Review Article

Detoxification Mechanisms of Mercury Toxicity in Plants: A Review
Detoxification Mechanisms of Mercury Toxicity in Plants: A Review

Shilpa Shrivastava¹, Archana Shrivastava², Jot Sharma³

¹Department of Biotechnology, College of Life Sciences, CHRI Campus, Gwalior, Madhya Pradesh, India. ²Department of Microbiology, College of Life Sciences, CHRI Campus, Gwalior, Madhya Pradesh, India. ³Department of Biotechnology, Jiwaji University, Gwalior, Madhya Pradesh, India.

*Correspondence: shilpa_biotech44@yahoo.com; jotsharma_68@yahoo.com

Received: Sep 20, 2015; Accepted: Nov 6, 2015; Published: Dec 15, 2015

Abstract
Mercury is one of the most toxic heavy metals present in the earth’s crust. It has been considered as environmental pollutant because of its potent toxicity to plants and humans. In this review, we discuss mercury toxicity responses on plant metabolism and its detoxification mechanism by phytochelatins and antioxidant enzymes. Some light is also shed on selenium antagonistic study with mercury. Due to its potential toxicity, it has attracted attention in fields of soil science and plant nutrition. Mercury has harmful toxic effects on the molecular and physiobiochemical behavior of plants. Mostly research work has been done on seed germination, and shoot, root, and leaf morphology. Enzyme responses with respect to mercury as a result Hg accumulated in food chain is also reviewed here. Hence, this review may provide a compiled data for other researches in this direction, to provide a better mechanism or details about mercury’s noxious effect in the ecosystem.

Keywords: Mercury; Phytochelatins; Ecosystem; Physiobiochemical behavior; Selenium; Toxicity; Plants.

1. INTRODUCTION

The rapid industrial and technological advancement by man has become a menace for himself. Heavy metals are emitted into the environment by both natural and anthropogenic causes. The major causes of emission are the anthropogenic sources, specifically mining operations (Hutton and Symon, 1986; Battarbee et al., 1988; Nriagu, 1989). The biotoxic effects of heavy metals refer to the harmful effects of heavy metals to the body when consumed above the bio-recommended limits. Heavy metals are present in soils as natural components or as a result of human activity. Metal-rich mine tailings, metal smelting, electroplating, gas exhausts, energy and fuel production, downwash from power lines, intensive agriculture, and sludge dumping are the most important human activities that contaminate soils and aqueous streams with large quantities of toxic metals (Seaward and Richardson, 1990).

Heavy metals occur as natural constituents of the earth crust. They are persistent environmental contaminants since they cannot be degraded or destroyed. Metalloids enter the body system through food, air, and water and bio-accumulate over a period of time (Lenntech, 2004; UNEP/GPA, 2004).

Genomic protection of our biota from environmental or global pollution is the key for conservation of earth’s biodiversity. Metals constitute one of the major groups of genotoxic environmental pollutants, posing serious threat to human as well as environmental well-being (Nriagu, 1990; Panda and Panda, 2002).

2. MERCURY

Mercury poisoning has become a problem of current interest as a result of environmental pollution on a global scale. Mercury is a strong phytotoxic as well as genotoxic metal (Fridovich, 1986; Suszeynsky & Shann, 1995). It is ubiquitous in the environment and is inevitable for both humans and animals to avoid its exposure in some form or forms on a regular basis. Mercury occurs widely in the biosphere (Clarkson, 1987).

Mercury occurs naturally in the environment. It is considered as one of the most toxic elements for human health and environment (Tuzen and Soyak, 2005; Ghaedi, 2006; Meucci et al., 2009; Niazi et al., 2009). Mercury pollution is one of the most serious environmental problems in the world (Pilon-Smits and Pilon, 2000). Its exposure in both organic and inorganic forms is the second-most common cause of toxic metal poisoning (Storelli et al., 2000). Moreover, it exhibits biomagnifications in aquatic food chains among pollutants (Ribeiro et al., 1996). The reported mercury content of food is rather low, about 0.02 pg/g, but there is a high variability in the content depending on the kind of product, its geographic origin, and the agricultural and industrial techniques of the area taken into account (Hugunin & Bradley, 1975).

3. CONSEQUENCES ON PLANTS

Mercury is not essential to living cells and performs no known biological function. It has a strong affinity for sulfur, and mercury’s primary mode of toxic action in living organisms is thought to be the interference of enzyme function...
and protein synthesis by binding to sulfhydryl (SH) groups (Sharma, 1985; Garcia and Reyes, 2001; Patra et al., 2004). Maximum work has been carried out on seed germination and seedling growth of different plant species in fields exposed to mercury (Sharma, 1985; Bonifacio and Montano, 1998; Al-Yemeni, 2001; Umadevi et al., 2009).

Mercury accumulation in plants has been studied in several plant species: pea (Pisum sativum L.), ryegrass (Lolium perenne), spearmint (Mentha spicata L.), Norway spruce (Picea abies L.), spinach (Spinacia oleracea), rice (Oryza sativa L.), and willow (Salix) (Beauford et al., 1977; Al-Attar et al., 1988; Godbold and Huettermann, 1988; Chunilall et al., 2004; Wang and Greger, 2004; Du et al., 2005).

Toxic effects of mercury in plants include abscission of older leaves, growth reduction, decreased, vigor inhibition of root and leaf development, and decreased chlorophyll content and nitrate reductase activity (Vyas and Puranik, 1993). Other adverse effects caused by excessive mercury accumulation include inclusion structure’s integrity disruption (Ma, 1998), mineral nutrient uptake reduction (Cho and Park, 2000; Patra and Sharma, 2000), and photosynthesis and transpiration reduction (Krupa and Baszynski, 1995). Higher concentrations (>1–2 mg/l) of mercury decreased the growth of pea (Beauford et al., 1977), tobacco (Suszynsky and Shann, 1995), tomato (Cho and Park, 2000), and alfalfa (Zhou, 2007). Mercury also inhibited water uptake through aquaporins in plasma membranes of wheat (Zhang and Tyerman, 1999). Inhibition of enzymes of different metabolic pathways has also been reported because of mercury toxicity (Basak et al., 2001; Lenti et al., 2002; Morch et al., 2002; Shaw and Rout, 2002).

Considerable amounts of mercury may be added to agricultural land with fertilizers, lime, and manures. The most important sources of contamination of agricultural soils have been the use of organic forms of mercury as a seed-coat dressing to prevent fungal diseases in seeds. The absorption of organic and inorganic mercury from soil by plants is low, and there is a barrier to mercury translocation from plant roots to shoots. Thus, large increase in mercury levels in soil produce only modest increase in mercury levels in plants by direct uptake from soil (Patra and Sharma, 2000). Higher plants receive inorganic nitrogen mostly in the form of nitrate. The nitrate in plant tissues is reduced to ammonium via nitrite by the sequential action of enzymes.

4. CONSEQUENCES ON ENZYMES

The enzymes glutamine synthetase (GS), glutamate synthase (GOGAT), glutamate dehydrogenase (GDH), and nitrate reductase (NR) are responsible for the biosynthesis of nitrogen-carrying amino acids (Lam, 1996).

Nitrate reductase (NR, EC 1.6.6.1) is a complex enzyme characterized as a SH containing molybdoflavohemoprotein (Hewitt and Nottan, 1979). Nitrate is currently one of the most hazardous pollutants (Awasthi and Rai, 2005). NR is substrate inducible and involves de novo synthesis of the enzyme in response to nitrate (Zielke and Filner, 1971; Somers et al., 1983). Nitrate reduction catalyzed by this enzyme is considered as the rate-limiting step in the overall process of nitrate assimilation pathway (Srivastava, 1980). Supply of inorganic mercury inhibited substantially in vivo as well as in vitro NR activity and endogenous nitrate pool in excised bean leaf segments. Though in vitro specific activity of the enzyme remains unchanged, it has been suggested that mercury has an inhibitory role on NR activity in bean leaf segments (Vyas and Puranik, 1993).

Application of mercury to excised bean leaf segments increased glutamate dehydrogenase (NADH-GDH, EC 1.4.1.3) activity substantially. However, specific activity of the enzyme decreased at lower concentration of mercury and increased to a lesser extent at higher concentration of mercury. Eventually, pronounced increase in mercury activity indicated the possible role of the enzyme under mercury stress (Gupta and Gadre, 2005). Mercury supply increased glutamate synthase [NAD (P) H-GOGAT, EC 1.4.1.14] (Basak et al., 2001) activity. It has been suggested that mercury activates the NADH-GDH enzyme by binding to thiol groups of protein.

In the presence of mercury (HgCl₂) demonstrates increase in NR activity by glutathione (GSH) involves its thiol groups (Vyas and Puranik, 1993).

GDH is found in all higher plants examined and is often present at high levels in senescing and root tissues (Loyolevargas and Jimenez, 1984). One of these alternative pathways is the reaction catalyzed by the mitochondrial NAD(H)-dependent glutamic acid dehydrogenase (GDH; EC 1.4.1.2), which possesses the capacity to assimilate ammonium in vitro utilizing the organic molecule 2-oxoglutarate to synthesize glutamic acid.

This observation led a number of authors to propose that GDH could operate in the direction of ammonium assimilation (Yamaya and Oaks, 1987; Oaks, 1995; Melo-Oliveira et al., 1996), although all the 15N labeling experiments performed in vivo on a variety of plants demonstrated that GDH operates in the direction of glutamic acid deamination (Robinson et al., 1992; Aubert et al., 2001). It was concluded that GDH is involved in the supply of 2-oxoglutarate rather than in the assimilation of ammonium when carbon becomes limiting (Robinson et al., 1992; Aubert et al., 2001; Miélin and Habash, 2002). The physiological role of GDH in the whole-plant context remains speculative given the recent finding that the majority of the GDH protein is located in the mitochondria of companion cells (Dubois et al., 2003). GDH was increased in the mitochondria and appeared in the cytosol of companion cells. Taken together, our results suggest that the enzyme plays a dual role in companion cells, either in the mitochondria when mineral nitrogen availability is low or in the cytosol when ammonium concentration increases above a certain
threshold (Tercé-Laforgue et al., 2004). Inhibition of NADH-GDH by arsenate in excised bean leaf segment (Jot and Gadre, 1995).

Ammonia in higher plants is believed to be assimilated primarily by the glutamine synthetase–glutamate pathway (Miflin and Lea, 1980). The GDH enzyme could operate primarily in the assimilation or reassimilation of ammonium and play a complementary role to the GOGAT cycle (Srivastava and Singh, 1987). Two isozymic forms of GOAT (i.e., Fd-GOAT and NAD(P)H-GOAT) are of common occurrence in the tissues of higher plants and are involved in the assimilation of primary ammonia as well as of photo-respiratory ammonia (Miflin and Lea, 1980). The GS and GOAT cyclic mechanism is largely active when exogenous nitrogen concentrations are limiting, due to the high affinity of GS for ammonium.

This pathway utilizes approximately 15% of the cells’ adenosine triphosphate (ATP) requirement (Reitzer, 2003) for the production of glutamine and its activity is, therefore, strictly regulated at both transcriptional and post-translational levels in order to prevent energy wastage. Thus the enzyme seems to be playing a pivotal role in linking the enzyme activity in plants, but the effect seems to be dependent on the isoform and the plant species analyzed (Puranik and Srivastava, 1994).

5. DETOXIFICATION MECHANISMS

Plants have developed defense mechanisms that ensure tolerance characteristics to cope with the negative effect of toxic metals or metalloids. These mechanisms include chelation, compartmentalization, biotransformation, and cellular repair (Salt et al., 1998).

The toxicity of inorganic Hg forms (e.g., HgCl₂) is at least in part explained by the element’s great affinity for biomolecules containing SH groups (Goyer, 2001). Phytochelatins (PCs) are cysteine-rich polypeptides of general structure [γ(-Glu-Cys)₂₅₋₃₅ Gly], which play an essential role in the detoxification of some heavy metals (cadmium [Cd], copper [Cu], zinc [Zn], mercury [Hg], and lead [Pb]) and metalloids (arsenic) in fungi, plants, nematodes, and other organisms (Grill et al., 1987; Clemens et al., 1999; Cobbett and Goldsborough, 2002; Vivares et al., 2005). The inhibitory effect of heavy metals may be due to the (a) blocking of the supply of reducing equivalents of nitrate reduction, (b) formation of mercurial derivatives of –SH of NR, and (c) synthesis of PCs (Subhadra and Sharma, 2007). The strength of mercury (II) binding to GSH and PC follows the given order: Y Glu-Cys-Gly(y Glu-Cys)₂ Gly(y Glu-Cys)₃ Gly(y Glu-Cys)₄ Gly (Patra and Sharma, 2000).

The transport of both PCs and Cd as its peptide complex, from the cytoplasm into the vacuole (Saltz and Rauser, 1995). In a second detoxification step, the PC–heavy metal complex is transported to the vacuole.

An (ATP-binding cassette)- ABC transporter, Hmt1, accepting low-molecular-weight PC–heavy metal complexes as substrate, has been identified in Schizosaccharomyces pombe (Ortiz et al., 1995), and phytochelatins (PCn) an Mg ATP-dependent transport activity for PC3 and PC3 ± Cd complexes has also been demonstrated in plants (Salt and Rauser, 1995).

The high stability of the PC-Hg multicomplexes (mPC-nHg) seems to be the main reason for the lack of previous Hg-PC characterization studies. A modified method to detect and quantify unbound PC of Hg in plant extracts via high-performance liquid chromatography coupled to electrospray tandem mass spectrometry and inductively coupled plasma mass spectrometry in parallel. Iglesia-Turin (2006) separated PC from Hg by adding the chelating agent sodium 2, 3-dimercaptopropanesulfonate monohydrate. PC2 was observed in plant samples. The best activator tested was Cd followed by Ag, Bi, Pb, Zn, Cu, Hg, and Au cations; these metals also induce PC biosynthesis in vivo in plant cell cultures (Cobbett and Goldsborough, 2002; Vivares et al., 2005). The transport of both PCs and Cd as its peptide complex, from the cytoplasm into the vacuole (Saltz and Rauser, 1995). In a second detoxification step, the PC–heavy metal complex is transported to the vacuole.

Mercury inactivates the GSH enzyme by binding to the thiol (–SH) groups of protein. In addition, it also provides evidence that GSH serves as a precursor for PC, and this was confirmed by using Buthionine sulphoximine (BSO), an inhibitor of γ-glutamyl-cysteine synthetase and a key enzyme of synthesis pathway (Jot and Subhadra, 2010). Studies employing the GSH biosynthetic inhibitor, buthionine sulfoximine, suggested an increase in the level of PCs and maintenance of GSH homeostasis in transgenic plants during exposure to excess zinc as the possible mechanism behind this tolerance (Singla-Pareek et al., 2006). PC plays a key role in protecting macromolecules from damage by free radicals by trapping them in an aqueous phase (Freedman et al., 1989).

When the non-PC-based mechanism of detoxification gets exhausted and free metal ions become available to induce PC synthesis (Schat et al., 2002). Exposure to excess Cu is capable of stress induction, in which the role of oxidative stress and reactive oxygen species (ROS) production may be involved (Stadtman and Oliver, 1991, Waldemar et al., 1994). On the other hand, under Cu toxicity, excess copper is an efficient generator of ROS in Fenton-type reactions, leading to disturbances in metabolic pathways and macromolecule damages (Hegedus et al., 2001).

The oxidative stress is bound up with the increased metal accumulation in plants and decreased efficiency of the ascorbate–GSH cycle under the metal stress (Wang et al., 2009). Exposure to toxic metals also induces plants to accumulate high amounts of proline (Štefl and Vašáková, 1982). Increased accumulation of proline leads to the increase of glutamate kinase activity and creates a possibility for an increase in glutamic acid content due to the synthesis of GSH and PCs in plant cells (Pavlíková et al., 2007). An increase of free proline inhibits biosynthesis of its excessive amounts

HATASO
in plants under heavy metal excess, and this results in the preferred utilization of glutamate for the metabolic route leading to PC synthesis (Pavliková et al., 2008).

To control the level and effects of ROS, cells have developed various antioxidant defenses, including antioxidant enzymes and low-molecular-mass radical scavengers. They regenerate the active form of antioxidants and eliminate or reduce the damage caused by ROS (Alschere et al., 1997). An increase or decline in the activity of antioxidants is a direct indication of the adaptive response of plants to avoid metal toxicity (Srivastava et al., 2004).

Antioxidants are important substances that possess the ability of protecting organisms from damages caused by free radical-induced oxidative stress (Canadianovic-Brunet et al., 2005). The antioxidative system includes both enzymatic and nonenzymatic systems.

The nonenzymatic system includes ascorbic acid (vitamin C), α-tocopherol, carotenoids, and so forth, and the enzymatic system include superoxide dismutase (SOD), catalase (CAT), peroxidase (POX), ascorbate peroxidase (APX), glutathione reductase (GR), and polyphenol oxidase (PPO). SOD, the first major enzyme found in all aerobes, catalyzes dismutation of superoxide anion to $\text{H}_2\text{O}_2$ and molecular oxygen. Glutathione peroxidase (GSH-POX) protects membrane lipids from oxidative damage and detoxifies organic peroxides; it can also act on organic hydroperoxides (Kantol et al., 1998). The intracellular level of $\text{H}_2\text{O}_2$ is regulated by a wide range of enzymes, the most important being CAT and peroxidase. CAT inactivates $\text{H}_2\text{O}_2$ to oxygen and water (Rusina et al., 2004).

Ali et al. (2010) demonstrated that the enhanced production of SOD, POD, and CAT may serve as useful biomarkers for Cu tolerance in Carthamus tinctorius. Free radicals and intermediate products of peroxidation are capable of damaging the integrity and altering the function of biomembranes, which can lead to the development of many pathological processes (Gutteridge, 1993). Various specific enzymes that limit free-radical formation, such as SOD, CAT, and GSH-POX, play an important role in the protection of cell membranes against oxidative damage (Faix et al., 2003).

Reactive oxygen intermediates (ROI: $\text{H}_2\text{O}_2$, $\text{O}^•−$, $\text{OH}^−$, and $\text{O}_2^•−$) ROI damages a number of cellular targets, including DNA, resulting in genotoxic stress, leading to mutation, genomic instability, or apoptosis (Achary and Panda, 2010).

Mostly heavy metals at excess concentrations damage plant tissues by producing free-radical oxygen species or directly interacting with the DNA, and thus their accumulation within the cell causes deleterious effects (Al-Qurainy, 2009). The plant Cleome gynandra was exposed to the sublethal and half of sublethal concentration of cadmium and copper metal contaminated soils; it was clearly indicated that C. gynandra has the highest values of total phenolics, percent antioxidant activity, and enzymatic antioxidants, which help the plant to cope with oxidative stress (Haribabu and Sudha, 2011).

An enhancement of the amount of phenolic compounds can be observed under different environmental factors and stress conditions (Sakihama & Yamasaki, 2002). An increase of phenolics correlated to the increase in activity of enzymes involved in the metabolism of phenolic compounds was reported (Michalak, 2006), suggesting the synthesis of phenolics under heavy metal stress. Phenolics are generally thought to prevent oxidative damage by scavenging active oxygen species and by breaking the radical chain reactions during lipid peroxidation; these antioxidative effects require the reduced form of phenolics, in the oxidized form act as prooxidants (Sakihama and Yamasaki, 2002). The antioxidative activity of phenolics is mainly due to their redox properties, which allow them to act as reducing agents, hydrogen donors, singlet oxygen quenchers, and metal chelators (Sakihama et al., 2002). Studies indicate that increasing levels of lead treatment markedly increased the phenolic content of Phaseolus vulgaris (Hamid et al., 2010).

Phytoremediation defines the use of plants to extract, sequester, and/or detoxify various kinds of environmental pollutants (Salt et al., 1998). The physicochemical techniques for soil remediation render the land useless for plant growth as they remove all biological activities, including useful microbes, such as nitrogen-fixing bacteria, mycorrhiza, and fungi, as well as fauna in the process of decontamination (Burns et al., 1996). The most commonly used methods for dealing with heavy metal pollution are still extremely costly.

Phytoremediation is the use of plants to extract, sequester, and/or detoxify pollutants and is a new and powerful technique for environmental clean-up. The mechanism(s) of heavy metal effect on nitrate assimilation has not been worked out so far.

5.1. Selenium
Selenium (Se) is an essential micronutrient and trace metal. Plants require little amount, and when it exceeds the limit, it causes toxicity in humans and plants (Ellis and Salt, 2003; Sager, 2006).

Pyrzynska (2009) provides data on selenium speciation in enriched vegetables. Metabolic importance of selenium for plants and in higher plants described by Germ et al. (2007) and Terry et al. (2000).

5.1.1. Selenium Toxicity
The toxicity of selenium in wheat and buckwheat plants was proportional to the concentration added as sodium selenite to soil cultures and solution cultures studied by Martin (1936).

The toxicity of selenate ($\text{SeO}_4^{2−}$) and selenite ($\text{SeO}_3^{−}$) to most plants can be attributed to a combination of three factors. First, selenate and selenite are readily absorbed from the soil by roots and translocated to other parts of the plant. Second, metabolic reactions convert these anions into organic forms of selenium. Third, organic selenium
metabolites, which act as analogues of essential sulfur compounds, interfere with cellular biochemical reactions Brown and Shrift (1982).

5.1.2. Selenium Antagonistic Effect
Dietary selenium (Se) status is inversely related to vulnerability to methylmercury (MeHg) toxicity (Ralston and Raymond, 2010).

According to the authors, Se has been shown to counteract the toxicity of heavy metals, such as cadmium, inorganic mercury, methylmercury, thallium, and, to a limited extent, silver. Vitamin E is very effective against lead toxicity but Se has little effect. The presumed protective effect of Se against cadmium and mercury toxicity is through the diversion in its binding from low-molecular-weight proteins to higher-molecular-weight ones (Whanger, 1992).

Exploring the structural basis for selenium/metal antagonism in *Allium fistulosum* studied by McNear et al. (2012). The type of interaction of selenium and mercury and selenium and copper on the cell growth of the planktonic alga *Dunaliella minuta* Lerche had been studied by Gotsis (1982). An antagonism study in *Glycine max* (soybean) roots by size exclusion and reversed phase HPLC-ICPMS. The result shows that the Se distribution pattern was found to be unaffected by the presence of Hg, but the amount of Se assimilated was found to be higher in plants co-exposed to Hg (Yathavakilla and Caruso, 2007).

6. CONCLUSION
Mercury pollution is all over the world. Despite above all known facts, there are many unknown facts. It is important to know the uptake mechanism, target and the extent to which it affects flora and fauna. In this review, findings support that mercury causes inhibition of enzyme activity namely nitrate, GDH and GOGAT etc. as previous results show that mercury causes toxicity in plants. Natural heavy metal accumulation could be a potential source for genetic manipulation of important agricultural crop plants.

Author Contributions
The tasks and responsibilities of this work were equally shared by all the authors.

Acknowledgment
Sincerely obliged to Dr. Jot Sharma (supervisor) and Dr. Archana Shrivastava (cosupervisor) for their encouragement and providing necessary administrative and research facility.

References


Godbold DL, Huettermann A (1988). Effect of zinc, cadmium and Hg on root elongation Picea (Karst) seedlings and the significance of these metals to forest die-back. Environmental Pollution, 53: 75-381.


HATASO


Tercé-Laforgue T, Dubois F, Ferrario-Méry S, et al. (December 2004). Glutamate dehydrogenase of tobacco is mainly induced in the cytosol of phloem companion cells when ammonia is provided either externally or released during photorespiration. Plant Physiology, 136: 4308-4317.


Citation: Shrivastava S, Shrivastava A, Sharma J (2015). Detoxicification Mechanisms of Mercury Toxicity in Plants: A Review. Recent Advances in Biology and Medicine, 1: 60-68.