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Full Length Research Paper

Antioxidative and physiological studies on *Colocasia esculentum* in response to arsenic stress

Mandakini J. Patel, Pritesh Parmar*, Bhaumik Dave and R. B. Subramanian

Lab No. 103, B R D School of Biosciences, Sardar Patel University, SPU Campus, Vallabh Vidyanagar - 388120 (Gujarat), India.

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The current study was undertaken to determine the effects of arsenic on *Colocasia esculentum*. Rhizomes were grown in pots containing 2.5 kg of garden soil with increasing concentration of arsenic. Arsenic accumulation was more in shoots compared to roots at higher concentrations. High arsenic concentration caused reduction in plant growth along with induction of few antioxidants. *C. esculentum* has a strong antioxidative and physiological defense mechanism. Under arsenic stress, an increase in catalase, peoxidase, few non-enzymatic antioxidants and an induction of few stress induced protein were observed, along with some anatomical changes in roots. The increase in antioxidant stress enzyme activities in response to arsenic exposure may be taken as evidence for an enhanced detoxification capacity of *C. esculentum,* a herbaceous monocot plant, towards reactive oxygen species (and derivatives) that might be generated in the stressed plants.

Key words: Arsenic, *Colocasia esculentum*, antioxidant, metal, root.

INTRODUCTION

Arsenic compounds are often unstable and in many cases are not well-defined materials. High levels of arsenic may also be present in some coals (up to 1500 mg/kg). As a result of the presence of arsenic in the parent rock, arsenic is observed naturally in soil in various quantities. In addition, some regions used for agriculture are contaminated by extensive use of arsenic compounds such as pesticides (Bernstam et al., 2000; Mitchell et al.,1995). Arsenic is more strongly bound to soils that have high clay or high organic matter and in these circumstances, is less available to plants. Arsenic is phytotoxic and trivalent arsenic is, in general, more toxic than pentavalent arsenic. Plants take up arsenic in proportion to the soil concentration, except at very high soil concentrations. Plants growing on smelter wastes or mine have developed resistance to arsenic toxicity; such plants sometimes have concentrations of arsenic (6000 mg/kg) that may be toxic to animals eating the plants. Common symptoms of arsenic uptake in humans are nausea, vomiting, abdominal pain, rice-water diarrhea, progressive general weakness, and severe dehydration, leading to collapse and heart failure. Arsenic taken up by plants is distributed to all tissues.

Terrestrial plants are able to accumulate arsenic to a substantial extent. The hyper accumulator plant species take up more than 100 mg/kg dry weight of the pollutant (Brooks et al., 1977). Phytoextraction, the use of plants to remove contaminants from soil, is an emerging technology due to its cost-effectiveness and environmental friendliness (Terry and Banuelos, 2000; Brooks, 1998; Cunningham et al., 1995). Plant cultivation and harvesting are inexpensive processes compared with traditional engineering approaches involving intense soil manipulation, and minimize the amount of secondary waste generated compared with soil heaping, leaching, or washing. Furthermore, this technology creates minimal environmental disturbance. Heavy metal stress results in the pro-

*Corresponding author. E-mail: priteshpar@gmail.com. Tel: +91-2692-234412 ext. 207 (O), +91-2692-238581 (R). Fax: +91-2692-231041.

Abbreviations: ICP-AAS, Inductively coupled plasma atomic absorption spectroscopy; **DTPA-TEA,** diethyltriamine penta acetic acid - triethanol amine; PVP, poly vinyl pyrrolidone.

duction of O_2^-, H_2O_2 and OH^-, which affect various cellular processes, mostly the functioning of membrane systems (Weckx and Clijsters, 1996, 1997).

Cells are normally protected against free oxy-radicals by the operation of intricate antioxidant systems, comprising both enzymatic systems such catalase and peroxidase and other non enzymatic systems, acting as free radical scavengers such as ascorbate and phenolic compounds. Ascorbate is involved, in addition, as a cofactor in the detoxifying enzymatic processes (Foyer et al., 1993). The basic mechanism underlying the metal detoxification process by metal hyper accumulators has been understood to a certain extent. Two classes of proteins, phytochelatins (PCs) and metallothioneins (MTs) are reported to be involved in the sequestration of the toxic metal ions into the plant vacuole by absorption and transportation (Grill et al., 1985; Zenk, 1996). The PCs are small proteins of 1.5 to 4.0 KD synthesized by phytochelatin synthase, while MTs are relatively large proteins (14 KD) synthesized through RNA translation (Vatamaniuk et al.,1999).

The objective of the present investigation was to study the effect of heavy metal arsenic on the growth and physiology of *C. esculentum* and to explore the role of antioxidants, total proteins and anatomical changes for metal tolerance in plant.

MATERIALS AND METHODS

Experimental design

Rhizomes of wild *C. esculentum* belonging to Araceae family were collected from Mogri, near Vallabh Vidyanagar, Gujarat, India. The rhizomes were transferred to black plastic pots containing garden soil (1 rhizome/2.5 kg soil/pot) after washing under running tap water in the botanical garden of the Sardar Patel University, Vallabh vidyanagar, Gujarat, India. All pots were watered regularly with tap water to field capacity. In order to prevent the loss of element out of the pots, plastic trays were placed under each pot, and the drained-out water collected was put back in the respective pots. After acclimatization, three days old rhizomes were exposed to arsenic applied in the form of sodium arsenate salt ($Na_3AsO_4.12H_2O$) (Hi-Media, Mumbai, India) in concentrations 50, 100, 150, 200 and 250 mg per kg of soil. Metal treatment was given as per the directions of Hamid et al. (2010).

Heavy metal analysis

After exposure to arsenic for 10 weeks, *C. esculentum* plants were harvested from each of the three replicate treatments, washed with water and were oven dried for three days. Samples (root, stem and leaf) of treated and untreated plants and the soil were acidified in a mixture of hydrochloric acid and nitric acid. For the determination of total arsenic contents, the samples were subjected to high pressure (for plant samples) and medium pressure (for soil) microwave digestion (Milestone, mls-1200). The digested samples were placed in 25 ml calibrated flasks and filled up to the mark with water. Arsenic hydride was produced by a pre-reduction of As (V) with potassium iodide. Later, the samples were again subjected to hydride generator. The determination of total arsenic (Francesconi and Edmonds, 1997) was carried out by inductively coupled plasma-atomic absorption spectroscopy (ICP-AAS) (GBC, plasmalab 8440M along with Hydride generator).

Biochemical analysis

Chlorophyll content

Chlorophyll estimation of *C. esculentum* leaves was carried out spectrophotometrically (Arnon, 1949).

Enzyme assay

Fresh root tissues of metal treated (five days) and control plants were homogenized in an ice-cooled mortar in phosphate buffer (pH 6.8) using a prechilled mortar and pestle in an ice bath. The supernatant after centrifugtion at 12000 *g* for 20 min at 4°C was used for catalase and peroxidase activity. Catalase activity was estimated by permanganate method (Povolotskaya and Sedenka, 1956; Gopalachari, 1963) calculated as mg H_2O_2 destroyed in 5 min by 1 g plant tissue. Peroxidase activity was determined following the method described by Kar and Mishra (1976) expressed as absorbance units (0.1 difference in absorbance value was taken as one unit of enzyme activity) per mg protein.

Total protein content

Fresh root tissues of the arsenic treated plants and the control plants were homogenized in an ice cooled mortar in extraction buffer containing 20 mM Tris (pH 8.0), 0.25 M sucrose, 5% poly vinyl pyrrolidone (PVP) and 3 µl of β-mercaptoethanol. The homogenate was centrifuged at 12000 *g* for 20 min and the supernatant was used for protein assays. Soluble root extracts of *C. esculentum* plants were used for total soluble protein by Lowry's et al. (1951) method using bovine serum albumin as standard (1 mg/ml) to compare the total protein profile of control and arsenic treated plants.

Proline

Free proline content was estimated following the procedure of Bates et al. (1973). Fresh root tissues were homogenized in 3% aqueous sulphosalicylic acid and the homogenate was filtered. An aliquote of 2 ml filtrate was used for proline estimation. The absorbance was measured at 520 nm. The amount of proline in the sample was calculated using a standard curve prepared from pure proline.

Lipid peroxidation

Lipid peroxidation in fresh roots was determined by estimating the malondialdehyde content following the method of Heath and Packer (1968). Tissues were homogenized in 0.1% trichloroacetic acid (TCA), centrifuged at 10000 *g* for 5 min and the absorbance of the supernatant was measured at 532 nm. Measurements were corrected for unspecific turbidity by subtracting the absorbance at 600 nm. The concentration of malondialdehyde was calculated using extinction coefficient of 155 mM^{-1} cm^{-1}.

Ascorbic acid content

Fresh plant roots were homogenized in 6% TCA at 4°C and centrifuged at 10000 *g* for 20 min. The supernatant was analyzed by dinitrophenylhydrazine method (Mukherjee and Chaudhuri, 1983)

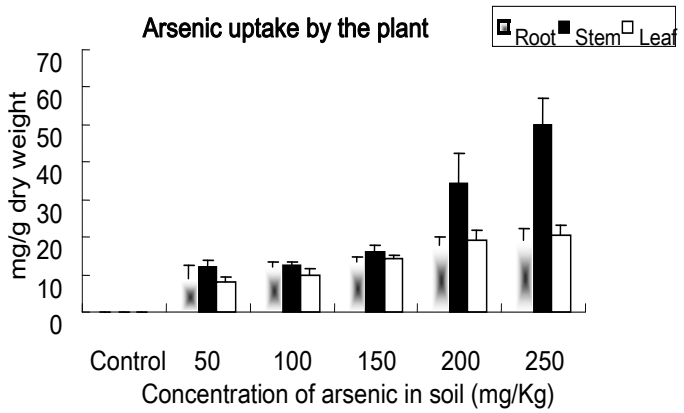

Figure 1. Arsenic contents in root, stem and leaves of *C. esculentum* treated with increasing concentrations of Cd.

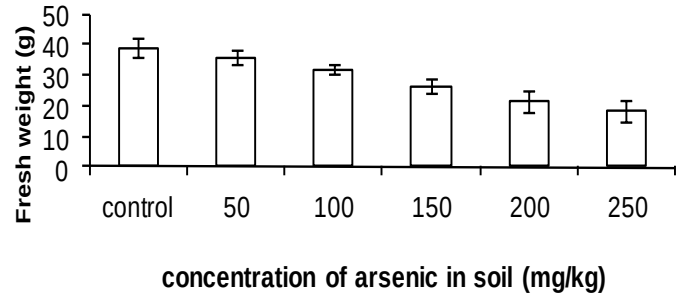

Figure 2. Effect of increasing concentrations of As on fresh weight of *C. esculentum.*

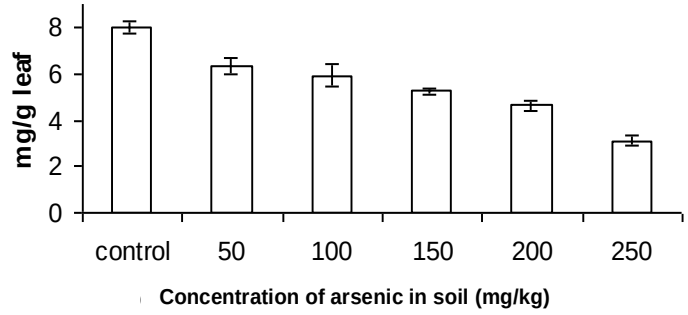

Figure 3. Effect on total chlorophyll content of *C. esculentum* treated with increasing concentrations of As

using standard ascorbic acid to compare the changes in vitamin C content of control and arsenic treated plants.

Anatomical studies

To study the anatomical changes, temporary slides of cross section of roots of untreated and 100 mg/kg arsenic treated plant of *C. esculentum* were observed and photographed using a Carl-Zeiss Image Analyzer at a magnification of 320×.

Statistics

All values reported in this work are mean ± standard deviation (SD) of three replicates.

RESULTS

Figure 1 shows the uptake of arsenic by *C. esculentum* exposed to different concentrations of arsenic. The shoots appear to accumulate most of the arsenic, with low levels of arsenic detected in the roots. At 50 and 100 mg/kg of soil arsenic, the metal content in the root was higher as compared to leaf, but with the increase in metal concentrations, arsenic content in shoots increased. The arsenic accumulation in the plant was highest in stem, followed by leaf and root. The heavy metal uptake in stem was 38 and 42% more than leaf and root, respectively. On exposure to different concentrations of arsenic for two months, *C. esculentum* showed pronounced effect on the whole plant biomass. The metal accumulation caused a conspicuous decrease of fresh weight in plants, and this became steeper with increasing concentrations of arsenic (Figure 2). The ability of the plant to continue growing in the presence of arsenic, a highly toxic heavy metal, resulted in the removal of metal from the soil. The growth showed a negative correlation with increased arsenic content. Exposure to different concentrations of arsenic showed a progressive reduction of total chlorophyll pigment of *C. esculentum* (Figure 3). The chlorophyll content of the plant declined with increasing concentrations of arsenic.

Arsenic assimilation induces a sharp increase in the enzymatic activity of peroxidase and catalase (Figures 4 and 5) in *C. esculentum* with respect to control. Activities of redox enzymes in *C. esculentum* exhibited a rise in relation to increasing concentrations of externally supplied arsenic to soil, and showed higher values as compared to untreated plant. Treatment with arsenic showed a marked increase in the total proteins in roots of *C. esculentum* (Figure 6). The total soluble proteins in metal treated plants increased to almost twice the concentration in control plant. *C. esculentum* plants exposed to arsenic showed progressive increase in free proline content. The increase in proline content of root was about two fold more than control treatment (Figure 7). The formation of malondialdehyde content was considered as a measure of lipid peroxidation. The lipid peroxidtion of roots of *C. esculentum* progressively increased by about three fold with increasing concentrations of externally supplied arsenic (Figure 8).

Ascorbic acid content of arsenic treated plant also showed an increase with increased metal concentration. The increase in ascorbic acid content is about two fold high under arsenic stress (Figure 9). Significant changes were observed in the root of arsenic treated *C. esculentum* against control. The roots showed a vast decrease of cell numbers in the cortex region of arsenic treated plants

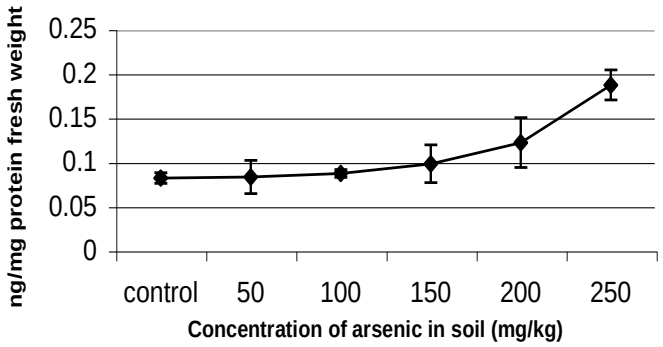

Figure 4. Peroxidase specific activity in roots of *C. esculentum* treatd with As.

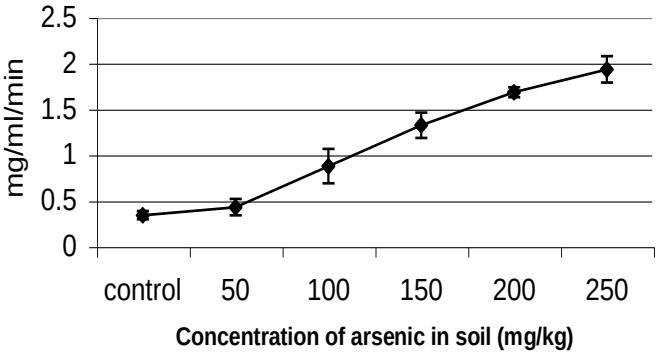

Figure 5. Catalse activity in roots of *C.esculentum* treatd with As.

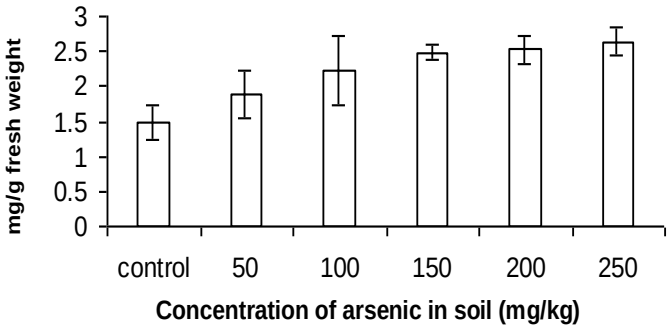

Figure 6. Effect of increasing concentrations of As on total soluble proteins of *C. esculentum.*

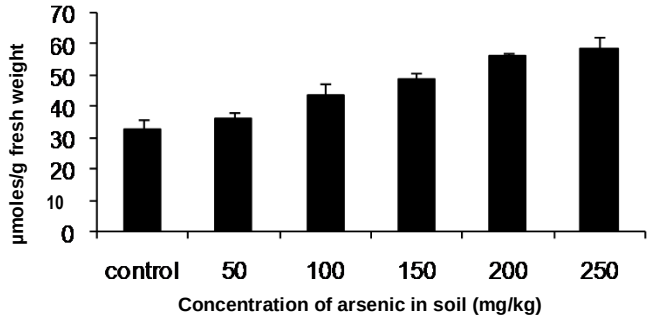

Figure 7. Effect on proline of *C. esculentum* treated with increasing concentrations of As.

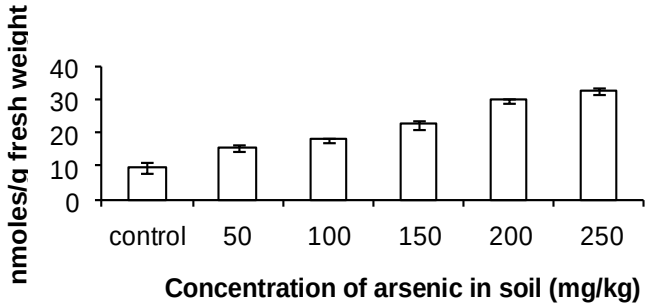

Figure 8. Effect of increasing As concentrations on TBARS of *C. esculentum* treated with increasing concentrations of As.

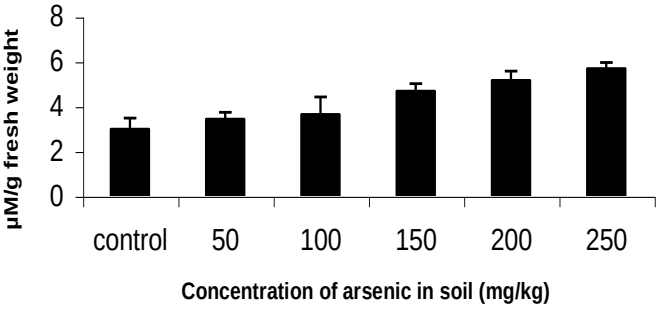

Figure 9. Effect on ascorbic acid content of *C. esculentum* treated with increasing concentrations of As.

(Figure 10). It also appeared that the cortex region consisted of elongated parenchyma cells instead of the normal parenchymatous tissue in the control plant.

DISCUSSION

The data presented here shows that arsenic accumulation was more in shoots as compared to the roots. We found that with the increase in metal concentrations in soil, arsenic translocation increased from roots to shoots. *C. esculentum* cannot uptake much of arsenic and hence cannot be categorized as a hyperaccumulator for arsenic but *C. esculentum* is already reported as a hyperaccumulator for cadmium with strong antioxidative response (Patel et al., 2005). Though the plant was unable to uptake high concentration of arsenic, it is equipped with superior antioxidative defenses, and the arsenic uptake in this plant can be enhanced by genetic engineering.

The observed decrease in fresh weight as a result of arsenic accumulation suggests a change in the plant's water status, which may be the result of decreased water

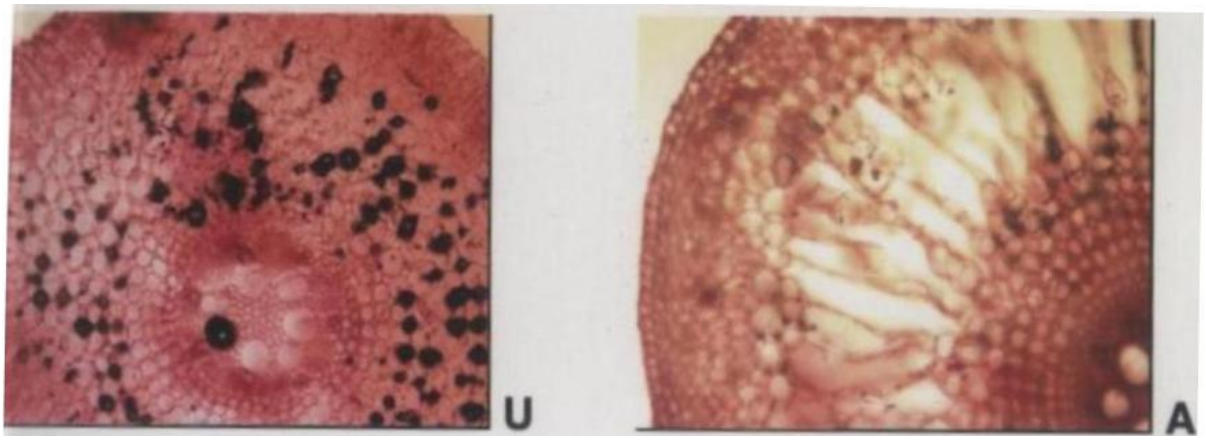

Figure 10. Anatomical changes observed in the roots of *C. esculentum* treated with 100 mg/kg of arsenic heavy metal (320×). U, Untreated root; A, arsenic treated root.

uptake or enhanced water loss, both of which may occur following membrane damage. Plant cell membranes are generally considered primary sites of metal injury (Barcelo and Poschenrieder, 1990). Both decrease in biomass and reduction in chlorophyll pigment of *C. esculentum* as seen in the present study under arsenic stress may be due to degradation of chlorophyll resulting in inhibition of photosynthesis (Salt et al., 1995). Photosynthesis is also sensitive to excessive Cu, and the pigment and protein components of photosynthetic membranes are the targets (Patsikka et al., 2002). Cu-induced generation of hydrogen peroxide, hydroxyl radicals or other reactive oxygen species (ROS) have been directly correlated with the damage to proteins and lipids (De Vos et al., 1991; Murphy and Taiz, 1997). It is known that environmental stresses often induce activity of free-radical detoxification enzymes such as catalase and peroxidase in plants. Therefore, catalase and peroxidase are two of the best candidate enzymes for testing plant responses to stress at the enzyme activity level. Catalase is one of the major antioxidant enzymes that eliminates hydrogen peroxide by converting it into oxygen and water. So, the increase in catalase enzyme protects the cell from oxidative damage.

Treatment with arsenic showed an increase in the total proteins in roots of *C. esculentum* after 20 h (Figure 8).

The increase in total protein content of roots suggests the expression of some low molecular weight proteins involved in the metal ion homeostasis known as metallothionein or phytochelatin (class III metallothionein), which are assumed to be involved in the accumulation, detoxification and metabolism of metal ions. Metallothioneins are thought to sequester excess amounts of certain metal ions, which vary for the structurally distinct proteins/polypeptides occurring in different organisms (Kagi and Schaffer., 1988) with final sequestration in the storage organelle of the cell. The increase in proline content of roots of arsenic treated plants indicate the response of plants to heavy metals stress. Proline has been shown to play an important role in ameliorating environmental stress in plants and microorganisms, including heavy metal stress. Surasak et al. (2002) showed that trans-genic algae expressing the mothbean *P5CS* gene have 80% higher free-proline levels than wild-type cells, and grow more rapidly in toxic Cd concentrations.

Lipid peroxidation is a process by which the functionality and integrity of the membrane is affected, and can produce irreversible damage to cell function (Mishra and Choudhuri, 1996). The increase of thiobarbituric acid reactive substances (TBARS) in *C. esculentum* may be considered as an index of oxidative damage due to inadequate response of the antioxidative systems, as observed in several other crops (Sudhakar et al., 2001). We presume that As facilitates lipid peroxidation by disorganizing the membrane structure (Cakmak and Horst, 1991). Enhanced lipid peroxidation, occurring in response to arsenic, indicates that arsenic toxicity resulted in the increased production of ROS, which in turn caused membrane damage.

C. esculentum showed increased ascorbic activity under metal stress. Ascorbic acid is a primary cellular antioxidant and also functions as secondary antioxidant because it represents a cellular reservoir to regenerate α-tocopherol, which scavanges lipid peroxide radicals (Foyer, 1993; Alscher et al., 1997). Increase in ascorbate with glutathione might restrict heavy metal-induced lipid peroxidation and oxidative stress (Schat et al., 1997).

The results of the anatomical studies indicate that the thickening of the cell wall and the reduction in number of cells in the cortical region of the roots may be due to accumulation of arsenic in the intercellular spaces of the internal cortex and the cell wall of the root. STEM data showed the electron dense granules contained cadmium in the cytoplasm and vacuoles of differentiating cortical cells and mature cells and in nuclei of undifferentiated cells in *Agrostis* and maize roots exposed to cadmium

(Rauser and Ackerley, 1987).

REFERENCES

Alscher RG, Donahue JL, Cramer CL (1997). Reactive oxygen species and antioxidants. Relationship in green cells. Plan. Physiol. 100: 224-233.

Arnon DI (1949). Copper enzymes in isolated Chloroplasts. Polyphenol oxidase in *Beta vulgaris*. Plan. Physiol. 24: 1-15.

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

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

Bernstam L, Nriagu J (2000). Molecular aspects of arsenic stress. J. Toxicol. Environ. Health B. 3: 293-322.

Brooks RR, Lee J, Reeves RD, Jaffre T (1977). Detection of nickeliferous rocks by analysis of herbarium species of indicator plants. J. Geochem. Explor. 7: 49-57.

Brooks RR (1998). Geobotany and hyperaccumulators. In: Brooks R R (ed) Plants that hyperaccumulate heavy metals. CAB International, Wallingford, UK, 55-94.

Cakmak I, Horst WJ (1991). Effect of aluminum on lipid peroxidation, superoxide dismutase, catalase and peroxidase activities in root tips of soybean (*Glycine max*). Physiol. Plant. 83: 463-468.

Cunningham SD, Berti WR, Huang JW (1995). Phytoremediation of contaminated soils. Trends Biotechnol. 13: 393-397.

De Vos CHR, Schat H, De Waal MAM, Vooijs R, Ernst WHO (1991). Increased resistance to copper-induced damage of the root cell plasmalemma in copper tolerant *Silene cucubalus*. Physiol. Plant. 82: 523-528.

Foyer CH (1993). Ascorbic acid. Antioxidants in Higher Plants (eds Alscher, R G and Hess J L), CRC Press, Boca Raton, pp 31-58.

Francesconi KA, Edmonds JS (1997). Arsenic and marine organisms. Adv. Inorg. Chem. 44: 147-189.

Gopalachari NC (1963). Changes in the activities of certain oxidizing enzymes during germination and seedling development of *Phaseolus mungo* and *Sorghum vulgare*. Indian J. Exp. Biol. 1: 98-100.

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

Hamid N, Bukhari N, Jawaid F (2010) Physiological response of *Phaseolus vulgaris* to different lead concentrations. Pak. J. Bot. 42(1) 239-246.

Heath RL, Packer L (1968). Photoperoxidation in isolated chloroplasts. I. Kinetics and stoichiometry of fatty acid peroxidation. Arch. Biochem. Biophys. 125: 189-198.

Kagi JHR, Schaffer A (1988). Biochemistry of Metallothionein Biochem. 27(23): 8509-8515.

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

Lowry OH, Rosebrough NJ, Farr AL, Randall RL (1951). Protein measurement with the folin reagent. J. Biol. Chem. 193: 265-275.

Mishra A, Choudhuri MA (1996). Possible implication of heavy metals (Pb^{+2} and Hg^{+2}) in free radical mediated membrane damage in two rice cultivars. Indian. J. Plan. Physiol. 1: 43-47.

Mitchell P, Barr D (1995). The nature and significance of public exposure to arsenic: a review of its relevance to South West England. Environ. Geochem. Health. 17: 57-82.

Mukherjee SP, Chaudhuri HA (1983). Implications of water stress induced changes in the levels of endogenous ascorbic acid and H_20_2 in *Vigna* seedlings. Physiol. Plant. 58: 166-170.

Murphy A, Taiz L (1997). Correlation between potassium efflux and copper sensitivity in ten Arabidopsis ecotypes. New Phytol. 136: 211-222.

Patel, Mandakini J, Patel , Jaymini N, Subramanian RB (2005). Effect of cadmium on growth and the activity of H_2O_2 scavenging enzymes in *Colocasia esculentum*. Plant. Soil. 273: 183–188.

Patsikka E, Kairavuo M, Sersen F, Aro EM, Tyystjärvi E (2002). Excess copper predisposes photosystem II to photoinhibition in Vivo by out competing iron and causing decrease in leaf chlorophyll. Plan. Physiol. 129: 1359-1367.

Povolotskaya KL, Sedenka DM (1956). A method for collective determination of ascorbic, polyphenol and proxidase activities. Biochimie. 21: 133-136.

Rauser WE, Ackerley CA (1987). Localization of cadmium in granules within differentiating and mature root cells. Can. J. Bot. 65: 643-646.

Salt DE, Blaylock M, Kumar PBAN, Dushenkov E, Ensley BB, Chet I, Raskin I (1995). Phytoremediation: A novel strategy for removal of toxic metals from environment using plants. Biotech. 13: 468-475.

Schat H, Sharma SS, Vooijs R (1997). Heavy metal induced accumulation of free proline in metal tolerant and non-tolerant eco- types of *Silene vulgaris*. Plan. Physiol. 101: 477-482.

Sudhakar C, Lakshmi A, Giridarakumar S (2001). Changes in the antioxidant enzyme efficacy in two high yielding genotypes of mulberry (*Morus alba* L.) under NaCl salinity. Plan. Sci. 161: 613-619.

Surasak Si, Samuel T, Desh Pal S. Verma, Richard T. Sayre (2002). Molecular Mechanisms of Proline-Mediated Tolerance to Toxic Heavy Metals in Transgenic Microalgae. Plant.Cell.14: 2837-2847.

Terry N, Banuelos G (2000). Phytoremediation of contaminated soil and water. Lewis Publishers, Boca Raton, FL. pp. 389.

Vatamaniuk OK, Mari S, Lu YP, Rea PA (1999). AtPCS1, a phytochelatin synthase from *Arabidopsis*: isolation and *in vitro* reconstitution. Proc. Natl. Acad. Sci. USA 96:7110-7115.

Weckx JEJ, Clijsters H (1996). Oxidative damage and defense mechanisms in primary leaves of *Phaseolus vulgaris* as a result of root assimilation of toxic amounts of copper. Physiol. Plant. 96: 506-512.

Weckx JEJ, Clijsters H (1997). Zinc phytotoxicity induces oxidative stress in primary leaves of *Phaseolus vulgaris*. Plant Physiol. Biochem. 35: 405-410.

Zenk MH (1996). Heavy metal detoxification in higher plants: a review. Gene. 179: 21-30.