**: 28- 33.

Singh, S., Seaxena, R., Pandey, K., Bhatt, K. and , Singha, S. (2004) Response of antioxidant in sunflower (*Helianthus annuus* L.) grown on different amendments of tannery sludge: its metal accumulation potential. *Chemosphere* **57**: 1663- 1673.

Singh, V. and Pandey, R. P. (1980) Medicinal plantlore of the tribals of eastern Rajasthan. *J. Econ. Taxon. Bot.* 137- 147.

Sinha, S. (1999) Accumulation of Cu, Cd, Cr, Mn, and Pb from artificially contaminated by soil *Bacopa monnieri*. *Environ. Monit. Assess*. **57**: 253-264.

Sinha, S. and Saxena, R., (2006) Effect of iron on lipid peroxidation and enzymatic and non-enzymatic antioxidant and bacoside content in medicinal plant *Baccopa monnieri* L. *Chemosphere* **62**: 1340- 1350.

Sinha, S., Gupta, M. and Chandra, P. (1996) Bioaccumulation and biochemical effect of mercury in the plant *Bacopa monnieri* (L.) *Environ. Toxicol. Wat. Qual*. **11**: 105-112.

Sinha, S., Saxena, R., (2006) Effect of iron on lipid peroxidation and enzymatic and non – enzymatic antioxidant and bacoside content in medicinal plant *Baccopa monnieri* L. Chemosphere **62**: 1340- 1350.

Sivarajan, V. V. and Balachandran, I. (1994) *Ayurvedic Drugs and Their Plant Sources*. Oxford and IBH Publishing Co., New Delhi.

Skinner, K., Wright, N. and Porter-Goff, E. (2007) Mercury uptake and accumulation by four species of aquatic plants. *Environ. Pollut*. **145**: 234-237.

Smeets, K. Cuypers, A., Lambrechts, A., Semane, B., Hoet, P., Van Lare, A. and Vangronsveld, J. (2005) Induction of oxidative stress and antioxidative mechanism in *Phaseolus vulgaris* after Cd application. *Plant. Physiol. Biochem*. **43**: 437- 444.

Song, W. Y., Martinoia, E., Lee, Y., Kim, D., Kim, D. Y., Vogt, E., Shim, D., Choi, K.S., Hwang, I. and Lee, Y. (2004) A novel family of Cys- rich membrane proteins mediates cadmium resistance in *Arabidopsis*. *Plant Physiol*. **135**: 1027- 1039.

Sonmez, O., Bukun, B., Kaya, C. and Aydemir, S. (2008) The assessment of tolerance to heavy metals (Cd, Pb and Zn) their accumulation in three weed species. *Pak. J. Bot*. **40**: 747-754.

Srivastava, H. S. (1974) In vivo nitrate reductase activity in maize seedlings *Indian . Biochem. Biophys*. **11**: 230- 232.

Srivastava, M. M., Juneja, A., Das, S., Srivastava, R., Srivastava, S., and Mishra, S. (1994) Studies on the uptake of trivalent and hexavalent chromium by onion (*Allium cepa*). *Chem. Speciat. Bioavailab*. **6**: 27- 30.

Stefanov, K. L., Popova, I., Kamburova, E., Pancheva., Kimenov, G., Kuleva, L. and Popv, S. (1993) Lipid and sterol changes in *Zea mays* caused by lead ions. *Phytochem.istry*. **33**: 47-51.

Steffens, J. C. (1990) The heavy metal binding peptides of plants. *Annu. Rev. Plant Physiol. Plant Mol. Biol*. **41**: 533-575.

Stobart, A. K., Griffiths, W. T., Ameen- Bukhari, I. and Sherwood, R. P. (1985) The effect of Cd^{2+} on the biosynthesis of chlorophyll in leaves of barley. *Physiol. Plant*. **63**: 293-298.

Stohs, S. J. and Bagchi, D. (1995) Oxidative mechanisms in the toxicity of metal ions. *Free Radicals Biol. Med.* **18:** 321-336**.**

Stolt, J. P., Sneller, F. E. C., Bryngelsson, T., Lundborg, T. and Schat, H. (2003) Phytochelatin and cadmium accumulation in wheat. *Environ. Exp. Bot*. **49:** 21-28.

Stolt, P., Asp, H. and Hultin, S. (2006) Genetic variation in wheat cadmium accumulation on soils with different cadmium concentrations. *J. Agron. Crop Sci.* **192**: 201-208.

Stroindski, A. (1999) Some physiological and Biochemical aspects of plant resistance to cadmium effect. Aantioxidative system *Acta. Physiol. Plant*. **21**: 175- 188.

Stroinski, A. (1999) Some physiological and biochemical aspects of plant resistance to cadmium effect. I: antioxidative system. *Acta Physiol.*

Plant. **21:** 175-188.

Stroinski, A.; and Zielezinska, M. (2001) Cadmium and oxidative stress influence on phytochelatin synthase activity in potato tuber *Acta. Physiol. Plant*. **23**: 157-160.

Sun, Q., Wang, X. R., Ding, S. M. and Yuan, X. F. (2005) Effects of interactions between cadmium and zinc on phytochelatin and glutathione production in wheat (*Triticum aestivum* L.) *Environ. Toxicol.* **20**: 195- 201.

Sun, Y. B., Zhou, Q. X., Wang, L. and Liu W. T. (2008) Cadmium tolerance and accumulation characteristics of *Bidens pilosa* L. as a potential Cd-hyperaccumulator. *Hazard. Mater **161**:* 808- 814.

Sun, Q., Wang, X. R., Ding, S. M. and Yuan, X. F. (2005) Effects of interactions between cadmium and zinc on phytochelatin and glutathione production in wheat (*Triticum aestivum* L.) *Environ. Toxicol.* **20**: 195- 201.

Sun, Y. B., Zhou, X. Q. and Ren, L. P.(2007) Growth responses of *Rorippa globosa* and its accumulation characteristics of Cd and As under the Cd-as combined pollution, *Environ. Sci*. **28**: 1355- 1360.

~~Sun, Y. B., Zhou, Q. X., Wang, L. and Liu W. T. (2008) Cadmium tolerance and accumulation characteristics of *Bidens pilosa* L. as a potential Cd-hyperaccumulator. *Hazard. Mater. **161**:* 808- 814.~~Sun, Y., Zhou, Q., Wang, L. and Liu, W. (2009) Cadmium tolerance and accumulation

characteristic of *Bidens pilosa* L. as potential Cd- hyperaccumulator. J. *Hazard. Mater.* **161**: 808- 814.

Szollosi, R., Varga, I. S., Erdei, L. and Mihalik, E. (2009) Cadmium –induced oxidative stress and antioxidative mechanisms in germinating Indian mustard (*Brassica juncea* L.) seeds. *Ecotoxicol. Environ. Saf.ety*. **72**: 1337- 1342.

Taiz, L. and Zeiger, E. (2002) *Plant Physiology:* (III[rd] Edn.) Sinauer Associates, Inc. Publishers, Sunderland, Massachussetts.

Tanaka, K., Fujimaki, S., Fujiwara, T., Yoneyama, T. and Hayashi, H. (2007) Quantitative estimation of the contribution of the phloem in cadmium transport to grains in rice plants (*Oryza sativa* L.). *Soil Sci. Plant Nutr.* **53**: 72-77.

Teisseire, H. and V. Guy, V. . (2000). Copper-induced changes in antioxidant enzymes activities in fronds of duckweed (*Lemna minor*). *Plant Sci.*, **153**: 65-72.

Tomar, M., Kaur, I., Neelu, A. and Bhatnagar, A. K. (2000) Effect of enhanced lead in soil on growth and development of *Vigna radiata* (Linn.) Wilczek. *Indian J. Plant Physiol.* **5**: 13-18.

Tomsig, J. L. and Suszkiw, J. B (1991) Permeation of Pb^{2+} through calcium channels: fura -2 measurements of voltage – and dihydropyridine

sensitive Pb^{2+} entry in isolated bovine chromaffin cells. *Biochem. Biophys. Acta.* **1069**: 197-200.

Tong, S., von Schirnding, Y. E. and , Prpaamontol, T. (2000) Environmental lead exposure: a public problem of global dimension. *Bull.*etin *of the World Health Org.*anization **78**: 1068- 1077.

Tripathi, A. K., Tripathi, S. and Tripathi, S. (1999) Changes in some physiological and biochemical characters in *Albezia lebbeck* as bio-indicators of heavy metal toxicity. *J. Environ. Biol.* **20**: 93-98.

Tukendorf, A. and Rauser, W. E. (1990) Changes in glutathione and phytochelation in roots of maize seedlings exposed to cadmium. *Plant Sci*. **70**: 155-166.

Turner, A. P. (1994) The responses of plants to heavy metals: In: S. M. Ross (Ed.). *Toxic Metals in Soil- Plant Systems*. John Wiley & Sons Ltd., 154-187.

Turner, A. P. and Dickinson, N. M. (1993) Survival of *Acer pseudoplatanus* L. (Sycamore) seedling on metalliferous soils. *New Phytol*. **123**: 509-521.

Vajpayee P., Tripathi, R. D., Rai. U. N., Ali, M. B., Singh, S. N. (2000) Chromium (VI) accumulation reduces chlorophyll biosynthesis, nitrate reductase activity and protein content in *Nymphaea alba* L. *Chemosphere* **41**: 1075- 1082.

Vajpayee, P., Sharma, S. C., Tripathi, R. D., Rai, U. N., Yunus, M. (1999) Bioaccumulation of chromium and toxicity to photosynthetic pigments, nitrate reductase activity and protein content of *Nelumbo nucifera* Gaertin. *Chemosphere* **39**: 2159- 2169.

Vajpayee P., Tripathi, R. D., Rai. U. N., Ali, M. B. and Singh, S. N. (2000) Chromium (VI) accumulation reduces chlorophyll biosynthesis, nitrate reductase activity and protein content in *Nymphaea alba* L. *Chemosphere* **41**: 1075- 1082.

Vallee, B. L. and Ulmer, D. D. (1972) Biochemical effects of mercury, cadmium and lead. *Annu. Rev. Biochem.* **41**: 91- 128.

Van Assche, F. and Clijsters, H. (1990) Effect of metals on enzyme activity in plants. *Plant Cell Environ.* **13**: 195-206.

Varga, A., Martinez, R. M. G., Zaray, G. and Fodor, F. (1999) Investigation of effects of cadmium, lead, nickel and vanadium contamination on the uptake and transport process in cucumber plants by TXRF spectrometry. *Spectrochiem. Act.* Part *B.* **54**: 1455-1462.

Vazques, M. D., Poschenrieder, C. H. and Barcelo, J. (1987) Chromium (VI) induced structural changes in bush bean plants. *Ann. Bot.* **59**: 427-438.

Vazques, M. D., Poschenrieder, C. H. and Barcelo, J. (1992) Ultra structural effects and localization of low cadmium concentration in bean roots. *New*

Phytol. **120**: 215-226.

Velasco- Alinsug, M. P., Rivero, G. C. and Quibuyen, T. A. O. (2005) Isolation of mercury-binding peptides in vegetative parts of *Chromolaena odorata*. Z. *Naturforsch*. **60**: 252-259.

Verkleij, J. A. C. and Schat, H. (1990) Mechanisms of metal tolerance in higher plants. In : A. J. Shaw (Ed.) *Heavy Metal Tolerance in Plants*: *Evolutionary Aspects*. CRC Press, Florida. 179-193.

Wagner, G. J. (1993) Accumulation of cadmium in crop plants and its consequences to human health *Adv*. *Agron*. **51**: 173- 212.

Wang, Q., Cui, Y. and Dong, Y. (2002) Phytoremediation of polluted waters: potentials and prospects of wetland plants. *Acta Biotechnol*. **22**: 199- 208.

Wang, Z., Zhang, Y., Huang, Z. and Huang, L. (2008) Antioxidative response of metal accumulator and non – accumulator plants under cadmium stress. *Plant Soil*. **310**: 137- 149.

Weber, M., Trampczynska, A. and Clemens, S. (2006) Comparative transcription analysis of toxic metal responses in *Arabidopsis thaliana* and the Cd^{2+} hyper tolerant facultative metallophyte *Arabidopsis halleri*. *Plant Cell Environ*. **29**: 950- 963.

Wei, C. Y., Sun, X., Wang, C., and Wang, W. Y. (2006) Factors influencing arsenic accumulation by *Pteris vittata*: A comparative field study at two sites. *Environ*. *Pollu*. **141**: 488- 493.

Weis, J. S. and Weis, P., (2004) Review of metal uptake, transport and release by wetland plants: implications for phytoremediation and restoration. *Environ. Int.ernational*. **30**: 685- 700.

Wenzel, W. W. and Jockwer, F. (1999) Accumulation of heavy metals in plants growing on mineralized soils of the Austrian Alps. *Environ. Pollut*. **104**: 145- 155.

WHO, (1999) Monographs on selected medicinal plants. Vol. 1. *World Health Organization*. Geneva.

WHO, (2002) Drug Information. Herbal Medicines. **16**: *World Health Organization*, Geneva.

WHO, (2003) Elementary mercury and inorganic mercury compounds: Human Health Aspects. Concise International Chemical Assessment Document. **50**: *World Health Organization* Geneva.

WHO, Contaminants, In Codex Alimentarius (1984) XVII, Edn. 1. FAO/WHO. Codex Alimentarius Commission, Rome.

Wierzbicka, M. (1998) Lead in the apoplast of *Allium cepa* L. root tips- ultra structural studies. *Plant Sci*. **133**: 105-119.

Wilkins, (1978) The measurement of tolerance to edaphic factors by means of root growth. *New Phytol*. **80**: 623-633.

Winkel-Shirley, B. (2002) Biosynthesis of flavonoids and effects of stress. *Curr. Opin. Plant Biol*. **5**: 218-223.

Wojcik, M. and Tukendorf, A. (1999) Cadmium tolerance of maize, rye and wheat seedlings. *Acta Physiol. Plant.* **21**: 99-107.

Wong, M. H. and Bradshaw, A. W. (1982) A comparison of the toxicity of heavy metals, using root elongation of rye grass, *Lolium perenne*. *New Phytol*. **91**: 255- 261.

Woods, P. W., (1999) Herbal healing. *Essence* **30**: 42- 46.

Woolhouse, H. W. (1983) Toxicity and tolerance in the response of plants to metal. In: O. L. Lange, P. S. Nobel, C. B. Osmond and H. Ziegler (Eds.). *Encyclopedia of Plant Physiology (New Series)* **12C** *Physiological Plant Ecology* III **12C**. Springer-Verlag, New York. **12C**: 245-302.

Wozny, A. and Jerczynska, E. (1991) The effect of lead on early stages of *Phaseolus vulgaris* L. growth *in vitro* conditions. *Biol. Plant*. **33**: 32-39.

Wozny, A., Stroinski, J. and Gwozdz, E. A. (1990) Plant cell responses to cadmium. *Seria Biol.ogia* 44 *Adam Mickiewicz University, Poznam*. **44:** 801-816

Wu, F. and Zhang, G. (2002) Alleviation of cadmium toxicity by application of zinc and ascorbic acid in barley. *J. Plant. Nutr*. **25**: 2745-2761.

Xiong, Z. T. (1998) Heavy metal contamination of urban soils and plants in relation to traffic in Wuhan City, China, *Toxicol. Environ. Chem*., **65**: 31-39.

Yadav, S., Sukla, O.P. and Rai, U. N. (2005) Chromium pollution and bioremediation. *Environ. News Arch.ives* **11:** 1-4.

Ye, Z. H., Baker, A. J. M., Wong, M. H. and Willis, A. J. (1997) Zinc, lead and cadmium tolerance, uptake and accumulation by *Typha latifolia*. *New Phytol*. **136**: 469-480.

Yoon, J., Cao, X., Zhou, Q. and Ma, L. Q. (2006) Accumulation of Pb, Cu and Zn in native plants growing on a contaminated Florida site. *Sci. Total Environ*. **368**: 456-464.

Zayed, A., Gowthaman, S. and Terry N. (1998) Phytoaccumulation of trace elements by Wwetland pPlants: 1 Duckweed. *J. Environ. Qual*. **27**: 715-721.

Zeid, I. M. (2001) Responses of *Phaseolus vulgaris* to chromium and cobalt treatments. *Biol. Plant*. **44**: 111-115.

Zengin and F. K. and Munzuroglu, O. (2005) Effects of some heavy metals on content of chlorophyll, proline and some antioxidant chemicals in bean (*Phaseolus vulgaris* L.) seedlings. *Acta Biol. Cracov. Ser. Bot*. **47**: 157-164.

Zenk, M. H. (1996) Heavy metal detoxification in higher plants–A review. *Gene* . **179:** 21-30.

Zhang, F., Li, X., Wang, C. and Shen, Z. (2000) Effect of cadmium on antioxidant rate of tissue and inducing accumulation of free proline in seedlings of mung bean *Plant Nutr*. **23**: 356- 368.

Zhang, G. P., Fukami, H. and Sekimoto, M. (2002) Influence of cadmium on mineral concentrations and yield components in wheat genotypes differing in Cd tolerance at seedling stage. *Field Crops Res*. **40**: 1–7.

Zhang, F. Q., Wang, Y. S., Lou, Z. P. and Dong, J. D. (2007) Effect of heavy metal stress on antioxidative enzymes and lipid peroxidation in leaves and roots of two mangrove plant seedlings (*Kandelia candel* and *Bruguiera gymnorrhiza*). *Chemosphere* **67**: 44-50.

Zheljazkov, V. D. and Warman, P. R. (2003) Application of high Cu compost to Swiss chard and basil. *Sci. Total Environ*. **302**: 13- 26.

Zheljazkov, V.D., Craker, L. E. and Xing, B. (2006) Effects of Cd, Pb and Cu on growth and essential oil contents in dill, Peppermint and basil. *Environ. Exp. Bot*. **58**: 9-16.

Zhang, F., Li, X., Wang, C. and Shen, Z. (2000) Effect of cadmium on antioxidant rate of tissue and inducing accumulation of free proline in seedlings of mung bean *Plant. Nutr*. **23**: 356- 368.

Zheljazkov, V. D. and , Ekaterina, A., Jeliazkova, A., Kovacheva, N. and Dzhurmanski, A. (2008) Metal uptake by medicinal plant species grown in soil soils contaminated by smelter. *Environ. Exp. Bot*. **64**: 207- 216.

Zheljazkov, V.D. Warman, P. R. (2003) Application of high Cu compost to Swiss chard and basil. *Sci. Total Environ.* **302**: 13- 26.

Zheljazkov, V.D., Craker, L. E. and Xing, B. (2006) Effects of Cd, Pb and Cu on growth and essential oil contents in dill, Peppermint and basil. *Environ. Exp. Bot.* **58**: 9-16.

Zimmels, Y., Kirzhner, F. and Roitman, S. (2004) Use of naturally growing aquatic plants for waste water purification. *Water Environ. Res. Found.* **76**: 220-230.

Zou, J. H., Wang, W. S., Jiang, W. and Liu, D. H. (2006) Effect of hexavalent chromium (VI) on root growth and cell division in root tip cells of *Amaranthus viridis* L. *Pak. J. Bot.* **38**: 673- 681.

Zou, J. H., Xu, P., Lu, X., Jiang, W., and Liu, D. H. (2008) Accumulation of cadmium in three sunflower (*Helianthus annuus* L.) cultivars. *Pak. J. Bot.* **40**: 759-765.

Zurayk, R., Sukkariyah, B. and Baalbaki, R. (2001) Common hydrophytes as bioindicators of nickel, chromium and cadmium pollution. *Water Air Soil Pollut.* **127**: 373- 388.

~~Abdel-Latif, A. (2008) Cadmium induced changes in pigment content, ion uptake, proline content and phosphoenolpyruvate carboxylase activity in *Triticum aestivaum* seedlings. *Australian Journal of Basic and Applied Sciences* **2**: 57- 62.~~

Aslam, M., Davis, S. S. Healy, M. A. (1977) Heavy metals in some Asian medicines and cosmetics. *Public Health*. **93**: 274-284.

Ernst, E. (2005) Contamination of herbal medicines. *Pharmaceut*. **275**: 167.

Abe, T., Fukami, M., Ichizen, N. and Ogasawara, M. (2006) Susceptibility of weed species to cadmium evaluated in a sand culture. *Weed Biol. Manage*. **6**: 107-114.

Adriano, D.C. (1986) *Trace Elements in the Terrestrial Environment*. Springer, Berlin.

Ahmed, A. and Tajmir-Riahi, H.A. (1993) Interaction of toxic metal ions Cd^{2+},Hg^{2+},and Pb^{2+} with light harvesting proteins of chloroplast thylakoid membranes: An FTIR spectroscopic study. *J. Inorg. Biochem*. **50:** 235-243.

Alia, K. V., Prasad, S. K. and Saradhi, P. P., (1995) Effect of zinc on free radicals and proline in *Brassica* and *Cajanus*. *Phytochem*. 39: 45- 47.

Allan, J.E. (1969) The Preparation of agricultural samples for analysis by Atomic Absorption Spectrometry. *Varian Techtron Bulletin* 12-69 (S.I.S. Edn.).

Alloway, B. J. (1990) Heavy Metals in Soils. John Wiley & Sons, New York.

and Tomaro, M. L. (1996) Heavy metal phytotoxicity induces oxidative stress in a moss, *Taxithellium* sp. *Plant Sci*. **121**: 151-159.

Aneesh, T. P., Hisham, M., Sekhar, S. M., Madhu, M. and Deepa, T. V. (2009) International market scenario of traditional Indian herbal drugs- India declining. *Inter. J. Green Pharmacy*. **10**: 184-190.

Angelova, V., Ivanov, K. and Ivanova, R. (2006) Heavy metal content in plants form family Lamiaceae cultivated in industrially polluted region. *J. Herb. Species Med. Plants* **11**: 37-46.

Anonymous, (1979) Effects of mercury in the Canadian environment. Associate Committee on Scientific Criteria for Environmental Quality, National Research Council of Canada, Canada. NRC Publication No. 16739.

Apel, K. and Hirt, H. (2004) Reactive oxygen species: metabolism, oxidative stress and signal transduction. *Annu. Rev. Plant. Biol.* **55**: 373- 399.

Arazi, T., Sunkar, R., Kaplan, B. and Formm, H. (1999) A tobacco plasma membrane calmodulin- binding transporter confers Ni^{2+} tolerance and Pb^{2+} hypersensitivity in transgenic plants. *Plant J.* **20**: 171-182.

Arnon, D. I. (1949) Copper enzymes in isolated chloroplasts. Polyphenoloxidase in *Beta vulgarirs*. *Plant Physiol.* **24:** 1-5.

Awasathi, L. P. and Menzel, G. (1986) Effect root extract from *Boerhavia diffusa* containing an antiviral principle upon plaque formation of RNA bacteriophages. Zentralblatt fur Bakteriologie **141**: 415-419.

Awasthi, L. P., Kumar, P., and Singh, R. V., (2003) Effect of *Boerhavia diffusa* inhibitor on the infection and multiplication of cucumber green mottle mosaic virus in musk melon plants *Inidan Phytopathology* **56**: 362.

Awasthi, L. P., and Kumar, P. (2003) Protection of some cucurbitaceous crops against natural infection of virusese through *Boerhavia diffusa* plants. *Indian Phytopathology* **56**: 318.

Awsathi, L. P. and Rizvi, S. M. A. (1998) Prevention of infection of a vector borne virus of tomato by *Boerhavia diffusa* glycoprotein. In: Abstracts, XIII Annual convention of the Indian Virological Society and National Symposium on Characterization on and Management of Viruses. NBRI and Lucknow University, Lucknow, Uttar Pradesh, India. Abstract no. 27.

Awsathi, L. P. and Rizvi, S. M. A. (1999) Effect of *Boerhavia diffusa* glycoprotein on the transmition of tomato yellow leaf curl virus by Bemisia tabaci Gen. In: Abstracts, National Symposium on Vectors of Plant diseases, Narendra Dev University of Agriculture and Technology, Kumarganj, Faizabad, Uttar Pradesh, India 56.

Axtell, N.R., Sternberg, S.P.K., and Claussen, K. (2003) Lead and nickel removal using *Microspora* and *Lemna minor*. *Bioresource Technol.* **89:** 41- 48.

Badawi, G. H., Yamauchi, Y., Shimada, F., Sasaki, R., Kawano, N., Tanaka, K. and Tanaka, K. (2004) Enhanced tolerance to salt stress and water

deficit by over expressing superoxide dismutase in tobacco (*Nicotiana tabacum*) chloroplasts. Plant Sci. **166**: 919- 928.

Badawi, G. H., Yamauchi, Y., Shimada, E., Sasaki, R., Kawano, N., Tanaka, K. and Tanaka, K. (1984) Enhanced tolerance to salt stress and water deficit by over expressing superoxide dismutase in tobacco (*Nicotiana tabacum*) *Chloroplasts. Plant Sci*. **166**: 919- 928.

Baker, A.J.M., Reeves, R. D., and Hajar, A.S.M (1994) Heavy metal accumulation and tolerance in British populations of the metallophyte *Thlaspi caerulescens* . J. & C. Presl (Brassicaceae) . *New Phytol.* **127**: 61-68.

Baker, A. J. M. and Walker, P. L. (1989) Physiological responses of plants to heavy metals and the quantification of tolerance and toxicity. *Chem. Specia. Bioavai.* **1**: 7-17.

Baker, R., Lavie, B. and Novo, E. (1985) Natural selection for resistance to the pollution. *Experientia* **41**: 697-699.

Bala, R. and Setia, R. C. (1990) Some aspects of cadmium and lead toxicity in plants. In: C. P. Malik, D. S. Bhatta, R. C. Setu and P. Singh (Eds.). *Advances in Frontier Areas of Plant Science*. Narendra Publishing House, Delhi. 167- 180.

Barcelo, J. and Poschenrieder, (1990) Plant water relations as affected by heavy metal stress: A review . *J. Plant Nutr*. **13**: 1-37.

Barcelo, J. and Poschenrieder, C. (1997) Chromium in plants. In: S. Carati, F. Tottarelli and P. Seqmi, (Eds.). *Chromium Environmental Issue*. Francotangata Press, Milan. 101-129.

Barman, S.C., Kisku, G.C. and Bhargava, S.K. (1999) Accumulation of heavy metals in vegetables, pulse, and wheat grown in fly ash amended soil. *Environ. Biol.* **20:** 15-18.

Barthwal, J. Nair, S. and Kakkar, P. (2008) Heavy metal accumulation in medicinal plants collected form environmentally different sites. *Biomed. Environ. Scie* . **21**: 319- 324.

Bates, L. S., Waldren, R. P. and Teare, I. D., (1973) Rapid determination of free proline for water stress studies. *Plant Soil*. **39**: 205- 208.

Beauford, W., Barber, J. and Barringer, A.R. (1977) Uptake and distribution of mercury within higher plants. *Physiol. Plant.* **39:** 261-265.

Berlyn, G. P. and Miksche, J. P. (1976) Botanical Microtechnique and *Cytochemistry*. The Iowa State University Press. Ames, Iowa. 326.

Bernier, M., Popovic, R. and Carpentier, R. (1993) Mercury inhibition at the donor side of photosystem II is reversed by chloride. FEBS Lett. **321:** 19-23.

Bharagava, R. N., Chandra, R. and Rai, V. (2008) Phytoextraction of trace elements and physiological changes in Indian mustard plants (*Brassica juncea* L.) grown in post methanated distillery effluent (PMDE) irrigated soil. *Biores. Technol.* **99**: 8316-8324.

Bhoomik, N. and Sharma, A. (1999) Lead toxicity in plant systems: Some aspects. *The Nucleus* **42**: 131- 181.

Bishnoi, N.R., Sheoran, I.S. and Singh, R. (1993) Effects of chromium on photosynthesis, respiration and nitrogen fixation in Pea (*Pisum sativum* L.) seedlings. *J. Plant Physiol.* **142**: 25-30.

Blaylock, M.J., Salt, D.E., Dushenkov, S., Zakharov, O., Gussman, C., Kapulink, Y., Ensley, B.D. and Raskin, I. (1997) Enhanced accumulation of Pb in Indian mustard by soil-applied chelating agents. *Environ. Sci. Technol.* **31**: 860-685.

Boominathan, R. and Doran, P. M. (2003) Cadmium tolerance and antioxidative defenses in hairy roots of the cadmium hyperaccumulator, *Thlaspi caerulescens*, *Biotehnol. Bioeng.* **83**: 158-167.

Borovik, A. J. (1990) Characteristics of Metals in Biological System. In: A. J. Shaw (Ed.). *Heavy Metal Tolerance in Plants: Evolutionary Aspects*. .CRC Press, Florida. 3-5.

Bradley, D. E. and Min, D. B. (1992) Singlet oxygen oxidation of foods. *Cat. Rev. Food Sci. Nutr*. **31**: 211- 236.

Brennan, N.A. and Shelly, M.L. (1999) A model of the uptake, translocation and accumulation of lead (Pb) by maize for the purpose of phytoextraction. *Ecol. Eng*. **12**: 271-297.

Brooks, R.R., Lee, J., Reeves, R.D. and Jaffre, T., (1977) Detection of nickeliferous rocks by analysis of herbarium specimens of indicator plants. *J. Geochem. Explor*. **7**: 49-58.

Brown, S. L., Chaney R. L., Angel, J. S. and Baker, A. J. M. (1994) Phytoremediation potential of *Thlaspi caerulescens* and bladder campion for zinc and cadmium – contaminated soil. *J. Environ. Qual*. **23**: 1151-1157.

Brown, S.L., Chaney, R.L., Angle, J.S. and Baker, A.J.M (1995) Zinc and cadmium uptake by hyper accumulator *Thalaspi caerulescens* grown in nutrient solution. *Soil. Sci. Soc. Am. J*. **59**: 125-133.

Brzyska, M., Ginalska, G. and Lobarzewski, J. (1991) Combined influence of lead, mercury and cadmium ions in the presence of magnesium on endopolygalacturonase activity. *Biotechnol. Appl. Biochem*. **13:** 83-89.

Buchanan, B. B., Gruissem, W. and Jones, R. L. (2000) *Biochemistry and Molecular Biology of Plants*. American Society of Plant Physiologists. Rockville, Maryland.

Burzynski, M. and Buczek, J. (1994) The influence of Cd, Pb, Cu and Ni on NO_3 uptake by cucumber seedlings. II. *in vitro* and *in vivo* effects of Cd, Cu, Pb and Ni on the plasmalemma ATPase and oxidoreductase from cucumber seedling roots. *Acta Phys. Plant*. **16**: 297-302.

Chan, T. Y. K. , Tomlinson, B., Critichley, A. J. H. (1993) Chinese herbal medicines revisited: a Hong Kong perspective. *The Lancet* **342**: 1532-1534.

Chandan, B. K., Sharma, A. K., and Anand, K. K. (1991) *Boerhavia diffusa*: A study of its hepatoprotective activity. *J. Ethnopharmacol.* **31**: 299- 307.

Chandra, R., Bharagava, R. N., Yadav, S. and Mohan, D. (2009) Accumulation and distribution of toxic metals in wheat (*Triticum aestivum* L.) and Indian mustard (*Brassica campestris* L.) irrigated with distillery and tannery effluents. J. Hazard. Mater. **162**: 1514-1521.

Chaney, R., M., Malik, Y. M., Li, S. L., Brown, E. P., Brewer, J. S. Angle, A. J. M. and Baker (1997) Phytoremediation of soil metals *Curr. Opin. Biotechnol*. **8**: 279-284.

Chatterjee, J. and Chatterjee, C. (2000) Zinc stress changes in the metabolism of

blackgram (*Phaseolus mungo*) *Indian J. Agri. Sci*. **70**: 250-252.

Chen, Z., Zhu, Y. G., Liu, W. J. and Meharg, A. A (2005) Direct evidence showing the effect of root surface iron plaque on arsenite and arsenate uptake into rice (*Oryza sativa*) roots. *New Phytol*. 165: 91-97.

Cheraghi, M., Lorestani, B. and Yousefi, N. (2009) Effect of waste water on heavy metal accumulation in Hamedan province vegetables. *Int. J. Bot*.**5**: 190-193.

Chopra, G. L. (1969) Angiosperms. *Systematics and Life Cycles*. S. Nagin & Co. Jalandhar, Punjab, India 361- 365.

Choudhury, S., Panda, S. K., (2005) Toxic effect, oxidative stress and ultra structural changes in moss *Taxitheelium nepalense* (Schwaegr.) Broth. Under lead and chromium toxicity. *Water Air Soil Pollut*. 167: 73- 90.

Ciura, J., Poniedzialek, M., Sekara, A. and Jedrszczyk, E. (2005) The possibility of using crops as metal phytoremediants. *Polish J. Environ. Stud.* **14**: 17-22.

Clemens, S., Palmagren, M. G. and Kraemer, U. (2002) A long way ahead: understanding the engineering plant metal accumulation. *Trends Plant Sci*. **7**: 309-315.

Clemens, S. (2006)Toxic metal accumulation, responses to exposure and mechanisms of tolerance in plants (2006). *Biochimie* **88**: 1707-1719.

Clijsters, H. and Van Assche, F. (1985) Inhibition of photosynthesis by heavy metals. *Photosynth. Res.* **7**: 31-40.

Cobbet, C. S. (2003) Heavy metals and plants- model systems and hyper-accumulators. *New Phytol.* **159**: 289- 293.

Cobbett, C.S. (2000) Phytochelatins and their roles in heavy metal detoxification. *Plant Physiol.* **123:** 825-832.

Cobbett, C.S. and Goldsbrough, P. (2002) Phytochelatins and metallothioneins: Roles in heavy metal detoxification and homeostasis. *Annu. Rev. Plant Biol.*. **53**: 159-182.

Cohen, C.K., Fox, T.C., Garvin, D.F. and Kochian, L.V. (1998) The role of iron-deficiency stress responses stimulating heavy-metal transport in plants. *Plant Physiol.* **116:** 1063-1072.

Corradi, M.G., Bianchi, A.K. and Albasini, A. (1993) Chromium toxicity in *Salvia sclarea*: 1. Effect of hexavalent chromium on seed germination and seedling development. *Environ. Expt. Bot.* **33**: 405-413.

Costa, G. and Morel, J. L. (1994) Water relations, gas exchange and amino acid content in Cd- treated lettuce. *Plant Physiol. Biochem.* **32-** 561-570.

Cseh, E. (2002) Metal permeability, transport and efflux in plants. In: M.N.V. Prasad and K. Strzalka (Eds.). *Physiology and Biochemistry of Metal Toxicity and Tolerance in Plants*. Kluwer *Academic, Dordrecht* 1-36.

Cseh, E., Fodor, F., Varga, A. and Zaray, G. (2000) Effect of lead treatment on the distribution of essential elements in cucumber, *J. Plant Nutr.* **23:**

1095-1105.

Cunningham, C. D. and Berti, W. R. (1993) Remediation of contaminated soils with green plants: An overview. *In vitro Cell Dev. Biol.* **29**: 207- 212.

Cunningham, S. D., Berti, W. R. and Huang, J. W. (1995) Phytoremediation of contaminated soils. *Trends Biotechnol.* **13**: 398- 403.

Daniel, G., Hussain, K. and Nabeesa- Salim, (2009) Bioaccumulation and effect of lead, chromium, and cadmium on peroxidase activity in bitter gourd (*Momordica charantia* L. Walp) cv. PRIYA *Inter. J. Plant. Sci.* **4**: 239-244

Davies, F.T., Puryear, J.D., Newton, R.J., Egilla, J.N. and Grossi, J.A.S. (2002) Mycorrhizal fungi increase chromium uptake by sunflower plants: influence on tissue mineral concentration, growth and gas exchange. *J. Plant Nutr.* **25**: 2389-2407.

Davies, K. L., Davies, M. S. and Francis, D. (1991) The influence of an inhibitor of phytochelatin synthesis on root growth and root meristematic activity in *Festuca rubra* L. in response to zinc. *New Phytol.* **118**: 565- 570.

De Fillippis, L. F. (1979) The effect of heavy metal compound on the permeability of *Chlorella* cells. *Z. Pflanzenphysiol.* **92**: 39-43.

De Vos, C.H.R., Vonk, M.J. and Schat, H. (1992) Glutathionine depletion due to copper induced phytochelatin synthesis causes oxidative stress in *Silene cucubalus*. *Plant Physiol.*, **98**: 853-858.

De, A.K., Sen, A.K., Modak, D.P. and Jana, S. (1985) Studies of toxic effect of Hg (II) on *Pistia stratiotes*. *Water Air Soil Pollut.* **24:** 351-360.

Delnomdedien, M., Boudou, A., Desmager, J.P. and Georgescauld, D. (1989) Interaction of $HgCl_2$ with the primary amino group of model membranes containing phosphatidyl serine and phosphatidyl ethanolamine. *Biochem. Biophys. Acta*. **986**: 191-199.

Dhar, M. L., Dha, M. M., Dhawan, B. N., Mehrotra, B. N. and Ray, C. (1968) Screening of Indian plants for biological activity: Part I *Indian J. Exp. Biol.* **6**: 232- 247.

Dixit, V., Pandey, V. and Shyam, R. (2002) Chromium ions inactivate electron transport and enhance superoxide generation *in vivo* in pea (*Pisum sativum* L. cv. Azad) root mitochondria. *Plant Cell Environ.* **25**: 687-690.

Dominguez- Solis, J. R., Lopez-Martin, M., Ager, F. J., Ynsa, M. D., Romero, L. C. and Gotor, C. (2004) Increased cysteine availability is essential for cadmium tolerance and accumulation in *Arabidopsis thaliana*. *Plant Biotechnol. J.* **2**: 469-476.

Driscoll, C. T., Yan, C., Schofield, C. L., Munson, R. and Holsapple, H. (1994) The chemistry and bioavailability of mercury in Adirandack lakes. *Environ. Sci. Technol.* 28: 136-143.

Dunbabin, J. S. and Bowner, K. H. (1992) Potential use of constructed wetlands for treatment of industrial waste waters containing metals. *Sci. Total Environ.* **111**: 151-168.

Ebbs, S. D. and Kochian, I. V. (1998) Phytoextraction of zinc by oat (*Avena sativa*), barley (*Hordeum vulgare*) and Indian mustard (*Brassica juncea*) *Environ. Sci. Technol.* **32**: 802-806.

Ebbs, S. D. and Kochian, L. V. (1997) Toxicity of zinc and copper to *Brassica* species: implications for phytoremediation. *Environ. Qual.* **26**: 776-778.

Ebinghaus, R., Tripathi, R. M. and Wallschlager, L. S.E (1999) Natural and anthropogenic mercury sources and their impact on the air- surface exchange of mercury on regional and global scales. In: R., Ebinghaus, R. R., Turner, L. D. Lacerda, O., Vasilev and W. Salomons, (Eds.) *Mercury Contaminated Sites: Characterisation, Risk Assessment and Remediation*. New York, Springer- Verlag, Berlin Heidelberg.

Ederli, L., Reale, L., Ferrauti, F. and Pasqualini, S. (2004) Responses induced by high concentration of cadmium in *Phragmites australis* roots. *Physiol. Plant.* **121:** 66-74.

Epstein, E. (1972) *Mineral Nutrition of Plants: Principles and Perspectives.* John Wiley and Sons, New York.

Escarre, J., Lefebure, C., Gruber, W., Leblanc, M., Lepart, J., Riviere, Y. and Delay, B. (2000) Zinc and cadmium hyperaccumulation by *Thlaspi caerulescens* from metalliferous and non metalliferous sites in the Mediterranean area: Implications for phytoremediation. *New Phytol*. **145**: 429- 437.

Falchuk, K.H., Goldwater, L. J. and Vallee, B. L. (1977) The biochemistry and toxicology of mercury. In: C. A. Mc Aulifee (Ed.). *The Chemistry of Mercury*. Mac Millan, London, 259-283.

Falk, H., Walling, R. and Au, W. (2003) A global approach to childhood lead poisoning prevention. *Int. J. Hyg. Environ. Health* **206**: 363-369.

Fett, J.P., Cambraia, J., Oliva, M.A. and Jorado, C.P. (1994) Cadmium uptake and growth inhibition in water hyacinth: Effect of nutrient solution factors. *J. Plant. Nutr*. **17**: 1205-1217.

Fitter, A.H. and Hay, R.K.M. (1983) *Environmental Physiology of Plants*. Academic Press, London.

Fitz, W. J. and Wenzel, W. W. (2002) Arsenic transformation in the soil rhizosphere plant system, fundamentals and potential application of Phytoremediation. *Biotechnol*. **99**: 259- 278.

Fodor, F. (2002) Physiological responses of vascular plants to heavy metals. In: M.N.V. Prasad and K. Strzalka (Eds.). *Physiology and Biochemistry of Metal Toxicity and Tolerance in Plants*. Kluwer Academic. Dordrecht. 149-177.

Folin, O. and Denis, W. (1915) A Calorimetric method for the determination of phenols (and phenol derivatives) in urine. *J. Biol. Chem.* **22**: 305- 308.

Foy, C. D. Chaney, R.L. and White, M.C. (1978) The Physiology of metal toxicity in plants. *Annu. Rev. Plant Physiol.* **29:** 511-566.

Foyer, C. H., Lopez- Delgado, H., Dat, J. F. and Scot, I. M. (1997) Hydrogen peroxide and glutathione associated mechanisms of acclamatory stress tolerance and signalling. *Physiol. Plant.* **100**: 241- 245.

Friedland, A. J. (1990) The movement of metals through soils and ecosystems. In: A. J. Shaw (Ed.). *Heavy Metal Tolerance in Plants: Evolutionary Aspects*. .CRC Press, Florida. 7-19.

Gaal, O., Medgyesi, G.A. and Vereczkey, L. (1980) *Electrophoresis in the Separation of Biological Macromolecules*. John Wiley and Sons, Chichester.

Gallego, S. M., Benavioas, M. P. and Tomaro, M. L. (1996) Effect of heavy metal ion excess on sunflower leaves: evidence for involvement of oxidative stress. *Plant Sci.* **121**: 151-159.

Gasic, O., Kastori, R. Petrovic, N. and Stagner, D. (1992) Effect of lead on some biochemistry and physiological parameters in corn (*Zea mays* L.) *Agric. Meditarania.* **122**: 257-261.

Gbaruko, B. C., and Friday, O. U., (2007) Bioaccumulation of heavy metals in some fauna and flora. *Int. J. Environ. Sci. Tech.* **4**: 197-202.

Giannopolitis, C. N. and Ries, S. K. (1977) Superoxide Dismutase I. Occurance in higher plants. *Plant Physiol.* **59**: 309- 314.

Gleba, D., Borisjuk, N., Borisjuk, L., Kneer, R., Poulev, A., Skarzhinskaya, M., Dushenkov, S., Logendra, S., Gliba, Y. and Raskin, I. (1999) Use of plant roots for phytoremediation and molecular farming. *Proc. Natl. Acad. Sci.* **96**: 5973- 5977.

Glick, B. R. (2003) Phytoremediation: synergistic use of plants and bacteria to clean up the environment. *Biotechnol. Adv.* **21**: 383- 393.

Goldbold D. L., Kettner, C. (1991) Use of root elongation studies to determine aluminium and lead toxicity in *Piea abide* seedlings J. *Plant Physiol.* **5**: 138-231.

Guo-sheng, S. Ming-xue, C., Xiu-fu, Z., Chun-mei, X.U., Dan-ying, W. Qian, Q. and Guo-ping, Z. (2007) Cadmium accumulation and its toxicity in Brittle culm 1(bc1), a fragile Rice mutant. *Rice Sci.* **14**: 217-22.

Gzyl, J., Przymusinski, R. and Wozny, A. (1997) Organospecific reaction of yellow lupin seedlings to lead. *Acta Soc. Bot. Pol.* **66**: 61-66.

Haag-Kerwer, A., Schafer, H. J., Heiss, S., Walter, C. and Rausch, T. (1999) Cadmium exposure in *Brassica juncea* causes decline in transpiration rate and leaf expansion without effect on photosynthesis. *J. Exp. Bot.* **50**: 1827-1835.

Hadjiliadis, N.D. (1997) Cytotoxicity, mutagen and carcinogenic potential of heavy metals related to human environment. In: *Environment* NATO- ASI Series, Kluwer, Dordrecht.

Hageman, R. H. and Reed, A. J. (1980) Nitrate reductase from higher plants. In: A. Sanpietra (Ed.) Methods in Enzymology. Academic Press. New York. 270- 280.

Hagemayor, J. (1993) Indicators for heavy metals in the terrestrial environment. In: B. Market (Ed.) *Plants as Biomonitors*. V.C.H. Weinheim, New York, 541-556.

Hale, K.L., McGrath, S.P., Lombi, E., Stack, S.M., Terry, N., Pickering, I.J., George, G.M. and Pilon-Smits, E.A.H. (2001) Molybdenum sequestration in *Brassica* species. A Role for Anthocyanins? *Plant Physiol.* **126:** 1391-1402.

Hall, J.L. (2002) Cellular mechanisms for heavy metal detoxification and tolerance. *J. Exp. Bot.* **53:** 1-11.

Halliwell, B. and Gutterodge, J. M. C. (1999) Free radicals in biology and medicine, Oxford, U K, Clarendon Press.

Hamlin, R. L., Schatz, C. and Baker, A. V. (2003) Zinc accumulation in Indian mustard as influenced by nitrogen and phosphorus nutrition. *J. Plant Nutr*. **26**: 177-190.

Han, F.X., Sridhar, B.B.M., Monts, D.L. and Su, Y. (2004) Phytoavailiability and toxicity of trivalent and hexavalent chromium to *Brassica juncea*, *New Phytol*. **162**: 489.

Harborne, J. B. (1980) Plant Phenolics In: E. A. Bell and B. V. Charlwood (Eds.). *Secondary Plant Products*. 329-395. Encyclopedia of Plant Physiology, New Series Vol. 8. Springer- Verlag Berlin Heidelberg New York.

Hattori, H., Kuniyasu, K., Chiba, K. and Chino, M. (2006) Effect of chloride application and low soil pH on cadmium uptake from soil by plants. *Soil Sci. Plant Nutr*. **52**: 89-94.

Heath, R. L. and Packer, L., (1968) Photo-peroxidation in isolated chloroplast. I. Kinetics and stoichiometry of fatty acid peroxidation. *Arc. Biochem. Biophys*. **125**: 189- 198.

Hendry, G.A.F., Baker, A.J.M. and Ewart, C.F. (1992) Cadmium tolerance and toxicity, oxygen radical processes and molecular damage in cadmium- tolerant and cadmium sensitive- clones of *Holeus lanatus* L. *Acta Bot. Neerl*. **41**: 271-281.

Henry, J. R. (2000) Phytoremediation of mercury In: An overview of the phytoremediation of lead and mercury. A report for the U. S. *Environmental Protection Agency*. *Washington*, D. C., USA. 44-46.

Hiruma – Lima, C. A., Grazioso, J. S., Bighetti, E.J., Germonsen Robineou, L. and Souza- Brito, A. R. (2000) The juice of fresh leaves of *Boerhavia diffusa* L. (Nyctaginaceae) markedly reduces pain in mice. *J. Ethnopharmacol*. **71**: 267-274.

Hopkins L., Malcolm, J., Hawkesford, (2000) J. S- supply, free sulphur and free aminoacids pools in Potato (*Solanum tuberosum* I. Cv Desiree) In: Brunold C, editor. Sulphur Nutrition and Sulfur Assimination in Higher Plants 259- 261.

Hosseini, R. H., Khanlarian, M., and Ghorbanli, M. (2007) Effect of lead on germination, growth and activity of catalase and peroxidase enzyme in root and shoot of two cultivars of *Brassica napus* L. *J. Biol. Sci.* **4**: 592-598.

Houston, M.C. (2007) The role of mercury and cadmium heavy metals in vascular disease, hypertension, coronary heart disease and myocardial infarction. *Altern. Ther. Health Med*. **13**: 128-133.

Huang, J.W. and Cunningham, S.D. (1996) Lead phytoextraction: species variation in lead uptake and translocation. *New Phytol*. **134**: 75-84.

Huang, J.W., Chen, J., Berti, W.R. and Cunningham, S.D. (1997)

Phytoremediation of lead–contaminated soils: role of synthetic chelators in lead phytoextraction. *Environ. Sci. Technol.* **31**: 800-805.

Hussain, I., Khan, F., Khan, I., Khan, L. and Wali-Ullah (2006) Determination of heavy metals in medicinal plants. *Chem. Soc. Pak.* **28**: 347-351.

Hussain, K. (2007) *Ecophysiological Aspects of Bacopa monnieri* (L.) Pennell. Ph.D. Thesis submitted to the University of Calicut.

Hussain, K., Abdussalam, A. K., Ratheesh- Chandra, P. and Nabeesa-Salim (2010) Bioaccumulation of heavy metals in *Bacopa monnieri* (L.) Pennell growing under different habitat. *Int. J. Ecol. Devel.* **10**:66-73.

Iqbal, M.Z., Saeeda, S. and Muhammed, S. (2001) Effects of chromium on an important arid tree (*Caesalpinia pulcherima*) of Karachi city, Pakistan. *Ekol Bratislava*. **20**: 414-22.

Ishikawa, S., Ae, N., Murakami, M. and Wagatsuma, T. (2006) Is *Brassica juncea* a suitable plant for phytoremediation of cadmium in soils with moderately low cadmium contamination?- Possibility of using other plant species for Cd- phytoextraction. *Soil Sci. Plant Nutr*. **52**: 32-42.

Jain, M. and Puranik, M. (1993) Protective effect of reduced glutathione on inhibition of chlorophyll biosynthesis by mercury in excised greening maize leaf segments. *Indian J. Exp. Biol.* **31**: 708-710.

Jalil, A., Selles, F. and Clarke, J.M. (1994) Effect of cadmium on growth and uptake of cadmium and other elements by Durum Wheat. *J. Plant Nutr*. **17**: 1839-1858.

Jamal, S.N., Iqbal, M.Z. and Athar, M. (2006) Effect of aluminum and

chromium on the germination and growth of two *Vigna species*. *Int. J. Environ. Sci. Techol*. **3**: 53-58.

Jeana, S. and Chaudhuri, M.A. (1982) Senescence in submerged aquatic angiosperms: Effect of heavy metals. *New phytol*. **90**: 477-484.

Jiang, W., Liu, D. and Hou, W. (2000) Hyperaccumulation of lead by roots, hypocotyls and shoots of *Brassica juncea*. *Biol. Plant*. **43:** 603-606.

Johansen, O. A. (1940) Plant Microtechnique. Mc Graw Hill Book Company, Inc. New York.

Joop, A. K., Marjolein, D., Paul, L. M. K., Henk, S., Jos, A. C. V. and Wilfried, H. O. E. (1994) Phytochelatin in cadmium sensitive and cadmium tolerant *Silene vulgaris*. *Plant Physiol*. **104**: 255-261.

Kabata- Pendias A. and Pendias, H. (1991) *Trace Elements in Soil and Plants* (3rd Edn). CRC Press. LLC. Boka Raton, FL.

Kabata- Pendias, A. and Pendias, H. (2001) Trace elements in soils and plants., 3rd ed. CRC. Press. 365.

Kabata-Pendias, A. and Pendias, H. (1999) *Biogeochemistry of Trace Elements* in (Polish) PWN. Warszawa. Poland.

Kagi, J.H.R. and Hapke, H.J. (1984) Biochemical interactions of mercury, cadmium and lead. In: J.O. Nriagu (Ed.). *Changing Metal Cycles and Human Health*. Springer – Verlag, New York. 237-250.

Kahle, H. (1993) Response of roots of trees to heavy metals. *Environ. Exp. Bot*.

33: 99-119.

Kakosy, T., Hudak, A., Naray, M. (1996) Lead intoxication epidemic caused by ingestion of contaminated ground paprika. J. *Toxicology- Clinical* Toxicology **34**: 507-511.

Kamal, M., Ghaly, A. E., Mahmoud, N. and Cote, R. (2004) Phytoaccumulation of heavy metals by aquatic plants. *Environ. Int.* **29**: 1029- 1039.

Kar, M. and Mishra, D. (1976) Catalse, peroxidase and polyphenol oxidase activities during rice leaf senescence. *Plant Physiol.* **57**: 315-319.

Kastori, R., Petrovic, N. and Arsenijevic, M.I. (1995) Effect of lead on the activities of some enzymes of nitrogen metabolism in sugar beet (*Beta vulgaris* L.). Glasnik Instituta Za Botanika I Botanicke Baste Univerziteta u Beograda. **29**: 77-84.

Kastori, R., Plenicar, M., Sakai, Z., Pankovic, D. and Maksimovic, A.I. (1998) Effect of excess lead on sunflower growth and photosynthesis. *J. Plant Nutr*. **21**: 75-85.

Khan, S. A., Khan, L. and Hussain, I. (2008) Profile of heavy metals in selected medicinal plants *Pak. J. Weed Sci. Res.* **14**: 101- 110.

Khatun, S. Ali, M. B., Hahn, E. and Paek, K. (2008) Copper toxicity in *Withania somnifera*: Growth and antioxidant enzymes responses of *in vitro* grown plants. *Environ. Exp. Bot.* **64**: 279- 285.

Kim, I. S., Kang, K. H., Jhnson-Green, P. and Lee, E. J. (2003) Investigation of heavy metal accumulation in *Polygonum thunbergii* for phytoextraction. *Environ. Pollut.* **126**: 235-243.

Kim, Y. Y., Yang, Y.Y. and Lee, Y. (2002) Pb and Cd uptake in rice roots. *Physiol. Plant.* **116**: 368-372.

Kiran, Y., and Sahin, A., (2006) The effects of cadmium on seed germination, root development and mitosis of root tip cells of lentil (*Lens culinaris* Medik). *World J. Agri. Sci.* **2** 196-200.

Kochian, L.V. (1991) Mechanisms of micronutrient uptake and translocation in plants. In: J.J. Mordtnet, F.R. Cox, L.M. Shuman and R.M. Welch (Eds.) *Micronutrients in Agriculture*. Soil Science Society of America, Madison, 229-296.

Kochian, L.V. (1995) Cellular mechanisms of aluminum toxicity and resistance in plants. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **46**: 237-260.

Koeppe, D.E. (1981) Lead: understanding the minimal toxicity of lead in plants. In: N.W. Lepp (Ed.) *Effects of Heavy Metal Pollution on Plants*. Vol. 1. Applied Science Publishers, London and New Jersey. 55-76.

Kos, B., Greman, H. and Lestan, D. (2003) Phytoextraction of lead, zinc and cadmium from soil by selected plants. *Plant. Soil. Environ.* 49: 548-553.

Krupa, Z. and Baszynski, T. (1995) Some aspects of heavy metals toxicity towards photosynthetic apparatus: Direct and indirect effects on light and dark reactions. *Acta. Physiol. Plant*. **17**: 177-190.

Krupa, Z., Ruszkowski, M. and Gilowska-Juny, E. (1982) The effect of chromate on the synthesis of plastid pigments and lipoquinones in *Zea mays* seedlings. *Acta Soc. Bot. Pol*. **51**: 275-281.

Kumar, N.P.B.A., Dushenkov, V., Motto, H. and Raskin, I. (1995) Phytoextraction: The use of plants to remove heavy metals from soil. *Environ. Sci. Technol*. **29:** 1232-1238.

Kupper, H., Kupper, F. and Spiller, M. (1996) Environmental relevance of heavy metal substituted chlorophylls using the example of water plants. *Experi. Botany*. **47**: 259- 266.

Kupper, H., Kupper, F. and Spiller, M. (1998) *In Situ* detection of heavy metal substituted chlorophylls in water plants. *Photosynth. Res*. **58:** 123-133.

Kupper, H., Zhao, F.J. and McGrath, S.P. (1999) Cellular compartmentation of zinc in leaves of the hyper accumulator, *Thlaspi caerulescens*. *Plant Physiol*. **119:** 305-311.

Landrigan, P. J. (1999) Risk assessment for children and other sensitive populations. *Ann. N.Y.* Acad. Sci., **895**: 1-9.

Lang, F., Sarvari, E., Szigeti, Z., Fodor, F. and Cseh, E. (1995) Effect of heavy metals on the photosynthetic apparatus in cucumber. In: P. Mathis

(Ed.). *Photosynthesis: From Light to Biosphere*. Vol. IV. Kluwer Academic, Netherlands. 533-536.

Lata, S. (1989) Effect of cadmium on seedlings growth, mobilization of food reserves and activity of hydrolytic enzymes in *Phaseolus aureus* L. seeds. *Acta Bot*. **17**: 290-293.

Lavid, N., Schwartza, A., Yarden, O. and Tel-Or, E. (2001) The involvement of polyphenols and peroxidase activities in heavy metal accumulation by epidermal glands of waterlily (Nymphaeceaea) *Planta* **212**: 323.

Lee, J. H., Hossner, L. R., Attrep, Jr. M., Kung, K. S., (2002) Uptake and translocation of plutonium, in two plant species using hydroponics. *Environ. Pollut*. 117: 61- 68.

Lee, Y. P. and Takahashi, T. (1966) An improved colorimetric determination of amino acids with the use of ninhydrin. *Anal. Biochem*. **14**: 71-77.

Lenka, M., Das, B.L., Panda, K.K. and Panda, B.B. (1993) Mecury-tolerance of *Chloris barbata* Sw. and *Cyperus rotundus* L. isolated from contaminated sites. *Biol. Plant*. **35:** 443-446.

Levitt, C. M. D., (1984) Sources of lead poisoning. J. American Medical Association **252**: 3127- 3128.

Levitt, J. (1980) *Responses of Plants to Environmental Stress*. (2nd Edn. Vol.2) *Academic Press*, New York.

Liang, Y., Chen, Q., Liu, Q. and Zhang, W., R., (2003) Exogenous silicon (Si) increases antioxidant enzyme activity and reduces lipid peroxidation in roots of salt-stressed barley (*Hordeum vulgarae* L.) *Plant Phsiol.* **160**: 1157-1164.

Lidsky, T.I., and Schneider, J.S (2006) Adverse effects of childhood lead poisoning: the clinical neuropsychological perspective. *Environ. Res.* **100**: 284-293.

Linger, P., Ostwald, A. and Haensler, J. (2005) *Cannabis sativa* L., growing on heavy metal contaminated soil: Growth, cadmium uptake and photosynthesis. *Biol. Plant*. 49: 567-576.

Liu, Z., He, X., Chen, W., Yuan, F., Yan, K. and Tao, D. (2009) Accumulation and tolerance characteristics of cadmium in a potential hyperaccumulator- *Lonicera japonica* Thumb. *Hazardous Materials*. **10**: 81-97.

Lowry, O.H., Rosenbrough, N.J., Farr, A.L. and Randall, R.J. (1951) Protein measurement with Folin-Phenol reagent. *J. Biol. Chem.* **193:** 265-275.

Maitani, T., Kubota, H., Satoh, K. and Yamada, T. (1996) The composition of metals bound to Class III metallothionein (Phytochelatin and its desglycyl peptide) induced by various metals in root cultures of *Rubia tinctorium*. *Plant Physiol*. **110**: 1145-1150.

Malajczuk, N. and Dell, B. (1995) Heavy metals in Philipines soils affect growth and establishment of trees. *ACIR-For. News Letter*. **18**: 2-3.

Malkowski, E., Kitta, A., Galas, W., Karcz, W. and Kuperberg, M.J. (2002) Lead distribution in corn seedlings (*Zea mays* L.) and its effect on growth and the concentrations of potassium and calcium. *Plant Growth Regul.* **37**: 69-76.

Markowitz, S. B., Nenez, C. M., Klitzman, S. (1994) Lead poisoning due to haige fen: the porphyry content of individual erythrocytes. J. American Medical Association **271**: 932- 934.

Marschner, H. (1983). General introduction to the mineral nutrition of plants. In: A. Lauchli and R.L. Bieleski (Eds.). *Encyclopedia of Plant Physiology (New Series) Inorganic Plant Nutrition vol. 15 A.*. Springer Verlag, New York. 5-60.

Marschner, H. (1986) *Mineral Nutrition of Higher Plants*. Academic Press. England.

Mazen, A.M.A., and Maghraby, O.M.O. (1997) Accumulation of cadmium, lead, strontium and role of calcium oxalate in water hycianth tolerance. *Biol. Plant*. **40**: 411-417.

McGrath, S. P. and Zhao, F. J. (2003) Phytoextraction of metals and metalloids from contaminated soils. *Curr. Opin. Biotechnol.* **14**: 1-6.

McGrath, S. P., Shen, Z. G. and Zhao, F. J. (1997) Heavy metal uptake and chemical changes in the rhizosphere of *Thlaspi caerulescens* and

Thlaspi ochroleucum grown in contaminated soils. *Plant Soil* **188**: 153-159.

McGrath, S.P. (1982) The uptake and translocation of tri- and hexavalent chromium and effects on the growth of oat in flowing nutrient solution and in soil. *New Phytol*. **92**: 381-390.

McNeilly, T. (1994) Metal toxicity. In: A. R. Yeo and T. J. Flowers (Eds.). *Soil Mineral Stresses: Approaches to Crop Improvement*. Springer-Verlag, New York, 145-174.

Meers, E., Vaudecasteele, B., Ruttens, A., Vangrousveld, J. and Tack, F.M.G., (2007). Potential of five Willo species (*Salix* spp.) for phytoextraction of heavy metals. *Environ. Exp. Bot*. **60**: 57-68.

Mehrotra, S., Mishra, K. P., Maurya, R., Srimal and Singh, V. K. (2002) Immunomodulation by ethanolic extract of *Boerhavia diffusa* roots. *Int. Immunopharmacol* **2**: 987-996.

Meidner, H. and Mansfield, T. A. (1968) *Physiology of Stomata*. McGraw Hill, Lindon.

Meuwly, P., Thibault, P., Schwan, A.L. and Rauser, W.R. (1995) Three families of thiol peptides are induced by Cd in maize. *Plant J.* **7:** 391-400.

Michalak, A. (2006) Phenolic compounds and their antioxidant activity in plants growing under heavy metal stress (2006) *Polish J. Environ. Stud*. **15**: 523-530.

Michalak, E. and Wierzbicka, M. (1998) Differences in lead tolerance between *Allium cepa* plants developing from seeds and bulbs. *Plant Soil*. **199**: 251-260.

Migocka, M. and Klobus, G. (2007) The properties of the Mn, Ni and Pb transport operating at plasma membranes of cucumber roots. *Physiol. Plant.* **129:** 578-587.

Mishra, A. and Choudhuri, M.A. (1999) Monitoring or phytotoxicity of lead and mercury from germination and early seedling growth induces two rice cultivars. *Water Air Soil Pollut.* **114**: 339-346.

Monni, S., Uhlig, C., Hunttila, O., Hausen, E. and Hynynen, J. (2001) Chemical composition and ecophysiological responses of *Eupatorium nigrum* to above ground element application. *Environ. Pollut.* **112:** 417-426.

Moodley, K. G., Baijnath, H., Southway- Ajulu, F. A., Maharaj, S. and Chetty, S. R. (2007) Determination of Cr, Pb and Ni in water, sludge and plants from settling ponds of a sewage treatment works. *Water* SA **33**: 723- 728.

Moral, R., Gomes, I., Pedreno, J.N. and Mataix, J. (1996) Absorption of chromium and effects on micronutrient content in tomato plants (*Lycopersicon esculentum*). *Agrochimica* **11**: 138-145.

Moral, R., Palacios, G., Gomes, I., Pedreo, J.N. and Mataix, J. (1994) Distribution and accumulation of heavy metals (Cd, Ni and Cr) in tomato plant. *Fresnius Environ. Bull*. **3**: 395-399.

Moral, R., Pedreno, J.N., Gomes, I. and Mataix, J. (1995) Effects of chromium on the nutrient element content and morphology of tomato. *J. Plant Nutr*. **18**: 815-822.

Moreno, F. N., Anderson, C.W. N., Stewart, R. B. and Robinson, B. H. (2004) Phytoremediation of mercury- contaminated mine tailings by induced plant- mercury accumulation . *Environ. Practice* **6**: 165- 175.

Moreno, F. N., Anderson, C. W. N., Stewart, R. B. and Robinson, B. H. (2005a) Mercury volatilisation and phytoextraction from base- metal mine tailings. *Environ. Pollut.* **136**: 341- 352.

Moreno, F. N., Anderson, C. W. N., Stewart, R. B., Robinson, B. H. Ghomshei, M. and Meech, J. A. (2005b) Induced plant uptake and transport of mercury in the presence of sulphur containing ligands and humic acid. *New Phytol*. **166**: 445-454.

Moreno, F. N., Anderson, C. W. N., Stewart, R. B., Robinson, B. H., Nomura, R., Ghomshei, M. and Meech, J. A. (2005) Effect of thioglands on plant- Hg accumulation and volatilisation from mercury – contaminated mine tailings. *Plant soil* **275**: 233-246.

Moreno, F. N., Anderson, C.W.N., Stewart, R. B. and Robinson, B. H. (2008) Phytofiltration of mercury – contaminated water: Volatilisation and plant – accumulation aspects. *Environ. Exp. Bot*. **62**: 78-85.

Moreno-Caselles, J., Moral, R., Perez- Espinosa, A. and Perez-Murcia, M.D. (2000) Cadmium accumulation and distribution in cucumber plant. *J.*

Plant Nutri., **2**: 29-34.

Morgan, J. F., Klucas, R. V., Grayer, R. J., Abian, J. and Becana, M. (1997) Complexes of iron with phenolic compounds from soybean nodules and other legume tissues: prooxidant and antioxidant properties. Free *Radic*. *Biol. Med*. **22**: 861.

Mysliwa-kurdziel, B. and Strzalka, K. (2002) Influence of metals on biosynthesis of photosynthetic pigments. In: M.N.V. Prasad and K. Strzalka (Eds.). *Physiology* and *Biochemistry of Metal Toxicity* and *Tolerance in Plants*. Kluwer Academic. The Netherlands. 201-227.

Nag, P., Paul, A.K and Mukherji, S. (1980) Effects of mercury, copper and zinc on the growth, cell division, GA. induced amylase synthesis and membrane permeability of plant tissues. *Indian J. Exp. Biol*. **18**: 822-827.

Nagajyoti, P. C., Dinakar, N., Prasad, T. N. V. K. V., Suresh, C. and Damodharam, T. (2008) Heavy metal toxicity: Industrial effluent effect on groundnut (*Arachis hypogaea* L.) seedlings. *J. Appl*. *Sciences Research*. **4**: 110-121.

Nath, S., Chaudhury, J., Pahan, K. and Mandal, A. (1993) Mercury and organomercurial resistance in rhizobial strains. *Indian J. Exp. Biol*. **31:** 50-53.

Noctor, G., Foyer, C. H., (1998) Ascorbate and glutathione: Keeping active oxygen under control. *Annu. Rev. Plant. Physiol. Plant. Mol. Biol.* **49**: 249- 279.

Obata, H., Inoue, N., Imai, K. and Umebayashi, M. (1994) Cadmium tolerance of calli induced from roots of plants with difference in cadmium tolerance. *Soil Sci. Plant Nutr*. **40**: 351-354.

Olujohungbe, A., Fields, P. A., Sandford, A. F., (1994) Heavy metal intoxication from homeopathic and herbal remedies. Postgraduate Medical Journal **70**: 764- 769.

Orcutt, D. M. and Nilsen, E. T. (2000) *Physiology of Plants Under Stress: Soil and Biotic Factors*. John Wiley & Sons, Inc. New York.

Ouariti, O., Boussama, N., Zarrouk, M., Cherif, A. and Ghorbal, M.H. (1997) Cadmium and copper induced changes in tomato membrane lipids. *Phytochemistry*. **45**: 1343-1350.

Pance, N.S., Larsen, P.B., Ebbs, S.D., Letham, D.L., Lasat, M.M., Garvin, D.F., Eide, D. and Kochian, L.V. (2000) The molecular physiology of heavy metal transport in the Zn/Cd hyperaccumulator *Thlaspi caerulescens*. *Proc. Natl. Acad. Sci.* U.S.A. **97:** 4956-4960.

Panda, S. K., (2007) Chromium – mediated oxidative stress and ultra structural changes in root cells of developing rice seedlings. *Plant. Physiol.* **164**: 1419-1428.

Panda, S.K. (2003) Heavy metal phytotoxicity induces oxidative stress in

Taxithelium sp. *Curr. Sci.* **84**: 631-633.

Panda, S.K. and Choudhury, S. (2005) Chromium stress in plants. *Braz. J. Plant Physiol.* **17**: 95-102.

Panda, S.K. and Patra, H.K. (1997) Physiology of chromium toxicity in plants. A Review. *Plant Physiol. Biochem*. **24**: 10-17.

Panda, S.K. and Patra, H.K. (1998) Alteration of nitrate reductase activity by chromium ions in excised wheat leaves. *Ind. J. Angio. Biochem*. **2**: 56-57.

Panda, S.K. and Patra, H.K. (2000) Does Cr. (III) produce oxidative damage in excised wheat leaves. *J. Plant. Biol*. **27**: 105-110.

Panda, S.K., Chaudhury, L. and Khan, M.H. (2003) Heavy metals induce lipid peroxidation and affects antioxidants in wheat leaves. *Biol. Plant.* **46**: 289-294.

Parekh, D., Puranik, R. M. and Srivastva, H. S. (1990) Inhibition of chlorophyll biosynthesis by cadmium in greening maize leaf segments. *Biochem. Physiol. Pflanzen*. **186**: 239- 242.

Parmar, N.G. and Chanda, S.V. (2005) Effects of mercury and chromium on peroxidase and IAA oxidase enzymes in the seedlings of *Phaseolus vulgaris*. *Tur. J. Biol.* **29**: 15-21.

Parmer, N. G. Vithalani, S. D. and Chanda, S. V. (2002) Alteration in growth and peroxidase activity by heavy metals in *Phaseolus* seedlings. *Acta Physiol. Plant*. **24**: 89-95.

Peralta, J. R., Gardea-Torresdey, J. L., Tiemann, E., Gomez, S., Arteaga, E., Rascon, and Parsons, J. G. (2000) Study of the effects of heavy

metals on seed germination and plant growth on alfalfa plant (*Medicago sativa*) grown in solid media. *Proceedings of the conference on hazardous waste research*. 135- 140.

Peralta, J.R., Gardea-Torresdey, J.L., Tiemann, K.J., Arteaga, S. and Rascon, E. (2001) Uptake and effects of five heavy metals on seed germination and plant growth in alfalfa (*Medicago sativa* L.). *Bull. Environ. Contam. Toxicol.* **66**: 727-734.

Perfus-Barbeoch, L., Leonhardt, N., Vavasseur, A. and Forestier, C. (2002) Heavy metal toxicity: Cadmium permeates through calcium channels and disturbs the plant water status. *The Plant J.* **32:** 539-548.

Petr, J., Skerik, J. and Dlouby, J. (1999) Cadmium, lead and mercury contents in ecologically grown crops. *Sci. Agri. Bohemica* **30:** 285-299.

Pilon-Smits, E. (2005) Phytoremediation. *Annu. Rev. Plant Biol.* **56:** 15-39.

Podar, D., Ramsey, M. H. and Hutchings, M. J. (2004) Effect of cadmium, zinc and substrate heterogeneity on yield, shoot metal concentration and metal uptake by *Brassica juncea*: Implications for human health risk assessment and phytoremediation. *New Phytol.* **163**: 313-324.

Poschenrieder, C., Vazquez, M.D., Bonet, A. and Barcelo, J. (1991) Chromium III iron interaction in iron sufficient and iron deficient bean plants. II Ultra structural aspects. *J. Plant Nutr.* **14**: 415-428.

Posmyk, M. M., Kontek, R. and Janas, K. M (2009) Antioxidant enzymes activity and phenolic compounds content in red cabbage seedlings exposed to copper stress. *Ecotoxicol. Environ. Saf.* **72**: 596-602.

Prasad, D.D.K. and Prasad, A.R.K. (1987) Effect of lead and mercury on chlorophyll synthesis in mung bean seedlings. *Phytochem.* **26:** 881-883.

Prasad, M.N.V. (1997) Trace metals. In: M.N.V. Prasad (Ed.). *Plant Ecophysiology*. John Wiley & Sons, Inc. 207-249.

Prasad, M.N.V. (1998) Metal-biomolecule complexes in plants: occurrence, functions and applications. *Analysis*. 26-28.

Prasad, S.M., Singh, J.B., Rai, L.C. and Kumar, H.D. (1991) Metal induced inhibition of photosynthetic electron transport chain of cyanobacterium, *Nostoc muscarum*. *FEMS Microbiol. Lett.* **82:** 95-100.

Pratima, N.P., Paul, A.K. and Mukherji, S. (1989) The effects of heavy metals, Zn and Hg on the growth and biochemical constituents of mung bean (*Vigna radiata*) seedlings. *Bot. Bull. Acad. Sin.* (Taipei) 30: 241-250.

Pulford, I.D., Watson, C. and McGregor, S.D. (2001) Uptake of chromium by trees: prospects for phytoremediation. *Environ. Geochem. Health* **23**: 307-311.

Radiotic, K., Ducic, T. and Mutavdzic, D. (2000) Changes in peroxidase activity

and isoenzymes in spruce needles after exposure to different concentration of cadmium. *Environ. Exp*. **44**: 105- 113.

Rai, U.N., Tripathi, R.D., Vajpayee, P., Pandey, N., Ali, M.B. and Gupta, D.K. (2003) Cadmium accumulation and its phytotoxicity in *Potamogeton pectinatus* L. (Potamogetonaceae). *Bull. Environ. Contam. Toxicol*. **70:** 566-575.

Rai, V., Vajpayee, P., Singh, S. N. and Mehrotra, S. (2004) Effect of chromium accumulation on photosynthetic pigments, oxidative stress defense system, nitrate reduction, proline level and eugenol content of *Ocimum tenuiiflorum* L. *Plant Sci*. **167**: 1159- 1169.

Ramabhimaiah, S., Stalin, D. and Kalaskar, N. J (1984) Pharmacological investigations of the water soluble fraction of the methanol extracts of *Boerhavia diffusa* roots. *Indian Drugs* **21**: 343- 344.

Raskin, I. and Ensley, B.D. (2000) *Phytoremediation of Toxic Metals Using Plants to Clean up the Environment.* John Wiley & Sons. Inc. New York.

Raskin, I., Kumar, N. P. B. A., Dushenkov, S. and Salt, D. E.(1994) Bioconcentration of heavy metals by plants. *Curr. Opin. Biotechnol.* **5**: 285-290.

Ratheesh Chandra, P., Abdussalam, A. K. Salim, N. and Puthur, J. T. (2010) Distribution of bio-accumulated Cd and Cr in two *Vigna* species and

the associated histological variations. *Stress physiology & Biochemistry* **6**: 4-12.

Rauser, W.E. (1987) Changes in glutathione content of maize seedlings exposed to cadmium. *Plant Sci.* **51:** 171-175.

Rauser, W. E. (1990) Phytochelatins. *Annu Rev. Biochem.* **59**: 61-86.

Rauser, W. E. (1995) Phytochelatins and related peptides: Structure, biosynthesis and function. *Plant Physiol.* **109**: 1141-1149.

Rawat, A. K. S., Mehrotra, S., Tripathi, S. K., and Sharma, U. (1997) Hepatoprotective activity in Punarnava - a popular Indian ethnomedicine. J. *Ethnopharmacology*. 56: 61- 68.

Reed, R. H. and Gadd, G. M. (1990) Metal tolerance in eukaryotic and prokaryotic algae. In: A. J. Shaw (Ed.). *Heavy Metal Tolerance in Plants: Evolutionary Aspects*. CRC Press, Florida. 105-118.

Reid, R.J., Dunbar, K.R. and McLaughlin, M.J. (2003) Cadmium loading into potato tubers: the roles of the periderm, xylem and phloem. *Plant Cell Environ.* **26:** 201-206.

Rice-Evans, C. A., Miller, N. J. and Paganga, G. (1997) Antioxidant properties of phenolic compounds. *Trends Plant Sci.* 2: 152.

Riedel, G.F. (1985) The relationship between chromium (IV) uptakes, sulphate uptake and chromium (IV) toxicity in the estuarine diatom *Thalassionira pseudonama*. *Aquatic Toxicol.* **7**: 191-204.

Ross, H.B. (1994) On the use of mosses (*Hylocomium splendens* and

Pleurozium schreberi) for estimating atmospheric trace metal deposition. *Water Air Soil Pollut.* **50**: 63-76.

Rout, G.R., Samantaray S. and Das, P. (1997) Different chromium tolerance among eight mung bean cultivars grown in nutrient culture. *J. Plant Nutr.* **20**: 341-347.

Rout, G.R., Sanghamitra, S. and Das, P. (2000) Effect of chromium and nickel on germination and growth in tolerant and non-tolerant populations of *Echinochola colona* (L.) *Chemosphere* **40**: 855-859.

Sadasivam, S. and Manikam, A. (1992) Biochemical Methods for Agricultural Sciences. Wiley Eastern Ltd., New Delhi and Tamilnadu Agricultural University, Coimbatore.

Sahadevan, K.K. (2001) Physiological and biochemical studies on mercury toxicity in *Vigna mungo* (L.) Hepper seedlings. Ph. D. Thesis submitted to the University of Calicut.

Sakihamay, Y. and Yamasaki, H. (2002) Lipid peroxidation induces by phenolics in cinjunction with aluminium ions. *Biol. Plantarum* **45**: 249.

Salati, E., Eneida, S. and Salati, E. (1999) Wetlands Projects Developed in Brazil. *Water Sci. Technol.* **40**: 19.

Salt, D. E., Smith, R. D. and Raskin, I. (1998) Phytoremediation. *Annu. Rev. Plant Physiol. Mol. Biol.* **49**: 643-668.

Salt, D.E. and Rauser, W.E. (1995) Mg ATP-dependent transport of

phytochelatins across the tonoplast of oat roots. *Plant Physiol.* **107:** 1293-1301.

Samantary, S. and Das, P. (1997) Accumulation and distribution of chromium, nickel and iron in the mung bean plant. *Fresnius Envir. Bull.* **6**: 633-641.

Samantary, S., Rout, G.R. and Das, P. (1996) A study of soil plant and root-shoot relationship in rice (*Oryza sativa* L. cv. Pathan) grown on chromiferous mine soil. *Proc. Natl. Acad. Sci.* USA. **66**: 349-357.

Samantary, S., Rout, G.R. and Das, P. (1998) Role of chromium on plant growth and metabolism. *Acta Physiol. Plant.* **20**: 201-212.

Sandmann, G. and Boger, P. (1983). The enzymological function of heavy metals and their role in electron transfer process of plants. In: A. Lauchli and R.L. Bieleski (Eds.). *Encyclopedia of Plant Physiology*, (New Series) Vol. **15B**. *Inorganic Plant Nutrition*. 563-596.

Sanità di Toppi, L., Prasad, M.N.V. and Ottonello, S. (2002) Metal chelating peptides and proteins in plants. In: M.N.V. Prasad and K. Strzalka (Eds.) *Physiology and Biochemistry of Heavy Metal Detoxification and Tolerance in Plants*. Kluwer Academic Publishers, Dordrecht. 59-93.

Sanita-di-Toppi, L. and Gabbrielli, R. (1999) Response to cadmium in higher plants. *Environ. Exp. Bot.* **41:** 105-130.

Saper, R. B., Kales, S. N., Paquin, J. Burns, M. J., Eisenberg, D. M. and Davis, R. B. (2004) Heavy metal content of ayurvedic herbal medicine products *Jama*, **292**: 2868- 2873.

Satoh, M., Karaki, E., Kakehashi, M., Okazaki, E., Gotoh, T. and Oyama, Y. (1999) Heavy-metal induced changes in non proteinaceous thiol levels and heavy metal binding peptides in *Tetraselmis tetrathele* (Prasinophyceae). *J. Phycol*. **35**: 989-928.

Scheller, H.V., Huang, B., Hatch. E. and Goldsbrough, P.B. (1987) Phytochelatin synthesis and glutathione levels in response to heavy metals in tomato cells. *Plant Physiol*. **85**: 1031-1035.

Sergin, I. V. and Ivanov, V. B. (2001) Physiological aspects of cadmium and lead toxic effects on higher plants. *Russ.J. Plant Physiol*. **48**: 523-544.

Sersen, F., Clik, G., Havranek, E. and Sykorova, M. (2005) Bioremediation by natural zeolite in plants cultivated in a heavy metal contaminated medium. *Fresenius Environ. Bull*. **14**: 13- 17.

Setia, R.C. and Bala, R. (1994) Anatomical changes in root and stem of wheat (*Triticum aestivum* L.) in response to different heavy metals. *Phytomorph*. **44**: 95-104.

Shanker, A.K., Cervantes, C., Loza-Tavera, H. and Avudainayagam, S. (2005) Chromium toxicity in plants. *Environ. Intl*. **31**: 739-753.

Shanker, A.K., Djnaguiraman, M., Sudhagar, R., Chandrashekar, C.N. and Pathmanabhan, G. (2004) Differential antioxidative response of ascorbate glutathione pathway enzymes and metabolites to chromium speciation stress in green gram (*Vigna radiata* (L.) R. Wilczek. cv CO 4) roots. *Plant Sci.* **166**: 1035-1043.

Shaw, B. P. and Rout, N. P. (1998) Age-dependent responses of *Phaseolus aureus* Roxb. to inorganic salts of mercury and cadmium. *Acta Physiol. Plant.* **20**: 85-90.

Shaw, B.P. (1995) Effect of mercury and cadmium on the activities of antioxidative enzymes in the seedlings *of Phaseolus aureus*. *Biol. Plant*. **37**: 587-596.

Shen, Z. G., Zhao, F. J. and McGrath, S. P. (1997) Uptake and transport of zinc in the hyperaccumulator *Thlaspi caerulescens* and the nonhyperaccumulator of *Thlaspi ochroleucum*. *Plant Cell Environ.* **20**: 898- 906.

Sheoran, I.S., Aggarwal, H. and Singh, R. (1990) Effect of cadmium and nickel on *in vivo* carbon dioxide exchange rate of pigeon pea. *Plant Soil* **129:** 243-249.

Shinmachi, F., Kumanda, Y., Noguchi, A. and Hasegawa, I. (2003) Translocation and accumulation of cadmium in cadmium tolerant *Polygonum thunberjii*. *Soil Sci. Plant Nutr*. **49**: 355- 361.

Shu, W.S., Ye, Z.H., Lan, C.Y., Zhang, Z.Q. and Wong, M.H. (2002) Lead, zinc and copper accumulation and tolerance in populations of *Paspalum*

distichum and *Cynodesmon dactylon*. *Environ. Pollut.* **120:** 445-453.

Siedlecka, A. and Krupa, Z. (2002) Functions of enzymes in heavy metal treated plants. In: Prasad M. N.V., Kazimierz, S. (Eds.) Physiology and biochemistry of metal toxicity and tolerance in plants. *Kluwer, Netherlands*, 314- 317.

Siedlecka, A. and Krupa, Z. (1997) Cd/Fe interaction in higher plants- its consequences for the photosynthetic apparatus. *Photosynthetica* **36**: 321- 331.

Singh, A.K. (2001) Effect of trivalent and hexavalent chromium on spinach (*Spinacea oleracea* L.) *Environ. Ecol*. **19**: 807-810.

Singh, K. P., Mohan, D., Sinha, S. and Dalwani, R. (2004) Impact assessment of treated/ untreated wastewater toxicants discharged by sewage treatment plants on health, agricultural and environmental quality in the wastewater disposal area. *Chemosphere* **55**: 227-255.

Singh, R. H. and Udupa, K. N. (1972) Studies on the Indian indigenous drug punarnava (*Boerhavia diffusa* Linn.) Part IV: Preliminary controlled clinical trial in nephritic syndrome. *Res. Indian Med.* **7**: 28- 33.

Singh, S., Seaxena, R., Pandey, K., Bhatt, K., Singha, S. (2004) Response of antioxidant in sunflower (*Helianthus annus* L.) grown on different

amendments of tannery sludge: its metal accumulation potential. *Chemosphere* **57**: 1663- 1673.

Singh, V. and Pandey, R. P. (1980) Medicinal plantlore of the tribals of eastern Rajasthan. *J. Econ. Taxon. Bot.* 137- 147.

Sinha, S. (1999) Accumulation of Cu, Cd, Cr, Mn, and Pb from artificially contaminated by soil *Bacopa monnieri*. *Environ. Monit. Assess.* **57**: 253-264.

Sinha, S., Gupta, M. and Chandra, P. (1996) Bioaccumulation and biochemical effect of mercury in the plant *Bacopa monnieri* (L.) *Environ. Toxicol. Wat. Qual.* **11**: 105-112.

Sinha, S., Saxena, R., (2006) Effect of iron on lipid peroxidation and enzymatic and non – enzymatic antioxidant and bacoside content in medicinal plant *Baccopa monnieri* L. Chemosphere 62: 1340- 1350.

Sivarajan, V. V. and Balachandran, I. (1994) *Ayurvedic Drugs and Their Plant Sources*. Oxford and IBH Publishing Co., New Delhi.

Skinner, K., Wright, N. and Porter-Goff, E. (2007) Mercury uptake and accumulation by four species of aquatic plants. *Environ. Pollut.* **145**: 234-237.

Sonmez, O., Bukun, B., Kaya, C. and Aydemir, S. (2008) The assessment of tolerance to heavy metals (Cd, Pb and Zn) their accumulation in three weed species. Pak. J.Bot. **40**: 747-754.

Stefanov, K.L., Popova, I., Kamburova, E., Pancheva., Kimenov, G., Kuleva, L. and Popv, S. (1993) Lipid and sterol changes in *Zea mays* caused by lead ions. *Phytochemistry*. **33**: 47-51.

Steffens, J.C. (1990) The heavy metal binding peptides of plants. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **41**: 533-575.

Stobart, A. K., Griffiths, W. T., Ameen- Bukhari, I. and Sherwood, R. P. (1985) The effect of Cd^{2+} on the biosynthesis of chlorophyll in leaves of barley. Physiol. Plant. 63: 293-298.

Stohs, S.J. and Bagchi, D. (1995) Oxidative mechanisms in the toxicity of metal ions. *Free Radicals Biol. Med.* **18:** 321-336.

Stolt, P., Asp, H. and Hultin, S. (2006) Genetic variation in wheat cadmium accumulation on soils with different cadmium concentrations. *J. Agron. Crop Sci.* **192**: 201-208.

Stroinski, A., and Zielezinska, M (2001) Cadmium and oxidative stress influence on phytochelatin synthase activity in potato tuber *Acta. Physiol. Plant.* **23**: 157-160.

Sun, Y. B., Zhou, X. Q. and Ren, L. P.(2007) Growth responses of *Rorippa globosa* and its accumulation characteristics of Cd and As under the Cd- As combined pollution, *Environ. Sci*. **28**: 1355- 1360.

Szollosi, R., Varga, I. S., Erdei, L. and Mihalik, E. (2009) Cadmium –induced oxidative stress and antioxidative mechanisms in germinating Indian

mustard (*Brassica juncea* L.) seeds. *Ecotoxicol. Environ. Safety*. 72: 1337- 1342.

Taiz, L. and Zeiger, E. (2002) *Plant Physiology:* (III[rd] Edn.) Sinauer Associates, Inc. Publishers, Sunderland, Massachussetts.

Tanaka, K., Fujimaki, S., Fujiwara, T., Yoneyama, T. and Hayashi, H. (2007) Quantitative estimation of the contribution of the phloem in cadmium transport to grains in rice plants (*Oryza sativa* L.). *Soil Sci. Plant Nutr*. **53**: 72-77.

Tomar, M., Kaur, I., Neelu, A. and Bhatnagar, A. K. (2000) Effect of enhanced lead in soil on growth and development of *Vigna radiata* (Linn.) Wilczek. *Indian J. Plant Physiol*. **5**: 13-18.

Tomsig, J.L. and Suszkiw, J.B (1991) Permeation of Pb^{2+} through calcium channels: fura -2 measurements of voltage – and dihydropyridine sensitive Pb^{2+} entry in isolated bovine chromaffin cells. *Biochem.Biophys. Acta.* **1069**: 197-200.

Tong, S., von Schirnding, Y. E., Prpaamontol, T. (2000) Environmental lead exposure: a public problem of global dimension. Bulletin of the World Health Organization **78**: 1068- 1077.

Tripathi, A. K., Tripathi, S. and Tripathi, S. (1999) Changes in some physiological and biochemical characters in *Albezia lebbek* as bio-indicators of heavy metal toxicity. *J. Environ. Biol.* **20**: 93-98.

Tukendorf, A. and Rauser, W. E. (1990) Changes in glutathione and phytochelation in roots of maize seedlings exposed to cadmium. *Plant Sci.* **70**: 155-166.

Turner, A. P. (1994) The responses of plants to heavy metals: In: S. M. Ross (Ed.). *Toxic Metals in Soil- Plant Systems*. John Wiley & Sons Ltd., 154-187.

Turner, A.P. and Dickinson, N.M. (1993) Survival of *Acer pseudoplatanus* L. (Sycamore) seedling on metalliferous soils. *New Phytol.* **123**: 509-521.

Vajpayee P., Tripathi, R. D., Rai. U. N., Ali, M. B., Singh, S. N. (2000) Chromium (VI) accumulation reduces chlorophyll biosynthesis, nitrate reductase activity and protein content in *Nymphaea alba* L. *Chemosphere* **41**: 1075- 1082.

Vajpayee, P., Sharma, S. C., Tripathi, R. D., Rai, U. N., Yunus, M. (1999) Bioaccumulation of chromium and toxicity to photosynthetic pigments, nitrate reductase activity and protein content of *Nelumbo nucifera* Gaertin. *Chemosphere* 39: 2159- 2169.

Vallee, B. L. and Ulmer, D. D. (1972) Biochemical effects of mercury, cadmium and lead. *Annu. Rev. Biochem.* **41**: 91- 128.

Van Assche, F. and Clijsters, H. (1990) Effect of metals on enzyme activity in plants. *Plant Cell Environ.* **13**: 195-206.

Vazques, M.D., Poschenrieder, C.H. and Barcelo, J. (1987) Chromium (VI) induced structural changes in bush bean plants. *Ann. Bot.* **59**: 427-438.

Vazques, M.D., Poschenrieder, C.H. and Barcelo, J. (1992) Ultra structural effects and localization of low cadmium concentration in bean roots. *New Phytol*. **120**: 215-226.

Velasco- Alinsug, M. P., Rivero, G. C. and Quibuyen, T. A. O. (2005) Isolation of mercury –binding peptides in vegetative parts of *Chromolaena odorata*. Z. *Naturforsch*. **60**c: 252-259.

Verkleij, J. A. C. and Schat, H. (1990) Mechanisms of metal tolerance in higher plants. In : A. J. Shaw (Ed.) *Heavy Metal Tolerance in Plants: Evolutionary Aspects*. CRC Press, Florida. 179-193.

Wagner, G. J. (1993) Accumulation of cadmium in crop plants and its consequences to human health *Adv. Agron*. **51**: 173- 212.

Wang, Q., Cui, Y. and Dong, Y. (2002) Phytoremediation of polluted waters: potentials and prospects of wetland plants. *Acta Biotechnol*. **22**: 199-208.

Wang, Z., Zhang, Y., Huang, Z. and Huang, L. (2008) Antioxidative response of metal accumulator and non – accumulator plants under cadmium stress. *Plant Soil*. **310**: 137- 149.

Wei, C. Y., Sun, X., Wang, C., and Wang, W. Y. (2006) Factors influencing arsenic accumulation by *Pteris vittata*: A comparative field study at two sites. *Environ. Pollu.* **141**: 488- 493.

Wenzel, W. W. and Jockwer, F. (1999) Accumulation of heavy metals in plants growing on mineralized soils of the Austrian Alps. *Environ. Pollut.* **104**: 145- 155.

WHO, (1999) Monographs on selected medicinal plants. Vol. 1. *World Health Organization*.Geneva.

WHO, (2002) Drug Information. Herbal Medicines. **16**: *World Health Organization*, Geneva.

WHO, (2003) Elementary mercury and inorganic mercury compounds: Human Health Aspects. Concise International Chemical Assessment Document **50**: *World Health Organization* Geneva.

WHO, Contaminants, In Codex Alimentarius (1984) XVII, Edn. 1. FAO/WHO. Codex Alimentarius Commission, Rome.

Wierzbicka, M. (1998) Lead in the apoplast of *Allium cepa* L. root tips- ultra structural studies. *Plant Sci.* **133**: 105-119.

Wilkins, (1978) The measurement of tolerance to edaphic factors by means of root growth. *New Phytol.* **80**: 623-633.

Wojcik, M. and Tukendorf, A. (1999) Cadmium tolerance of maize, rye and wheat seedlings. *Acta Phys. Plant.* **21**: 99-107.

Woods, P. W., (1999) Herbal healing. *Essence* **30**: 42-46.

Woolhouse, H.W. (1983) Toxicity and tolerance in the response of plants to metal. In: O.L. Lange, P.S. Nobel, C.B. Osmond and H. Ziegler (Eds.). *Encyclopedia of Plant Physiology (New Series) Physiological Plant Ecology III* **12C**. Springer-Verlag, New York. **12C**: 245-302.

Wozny, A. and Jerczynska, E. (1991) The effect of lead on early stages of *Phaseolus vulgaris* L. growth *in vitro* conditions. *Biol. Plant.* **33**: 32-39.

Wozny, A., Stroinski, J. and Gwozdz, E.A. (1990) Plant cell responses to cadmium. *Seria Biologia* 44. Adam Mickiewicz University, Poznam.

Xiong, Z. T. (1998) Heavy metal contamination of urban soils and plants in relation to traffic in Wuhan City, China, *Toxicol. Environ. Chem.*, **65**: 31-39.

Yadav, S., Sukla, O.P. and Rai, U.N. (2005) Chromium pollution and bioremediation. *Environ. News Archives* **11:** 1-4.

Ye, Z. H., Baker, A.J.M., Wong, M. H. and Willis, A. J. (1997) Zinc, lead and cadmium tolerance, uptake and accumulation by *Typha latifolia*. *New Phytol.* **136**: 469-480.

Yoon, J., Cao, X., Zhou, Q. and Ma, L. Q. (2006) Accumulation of Pb, Cu and Zn in native plants growing on a contaminated Florida site. *Sci. Total Environ.* **368**: 456-464.

Zayed, A., Gowthaman, S. and Terry N. (1998) Phytoaccumulation of trace elements by Wetland Plants: 1 Duckweed. *J. Environ. Qual.* **27**: 715-721.

Zeid, I.M. (2001) Responses of *Phaseolus vulgaris* to chromium and cobalt treatments. *Biol. Plant*. **44**: 111-115.

Zengin and F. K. and Munzuroglu, O. (2005) Effects of some heavy metals on content of chlorophyll, proline and some antioxidant chemicals in bean (*Phaseolus vulgaris* L.) seedlings. *Acta Biol. Cracov. Ser. Bot.* **47**: 157-164.

Zenk, M. H. (1996) Heavy metal detoxification in higher plants-A review. *Gene*. **179:** 21-30.

Zhang, F. Q., Wang, Y. S., Lou, Z. P. and Dong, J. D. (2007) Effect of heavy metal stress on antioxidative enzymes and lipid peroxidation in leaves and roots of two mangrove plant seedlings (*Kandelia candel* and *Bruguiera gymnorrhiza*). *Chemosphere* **67**: 44-50.

Zheljazkov, V.D., Craker, L. E. and Xing, B. (2006) Effects of Cd, Pb and Cu on growth and essential oil contents in dill, Peppermint and basil. *Environ. Exp. Bot.* **58**: 9-16.

Zheljazkov, V. D., Ekaterina, A., Jeliazkova, A., Kovacheva, N. and Dzhurmanski, A. (2008) Metal uptake by medicinal plant species grown in soil soils contaminated by smelter. *Environ. Exp. Bot.* **64**: 207- 216.

Zimmels, Y., Kirzhner, F. and Roitman, S. (2004) Use of naturally growing aquatic plants for waste water purification. *Water Environ. Res. Found.* **76**: 220-230.

Zou, J. H., Wang, W. S., Jiang, and Liu, D. H. (2006) Effect of hexavalent chromium (VI) on root growth and cell division in root tip cells of *Amaranthus viridis* L. *Pak. J. Bot.* **38**: 673- 681.

Zou, J., Xu, P., Lu, X., Jiang, W., and Liu, D. (2008) Accumulation of cadmium in three sunflower (*Helianthus annuus* L.) cultivars. *Pak. J. Bot.* **40**: 759-765.

Mohan, B. S. and Hosetti, B. B. (1997) Potential phytoxicity of lead and cadmium to *Lemna minor* grown in sewage stabilization ponds. *Environ. Res.* 98: 233- 238.

Eun, S. O., Youn, H. S. and Lee, Y. (2000) Lead disturbs microtubule organization in the root meristem of *Zea mays*. *Physiol. Plant.* **110**: 357-365.

Sahu, A. N., Damiki, L., Nilanjan, G. and Dubey, S. (2008) Phytopharmacological review of *Boerhavia diffusa* Linn.(Punarnava). *Phcog. Rev.* **2**: 14-22.

Liu, D., Jiang, W. and Gao, X. (2003) Effects of cadmium on root growth, cell division and nucleoli in root tip cells of garlic. *Biol. Plant.* **47**: 79-83.

Linger, P., Mossig, J., Fischer, H. and Kobert, J. (2002) Industrial hemp (*Cannabis sativa* L.) growing on heavy metal contaminated soil: fibre quality and phytoremediation potential. Ind. *Crops Prod*. **16**: 33- 42.

Chakraborti, K. K. and Handa, S. S. (1989) Antihepatotoxic investigations of *Boerhavia diffusa* L. Indian Drugs **27**: 161- 166.

Gopal, G. V. and Shah, G. L. (1985) Some folk medicinal plants used for jaundice in Gujarat, India. *J. Res. Edu. Indian Med*.**4**: 44-49.

Kirtikar, K. R. and Basu, B. D. (1956) *Indian Med. Plant*. Lalit Mohan Basu Allhabad, India.

Pari, L. and Satheesh, A. M. (2004) Antidiabetic activity of *Boerhavia diffusa* L. effect on hepatic key enzymes in experimental diabetes *J. Ethnopharmacol*. **9**: 109- 113.

REFERENCES

Abe, T., Fukami M., Ichizen, N. and Ogasawara, M. (2006) Susceptibility of weed species to cadmium evaluated in a sand culture. *Weed Biol. Manage*. **6**: 107-114.

Adriano, D.C. (1986) *Trace Elements in the Terrestrial*

Environment. Springer, Berlin.

Ahmed, A. and Tajmir-Riahi, H.A. (1993) Interaction of toxic metal ions Cd^{2+},Hg^{2+},and Pb^{2+} with light harvesting proteins of chloroplast thylakoid membranes: An FTIR spectroscopic study. *J. Inorg. Biochem*. **50:** 235-243.

Allan, J.E. (1969) The Preparation of agricultural samples for analysis by Atomic Absorption Spectrometry. *Varian Techtron Bulletin* 12-69 (S.I.S. Edn.).

Alloway, B. J. (1990) Heavy metals in soils. John Wiley& Sons, New York.

Aneesh, T. P., Hisham, M., Sekhar, S. M., Madhu, M. and Deepa, T. V. (2009) International market scenario of traditional Indian herbal drugs- India declining. *Inter. J. Green Pharmacy*. **10**: 184-190.

Angelova, V., Ivanov, K. and Ivanova, R. (2006) Heavy metal content in plants form family Lamiaceae cultivated in industrially polluted region. *J. Herb. Species Med. Plants* **11**: 37-46.

Anonymous, (1979) Effects of mercury in the Canadian environment. Associate Committee on Scientific Criteria for Environmental Quality, National Research Council of Canada, Canada. NRC Publication No. 16739.

Arazi, T., Sunkar, R., Kaplan, B. and Formm, H. (1999) A tobacco plasma membrane calmodulin- binding transporter confers Ni^{2+} tolerance and Pb^{2+} hypersensitivity in transgenic

plants. *Plant J.* **20**: 171-182.

Arnon, D.I. (1949) Copper enzymes in isolated chloroplasts. Polyphenoloxidase in *Beta vulgarirs*. *Plant Physiol.* **24:** 1-5.

Awasathi, L. P. and Menzel, G. (1986) Effect root extract from *Boerhavia diffusa* containing an antiviral principle upon plaque formation of RNA bacteriophages. *Zentralblatt fur Bakteriologie* **141**: 415-419.

Awsathi, L. P. and Rizvi, S. M. A. (1998) Prevention of infection of a vector borne virus of tomato by *Boerhavia diffusa* glycoprotein. In: Abstracts, XIII Annual convention of the Indian Virological Society and National Symposium on Characterization on and Management of Viruses. NBRI and Lucknow University, Lucknow, Uttar Pradesh, India. Abstract no. 27.

Awsathi, L. P. and Rizvi, S. M. A. (1999) Effect of *Boerhavia diffusa* glycoprotein on the transmition of tomato yellow leaf curl virus by Bemisia tabaci Gen. In: Abstracts, National Symposium on Vectors of Plant diseases, Narendra Dev University of Agriculture and Technology, Kumarganj, Faizabad, Uttar Pradesh, India 56.

Axtell, N.R., Sternberg, S.P.K., and Claussen, K. (2003) Lead and nickel removal using *Microspora* and *Lemna minor*. *Bioresource Technol.* **89:** 41- 48.

Baker, A.J.M., Reeves, R. D., and Hajar, A.S.M (1994) Heavy metal accumulation and tolerance in British populations of the

metallophyte *Thlaspi caerulescens* . J. & C. Presl (Brassicaceae) . *New Phytol.* **127**: 61-68.

Baker, A. J. M. and Walker, P. L. (1989) Physiological responses of plants to heavy metals and the quantification of tolerance and toxicity. *Chem. Specia. Bioavai.* **1**: 7-17.

Baker, R., Lavie, B. and Novo, E. (1985) Natural selection for resistance to the pollution. *Experientia* **41**: 697-699.

Bala, R. and Setia, R. C. (1990) Some aspects of cadmium and lead toxicity in plants. In: C. P. Malik, D. S. Bhatta, R. C. Setu and P. Singh (Eds.). *Advances in Frontier Areas of Plant Science*. Narendra Publishing House, Delhi. 167- 180.

Barcelo, J. and Poschenrieder, (1990) Plant water relations as affected by heavy metal stress: A review . *J. Plant Nutr*. **13**: 1-37.

Barcelo, J. and Poschenrieder, C. (1997) Chromium in plants. In: S. Carati, F. Tottarelli and P. Seqmi, (Eds.). *Chromium Environmental Issue*. Francotangata Press, Milan. 101-129.

Barman, S.C., Kisku, G.C. and Bhargava, S.K. (1999) Accumulation of heavy metals in vegetables, pulse, and wheat grown in fly ash amended soil. *Environ. Biol*. **20:** 15-18.

Beauford, W., Barber, J. and Barringer, A.R. (1977) Uptake and distribution of mercury within higher plants. *Physiol. Plant.* **39:** 261-265.

Bernier, M., Popovic, R. and Carpentier, R. (1993) Mercury inhibition at the donor side of photosystem II is reversed by chloride. FEBS Lett. **321:** 19-23.

Bharagava, R. N., Chandra, R. and Rai, V. (2008) Phytoextraction of trace elements and physiological changes in Indian mustard plants (*Brassica juncea* L.) grown in post methanated distillery effluent (PMDE) irrigated soil. *Biores. Techno.* **99**: 8316-8324.

Bhoomik, N. and Sharma, A. (1999) Lead toxicity in plant systems: Some aspects. *The Nucleus* **42**: 131- 181.

Bishnoi, N.R., Sheoran, I.S. and Singh, R. (1993) Effects of chromium on photosynthesis, respiration and nitrogen fixation in Pea (*Pisum sativum* L.) seedlings. *J. Plant Physiol.* **142**: 25-30.

Blaylock, M.J., Salt, D.E., Dushenkov, S., Zakharov, O., Gussman, C., Kapulink, Y., Ensley, B.D. and Raskin, I. (1997) Enhanced accumulation of Pb in Indian mustard by soil-applied chelating agents. *Environ. Sci. Technol.* **31**: 860-685.

Borovik, A. J. (1990) Characteristics of Metals in Biological System. In: A. J. Shaw (Ed.). *Heavy Metal Tolerance in Plants: Evolutionary Aspects*. .CRC Press, Florida. 3-5.

Brennan, N.A. and Shelly, M.L. (1999) A model of the uptake, translocation and accumulation of lead (Pb) by maize for the purpose of

phytoextraction. *Ecol. Eng*. **12**: 271-297.

Brooks, R.R., Lee, J., Reeves, R.D. and Jaffre, T., (1977) Detection of nickeliferous rocks by analysis of herbarium specimens of indicator plants. *J. Geochem. Explor*. **7**: 49-58.

Brown, S. L., Chaney R. L., Angel, J. S. and Baker, A. J. M. (1994) Phytoremediation potential of *Thlaspi caerulescens* and bladder campion for zinc and cadmium – contaminated soil. *J. Environ. Qual*. **23**: 1151-1157.

Brown, S.L., Chaney, R.L., Angle, J.S. and Baker, A.J.M (1995) Zinc and cadmium uptake by hyper accumulator *Thalaspi caerulescens* grown in nutrient solution. *Soil. Sci. Soc. Am. J*. **59**: 125-133.

Brzyska, M., Ginalska, G. and Lobarzewski, J. (1991) Combined influence of lead, mercury and cadmium ions in the presence of magnesium on endopolygalacturonase activity. *Biotechnol. Appl. Biochem.* **13:** 83-89.

Buchanan, B. B., Gruissem, W. and Jones, R. L. (2000) *Biochemistry and Molecular Biology of Plants*. *American Society of Plant Physiologists*.

Burzynski, M. and Buczek, J. (1994) The influence of Cd, Pb, Cu and Ni on NO_3 uptake by cucumber seedlings. II. *in vitro* and *in vivo* effects of Cd, Cu, Pb and Ni on the plasmalemma ATPase and oxidoreductase from

cucumber seedling roots. *Acta Phys. Plant*. **16**: 297-302.

Chandan, B. K., Sharma, A. K., and Anand, K. K. (1991) *Boerhavia diffusa*: A study of its hepatoprotective activity. *J. Enthnopharmacol*. **31**: 299- 307.

Chandra, R., Bharagava, R. N., Yadav, S. and Mohan, D. (2009) Accumulation and distribution of toxic metals in wheat (*Triticum aestivum* L.) and Indian mustard (*Brassica campestris* L.) irrigated with distillery and tannery effluents. J. Hazard. Mater. **162**: 1514-1521.

Chatterjee, J. and Chatterjee, C. (2000) Zinc stress changes in the metabolism of blackgram (*Phaseolus mungo*) *Indian J. Agri. Sci*. **70**: 250-252.

Chen, Z., Zhu, Y. G., Liu, W. J. and Meharg, A. A (2005) Direct evidence showing the effect of root surface iron plaque on arsenite and arsenate uptake into rice (*Oryza sativa*) roots. *New Phytol*. 165: 91-97.

Cheraghi, M., Lorestani, B. and Yousefi, N. (2009) Effect of waste water on heavy metal accumulation in Hamedan province vegetables. *Int. J. Bot*.**5**: 190-193.

Chopra, G. L. (1969) Angiosperms Systematics and Life Cycles. S. Nagin and Jalandhar, Punjab, India 361- 365.

Ciura, J., Poniedzialek, M., Sekara, A. and Jedrszczyk, E. (2005) The possibility of using crops as metal phytoremediants. *Polish J. Environ. Stud.* **14**: 17-22.

Clemens, S., Palmagren, M. G. and Kraemer, U. (2002) A long way ahead: understanding the engineering plant metal accumulation. *Trends Plant Sci*. **7**: 309-315.

Clemens, S., Toxic metal accumulation, responses to exposure and mechanisms of tolerance in plants (2006). *Biochimie* **88**: 1707-1719.

Cobbet, C. S. (2003) Heavy metals and plants- model systems and hyper accumulators. *New Phytol*. **159**: 289- 293.

Cobbett, C.S. (2000) Phytochelatins and their roles in heavy metal detoxification. *Plant Physiol.* **123:** 825-832.

Cobbett, C.S. and Goldsbrough, P. (2002) Phytochelatins and metalothioneins: Roles in heavy metal detoxification and homeostasis. *Annu. Rev. Plant Biol.*. **53**: 159-182.

Cohen, C.K., Fox, T.C., Garvin, D.F. and Kochian, L.V. (1998) The role of iron-deficiency stress responses stimulating heavy-metal transport in plants. *Plant Physiol.* **116:** 1063-1072.

Corradi, M.G., Bianchi, A.K. and Albasini, A. (1993) Chromium toxicity in *Salvia sclarea*: 1. Effect of hexavalent chromium on seed germination and seedling development. *Environ. Expt. Bot*. **33**: 405-413.

Costa, G. and Morel, J. L. (1994) Water relations, gas exchange and amino acid content in Cd- treated lettuce. *Plant Physiol. Biochem*. **32-** 561-570.

Cseh, E. (2002) Metal permeability, transport and efflux in plants. In: M.N.V. Prasad and K. Strzalka (Eds.). *Physiology and Biochemistry of Metal*

Toxicity and Tolerance in Plants. Kluwer *Academic Publishers, Dordrecht* 1-36.

Cseh, E., Fodor, F., Varga, A. and Zaray, G. (2000) Effect of lead treatment on the distribution of essential elements in cucumber, *J. Plant Nutr*. **23**: 1095-1105.

Cunningham, C. D. and Berti, W. R. (1993) Remediation of contaminated soils with green plants: An overview. *In vitro Cell Dev. Biol*. **29**: 207- 212.

Daniel, G., Hussain, K. and Nabeesa- Salim, (2009) Bioaccumulation and effect of lead, chromium, and cadmium on peroxidase activity in bitter ground (*Momordica charantia* L. walp) cv. PRIYA *Inter. J. Plant. Sci.* **4**: 239-244

Davies, F.T., Puryear, J.D., Newton, R.J., Egilla, J.N. and Grossi, J.A.S. (2002) Mycorrhizal fungi increase chromium uptake by sunflower plants: influence on tissue mineral concentration, growth and gas exchange. *J. Plant Nutr*. **25**: 2389-2407.

Davies, K. L., Davies, M. S. and Francis, D. (1991) The influence of an inhibitor of phytochelatin synthesis on root growth and root meristematic activity in *Festuca rubra* L. in response to zinc. *New Phytol*. **118**: 565- 570.

De Fillippis, L. F. (1979) The effect of heavy metal compound on the permeability of *Chlorella* cells. *Z. Pflanzenphysiol*. **92**: 39-43.

De Vos, C.H.R., Vonk, M.J. and Schat, H. (1992) Glutathionine depletion due

to copper induced phytochelatin synthesis causes oxidative stress in *Silene cucubalus*. *Plant Physiol*., **98**: 853-858.

De, A.K., Sen, A.K., Modak, D.P. and Jana, S. (1985) Studies of toxic effect of Hg (II) on *Pistia stratiotes*. *Water Air Soil Pollut*. **24:** 351-360.

Delnomdedien, M., Boudou, A., Desmager, J.P. and Georgescauld, D. (1989) Interaction of $HgCl_2$ with the primary amino group of model membranes containing phosphatidyl serine and phosphatidyl ethanolamine. *Biochem. Biophys. Acta*. **986**: 191-199.

Dhar, M. L., Dha, M. M., Dhawan, B. N., Mehrotra, B. N. and Ray, C. (1968) Screening of Indian plants for biological activity: Part I *Indian J. Exp. Biol*. **6**: 232- 247.

Dixit, V., Pandey, V. and Shyam, R. (2002) Chromium ions inactivate electron transport and enhance superoxide generation *in vivo* in pea (*Pisum sativum* L. cv. Azad) root mitochondria. *Plant Cell Environ*. **25**: 687-690.

Dominguez- Solis, J. R., Lopez-Martin, M., Ager, F. J., Ynsa, M. D., Romero, L. C. and Gotor, C. (2004) Increased cysteine availability is essential for cadmium tolerance and accumulation in *Arabidopsis thaliana*. *Plant. Biotechnol. J.* **2**: 469-476.

Dunbabin, J. S. and Bowner, K. H. (1992) Potential use of constructed wetlands for treatment of industrial waste waters containing metals. *Sci. Total Environ.* **111**: 151-168.

Ebbs, S. D. and Kochian, I. V. (1998) Phytoextraction of zinc by oat (*Avena sativa*), barley (*Hordeum vulgare*) and Indian mustard (*Brassica juncea*) *Environ. Sci. Technol.* **32**: 802-806.

Ebinghaus, R., Tripathi, R. M. and Wallschlager, L. S.E (1999) Natural and anthropogenic mercury sources and their impact on the air- surface exchange of mercury on regional and global scales. In Ebinghaus, R., Turner, R. R., Lacerda, L. D., Vasilev O. and Salomons, W. (Eds.) *Mercury contaminated sites: Characterisation, Risk Assessment and Remediation*. New York, Springer- Verlag Berlin Heidelberg.

Ederli, L., Reale, L., Ferrauti, F. and Pasqualini, S. (2004) Responses induced by high concentration of cadmium in *Phragmites australis* roots. *Physiol. Plant.* **121:** 66-74.

Epstein, E. (1972) *Mineral Nutrition of Plants: Principles and Perspectives*. John Wiley and Sons, New York.

Escarre, J., Lefebure, C., Gruber, W., Leblanc, M., Lepart, J., Riviere, Y. and Delay, B. (2000) Zinc and cadmium hyperaccumulation by *Thlaspi caerulescens* from metalliferous and non metalliferous sites in the Mediterranean area: Implications for phytoremediation. *New Phytol.* **145**: 429- 437.

Falchuk, K.H., Goldwater, L. J. and Vallee, B. L. (1977) The biochemistry and toxicology of mercury. In: C. A. Mc Aulifee (Ed.). *The Chemistry of Mercury*. Mac Millan, London, 259-283.

Falk, H., Walling, R. and Au, W. (2003) A global approach to childhood lead poisoning prevention. *Int. J. Hyg. Environ. Health* **206**: 363-369.

Fett, J.P., Cambraia, J., Oliva, M.A. and Jorado, C.P. (1994) Cadmium uptake and growth inhibition in water hyacinth: Effect of nutrient solution factors. *J. Plant. Nutr*. **17**: 1205-1217.

Fitter, A.H. and Hay, R.K.M. (1983) *Environmental Physiology of Plants*. Academic Press, London.

Fitz, W. J. and Wenzel, W. W. (2002) Arsenic transformation in the soil rhizosphere plant system, fundamentals and potential application of

Fodor, F. (2002) Physiological responses of vascular plants to heavy metals. In: M.N.V. Prasad and K. Strzalka (Eds.). *Physiology and Biochemistry of Metal Toxicity and Tolerance in Plants.* Kluwer Academic Publishers, Dordrecht. 149-177.

Folin, O. and Denis, W. (1995) A Calorimetric method for the determination of phenols (and phenol derivatives) in urine. *J. Biol. Chem.* **22**: 305- 308.

Foy, C. D. Chaney, R.L. and White, M.C. (1978) The Physiology of metal toxicity in plants. *Annu. Rev. Plant Physiol*. **29:** 511-566.

Friedland, A. J. (1990) The movement of metals through soils and ecosystems. In: A. J. Shaw (Ed.). *Heavy Metal Tolerance in Plants: Evolutionary Aspects*. .CRC Press,

Florida. 7-19.

Gaal, O., Medgyesi, G.A. and Vereczkey, L. (1980) Electrophoresis in the separation of biological macromolecules. John Wiley and Sons, Chichester. 83-87.

Gbaruko, B. C., and Friday, O. U., (2007) Bioaccumulation of heavy metals in some fauna and flora. Int. J. Environ. Sci. Tech. **4**: 197-202.

Gleba, D., Borisjuk, N., Borisjuk, L., Kneer, R., Poulev, A., Skarzhinskaya, M., Dushenkov, S., Logendra, S., Gliba, Y. and Raskin, I. (1999) Use of plant roots for phytoremediation and molecular farming. *Proc. Natl. Acad. Sci.* **96**: 5973- 5977.

Glick, B. R. (2003) Phytoremediation: synergistic use of plants and bacteria to clean up the environment. *Biotechnol. Adv.* **21**: 383- 393.

Haag-Kerwer, A., Schafer, H. J., Heiss, S., Walter, C. and Rausch, T. (1999) Cadmium exposure in *Brassica juncea* causes decline in transpiration rate and leaf expansion without effect on photosynthesis. *J. Exp. Bot.* **50**: 1827-1835.

Hadjiliadis, N.D. (1997) Cytotoxicity, mutagen and carcinogenic potential of heavy metals related to human environment. In: *Environment* NATO- ASI Series, Kluwer, Dordrecht.

Hagemayor, J. (1993) Indicators for heavy metals in the terrestrial environment. In: B. Market (Ed.) *Plants as Biomonitors*. V.C.H. Weinheim, New York, 541-556.

Hale, K.L., McGrath, S.P., Lombi, E., Stack, S.M., Terry, N., Pickering, I.J., George, G.M. and Pilon-Smits, E.A.H. (2001) Molybdenum sequestration in *Brassica* species. A Role for Anthocyanins? *Plant Physiol.* **126:** 1391-1402.

Hall, J.L. (2002) Cellular mechanisms for heavy metal detoxification and tolerance. *J. Exp. Bot.* **53:** 1-11.

Hamlin, R. L., Schatz, C. and Baker, A. V. (2003) Zinc accumulation in Indian mustard as influenced by nitrogen and phosphorus nutrition. *J. Plant Nutr*. **26**: 177-190.

Han, F.X., Sridhar, B.B.M., Monts, D.L. and Su, Y. (2004) Phytoavailiability and toxicity of trivalent and hexavalent chromium to *Brassica juncea*, *New Phytol*. **162**: 489.

Hattori, H., Kuniyasu, K., Chiba, K. and Chino, M. (2006) Effect of chloride application and low soil pH on cadmium uptake from soil by plants. *Soil Sci. Plant Nutr*. **52**: 89-94.

Hendry, G.A.F., Baker, A.J.M. and Ewart, C.F. (1992) Cadmium tolerance and toxicity, oxygen radical processes and molecular damage in cadmium- tolerant and cadmium sensitive- clones of *Holeus lanatus* L. *Acta Bot. Neerl*. **41**: 271-281.

Henry, J. R. (2000) Phytoremediation of mercury In: An overview of the phytoremediation of lead and mercury. A report for the U. S. *Environmental Protection Agency*. *Washington*, D. C., USA. 44-46.

Hiruma – Lima, C. A., Grazioso, J. S., Bighetti, E.J., Germonsen Robineou, L. and Souza Brito, A. R. (2000) The juice of fresh leaves of *Boerhavia diffusa* L. (Nyctaginaceae) markedly reduces pain in mice. *J. Ethnopharmacol*. **71**: 267-274.

Hosseini, R. H., Khanlarian, M., and Ghorbanli, M. (2007) Effect of lead on germination, growth and activity of catalase and peroxidase enzyme in root and shoot of two cultivars of *Brassica napus* L. *J. Biol. Sci.* **4**: 592-598.

Houston, M.C. (2007) The role of mercury and cadmium heavy metals in vascular disease, hypertension, coronary heart disease and myocardial infarction. *Altern. Ther. Health Med*. **13**: 128-133.

Huang, J.W. and Cunningham, S.D. (1996) Lead phytoextraction: species variation in lead uptake and translocation. *New Phytol*. **134**: 75-84.

Huang, J.W., Chen, J., Berti, W.R. and Cunningham, S.D. (1997) Phytoremediation of lead–contaminated soils: role of synthetic chelators in lead phytoextraction. *Environ. Sci. Technol*. **31**: 800-805.

Hussain, K. (2007) *Ecophysiological Aspects of Bacopa monnieri* (L.) Pennell. Ph.D. Thesis submitted to the University of Calicut.

Hussain, K., Abdussalam, A. K., Ratheesh- Chandra, P. and Nabeesa-Salim (2010) Bioaccumulation of heavy metals in *Bacopa monnieri* (L.) Pennell growing under different habitat. *Int. J. Ecol. Devel.* **10**:66-73.

Iqbal, M.Z., Saeeda, S. and Muhammed, S. (2001) Effects of chromium on an important arid tree (*Caesalpinia pulcherima*) of Karachi city, Pakistan. *Ekol Bratislava*. **20**: 414-22.

Ishikawa, S., Ae, N., Murakami, M. and Wagatsuma, T. (2006) Is *Brassica juncea* a suitable plant for phytoremediation of cadmium in soils with moderately low cadmium contamination?- Possibility of using other plant species for Cd- phytoextraction. *Soil Sci. Plant Nutr*. **52**: 32-42.

Jain, M. and Puranik, M. (1993) Protective effect of reduced glutathione on inhibition of chlorophyll biosynthesis by mercury in excised greening maize leaf segments. *Indian J. Exp. Biol*. **31**: 708-710.

Jalil, A., Selles, F. and Clarke, J.M. (1994) Effect of cadmium on growth and uptake of cadmium and other elements by Durum Wheat. *J. Plant Nutr*. **17**: 1839-1858.

Jamal, S.N., Iqbal, M.Z. and Athar, M. (2006) Effect of aluminum and chromium on the germination and growth of two *Vigna species*. *Int. J. Environ. Sci. Techol*. **3**: 53-58.

Jeana, S. and Chaudhuri, M.A. (1982) Senescence in submerged aquatic angiosperms: Effect of heavy metals. *New phytol*. **90**: 477-484.

Jiang, W., Liu, D. and Hou, W. (2000) Hyperaccumulation of lead by roots, hypocotyls and shoots of *Brassica juncea*. *Biol. Plant*. **43:** 603-606.

Joop, A. K., Marjolein, D., Paul, L. M. K., Henk, S., Jos, A. C. V. and Wilfried, H. O. E. (1994) Phytochelatin in cadmium sensitive and cadmium tolerant *Silene vulgaris*. *Plant Physiol*. **104**: 255-261.

Kabata- Pendias A. and Pendias, H (1991) *Trace Elements in Soil and Plants* (3rd Edn). CRC Press. LLC. Boka Raton, FL.

Kabata-Pendias, A. and Pendias, H. (1999) *Biogeochemistry of Trace Elements* in (Polish) PWN. Warszawa. Poland.

Kagi, J.H.R. and Hapke, H.J. (1984) Biochemical interactions of mercury, cadmium and lead. In: J.O. Nriagu (Ed.). *Changing Metal Cycles and Human Health*. Springer – Verlag, New York. 237-250.

Kahle, H. (1993) Response of roots of trees to heavy metals. *Environ. Exp. Bot.* **33**: 99-119.

Kamal, M., Ghaly, A. E., Mahmoud, N. and Cote, R. (2004) Phytoaccumulation of heavy metals by aquatic plants. *Environ. Int*. **29**: 1029- 1039.

Kastori, R., Petrovic, N. and Arsenijevic, M.I. (1995) Effect of lead on the activities of some enzymes of nitrogen metabolism in sugar beet (*Beta vulgaris* L.). Glasnik Instituta Za Botanika I Botanicke Baste Univerziteta u Beograda. **29**: 77-84.

Kastori, R., Plenicar, M., Sakai, Z., Pankovic, D. and Maksimovic, A.I. (1998) Effect of excess lead on sunflower growth and photosynthesis. *J. Plant Nutr*. **21**: 75-85.

Khatun, S. Ali, M. B., Hahn, E. and Paek, K. (2008) Copper toxicity in *Withania somnifera*: Growth and antioxidant enzymes responses of *in vitro* grown plants. *Environ. Exp. Bot*. **64**: 279- 285.

Kim, Y. Y., Yang, Y.Y. and Lee, Y. (2002) Pb and Cd uptake in rice roots. *Physiol. Plant.* **116**: 368-372.

Kiran, Y., and Sahin, A., (2006) The effects of cadmium on seed germination, root development and mitotic of root tip cells of lentil (*Lens culinaris* Medik). *World J. Agri. Sci.* **2** 196-200.

Kochian, L.V. (1991) Mechanisms of micronutrient uptake and translocation in plants. In: J.J. Mordtnet, F.R. Cox, L.M. Shuman and R.M. Welch (Eds.). *Micronutrients in Agriculture*. Soil Science Society of America, Madison, WI. 229-296.

Kochian, L.V. (1995) Cellular mechanisms of aluminum toxicity and resistance in plants. *Ann. Rev. Plant Physiol. Plant Mol. Biol.* **46**: 237-260.

Koeppe, D.E. (1981) Lead: understanding the minimal toxicity of lead in plants. In: N.W. Lepp (Ed.) *Effects of Heavy Metal Pollution on Plants*. Applied Science Publishers, London and New Jersey. **1**: 55-76.

Kos, B., Greman, H. and Lestan, D. (2003) Phytoextraction of lead, zinc and cadmium from soil by selected plants. *Plant. Soil. Environ.* 49: 548-553.

Krupa, Z. and Baszynski, T. (1995) Some aspects of heavy metals toxicity towards photosynthetic apparatus: Direct and indirect effects on light and dark reactions. *Acta. Physiol. Plant.* **17**: 177-190.

Krupa, Z., Ruszkowski, M. and Gilowska-Juny, E. (1982) The effect of

chromate on the synthesis of plastid pigments and lipoquinones in *Zea mays* seedlings. *Acta Soc. Bot. Pol*. **51**: 275-281.

Kumar, N.P.B.A., Dushenkov, V., Motto, H. and Raskin, I. (1995) Phytoextraction: The use of plants to remove heavy metals from soil. *Environ. Sci. Technol*. **29:** 1232-1238.

Kupper, H., Kupper, F. and Spiller, M. (1998) *In Situ* detection of heavy metal substituted chlorophylls in water plants. *Photosynth. Res*. **58:** 123-133.

Kupper, H., Zhao, F.J. and McGrath, S.P. (1999) Cellular compartmentation of zinc in leaves of the hyper accumulator, *Thlaspi caerulescens*. *Plant Physiol*. **119:** 305-311.

Landrigan, P. J. (1999) Risk assessment for children and other sensitive populations. *Ann. N.Y.* Acad. Sci., **895**: 1-9.

Lang, F., Sarvari, E., Szigeti, Z., Fodor, F. and Cseh, E. (1995) Effect of heavy metals on the photosynthetic apparatus in cucumber. In: P. Mathis (Ed.). *Photosynthesis: From Light to Biosphere*. Vol. IV. Kluwer Academic Publishres, Netherlands. 533-536.

Lata, S. (1989) Effect of cadmium on seedlings growth, mobilization of food reserves and activity of hydrolytic enzymes in *Phaseolus aureus* L. seeds. *Acta Bot*. **17**: 290-293.

Lee, Y. P. and Takahashi, T. (1966) An improved colorimetric determination of amino acids with the use of ninhydrin. *Anal. Biochem*. **14**: 71-77.

Lenka, M., Das, B.L., Panda, K.K. and Panda, B.B. (1993) Mecury-tolerance of

Chloris barbata Sw. and *Cyperus rotundus* L. isolated from contaminated sites. *Biol. Plant*. **35:** 443-446.

Levitt, J. (1980) *Responses of Plants to Environmental Stress*. (2nd Edn. Vol.2) *Academic Press*, New York.

Liang, Y., Chen, Q., Liu, Q. and Zhang, W., R., (2003) Exogenous silicon (Si) increases antioxidant enzyme activity and reduces lipid peroxidation in roots of salt-stressed barley (*Hordeum vulgarae* L.) *Plant Phsiol.* **160**: 1157-1164.

Lidsky, T.I., and Schneider, J.S (2006) Adverse effects of childhood lead poisoning: the clinical neuropsychological perspective. *Environ. Res.* **100**: 284-293.

Linger, P., Ostwald, A. and Haensler, J. (2005) *Cannabis sativa* L., growing on heavy metal contaminated soil: Growth, cadmium uptake and photosynthesis. *Biol. Plant*. 49: 567-576.

Lowry, O.H., Rosenbrough, N.J., Farr, A.L. and Randall, R.J. (1951) Protein measurement with Folin-Phenol reagent. *J. Biol. Chem.* **193:** 265-275.

Maitani, T., Kubota, H., Satoh, K. and Yamada, T. (1996) The composition of metals bound to Class III metallothionein (Phytochelatin and its desglycyl peptide) induced by various metals in root cultures of *Rubia tinctorium*. *Plant Physiol*. **110**: 1145-1150.

Malajczuk, N. and Dell, B. (1995) Heavy metals in Philipines soils affect growth and establishment of trees. *ACIR-For. News Letter*. **18**: 2-3.

Malkowski, E., Kitta, A., Galas, W., Karcz, W. and Kuperberg, M.J. (2002) Lead distribution in corn seedlings (*Zea mays* L.) and its effect on growth and the concentrations of potassium and calcium. *Plant Growth Regulation*. **37**: 69-76.

Marschner, H. (1983). General introduction to the mineral nutrition of plants. In: A. Lauchli and R.L. Bielesko (Eds.). *Encyclopedia of Plant Physiology (New Series) Inorganic Plant Nutrition*. Springer Verlag, New York. **15A**: 5-60.

Marschner, H. (1986) *Mineral Nutrition of Higher Plants*. Academic Press. England.

Mazen, A.M.A., and Maghraby, O.M.O. (1997) Accumulation of cadmium, lead, strontium and role of calcium oxalate in water hycianth tolerance. *Biol. Plant*. **40**: 411-417.

McGrath, S. P. and Zhao, F. J. (2003) Phytoextraction of metals and metalloids from contaminated soils. *Curr. Opin. Biotechnol*. **14**: 1-6.

McGrath, S. P., Shen, Z. G. and Zhao, F. J. (1997) Heavy metal uptake and chemical changes in the rhizosphere of *Thlaspi caerulescens* and *Thlaspi ochroleucum* grown in contaminated soils. *Plant and Soil* **188**: 153- 159.

McGrath, S.P. (1982) The uptake and translocation of tri- and hexavalent chromium and effects on the growth of oat in flowing nutrient solution and in soil. *New Phytol*. **92**: 381-390.

McNeilly, T. (1994) Metal toxicity. In: A. R. Yeo and T. J. Flowers (Eds.). *Soil Mineral Stresses: Approaches to Crop Improvement*. Springer-Verlag, New York, 145- 174.

Meers, E., Vaudecasteele, B., Ruttens, A., Vangrousveld, J., Tack, F.M.G., (2007). Potential of five Willo species (*Salix* spp.) for phytoextraction of heavy metals. *Environ. Exp. Bot.* **60**: 57-68.

Mehrotra, S., Mishra, K. P., Maurya, R., Srimal and Singh, V. K. (2002) Immunomodulation by ethanolic extract of *Boerhavia diffusa* roots. *Int. Immunopharmacol* **2**: 987- 996.

Meidner, H. and Mansfield, T. A. (1968) *Physiology of Stomata*. McGraw Hill, Lindon.

Meuwly, P., Thibault, P., Schwan, A.L. and Rauser, W.R. (1995) Three families of thiol peptides are induced by Cd in maize. *Plant J.* **7:** 391-400.

Michalak, A., Phenolic compounds and their antioxidant activity in plants growing under heavy metal stress (2006) *Polish J. Environ. Stud*. **15**: 523-530.

Michalak, E. and Wierzbicka, M. (1998) Differences in lead tolerance between *Allium cepa* plants developing from seeds and bulbs. *Plant Soil*. **199**: 251-260.

Migocka, M. and Klobus, G. (2007) The properties of the Mn, Ni and Pb transport operating at plasma membranes of cucumber roots.

Physiol. Plant. **129:** 578-587.

Mishra, A. and Choudhuri, M.A. (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.

Monni, S., Uhlig, C., Hunttila, O., Hausen, E. and Hynynen, J. (2001) Chemical composition and ecophysiological responses of *Eupatorium nigrum* to above ground element application. *Environ. Pollut.* **112:** 417-426.

Moodley, K. G., Baijnath, H., Southway- Ajulu, F. A., Maharaj, S. and Chetty, S. R. (2007) Determination of Cr, Pb and Ni in water, sludge and plants from settling ponds of a sewage treatment works. *Water* SA **33**: 723- 728.

Moral, R., Gomes, I., Pedreno, J.N. and Mataix, J. (1996) Absorption of chromium and effects on micronutrient content in tomato plants (*Lycopersicon esculentum*). *Agrochimica* **11**: 138-145.

Moral, R., Palacios, G., Gomes, I., Pedreo, J.N. and Mataix, J. (1994) Distribution and accumulation of heavy metals (Cd, Ni and Cr) in tomato plant. *Fresnius Environ. Bull*. **3**: 395-399.

Moral, R., Pedreno, J.N., Gomes, I. and Mataix, J. (1995) Effects of chromium on the nutrient element content and morphology of tomato. *J. Plant Nutr*. **18**: 815-822.

Moreno, F. N., Anderson, C. W. N., Stewart, R. B. and Robinson, B. H. (2005) Mercury volatilisation and phytoextraction from base- metal mine tailings. *Environ. Pollut.* **136**: 341- 352.

Moreno, F. N., Anderson, C. W. N., Stewart, R. B., Robinson, B. H. Ghomshei, M. and Meech, J. A. (2005) Induced plant uptake and transport of mercury in the presence of sulphur containing ligands and humic acid. *New Phytol*. **166**: 445-454.

Moreno, F. N., Anderson, C. W. N., Stewart, R. B., Robinson, B. H., Nomura, R., Ghomshei, M. and Meech, J. A. (2005) Effect of thioglands on plant- Hg accumulation and volatilisation from mercury – contaminated mine tailings. *Plant soil* **275**: 233-246.

Moreno, F. N., Anderson, C.W. N., Stewart, R. B. and Robinson, B. H. (2004) Phytoremediation of mercury- contaminated mine tailings by induced plant- mercury accumulation . *Environ. Practice* **6**: 165- 175.

Moreno, F. N., Anderson, C.W.N., Stewart, R. B. and Robinson, B. H. (2008) Phytofiltration of mercury – contaminated water: Volatilisation and plant – accumulation aspects. *Environ. Exp. Bot*. **62**: 78-85.

Moreno-Caselles, J., Moral, R., Perez- Espinosa, A. and Perez-Murcia, M.D. (2000) Cadmium accumulation and distribution in cucumber plant. *J. Plant Nutri*., **2**: 29-34.

Mysliwa-kurdziel, B. and Strzalka, K. (2002) Influence of metals on

biosynthesis of photosynthetic pigments. In: M.N.V. Prasad and K. Strzalka (Eds.). *Physiology* and *Biochemistry of Metal Toxicity* and *Tolerance in Plants*. Kluwer Academic Publishers, The Netherlands. 201-227.

Nag, P., Paul, A.K and Mukherji, S. (1980) Effects of mercury, copper and zing on the growth, cell division, GA. induced amylase synthesis and membrane permeability of plant tissues. *Indian J. Exp. Biol*. **18**: 822-827.

Nagajyoti, P. C., Dinakar, N., Prasad, T. N. V. K. V., Suresh, C. and Damodharam, T. (2008) Heavy metal toxicity: Industrial effluent effect on groundnut (*Arachis hypogaea* L.) seedlings. *J. Appl. Sciences Research*. **4**: 110-121.

Nath, S., Chaudhury, J., Pahan, K. and Mandal, A. (1993) Mercury and organomercurial resistance in rhizobial strains. *Indian J. Exp. Biol*. **31:** 50-53.

Obata, H., Inoue, N., Imai, K., Umebayashi, M. (1994) Cadmium tolerance of calli induced from roots of plants with difference in cadmium tolerance. *Soil Sci. Plant Nutr*., **40**: 351-354.

Orcutt, D. M. and Nilsen, E. T. (2000) *Physiology of Plants Under Stress: Soil and Biotic Factors*. John Wiley & Sons, Inc. New York.

Ouariti, O., Boussama, N., Zarrouk, M., Cherif, A. and Ghorbal, M.H. (1997)

Cadmium and copper induced changes in tomato membrane lipids. *Phytochemistry*. **45**: 1343-1350.

Pance, N.S., Larsen, P.B., Ebbs, S.D., Letham, D.L., Lasat, M.M., Garvin, D.F., Eide, D. and Kochian, L.V. (2000) The molecular physiology of heavy metal transport in the Zn/Cd hyperaccumulator *Thlaspi caerulescens. Proc. Natl. Acad. Sci.* U.S.A. **97:** 4956-4960.

Pance, N.S., Larsen, P.B., Ebbs, S.D., Letham, D.L., Lasat, M.M., Garvin, D.F., Eide, D. and Kochian, L.V. (2000) The molecular physiology of heavy metal transport in the Zn/Cd hyperaccumulator *Thlaspi caerulescens. Proc. Natl. Acad. Sci.* U.S.A. **97:** 4956-4960.

Panda, S.K. (2003) Heavy metal phytotoxicity induces oxidative stress in *Taxithelium* sp. *Curr. Sci.* **84**: 631-633.

Panda, S.K. and Choudhury, S. (2005) Chromium stress in plants. *Braz. J. Plant Physiol*. **17**: 95-102.

Panda, S.K. and Patra, H.K. (1997) Physiology of chromium toxicity in plants. A Review. *Plant Physiol. Biochem*. **24**: 10-17.

Panda, S.K. and Patra, H.K. (1998) Alteration of nitrate reductase activity by chromium ions in excised wheat leaves. *Ind. J. Angio. Biochem*. **2**: 56-57.

Panda, S.K. and Patra, H.K. (2000) Does Cr. (III) produce oxidative damage in excised wheat leaves. *J. Plant. Biol*. **27**: 105-110.

Panda, S.K., Chaudhury, L. and Khan, M.H. (2003) Heavy metals induce lipid peroxidation and affects antioxidants in wheat leaves. *Biol. Plant.* **46**: 289-294.

Parekh, D., Puranik, R. M. and Srivastva, H. S. (1990) Inhibition of chlorophyll biosynthesis by cadmium in greening maize leaf segments. *Biochem.*

Physiol. Pflanzen. **186**: 239- 242.

Parmar, N.G. and Chanda, S.V. (2005) Effects of mercury and chromium on peroxidase and IAA oxidase enzymes in the seedlings of *Phaseolus vulgaris*. *Tur. J. Biol.* **29**: 15-21.

Parmer, N. G. Vithalani, S. D. and Chanda, S. V. (2002) Alteration in growth and peroxidase activity by heavy metals in *Phaseolus* seedlings. *Acta Physiol. Plant*. **24**: 89-95.

Perfus-Barbeoch, L., Leonhardt, N., Vavasseur, A. and Forestier, C. (2002) Heavy metal toxicity: Cadmium permeates through calcium channels and disturbs the plant water status. *The Plant J.* **32:** 539-548.

Perfus-Barbeoch, L., Leonhardt, N., Vavasseur, A. and Forestier, C. (2002) Heavy metal toxicity: Cadmium permeates through calcium channels and disturbs the plant water status. *The Plant J.* **32:** 539-548.

Petr, J., Skerik, J. and Dlouby, J. (1999) Cadmium, lead and mercury contents in ecologically grown crops. *Sci. Agri. Bohemica* **30:** 285-299.

Pilon-Smits, E. (2005) Phytoremediation. *Annu. Rev. Plant Biol.* **56:** 15-39.

Podor, D., Ramsey, M. H. and Hutchings, M. J. (2004) Effect of cadmium, zinc and substrate heterogeneity on yield, shoot metal concentration and

metal uptake by *Brassica juncea*: Implications for human health risk assessment and phytoremediation. *New Phytol*. **163**: 313-324.

Poschenrieder, C., Vazquez, M.D., Bonet, A. and Barcelo, J. (1991) Chromium III iron interaction in iron sufficient and iron deficient bean plants. II Ultra structural aspects. *J. Plant. Nutr*. **14**: 415-428.

Posmyk, M. M., Kontek, R. and Janas, K. M (2009) Antioxidant enzymes activity and phenolic compounds content in red cabbage seedlings exposed to copper stress. Ecotoxicol. Environ. Saf. **72**: 596-602.

Prasad, D.D.K. and Prasad, A.R.K. (1987) Effect of lead and mercury on chlorophyll synthesis in mung bean seedlings. *Phytochem.* **26:** 881-883.

Prasad, M.N.V. (1997) Trace metals. In: M.N.V. Prasad (Ed.). *Plant Ecophysiology*. John Wiley & Sons, Inc. 207-249.

Prasad, M.N.V. (1998) Metal-biomolecule complexes in plants: occurrence, functions and applications. *Analysis*. 26-28.

Prasad, S.M., Singh, J.B., Rai, L.C. and Kumar, H.D. (1991) Metal induced inhibition of photosynthetic electron transport chain of cyanobacterium, *Nostoc muscarum*. *FEMS Microbiol. Lett*. **82:** 95-100.

Pratima, N.P., Paul, A.K. and Mukherji, S. (1989) The effects of heavy metals, Zn and Hg on the growth and biochemical constituents of mung bean (*Vigna radiata*) seedlings. *Bot. Bull. Acad. Sin.* (Taipei) 30: 241-250.

Pulford, I.D., Watson, C. and McGregor, S.D. (2001) Uptake of chromium by trees: prospects for phytoremediation. *Environ. Geochem. Health*. **23**: 307-11.

Radiotic, K., Ducic, T. and Mutavdzic, D. (2000) Changes in peroxidase activity and isoenzymes in spruce needles after exposure to different concentration of cadmium. *Environ. Exp*. **44**: 105- 113.

Rai, U.N., Tripathi, R.D., Vajpayee, P., Pandey, N., Ali, M.B. and Gupta, D.K. (2003) Cadmium accumulation and its phytotoxicity in *Potamogeton pectinatus* L. (Potamogetonaceae). *Bull. Environ. Contam. Toxicol*. **70:** 566-575.

Rai, V., Vajpayee, P., Singh, S. N. and Mehrotra, S. (2004) Effect of chromium accumulation on photosynthetic pigments, oxidative stress defense system, nitrate reduction, proline level and eugenol content of *Ocimum tenuiiflorum* L. Plant Science **167**: 1159- 1169.

Ramabhimaiah, S., Stalin, D. and Kalaskar, N. J (1984) Pharmacological investigations of the water soluble fraction of the methanol extracts of *Boerhavia diffusa* roots. *Indian Drugs* **21**: 343- 344.

Raskin, I. and Ensley, B.D. (2000) *Phytoremediation of Toxic Metals Using Plants to Clean up the Environment.* John Wiley & Sons. Inc. New York.

Raskin, I. and Ensley, B.D. (2000) *Phytoremediation of Toxic Metals Using Plants to Clean up the Environment.* John

Wiley & Sons. Inc. New York.

Raskin, I., Kumar, N. P. B. A., Dushenkov, S. and Salt, D. E.(1994) Bioconcentration of heavy metals by plants. *Curr. Opin. Biotechnol.* **5**: 285-290.

Rauser, W. E. (1990) Phytochelatins. *Annu Rev. Biochem*. **59**: 61-86.

Rauser, W. E. (1995) Phytochelatins and related peptides: Structure, biosynthesis and function. *Plant Physiol*. **109**: 1141-1149.

Rauser, W.E. (1987) Changes in glutathione content of maize seedlings exposed to cadmium. *Plant Sci*. **51:** 171-175.

Reed, R. H. and Gadd, G. M. (1990) Metal tolerance in eukaryotic and prokaryotic algae. In: A. J. Shaw (Ed.). *Heavy Metal Tolerance in Plants: Evolutionary Aspects*. CRC Press, Florida. 105-118.

Reid, R.J., Dunbar, K.R. and McLaughlin, M.J. (2003) Cadmium loading into potato tubers: the roles of the periderm, xylem and phloem. *Plant Cell Environ.* **26:** 201-206.

Riedel, G.F. (1985) The relationship between chromium (IV) uptakes, sulphate uptake and chromium (IV) toxicity in the estuarine diatom *Thalassionira pseudonama*. *Aquatic Toxicol*. **7**: 191-204.

Ross, H.B. (1994) On the use of mosses (*Hylocomium splendens* and *Pleurozium schreberi*) for estimating atmospheric trace metal deposition. *Water Air Soil Pollut*. **50**: 63-76.

Rout, G.R., Samantaray S. and Das, P. (1997) Different chromium tolerance

among eight mung bean cultivars grown in nutrient culture. *J. Plant Nutr*. **20**: 341-347.

Rout, G.R., Sanghamitra, S. and Das, P. (2000) Effect of chromium and nickel on germination and growth in tolerant and non-tolerant populations of *Echinochola colona* (L.) *Chemosphere*. **40**: 855-859.

Sahadevan, K.K. (2001) Physiological and biochemical studies on mercury toxicity in *Vigna mungo* (L.) Hepper seedlings. Ph. D. Thesis submitted to the University of Calicut.

Salati, E., Eneida, S. and Salati, E. (1999) Wetlands Projects Developed in Brazil. *Water Sci. Technol*. **40**: 19.

Salt, D. E., Smith, R. D. and Raskin, I. (1998) Phytoremediation. *Annu. Rev. Plant. Physiol. Mol. Biol*. **49**: 643-668.

Salt, D.E. and Rauser, W.E. (1995) Mg ATP-dependent transport of phytochelatins across the tonoplast of oat roots. *Plant Physiol*. **107:** 1293-1301.

Samantary, S. and Das, P. (1997) Accumulation and distribution of chromium, nickel and iron in the mung bean plant. *Fresnius Envir. Bull*. **6**: 633-641.

Samantary, S., Rout, G.R. and Das, P. (1996) A study of soil plant and root-shoot relationship in rice (*Oryza sativa* L. cv. Pathan) grown on chromiferous mine soil. *Proc. Natl. Acad. Sci*. USA. **66**: 349-357.

Samantary, S., Rout, G.R. and Das, P. (1998) Role of chromium on plant growth

and metabolism. *Acta Physiol. Planta*. **20**: 201-212.

Sandmann, G. and Boger, P. (1983). The enzymological function of heavy metals and their role in electron transfer process of plants. In: A. Lauchli and R.L. Bieleski (Eds.). *Encyclopedia of Plant Physiology*, (New Series) Vol. **15B**. *Inorganic Plant Nutrition*. 563-596.

Sanita-di-Toppi, L. and Gabbrielli, R. (1999) Response to cadmium in higher plants. *Environ. Exp. Bot.* **41:** 105-130.

Satoh, M., Karaki, E., Kakehashi, M., Okazaki, E., Gotoh, T. and Oyama, Y. (1999) Heavy-metal induced changes in non proteinaceous thiol levels and heavy metal binding peptides in *Tetraselmis tetrathele* (Prasinophyceae). *J. Phycol.* **35**: 989-928.

Scheller, H.V., Huang, B., Hatch. E. and Goldsbrough, P.B. (1987) Phytochelatin synthesis and glutathione levels in response to heavy metals in tomato cells. *Plant Physiol*. **85**: 1031-1035.

Sergin, I. V. and Ivanov, V. B. (2001) Physiological aspects of Cadmium and lead toxic effects on higher plants. *Russ.J. Plant Physiol*. **48**: 523-544.

Sersen, F., Clik, G., Havranek, E. and Sykorova, M. (2005) Bioremediation by natural zeolite in plants cultivated in a heavy metal contaminated medium. *Fresenius Environ. Bull*. **14**: 13- 17.

Setia, R.C. and Bala, R. (1994) Anatomical changes in root and stem of wheat (*Triticum aestivum* L.) in response to different heavy metals.

Phytomorph. **44**: 95-104.

Shanker, A.K., Cervantes, C., Loza-Tavera, H. and Avudainayagam, S. (2005) Chromium toxicity in plants. *Environ. Intl*. **31**: 739-753.

Shanker, A.K., Djnaguiraman, M., Sudhagar, R., Chandrashekar, C.N. and Pathmanabhan, G. (2004) Differential antioxidative response of ascorbate glutathione pathway enzymes and metabolites to chromium speciation stress in green gram (*Vigna radiata* (L.) R. Wilczek. cv CO 4) roots. *Plant Sci*. **166**: 1035-1043.

Shaw, B. P. and Rout, N. P. (1998) Age-dependent responses of *Phaseolus aureus* Roxb. to inorganic salts of mercury and cadmium. *Acta Physiol. Plant*. **20**: 85-90.

Shaw, B.P. (1995) Effect of mercury and cadmium on the activities of antioxidative enzymes in the seedlings *of Phaseolus aureus*. *Biol. Plant*. **37**: 587-596.

Shen, Z. G., Zhao, F. J. and McGrath, S. P. (1997) Uptake and transport of zinc in the hyperaccumulator *Thlaspi caerulescens* and the nonhyperaccumulator of *Thlaspi ochroleucum*. *Plant Cell Environ*. **20**: 898- 906.

Sheoran, I.S., Aggarwal, H. and Singh, R. (1990) Effect of cadmium and nickel on *in vivo* carbon dioxide exchange rate of pigeon pea. *Plant Soil* **129:** 243-249.

Shinmachi, F., Kumanda, Y., Noguchi, A. and Hasegawa, I. (2003) Translocation and accumulation of cadmium in cadmium tolerant *Polygonum thunberjii*. *Soil Sci. Plant Nutr*. **49**: 355- 361.

Shu, W.S., Ye, Z.H., Lan, C.Y., Zhang, Z.Q. and Wong, M.H. (2002) Lead, zinc and copper accumulation and tolerance in populations of *Paspalum distichum* and *Cynodesmon dactylon*. *Environ. Pollut*. **120:** 445- 453.

Siedlecka, A. and Krupa, Z. (1997) Cd/Fe interaction in higher plants- its consequences for the photosynthetic apparatus. *Photosynthetica* **36**: 321- 331.

Singh, A.K. (2001) Effect of trivalent and hexavalent chromium on spinach (*Spinacea oleracea* L.) *Environ. Ecol*. **19**: 807- 810.

Singh, R. H. and Udupa, K. N. (1972) Studies on the Indian indigenous drug punarnava (Boerhavia diffusa Linn.) Part IV: Preliminary controlled clinical trial in nephritic syndrome *J. Research Indian Medicine* **7**: 28- 33.

Singh, V. and Pandey, R. P. (1980) Medicinal plantlore of the tribals of eastern Rajasthan. *J. Economoic Taxonomic Botany* 137- 147.

Sinha, S. (1999) Accumulation of Cu, Cd, Cr, Mn, and Pb from artificially contaminated by soil *Bacopa monnieri*. *Environ. Monit. Assess*. **57**: 253-264.

Sinha, S., Gupta, M. and Chandra, P. (1996) Bioaccumulation and biochemical

effect of mercury in the plant *Bacopa monnieri* (L.) *Environ. Toxicol. Wat. Qual*. **11**: 105-112.

Sivarajan, V. V. and Balachandran, I. (1994) *Ayurvedic Drugs and Their Plant Sources*. Oxford and IBH Publishing Co., New Delhi.

Skinner, K., Wright, N. and Porter-Goff, E. (2007) Mercury uptake and accumulation by four species of aquatic plants. *Environ. Pollut*. **145**: 234-237.

Sonmez, O., Bukun, B., Kaya, C. and Aydemir, S. (2008) The assessment of tolerance to heavy metals (Cd, Pb and Zn) their accumulation in three weed species. Pak. J.Bot. **40**: 747-754.

Stefanov, K.L., Popova, I., Kamburova, E., Pancheva., Kimenov, G., Kuleva, L. and Popv, S. (1993) Lipid and sterol changes in *Zea mays* caused by lead ions. *Phytochemistry*. **33**: 47-51.

Stefanov, K.L., Popova, I., Kamburova, E., Pancheva., Kimenov, G., Kuleva, L. and Popv, S. (1993) Lipid and sterol changes in *Zea mays* caused by lead ions. *Phytochemistry*. **33**: 47-51.

Steffens, J.C. (1990) The heavy metal binding peptides of plants. *Annu. Rev. Plant Physiol. Plant Mol. Biol*. **41**: 533-575.

Stobart, A. K., Griffiths, W. T., Ameen- Bukhari, I. and Sherwood, R. P. (1985) The effect of Cd^{2+} on the biosynthesis of chlorophyll in leaves of barley. Physiol. Plant. 63: 293-298.

Stohs, S.J. and Bagchi, D. (1995) Oxidative mechanisms in the toxicity of metal ions. *Free Radicals Biol. Med.* **18:** 321-336.

Stolt, P., Asp, H. and Hultin, S. (2006) Genetic variation in wheat cadmium accumulation on soils with different cadmium concentrations. *J. Agron. Crop Sci.* **192**: 201-208.

Stroinski, A., and Zielezinska, M (2001) Cadmium and oxidative stress influence on phytochelatin synthase activity in potato tuber *Acta. Physiol. Plant*. **23**: 157-160.

Taiz, L. and Zeiger, E. (2002) *Plant Physiology:* (IIIrd Edn.) Sinauer Associates, Inc. Publishers, Sunderland, Massachussetts.

Tanaka, K., Fujimaki, S., Fujiwara, T., Yoneyama, T. and Hayashi, H. (2007) Quantitative estimation of the contribution of the phloem in cadmium transport to grains in rice plants (Oryza sativa L.). *Soil Sci. Plant Nutr*. **53**: 72-77.

Tomar, M., Kaur, I., Neelu, A. and Bhatnagar, A. K. (2000) Effect of enhanced lead in soil on growth and development of *Vigna radiata* (Linn.) Wilczek. *Indian J. Plant Physiol*. **5**: 13-18.

Tripathi, A. K., Tripathi, S. and Tripathi, S. (1999) Changes in some physiological and biochemical characters in *Albezia lebbek* as bio-indicators of heavy metal toxicity. *J. Environ. Biol.* **20**: 93-98.

Tukendorf, A. and Rauser, W. E. (1990) Changes in glutathione and phytochelation in roots of maize seedlings exposed to cadmium. *Plant Sci*. **70**: 155-166.

Turner, A. P. (1994) The responses of plants to heavy metals: In: S. M. Ross (Ed.). *Toxic Metals in Soil- Plant Systems*. John Wiley & Sons Ltd., 154-187.

Turner, A.P. and Dickinson, N.M. (1993) Survival of *Acer pseudoplatanus* L. (Sycamore) seedling on metalliferous soils. *New Phytol*. **123**: 509-521.

Vallee, B. L. and Ulmer, D. D. (1972) Biochemical effects of mercury, cadmium and lead. *Annu. Rev. Biochem*. **41**: 91- 128.

Van Assche, F. and Clijsters, H. (1990) Effect of metals on enzyme activity in plants. *Plant Cell Environ*. **13**: 195-206.

Vazques, M.D., Poschenrieder, C.H. and Barcelo, J. (1987) Chromium (VI) induced structural changes in bush bean plants. *Ann. Bot.* **59**: 427-438.

Vazques, M.D., Poschenrieder, C.H. and Barcelo, J. (1992) Ultra structural effects and localization of low cadmium concentration in bean roots. *New Phytol*. **120**: 215-226.

Velasco- Alinsug, M. P., Rivero, G. C. and Quibuyen, T. A. O. (2005) Isolation of mercury –binding peptides in vegetative parts of *Chromolaena odorata*. Z. *Naturforsch*. **60**c: 252-259.

Verkleij, J. A. C. and Schat, H. (1990) Mechanisms of metal tolerance in higher plants. In : A. J. Shaw (Ed.) *Heavy Metal Tolerance in Plants: Evolutionary Aspects*. CRC Press, Florida. 179-193.

Wagner, G. J. (1993) Accumulation of cadmium in crop plants and its consequences to human health *Adv. Agron.* **51**: 173- 212.

Wang, Q., Cui, Y. and Dong, Y. (2002) Phytoremediation of polluted waters: potentials and prospects of wetland plants. *Acta Biotechnologica* **22**: 199- 208.

Wang, Z., Zhang, Y., Huang, Z. and Huang, L. (2008) Antioxidative response of metal accumulator and non – accumulator plants under cadmium stress. *Plant Soil*. **310**: 137- 149.

Wei, C. Y., Sun, X., Wang, C., and Wang, W. Y. (2006) Factors influencing arsenic accumulation by *Pteris vittata*: A comparative field study at two sites. *Environ. Pollu*. **141**: 488- 493.

Wenzel, W. W. and Jockwer, F. (1999) Accumulation of heavy metals in plants growing on mineralized soils of the Austrian Alps. *Environ. Pollut.* **104**: 145- 155.

WHO, (2002) Drug Information. Herbal Medicines. **16**: *World Health Organization*, Geneva.

Wierzbicka, M. (1998) Lead in the apoplast of *Allium cepa* L. root tips- ultra structural studies. *Plant Sci*. **133**: 105-119.

Wilkins, (1978) The measurement of tolerance to edaphic factors by means of root growth. *New Phytol*. **80**: 623-633.

Wojcik, M. and Tukendorf, A. (1999) Cadmium tolerance of maize, rye and wheat seedlings. *Acta Phys. Plant* **21**: 99-107.

Woods, P. W., (1999) Herbal healing. *Essence* **30**: 42- 46.

Woolhouse, H.W. (1983) Toxicity and tolerance in the response of plants to metal. In: O.L. Lange, P.S. Nobel, C.B. Osmond and H. Ziegler (Eds.). *Encyclopedia of Plant Physiology (New Series) Physiological Plant Ecology III.* Springer-Verlag, New York. **12C**: 245-302.

Wozny, A. and Jerczynska, E. (1991) The effect of lead on early stages of *Phaseolus vulgaris* L. growth *in vitro* conditions. *Biol. Plant*. **33**: 32-39.

Wozny, A., Stroinski, J. and Gwozdz, E.A. (1990) Plant cell responses to cadmium. *Seria Biologia* 44. Adam Mickiewicz University, Poznam.

Xiong, Z. T. (1998) Heavy metal contamination of urban soils and plants in relation to traffic in Wuhan City, China, *Toxicol. Environ. Chem*., **65**: 31- 39.

Yadav, S., Sukla, O.P. and Rai, U.N. (2005) Chromium pollution and bioremediation. *Environ. News Archives* **11:** 1-4.

Ye, Z. H., Baker, A.J.M., Wong, M. H. and Willis, A. J. (1997) Zinc, lead and cadmium tolerance, uptake and accumulation by *Typha latifolia*. *New Phytol*. **136**: 469-480.

Yoon, J., Cao, X., Zhou, Q. and Ma, L. Q. (2006) Accumulation of Pb, Cu and Zn in native plants growing on a contaminated Florida site. *Sci. Total Environ.* **368**: 456-464.

Zayed, A., Gowthaman, S. and Terry N. (1998) Phytoaccumulation of trace elements by Wetland Plants: 1 Duckweed. *J. Environ. Qual.* **27**: 715-721.

Zengin and F. K. and Munzuroglu, O. (2005) Effects of some heavy metals on content of chlorophyll, proline and some antioxidant chemicals in bean (Phaseolus vulgaris L.) seedlings. *Acta Biol. Cracov. Ser. Bot.* **47**: 157-164.

Zenk, M. H. (1996) Heavy metal detoxification in higher plants-A review. *Gene*. **179:** 21-30.

Zhang, F. Q., Wang, Y. S., Lou, Z. P. and Dong, J. D. (2007) Effect of heavy metal stress on antioxidative enzymes and lipid peroxidation in leaves and roots of two mangrove plant seedlings (*Kandelia candel* and *Bruguiera gymnorrhiza*). *Chemosphere*, **67**: 44-50.

Zhang, F. Q., Wang, Y.S., Lou, Z. P. and Dong, J. D. (2007) Effect of heavy metals stress on antioxidative enzymes and lipid peroxidation in leaves and roots of two mangrove plant seedlings (*Kandelia candel* and *Bruguiera gymnorrhiza*) Chemosphere 67: 44- 50.

Zheljazkov, V.D., Craker, L. E. and Xing, B. (2006) Effects of Cd, Pb and Cu on growth and essential oil contents in dill, Peppermint and basil. *Environ. Exp. Bot.* **58**: 9-16.

Zimmels, Y., Kirzhner, F. and Roitman, S. (2004) Use of naturally growing aquatic plants for waste water purification. *Water Environ. Research Found.* **76**: 220-230.

Zou, J., Xu, P., Lu, X., Jiang, W., and Liu, D. (2008) Accumulation of cadmium in three sunflower (*Helianthus annuus* L.) cultivars. *Pak. J. Bot.* **40**: 759-765.

Chan, T. Y. K. , Tomlinson, B., Critichley, A. J. H. (1993) Chinese herbal medicines revisited: a Hong Kong perspective. *The Lancet* **342**: 1532-1534.

Kakosy, T., Hudak, A., Naray, M. (1996) Lead intoxication epidemic caused by ingestion of contaminated ground paprika. J. *Toxicol*ogy- *Clinical* Toxicology **34**: 507-511.

Levitt, C. M. D., (1984) Sources of lead poisoning. J. American Medical Association **252**: 3127- 3128.

Markowitz, S. B., Nenez, C. M., Klitzman, S. (1994) Lead poisoning due to hai ge fen: the porphyry content of individual erythrocytes. J. American Medical Association **271**: 932- 934.

Tong, S., von Schirnding, Y. E., Prpaamontol, T. (2000) Environmental lead exposure: a public problem of global dimension. Bulletin of the World Health Organization **78**: 1068- 1077.

WHO, (1999) Monographs on selected medicinal plants. Vol. 1. *World Health Organization*.Geneva.

WHO, (2003) Elementary mercury and inorganic mercury compounds: Human Health Aspects. Concise International Chemical Assessment Document **50**: *World Health Organization* Geneva.

Olujohungbe, A., Fields, P. A., Sandford, A. F., (1994) Heavy metal intoxication from homeopathic and herbal remedies. Postgraduate Medical Journal **70**: 764- 769.

Goldbold D. L., Kettner, C. (1991) Use of root elongation studies to determine aluminium and lead toxicity in *Piea abide* seedlings J. *Plant Physiol.* **5**: 138-231.

Hopkins L., Malcolm, J., Hawkesford, (2000) J. S- supply, free sulphur and free aminoacids pools in Potato (*Solanum tuberosum* I. Cv Desiree) In: Brunold C, editor. Sulphur Nutrition and Sulfur Assimination in Higher Plants 259- 261.

Vajpayee, P., Sharma, S. C., Tripathi, R. D., Rai, U. N., Yunus, M. (1999) Bioaccumulation of chromium and toxicity to photosynthetic

pigments, nitrate reductase activity and protein content of *Nelumbo nucifera* Gaertin. *Chemosphere* 39: 2159- 2169.

Vajpayee P., Tripathi, R. D., Rai. U. N., Ali, M. B., Singh, S. N. (2000) Chromium (VI) accumulation reduces chlorophyll biosynthesis, nitrate reductase activity and protein content in *Nymphaea alba* L. *Chemosphere* **41**: 1075- 1082.

Awasthi, L. P., and Kumar, P. (2003) Protection of some cucurbitaceous crops against natural infection of virusese through *Boerhavia diffusa* plants. *Indian Phytopathology* **56**: 318.

Awasthi, L. P., Kumar, P., and Singh, R. V., (2003) Effect of *Boerhavia diffusa* inhibitor on the infection and multiplication of cucumber green mottle mosaic virus in musk melon plants *Inidan Phytopathology* **56**: 362.

Rawat, A. K. S., Mehrotra, S., Tripathi, S. K., and Sharma, U. (1997) Hepatoprotective activity in Punarnava - a popular Indian ethnomedicine. J. *Ethnopharmacology*. 56: 61- 68.

Alia, K. V., Prasad, S. K., Saradhi, P. P., (1995) Effect of zinc on free radicals and proline in *Brassica* and *Cajanus*. *Phytochem*. 39: 45- 47.

Lee, J. H., Hossner, L. R., Attrep, Jr. M., Kung, K. S., (2002) Uptake and translocation of plutonium, in two plant species using hydroponics. *Environ. Pollut*. 117: 61- 68.

Singh, S., Seaxena, R., Pandey, K., Bhatt, K., Singha, S. (2004) Response of antioxidant in sunflower (*Helianthus annus* L.) grown on different amendments of tannery sludge: its metal accumulation potential. *Chemosphere* **57**: 1663- 1673.

Sinha, S., Saxena, R., (2006) Effect of iron on lipid peroxidation and enzymatic and non – enzymatic antioxidant and bacoside content in medicinal plant *Baccopa monnieri* L. Chemosphere 62: 1340- 1350.

Zou, J. H., Wang, W. S., Jiang, and Liu, D. H. (2006) Effect of hexavalent chromium (VI) on root growth and cell division in root tip cells of *Amaranthus viridis* L. *Pak. J. Bot*. **38**: 673- 681.

Panda, S. K., (2007) Chromium – mediated oxidative stress and ultra structural changes in root cells of developing rice seedlings. *Plant. Physiol.* **164**: 1419-1428.

Choudhury, S., Panda, S. K., (2005) Toxic effect, oxidative stress and ultra structural changes in moss *Taxitheelium nepalense* (Schwaegr.) Broth. Under lead and chromium toxicity. *Water Air Soil Pollut*. 167: 73- 90.

Foyer, C. H., Lopez- Delgado, H., Dat, J. F. and Scot, I. M. (1997) Hydrogen peroxide and glutathione associated mechanisms of acclamatory stress tolerance and signalling. *Physiol. Plant*. **100**: 241- 245.

Barthwal, J. Nair, S. and Kakkar, P. (2008) Heavy metal accumulation in medicinal plants collected form environmentally different sites. *Biomed. Environ. Scie* . **21**: 319- 324.

Noctor, G., Foyer, C. H., (1998) Ascorbate and glutathione: Keeping active oxygen under control. *Annu. Rev. Plant. Physiol. Plant. Mol. Biol.* **49**: 249- 279.

Sun, Y. B., Zhou, X. Q. and Ren, L. P.(2007) Growth responses of *Rorippa globosa* and its accumulation characteristics of Cd and As under the Cd- As combined pollution, *Environ. Sci*. **28**: 1355- 1360.

Abdel-Latif, A. (2008) Cadmium induced changes in pigment content, ion uptake, proline content and phosphoenolpyruvate carboxylase activity in *Triticum aestivaum* seedlings. *Australian Journal of Basic and Applied Sciences* **2**: 57- 62.

Liu, Z., He, X., Chen, W., Yuan, F., Yan, K. and Tao, D. (2009) Accumulation and tolerance characteristics of cadmium in a potential hyperaccumulator- *Lonicera japonica* Thumb. *Hazardous Materials.* **10**: 81-97.

Boominathan, R. and Doran, P. M. (2003) Cadmium tolerance and antioxidative defenses in hairy roots of the cadmium hyperaccumulator, *Thlaspi caerulescens*, *Biotehnol. Bioeng*. **83**: 158- 167.

Ratheesh Chandra, P., Abdussalam, A. K. Salim, N. and Puthur, J. T. (2010) Distribution of bio-accumulated Cd and Cr in two *Vigna* species and the associated histological variations. *Stress physiology & Biochemistry* **6**: 4-12.

Saper, R. B., Kales, S. N., Paquin, J. Burns, M. J., Eisenberg, D. M. and Davis, R. B. (2004) Heavy metal content of ayurvedic herbal medicine products *Jama*, **292**: 2868- 2873.

Halliwell, B. and Gutterodge, J. M. C. (1999) Free radicals in biology and medicine, Oxford, U K, Clarendon Press.

Hussain, I., Khan, F., Khan, I., Khan, L. and Wali-Ullah (2006) Determination of heavy metals in medicinal plants. *Chem. Soc. Pak.* **28**: 347-351.

Cunningham, S. D., Berti, W. R. and Huang, J. W. (1995) Phytoremediation of contaminated soils. *Trends Biotechnol.* **13**: 398- 403.

Kabata- Pendias, A. and Pendias, H. (2001) Trace elements in soils and plants, 3rd -

Table 20 a: BCF and TF values of Heavy Metal bioaccumulation in Twenty Medicinal Plants

Name of the plant	Treatments	Heavy metal applied (mg)	Accumulation		BCF	TF
			Root	Shoot		
Aerva lanata	Cadmium	1.979	298.0	39.51	0.15	0.13
	Chromium	70.56	585.0	63.30	0.008	0.10
	Mercury	0.203	54.16	4.630	0.26	0.08
	Lead	182.0	61.80	19.39	0.002	0.31
Alternanthera tenella	Cadmium	0.593	227.0	42.10	0.46	0.07
	Chromium	21.16	567.3	52.90	0.02	0.09
	Mercury	0.025	6.300	2.650	0.25	0.42
	Lead	54.62	723.4	149.9	0.01	0.20
Amaranthus spinosus	Cadmium	2.639	371.0	45.40	0.14	0.12
	Chromium	94.08	815.3	554.5	0.008	0.69
	Mercury	0.135	33.75	3.540	0.25	0.10
	Lead	24.27	763.24	441.0	0.03	0.57
Andrographis paniculata	Cadmium	0.650	133.5	61.50	0.20	0.46
	Chromium	23.52	254.1	76.30	0.01	0.26
	Mercury	0.025	5.720	2.740	0.21	0.47
	Lead	60.69	924.0	229.5	0.01	0.23
Beloperon plumbaginifolia	Cadmium	2.640	217.8	124.0	0.08	0.56
	Chromium	94.08	585.9	58.20	0.006	0.09
	Mercury	0.135	44.78	7.090	0.33	0.17
	Lead	24.27	286.2	267.9	0.01	0.93
Blepharis maderaspatensis	Cadmium	1.978	362.9	138.8	0.18	0.38
	Chromium	70.56	50.0	271.7	0.0007	5.43
	Mercury	0.101	54.28	6.040	0.5	0.11
	Lead	182.0	842.7	766.6	0.004	0.79
Boerhavia diffusa	Cadmium	2.639	484.3	342.7	0.05	0.7
	Chromium	94.09	906.3	502.9	0.009	0.55
	Mercury	0.135	77.26	8.270	0.57	0.1

	Lead	24.27	854.9	459.5	0.03	0.55
Catharanthus roseus	Cadmium	0.593	208.6	123.4	0.35	0.59
	Chromium	21.16	383.9	342.4	0.01	0.89
	Mercury	0.025	3.260	0.576	0.13	0.17
	Lead	54.62	428.6	413.6	0.007	0.96
Eclipta prostrata	Cadmium	0.593	192.1	147.1	0.32	0.76
	Chromium	21.16	414.2	300.5	0.01	0.73
	Mercury	0.025	9.160	2.280	0.36	0.24
	Lead	54.62	260.2	41.46	0.003	0.15
Eupatorium triplinerve	Cadmium	2.639	589.8	195.0	0.22	0.33
	Chromium	94.08	114.2	121.8	0.001	1.06
	Mercury	0.135	70.35	8.920	0.52	0.12
	Lead	24.28	54.73	111.7	0.002	2.04
Kyllinga nemoralis	Cadmium	3.300	334.37	369.4	0.10	1.1
	Chromium	117.6	414.8	192.0	0.003	0.43
	Mercury	0.169	79.23	12.88	0.46	0.16
	Lead	303.4	653.1	662.1	0.002	1.0
Leucas lavandulifolia	Cadmium	0.593	248.8	23.03	0.41	0.09
	Chromium	21.16	406.9	46.00	0.001	0.11
	Mercury	0.025	7.340	5.336	0.29	0.72
	Lead	54.62	273.8	89.50	0.005	0.32
Oxalis corniculata	Cadmium	0.656	124.26	20.17	0.18	0.16
	Chromium	23.56	462.7	294.8	0.01	0.63
	Mercury	0.027	7.760	6.180	0.28	0.79
	Lead	60.69	424.8	97.05	0.006	0.22
Phyllanthus amarus	Cadmium	0.593	97.50	83.70	0.16	0.85
	Chromium	21.16	100.2	3.910	0.004	0.8
	Mercury	0.024	5.930	1087	0.24	0.65
	Lead	54.62	705.0	40.45	0.01	1.54

Portulaca oleracea	Cadmium	0.590	151.3	40.45	0.25	0.26
	Chromium	21.16	341.5	299.8	0.01	0.87
	Mercury	0.024	6.220	5.630	0.25	0.9
	Lead	54.62	110.92	335.0	0.002	3.04
Rubia cordifolia	Cadmium	1.979	1273	270.0	0.64	0.21
	Chromium	70.60	714.5	43.73	0.01	0.06
	Mercury	0.101	32.25	7.830	0.31	0.25
	Lead	182.0	746.0	43.46	0.004	0.05
Scoparia dulcis	Cadmium	1.979	661.0	295.4	0.33	0.44
	Chromium	94.08	40.51	2.787	0.0004	0.06
	Mercury	0.135	75.32	9.330	0.55	0.13
	Lead	24.27	407.12	366.5	0.016	0.70
Sida acuta	Cadmium	1.979	789.1	366.5	0.39	0.46
	Chromium	70.56	372.1	267.6	0.003	0.71
	Mercury	0.203	127.0	3.310	0.62	0.026
	Lead	182.0	497.6	312.7	0.002	0.62
Sida alnifolia	Cadmium	1.970	517.3	239.6	0.26	0.46
	Chromium	70.56	553.8	126.4	0.007	0.22
	Mercury	0.203	99.13	1.115	0.48	0.02
	Lead	182.0	673.4	301.3	0.003	0.44
Vernonia cinerea	Cadmium	0.593	430.4	301.4	0.72	0.70
	Chromium	21.16	410.6	34.27	0.01	0.08
	Mercury	0.124	4.640	4.390	0.03	0.94
	Lead	54.26	663.9	390.5	0.001	0.61

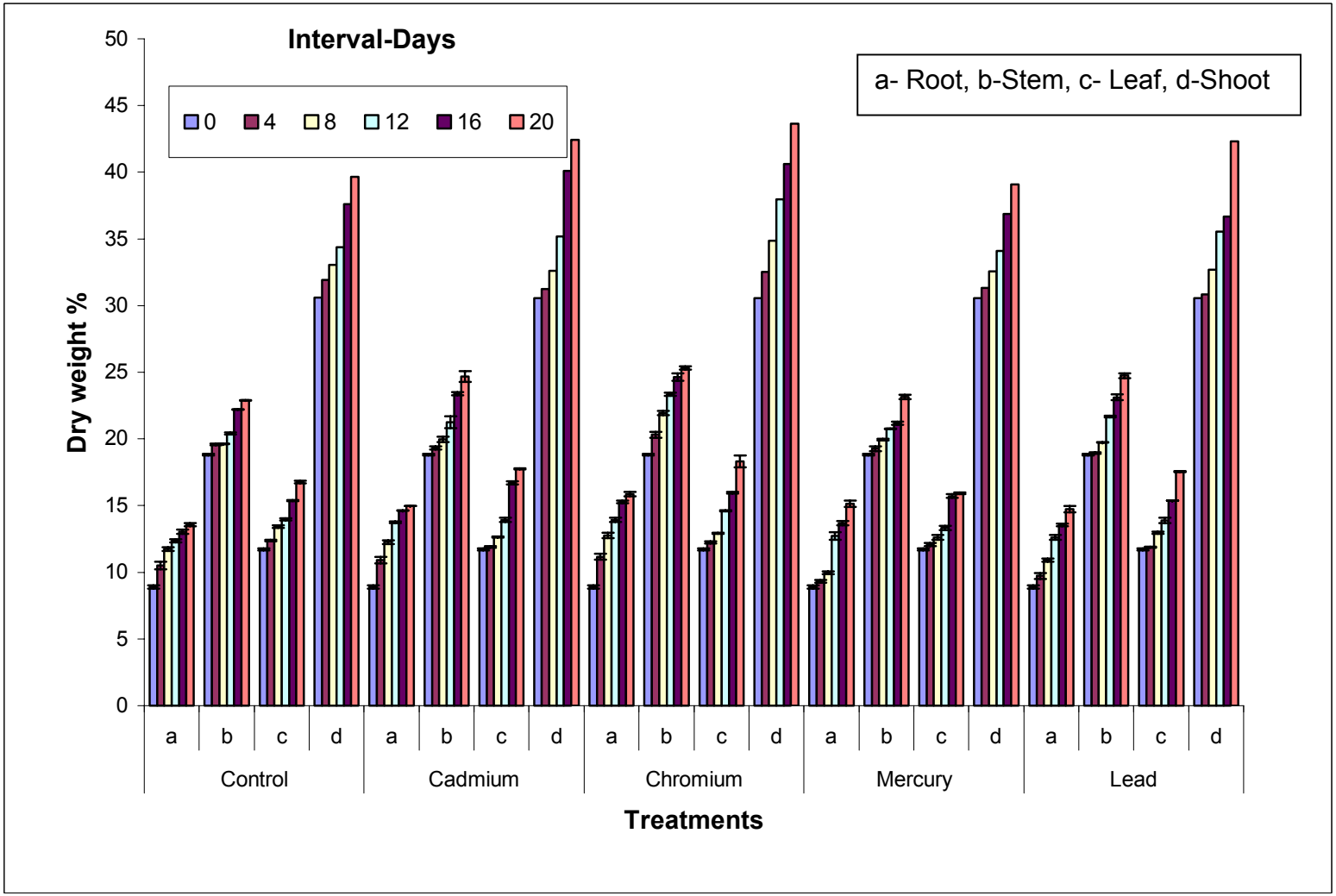


Fig. 7 Effect of Heavy Metals on the Dry weight percentage in *Boerhavia diffusa*

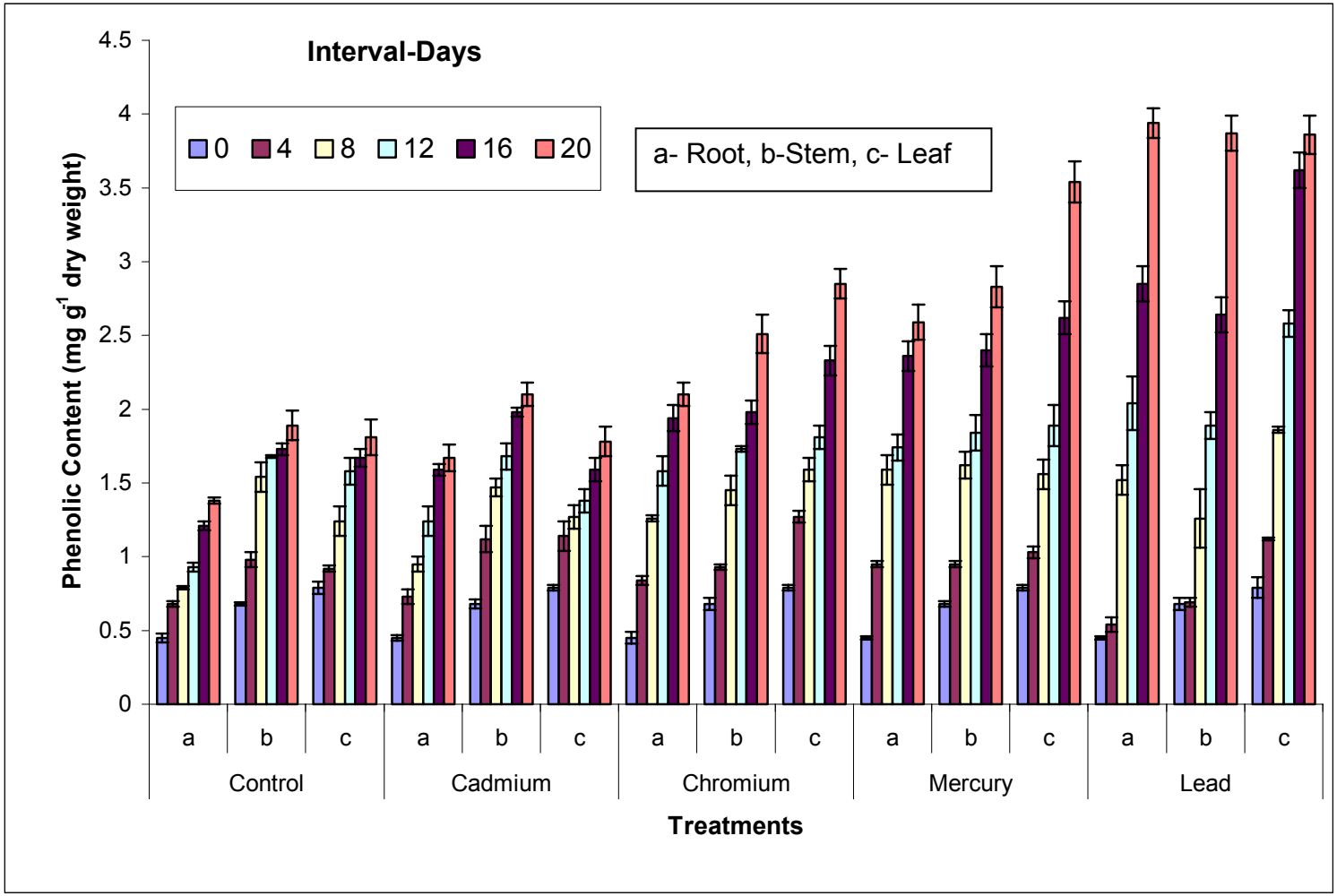


Fig. 19 Effect of Heavy Metals on Phenolics in *Boerhavia diffusa*

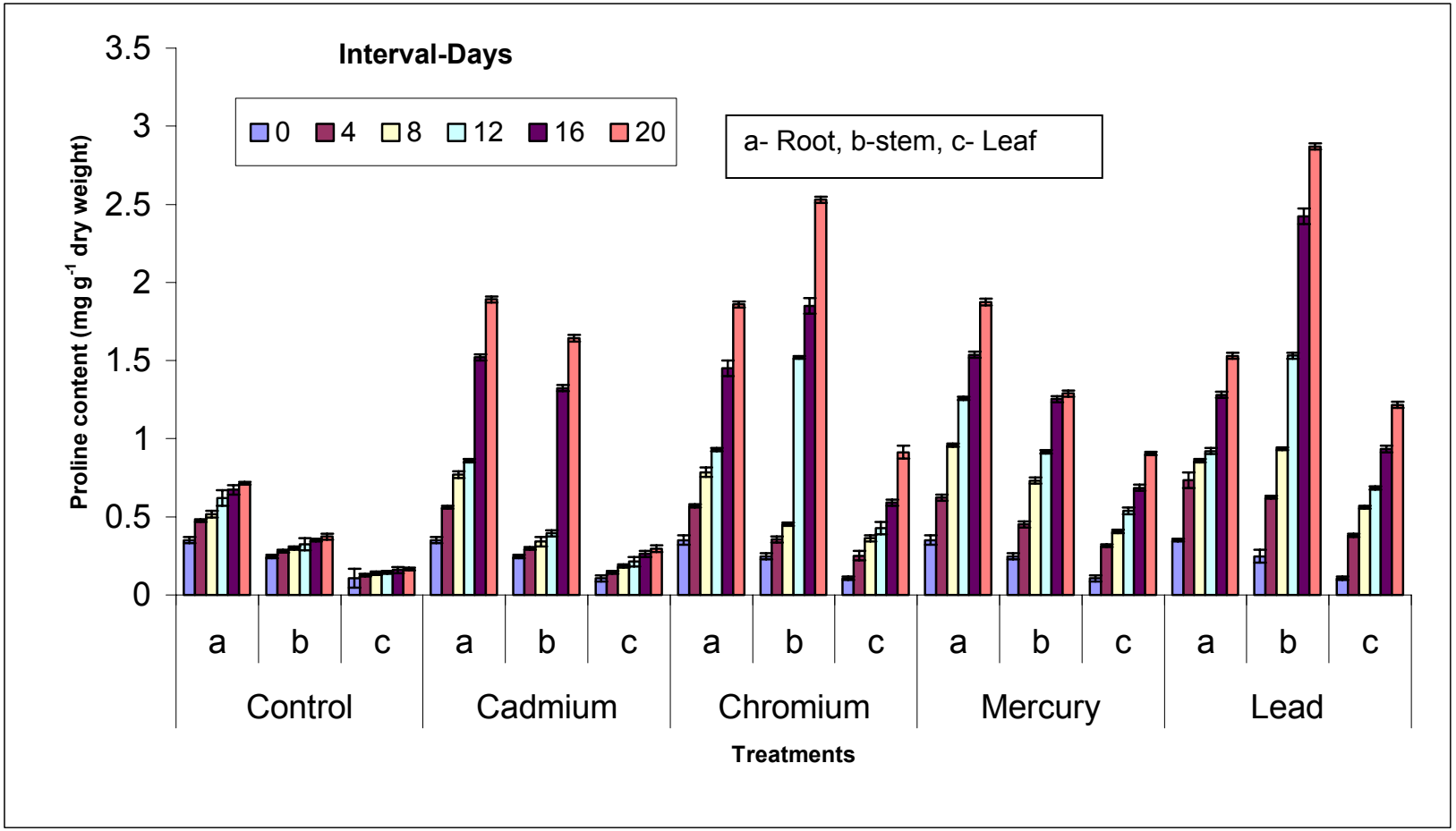


Fig. 14 Effect of Heavy Metals on Proline content in *Boerhavia diffusa* (mg g^{-1} dry weight)

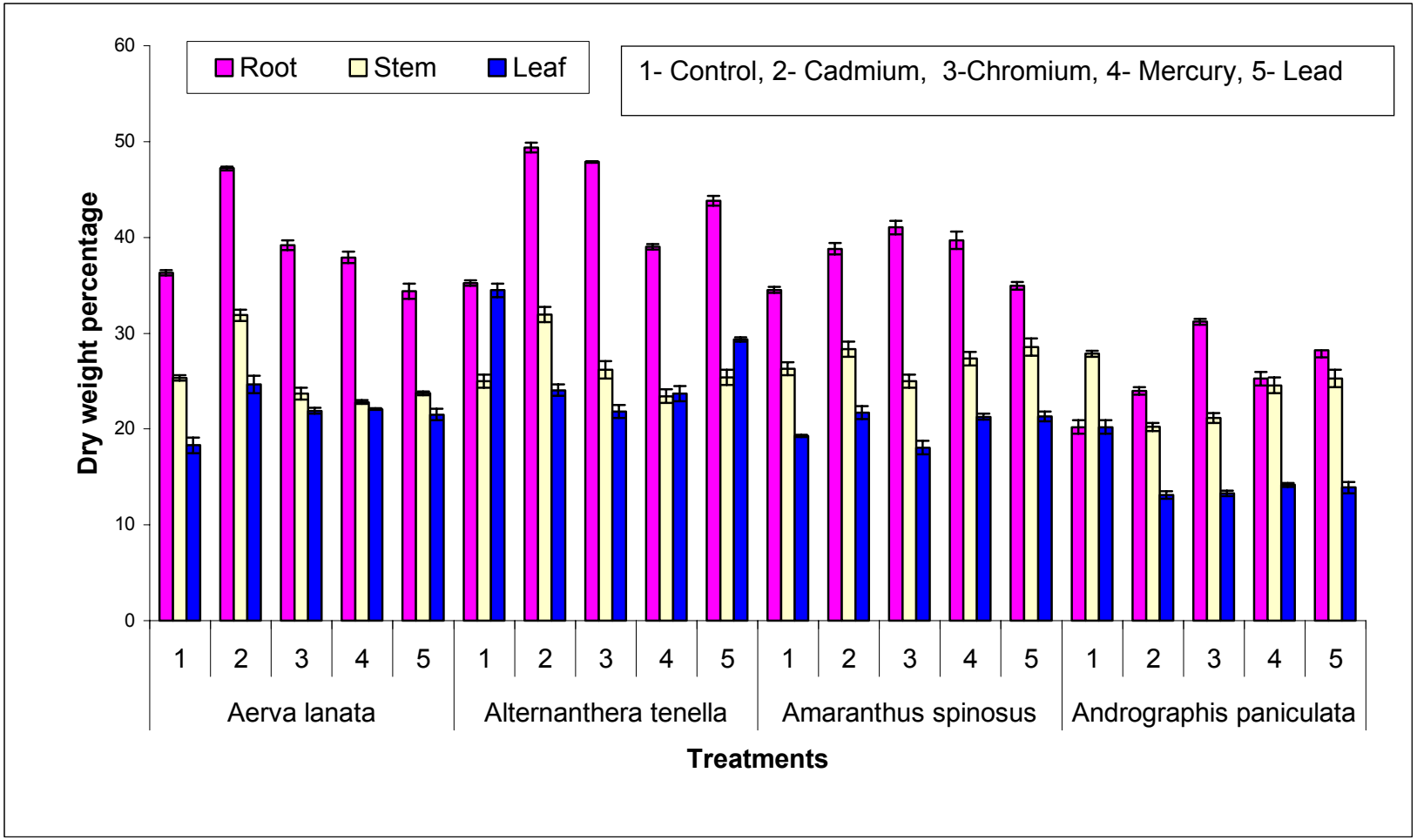


Fig. 46 Effect of Heavy Metal treatments on dry weight distribution in Medicinal plants

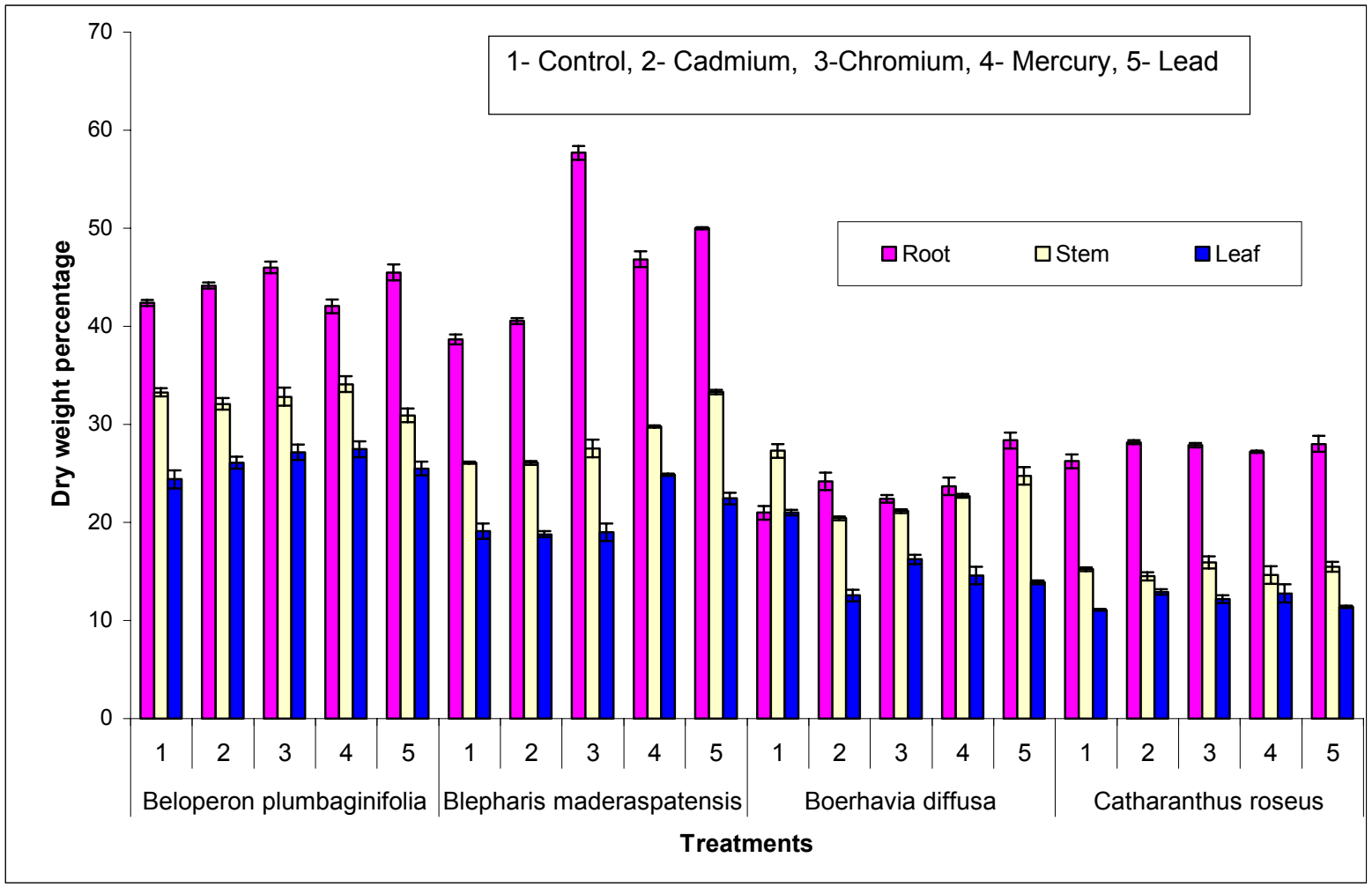


Fig. 47 Effect of Heavy Metal treatments on dry weight distribution in Medicinal plants

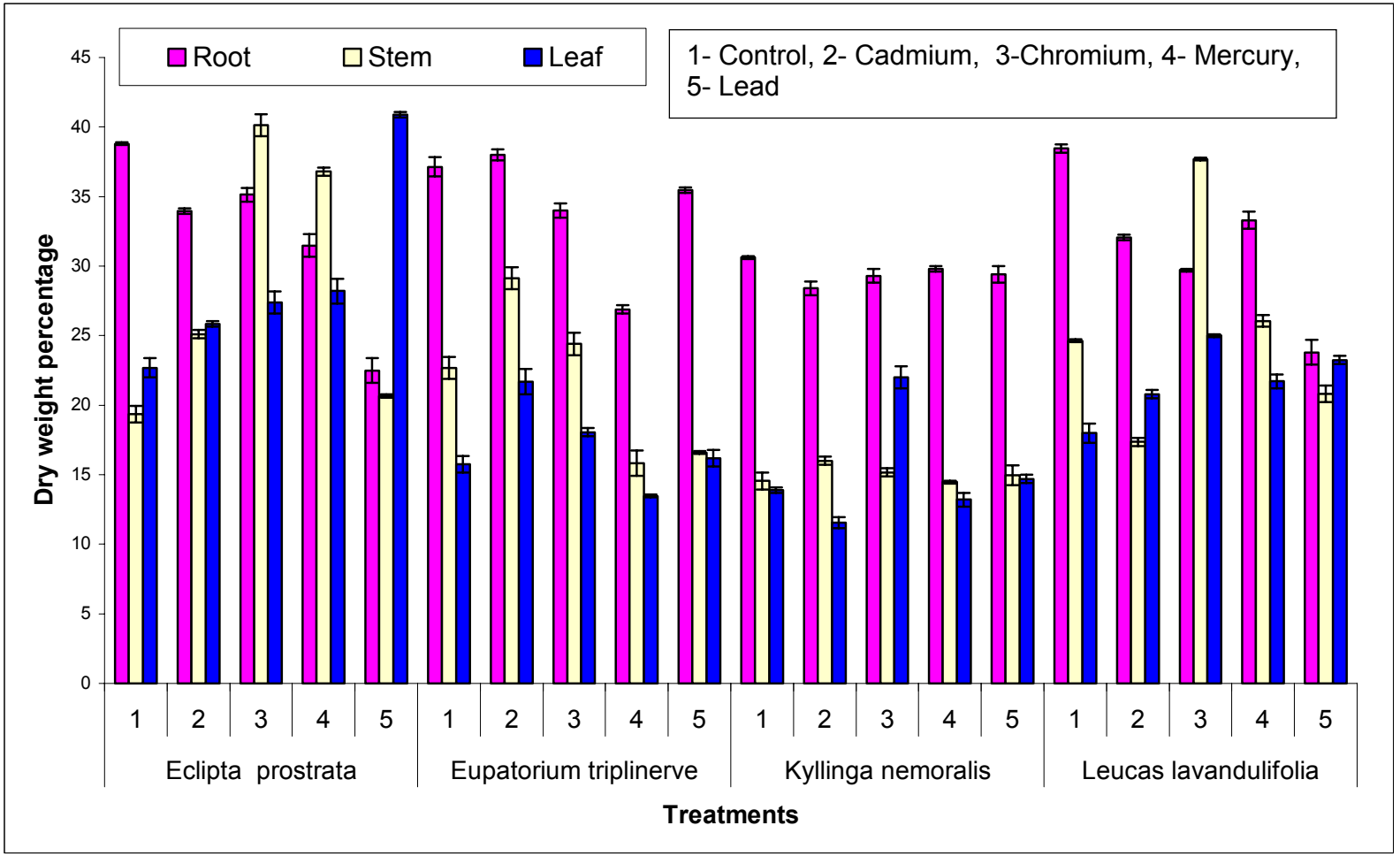


Fig. 48 Effect of Heavy Metal treatments on dry weight distribution in Medicinal plants

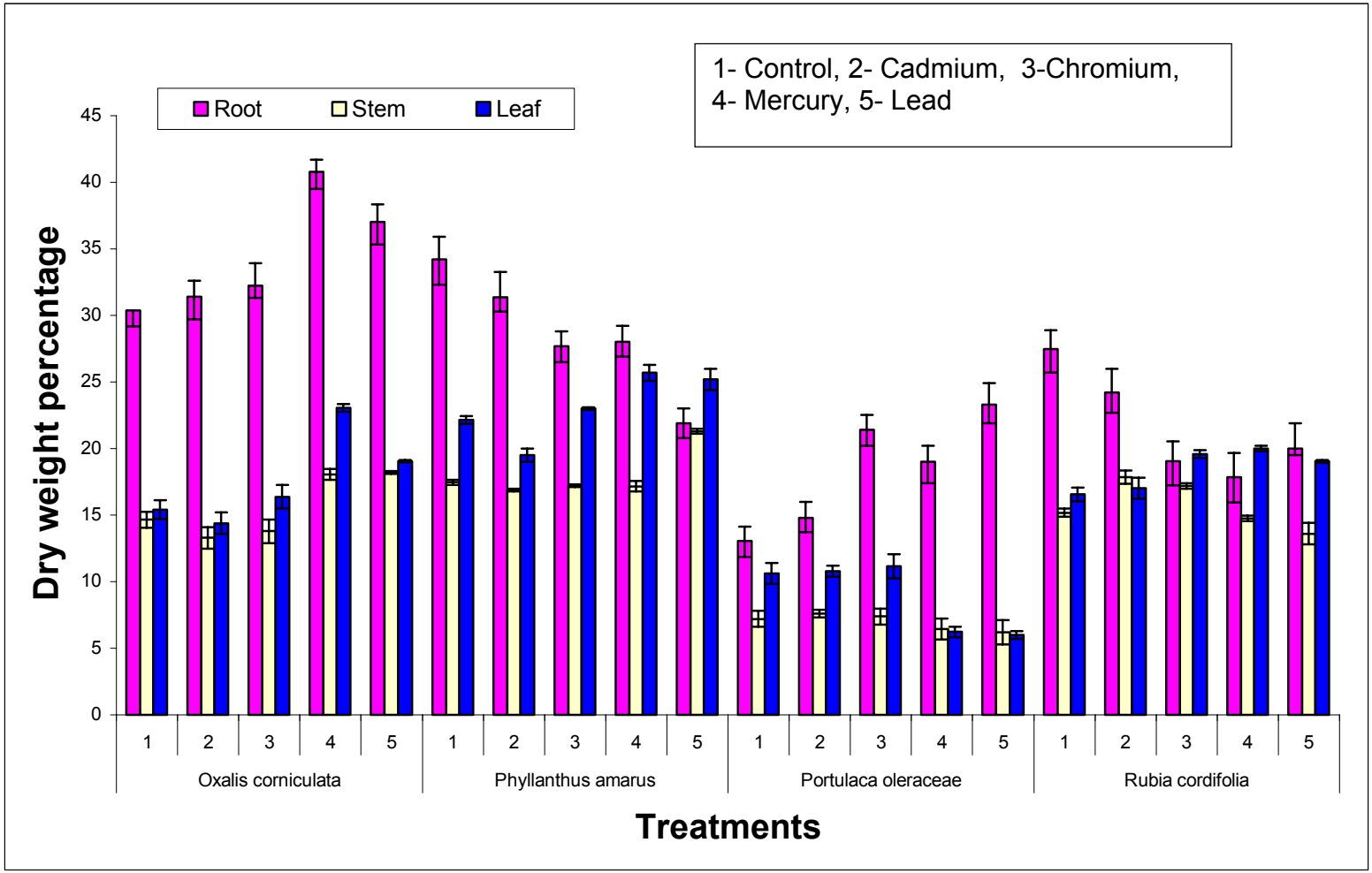


Fig. 49 Effect of Heavy Metal treatments on dry weight distribution in Medicinal plants

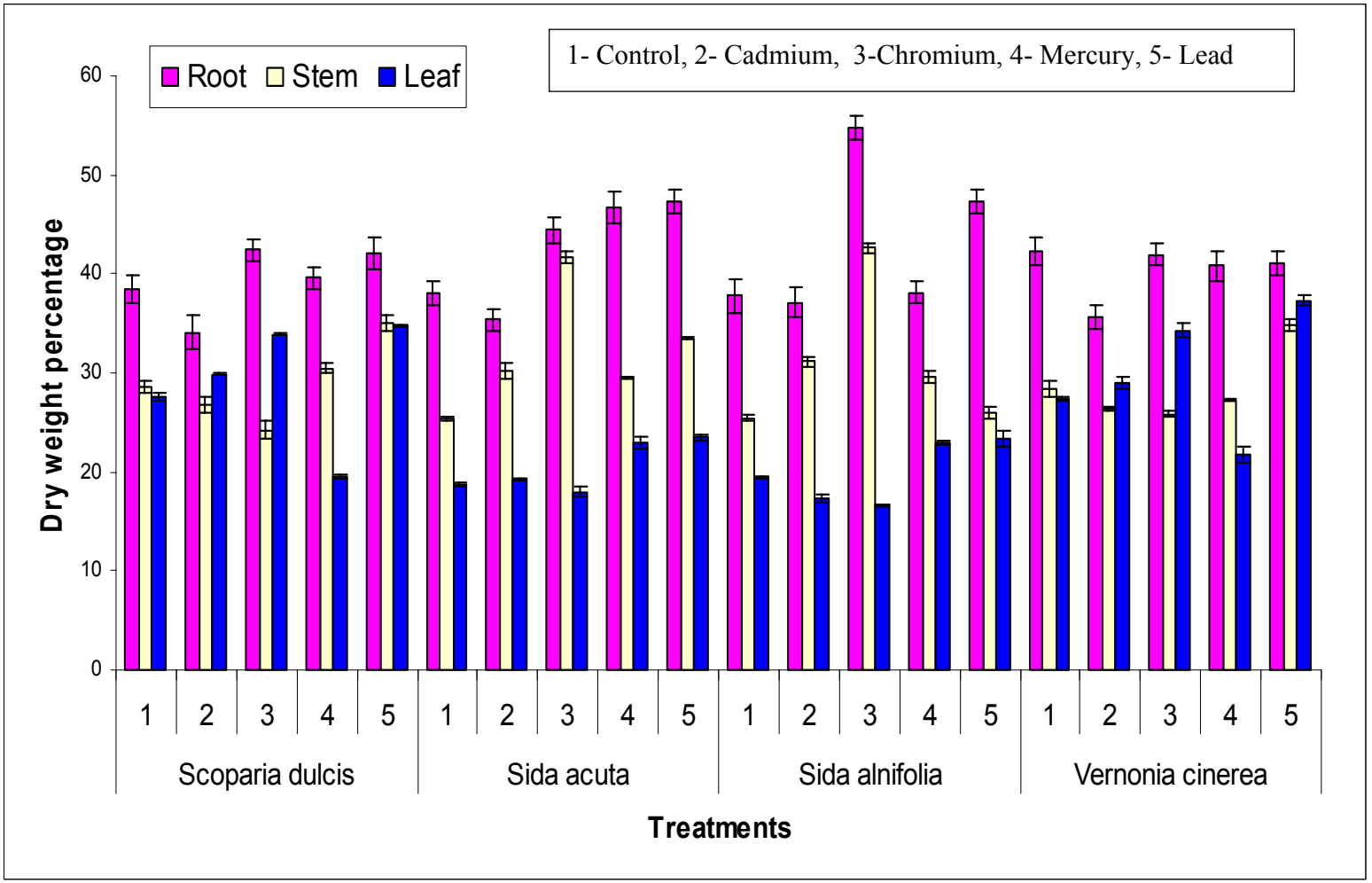


Fig. 50 Effect of Heavy Metal treatments on dry weight distribution in Medicinal plants

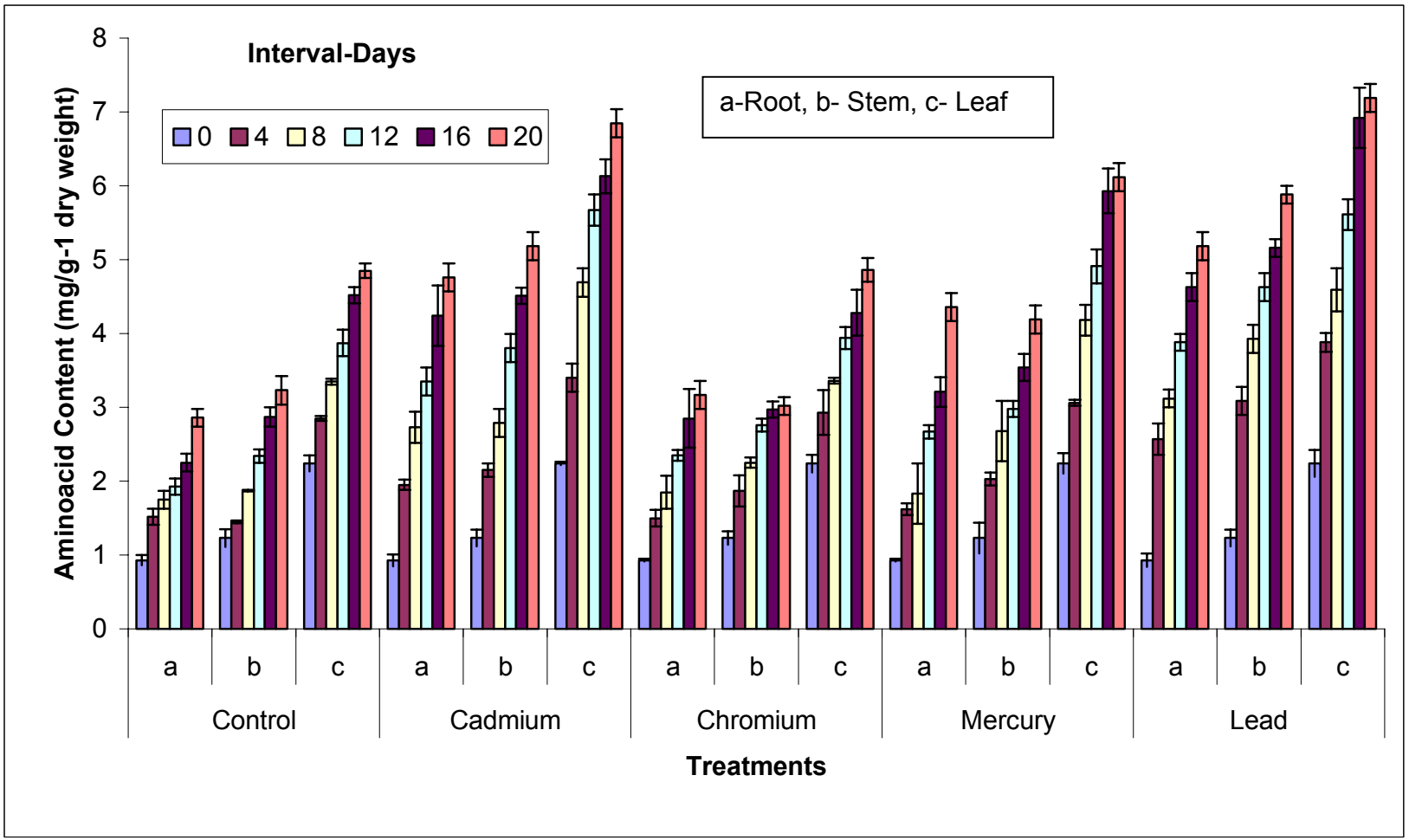


Fig.13 Effect of Heavy Metals on Total free Amino acid content in *Boerhavia diffusa*

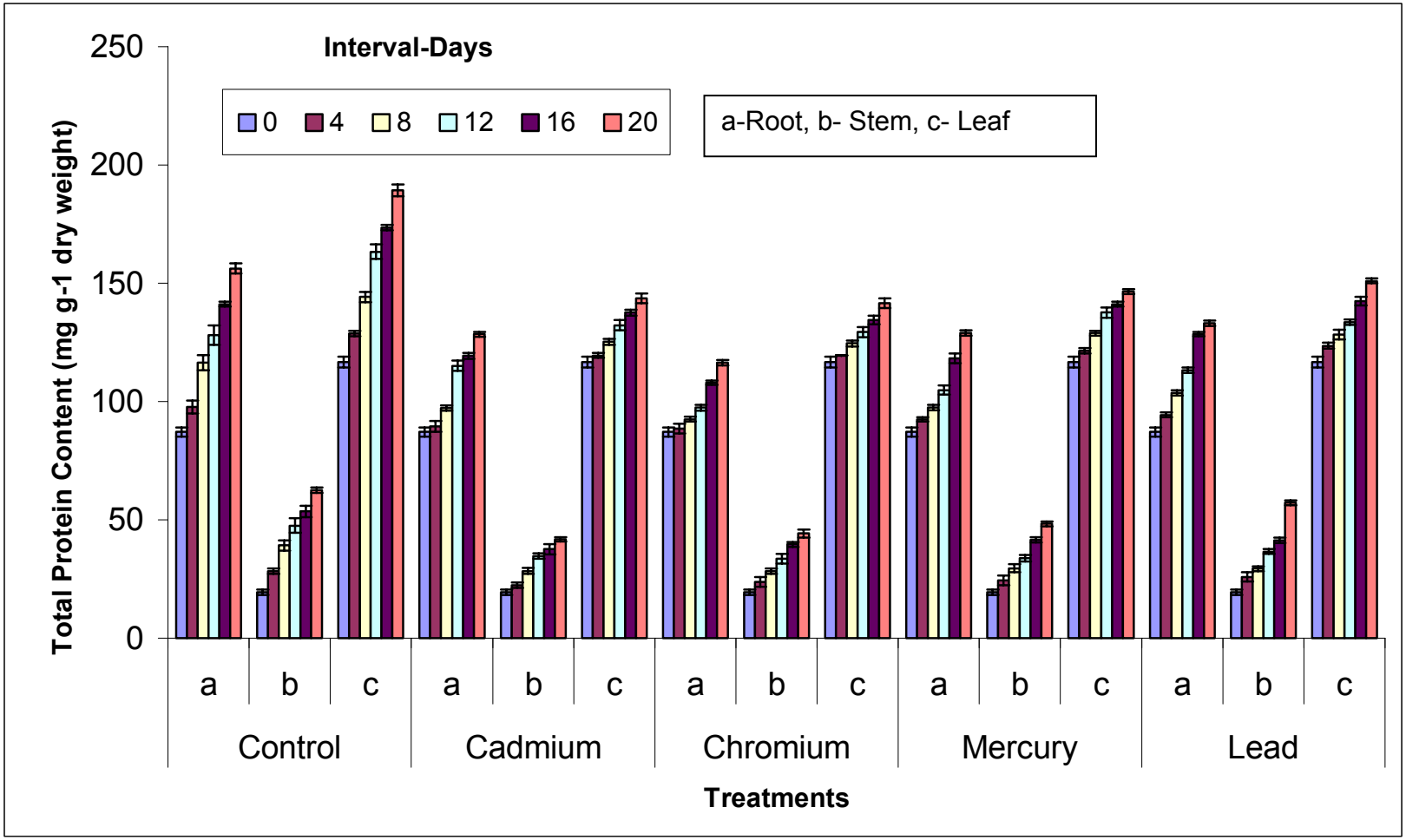


Fig. 8 Effect of Heavy Metals on Total Protein content in *Boerhavia diffusa*

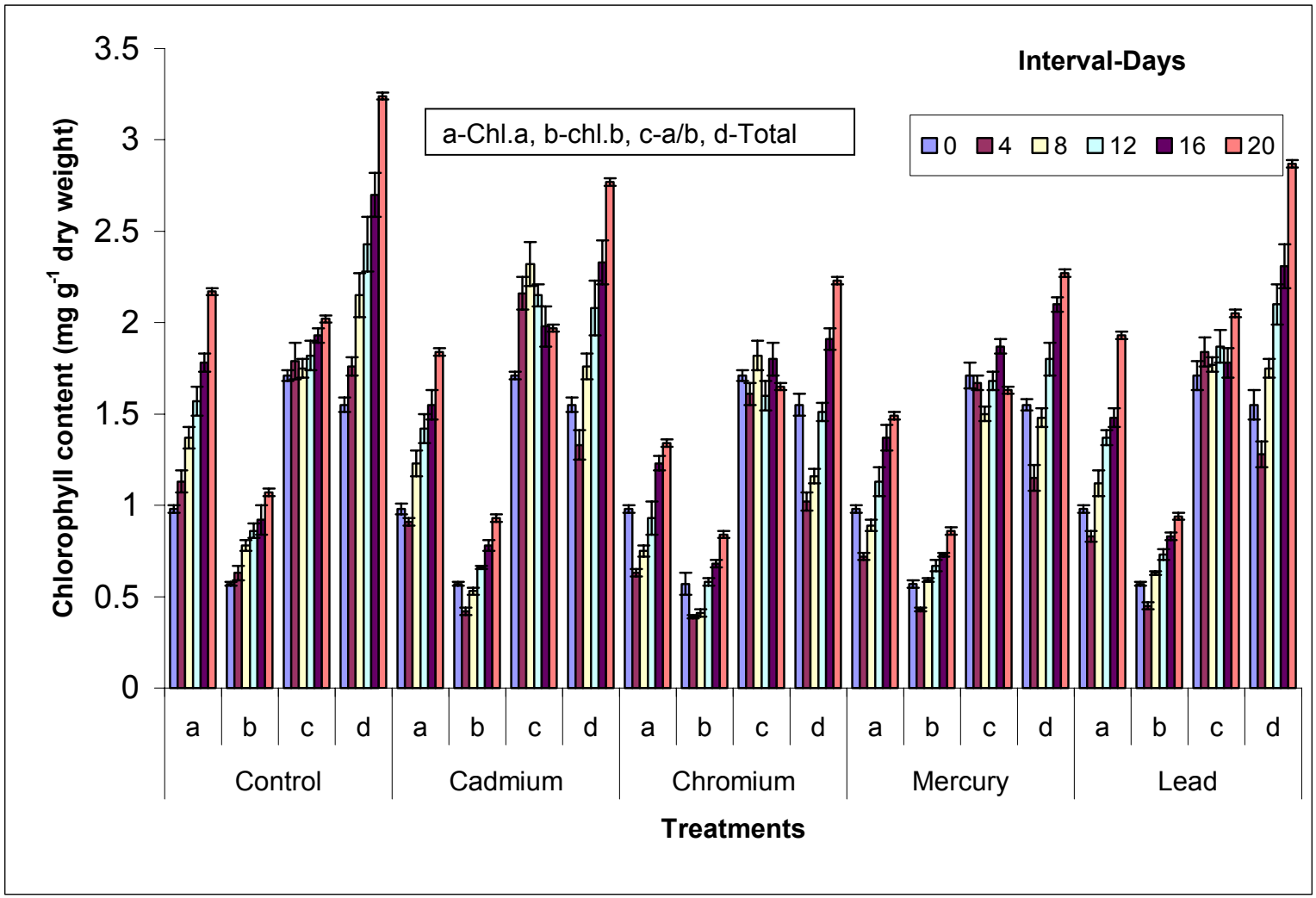


Fig. 15 Effect of Heavy Metals on Chlorophyll content in *Boerhavia diffusa*

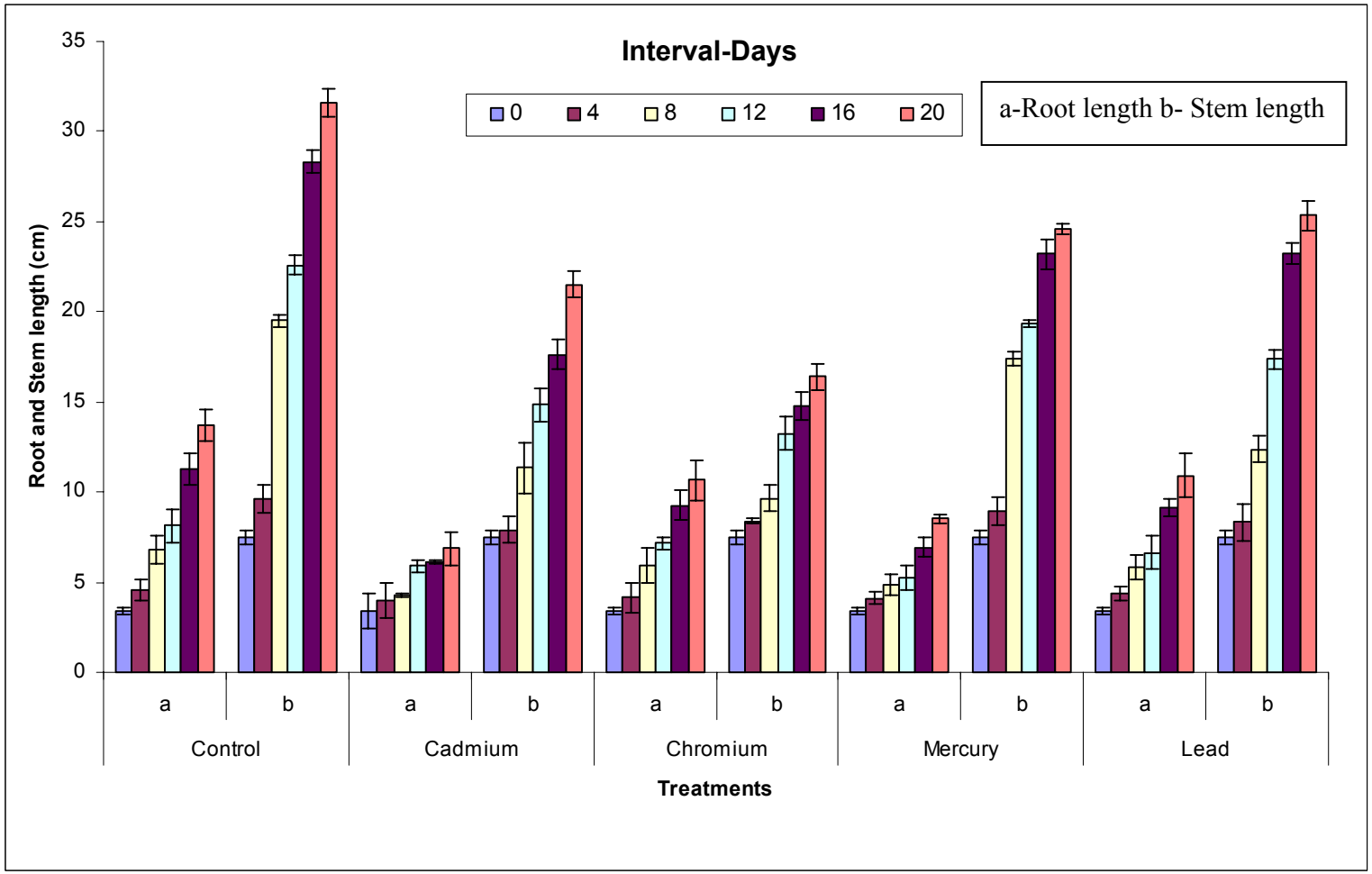


Fig. 3 Effect of Heavy Metals on root and stem length in *Boerhavia diffusa*

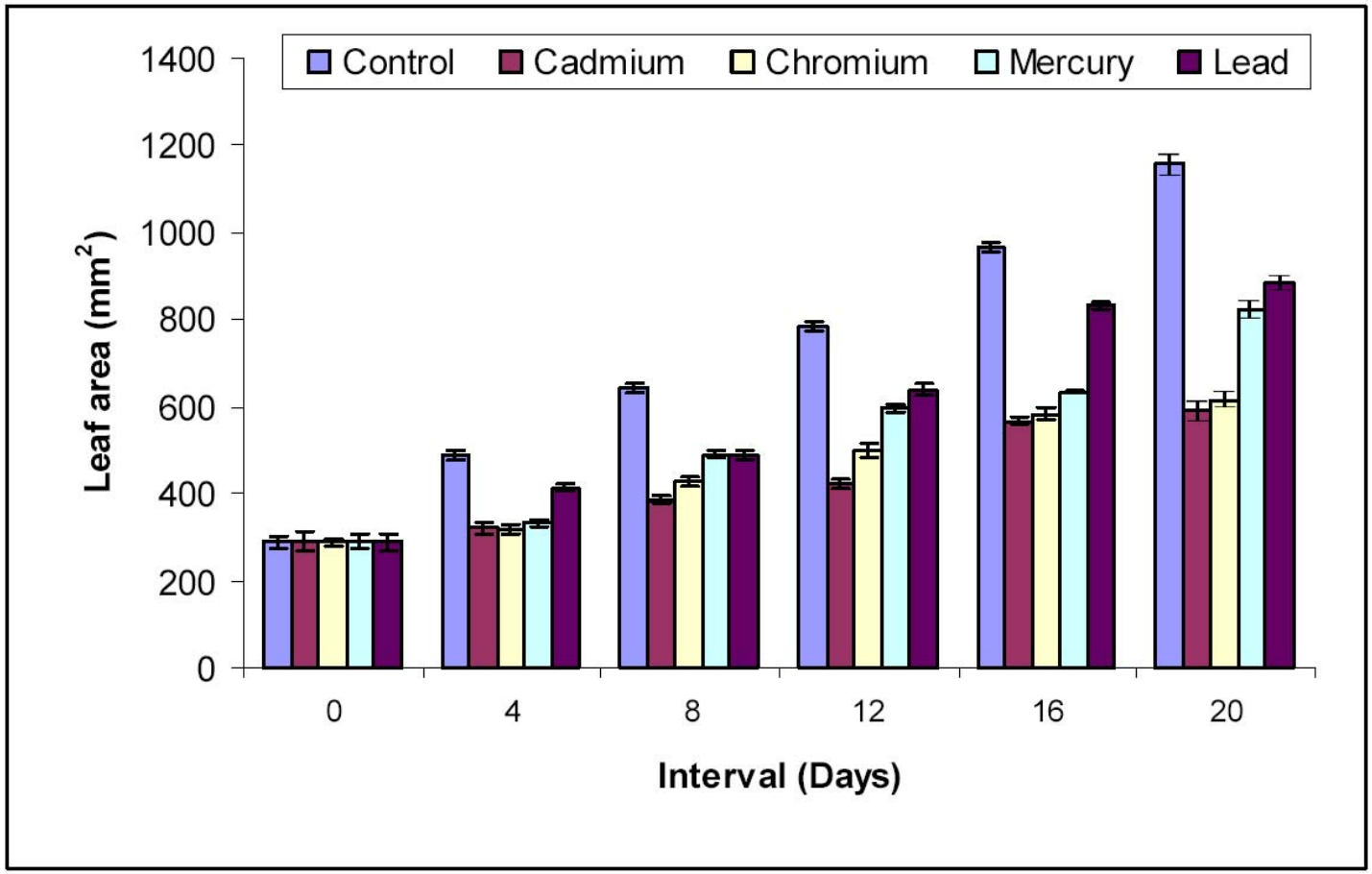


Fig. 4 Effect of Heavy Metals on leaf area in *Boerhavia diffusa*

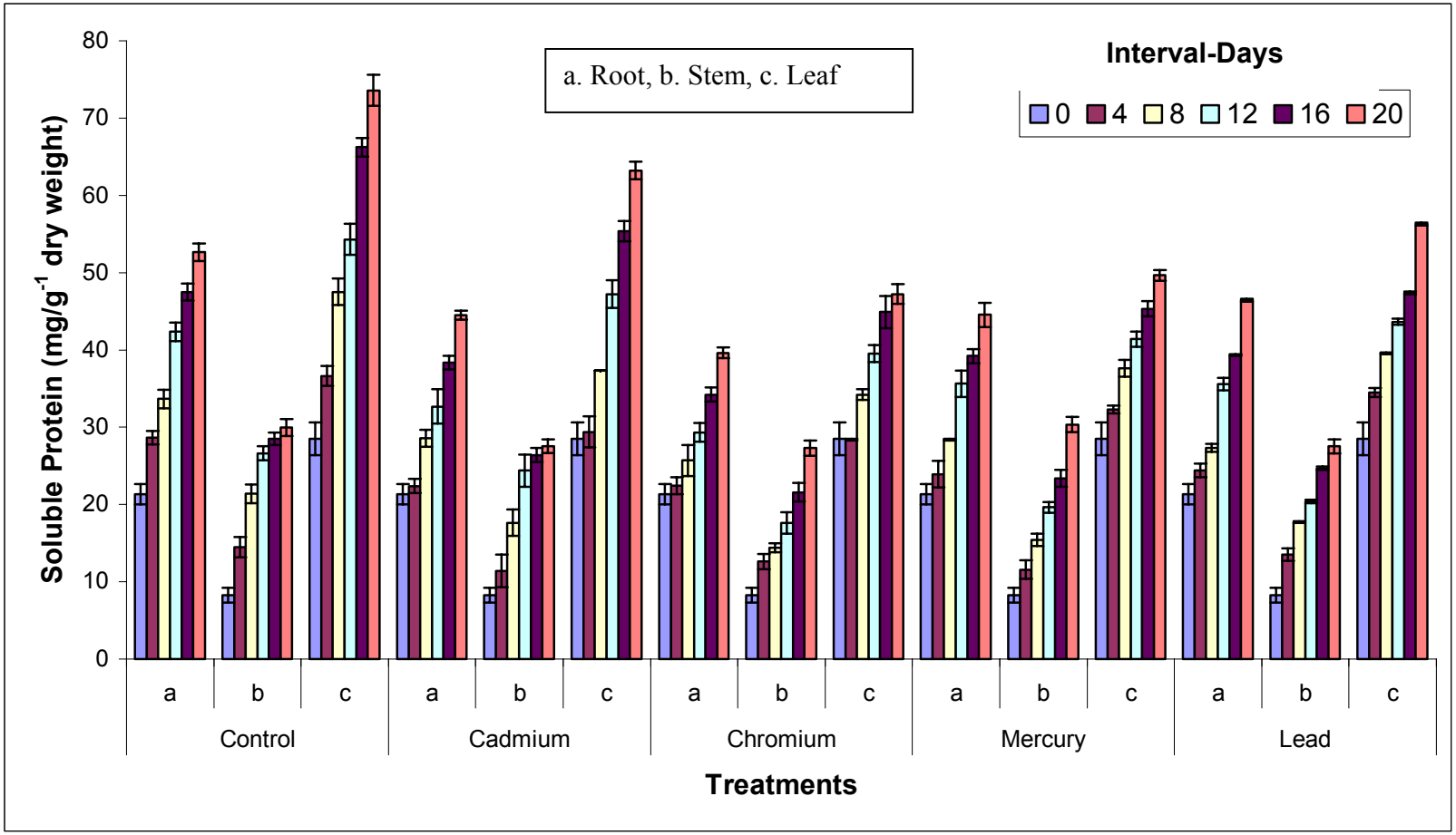


Fig. 9 Effect of Heavy Metals on Soluble Protein content in *Boerhavia diffusa*

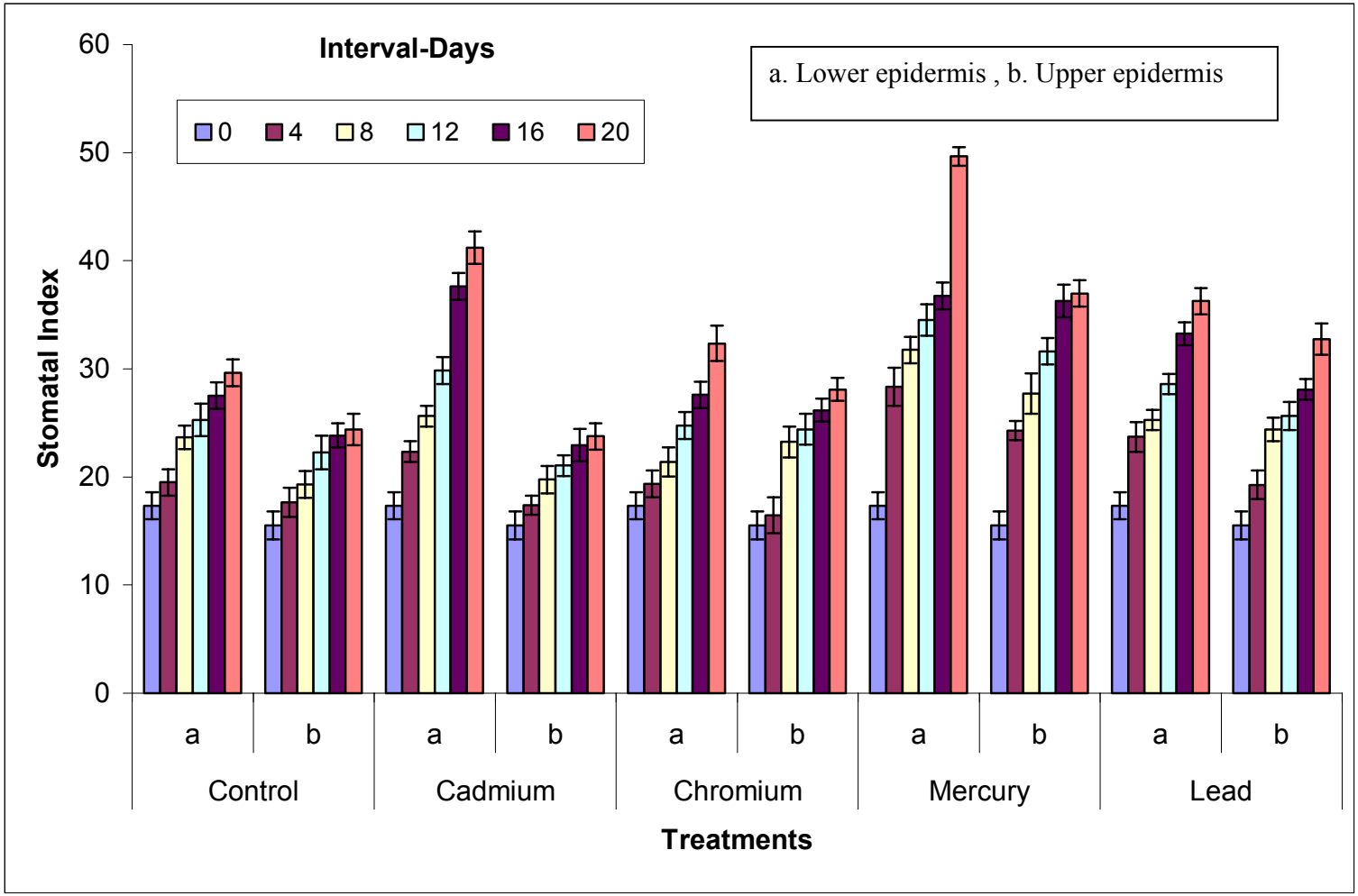


Fig. 6 Effect of Heavy Metals on the Stomatal Index in *Boerhavia diffusa*

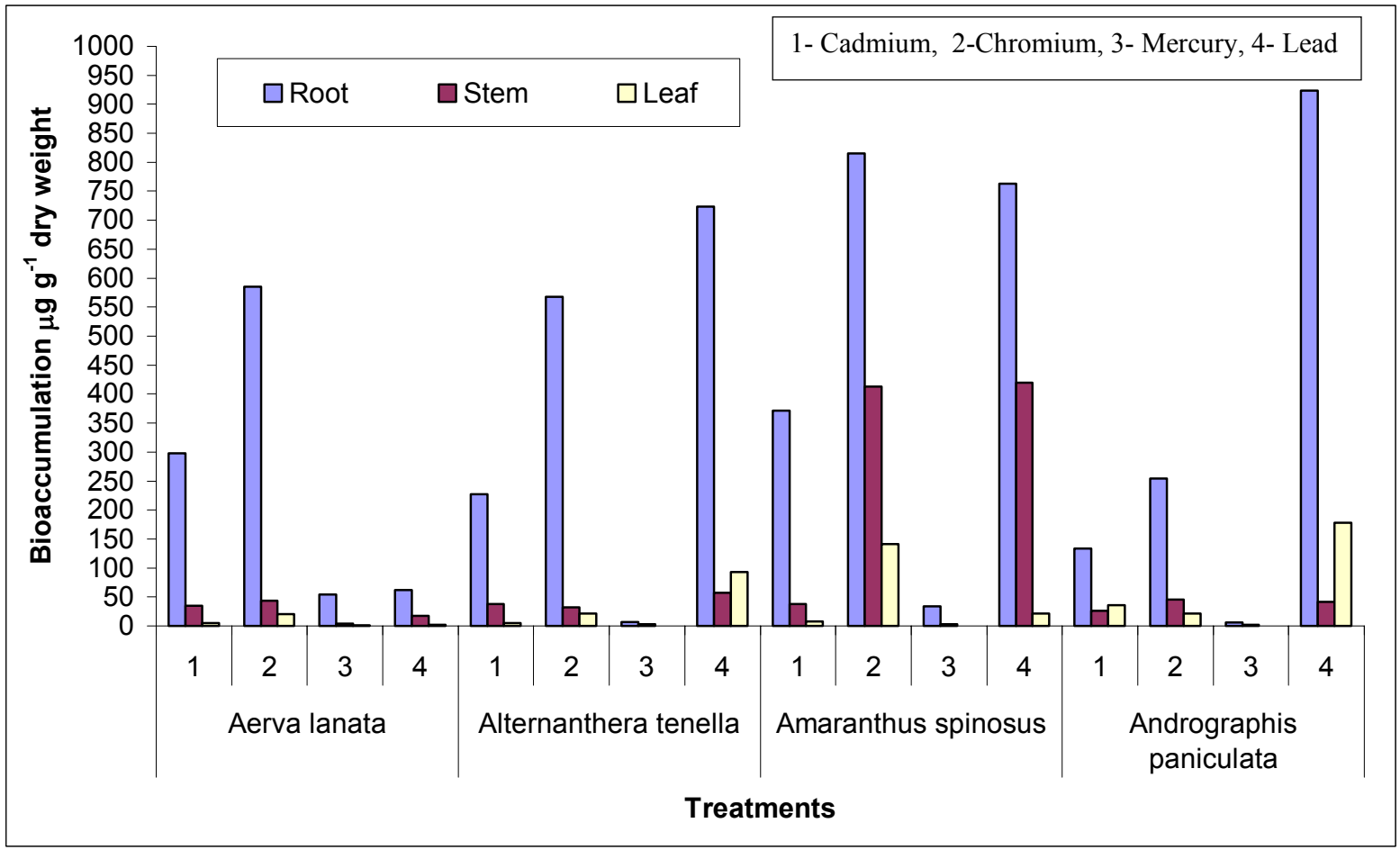


Fig. 51 Bioaccumulation pattern of Cadmium, Chromium, Mercury and Lead in Medicinal plants

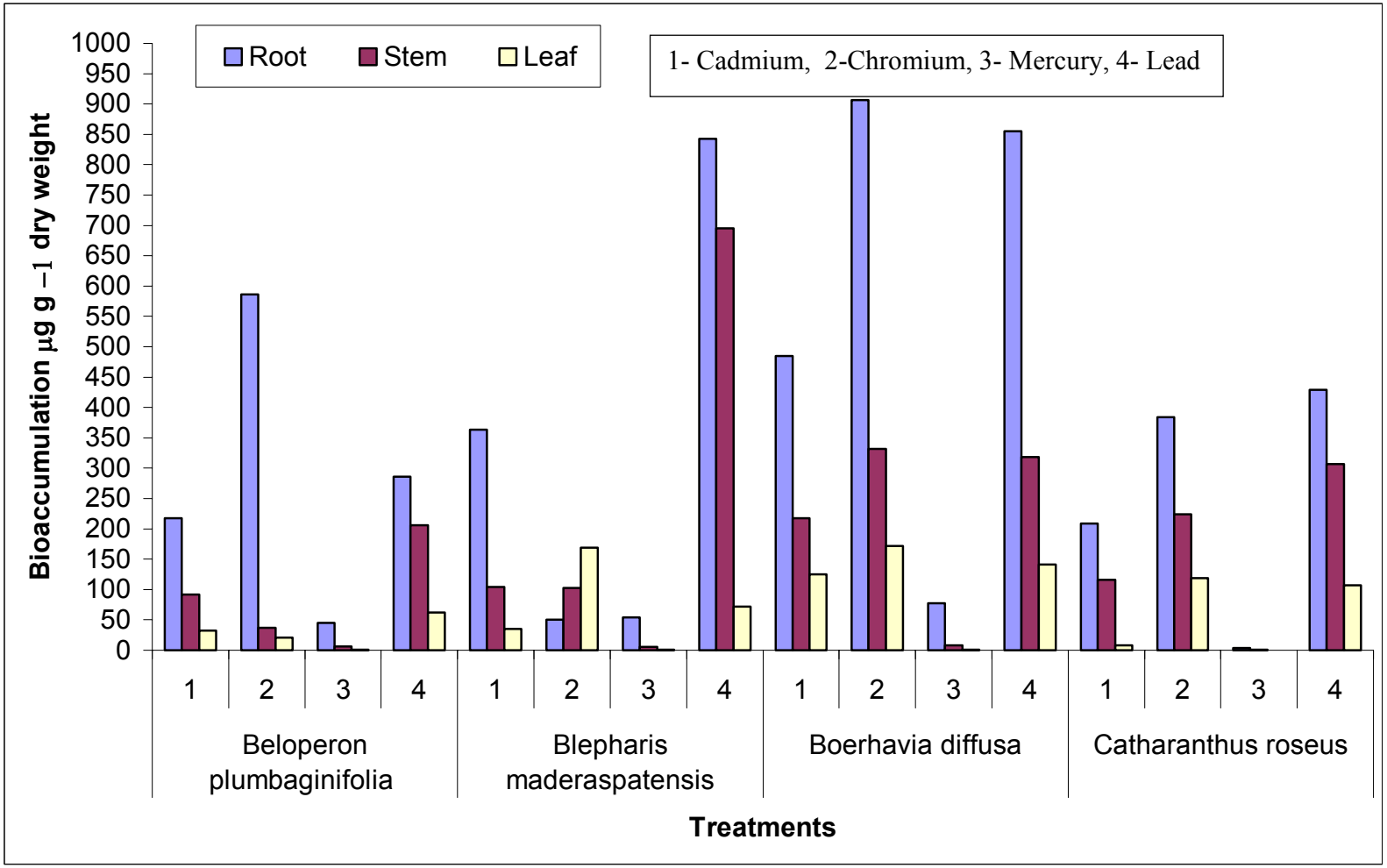


Fig. 52 Bioaccumulation pattern of Cadmium, Chromium, Mercury and Lead in Medicinal plants

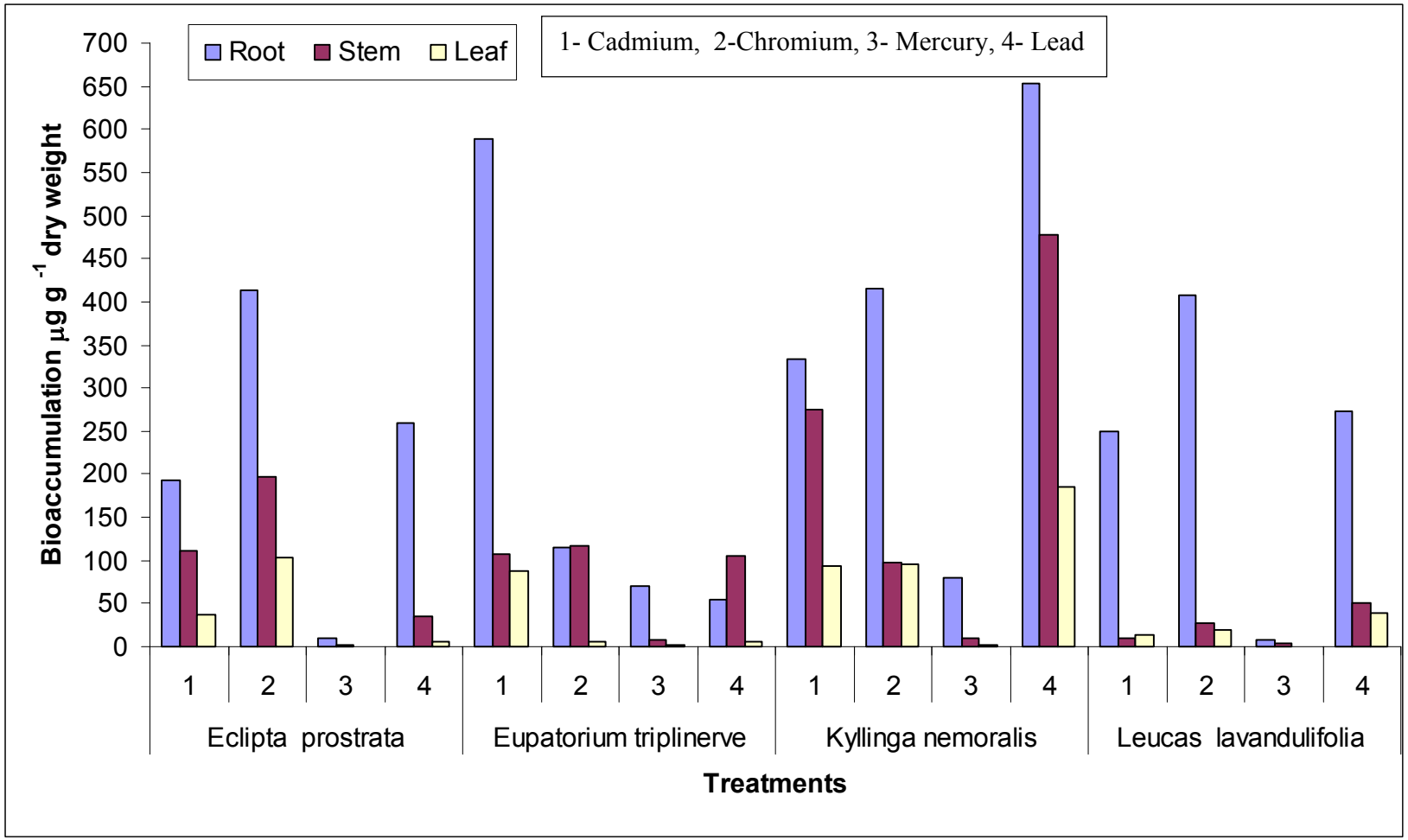


Fig. 53 Bioaccumulation pattern of Cadmium, Chromium, Mercury and Lead in Medicinal plants

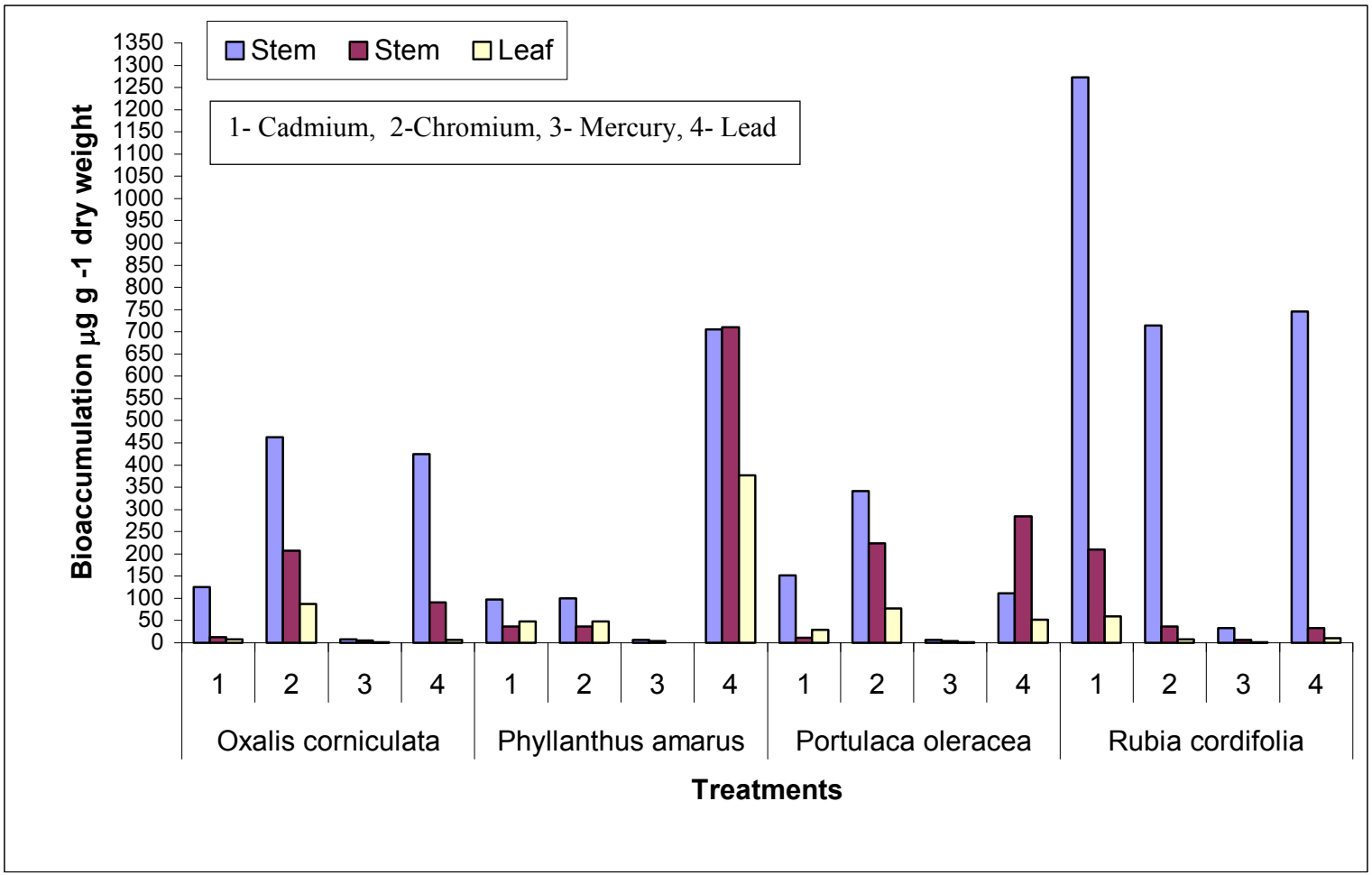


Fig. 54 Bioaccumulation pattern of Cadmium, Chromium, Mercury and Lead in Medicinal plants

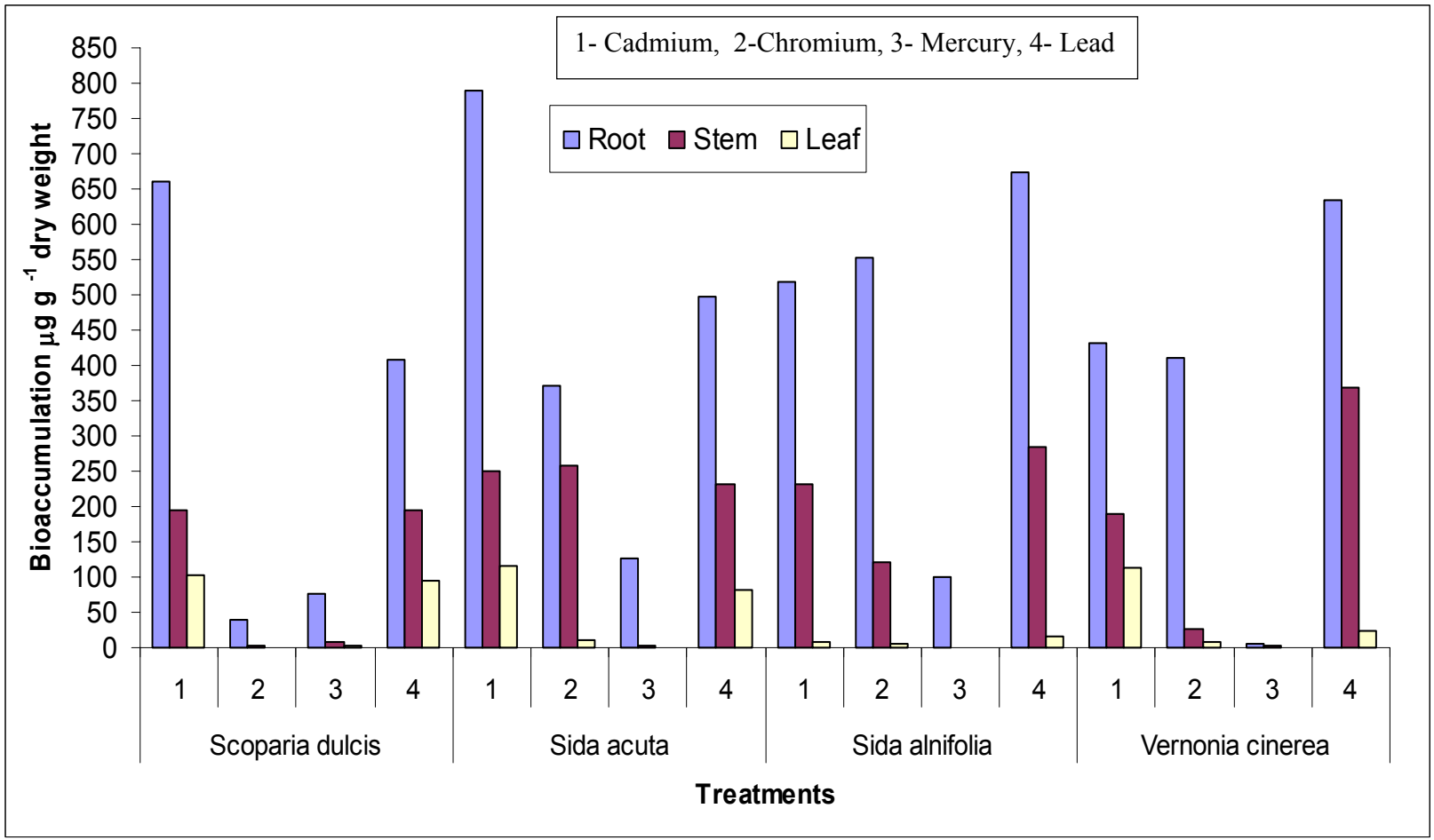


Fig. 55 Bioaccumulation pattern of Cadmium, Chromium, Mercury and Lead in Medicinal plants

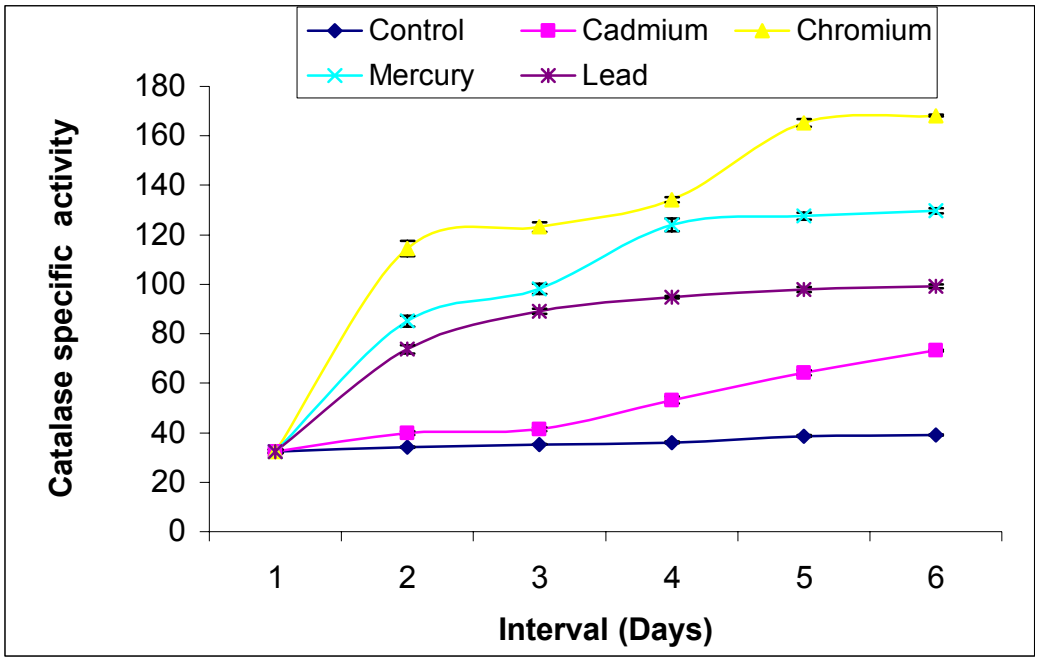


Fig. 35 Effect of Heavy Metals on Catalase specific activity in Root tissue of *Boerhavia diffusa*

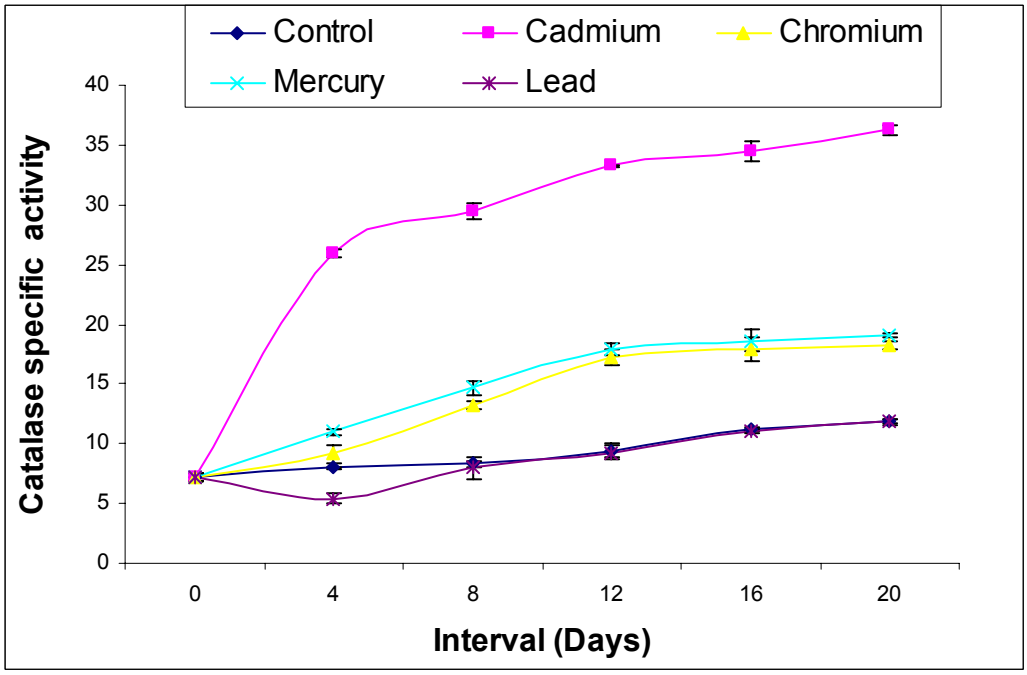


Fig. 36 Effect of Heavy Metals on Catalase specific activity in Stem tissue of *Boerhavia diffusa*

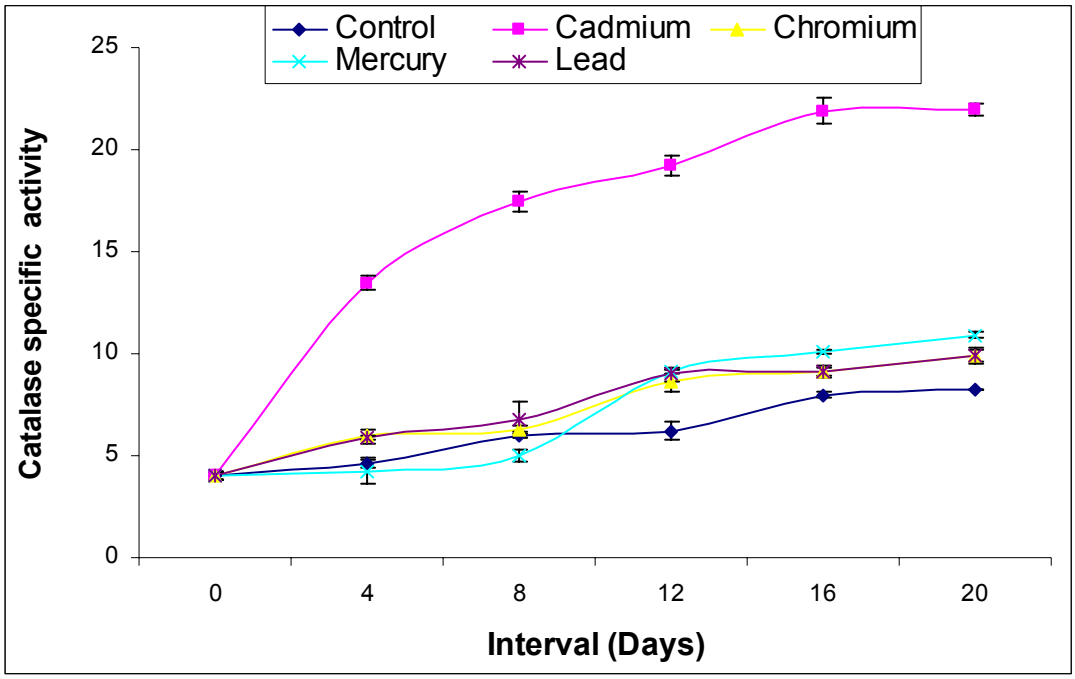


Fig. 37 Effect of Heavy Metals on Catalase specific activity in Leaf tissue of *Boerhavia diffusa*

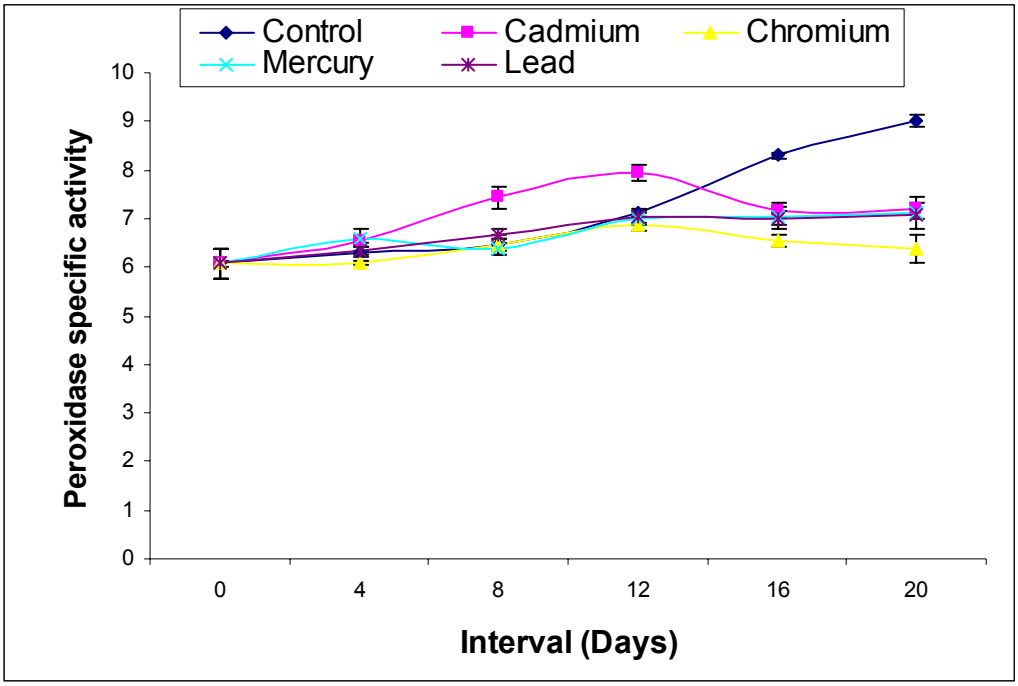


Fig. 26 Effect of Heavy Metals on Peroxidase specific activity in Root tissue of *Boerhavia diffusa*

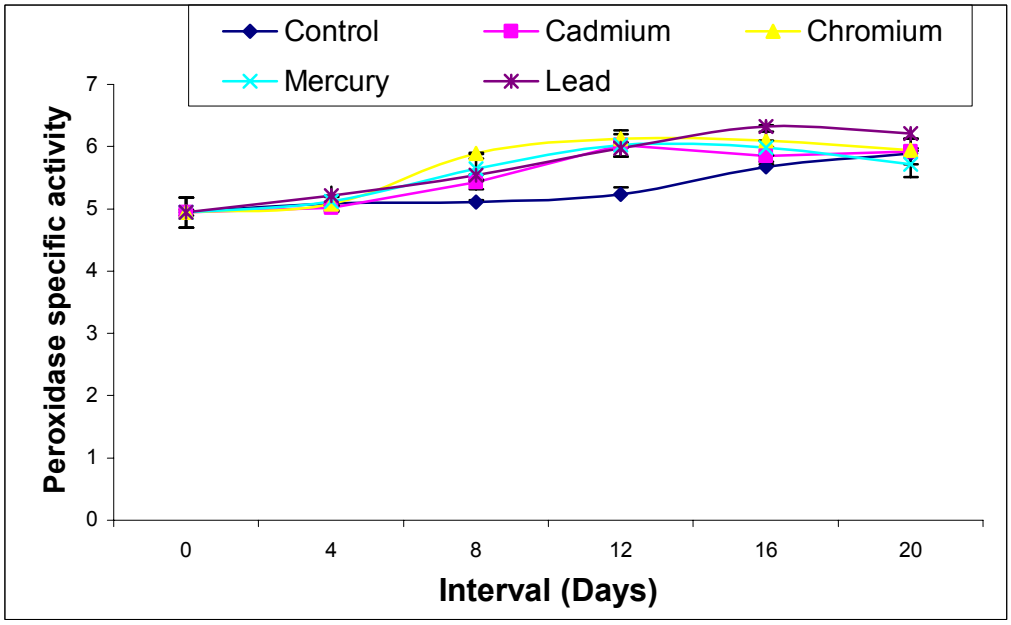


Fig. 27 Effect of Heavy Metals on Peroxidase specific activity in Stem tissue of *Boerhavia diffusa*

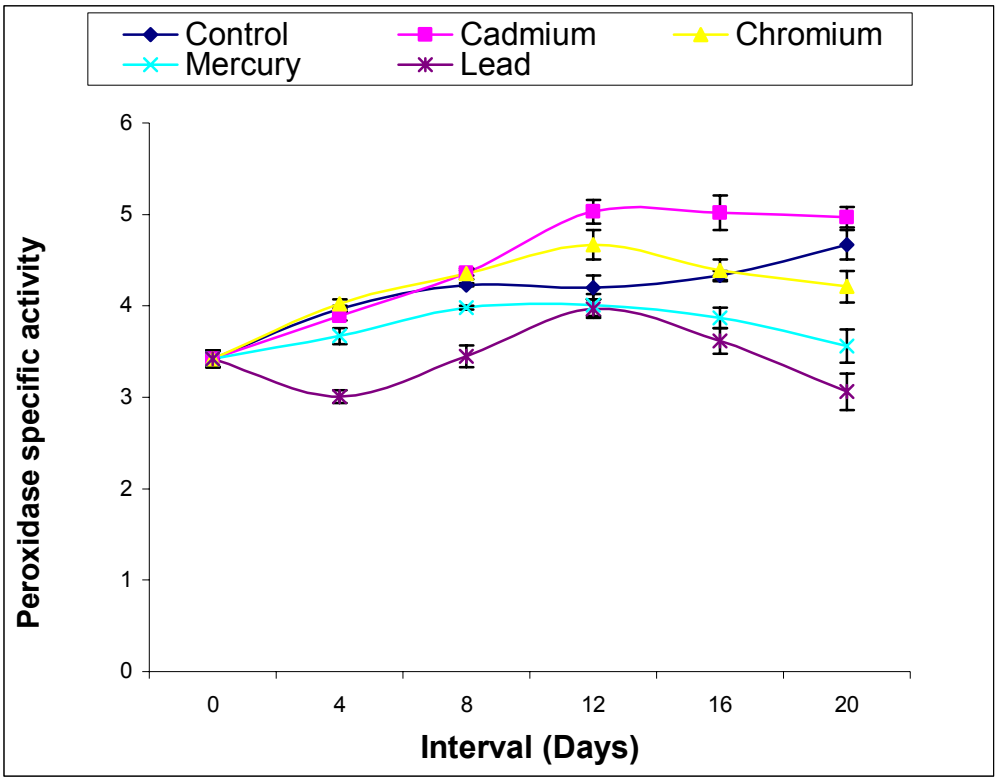


Fig. 28 Effect of Heavy Metals on Peroxidase specific activity in Leaf tissue of *Boerhavia diffusa*

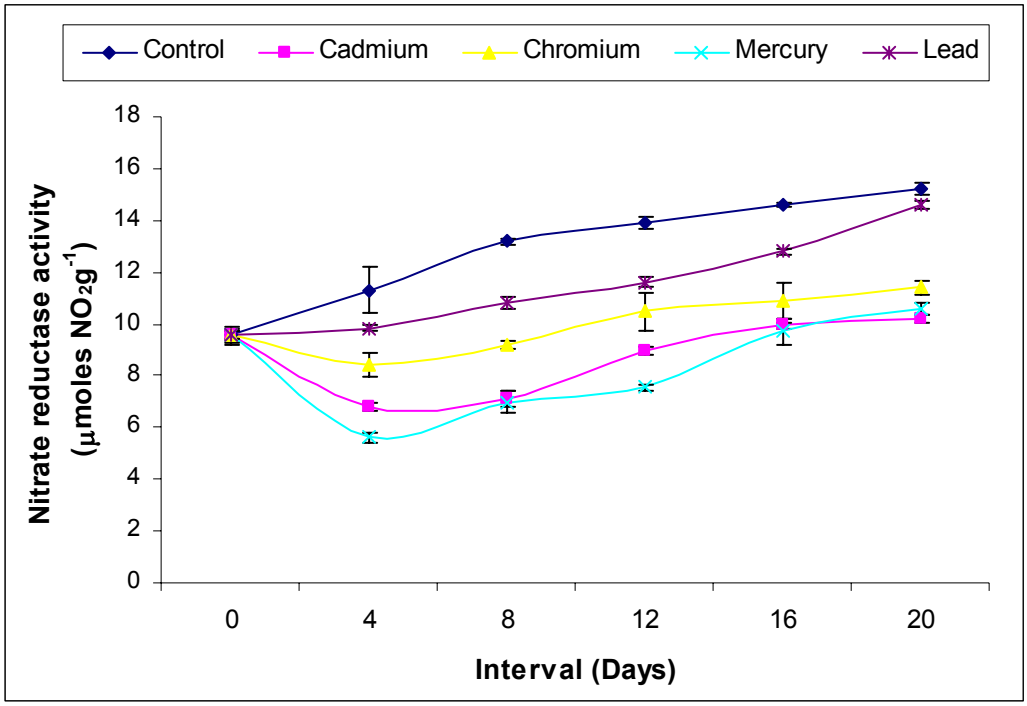


Fig. 16 Effect of Heavy metals on Nitrate reductase activity in root tissue of *Boerhavia diffusa*

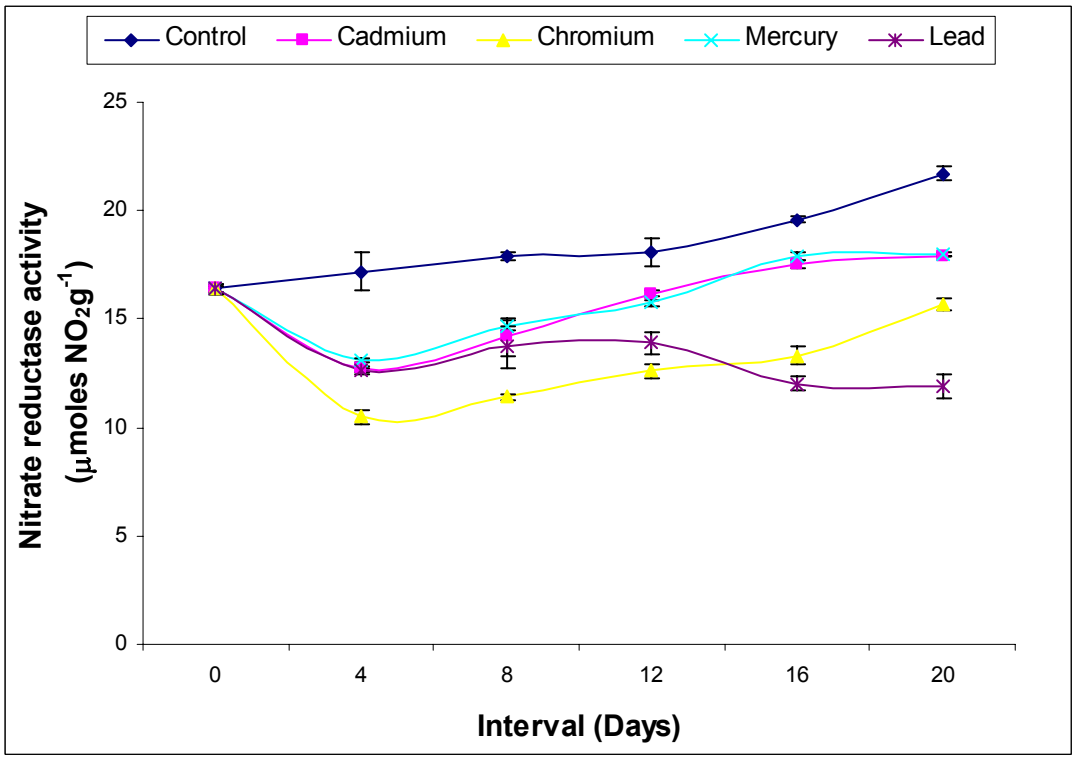


Fig. 17 Effect of Heavy metals on Nitrate reductase activity in stem tissue of *Boerhavia diffusa*

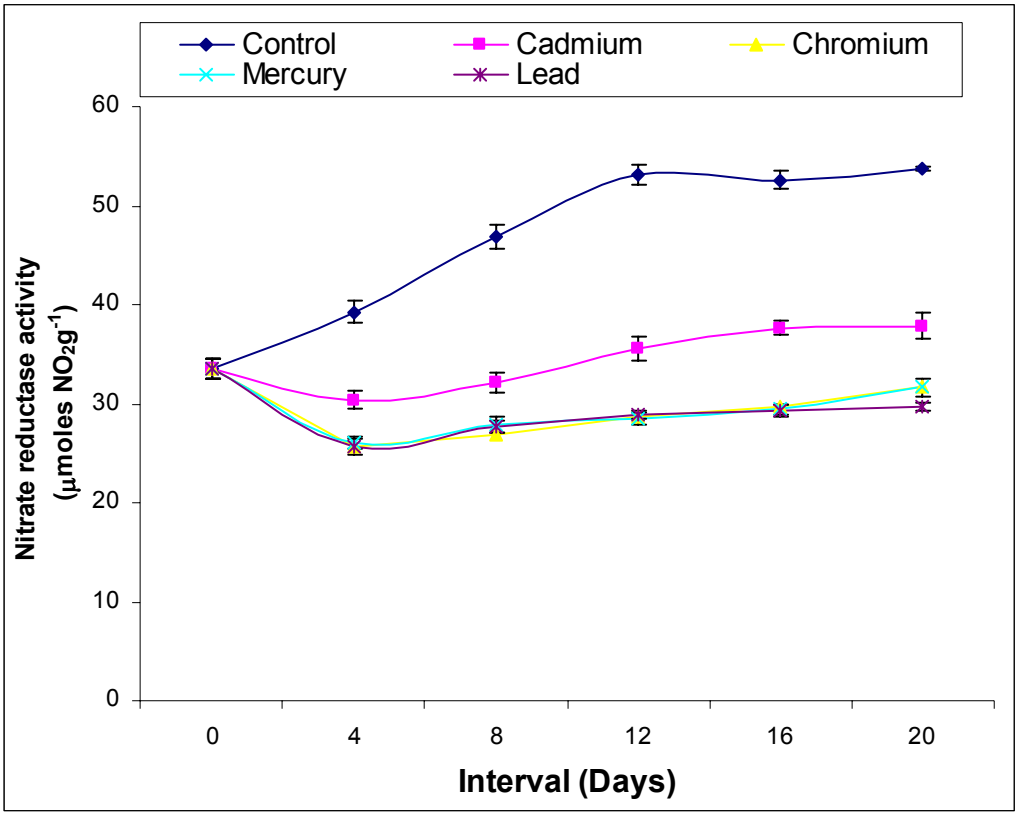


Fig. 18 Effect of Heavy metals on Nitrate reductase activity in leaf tissue of *Boerhavia diffusa*

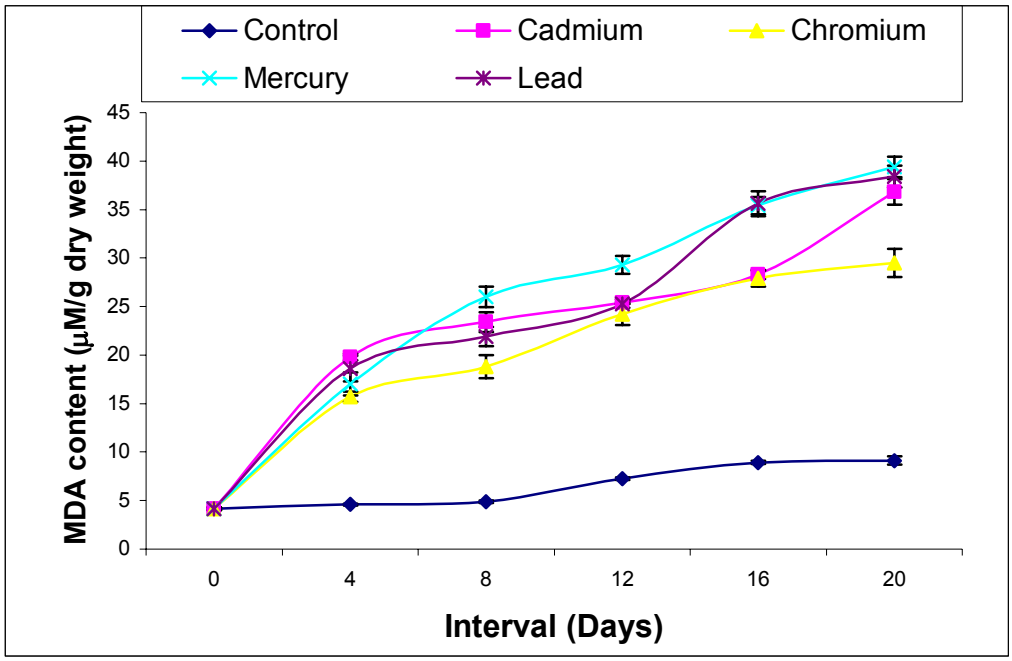


Fig. 20 Effect of Heavy metals on Lipid Peroxidation in root tissue of *Boerhavia diffusa*

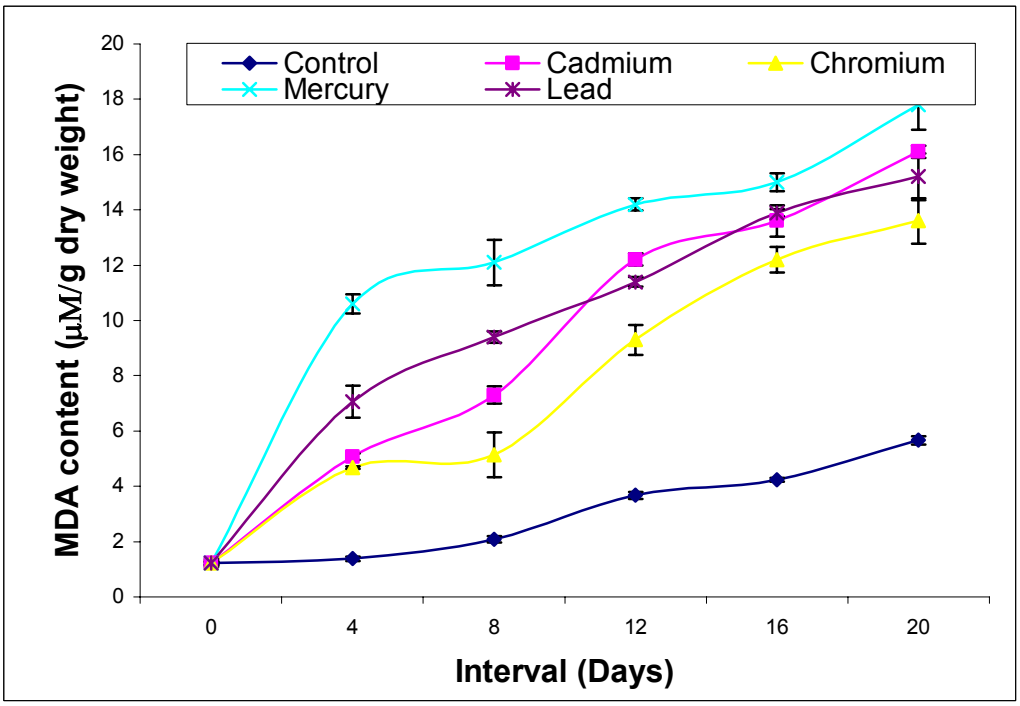


Fig. 21 Effect of Heavy metals on Lipid Peroxidation in stem tissue of *Boerhavia diffusa*

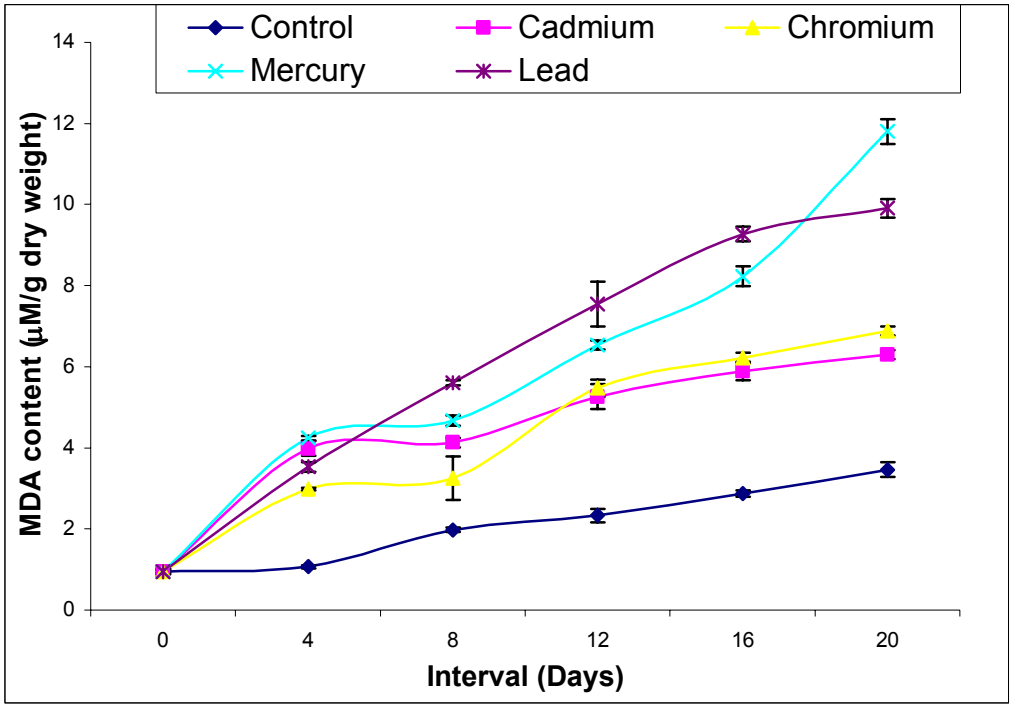


Fig. 22 Effect of Heavy metals on Lipid Peroxidation in Leaf tissue of *Boerhavia diffusa*

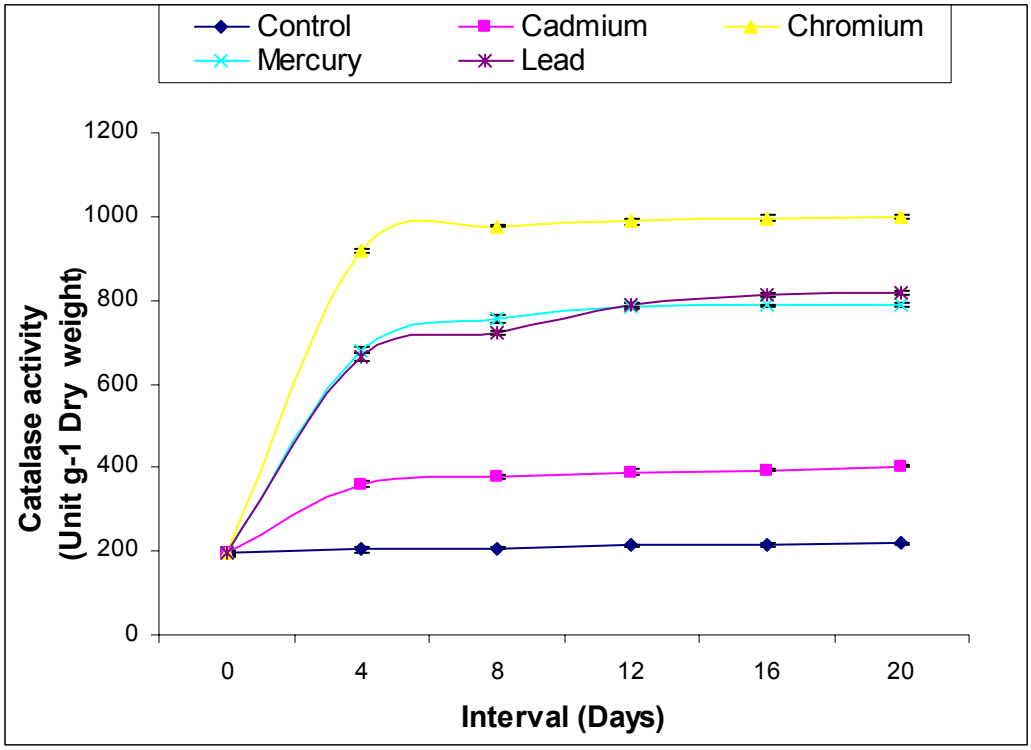


Fig. 32 Effect of Heavy Metals on Catalase unit activity in Root tissue of *Boerhavia diffusa*

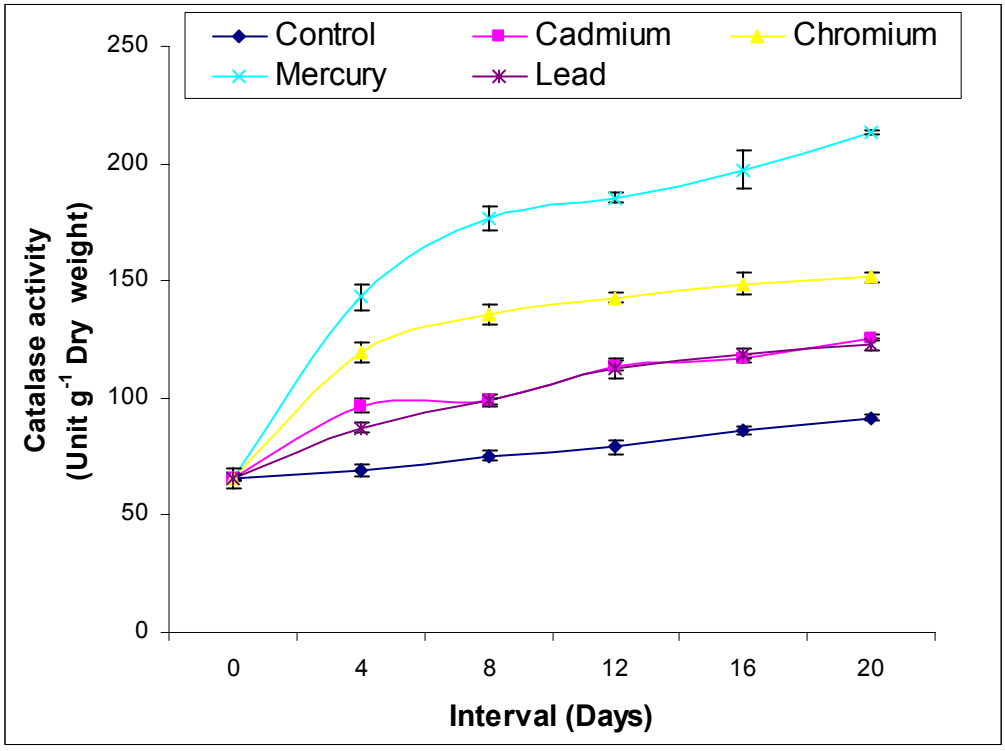


Fig. 33 Effect of Heavy Metals on Catalase unit activity in Stem tissue of *Boerhavia diffusa*

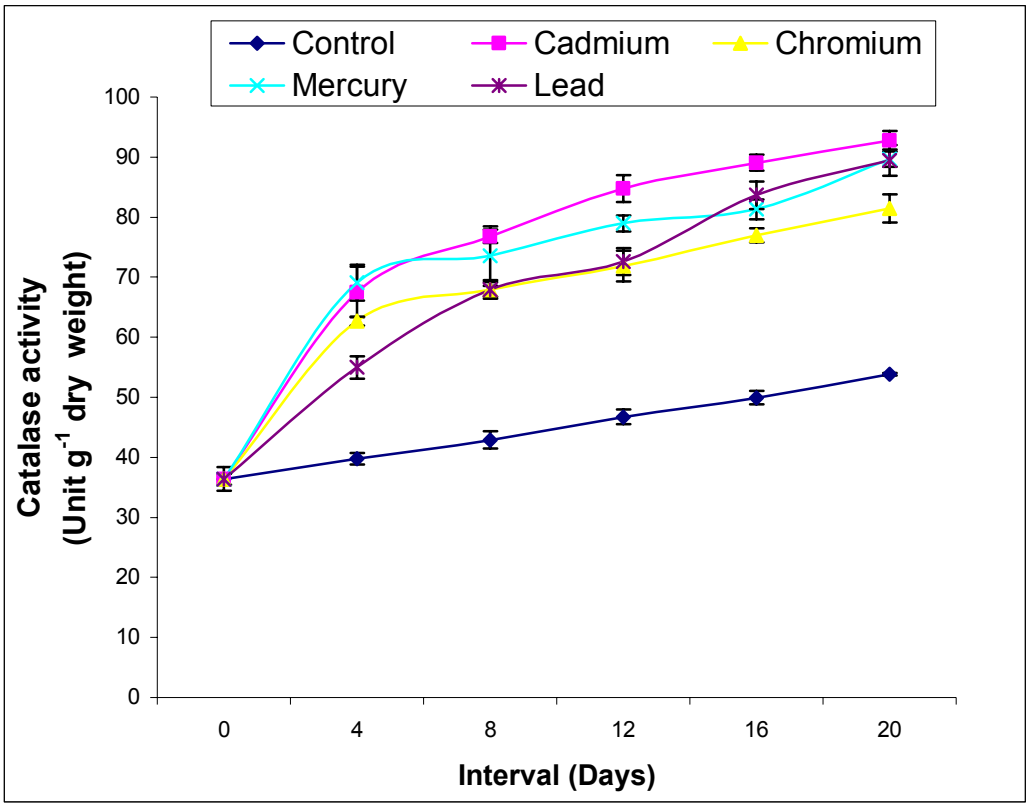


Fig. 34 Effect of Heavy Metals on Catalase unit activity in Leaf tissue of *Boerhavia diffusa*

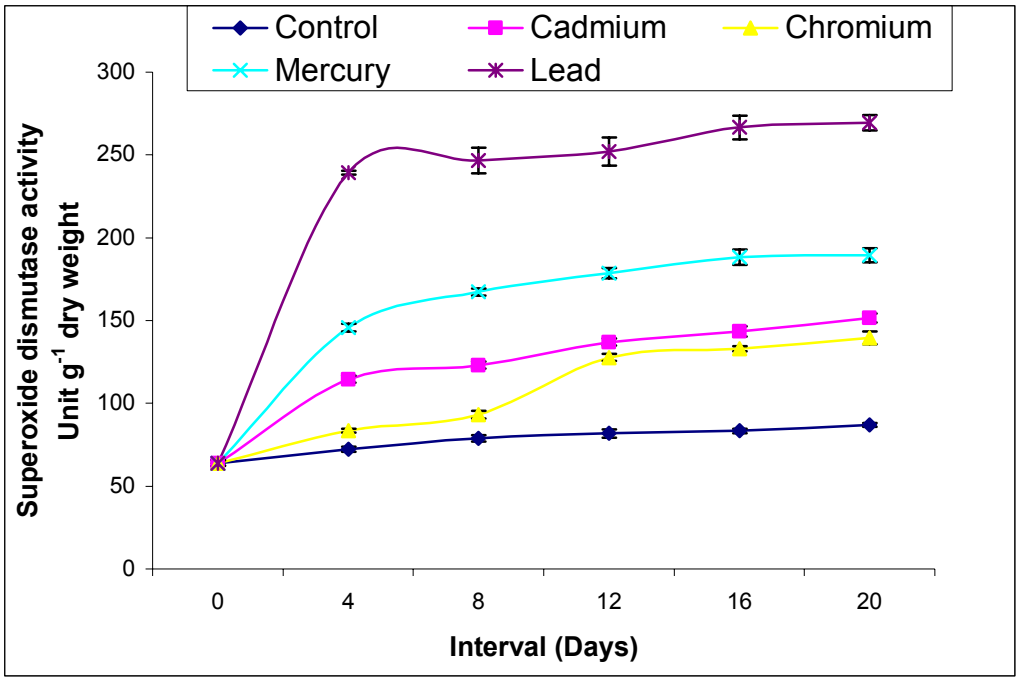


Fig. 29 Effect of Heavy Metals on Superoxide dismutase activity in Root tissue of *Boerhavia diffusa*

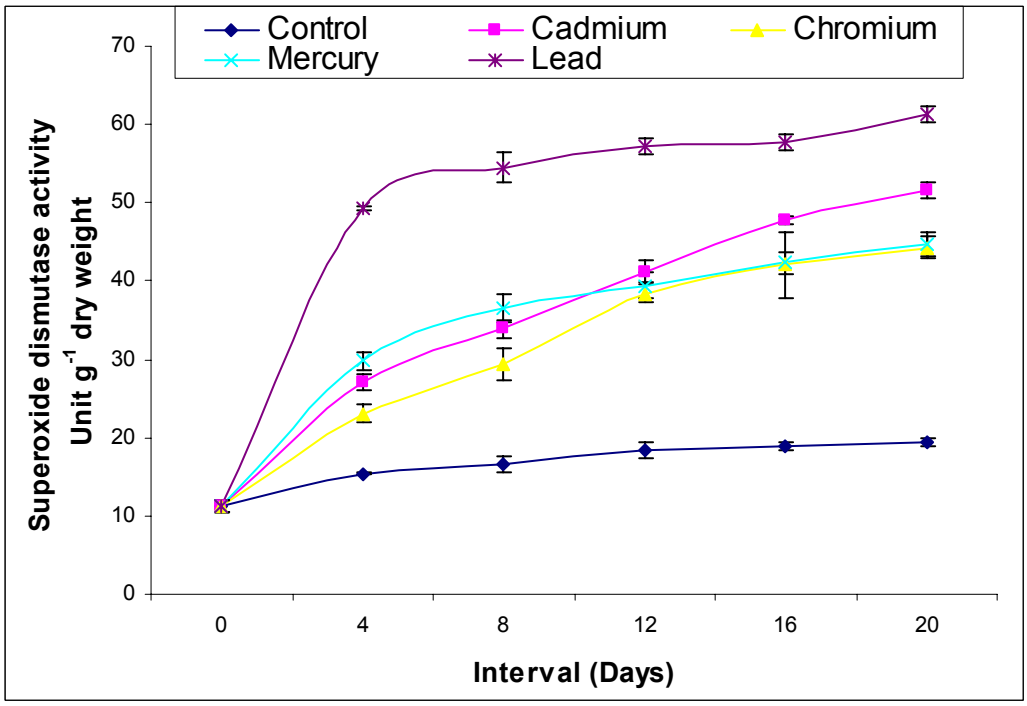


Fig. 30 Effect of Heavy Metals on Superoxide dismutase activity in Stem tissue of *Boerhavia diffusa*

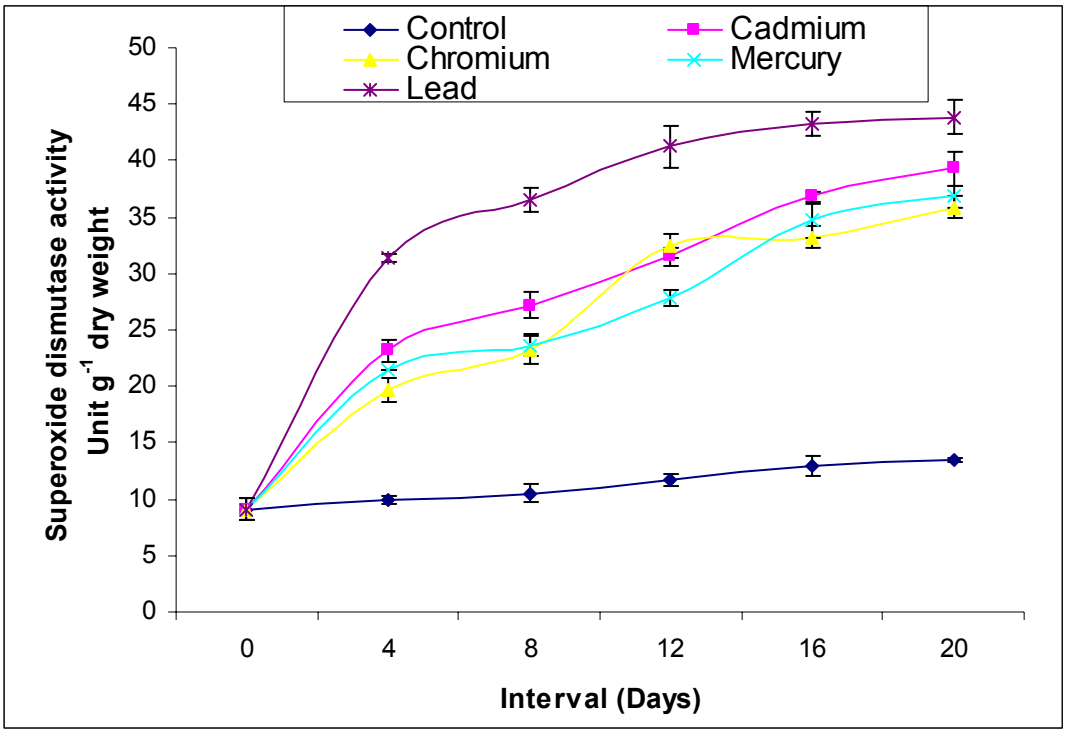


Fig. 31 Effect of Heavy Metals on Superoxide dismutase activity in Leaf tissue of *Boerhavia diffusa*

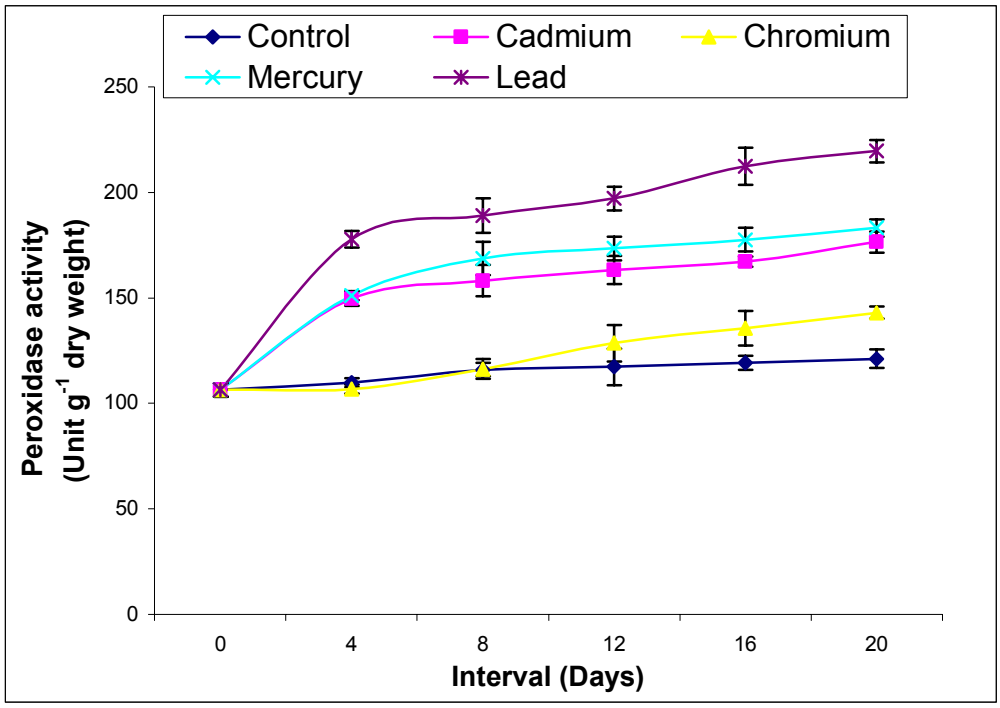


Fig. 23 Effect of Heavy Metals on Peroxidase activity in Root tissue of *Boerhaavia diffusa*

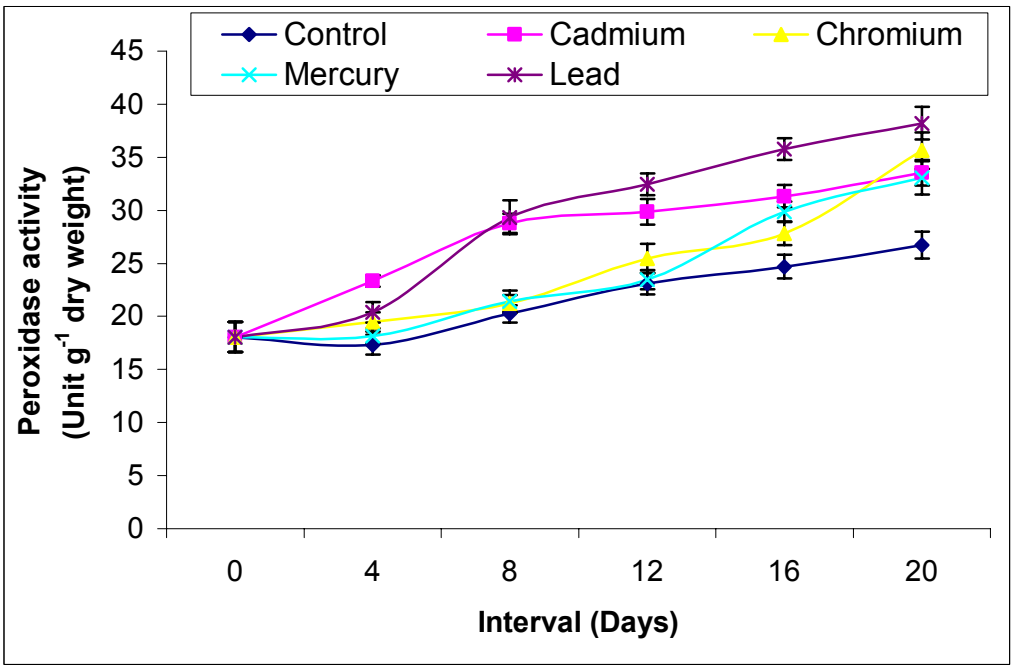


Fig. 24 Effect of Heavy Metals on Peroxidase activity in Stem tissue of *Boerhavia diffusa*

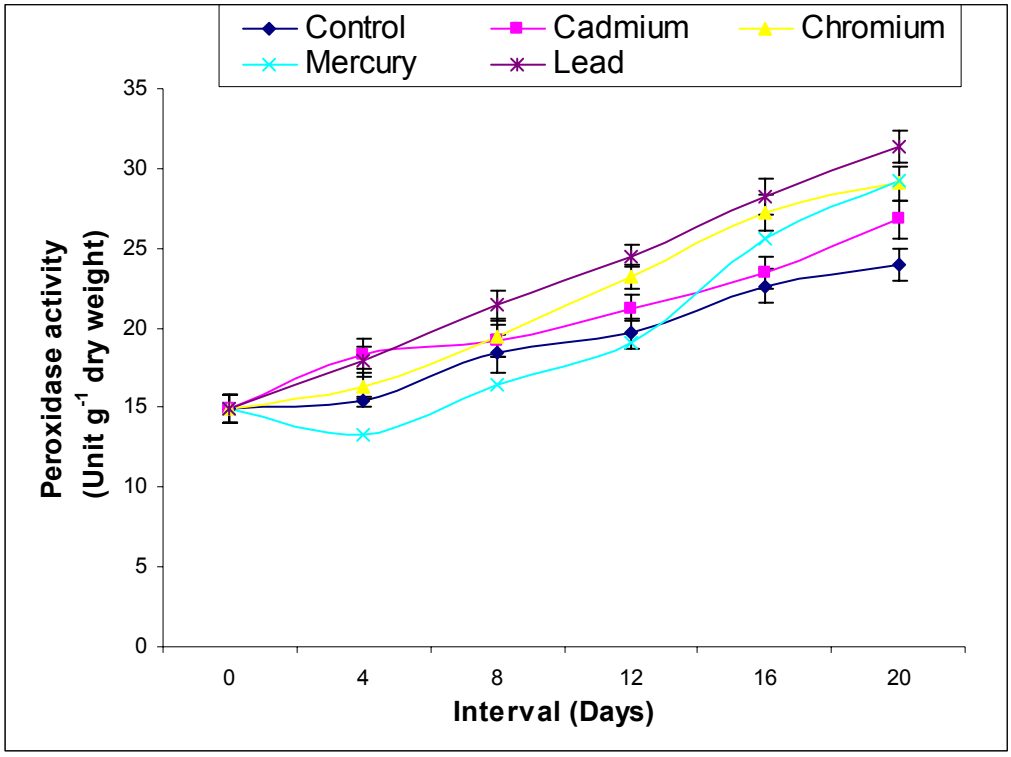


Fig. 25 Effect of Heavy Metals on Peroxidase activity in Leaf tissue of *Boerhavia diffusa*

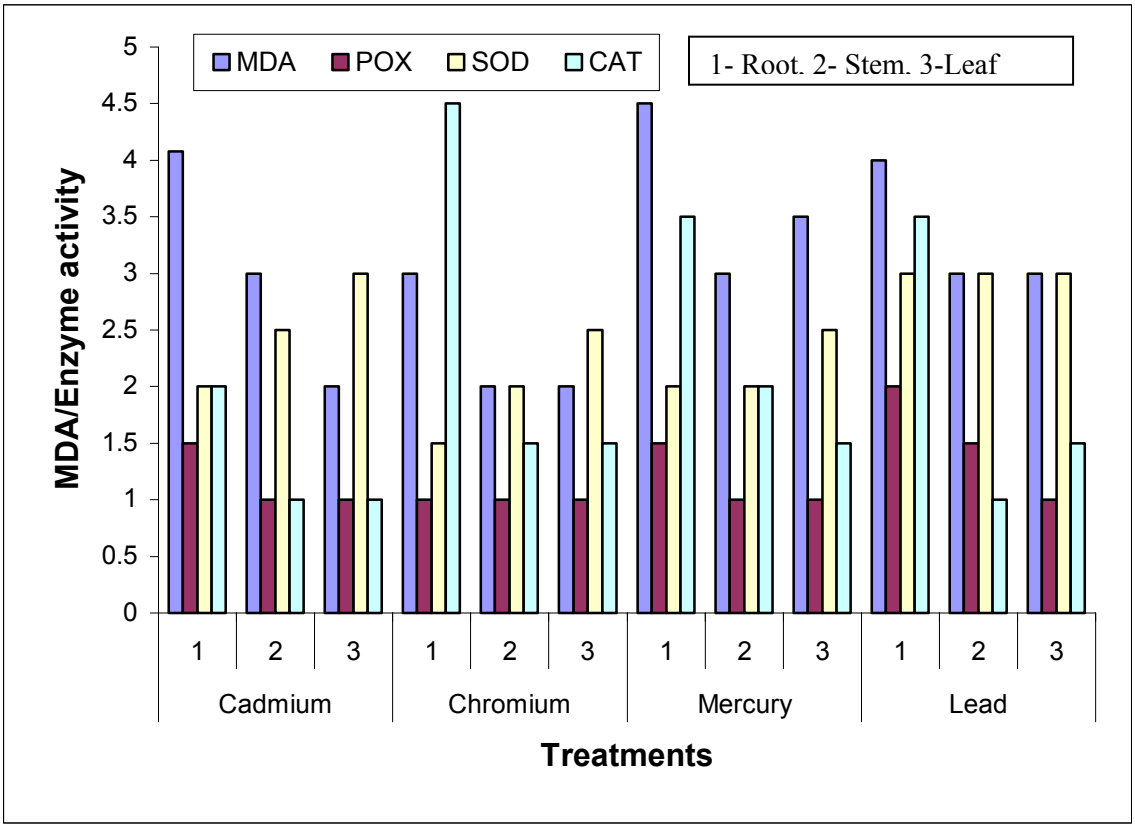


Fig. 38 Comparison between Malonedialdehyde and Stress enzymes activity

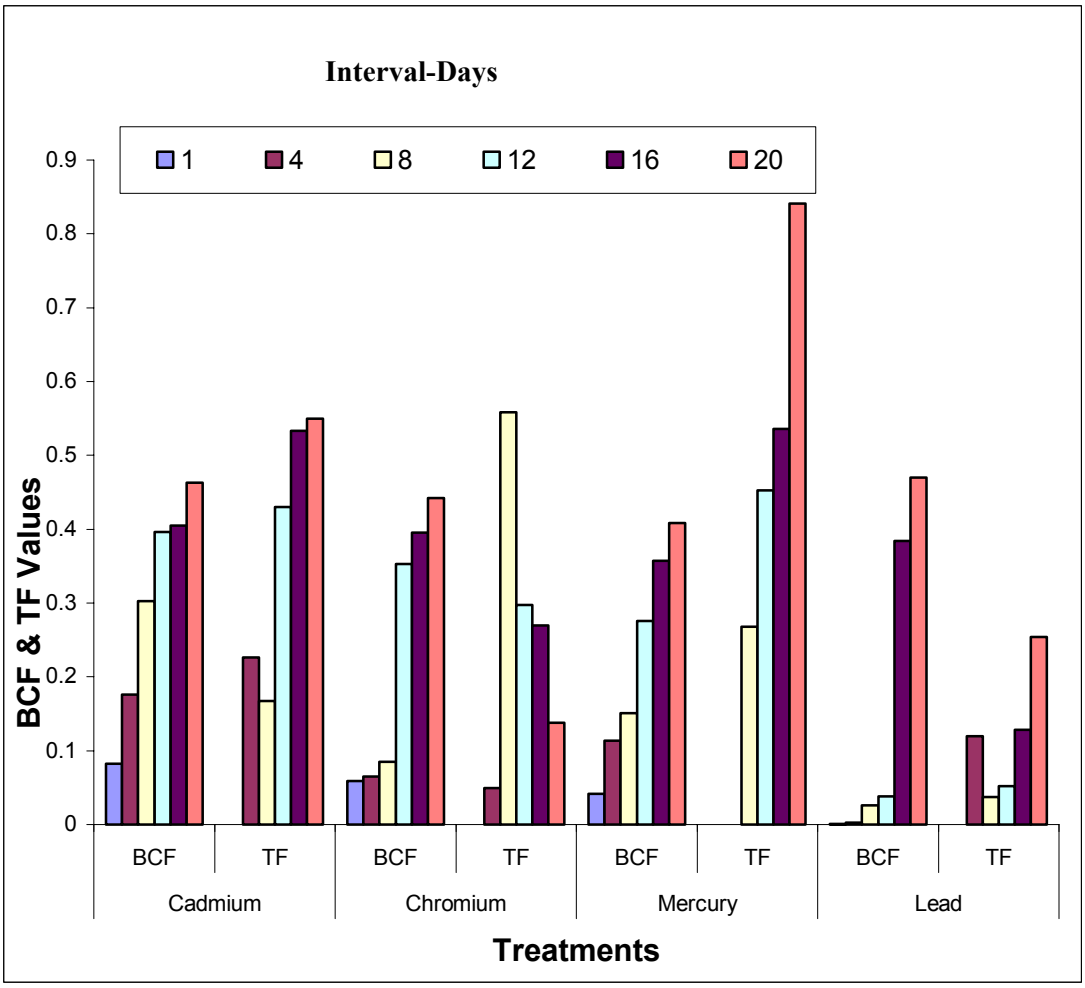


Fig. 41 Bioaccumulation pattern of Heavy Metals in terms of BCF and TF

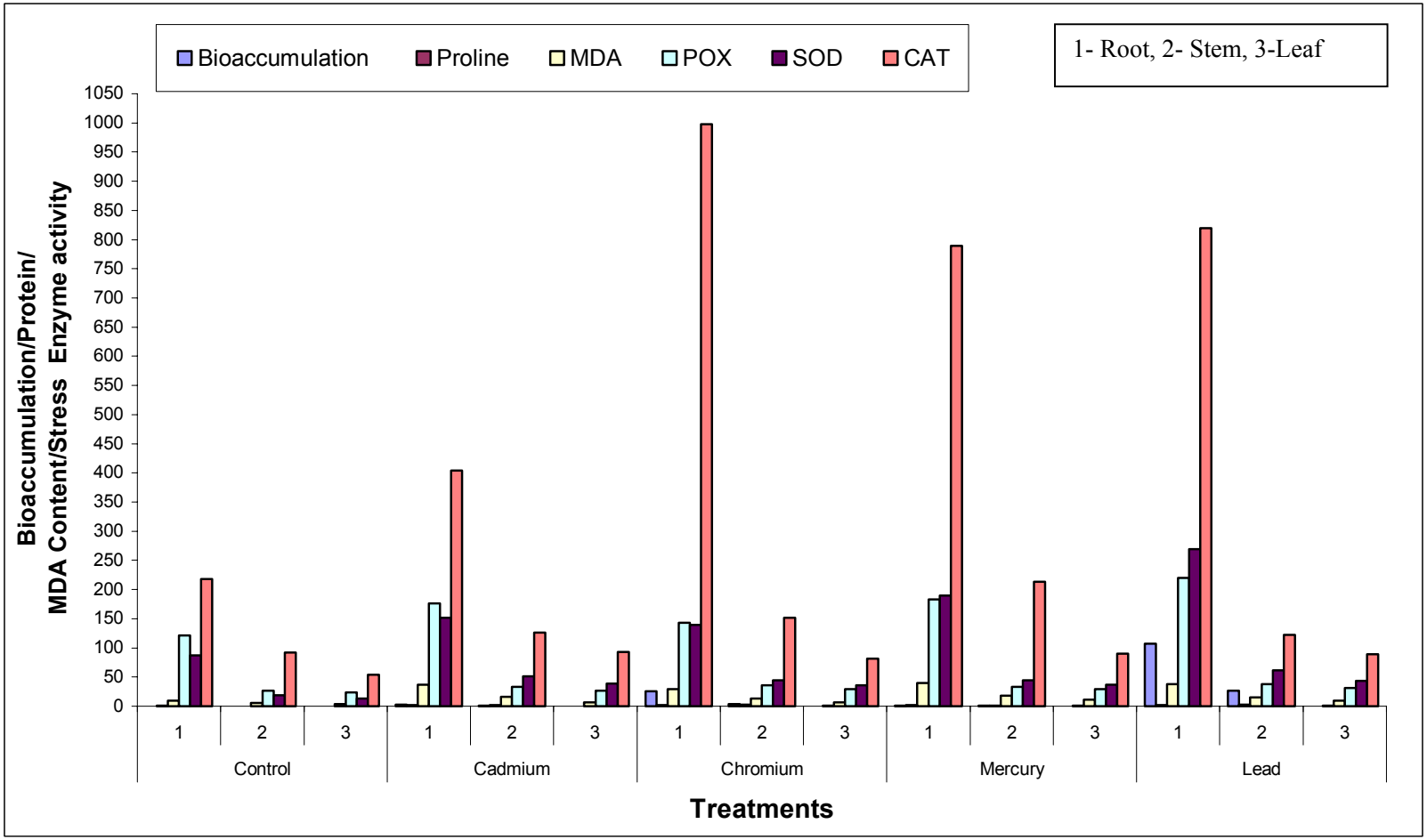


Fig. 39 Comparison between Bioaccumulation, protein and MDA Content and Stress enzymes activities of *Boerhaviadiffusa*

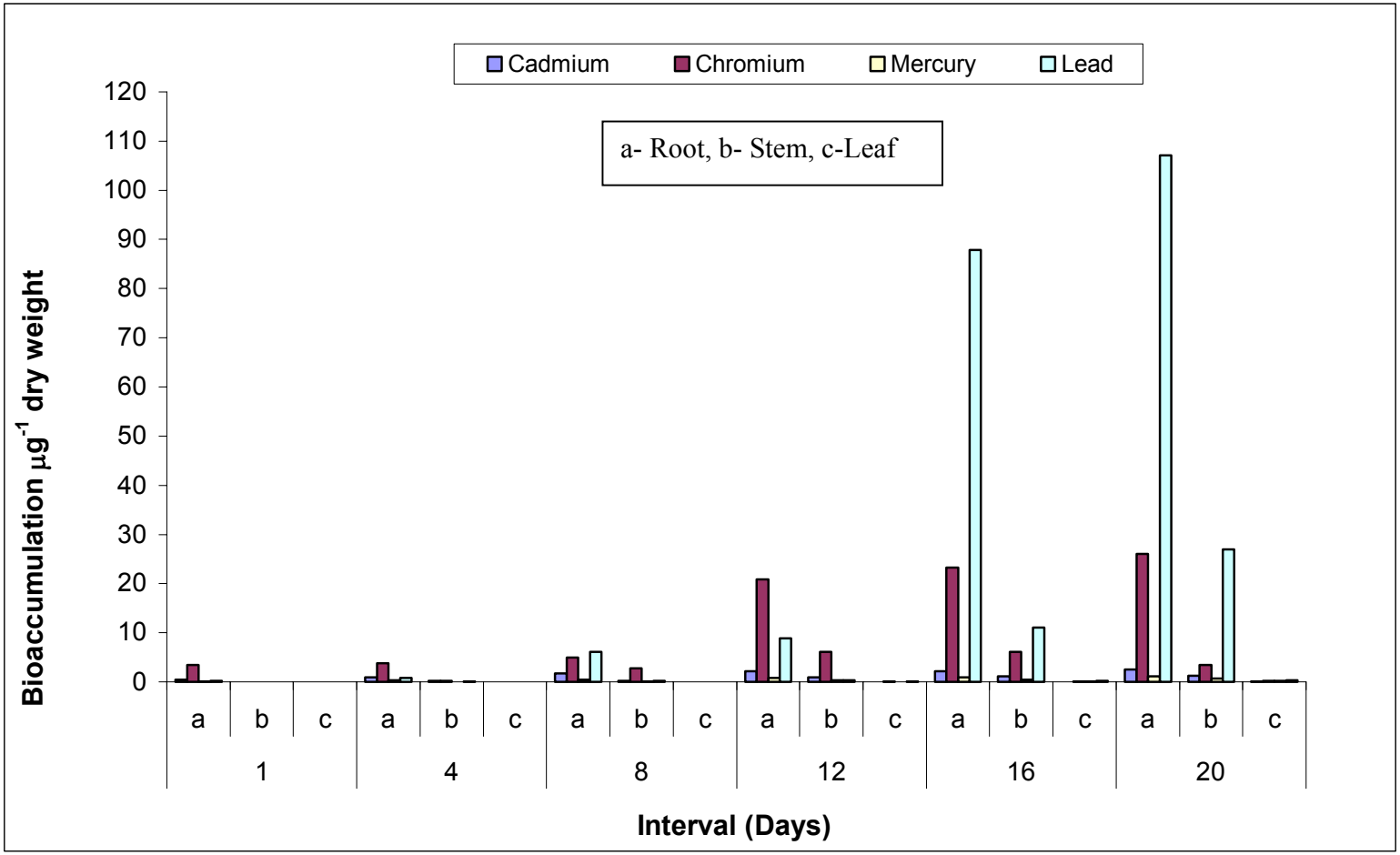


Fig. 40 Bioaccumulation pattern of Heavy Metals in *Boerhavia diffusa*

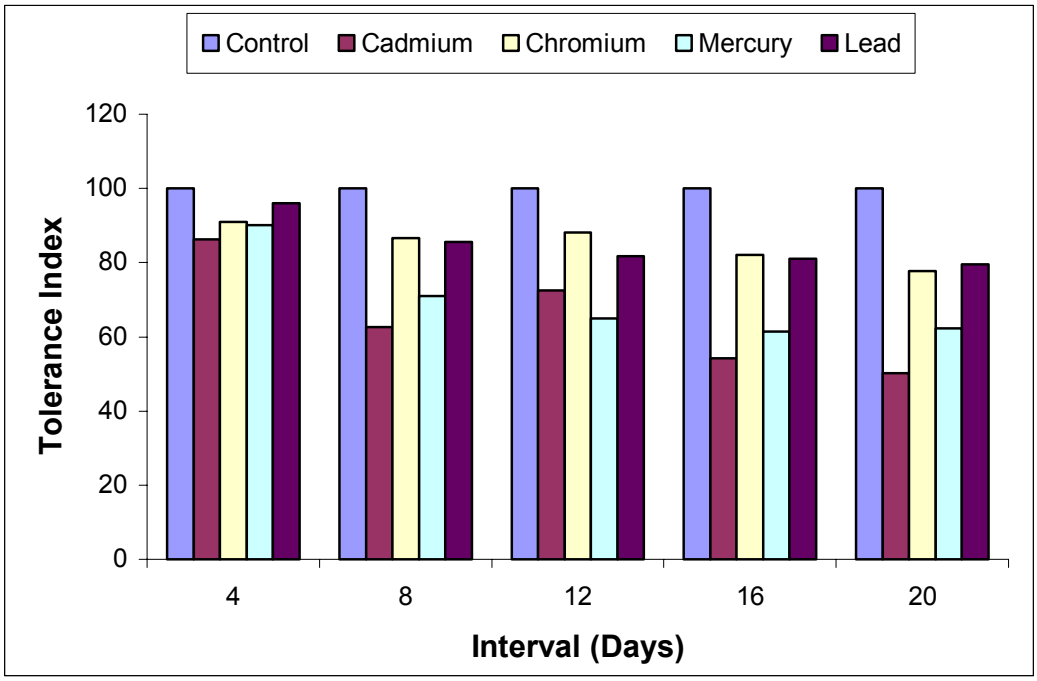


Fig. 5 Effect of Heavy Metals on Tolerance Index percentage pertaining to Root length in *Boerhavia diffusa* during growth

Fig. 1 Growth performance of *Boerhavia diffusa* in Hogland nutrient solution

Fig. 2 Effect of Heavy Metals on Morphology of *Boerhavia diffusa*

1. Control, 2. Cadmium, 3. Chromium, 4. Mercury, 5. Lead

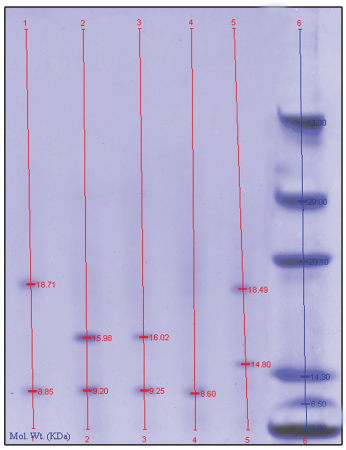


Fig. 10 SDS PAGE protein profile of *Boerhavia diffusa* Root treated with heavy metals

1. Cadmium
2. Chromium
3. Mercury
4. Lead
5. Control
6. Marker proteins

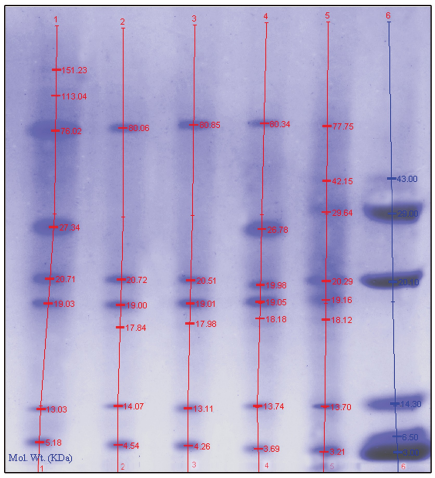


Fig. 11 SDS PAGE protein profile of *Boerhavia diffusa* Stem treated with heavy metals

1. Cadmium
2. Chromium
3. Mercury
4. Lead
5. Control
6. Marker proteins

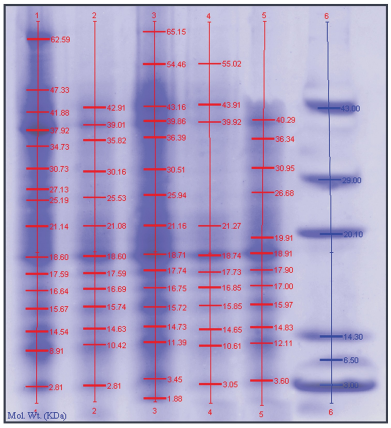
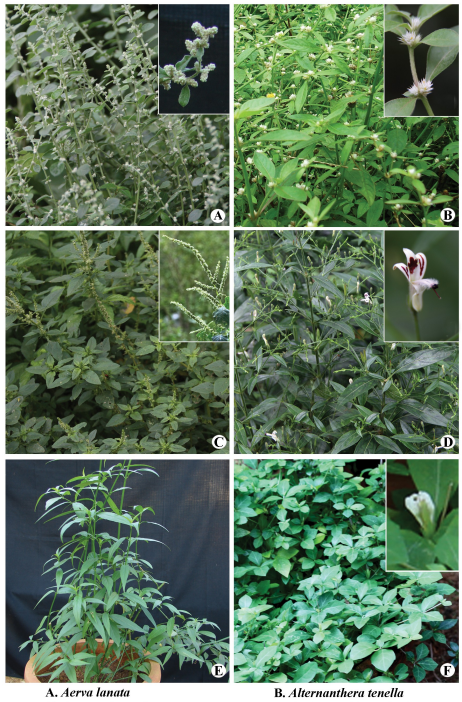


Fig. 12 SDS PAGE protein profile of *Boerhavia diffusa* Leaf treated with heavy metals

1. Cadmium
2. Chromium
3. Mercury
4. Lead
5. Control
6. Marker proteins

Fig. 56



A. ***Aerva lanata*** **B.** ***Alternanthera tenella***
C. ***Amaranthus spinosus*** **D.** ***Andrographis paniculata***
E. ***Beloperon plumbaginifolia*** **F.** ***Blepharis maderaspatensis***
Inset: Inflorescence/ Flowers

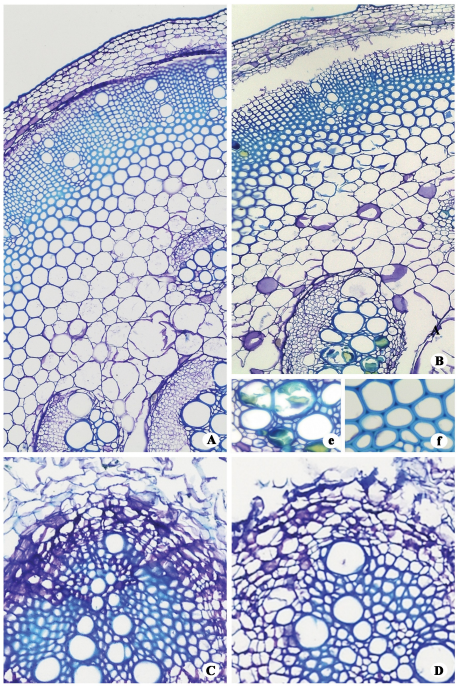


Fig. 42 Histochemical localisation of Cadmium in the Stem and Root of *Boerhavia diffusa*

A. Control Stem; B. Treated Stem; C.Control Root; D. Treated root.
e. Cadmoum localisation; f. Cell wall thickening.

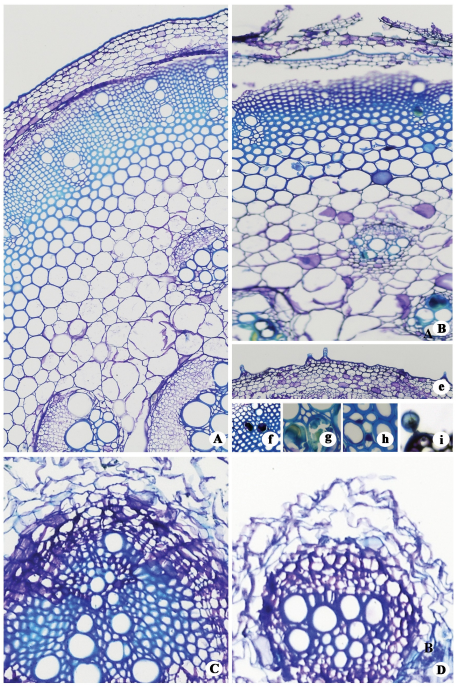


Fig. 43 Histochemical localisation of Chromium in the Stem and Root of *Boerhavia diffusa*

A. Control Stem; B. Treated Stem; C.Control Root; D. Treated root.
e & i. Epedermis with trichomes; f &g. Chromium localisation; h. Cell wall thickening

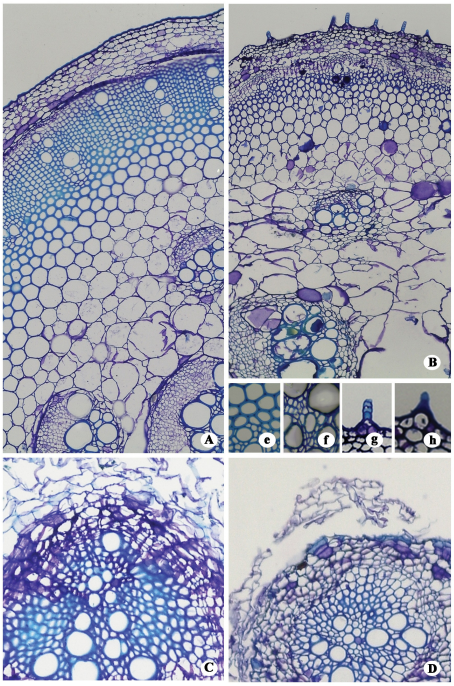


Fig. 44 Histochemical localisation of Mercury in the Stem and Root of *Boerhavia diffusa*
A. Control Stem; B. Treated Stem; C. Control Root; D. Treated Root
e & f. Cell wall thickening ; g & h. Epidermis with trichome

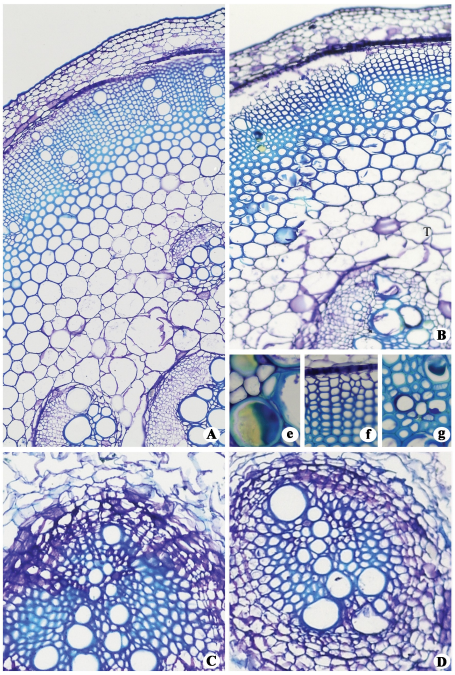
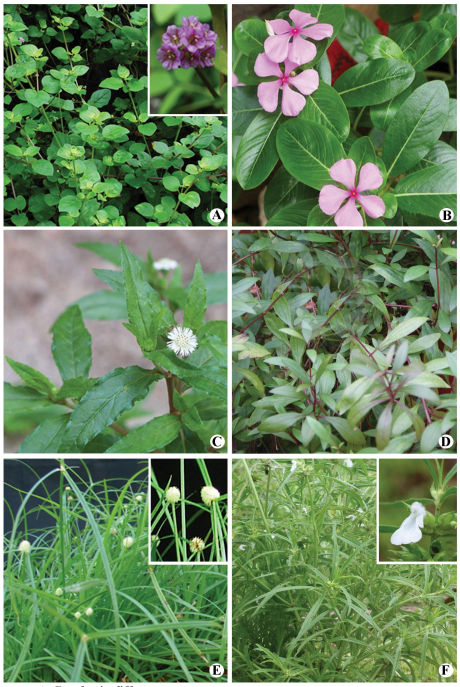


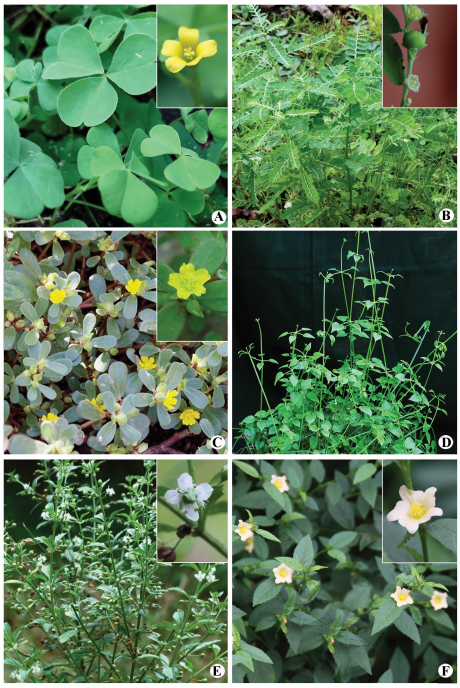
Fig. 45 Histochemical localisation of Lead in the Stem and Root of *Boerhavia diffusa*
A. Control Stem; B. Treated Stem; C. Control root; D. Treated root.
e & g. Localisation of lead; f. Cell wall thickening.

Fig. 57



A. *Boerhavia diffusa*
B. *Catharanthus roseus*
C. *Eclipta prostrata*
D. *Eupatorium triplinerve*
E. *Kyllinga nemoralis*
F. *Leucas lavandulifolia*
Inset: Inflorescence/ Flowers

Fig. 58

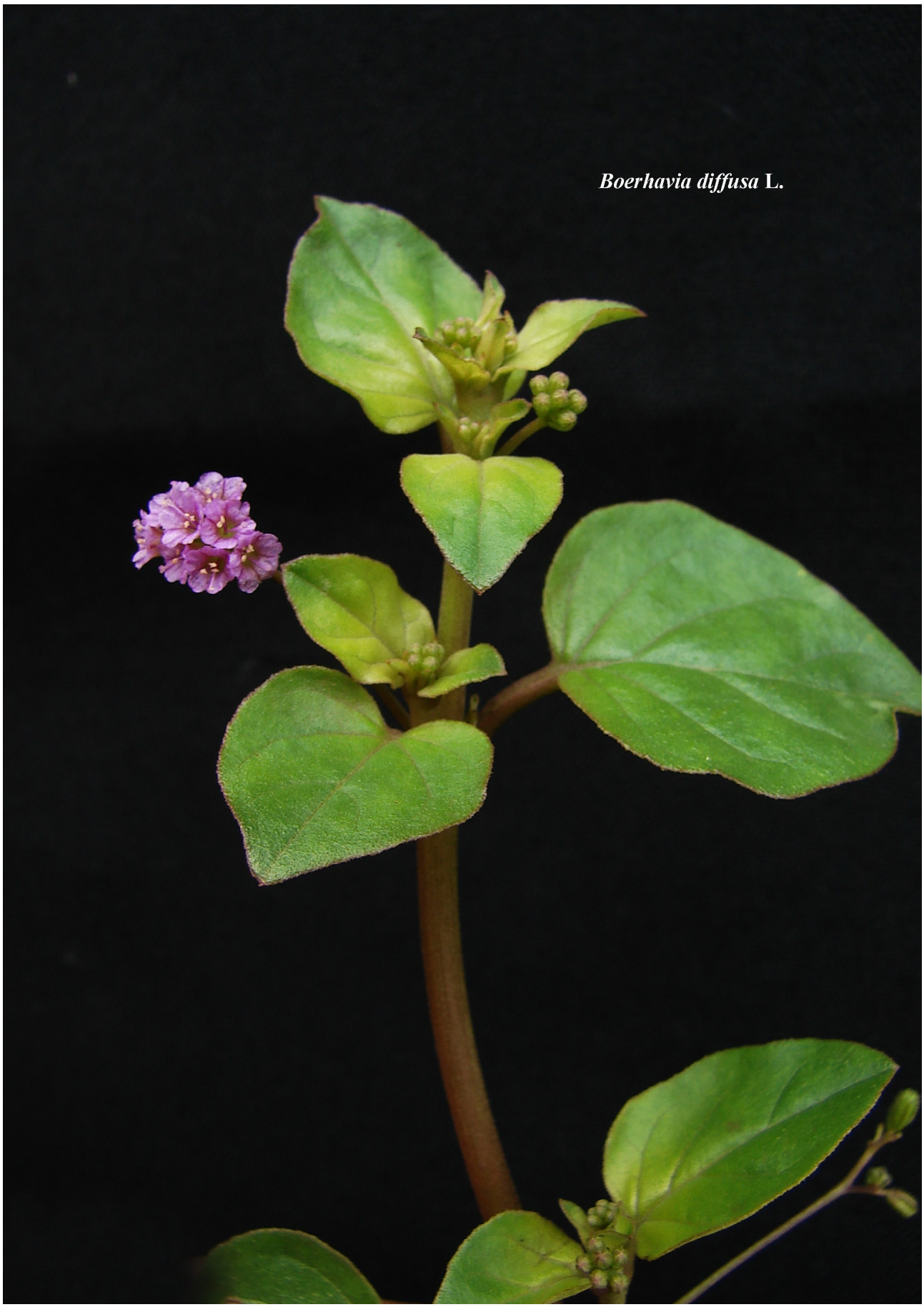


A. *Oxalis corniculata*
B. *Phyllantus amarus*
C. *Portulaca oleracea*
D. *Rubia cordifolia*
E. *Scoparia dulcis*
F. *Sida acuta*
Inset: Inflorescence/ Flowers

Fig. 59

A. ***Sida alnifolia*** **B.** ***Vernonia cinerea***
Inset: Inflorescence/ Flowers


Boerhavia diffusa L.
Boerhavia diffusa L.


Boerhavia diffusa **L.**

Source: ***Hortus Malabaricus*** **7:200**