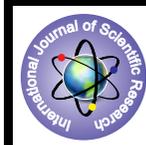


# A Journey From Heavy Metal To Metallothionein: A Critical Review of Heavy Metal Remediation By Plants



## Biosciences

**KEYWORDS :** Heavy metals, phytoremediation, metallothionein, hyperaccumulator

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### ABSTRACT

*The ruling idea of the present review is that plants are naturally capable of absorbing metals and ions from the land for their outgrowth. During evolution as soil is the lone source of nutrition for plants, they have accommodated themselves to heavy metals in soil, which otherwise are highly toxic to other living beings. Since plants are naturally adjusted to withstand toxic concentrations of minerals in soil, including heavy metals, it is legitimate to apply them to remediate such contamination. Under metal stress plant undergoes various physiological, biochemical and molecular alterations. Various proteins, including metallothionein protein plays an important role in the heavy metal intake and its compartmentalization in plants. The function parameter of this protein lies within its type and form which relying on its amino acid composition rich in cysteine amino acid. Promising metallothionein proteins are available that can be used in phytoremediation technology through transgenic plant.*

### 1. Heavy metal contamination:

Hard metals are specified as the metals with a density greater than 5 gm/cm<sup>3</sup>. Fifty three of the naturally occurring metals are heavy metals (Weast, 1984). The metals found in our environment come from the natural weathering process of the earth's crust, mining, soil erosion, urban runoff, sewage effluents, industrial discharge, pest or disease control agents and air pollution fallout. According to their chemical properties and biological function, heavy metals form a heterogeneous group; toxicity varies by metals and concentrations. Many of them (Cr, Hg, Cd, Pb, Co, Cu, Zn and Ni) are highly toxic both in soluble salt and elemental forms. Soil contaminated with excess amount of metal in varying combinations and concentrations adversely affect the health of mankind. These heavy metals are toxic because they cause DNA damage and their carcinogenic effects in animals and humans are probably caused by their mutagenic ability (Sanon, Ndoye, Baudoin, Prin, & Galiana, 2010). Potential threat is that heavy metals are not degradable and without intervention stay in soil for centuries. Elemental pollutants are particularly difficult to remediate from air, water and soil because, unlike organic pollutants that can be degraded to less or nontoxic small molecules because they are immutable by all biochemical reactions and hence remain in ecosystem (Taylor, Krauter, Jr, Dibley, & Pinkart, n.d.). One of the largest problem associated with the persistence of heavy metals is the potential for bioaccumulation and bio-magnification causing heavier exposure for some organisms than is present in the environment alone (Brett et al., 1997). The conventional remediation technologies (other than bioremediation) used for in situ and ex situ remediation are typically expensive and destructive. They include stabilization, solidification, soil flushing, chemical reduction/oxidation, electro kinetics, low temperature thermal desorption, soil washing, incineration, pneumatic fracturing, vitrification, excavation/retrieval, disposal and landfill.

### 2. Phytoremediation:

Phyto-technologies are a set of technologies using plants to remediate contaminants in soil, deposits, groundwater and surface water. Due to relatively low costs this technology has become an attractive alternatives to conventional clean-up technologies (Eloheid, 2008).

#### 2.1. Types of phytoremediation

According to Schwitzguébel (2000) several types of phytoremediation can be defined as:

1. Phytoextraction: The use of pollutant-accumulating plants to

remove pollutants like metals or organics from soil by concentrating them in harvestable plant parts.

2. Phytotransformation: The degradation of complex organic molecules to simple molecules or the incorporation of these molecules into plant tissues.

3. Phytostimulation: Plant-assisted bioremediation, microbial and fungal degradation stimulated by release of exudates/enzymes into the root zone (rhizosphere).

4. Phytovolatilization: The use of plants to volatilize pollutants or metabolites.

5. Rhizofiltration: Use of plant roots to adsorb pollutants and metal from aqueous waste streams.

5. Pump and tree (Dendroremediation): The use of trees to evaporate water and thus to extract pollutants from the soil.

6. Phytostabilisation: The use of plants to reduce the bioavailability and mobility of pollutants in the environment, thus their migration is prevented to groundwater or their entry into the food chain.

7. Hydraulic control: The control of the water table and the soil field capacity by plant canopies.

As mentioned above, these ways in which plants are used to clean up or remediate contaminated sites. To remove pollutants from air, sediment and/or water and soil plants can break down, or contain and stabilize inorganic contaminants or degradation of organic pollutants by acting as filters or traps. Amendable to a broad range of contaminants (organic and inorganic) including many metals with limited alternative options.

### 3. Hyperaccumulation:

Some plants which grow on metalliferous soils have developed the ability to accumulate massive amounts of the indigenous metals in their tissues without exhibiting symptoms of toxicity (Entry & Leep-, 2011). Chaney, 1983 (Taylor et al., 2008) was the first to suggest using these "hyperaccumulators" for the phytoremediation of metal polluted sites. So far, more than 400 species of natural metal hyperaccumulators belonging to 45 families have been documented in the world. Plants growing on metalliferous soils can be grouped into three categories according to Baker (Luo et al., 2007): Excluders, Accumulators and Indicators.

Hyperaccumulators are referred to the plants that accumulate > 1000mg/kg of Ni and Co(Brett et al., 1997), >1000mg/kg of Cr(Reeves, n.d.), >1000mg/kg of As(Cai, Su, & Ma, 2004) and >1500mg/kg of Mo(Zhao, Lombi, & McGrath, 2003). Following the harvest of metal-enriched plants, the weight and volume of the contaminated material can be further reduced by ashing or composting.(Alkorta et al., 2004).

#### 4. Effects of heavy metals:

Whenever there is metal stress to the plants there are physiological, anatomical, biochemical and molecular changes observed. Different species may have evolved different mechanism to tolerate excess metals ions and even within one plant species more than one mechanism could be in operation.

##### 4.1. Physiological effects:

Plant growth is a function of complex interplay between sources and sink limitations of the two main organs of a plant, the root system and the shoot. The reduction in plant height could be attributed to decline in the cell enlargement and more leaf senescence in the plant under water stress(Dalcorso, Farinati, & Furini, 2010). The most common effect of metal toxicity in plants is stunted growth, leaf chlorosis and alteration in the activity of many key enzymes of various metabolic pathways(Zaefarian, Rezvani, Rejali, & Ardakani, 2012). The reduction in plant growth during stress is due to low water potential, hampered nutrient uptake and secondary stress such as oxidative stress. Retarded shoot growth due to the presence of the root environment with excess of Pb has also been found by Seyyedi(Agrawal, 2010). A few cases of increase in the plant biomass due to metal pollutants have been reported(Ranieri et al., 2005).

##### 4.2. Anatomical effects:

Studies on metal affected plants revealed clotted depositions in roots and stem break down of parenchyma cells, and a decrease in starch content in leaves of plants treated with high concentrations of Zn. During stress like drought, anoxia, salinity, heavy metal or nutrient stresses, several plants produced a root exodermis (i.e. a hypodermis with Casparian bands). This exodermis constitutes a barrier to the water and solutes radials flows and may contribute substantially to the overall resistance(Najaphy, Niari, Mostafaie, & Mirzaee, 2010). Anatomic traits of leaves affect gas exchanges and heavy metals like Cd induce the development of xerophytes features in leaves(Andrew, Shi, Lindsay, Urwin, & Nigel, 1991). Generally, the cell-wall thickness in the leaf epidermis could increase or an important lignified hypodermis could be produced in order to limit the water loss and stress (Degenhardt & Gimmler, 2000).

##### 4.3. Biochemical effects:

The decline in chlorophyll content in plants exposed to Cd+ and Pb+ stress is believed to be due to inhibition of important enzymes. Sugars play a number of ecological roles in plant protection against wounds and infection as well as in the detoxification of foreign substances (Agrawal, 2010). Cadmium toxicity greatly impaired not only the breakdown of soluble sugars but also the translocation of soluble sugars to the growing embryonic axis(Nagendran, Selvam, & Joseph, 2002). When heavy metal toxicity crosses the threshold limit, the protein level decreases and this might be due to the breakdown of protein synthesis mechanism. The production of ROS in plants, known as the oxidative burst, is an early event of plant defence response. ROS can seriously damage plants by increasing lipid peroxidation, protein degradation, DNA fragmentation and ultimately cell death. ROS such as O<sub>2</sub><sup>-</sup>, H<sub>2</sub>O<sub>2</sub> and •OH radicals, can directly attack membrane lipids and increase lipid peroxidation(Flora, Mittal, & Mehta, 2008). ROS increases the content of malondialdehyde (MDA). To minimize the affections of oxidative stress, plants have evolved a complex enzymatic and non-enzymatic antioxidant system, such as low-molecular mass antioxidants

(glutathione, ascorbate, carotenoids) and ROS scavenging enzymes superoxide dismutase (SOD), peroxidase (POD), catalase (CAT), ascorbate peroxidase (APX)(Zaharah & Growth, 2009). O<sub>2</sub><sup>-</sup> can be dismutated into H<sub>2</sub>O<sub>2</sub> by SOD in the chloroplast, mitochondrion, cytoplasm and peroxisome. POD plays a key role in scavenging H<sub>2</sub>O<sub>2</sub> which was produces through dismutation of O<sub>2</sub><sup>-</sup>-catalyzed by SOD.

##### 4.4. Molecular effects:

ROS is capable of inducing damage to almost all cellular macromolecules including DNA which includes base deletion, pyrimidine dimers, cross-links, strand breaks and base modification, such as alkylation and oxidation. Molecular techniques such as the analysis of molecular variance of the random amplified polymorphic DNA markers are useful to investigate the genetic diversity and heavy metal tolerance in plant populations, providing the opportunity to investigate the first step in the differentiation of plant populations under severe selection pressure and to select plants for phytoremediation(Gonzalez-mendoza, Quiroz, & Zapata-perez, 2007). Plant systems also have genes that encode metallothionein which are a group of low molecular mass, cysteine rich, metal binding proteins(Evans, Gatehouse, Gatehouse, Robinson, & Croy, 1990). GSH is one of the crucial metabolites in plants which is considered as most important intracellular defence against ROS induced oxidative damage. It occurs abundantly in reduced form (GSH) in plant tissues and is localized in all cell compartments like cytosol, endoplasmic reticulum, vacuole, mitochondria, chloroplasts, peroxisomes as well as in apoplast. GSH is a precursor of PCs, which plays important role in controlling cellular heavy metal concentration(Gill & Tuteja, 2010).

##### 5. Genetic engineering:

Genetic engineering approach has successfully facilitated to alter the biological functions of plants through modification of primary and secondary metabolism and by adding new phenotypic and genotypic characters to plants with the aim of understanding and improving their phytoremediation properties(Ka, 2000). Analysis of molecular variance of the random amplified polymorphic DNA markers are also useful to investigate the genetic diversity and heavy metal tolerance in plant populations, providing the opportunity to investigate the first steps in the differentiation of plant populations under severe selection pressure and to select plants for phytoremediation(Flora et al., 2008). For example a genetic analysis of copper tolerance with Cu-tolerant and susceptible lines of *Mulius guttatus* showed that a modifier gene that is active only in presence of the tolerance gene is responsible for the difference in Cu-tolerance in this species(Smith & Schoeitle, 1991). Similar studies with Zn-hyperaccumulator *Arabidopsis halleri* and the non-accumulator *Arabidopsis petraea* suggested that Zn-tolerance is also controlled by a single major gene(Najaphy et al., 2010). Therefore, the desired characters for phytoremediation can be improved by identifying candidate protein, metal chelators, and transporter genes for transfer and/or over expression of particular gene.

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