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Response of Plants towards Heavy Metal Toxicity: An overview of Avoidance, Tolerance and Uptake Mechanism

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Abstract: Heavy metal contamination of soils is one of the serious environmental concerns due to metal's persistent nature and their biomagnification potential. The high concentrations of both essential and non-essential heavy metals affect the plant growth adversely and even lead to death in extreme conditions. Plants adopt various cellular mechanisms to minimize the metal toxicity. On exposure to heavy metals plant initially try to prevent entry of metal in to root cells by implementing avoidance strategy. When heavy metals manage to enter the root cells, they are detoxified intra-cellularly through processes like cell wall binding, organic acids, chelation, sequestration etc. Excess of heavy metal also activate oxidative stress defense mechanisms and the synthesis of stress-related proteins in plants. This inherent ability of plants to tolerate and accumulate toxic contaminants can be explored to treat the metal contaminated areas efficiently. The present review paper covers an overview of plant responses towards heavy metal toxicity and mechanisms of metal uptake, translocation and accumulation.

Keywords: Heavy metal, Avoidance, Tolerance, Chelation, Oxidative Stress Defense, Phytoremediation

Introduction

Heavy metal pollution due to various industrial, economic or social activities has become one of the most important global environmental problems. Metals and/ or metalloids having atomic density > 5 g/cm^3 and atomic weight > 20 are termed as heavy metal[1]. Some of the heavy metals like Cu, Co, Fe, Ni and Zn are essential micronutrients and are constituents of many plant enzymes and proteins. High concentrations of essential and non-essential heavy metals in the soils lead to toxicity symptoms inhibiting plant growth[2]. A variety of physiological and biochemical processes in plants are also affected by higher concentration of heavy metals. Severity of heavy metal contamination increases many fold due to their persistent nature and bio-magnification potential. Some plant species has naturally developed various extra and intra cellular mechanisms to tolerate and/or detoxify the heavy metals. According to Hall J.L., (2002)[2] these mechanisms mainly focus on avoiding toxic concentration build up to prevent damaging effects. In recent years, extensive research has been conducted to understand the cellular or molecular mechanisms involved in metal tolerance. The present paper summarizes plant response towards heavy metal toxicity and mechanism of metal uptake, translocation and accumulation in plants.

Plant Response to Heavy Metal Exposure

The plants utilize number of metal specific strategies to combat high external metal concentrations which are mainly classified into two categories, Avoidance (restriction to metal uptake) and Tolerance[3].

A) Avoidance:

This mechanism limits the uptake of heavy metals and prohibits their entry in plant tissues through root cells. The avoidance strategy is the first line of defense which mainly works at extracellular level through various mechanisms like immobilization by mycorrhizal association, complexation by root exudates, and modification of rhizosphere pH, exudation of metal-binding organic acids or formation of redox barrier.

I. **Immobilization by Mycorrhizal Associations:** The presence of mycorrhizal associations between fungi and roots of host plants in metal contaminated soils indicate an important relationship in plant tolerance and accumulation[4]. However, the exact role was not established. Ectomycorrhizas (ECM) and Arbuscular Mycorrhizas (AM) are the two most common mycorrhizal associations in plants growing on heavy metal contaminated soils[5]. Mycorrhizas adopt absorption, adsorption or chelation mechanisms to restrict the entry of heavy

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metals in to host plant[2]. They provide an effective exclusion barrier to metal uptake.

II. **Root Exudates:** Roots releases variety of diffusates (like amino or organic acids, water, inorganic ions, sugars etc.), excretions (like bicarbonates, protons, carbon dioxide etc.) and secretions (like mucilage, siderophores, allelopathic compounds etc.) which collectively termed as root exudates and helped the plant to survive in metal contaminated areas[6]. The root exudates form stable heavy metal-ligand complexes in the vicinity of the root thus making them unavailable and lessening the toxicity. Some root exudates increase the pH of rhizosphere which precipitates metals and limits their bioavailabilty.

B) Tolerance:

Tolerance mechanism is capable of accumulating, storing and immobilizing heavy metals by binding them with amino acids, proteins or peptides[7]. It is plant's second line of defense which mainly focuses on intra cellular metal detoxification. Metal tolerance is generally achieved by two strategies. Tong Y. et al., (2004)[8] reported that on exposure to heavy metals plant firstly tries to prevent metal transport across the plasma membrane by binding or modification of metal ions. Secondly metal ions which enter the plant body were detoxify through inactivation or converted into less toxic form.

I. **Metal Binding to Cell Wall:** Pectic sites, histidyl groups and extracellular carbohydrates present in cell wall caused immobilization of heavy metals and prevent their uptake in to cytosol[9]. Cell wall pectins consisting of polygalacturonic acid act as a cation exchanger. The heavy metals are bound to carboxylic groups of polygalacturonic acids restricting the plant uptake[10]. Studies indicated that the chemical properties of the cell wall might modulate metal uptake and consequently metal tolerance. The cell wall has little impact on metal tolerance due to inadequate number of metal absorption sites. However, the role of the cell wall in metal tolerance is not completely understood.

II. **Active Efflux Pumping at Plasma Membrane:** Reichman S.M., (2002)[3] showed that active efflux of the metal which lowers the intracellular concentration to subtoxic levels is a frequently utilized strategy to produce tolerance. This mechanism is well documented in bacteria[11] and animal cells[12]. P_{1B}-ATP_{ases} which belongs to P-type ATPase superfamily and ATP-binding cassette (ABC) transporters are mainly worked as heavy metal efflux pumps in plants[9,13]. The two subfamilies of ABC transporters namely Multidrug Resistance-associated Protein (MRP) and Pleiotropic Drug Resistance (PDR) are particularly active in the sequestration of chelated heavy metals[9]. Only a few evident indications of plasma membrane efflux transporters are reported in plants.

III. **Organic Acids:** Organic acids within cells act to detoxify metals by complexing and making them unavailable to the plant[3]. It acts as metabolic intermediates in the formation of ATP from carbohydrates in nitrogen metabolism and in ionic balance. Hence, metabolic abnormalities in any of these processes would be reflected by changes in the concentrations of organic acids. Therefore, an increase in organic acids with increasing supply of metals could imply a detoxification mechanism or conversely, disruption of metabolism resulting in the production of organic acids as a stress response to excess metal.

IV. **Inactivation of Toxic Metals**: At cytoplasmic level phytochelatins and /or metallothioneins play major role to provide metal tolerance. The metal-phytochelatins/ metallothioneins complex is actively transported from the cytosol across the tonoplast into the vacuole where it is store without any toxicity[8].

a) Phytochelatins (PCs):

Phytochelatins are derived from glutathione (GSH) and possess the general structure (γ-Glu-Cys) n-Gly (where n=2 to 11)[14]. On activation in presence of heavy metals PC synthase enzyme produced PCs by trans-peptidation of γ-glutamyl-cysteinyl dipeptides from GSH[15]. The cystein part of PCs carry out metal co-ordination while thiol group and gluatmic acid provides water solubility to PCs[16]. Among the various heavy metals Cd is strongest inducer of PCs. Not all the metals which trigger the PCs synthesis are able to form complexes with it. Generally

PCs are associated with both homeostasis and trafficking of essential metals as well as detoxification of heavy metals[17,18].

Once the PC-metal complex was formed, it transported to vacuoles by metal/H+ antiporters or ABC transporters isolating the toxic metals from metal sensitive enzymes[14,19]. In vacuoles, PC metal complexes become more resistant to proteolytic degradation on incorporation of inorganic sulfide and sulfite ions[16,20]. Under favorable conditions metals from PC-metal-sulfide complexes are liberated causing PC degradation.

b) Metallothioneins (MTs):

Metallothioneins are cysteine rich proteins induced by various abiotic stresses having molecular weight between 5-20 kDa and mercaptide groups which causes metal ion binding with thiols. Based on structure and content of cysteine they are divided in to MT1 and MT2[9,21,22,23]. Class 1 MTs contain cysteine motifs that align with mammalian MTs, whereas Class 2 MTs contain similar cysteine clusters but they do not align with Class1 MTs. Hamer D.H., (1986)[24] mentioned that MTs showed varied response to different heavy metals. It was observed that they participate in antioxidant protection and plasma membrane repair mechanism. There is no much information about exact role of MTs in detoxification of several of metals[25].

C) Other Alternatives:

Under heavy metal stress plants also activate oxidative stress defense mechanisms and the synthesis of stress-related proteins such as heat shock proteins, reactive oxygen species and hormones.

I. **Stress related proteins:** With heavy metal exposure, most of the plants trigger the synthesis of sets of novel proteins[23]. Most of the proteins endowed plasma membranes to act as barrier for metal inflow which leads to metal homeostasis and detoxification[26]. The common stress related Heat Shock Proteins (HSP), act as molecular chaperones and help in normal protein folding and assembly. It may also function in the protection and repair of proteins under stressful conditions[23]. The synthesis of HSP under heavy metal stress has been observed in different plants[27] but its role is largely unknown.

II. **Antioxidant Defense and Reactive Oxygen Species:** The increased synthesis of reactive oxygen species (ROS) like superoxide radicals ($O_2^{\cdot -}$), hydroxyl radicals ($OH^{\cdot}$) and hydrogen peroxide (H_2O_2) is one of the initial responses to heavy metal stress. These species are continuously produced at low level during normal metabolic processes[28]. ROS, particularly H_2O_2 plays an important role as intermediate signaling molecules to regulate defense system[29,30]. ROS have a dual function: higher concentrations, damages the cells; but at moderate levels, they help to adapt stress by induction of an antioxidant response[31]. A complex ROS scavenging mechanism at the molecular and cellular levels decreases oxidative damage with increased resistance to heavy metals[32].

III. **Hormones:** Peleg Z. and Blumwald E., (2011)[33] suggest that the regulation of hormone synthesis in presence of heavy metals indicates that plant hormones play a crucial role in the adaptation to metal stress. The hormones such as salicylic acid, jasmonic acid, ethylene, gibberellic acid are implicated in plant defense signaling pathways. Jasmonic acid treatment increased the capacity for glutathione synthesis which plays central role in protecting plants from heavy metal stress[34]. Salicylic acid activates defense related genes either by H_2O_2 mediated signal transduction pathway or by directly affecting mechanisms of metal detoxification by inhibiting[35,36]. Salicylic acid inhibits two major H_2O_2 scavanging enzymes catalase and ascorbate peroxidase which causes cellular H_2O_2 concentration to rise and subsequently acts as second messenger. Heavy metal stress induces ethylene biosynthesis[37] acts as endogenous signal triggering the plant defense response. Summary of avoidance and tolerance mechanisms exhibited by plants exposed to elevated metal concentrations are depicted in Figure 1.

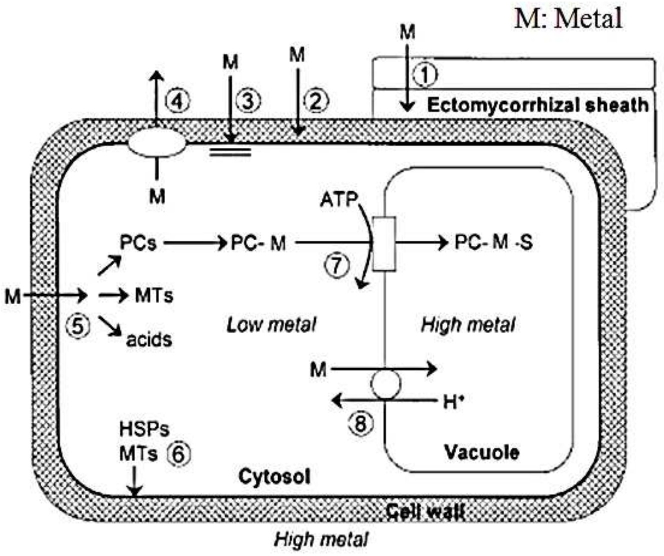

Figure.1: Summary of Heavy Metal Avoidance and Tolerance Mechanisms *(Source: Hall J.L., 2002)*[2]

1. Restriction of metal movement to roots by mycorrhizas,
2. Binding to cell wall and root exudates,
3. Reduced influx across plasma membrane,
4. Active efflux into apoplast,
5. Chelation in cytosol by various ligands,
6. Repair and protection of plasma membrane under stress conditions,
7. Transport of PC-M complex into the vacuole,
8. Transport and accumulation of metal in vacuole

Categorization of Plants based on Heavy metal Response:

Based on both avoidance and tolerance strategies, the plants are categorized into four different groups, such as Indicators, Excluder, Accumulator and Hyperaccumulator[38].

Indicators: Plants in which uptake and translocations reflect soil metal concentration and show toxic symptoms are known as Indicators. The growth of these plants reduced as soil concentrations increase.

Excluders: The plants that restrict the uptake of toxic metals into above ground biomass are known as Excluder. Excluder plant has high levels of heavy metals in the roots and transfer factor or shoot/root quotients are less than 1. They are used to stabilize the soil and avoid further contamination.

Accumulators: Plants in which uptake and translocation reflect background metal concentration without showing toxic symptoms are known as accumulators.

Hyperaccmulators: Hyperaccumulators are species capable of accumulating metals at levels 100-fold greater than those typically measured in common non-accumulator plants.

A plant is classified as a hyperaccumulator for heavy metals when it meets following four criteria:

1. Transfer factor or shoot/root quotient (level of heavy metal in the shoot divide by level of heavy metal in the root) is more than 1
2. Extraction coefficient (level of heavy metal in the shoot divide by total level of heavy metal in the growth medium) is more than 1
3. Higher levels of heavy metals of 10–500 times the levels in normal or uncontaminated plants
4. Metal accumulation exceeding a threshold value of shoot metal concentration of 1% or 10,000 (Zn, Mn), 0.1% or 1000 mg/kg (Ni, Co, Cr, Cu, Pb and Al), 0.01% or 100 mg/kg (Cd and Se) of the dry weight plant biomass[39].

Heavy metal Uptake, Translocation and Accumulation within Plant

To know the avoidance and tolerance mechanism in detail it is essential to study the heavy metal uptake in plants. Accumulation of metal is a function of uptake capacity and intracellular binding sites. Mobilization of metals, uptake from soil, compartmentation and sequestration, xylem loading, distribution in aerial parts and storage in leaf cells are the main steps involved in accumulation of metal in plants. At every level, concentration, affinities of chelating molecules and selectivity of transport activities affect metal accumulation rates[40]. Quantity and intensity factor along with reaction kinetics govern the transfer of heavy metals in plants[41].

For hyperaccumulation, the plant must possess the ability to solubilize metals from the soil, take up metal using specific ion transporter proteins and detoxify metal effects on cellular processes by chelation and compartmentation, thereby translocating metal even to sensitive regions of the plant, such as leaves, where many important metabolic processes occur[42]*.* The pathway of metal uptake in plants is presented in Figure 2.

I. **Mobilization of Metals:** Metals in soil mostly exists as insoluble bound fraction and needs to be mobilized into the solution to make it available for plants. Natural hyperaccumulators solubalizes the soil bound metals by secretion of root exudates which causes acidification of rhizosphere[43] and metal chelation by secretion of mugenic and aveic acid[44]. Though root exudates are important in metal mobilization and uptake, still its complete mechanism is not clear.

II. **Root Uptake:** Bioavailable metal enters in plant either through inter cellular spaces (apoplastic pathway) or by crossing plasma membrane (symplastic pathway)[45]. The extracellular negatively charged sites (COO-) of the root cell walls where most of the metal ions are adsorbed, act as initial barrier for metal translocation. Additionally impermeable suberin layers in the cell wall also reduced transport of metals from root apoplast to root xylem[46,47]. Therefore, to cross this barrier and to reach the xylem, metals must move symplastically. Ghosh M. and Singh S.P., (2005)[48] stated that inward movement of metals during symplastic pathway is possible due to strong electrochemical gradient provided by negative resting potential of 170 mV. Symplastic pathway is an energy dependent process mediated by specific or generic metal ion carriers or channels[45].

III. **Root to Shoot Transport:** Subsequent to metal uptake into the root symplasm, three processes govern the movement of metals from the root into the xylem: sequestration of metals inside root cells, symplastic transport into the stele and release into the xylem. The xylem loading is a tightly regulated process mediated by membrane transport proteins which remain to be identified[40]. Under normal condition the high cation exchange capacity of xylem cell walls restrict the further transport of metal ions, while in hyper accumulators complexation of metals with low molecular weight chelators allows easy translocation to shoot[43].

IV. **Metal Unloading, Trafficking and Storage in Leaves:** Mahmood T., (2010)[43] indicated that transportation and distribution of metal in leaves occurs via apoplast or symplast. Trafficking of metals occurs inside every plant cell, maintaining the concentrations within the specific physiological ranges in each organelle and ensuring delivery of metals to metal-requiring proteins[40]. In hyperaccumulators metal complexing with organic ligands provides high metal tolerance[45]. In the leaf tissues, metals are sequestered in extracellular or sub-cellular compartments. The cell types where metals are deposited vary with the metal as well as with the plant species.

V. **Sequestration:** Sequestration in plant vacuole which prevents free concentration of metal ions in cytosol is final step of metal accumulation[8]. Peer W.A et al., (2006)[45] indicate that metals may remain in cell wall due to interaction of polyvalent cations with negative charge sites.

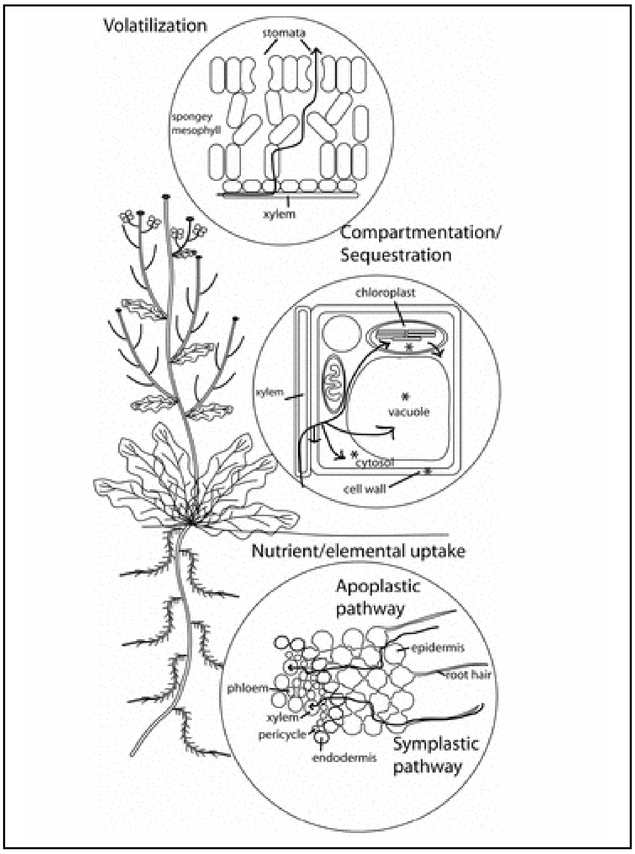

Figure.2: Pathway of Metal Uptake in Plants *(Sources: Peer W.A. et al., 2006)*[45]

Conclusion

Plants have developed various extra and intracellular defense mechanisms to fight against heavy metal toxicity. As first line of defense, plant attempts to prevent the entry of heavy metal in to root cells by immobilization through mycorrhizal association, complexation by root exudates or modification of rhizosphere pH. Even then if metal manage to enter the root cell, second line of defense activates which includes binding of metal ions with cell wall or plasma membrane, use of phytochelatins and metallothiones or sequestration in vacuole. For systematic improvements in heavy metal phytormediation better understanding of cellular mechanisms involved in heavy metal avoidance, uptake, transport and accumulation is essential. The multidisciplinary approach combining plant biology, genetic engineering, soil chemistry, soil microbiology as well as agricultural and environmental engineering will help in optimization of plants for heavy metal detoxification.

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References

1. Nagajyoti P.C., Lee K.D. and Sreekanth T.V.M., Heavy metals, occurrence and toxicity for plants: A review, *Environmental Chemistry Letters,* 2010, 8, 199-216.
2. Hall J.L., Cellular mechanisms for heavy metal detoxification and tolerance, *Journal of Experimental Botany,* 2002, 53(366), 1-11.
3. Reichman S.M., The responses of plants to metal toxicity: A review focusing on copper, manganese and zinc, Published as Occasional Paper No.14 (ISBN 1-876205-13-X) in *Australian Minerals and Energy Environment Foundation,* 2002, 1-40.
4. Jentschke G. and Godbold D., Metal toxicity and ectomycorrhizas, *Physiologia Plantarum,* 2000, 109(2), 107–116.
5. Leyval C., Turnau K. and Haselwandter K., Effect of heavy metal pollution on mycorrhizal colonization and function: physiological, ecological and applied aspects, *Mycorrhiza,* 1997, 7(3), 139-153.
6. Marschner H., Mineral nutrition of higher plants, 2nd edition, *Academic Press: London, UK.* 1997.
7. Pal M., Horvath E., Janda T., Paldi E. and Szalai G., Physiological changes and defense mechanisms induced by cadmium stress in maize, *Journal of Plant Nutrition and Soil Science,* 2006, 169(2), 239–246.
8. Tong Y., Kneer R. and Zhu Y., Vacuolar compartmentalization: A second generation approach to engineering plants for Phytoremediation, *Trends in Plant Science,* 2004, 9(1), 7-9.
9. Manara A., Plant responses to heavy metal toxicity in plants and heavy metals, Ed. A. Furini, Springer Briefs in Biometals, DOI: 10.1007/978-94-007-4441-7_2. 2012, 27-53.
10. Ernst W.H.O., Verkleij J.A.C. and Schat H., Metal tolerance in plants, *Acta Botanica Neerlandica,* 1992, 41, 229-248.
11. Silver S., Bacterial resistances to toxic metal ions - A review, *Gene,* 1996, 179(1), 9-19.
12. Palmiter R.D. and Findley S.D., Cloning and functional characterization of a mammalian zinc transporter that confers resistance to zinc, *EMBO Journal*, 1995, 14(4), 639-649.
13. Axelsen K.B. and Palmgren M.G., Inventory of the super family of P-Type ion pumps in Arabidopsis, *Plant Physiology,* 2001, 126(2), 696-706.
14. Cobbett C.S., Phytochelatin biosynthesis and function in heavy metal detoxification, *Current Opinion in Plant Biology,* 2000, 3(3), 211-216.
15. Chen J., Zhou J. and Goldsbrough P.B., Characterization of phytochelatin synthase from Tomato, *Physiologia Plantarum,* 1997, 101(1), 165-172.
16. Bertrand M. and Guary J.C., How plants adopt their physiology to an excess of metals, *In* Passarakli M. (Ed.) Handbook of Plant and Crop Physiology, New York Marcel Dekker, 2002, 751-761.
17. Thumann J., Grill E., Winnacker E.L. and Zenk M.H., Reactivation of metal requiring apoenzymes by phytochelatin metal complexes, *FEBS Letters,* 1991, 284(1), 66-69.
18. Ebbs S., Lau I., Ahner B. and Kochian L., Phytochelatin synthesis is not responsible for Cd tolerance in the Zn/Cd hyperaccumulator *Thlaspi caerulescens* (J.&C. Presl), *Planta,* 2002, 214(4): 635- 640.
19. Rea P.A., Li Z.S., Lu Y.P., Drozdowicz Y.M. and Mortinoia E., From VacuolarGS-X pumps to multi specific ABC transporters, *Annual Review of Plant Physiology and Plant Molecular Biology,* 1998, 49, 727-760.
20. Steffens J.C., The heavy metal binding peptides of plants, *Annual Review of Plant Physiology and Plant Molecular Biology,* 1990, 41, 553-575.
21. Robinson N.J., Tommey A.M., Kuske C. and Jackson P.J., Plant metallothioneins, *Biochemical Journal*, 1993, 295(pt 1), 1-10.
22. Rauser W.E., Structure and function of metal chelators produced by plants; the case for organic acids, amino acids, phytin and metallothioneins. *Cell Biochemistry and Biophysics,* 1999, 31 (1), 19-48.
23. Mishra S. and Dubey R.S., Heavy metal uptake and detoxification mechanisms in Plants, *International Journal of Agricultural Research,* 2006, 1(2), 122-141.
24. Hamer D.H., Metallothionein, *Annual Review of Bio Chemistry,* 1986, 55, 913-951.
25. Goldsborough P., Metal tolerance in plants: The role of phytochelatins and metallothioneins, *In* Terry N.,

Banuelos G. (Ed.), Phytoremediation of contaminated soil and water, *CRC Press LLC*, 2000; 221-233.

26. Suzuki N., Yamaguchi Y., Koizumi N. and Sano H., Functional characterization of a heavy metal binding protein Cdl19 from Arabidopsis, *The Plant Journal,* 2002, 32(2), 165.
27. Wollgiehn R. and Neumann D., Stress response of Tomato cell cultures to toxic metals and heat shock: differences and similarities, *Journal of Plant Physiology,* 1995; 146(5-6), 736-742.
28. Arora A., Sairam R.K., Srivastava G.C. Oxidative stress and antioxidative system in plants, *Current Science,* 2002, 82(10), 1227-1238.
29. Vranova E., Inze D. and Van Breusegem F., Signal transduction during oxidative stress, *Journal of Experimental Botany,* 2002, 53(372), 1227-1236.
30. Moura D.J., Peres V.F., Jacques R.A. and Saffi J. Heavy metal toxicity oxidative stress parameters and DNA repair, *In* Gupta D.K. and Sandalio L.M. (Ed.) Metal toxicity in plants: Perception, signaling and remediation, *Springer-Verlag Berlin Heidelberg publisher,* 2012, 187-205.
31. Chen S., Olbrich A., Langenfeld-Heyser R., Fritz E. and Polle A. Quantitative X-ray microanalysis of hydrogen peroxide within plant cells, *Microscopy Research and Technique.* 2009, 72(1), 49-60.
32. Rastgoo L. and Alemzadeh A., Biochemical responses of Gouan (*Aeluropus littoralis*) to heavy metals stress, *Australian Journal of Crop Science,* 2011, 5(4), 375-383.
33. Peleg Z. and Blumwald E., Hormone balance and abiotic stress tolerance in crop plants, *Current Opinion in Plant Biology,* 2011, 14(3), 290-295.
34. Xiang C. and Oliver D.J., Glutathione metabolic genes coordinately respond to heavy metals and jasmonic acid in Arabidopsis, *The Plant Cell,* 1998, 10(9), 1539-1550.
35. Chen Z., Ricigliano W. and Klessig D.F., Purification and characterization of a soluble salicylic acid binding protein from Tobacco, *Proceedings of National Academy Sciences*, 1993, 90(20), 9533-9537.
36. Metwally A., Finkemeler I., Georgi M. and Dietz K.F., Salicylic acid alleviates the cadmium toxicity in Barley seedlings, *Plant Physiology,* 2003; 132(1): 272-281.
37. Milone M.T., Sgherri C., Clijsters H. and Navari-Izzo F., Antioxidative responses of Wheat treated with realistic concentration of cadmium, *Environmental and Experimental Botany*, 2003, 50(3), 265-276.
38. Prasad M.N.V., Heavy metal stress in plants from biomolecules to ecosystems, Ed. 2nd, *Springer Verlag Berlin Publisher*, ISBN 3-540-40131-8, 2004.
39. Mganga N., Manoko M.L.K. and Rulangaranga Z.K., Classification of plants according to their heavy metal content around North Mara Gold Mine, Tanzania: Implication for Phytoremediation, *Tanzania Journal of Science,* 2011, 37, 109-119.
40. Clemens S., Palmgren M.G. and Kramer U., A long way ahead: Understanding and engineering plant metal accumulation, *Trends in Plant Science,* 2002, 7(7), 309-315.
41. Brummer G.W., Gerth J. and Herms U., Heavy metal species mobility and availability in soils. *Z. Pflanzenernähr, Bodenk,* 1986, 149(4), 382-398.
42. Rajakaruna N., Tompkins K.M. and Pavicevic P.G., Phytoremediation: An affordable green technology for the clean-up of metal-contaminated sites in Sri Lanka, *Ceylon Journal of Science (Biological Sciences),* 2006, 35(1), 25-39.
43. Mahmood T., Review phytoextraction of heavy metals the process and scope for remediation of contaminated soils, *Soil and Environment,* 2010, 29(2), 91-109.
44. Salt D.E., Blaylock M., Kumar P.B.A.N., Dushenkov V., Ensley B.D., Chet I. and Raskin I., Phytoremediation: A novel strategy for the removal of toxic metals from the environment using plants, *Biotechnology,* 1995, 13, 468-475.
45. Peer W.A., Baxter I.R., Richards E.L., Freeman J.L. and Murphy A.S., Phytoremediation and hyperaccumulator plants, *Molecular Biology of Metal Homeostasis and Detoxification*, *Topics in Current Genetics,* 2006, 14(84), 299-340.
46. Lasat M.M., Phytoextraction of toxic metals: A review of biological mechanisms, *Journal of Environmental Quality,* 2002, 31(1), 109-120.
47. Taiz L. and Zeiger E., Plant Physiology, 3rd edition, Sinauer Associates, Sunderland, MA, 2002.
48. Ghosh M. and Singh S.P., A comparative study of cadmium phytoextraction by accumulator and weed species, *Environmental Pollution,* 2005, 133(2), 365-371.

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