



Research review paper

Hyperaccumulators, arbuscular mycorrhizal fungi and stress of heavy metals

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ABSTRACT

Use of plants, with hyperaccumulating ability or in association with soil microbes including the symbiotic fungi, arbuscular mycorrhiza (AM), are among the most common biological methods of treating heavy metals in soil. Both hyperaccumulating plants and AM fungi have some unique abilities, which make them suitable to treat heavy metals. Hyperaccumulator plants have some genes, being expressed at the time of heavy metal pollution, and can accordingly localize high concentration of heavy metals to their tissues, without showing the toxicity symptoms. A key solution to the issue of heavy metal pollution may be the proper integration of hyperaccumulator plants and AM fungi. The interactions between the soil microbes and the host plant can also be important for the treatment of soils polluted with heavy metals.

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1. Introduction

Heavy metals (53 elements) are categorized into one group based on their density ($>5 \text{ g/cm}^3$) (Holleman and Wiberg, 1985). Heavy metals including iron (Fe), manganese (Mn), copper (Cu), zinc (Zn) and nickel (Ni) are necessary for plant growth and functioning. They catalyze different enzymatic and redox reactions, carry electron and are the main component of DNA and RNA metabolism (Zenk, 1996). However, some of the heavy metals such as Cd and Pb are not necessary to plant growth and, especially their high levels can adversely affect plant growth.

At high concentrations, heavy metals influence the structure of enzymes and hence their functionality by affecting the protein structure

or substituting a necessary element. As the structure of plasma membrane proteins such as H^+ -ATPases is sensitive to alteration by heavy metals; the toxic effects of heavy metals can influence the permeability and functioning of plasma membrane. In addition, heavy metals cause oxidative stress (production of reactive oxygen species), adversely affecting different cellular components and hence plant tissues (Sajedi et al., 2010; Schutzendubel and Polle, 2002).

At high concentrations of heavy metals, there are different mechanisms, utilized by plants, to keep ion homeostasis, and hence to detoxify their unfavorable effects on plant growth (Clemens, 2001). Root exudates are able to chelate heavy metals and cellular walls can bind heavy metals. Inside the cells, production of compounds such as phytochelatins and metallothioneins with high affinity for heavy metals can bind heavy metals and hence control their cytoplasmic concentration by transporting them across tonoplast and their subsequent sequestration in the vacuole (Hall, 2002).

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In addition to adversely affecting plant growth, heavy metals have unfavorable effects on the environment. There is a wide range of lands in the world contaminated with different sources of pollution affecting ecosystem health and human food chain. The important sources of heavy metals in soil are industrial activities, mines, polluted water, and sewage sludge (Lebeau et al., 2008). Different methods have been used for the remediation of polluted environment with heavy metals, however, use of biological methods have been proved to be more effective and less expensive (Lynch and Moffat, 2005; Salt et al., 1995a).

There are different methods of cleaning the environment from different sources of pollutants (such as heavy metals) including plant bioremediation, using soil as a filtering medium and use of different soil microbes such as AM fungi and plant growth promoting rhizobacteria (PGPR) (Lebeau et al., 2008). There are plants called hyperaccumulators, which are able to absorb high amounts of heavy metals, while their growth is not affected. This ability can be effective for the removal of heavy metals from the polluted soils. Some of these plants belong to the families such as *Brassicaceae*, in which a limited number of plant species are able to develop mycorrhizal symbiosis with their host plant (Assuncao et al., 2001).

In soil there are arbuscular mycorrhizal (AM) fungi developing symbiotic association with most of terrestrial plants. In this symbiosis AM fungi can significantly enhance the host plant ability to absorb water and nutrients in the exchange for carbon (C). AM spores are able to germinate in soil; however, for the symbiosis process to proceed the presence of host plant is necessary, indicating that AM fungi are obligate biotrophs (Smith and Read, 2008).

Through communicating signal molecules, AM spores and plant roots can develop their symbiotic association. Germinated spores produce hyphae, which grow toward the host plant roots and eventually enter the cellular cortex. The hyphal network is developed by plant C source, producing a very extensive hyphal network with some unique abilities, indicated in the following. 1) Enhancing plant water and nutrient uptake, 2) alleviating soil stresses including heavy metals, 3) improving soil structure, 4) controlling pathogens, and 5) interacting with other soil microbes. Accordingly, AM fungi are considered to be a very effective component of ecosystem increasing crop production and contributing to the environmental cleanness (Miransari 2011a,b; Smith and Read, 2008).

Numerous research works have indicated that AM fungi can significantly enhance their host plant ability to grow in soils polluted with heavy metals. Such effects are due to AM fungi abilities as well as their positive effects on plant growth. AM fungi are morphologically and physiologically unique and hence can perform efficiently under stress. In addition they can increase the growth of their host plant by enhancing water and nutrient uptake (Miransari 2011a,b). In the following parts the bioremediation methods of removing heavy metals from soil and the effects of AM symbiosis on the uptake of heavy metals as well as the related molecular mechanisms are mentioned.

2. Bioremediation

Bioremediation or phytoremediation is the process of using plants and soil microbes for the removal and cleaning of pollutants from soil including heavy metals. It includes the following subcategories: 1) phytoextraction: uptake of heavy metals by the harvestable parts of plant, 2) phytodegradation: decomposition of pollutants by plants and microbes, 3) rhizofiltration: absorption of metals from polluted waters, 4) phytostabilization: decreased mobility and immobilization of pollutants in soil by plant roots and microbes, and 5) phytovolatilization: volatilization of pollutant into the atmosphere by plant roots (Chaudhry et al., 1998; Khan, 2005).

Plants can use some mechanisms to alleviate the unfavorable effects of stress on their growth up to some extent. Under stress plant

tissues can function singly or together to modify the stress. The allocation of different compounds and ions into different plant tissues is among the strategies, which plants use to alleviate the stress and is called phytoremediation, or specifically phytoextraction. The method is more effective when the concentration of heavy metals does not exceed a certain amount, for example, for Pb the limit is equal to 1500 mg/kg.

Phytoremediation of agricultural soils can result in the sustainable production of crop plants by positively affecting soil properties (Audet and Charest, 2007a; Salt et al., 1998). There are different parameters influencing the effectiveness of phytoremediation including soil depth and the translocation rate of heavy metals from roots to shoots. Although at least five years is required for soil remediation, longer time is usually necessary for soil clean-up (Dickinson and Pulford, 2005; Khan et al., 2000).

With respect to disadvantages related to non-biological methods of treating polluted soils, use of biological methods including hyperaccumulator plants and soil microbes has been indicated to be promising to alleviate the stress of heavy metals (Glick, 2003; Hernandez-Allica et al., 2006; Kuiper et al., 2004; McGrath et al., 2006). There are two different methods by which soil microbes can assist the plant to alleviate the stress: 1) enhanced metal mobility by microbial production of biosurfactants (Herman et al., 1995), siderophores (Dubbin and Louise Ander, 2003) and organic acids (Di Simine et al., 1998), and/or 2) increasing plant growth by PGPR (Zhuang et al., 2007) and/or AM fungi (Khan, 2006). Under heavy metal stress plant allocates most of heavy metals to their roots so that plant shoots can function more efficiently. Other parameters including the rate of plant growth, and plant morphological and physiological properties can also affect plant performance under stress affecting plant abilities for bioremediation (Audet and Charest, 2008).

Bioremediation is the process by which soil pollutants are removed, decreased or transported using biological methods and is essential for sustainable development (BBSRC, 1999). It has been a research subject for microbiologists and molecular biologists. Use of soil microbes or plants with the ability to absorb or degrade the pollutant (phytoremediation) or plants to limit the movement of pollutant (phytostabilization) in soil are the common processes of bioremediation.

In the combined use of plant and soil microbes, plant supplies C source for microbes, which absorb, degrade or make the plant absorb the released elements. The energy source, used for such a process is usually up to 40% of plant photosynthates exuded by plant roots (Lynch and Moffat, 2005). Audet and Charest (2007a) indicated that the phytoextraction ability of plants is a very important character significantly affecting the bioremediation process.

3. Hyperaccumulators

There are plants, which have the ability to absorb high amounts of heavy metals, while their growth remains unaffected. The technology of using plants for removing heavy metals from soil is called "phytoremediation". It is rarely common that plants hyperaccumulate heavy metals as only less than 0.2% of angiosperms (450 species with the majority (75%) being Ni hyperaccumulators) are hyperaccumulators of heavy metals. Hyperaccumulators must have the ability of metal homeostasis while growing in a polluted environment (Verbruggen et al., 2009).

The *Thlaspi* family are hyperaccumulating plants among which 23 species hyperaccumulate nickel (Ni), 10 species hyperaccumulate Zn, just three species (*T. caerulescens*, *T. praecox* and *T. goesingense*) hyperaccumulate Cd and one species hyperaccumulate Pb (Lombi et al., 2000; Vogel-Mikuš et al., 2005, 2008). *T. caerulescens* is among the most well known hyperaccumulators (Assunção et al., 2003). Interestingly, this plant is able to grow in serpentine soils, which contain high levels of heavy metals including Zn, Co, Pb, Cr, Cd and Ni,

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