



## Association between heavy metals and arbuscular mycorrhizae-forming fungi

### Relación entre los metales pesados y los hongos formadores de micorrizas arbusculares

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**ABSTRACT:** In recent years, heavy metal contamination has been a topic that has aroused special interest worldwide, due to the toxicity of heavy metals. Heavy metals can reach the trophic chain by different routes, since many of them are essential nutrients and they are absorbed by plants. There are several technologies to remediate contaminated soils, including phytostabilization and phytoextraction, as part of phytoremediation strategies. Arbuscular mycorrhizal fungi (AMF) form symbiotic associations with plants and help those combat different stress types, including metal stress. Essential micronutrients are absorbed in greater quantities by mycorrhizal plants; however, when these elements are found in high concentrations in the soil as contaminants, mycorrhizal fungi can store these cations, avoiding toxicity in plants or hyperaccumulation, a phenomenon known as phytostabilization.

**Key words:** pollution, phytoremediation, phytostabilization.

**RESUMEN:** En los últimos años, la contaminación por metales pesados ha sido un tema que ha despertado especial interés a nivel mundial, por la toxicidad que presentan. Los metales pesados pueden llegar a la cadena trófica por diferentes vías, ya que muchos de ellos constituyen nutrientes esenciales y son absorbidos por las plantas. Existen varias tecnologías para remediar suelos contaminados, entre ellas están la fitoestabilización y la fitoextracción, como parte de estrategias de fitorremediación. Los hongos micorrízicos arbusculares (HMA) forman asociaciones simbióticas con las plantas y las ayudan a combatir distintos tipos de estrés, entre ellos el metálico. Los micronutrientes esenciales son absorbidos en mayor cuantía por las plantas micorrizadas; sin embargo, cuando estos elementos se encuentran en grandes concentraciones en el suelo constituyendo contaminantes, los hongos micorrízicos pueden almacenar estos cationes, evitando la toxicidad en las plantas o la hiperacumulación, a este fenómeno se le conoce como Fitoestabilización.

**Palabras clave:** contaminación, fitorremediación, fitoestabilización.

## INTRODUCTION

Heavy metals (HM) constitute a danger to mankind, because once the element reaches the soil, it follows two pathways leading to the trophic chain: by absorption by plants or by washing into groundwater, and can reach living organisms and cause acute damage, even death (1,2).

The polluting sources of heavy metals can be various, from the use of an old industrial land for agriculture to the indiscriminate use of agrochemicals in an agricultural ecosystem (3); however, plants have developed defense

mechanisms against this polluting situation, even achieving a hyperaccumulation of these metals in some cases (4).

In some plant species, tolerance to heavy metals, through the use of arbuscular mycorrhizae, constitutes an example of bioaccumulation and biosorption (5,6) and they can be used in phytoremediation programs, since it has been seen how these fungi are able to accumulate heavy metals in their spores and hyphae and decrease the content of these metals in accumulator plants (7).

For this reason, some aspects of the phytostabilization of heavy metals by arbuscular mycorrhizal fungi are discussed in this work.

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## HEAVY METALS IN THE AGROECOSYSTEM

Heavy metals are naturally present in soils, some to a greater and others to a lesser extent, depending on the material that gave rise to them, but there has been an anthropogenic accumulation due to industrial and agricultural activities and the disposal of waste of all kinds (3,6,8).

A heavy metal is considered to be that element that has a density equal to or greater than  $5 \text{ g cm}^3$  when in elemental form or whose atomic number is greater than  $20 \text{ g cm}^3$ , excluding alkaline and alkaline-earth metals (9). Due to the small quantities commonly handled, these are referred to as "trace elements" or "trace metals" and include aluminum, which cannot be qualified as a "heavy metal" because of the characteristics mentioned above, but because of its toxicity (6,10), although this definition also includes essential elements for plants such as Iron (Fe), Copper (Cu), Manganese (Mn), Zinc (Zn) and Nickel (Ni) or others essential for animals such as Cobalt (Co) and Chromium (Cr) (11).

Essential micronutrients are required in only a few milligrams or micrograms per day and when they pass a certain concentration threshold they become toxic, such is the case of Selenium and Zinc, which have very close limits between the required dose and the toxic one (4).

The non-essential heavy metals or those with no known biological function, whose presence in certain quantities in living beings leads to dysfunctions in organisms, are: antimony (Sb), arsenic (As), beryllium (Be), cadmium (Cd), strontium (Sr), mercury (Hg), lead (Pb) and titanium (Ti) (8). The main sources of soil contamination with heavy metals are irrigation with water from streams and wastewater contaminated by industry, the application of contaminated solid waste and the use of former industrial land contaminated by oil and industrial waste discharges, as well as poor irrigation and drainage practices and the intensive use of fertilizers and pesticides (9).

In soil, the mobility of heavy metals in their cationic forms decreases as pH increases, because they precipitate forming hydroxides, carbonates or by forming organic complexes that are not assimilated by organisms (10). The quantities of Ni in soils depend on the physical-chemical characteristics of the soil. This element is highly mobile and considered essential for plant metabolism, requiring minimal amounts ( $0.001 \text{ mg kg}^{-1}$ ) (12,13). It is deposited in leaves and seeds, and chlorosis is a common symptomatology associated with high levels (13).

Potentially toxic substances can also enter the plant through the foliar route, depending on the morphology and respiration rate of the plant that allows its entrance, from the accumulation and variation in concentration gradients. Metals from aerial sources of contamination, such as Pb, reach the plant as a result of wind drift. Likewise, foliar fertilizer applications generate reactions that facilitate the translocation of metals to the interior of the tissue (14,15). Pb

can cause damage to plant germination, reduce enzymatic activity, carbon assimilation, and photosynthesis, among others (16).

In the case of cadmium, given its high solubility in the soil, it is translocated from the roots to the aerial parts of plant, increasing the risk of its incorporation into the food chain, due to its similarity with calcium ( $\text{Ca}^{2+}$ ) (17). It has been reported that this element can cause leaf rolling and chlorosis, alter RNA synthesis, reduce photosynthetic activity, inhibit stomatal opening and decrease enzymatic activity in plants (18).

In a study carried out in Cuban soils of low or null anthropic activity, high natural contents of HM were found in  $\text{mg kg}^{-1}$ : Cu (163-138), Ni (68-676), Cr (78-1259), Zn (83-462), Pb (36-67) and Cd (2-10.9) which, when compared with the international norms, are much higher (19).

Soil is one of the matrices most closely linked to the pollution retention and its magnification in the trophic chain. The introduction of pollutants into the soil can result in damage to its structure, loss of vital ecological functions for the ecosystem homeostasis and consequently of its agricultural and environmental value (19,20). Agricultural soils require special attention, since they serve as a nutritional source for plants destined for human and animal consumption and must guarantee the safety of food generated in them (21). If toxic elements such as Cd and Pb reach crops, they can be a problem for the entire trophic chain (22). In this regard, a study carried out in China determined that there is a high risk for consumers of rice grown in areas near the Yangtze River, as high levels of heavy metals were found in soil, stalks and grains. According to the risk quotients, people were exposed to cancer risk mainly due to Cd, Pb and As concentrations (23).

The technological process applied to crops can itself be a source of heavy metal contamination in soils. This pollutant load generated by the technology can be transferred to the crops grown in these soils, due to the adaptive and intrinsic characteristics of each one of them (24). There are plant species that have the capacity to remove and store these elements from the soil in high concentrations; these plants are classified as heavy metal accumulators and hyperaccumulators (20).

Among plants that accumulate metals, species have been found that have the capacity to store extraordinary quantities, such as potatoes (*Solanum tuberosum* L.), tomatoes (*Solanum lycopersicum* L.), rice (*Oryza sativa* L.), among others; these concentrations are notably higher than the indexes considered toxic for the plant kingdom, which are called hyperaccumulator plants. The effect of heavy metals on plants results in a change in their biochemical activity, as well as in their functioning (25). The ability to accumulate metals is not a common characteristic of most plants; on the contrary, it is the result of an evolutionary response, since the natural occurrence of high levels of metals in the biosphere is sporadic (26).

## ARBUSCULAR MYCORRHIZAL SYMBIOSIS

Mycorrhizal symbiosis in ecosystems is a plant adaptation mechanism to different stressful conditions, since it facilitates increases in the absorption of nutrients and water, improves soil aggregates, causes a bioprotection effect against some pathogens, among other benefits (27,28).

This union is defined as a symbiotic association, since both organisms establish successive exchanges of nutritive substances, essential metabolites and hormonal substances, as well as lead to the creation of new structures, representing a mutual benefit for both symbionts (29).

The role of mycorrhizae in the absorption of nutrients is very complex, and may be the result of several possible mechanisms (30), such as:

Increase in the surface area of root absorption and soil exploration (physical effect). Increase in root absorption capacity (physiological effect).

Morphological and physiological modifications in mycorrhizal roots, in relation to non-mycorrhizal roots.

Absorption of available nutrients not accessible to non-mycorrhizal roots directly through hyphae or indirectly by favoring root development.

Utilization of forms not available to non-mycorrhizal roots through solubilization and mineralization in the case of ectomycorrhizae and modifications in the dynamics of the nutrient balance between the solid and liquid phase of the soil, in the case of AMF. Temporary storage of nutrients in the fungal biomass or roots avoiding their chemical and biological immobilization or leaching.

Establishment of mineralizing, nutrient solubilizing and diazotrophic microorganisms in the mycorrhizosphere.

Amortization or mitigation of the adverse effects of heavy metals, salinity, water stress and attack of root pathogens on nutrient uptake.

In recent years in Cuba, a wide group of positive results have been achieved on the management of arbuscular mycorrhizal symbiosis in agroecosystems, based on the existence of inoculants that are applied in low quantities and the knowledge of the bases for an effective management of these and integrated not only with mineral fertilizers, but also with green and organic fertilizers (31-34).

### AMF EFFECT AGAINST HEAVY METALS

Plants and fungi have developed strategies to obtain nutrients from soils of varied composition, using different mechanisms in the assimilation of metals, at the same time that they prevent toxicity, coordinate transport, chelation and sequestration of metallic elements at the cellular level, to maintain the ionic balance (35).

High amounts of metals can be accumulated in a variety of physiological processes, independently of the associated metabolic pathway, using biomass (live-dead), as well as cellular products (polysaccharides), in the removal of heavy metals (36). For this reason, the genome of eukaryotes encodes several families of metal transporters, which direct

the translocation process inside plants and fungi, differing in location at the cellular level, specific substrate on which expression patterns act (35).

Different microorganisms are capable of actively and passively concentrating metals at levels that are substantially higher than those of the surrounding environment, which is why for several decades there has been a growing interest in microorganism-metal interaction and its possible commercial applications. The immobilization of HM by active (energy-dependent) and passive (energy-independent) processes are known as bioaccumulation and biosorption, respectively, and include mechanisms such as chelation, ion exchange, and encapsulation (4).

Plants have developed mechanisms that modify their environment by excreting organic compounds through the root, as a rich substrate for the surrounding microbial community (37,38), involving basic aspects such as chelation and acidification of the rhizosphere, among others, which decrease the mobility of heavy metals (39). The most important structures of arbuscular mycorrhizal fungi involved in symbiosis and tolerance to heavy metals are the arbuscules, vesicles and hyphae (40).

Several investigations have shown the stabilizing capacity of these fungi on the absorption of metals by the plant. This buffering effect translates into an increase in the supply of micronutrients to the plant when it grows in soils deficient in these micronutrients, while reducing the incorporation of metals into plant tissues when plants grow in soils with high levels of these metals (40).

Other authors demonstrated that mycorrhizal fungi were able to increase the concentration of Zn by the plant under conditions of deficiency of this element in the soil, while, when levels of the metal in the soil exceeded a certain threshold, the concentration of Zn decreased considerably (41,42).

The phytostabilizing effect of mycorrhizae on the aerial part of the plant has been observed on multiple occasions, and protection against Al, U, Cs, Sr, Cd, Mn, Zn and Cu has been described (40). This protective effect is mainly due to the immobilization of the metal in the mycelium of the fungus, especially in cell walls (42), but also in internal mycelial structures such as vesicles or, at an intracellular level, polyphosphate granules (40). Metal accumulation in the fungus internal mycelium is what would explain its accumulation in roots of mycorrhizal plants considered as a whole (root + fungus) (41). In fact, the aerial biomass tissues of mycorrhizal plants have lower levels of heavy metals than those of non-mycorrhizal roots when the plant grows in soils with elevated levels of metals (43). When comparing the effect of fungi isolated from contaminated soils in relation to fungi isolated from non-contaminated soils, it is observed that the former are more effective, although their successive cultivation in non-contaminated soils may reduce this capacity (44). The fact that not all mycorrhizal fungi have the same tolerance to heavy metals could facilitate the use of the most sensitive isolates as biomarkers of contamination (45).

Other researchers obtained as a result that inoculation with AMF and salicylic acid increased the tolerance of bell pepper plants (*Capsicum annuum* L.) to high concentrations of Cu in the soil, inducing tolerance mechanisms to stress caused by heavy metals (46).

In Mexico, the capacity of the genus *Amaranthus* to accumulate Pb and Cd when inoculated with mycorrhizal fungi of the genus *Glomus* has been evaluated, showing a higher percentage of colonization of the fungus and greater extraction of the crop when concentrations of these metals increase, even when none of these are essential nutrients for plants (47).

Siderophores, apart from their ability to bind Fe, can also form complexes with other metals such as Al, Cd, Cu, Pb and Zn, which causes an increase in the solubility of these metals in the soil (48).

Glomalin, a glycoprotein produced by mycorrhizal hyphae, has shown its potential to establish links with highly toxic molecules such as heavy metals, which constitutes an effective barrier that accumulates them in the mycelial mass without allowing them to enter plant cells (49).

Heavy metals can be immobilized by secretion of glycoproteins or adsorbed on fungal cell walls, thus reducing their toxic effect on plants (50). Both the capacity to increase the acquisition of mineral micronutrients by plants growing in deficient soils, and to decrease it in those growing in soils contaminated with heavy metals, depend on the action of a set of processes to maintain homeostasis in arbuscular mycorrhizal fungi (40).

In Brazil, a study was conducted on the biochemical and nutritional changes induced by Pb in sunflower plants (*Helianthus annuus* L.). Pb toxicity generates changes in the nutritional balance of Ca and Mg, which can be used as a nutritional marker of Pb toxicity in sunflower. Proline concentrations, which are proportional to the increase of Pb concentrations in the plant, can also be used as a biochemical marker in this crop. This amino acid reduces the toxic effect of metals and can contribute as an available source of carbon and nitrogen (51).

Several studies have shown that phytostabilization of heavy metals generated by arbuscular mycorrhizal fungi in the plant can be a good management solution to the contamination problem (52); however, it is characterized by its great distance in practical terms.

For the application of this technique, each case must be considered as unique and unrepeatable, because the environmental characteristics of each crop exposed to these conditions, as well as the elements involved (heavy metal, plant, environment, mycorrhizae) infers particular features and in constant dynamics, which directly influences this technique success (4). Therefore, phytostabilization of heavy metals by means of AMF can constitute a phytoremediation strategy for contaminated soils.

## CONCLUSIONS

- Heavy metals in the agroecosystem constitute a risk for the food chain since some of them, despite being micronutrients necessary for plants and animals,

large concentrations of these can cause toxicity or other damage.

- Arbuscular mycorrhizal fungi are an alternative to counteract the toxic effect caused by heavy metals for plants, by phytostabilizing concentrations of these elements.

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