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Date Received:  
14/01/2015;  
Date Revised:  
23/02/2015;  
Date Published:  
25/02/2015:

# Phytotoxicity of Hg and its Detoxification through Microorganisms in Soil

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**How to Cite:**

Amin A, Latif Z.

Phytotoxicity of Hg and  
its Detoxification  
through  
Microorganisms in Soil  
(2015). Adv. Life Sci.  
2(2). pp: 98-105

**Abstract:**

Due to the advent of industrialization, pollution of terrestrial environment by heavy metals has emerged as a great issue. Therefore, it is an urgent need to realize the Hg-induced toxicity in plants and as well as in animals and the harmful effects by the consumption of contaminated nutrition. Mercury is considered as a hazardous contaminant that can be changed into various oxidation states easily and causes many deleterious effects in several physiological processes in both plants and animals. Microorganisms possess two extensively studied Hg-detoxification processes like *Mer* operon (*merT*PCFAD) and *Met* gene which encode the functional proteins for transportation (*merT*, *merP* and/or *merC*, *merF*), reduction (*merA*) and a secondary regulatory protein (*merD*) and sulfhydrylase enzymes (*met* gene) respectively to modify toxic Hg<sup>+2</sup> to nontoxic elemental state (Hg<sup>0</sup>). Due to the ever increase in Hg-pollution and very little information about its phytotoxic effects and detoxification mechanisms, the authors expect, the present article will make possibility in the provision of a comprehensive literature study about Hg-induced toxicity in plants and its detoxification processes to provoke for advance research in this field.



## Introduction

Mercury (Hg), also known as hydrargyrum or quicksilver is a group IIB element with an atomic weight of 200.59. Its property to remain liquid at room temperature and normal pressure makes it a unique metal. It rarely occurs in pure state in nature but is found most commonly as the ore cinnabar (HgS) [1]. Earlier records indicate that mercury is used in alchemy in China as early as the second century B.C and references to cinnabar mines and the medicinal use of mercury were made by Pliny the Elder in the first century. On average, the level of mercury in earth crust is around ~0.5 ppm but large deposits of Hg are found in the areas of volcanic activity. It has been released into the lithosphere, atmosphere and hydrosphere by geochemical process and therefore it is an important toxic element in the biosphere [2, 3]. Mercury tends to be found in three oxidation states as a Hg<sup>0</sup>(elemental or metallic), Hg<sub>2</sub><sup>2+</sup> (mercurous) and Hg<sup>2+</sup> (mercuric ion) [4]. Metallic mercury is relatively nontoxic as compared to oxidized states of Hg due to its low solubility, but it can be modified to highly toxic oxidized forms *in vivo* by catalase and peroxidase enzymes. The level to which mercury can prove hazardous strongly relies in its oxidation state. Different forms of mercury in chemical terms have been classified [5, 6] as

Volatile species	Hg <sup>0</sup> and (CH <sub>3</sub> ) <sub>2</sub> Hg
Reactive species	Hg <sup>+2</sup> , HgX <sup>+</sup> , HgX <sub>2</sub> <sup>-</sup> , HgX <sub>3</sub> <sup>-</sup> , HgX <sub>4</sub> <sup>2-</sup> (where X= OH, Cl <sup>-</sup> or Br <sup>-</sup> ), HgO on aerosol particles and complexes of Hg with organic acids.
Nonreactive species	CH <sub>3</sub> Hg <sup>+</sup> , CH <sub>3</sub> HgCl, CH <sub>3</sub> HgOH and other (HgCN) <sub>2</sub> , HgS and organomercurials.

The highly toxic forms of mercury are the reactive inorganic mercury ions (Hg<sup>+2</sup>) that possess great affinity for cysteine residues of proteins and to N-atoms of nuclear material of the cell and the organomercurial monomethylmercury (CH<sub>3</sub>Hg<sup>+</sup>; MMHg) which have high affinity for tissues of central nervous system, high lipid solubility, high uptake rate across biological membranes and longer residence/removal time in biological tissues [7]. The characteristics of MMHg contribute significantly to bioaccumulation and biomagnification in mercury toxicity. Bioaccumulation is defined as the increase in the total amount of Hg in an organism over time while biomagnification of Hg

(incremental increase of mercury concentration at each trophic level of the food chain) is as the increase in mercury concentrations in tissue through trophic transfer, from primary producer to terminal carnivorous consumers via food chain or food web [8]. MMHg is evenly distributed in body tissues while inorganic mercury is unevenly distributed because of its less efficiency than MMHg in crossing biological membranes [9]. Mercury uptake, distribution, and toxicity are therefore dependent on the speciation and transformations of mercury.

## Methods

### Literature search strategy and selection criteria

A peer-reviewed literature search was carried out by use of the key terms like “mercury toxicity”, “phytotoxicity”, “Hg-oxidation states”, “biogeochemical cycling of mercury”, “mercury detoxification systems”, “*mer* operon” and “*met* gene” from Google Scholar, Google Web Browser, PubMed Central, Springer Online Archives Collection and PubMed for this review. The retrieval of the search was done without applying any filter to limit the study type. Articles resulting from these searches and relevant references for this review were selected published from August, 1939 to December, 2014. Articles published in various languages like English, French, and German were also included. Some references are not falling in the above mentioned key terms due to their significant data or relevance to the specificity of this topic. In this comprehensive review, 72 peer reviewed research and review articles were selected.

## Discussion

### The Biogeochemical Cycle of Mercury

Mercury occurs naturally in biogeochemical system of earth, but centuries of anthropogenic activities [10] like mining and fossil fuel burning [11], have been mobilizing increasing amounts of mercury in the atmosphere [12-14], terrestrial environment [15] and aquatic systems [16-18]. Mercury can be transformed to different states by oxidation-reduction and methylation-demethylation processes. In redox reactions, the process of oxidation causes the mercury to lose electrons by changing its valency from Hg<sup>0</sup> to Hg<sup>2+</sup> while reduction causes the mercury to gain electrons by transforming to lower valence state [19-24].

The oxidation of mercury (Hg<sup>0</sup>) in the atmosphere is a significant phenomenon due its involvement in the

### Keywords:

Hg-oxidation states, Biogeochemical cycling of Hg, Hg-phytotoxicity, Hg-detoxification, *Met* gene, *Mer* operon

dethronement of mercury in both soil and water. Metallic mercury ( $Hg^0$ ) can be volatilized easily into vapors and discharge to the atmosphere where they can be transmitted in air currents for a year or more and again accumulate into the environment to initiate another cycle [25,26]. In contrast, toxic mercuric state ( $Hg^{2+}$ ) can remain in the atmosphere for less than two weeks because of its low tendency to evaporate, greater solubility and reactive nature. Therefore, when elemental mercury becomes ionic mercury, it tends to quickly infuse in water as rain and snow and consequently accumulate back in the environment [27,28].

In nature, mercury changes to methylmercury upon acquisition of the methyl group ( $CH_3$ ). This process ends up producing extremely lethal compounds including methylmercury ( $MeHg^+$ ) that tends to accumulate in living cells and pass all along the food chain, from smaller organisms i.e., microbes, then to aquatic organisms i.e., fish and ultimately to humans [29-32] (Figure 1).

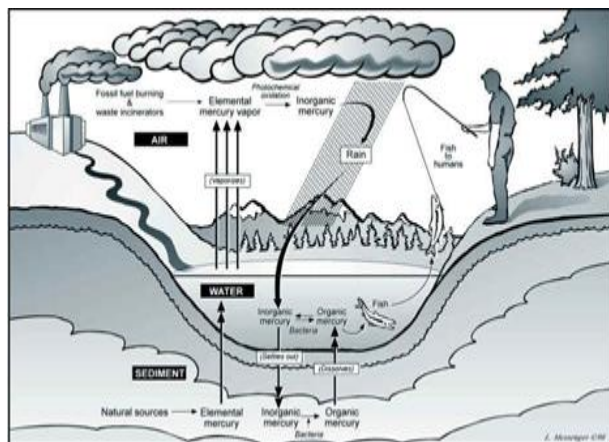


Figure 1: The global biogeochemical cycling of mercury [33].

### Uptake of Hg by Plants

Mercury toxicity has become an ever increasing challenge as a consequence of global heavy metal contamination. Considerable amount of mercury is being added to agricultural soil due to the usage of sewage sludge, chemical fertilizers, lime and manures [34-36]. The dynamic association between mercury in the soil and its absorption by the plants is not collinear and depends on many variable factors like cation-anion interchange capability, pH of soil, soil aeration and plant varieties. With the increase in pH of soil and in the

vicinity of lime and salts, the mercury absorption can be decreased [37, 38].

It has been reported that the absorption of Hg is specific to the bryophyte plants, lichens, mycorrhizae, wetland plants, tracheophyte plants and crop vegetation [39]. Various aspects that affects the absorption of Hg by plants include the organic matter of soil or sediments, the interchange capability of various atoms and molecules like carbon, oxides and carbonate content, redox potential, the formulation used and the total metal content. Generally, the absorption of mercury by plants could be directly correlated to the contamination level. The absorption of Hg in most of plants is due to its accumulation in the plant roots or its absorption through shoots in the form of vapors or by translocation [40, 41]. It is considered that plants absorb the elemental form ( $Hg^0$ ) and accumulate it in the shoots, but not translocated to the roots [42].

Toxic mercuric ions are considered to get entry into plant cells by same recruitment mechanisms as essential nutrients compete with these ions for uptake. Mercury (Hg), categorized as class B metal prefers to bind with sulfur and nitrogenous ligands and is considered to get entry into the cell through ion channels competing with other toxic and essential metals. Nonetheless, this knowledge is primarily a result of experiments in animal cells and the authors believe that there are some other mechanisms of mercury absorption that are still under consideration [43, 44].

### Phytotoxicity Induced by Hg

**General Effects:** The introduction of mercury in plant systems has principle importance due to its application in fertilizers, herbicides and seed disinfectants [45]. Few mercury species are being used on tree foliage as fungicides and they can be transferred, relocated and redistributed in plants.

At the cellular and subcellular level, the processes by which metals may prove lethal include obstruction of biologically significant molecules (e.g. enzymes, polynucleotides), transportation systems for micronutrients, displacement or substitution of metal ions from biomolecules (such as magnesium ( $Mg^{+2}$ ) from chlorophyll), deforming and inactivating enzymatic proteins, and compromising cell membrane integrity. The possible causal processes causing Hg-

induced phytotoxicity are modifications in the porosity of the outer cell envelop (cell membrane), high affinity for reactive groups like sulphhydryl, phosphate, adenosine diphosphate or adenosine triphosphate, and displacement of essential ions and its capability in the disruption of several functions involving critical proteins [37, 38].

Toxic mercuric ions also disrupt the antioxidant defense mechanism by altering the modulation of non-protein thiols, non-enzymatic antioxidants glutathione, ascorbate peroxidase and glutathione reductase and the antioxidant enzyme superoxide dismutase [46-49].

The evidence of mercury phytotoxicity has been studied in various grain crops like *Oryza sativa* and *Triticum aestivum*. The primary effects of Hg compounds are on the embryo and secondary on endosperm. Hg compounds cause the breakdown of -SH- system by interfering it in biological systems resulting in the production of -S-Hg-S- bridge which may influence germination and embryo development (rich in SH ligands). In *O. sativa* and *Zea mays*, HgCl<sub>2</sub> is involved in the obstruction of primary roots elongation as compared to shoots [37, 38].

Hg influences both light and dark reactions of photosynthesis by substituting the central atom of chlorophyll (Mg<sup>+2</sup>) by Hg *in vivo* which is an important damaging mechanism. It also reduces the transpiration rate, water uptake and chlorophyll synthesis. Toxic mercuric cations are involved in the loss of magnesium, potassium, manganese and deposition of iron which lead to the modifications in cell membrane porosity [50].

**Genotoxicity:** The cellular and molecular mechanisms that are involved in Hg-induced toxicity in plants are practically unknown due to scarce studies considering Hg genotoxicity. However, it has been shown that mercury can insert harmful genetic effects to different plant species [51].

In earlier experiments, multinucleated cells in the root tips of corn seedlings, exposed to solution of Ceresan (ethyl mercuric phosphate; a fungicide) resulted in the formation of polyploidy, aneuploidy and c-tumors through c-mitosis [52,53]. C-mitosis (colchicine treated), sister chromatid exchanges, chromosomal aberrations and spindle alterations can be stimulated by

several compounds at similar dosage but butyl mercury bromide is most notable in this respect [54]. It has been reported that inorganic mercury poisoning in *Allium cepa* (onion) and *Allium sativum* (garlic) resulted in reduction of mitotic index in the cells of root tip and an increment in chromosomal aberrations that depend on concentration and time of exposure. Mercuric chloride (HgCl<sub>2</sub>) was concluded as more cytotoxic as compared to mercurous chloride (Hg<sub>2</sub>Cl<sub>2</sub>) and lowest effective concentration tested (LECT) was measured as 10 ppm. The greater tolerance of *A. sativum* than *A. cepa* was attributed to the presence of high levels of heterochromatin in the former and low amount of sulfur in the later [37, 38].

### Detoxification of Hg in Soil through Microorganisms

Release of Hg from natural sources (volcanic activity and weathering of rocks) [55-57] and anthropogenic sources (fossil fuel combustions, electricity-generating grid stations, gold and mercury mining, production of chlorine, cement, caustic soda, pesticides, medical instruments, mirrors and industrial effluents etc) [58,59] poses a major menace to the soil environment [60]. Generally, heavy metals cannot be degraded by biological mechanisms and exist in the environment to an indefinite extent. After their accumulation in the soils, the lethal heavy metals adversely influence the soil microflora, including plant growth promoting rhizobacteria (PGPR) in the rhizosphere, and their physiological processes. Furthermore, the elevated concentrations of Hg and their uptake by plants also pose adverse affects on the plant growth [61], symbiotic association and ultimately the crop yields by disrupting cell organelles, and disintegrating the membranes, serving as genotoxic substance disrupting the photosynthetic and respiration processes [41,62]. Therefore, the remediation of Hg-polluted sites has become an urgent need, as these lands have covered large areas which have been interpreted inapplicable for sustainable agriculture.

Two extensively studied resistance or detoxification systems based on clustered genes on *Mer* operon and *Met* gene. The *mer* operons (*merTPCFAD*) possess variable structures and constitute a number of genes which encode various functional proteins. In *Staphylococcus aureus* and *Bacillus* sp., the *merR* genes

which are involved in the expression of functional proteins for metalloregulation, are transcribed in that direction as the structural genes of *mer* operon whereas in other species, it is transcribed separately and divergently from the structural genes. *merR* binds the promoter-operator site of *mer* operon and activates/represses the transcription of structural genes in the presence/absence of activating concentration of  $Hg^{2+}$  respectively [63,64]. The structural *mer* genes are involved in the expression of proteins which aid in the transportation like *merT*, *merP* and *merC*, *merF* and reduction (*merA*) of toxic form of mercury. *merB* genes confer resistance to many organomercurials by hydrolyzing the C-Hg bond [65]. The distal promoter gene, *merD* binds weakly the same operator-promoter site as and is involved in down-regulation of the *mer* operon [66].

The other mercury detoxification system is the expression of *met* gene which encodes sulfhydrylase (SHLase) enzymes. This enzyme regulates the methionine biosynthesis and results hydrogen sulfide ( $H_2S$ ) production.  $H_2S$  reacts with toxic form of mercury ( $Hg^{2+}$ ) and precipitate it into nontoxic mercuric sulfide ( $HgS$ ) [67-69]. Detoxification mechanisms that employ different microbes to take off environmental contaminants have obtained a profound interest in the recent years [70]. The commonly used bacterial and yeast genera in the bioremediation of Hg include *Bacillus*, *Pseudomonas*, *Citrobacter*, *Klebsiella*, *Rhodobacter* and *Saccharomyces*, *Candida* and *Pichia* respectively [37, 38, 71, 72].

Thus, by applying these microorganisms as a biofertilizers to Hg-contaminated soils, the toxicity of Hg can be reduced resulting in the enhancement of soil fertility and crop productivity which aids in sustainable agriculture.

### Conclusion

In conclusion, mercury is a hazardous contaminant associated with serious problems in plants and animals because it can be easily spread through many ecosystems. Unfortunately, very less information is available about phytotoxicity caused by Hg, processes by which Hg is absorbed by plant cells and detoxification mechanisms by which it is modified from toxic to nontoxic form in soil through microorganisms.

Although plants attribute a significant role as the base of several trophic levels in food chain particularly of humankind subsistence and thriftiness, therefore, it is an urgent necessity to step up the knowledge about the mechanisms of Hg uptake by plants, its phytotoxicity and detoxification mechanisms of this pollutant. The mini review presented here will provide a worthy route for other scientists engaged to research on Hg-induced phytotoxicity and its modification or detoxification processes to stimulate foster research in this field.

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