



Review Article

A Comparative Analysis Of Cadmium And Chromium Toxicity And Detection Of Their Genotoxic Effect On Plants By Rapd (Review Article)

Pal, S.

Assistant Professor, Department of Botany, Lady Brabourne College, Kolkata.

*Corresponding: **Suparna Pal**, Email : suparna_005@yahoo.co.in

ABSTRACT

Heavy metal pollution is an alarming environmental issue in today's world. Among these cadmium and chromium are most important as huge amount of these metals are released in the atmosphere by anthropogenic activity. Due to rapid urbanization and indiscriminate use of cadmium and chromium in various factories these metals are getting access in food chain. Due to high solubility divalent Cd and hexavalent Cr (VI) get accumulated in soil and taken up by plants resulting acute phytotoxicity. Being biologically non essential both these metals with teratogenic effect are injurious to animal leading to cancers also. Various plants with greater capacity of metal accumulation have been identified and categorised as hyperaccumulator. These plants are potential candidate for ecofriendly phytoremediation process or decontamination of heavy metal polluted soil. Various biochemical defence mechanism of these plants are of great interest in new plant research areas. Overexpression of enzymatic (superoxide dismutase, glutathione reductase, ascorbate peroxidase) and non enzymatic (non protein thiole content, proline, tocopherol) antioxidants in these Cd, Cr resistant plants has been well reported. The genotoxic effect of Cd, Cr on plants has been evaluated and established very efficiently by RAPD method. Cd and Cr alter the primer binding sites on DNA and also induce point mutation which is evidenced by the DNA banding profile polymorphism between control and treated plants. Appearance of new bands and disappearance of control bands have been reported by various workers to confirm the genotoxic effect of these metals by RAPD method.

Key words : cadmium, chromium, phytoremediation, genotoxicity, RAPD

INTRODUCTION

The soil environment is a major sink for multitude of chemicals and heavy metals, which inevitably leads to environmental contamination problems. On being discharged into soil, the heavy metals get accumulated and may disturb the soil ecosystem, plant productivity, and also pose threat to human health and environment. Being a serious threat to biota due to their acute toxicity and non biodegradable nature, heavy metal contamination of soil has become an increasing problem worldwide. Among these heavy metals cadmium and chromium are of increasing concern due to their indiscriminate use and wide presence in the environment. Cd is one of the most toxic heavy metals in plants due to its solubility and mobility. Cr has no verified biological role and has been classified as nonessential for plants and animals.

SOURCES, UPTAKE, ACCUMULATION AND EFFECTS of Cd ON PLANT

Divalent heavy metal cation Cd with long biological half life is one of the most hazardous elements and a major pollutant due to its greater water solubility and phytotoxicity. According to the

Agency for Toxic Substances and Disease Registry (2007), the position of Cd is 7th in the list of "Top twenty hazardous substances, 2007". Cd is released in the atmosphere by anthropogenic activity such as disposal of house hold and municipal wastes, electroplating, photocopying, dyeing, effluents of cadmium nickel battery manufacturing unit and metal smelting industries. Though Cd is biologically non essential, it is still observed that plants can accumulate this heavy metal, sometimes in excess. *Chromolaena odorata*, a siam weed was reported as a hyperaccumulator, (Tanhan et al., 2007) accumulating 102.3 mg/kg Cd in shoot and 1440.9 mg/kg Cd in roots. *Avena strigosa* and *Crotalaria juncea* possess the greater potential for Cd accumulation and tolerance (Uraguchi et al., 2006). *Pistia stratiotes* is a well known aquatic macrophyte capable of accumulating wide range of heavy metals. Its potential to tolerate higher metal concentration by the synthesis of metal binding peptides has been used for the treatment of urban sewage. Narain and his group (2011) explored the phytoaccumulation capacity of water hyacinth to clean and improve water quality of natural water bodies polluted with industrial and municipal effluents. Their report revealed the average removal efficiency of this

plant, which were 80.26% for Cr and 71.28 % for Cd. Singh and his colleagues (2010) surveyed the accumulation, translocation and subsequent uptake of Cd, Zn, Pb, Cu, Ni, Mn and Fe in eleven native plants grown in field contaminated with fly ash of a thermal power station. Their report showed highest accumulation of Cd in *Typha sp.*

In natural conditions the mobility of metals depends on the chemical form of the metal, the adsorptive properties of soil particles, the soil organic matter horizon, pH, redox potential, temperature and concentration of other elements. The chemistry of metal interaction with soil matrix is one of the important criteria for metal accumulation and uptake by the plants. Soil pH regulates not only the metal availability but also it affects the metal uptake by the roots. Solubility of heavy metals generally greater as pH decreases within the pH range of normal agricultural soil. The retention of metal to soil organic matter is weaker at low pH, resulting in more available metal in the soil solution for root absorption. The high pH value could have accounted for a low transfer of metal from soil to plants.

Regarding the amount of pollutant accumulated, three categories of plants were proposed by: (1) *excluders*: those that grow in metal-contaminated soil and maintain the shoot concentration at low level up to a critical soil value above which relatively unrestricted root-to-shoot transport results; (2) *accumulators*: those that concentrate metals in the aerial part; (3) *indicators*: where uptake and transport of metals to the shoot are regulated so that internal concentration reflects external levels, at least until toxicity occurs. The toxicity of metals, and of their compounds, largely depends on their bioavailability, i.e. the mechanisms of uptake through the cell's membrane, intracellular distribution and binding to cellular macromolecules. The relative toxicity of different metals to plants act through one of the following: changes in the permeability of the cell's membrane; reactions of sulphhydryl (–SH) groups with cations; affinity for reacting with phosphate groups and active groups of ADP or ATP; replacement of essential ions and oxidative stress. Most common unspecific symptoms of metals phytotoxicity are: growth inhibition, nutrient imbalance, disturbances in the ion and water regime, photosynthetic impairment and genotoxicity. One of the most important first visual symptoms due to the adverse effect of Cd and Cr on plant is the reduction of growth, gradual degradation of chlorophyll content i.e. induction of chlorosis and ultimate necrosis resulting decrease in photosynthesis rate. Loss of chlorophyll under Cd exposure may be due to the inhibition of its biosynthesis, enhanced enzymatic degradation, oxidative damage and decrease in chloroplast density per cell.

EFFECT OF Cr ON PLANTS

Untreated effluents of few industries are the prominent sources of Cr in the atmosphere. In soil Cr exists in trivalent (Cr III) and hexavalent (Cr VI) form. Being more mobile Cr VI is more phytotoxic than Cr III. Hexavalent Cr is used for metal plating, cooling tower water treatment, wood preservation, in paint and plastic industry and also as colouring agent of soft drinks. Leather tanning industry is the main source for high influx of Cr to the biosphere, accounting for 40% of total

industrial use. Redox behavior of Cr is attributed to the direct involvement of Cr inducing oxidative stress that initiates degradation of photosynthetic pigments and causing severe damage to cell membrane due to lipid peroxidation (Panda and Chowdhury, 2004). A common parameter affected by Cr is the amount of photosynthetic pigments, which tends to decrease when plants are exposed to high doses of this metal which is due to the suppression of light capture efficiency of PS II and interruption of electron transport rate (Rodriguez et al., 2011). Accumulation of Cr by plants can reduce growth, pigment content, induce chlorosis, and alter enzymatic function, damage root cells and cause ultrastructural modifications of chloroplast and cell membrane (Panda and Chowdhury, 2004; Subrahmanyum, 2008). Growth of aerial part growth has also been proven to be negatively affected by Cr in species like rice, wheat, oat and sorghum. Cr, due to its structural similarity with some essential elements, can affect mineral nutrition of plants in a complex way (Shanker et al., 2005). Cr induces ROS production causes structural damage to the pigment-protein complexes located in the thylakoid membrane (e.g. the destabilization and degradation of the proteins of the peripheral part of antenna complex), followed by the pheophytinization of the chlorophylls (substitution of Mg^{2+} by H^{+} ions), and destruction of the thylakoid's membranes. Cr damages the water oxidizing centres (WOC) associated to PSII and investigation of Henriques (2010) revealed that this could be explained by the reduction of Ca^{2+} and Mn^{2+} availability (caused by Cr), being that these elements are fundamental in the structure and functioning of the WOC. Dhir et al. (2009) found a significant decrease in RuBisCO activity induced by exposure to wastewater (rich in Cr) from an electroplating unit due to the substitution of Mg in the active site of RuBisCO subunits by metal ions; decline in RuBisCO content as a result of oxidative damage and by a shift in the enzyme's activity from carboxylation to oxygenation. Proline is an amino acid which increases with the increasing concentration of heavy metal ions, thus acting as an important biomarker for stress. Proline accumulation in stressed plant is a primary defence response that provides protection against oxidative damage is evident from earlier works. Various works have been conducted regarding the function of free proline accumulation such as: stabilization of sub cellular structure, energy storage as an osmotic adjustment mechanism, radical scavenger and constitution of cell wall protein. The high correlation between proline accumulation and heavy metal stress has also been described. Among the heavy metals, Cd is the strongest inducer of proline accumulation has been previously reported. The study carried out by Yildiz and Terzi (2012) on the effect of different concentration of Cr(VI) on dehydrogenase activity, total soluble protein, MDA and antioxidative enzymes of two barley cultivars revealed that decrease in dehydrogenase activity and protein content as well as increase in proline content, lipid peroxidation and SOD activity may be indicative of oxidative stress induced by Cr (VI). Cr induces lipid peroxidation coupled with K^{+} leakage and also reduces nitrogen reductase activity in *Ocimum tenuiflorum*. But hyperactivity of SOD, GR, CAT proved strong antioxidant properties of *Ocimum* against. Cr

acts on DNA causing genotoxicity directly. Cr can also form complexes which can react with hydrogen peroxide and generate significant amounts of hydroxyl radicals that may directly trigger DNA alterations and other effects. Besides degrading DNA, ROS can also affect Mitogen-Activated Protein Kinases (MAPK), which cause the deregulation of cell proliferation (tumor inducing effect), causing mutagenicity through an indirect pathway (Beyersmann and Hartwig, 2008).

HEAVY METAL TOLERANCE IN PLANTS

The adaptive responses of plants to heavy metal contamination are efficient processes that include many physiological, molecular, genetic and ecological traits. In general heavy metal stress symptoms can be divided into visible and only measurable ones. Cd and Cr accumulation in plants causes disturbance in membrane function enzyme activity, cell division and cellular redox homeostasis (Ortega-Villasante, 2005). Biomass production was extensively studied in split pots filled with soil spiked at different concentrations (0, 3, 6, 12, 25 and 50 mg/kg) of Cd by Selvam and Wong (2009) and revealed decline in biomass production which established that cadmium is toxic for biomass production. Cd has low redox potential so it cannot participate in biological redox reaction but there are some evidences that it could perform oxidation related disturbances, including lipid peroxidation (Vassilev et al., 2004). Enhancement of lipid peroxidation is the measurement of oxidative damage of biomembrane with increasing concentration of Cd and Cr is generally due to the formation of ROS. Aerobic organisms have developed enzymatic as well as nonenzymatic antioxidants to combat oxidative stress, among these; most important are low molecular antioxidants such as ascorbic acid, glutathione, thiol, tocopherols and carotenoids. The accumulation of ROS may be a consequence of the shift in the balance between their productivity and functioning of the antioxidant system comprising both enzymatic antioxidants (SOD: Superoxide dismutase, CAT: Catalase, GR: Glutathione reductase, APX: Ascorbate peroxidase) and non enzymatic ROS scavengers such as glutathione, carotenoids, ascorbate and proline. Among antioxidant enzymes SOD plays a pivotal role in the first line of defence against ROS, reducing the oxidative stress by the dismutation of superoxide radicals into H_2O_2 and O_2 . Dey and his co workers (2007) reported the excess production of MDA due to lipid peroxidation under Cd and Pb stress reflecting damaging effect of heavy metals on wheat seedlings. Martin and his colleagues (2011) studied the effect of Cd (10-100 μ M) on growth parameters, chlorophyll and proline content, enzymatic antioxidative response and lipid peroxidation of Tobacco (*Nicotiana tabacum*). Results showed gradual degradation of chlorophyll content with the increase in Cd concentration and enhanced lipid peroxidation which are indicative of oxidative stress but hyper activity of GPX, SOD scavenging H_2O_2 is attributed the Cd tolerance ability. Srivastava and his coworkers (2011) exposed Soybean (*Glycine max*) to different level of Cd to evaluate growth inhibition and recorded huge deterioration in chlorophyll content, nitrate reductase activity and total protein

contents, while elicitation in both non enzymatic (ascorbate, glutathione) and enzymatic (CAT, SOD, APX) antioxidants was also reported. The investigation of Vassilev and Lidon (2011) revealed enhanced membrane lipid peroxidation, K^+ leakage, chlorophyll degeneration, diminished content of soluble protein and glutamic acid in Barley (*Hordeum vulgare*) under 54 μ M Cd treatment. Glutathione alleviates tolerance capacity of plants to ROS by participating in the detoxification of ROS generated by Cd and Cr. It was reported by Ozturk and his coworkers (2003) that increasing Cd supply markedly enhanced the production of non protein thiole groups providing high Cd tolerance in durum wheat cultivars. Upregulation of the activity of glutathione reductase, one of the most important antioxidative enzymes was established in their study. Increased GR activity in roots exposed to Cd was also reported in various plants such as radish, sugarcane, *Arabidopsis* and alfalfa. Species with higher level of SH compounds were reported to be more tolerant to heavy metal stress than those non SH groups. Metal accumulators were equipped with a higher level of antioxidative enzyme activity for scavenging ROS. The investigation (Wang et al., 2008) revealed that the activity of antioxidative isozymes SOD and CAT were enhanced rapidly in metal accumulator species in response to Cd and Cr than nonaccumulator species. Among the enzymatic scavengers SOD, CAT, APX and GR are most effective and participate in a highly developed detoxification system named the ascorbate-glutathione cycle/ Halliwell-Asada cycle. Bah and his group (2011) studied the modulation of antioxidant defence system in *Typha angustifolia* after 30 days exposure to Cd, Cr reported the enhanced activity of SOD, CAT, APX under heavy metal stress. Pandey and Singh (2012) reported that pea plants exposed to different Cd concentration showed growth inhibition and induction of chlorosis and necrosis of young leaves but significant increase in SOD activity and low activity of POD and CAT explains the accumulation of H_2O_2 in cell causing oxidative damage. Cd tremendously affects chromosome morphology. Root tips and leaves of *Vigna radiata* treated with different levels of Cd showed chromosome abnormalities such as lagged chromosomes, anaphase bridge, undistributed chromosome (Muneer et al 2011). Not only resistance to toxic heavy metals, but metal cation homeostasis is essential for plant nutrition also. Many plant transporters have been identified at molecular levels facilitating Cd sensitivity and accumulation such as AtNramp cDNA from *Arabidopsis*.

METAL HYPERACCUMULATION AND PHYTOREMEDIATION

Metal hyperaccumulation is a fascinating phenomenon, which has interested scientists for over a century. Hyperaccumulators constitute a group of exceptional plant species and they possess genetically inherited traits of metal hyperaccumulation and tolerance. The understanding of metal hyperaccumulation physiology has recently improved as a result of the development of molecular tools. Researchers are going on to identify and clone the genes responsible for metal accumulation, detoxification and tolerance in plant tissues. Transgenic approaches successfully employed to promote

phytoextraction of metals (Cd, Pb, Cu) and metalloids (As, Se) from contaminated soil by their accumulation in the aboveground biomass involved mainly implementation of metal transporters, improved production of enzymes of sulfur metabolism and production of metal detoxifying chelators metallothioneins and phytochelatin. Non protein thiols (NPTs) which contain a high percentage of Cys sulfhydryl residues in plants, plays a pivotal role in heavy metal detoxification. Reduced form of glutathione (GSH) is one of the most important components of NPT metabolism. GSH, a sulfur containing tripeptide (γ -Glu- Cys- Gly), plays several roles in heavy metal tolerance and sequestration, protecting cells from oxidative damage in plants caused by heavy metals. GSH is the direct precursor of phytochelatin (PC), the metal binding peptide involved in heavy metal tolerance and sequestration. The roles of GSH and PC synthase in heavy metal tolerance were well illustrated in Cd sensitive *Arabidopsis* mutants. Over expression of an *Arabidopsis* PC synthase (*AtPCS1*) gene in transgenic *Arabidopsis* with the goal of increasing PC synthesis, metal accumulation and tolerance has been done. The overexpression of either γ - glutamylcysteine synthetase (γ -ECS) or glutathione synthetase (GS) in *Brassica juncea* transgenic was previously done to result in higher accumulation of GSH and phytochelatin (PC) to enhance Cd tolerance. Sun and his group (2010) reported that the variation in phytochelatin production in two Cd treated species *Rorippa globosa* and *R. islandica* may be used as biomarker of Cd hyperaccumulation and the synthesis of PCS for the enhanced uptake of Cd. A hydroponic experiment was conducted by Zeng and his coworkers (2012) to determine the possible effect of exogenous glutathione in rice seedling under Cr stress and revealed that addition of GSH in culture solution obviously alleviated the activity of antioxidative enzymes and reduction in MDA accumulation. Kupper and Kochain (2010) reported the differential cellular expression of ZNT1 and ZNT5, members of ZIP gene family, a novel transporter of plant under Cd, Fe toxicity. The genetic basis of Cd tolerance and hyperaccumulation was investigated in *Arabidopsis halleri* (Bert et al. 2003). Their results suggested that Cd tolerance may be governed by more than one gene and Cd tolerance and accumulation are independent characters.

Other than hyperaccumulators, Cd and Cr tolerant plants have been identified that are important for their detoxification mechanism and alleviating antioxidative defenses. Aquatic macrophytes were found to be the potential scavengers of heavy metals from aquatic environment. Various works have been done to study the effect of heavy metals and to evaluate tolerance potential of different weeds and aquatic macrophytes such as *Eichhornia crassipes* and *Pistia stratiotes* which revealed enhanced activity of antioxidative isozymes (CAT, POX, SOD).

In recent years, public concern related to ecological threats caused by heavy metal has led to intensive research of new economical plant based remediation technologies. Conventional methods used for reclamation of soil, namely chemical, physical and microbiological methods are costly to install and operate. An ecofriendly and low cost strategy for environment clean up is phytoremediation which mainly employs hyperaccumulators to

remove heavy metal from contaminated sites is now a day receiving more attention worldwide. The identification and selection of a promising hyperaccumulator plant is the key step and most important approach for successful phytoremediation. Hyperaccumulators accumulate appreciable quantities of metals in their tissue regardless of concentration of metal in the soil. Almost 500 plant species of 101 families has been identified as heavy metal accumulators till date. These are Asteraceae, Brassicaceae, Cryophyllaceae, Cyperaceae, Fabaceae, Lamiaceae, Poaceae, Violaceae etc. Metal hyperaccumulation occurs in approximately 0.2 % of all angiosperms and is particularly well represented in Brassicaceae. Alarming Cd and Cr pollution forced to screen for hyperaccumulator plants suitable for phytoremediation. First reported Cd hyperaccumulator is *Thlaspi caerulescens* (Baker et al. 2000) then *Sedum alfredii*, *Taraxacum monogolicum*, *Rorippa globosa* have been reported recently. *Leersia hexandra*, was reported (Zhang et al. 2007) as a hyperaccumulator of Cr. *Crotalaria juncea* and *C. dactylon* have been reported as suitable candidate for Cr and Ni remediation. Plant species have been recently used for heavy metal accumulation and most of the studies have been done on hyperaccumulator or metal tolerant species. Metal accumulator plants though useful to phytoextract metal contaminants from soil but have many shortcomings such as low biomass, edible nature and difficult to harvest. Considering the high above ground biomass production and Cd accumulation in shoot, *Brassica napus* has been declared as a potential candidate for Cd phytoextraction by Selvam and Wong (2009). Rezvani and Zaefarian (2011) investigated the growth, bioaccumulation and translocation factor of Cd and Pb in *Aeluropes littoralis*. The enhanced translocation of Cd to the shoot of this plant indicates its great performances for phytoextraction and was introduced as Cd hyperaccumulator. Mazid and his coworkers (2011) reported *Acacia mangium* with higher bioconcentration and translocation factor as an efficient phytoremediator for Cd, Cu and Zn contaminated soil to mitigate soil pollution.

DETECTION OF GENOTOXIC EFFECT OF Cd, Cr BY RAPD METHOD

Impact assessment of contaminants in soil is an important issue in environmental quality study and remediation of contaminated land. In recent years several tests have been developed to evaluate the toxicity of the environmental contaminants. Assessment of genotoxin induced DNA damage and mutation at molecular level is important in ecogenotoxicity study. In soil genotoxicity study, advances in molecular biology have led to the development of a number of selective and sensitive PCR based assay for DNA analysis. DNA alterations detected by RAPD analysis offered a useful biomarker assay for the evaluation of genotoxic effects of heavy metals (Aydin et al., 2013; Aslam, 2014). RAPD assay detects wide range of DNA damages (point mutations, inversions, deletions) and at the same time large number of samples can be studied. In RAPD studies, similarities and diversities are described by appearance of new bands, disappearance of bands, and variation in band intensities. Ecotoxicological literature displayed that RAPD assay is a

fundamental tool to evaluate the effects of toxicants on organisms under optimized conditions. The presence, absence and intensity of bands are related to DNA damages, mutations by genotoxicants. RAPD assay was successfully used to monitor DNA changes induced by heavy metals such as lead, cadmium, copper (Körpe and Aras, 2011), UV and x-ray. DNA damages and mutations may alter a primer binding site and thus genomic template stability changes and polymorphism occurs within dose-dependent treatments and untreated organisms. DNA damage in the root tip of maize seedling under Cd stress became evident by the presence and/or absence of DNA fragment in the treated samples compared to the control group was detected by RAPD analysis (Shahrtash et al. 2010). Qurainy and his coworkers (2010) have reported that RAPD polymorphism can be utilized to detect genotoxicity of Cd, Pb and Zn in *Eruca sativa*. DNA damage and polymorphism was detected by RAPD in Barley seedling treated with Cd (30-120 mg/L) (Liu et al. 2009). Their result showed variation in band intensity, loss of normal bands and appearance of new bands compared with normal seedling. *Hydrilla verticillata* and *Ceratophyllum demersum* treated with 10µM/L Cd showed change in chlorophyll content, protein content and DNA profile and DNA damage was investigated by RAPD analysis (Gupta et al., 2009). In their study Cenkci and his coworkers (2009) used RAPD to detect DNA damage in roots and leaves of *Phaseolus vulgaris* exposed to Cr. Qari (2010) investigated the genotoxic and antigenotoxic effect of aqueous extract of *Costus speciosus* in the *Allium cepa* root tip. Through RAPD banding pattern antigenotoxic capacity of *Costus speciosus* extract was established. Recently genotoxic effect of Cd was well established in *Capsicum annum* (Aslam et al., 2013), and okra seedling (Aydin et al., 2014) by RAPD method. Over the last few years there has been a noticeable increase in studies aimed at evaluating the genotoxic effects of drugs through RAPD. Results of the investigation of It is concluded from all these studies that RAPD analysis could be a useful tool for quick detection of genotoxic effect of heavy metals, air pollutant, UV radiance and herbicides in plants and comparison between treated and untreated genomes is necessary to evaluate how environmental pollutants modify the structure of DNA in living organisms.

REFERENCES

- Aslam, R., Ansari, M.Y.K., Choudhary, S., Bhat, T.M. and Jahan, N. (2014). Genotoxic effects of heavy metal cadmium on growth, biochemical, cyto-physiological parameters and detection of DNA polymorphism by RAPD in *Capsicum annum* L.- an important spice crop in India. *Saudi J. Bio. Sci.*, 21: 465- 472.
- Aydin, S.S., Basaran, E., Cansaran-Duman, D. and Aras, S. (2013). Genotoxic effect of cadmium in okra seedling: Comparative investigation with population parameters and molecular markers. *J Environ. Bio.*, 34: 985-990.
- Bah, A.M., Dai, H., Zhao, J., Sun, H., Cao, F., Zhang, G. and Wu, F. (2011). Effect of cadmium, chromium and lead on growth, metal uptake and antioxidative capacity in *Typha angustifolia*. *Biol. Trace. Elem. Res.*, 142:77-92.
- Baker, A.J.M., McGrath, S.P., Reeves, R.P. and Smith, J.A.C. (2000). Metal hyperaccumulator plants: a review of the ecology and physiology of a biochemical resource for phytoremediation of metal polluted soils. In: Terry N, Baelos G, eds. *Phytoremediation of contaminated soil and water*. Florida, USA: Lewis publishers, 85-107.
- Bert, V., Meerts, P., Laprade, P.S., Gruber, B. and Verbruggen, N. (2003). Genetic basis of cadmium tolerance and hyperaccumulation in *Arabidopsis halleri*. *Plant and Soil.*, 249 : 9-18.
- Bozari, S. and Aksakal, O. (2012). Application of RAPD to detect genotoxic effect of trifluran on maize (*Zea mays*). *Drug. Chem. Toxicol.*, 46(5):291-296.
- Beyersmann, D. and Hartwig, A. (2008). Carcinogenic metal compounds: recent insight into molecular and cellular mechanisms. *Arch. Toxicol.*, 82: 493-512.
- Cenkci, S., Yildiz, M., Cigerci, I., Konuk, M. and Bozdog, A. (2009). Toxic chemical induced genotoxicity detected by random amplified polymorphic DNA (RAPD) in bean (*Phaseolus vulgaris*) seedlings. *Chemosphere*, 76: 900-906.
- Dey, S., Dey, J., Patra, S. and Pothal, D. (2007). Changes in the antioxidative enzyme activities and lipid peroxidation in wheat seedlings exposed to cadmium and lead stress. *Braz. J. plant Physiol.*, 19(1):53-60.
- Dhir, B., Sharmila, P., Saradhi, P.P. and Nasim, S.A.(2009). Physiological and antioxidative responses of *Salvinia natans* exposed to Chromium-rich wastewater. *Ecotoxicol. Environ. Safe.*, 72: 1790-1797.
- Gupta, M. and Sarin, N.B. (2009). Heavy metals induced DNA changes in aquatic macrophytes: Random amplified polymorphism DNA analysis and identification of sequence characterized amplified region marker. *J. Environ. Sci.*, 21:686-690.
- Henriques, F.S. (2010). Changes in biomass and photosynthetic parameters of tomato plants exposed to trivalent and hexavalent Chromium. *Biol. Planta.*, 54:583-586.
- Korpe, D.A. and Aras, S. (2011). Evaluation of Cu induced stress on eggplant (*Solanum melongena* L.) seedlings at the molecular and population levels by use of various biomarkers. *Mut. Research.*, 719(2):29-34.
- Kupper, H. and Kochain, L.V. (2010). Transcriptional regulation of metal transport genes and mineral nutrition during acclimatization to cadmium and zinc in the Cd/Zn hyperaccumulator, *Thalpi caerulescens* (Ganges population). *New. Phytol.*, 185:114-129.
- Liu, W., Yang, Y.S., Zhou, Q.X., Xie, L.J. and Han, Y.P.(2009). Risk assessment of cadmium contaminated soil on plant DNA damage using RAPD and physiological indices. *J. Haza. Materials.*, 161:878-883.
- Martins, L.L., Mourato, M.P., Cardoso, A.I., Pinto, A.P., and Mota, A.M. (2011). Oxidative stress induced by Cd in *Nicotiana tabacum* L.: effects on growth parameters, oxidative damage and antioxidant responses in different plant parts. *Acta Physiol. Plantarum.*, 33(4): 1375-1383.

- Majid, N.M., Islam, M.M., Justin, V., Abdu A. and Ahmadpour, P. (2011). Evaluation of heavy metal uptake and translocation by *Acacia mangium* as a phytoremediator of copper contaminated soil. *Afr. J. Biotechnol.*, 10(42): 8373-8379.
- Muneer, S., Qadri, T.N., Mahmooduzzaffar, O. and Siddiqi, T.O. (2011). Cytogenetic and biochemical investigations to study the response of *Vigna radiata* to cadmium stress. *Afri. J. Plant Sci.*, 5(3):183-192.
- Narain, S., Ojha C.S.P., Mishra S.K., Chaube U. C. and Sharma P.K. (2011). Cadmium and Chromium removal by aquatic plants. *Int. J. Environ Sci.*, 1(6):1297-1304.
- Ortega- Villasante, C., Rellas, A.Z.Z., Ivarez, R., De, F.F., Campo, M. and Carpena-Ruiz, P.O. (2005). Cellular damage induced by Cd and Hg in *Medicago sativa*. *J. Exp. Bot.*, 56:2239-2251.
- Ozturk, L., Eker, S. and Ozturk, F. (2003). Effect of cadmium on growth and concentration of cadmium, ascorbic acid and sulphhydryl groups in Durum Wheat cultivars. *Turk. J. Agri.*, 27:161-168.
- Panda, S.K. and Chowdhury, S. (2004). Changes in nitrate reductase activity, lipid peroxidation and antioxidant system in moss *Polytrichum* sp. Subjected to hexavalent chromium treatment. *Braz. J. Plant Physiol.*, 31:179-184.
- Pandey, N. and Singh, G.K. (2012). Studies on antioxidative enzymes induced by cadmium in pea plants (*Pisum sativum*). *J. Environ. Biol.*, 33:201-206.
- Qari, S.H. (2010). DNA-RAPD fingerprinting and cytogenetic screening of genotoxic and antigenotoxic effect of aqueous extracts of *Costus speciosus* (Koen). *JKAU: Sci.*, 22:133-152.
- Qurainy, F., Alameri, A.A. and Khan, S. (2010). RAPD profile for the assessment of genotoxicity on a medicinal plant: *Eruca sativa*. *J. Med. Plants Research.*, 4(7):579-586.
- Rezvani, M. and Zaefarian, F. (2011). Bioaccumulation and translocation factor of cadmium and lead in *Aeluropus litorali*. *A.J.A.E.*, 2(4):114-119.
- Rodriguez, E., Santos, C., Lucas, E. and Pereira, M.J. (2011). Evaluation of Chromium (VI) toxicity to *Chlorella vulgaris* Beijerinck cultures. *Fresenius Environmental Bulletin.*, 20: 334-339.
- Selvam, A. and Wong, J.W. (2008). Phytochelatin synthesis and cadmium uptake of *Brassica napus*. *Environ. Technol.*, 29:765-773.
- Shahrtash, M., Zaden, S.M. and Mohabatkar, H. (2010). Cd induced genotoxicity detected by the random amplification of polymorphism DNA in the maize seedling root. *J. Cell and Mol. Research.*, 2(1): 42-48.
- Shanker, A.K, Cervanter, C. and Loza-Tavera, H.(2005). Chromium toxicity in plants. *Environ. Int.*, 31(5):739-753.
- Srivastava, R., Khan, R., Manzoor, N. and Mahmooduzzafar, N. (2011). Responses of cadmium exposures on growth, physio-biochemical characteristics and the antioxidative defence system of soybean (*Glycine max* L.). *J. of Phytology.*, 3(10): 20-25.
- Subrahmanyam, D. (2008). Effect of Cr toxicity on leaf photosynthetic characteristics and oxidative changes in wheat. *Photosynthetica.*, 46(3):339-340.
- Sun, R., Jin, C. and Zhou, Q. (2010). Characteristics of cadmium accumulation and tolerance in *Rorippa globosa* (Turcz.) Thell., a species with some characteristics of cadmium hyperaccumulation. *Plant Growth Regul.*, 61:67-74.
- Tanhan, P., Kruatrachu, M. and Pokethitiyook, P.(2007). Uptake and accumulation of Cadmium, Lead and Zinc by Siam weed [*Chromolaena odorata* King and Robinson]. *Chemosphere.*, 68:323-329.
- Uraguchi, S., Watanabu, I., Kiyono, M. and Kuno, K. (2006). Characteristics of Cadmium accumulation and tolerance in novel Cd- accumulating crops, *Avena strigosa* and *Crotalaria juncea*. *Exp. Botany.*, 57:2955-2965.
- Vassilev, A. and Lidon, F. (2011). Cadmium induced membrane damage and changes in soluble protein and free amino acid contents in young barley plants. *Emer. J. Food. Agri C.*, 23(2):130-136.
- Vassilev, A., Lidon, F., Scotti, P., Barreiro, M.G. and Yordanov, I. (2004). Cadmium-induced changes in chloroplast lipids and photosystem activities of barley plants. *Biol. Plant.*, 48(1):153-156.
- Wang L., Zhaou, D.L. and Sun, Y. (2008). Effect of cadmium toxicity on nitrogen metabolism in leaves of *Solanum nigrum* L. as a newly found cadmium hyperaccumulator. *J. Haza. Materials.*, 154: 818.
- Yildiz, M. and Terzi, H. (2012). Effect of hexavalent chromium stress in roots of Cr- tolerant and Cr- sensitive barley cultivars. *Acta Agromica Hungarica.*, 60(1): 29-36.
- Zeng, F., Qiu, B., Wu, X., Niu, S. and Zhang, G. (2012). Glutathione mediated alleviation of Cr toxicity in rice plants. *Researchgate.*, 124-134.
- Zhang, X. H., Liu, J., Huang, H.T., Chen, Z. and Wang, D. Q. (2007). Chromium accumulation by the hyperaccumulator plant *Leersia hexandra* Swartz. *Chemosphere.*, 67:1138-1143.