

## Review Article

# Phytotoxicity of Mercury in Plants: A Review

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With the advent of the industrial revolution, heavy metal contamination has become an ever increasing concern. Thus, it is of major importance to understand the extent of the toxicity in plants and animals and the consequences from the ingestion of contaminated food. Hg is easily modified into several oxidation states, and it can be spread in many ecosystems. Due to the recurrence of Hg pollution and due to the lack of knowledge about the effects of this heavy metal in plants, the aim of the present text is to provide a comprehensive review of the literature regarding Hg phytotoxicity.

## 1. Heavy Metal Pollution: Environmental Issue

Agriculture had a major impact in humans, being the major force behind the passage from a hunter-gatherer/forager society to a sedentary one, becoming a crucial tool for human sustainability and the development of economics worldwide. However, the passage to a sedentary and ever developing society was accompanied by a drastic transformation of the environment and the inherent exposure to new risks caused directly or indirectly by those transformations [1].

One of the many new risks that might have affected those early societies was the continuous exposure to pollutants, namely, heavy metals. The first cases of human exposure to heavy metals, beside the naturally occurring phenomena from Earth processes (e.g., volcanoes and rock constituents), were soon reinforced by the inadvertently discharge of these elements to the environment by human activities (e.g., from ore mining and smelting) [2, 3]. Since then, and with the advent of the industrial revolution, heavy metal contamination has become an ever increasing concern [4, 5]. Moreover, due to their stability and as they cannot be degraded, heavy metals tend to accumulate easily spread in a wide variety of ecosystems [6]. For most of the heavy metals, contamination arises from industrial applications, mining, smelters, combustion of fuel, and byproducts. From these sources, contaminants can be present in the ecosystem as airborne

particles, wastewaters, and sludge [7], polluting not only sites near the source but also locations thousands of kilometers apart.

Within the many occurrences of ecosystems' pollution provoked by heavy metals, it easily comes to mind the magnitude and reach of the Minamata disaster (1950), which caught the world unaware of the damage that long exposure to heavy metals can induce in organisms. Mercury (Hg) was the main pollutant, and the subsequent associated diseases caused 2,265 casualties just from direct exposure to Hg [8].

The uncontrolled release of highly polluting substances from mining wastes (e.g., cyanide, arsenic, Hg, and sulphurs) in the region of Oruro (Bolivia) transformed a unique ecosystem in a desert. Nearly 53,000 inhabitants suffer from water pollution, the salinization, and desertification of thousands of acres of land, beside the diseases and death (human and livestock) associated to exposure to these pollutants [9].

In the mid 80's, water used to extinguish a major fire carrying 30 tonnes of a fungicide containing Hg was used in the Upper Rhine. The substantial increase in Hg levels lead to massive fish death in an area over 100 km [10].

In 1998, a nature reserve in Spain was contaminated after the rupture of a dam, releasing sludge and contaminated wastewater. The wastewater entered the Guadamar River, polluting the river with heavy metals such as cadmium, lead, zinc, and copper. It affected an area of 4.634 hectares,

contaminating 2.703 hectares with sludge and 1.931 with acidic water. Experts estimated that Europe's largest bird sanctuary, as well as Spain's agriculture and fisheries, would suffer permanent damage from the pollution that was accumulated in this ecosystems (DPPR/SEI/BARPI—collapse of the dam of a tailings pond).

Due to the critical risks associated with metal toxicity to human health and environment [11, 12], scientists have been studying the effects of heavy metals with more emphasis on the bioremediation/chelation of the metal ions by organisms [13] and the severe effects in humans [3].

Among the different models available to study heavy metal toxicity, plants present some unique features that make them interesting subjects for this type of assays. Firstly, as primary producers of the food chain, understanding the toxic effect of this metal in the plant status as well as the risks of biomagnifications of these toxicants for consumers is highly important. Also, because plants lack the ability to escape from contaminated sites, these organisms evolved mechanisms to handle exposure to toxicants [14]. For instance, certain species can regulate the amount of pollutants which are taken from the surroundings, resort to sequestration and inactivation in subcellular compartments [15], or tolerate the deleterious effects of heavy metals. Baker 1981 [16] proposed the classification of plants, according to their capability to accumulate toxicants, in three categories: excluders, accumulators, and indicators. The excluders are all of those that can grow in contaminated soils while maintaining the concentration of toxicants at low levels, when compared to the concentrations in the soil. The accumulators are those species that can survive despite concentrating contaminants at high doses in the aerial portion. Plants considered to be indicators can regulate the uptake and transport of the pollutant to the aerial part, being that the internal concentration is often similar to the one observed in the soil's parent soil [17].

Among the heavy metals that have been deemed of highest concern by the European Union, some have been the target of many investigations (e.g., Cd) while, for others, the level of understanding about the mechanism and extent of their phytotoxicity (e.g., Hg) is insufficient.

The toxicity of metals and their compounds largely depends on their bioavailability, that is, the mechanisms of uptake through cell membranes, intracellular distribution, and binding to cellular macromolecules [18]. From the literature available, it is generally agreed that the mechanistic evolving the toxicity of heavy metals is originated by a complex pattern of interactions between cellular macromolecules and the metal ions. The entrance of the metal in the cell can mobilize several metabolic and signal transduction pathways and genetic processes to neutralize the source of toxicity [18]. Although the relative toxicity of different metals to plants can vary with the genotype and experimental conditions, most heavy metals act through one of the following: changes in the permeability of the cell 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 [13, 19].

Considerable attention has therefore been paid to understanding the structural, kinetic, and thermodynamic details

of these interactions in a number of laboratories, as a key requirement for unraveling and discussing the mechanisms of action and physiological roles of metal ions in living systems (e.g., [20, 21]).

In the case of Hg, it is known that this metal is easily modified into several oxidation states and it can also be spread through many ecosystems [22, 23]. These modifications can be resumed in two major cycles, one occurring in a global extent which involves the circulation of Hg as elemental Hg ( $Hg^0$ ) in the atmosphere. The other, with a low range, depends on the intervention of organisms that can methylate the inorganic Hg into organic Hg compounds, being the latter the most toxic for the living organisms [22].

Due to the recurrence of Hg pollution and also because of the lack of knowledge about the effects of this heavy metal in plants, it is urgent to evaluate and understand the extent of Hg-induced phytotoxicity. In the past, management and regulatory responses to the problem of bioaccumulation generally have been constrained by a lack of information on sources, methods of transport, chemical interaction, and biological significance of Hg in the environment. To comprehend the extent of the toxicity and the proprieties that make this heavy metal so interesting to study, the following sections of the introduction will be dedicated to elucidate the reader about the Hg, mainly, the inorganic form ( $Hg^{2+}$ ), which is the predominant form in agricultural soils and thus the most interesting for this paper.

## 2. Chemical Forms of Hg

Among metals, Hg is unique in that it is found in the environment in several physical and chemical forms: for example, elemental Hg ( $Hg^0$ ), inorganic Hg ( $Hg^{2+}$ ), associated with ions (SHg, ClHg<sub>2</sub>), mercurous chloride or calomel ( $Hg_2Cl_2$ ), and organic Hg (e.g.,  $CH_3$ -Hg) [8, 23, 24].

Hg is a metal with different and singular proprieties than those of the others transition metals, being the only one that, at room conditions, exists in liquid form. It is a good electric conductor, being applied in many technological areas, such as informatics, production of batteries, and light bulbs.

The high solubility in water and easiness with which Hg shifts to the gaseous phase [23] are two of the most important properties of this heavy metal. These proprieties explain the ability and effectiveness of Hg to move in several ecosystems and remain in the atmosphere for long periods, being later on deposited in the soil or water bodies [25].

Hg can form salts with oxygen, sulphur, chlorine, and amalgams (alloys) with most metals, except iron and platinum. The inorganic forms of Hg can include the vapour Hg and the liquid form and mercuric Hg. The liquid is volatile and releases a monatomic gas usually referred to as Hg vapour. This species plays a key role in the global cycling of the heavy metal because it can exist as a cation with an oxidation state of 1+ (mercurous) or 2+ (mercuric) [22, 23].

The first oxidation state ( $Hg^+$ ) is the mercurous Hg form, and it is encountered in the form of calomel and mercurous chloride ( $Hg_2Cl_2$ ). Like shown in Figure 1, mercuric mercury ( $Hg^{2+}$ ) forms the divalent state of the Hg cycle, and it is

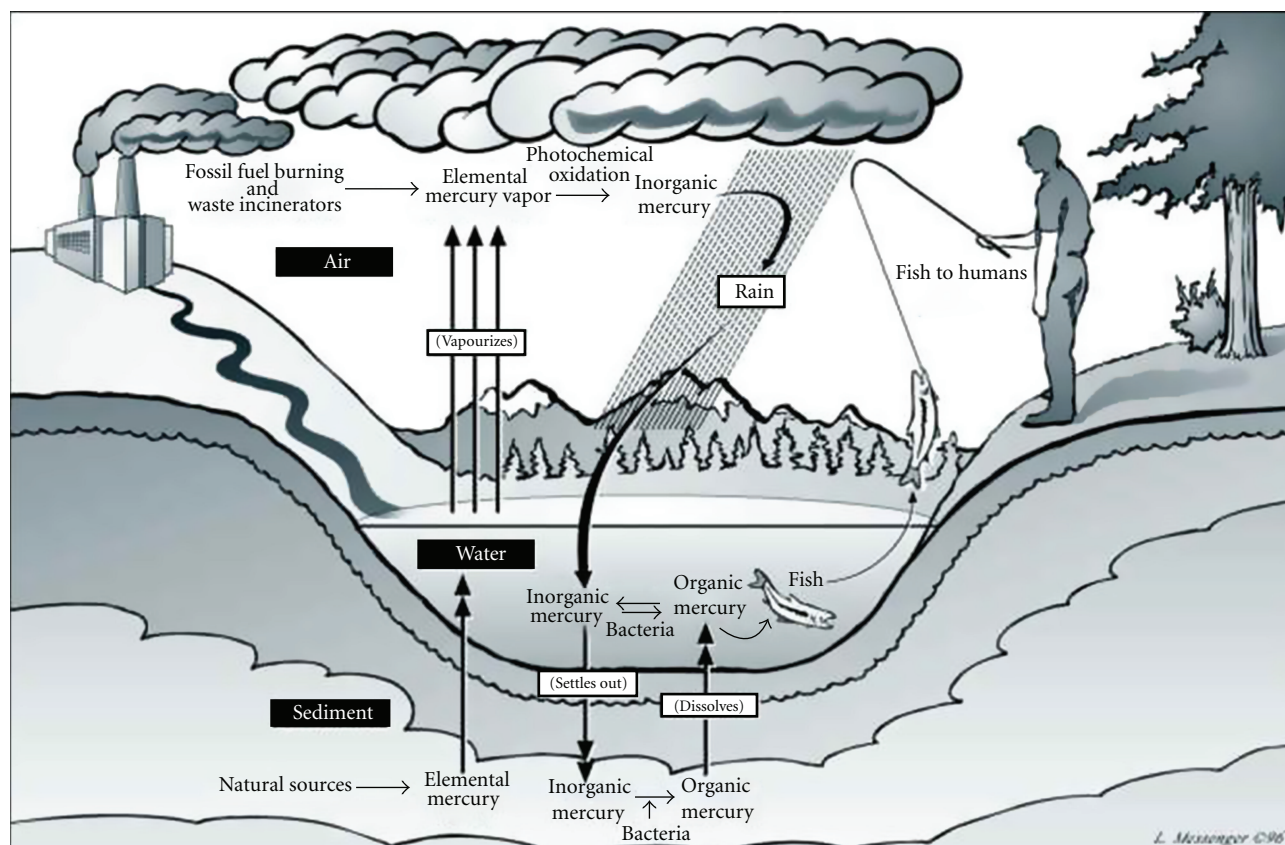


FIGURE 1: The Biogeochemical cycle of Hg. Briefly, it starts with the evaporation of Hg from natural and anthropogenic sources, which is then oxidized to inorganic Hg. This element is spread by the rain. Once in the soil, Hg can be transformed into organic compounds by bacteria (taken from [http://www.mercury.utah.gov/atmospheric\\_transport.htm](http://www.mercury.utah.gov/atmospheric_transport.htm)).

responsible for the formation of practically all the inorganic and organic form encountered in the environment and organisms.  $\text{Hg}^{2+}$  is a product of the metabolism of vapour Hg as well as, from the organic compounds of Hg. Due to these proprieties, this form plays a key role in the cycle of Hg and in the toxicology of this heavy metal in living organisms [23]. High levels of this form have strong phytotoxic effects; when present in toxic concentrations can induce visible injuries and physiological disorders in plant cells triggering the production of ROS (reactive oxygen species) leading to cellular disruption. Mercuric Hg has high affinity for thiol groups, special for the anions  $\text{R-S}^-$ . The easiness of the movement between thiol groups and in entering the cells is due to the high speed with which the reversible reactions between Hg's ionic forms occur. When mercuric Hg is in the form of water-soluble salts, as Hg chloride, it can be a highly potent poison. As a matter of fact, legends tell that the Chinese emperor Qin Shi Huang, the first to unify China, died after ingesting Hg pills concocted by his physicians in an attempt to obtain immortality.

Studies regarding Hg toxicity are mostly from animals and humans, being known that it is linked to autoimmune diseases [26]. The toxicity of inorganic Hg forms (e.g.,  $\text{HgCl}_2$ ) is at least in part explained by the element's great affinity for biomolecules containing sulfhydryl (SH) groups

[27] and by a lower affinity for carboxyl, amide, and amine groups [27]. Inorganic Hg arrives to the ecosystems as the result of the Hg cycling, and in the presence of the right conditions  $\text{Hg}^{2+}$  can be transformed in organic Hg forms.

Some organisms, like bacteria or fungus, can naturally modify the Hg available in the environment ( $\text{Hg}^{2+}$ ) by methylation of this ion, making the final product more dangerous and toxic than its precursor [5, 22, 23]. The more common organic forms are methyl-Hg ( $\text{CH}_3\text{-Hg}^+$ ) and ethyl-Hg, which despite the chemical differences have similar proprieties. Methyl-Hg is the most toxic of the organo-compounds [23, 28]. It is produced by biomethylation and it can be incorporated into trophic chains, mostly in the aquatic ones. The intact organomercurial cation is believed to be the toxic agent responsible for the damage provoked in cells; for instance, in humans, Hg can be modified to methyl-Hg that is capable of causing damage to the nervous system, liver, and ultimately cause death by multiorgan failure [29].

### 3. Ancient and Modern Applications of Hg

Hg and its compounds have been used by mankind since ancient recorded civilizations. Hg has been extracted from mines for centuries; Almaden (originated from the Arabic "the metal") is one of the biggest mines in the world, and



it has been used to extract Hg since the Roman Empire age, to produce explosives [23]. Egyptians used this heavy metal as a medicinal compound and in tombs, as a preservative. In medicine, mercurials use has been as vast as treatments for syphilis and various skin disorders, used as antiseptic or to treat diuretic and chemotherapeutic problems. Hg components were also employed to produce high-quality hats: Hg nitrate was applied to treat the fur used to make hats.

Since the industrialization era, Hg has been used as a compound of electric equipment, batteries, and explosives. It has also been used in medicine, cosmetics, and for agricultural purposes [19]. Activities like the smelting of copper and lead and the extraction of precious metals (e.g., gold and silver) contribute with a high percentage of the Hg pollution of aquatic systems [30]. The burning of fossil fuels, the chloralkali industries, production of electric equipment, and paint are the largest consumers of Hg [19, 31].

All of these anthropogenic activities promote an accumulation of this heavy metal in terrestrial and aquatic ecosystems, which can persist in these locations and in the living organism for over 100 years after the source of pollution has closed [23, 31].

It is understandable that Hg and its compounds present a big dilemma to those interested in making use of its many proprieties. If, on the one hand, Hg has great and useful advantages, on the other, it has great toxicity and is easily spread throughout diverse ecosystems.

#### 4. Uptake of Hg by Plants

Contamination of soils by Hg is often due to the addition of this heavy metal as part of fertilizers, lime, sludges, and manures. The dynamics between the amount of Hg that exist in the soil and its uptake by plants is not linear and depends on several variables (e.g., cation-exchange capacity, soil pH, soil aeration, and plant species). The uptake can be reduced when the soil's pH is high and/or there is an abundance of lime and salts [13, 19].

Another factor affecting the level of accumulation of Hg is the species and the variety [32] as a matter of fact; at least 45 plant families include metal-accumulating species [33]. Most of the plants that uptake Hg tend to accumulate it on the roots [31], and some are even able to accumulate moderate amounts in the shoots [34, 35] either due to translocation or direct absorption of the vapour form. The work done by Suszcynsky and Shann [36] showed that plants exposed to  $Hg^0$  can uptake and accumulate it in shoots, but there is no translocation to the roots.

Toxic metal ions are thought to enter plant cells by the same uptake process as micronutrients, competing with these elements for absorption. Hg, which is a class B metal [14], preferentially binds with sulphur and nitrogen ligands and is thought to enter the cell through ionic channels competing with other heavy metals like cadmium or essential metals like zinc, copper and iron [37]. However, this information is mostly based on experiments in animal cells and the authors believed that the uptake of Hg can occur via other processes which still remain unclear.

### 5. Hg-Induced Phytotoxicity

**5.1. General Effects.** The interaction between Hg and plant systems is of particular importance due to the highly employment in seed disinfectants, fertilizers, and herbicides [38]. Ross and Stewart 1962 [39] have shown that some Hg compounds used on tree foliage as fungicides can be translocated and redistributed in plants.

At the cellular level, the possible mechanisms which heavy metals can damage comprise the blocking of important molecules (e.g., enzymes and polynucleotides), the transport of essential ions, displacement or substitution of metal ions from molecules (such as Mg from chlorophyll), denaturing or inactivation of proteins, and disruption of cell membranes or organelles [13]. Relative to Hg, the possible mechanisms of its phytotoxicity can be through the change of the permeability of cells membrane, high affinity to react with the sulphhydryl (SH) groups, affinity for reacting with phosphate groups, and the replacement of essential ions and its ability to disrupt functions involving critical or nonprotected proteins [13, 19].

Hg is known to affect the antioxidant defence system, by interfering with the modulation of the nonenzymatic antioxidants glutathione (GSH) and nonprotein thiols (NPSH) and the enzymatic antioxidants superoxide dismutase (SOD), ascorbate peroxidase (APX), and glutathione reductase (GR) [2, 40, 41].

Many forms of Hg have been related to seed injuries and reducing seed viability. When Hg interacts with the SH groups to form the S–Hg–S bridge, disrupting the stability of the group, it can affect seed's germination and embryo's growth (tissues rich in SH ligands). Hg chloride has been shown to reduce elongation of *Zea mays*' primary roots as well as an inhibition of the gravimetric response of the seedlings [13]. The same author also discussed that, with increasing concentration of this heavy metal, the respiration rates of *Vigna radiata* seedlings declined, as did the total nitrogen and sugars content and the DNA and RNA content.

Exposure to Hg can also reduce photosynthesis, transpiration rate, and water uptake and chlorophyll synthesis. Both organic and inorganic Hg have been showed to cause loss of potassium, magnesium, and manganese and accumulation of iron [22]. These decreases explain the changes in the permeability of cell membrane by compromising its integrity.  $Hg^{2+}$  is one of the forms of Hg that can affect the plasma membrane and might explain the toxicity provoked in the aerial part of plants; however some authors believe that it is the damage in the roots that explain the toxicity observed in the shoots.

**5.2. Genotoxicity.** Studies considering Hg's genotoxicity are scarce, and the cellular and molecular mechanistic involving the toxicity of this metal are practically unknown. However, it has been demonstrated that this heavy metal can introduce deleterious errors in the genetic materials of crop plants' species.

Inside the cells, Hg ions tend to form covalent bonds, because of their easily deformable outer electron shells. A number of potentially reactive sites for Hg bonding are

present in DNA, depending on external conditions such as ionic strength, presence of different competing ions, and base composition [13].

The effects of Hg's forms depend of the concentration and time of exposure to plants, with very marked effects in S-phase, when it can induce damage leading to severe clastogenicity [13].

The binding of Hg to DNA results in potential toxic effects: chronic intake of methyl-Hg<sup>2+</sup> at subtoxic levels results in chromosomal damage in humans, presumably due to its direct interaction with DNA [8]. In plants, it has been demonstrated that low doses of Hg can provoke c-mitosis, sister chromatid exchanges, chromosomal aberrations, and spindle alterations [13].

Despite that the interaction of metal ions with the sulfur atoms of nucleosides and amino acids bearing the thiol group has provided the dominant mechanism for explaining Hg's toxicity, certain effects (e.g., mutagenic effects) cannot be totally explained by this process. Ribose/ribosephosphate groups and purine/pyrimidine bases present several N and O atoms, which might be potential binding sites for Hg. The ligation of Hg to nucleobases can lead to nucleobase impairing; this phenomenon has been suggested as being relevant to the mutagenic potential of Hg. By this way, Hg can induce alteration on the amino acid sequences of proteins to be synthesized [29].

Despite of all these, there still plenty of unknown aspects regarding Hg's genotoxicity, namely, the mechanistic, target, and extent of its effects in plants.

## 6. Concluding Remarks

As it has been explored throughout this paper, Hg is a critical pollutant which can be easily spread through many ecosystems causing several toxic effects in many biological processes. Unfortunately, very little is known about Hg-induced phytotoxicity, even though plants assume a fundamental role as the base of many trophy chains and in particular of mankind subsistence and economy. It is therefore necessary to increase the level of knowledge about the mechanism by which Hg is uptake by plants and which processes are targeted by this pollutant.

What little is known about Hg toxicity is presented in this paper, which the authors expect, could be a valuable source for other researchers working with Hg phytotoxicity and, possibly, to entice further investigation in this area of research.

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