

Metodologia (Methodology)

Phytohyperaccumulator-AMF (arbuscularmycorrhizal fungi) interaction in heavy metals detoxification of soil

Interação entre hiperacumuladores-FAM (fungos arbusculares micorrizais) na desintoxicação de solos metalíferos

AISHA UMAR¹

Soil fungi (micropartner) develop mycorrhizal interactions with 80 % of land plant species (macropartner) (WANG & QIU, 2006). Soil composed of non-degradable heavy metals toxicity that inhibits the growth (LOMBI *ET AL.*, 2001) and water status of plants (BARCELÓ AND POSCHENRIEDER, 1990). HMs in the soil causes vegetation free world. Phytohyperaccumulators gather more than 1 % Zn or Mn, 0.1 % Ni, Cu, Co or Pb and 0.01 % Cd in the shoots (COBETT, 2003) than roots (VÁZQUEZ *ET AL.*, 1994). These plants can cope with low levels of fertility and poor soil structure, which may induce mineral deficiencies (STANLEY AND ISABELLE, 2015). Mycorrhizae are the mutualistic symbiotic association (non-pathogenic) of a specific group of soil-borne fungi with the roots of higher plants (SIEVERDING, 1991). Arbuscular mycorrhizal fungi colonize the roots of approximately 65 % of all known land plant food and bio energy crops

¹ Lecturer at Departament of Botany, Minhaj University, Lahore, Pakistan.

(WANG & QIU, 2006) may interfere with heavy metal absorption (LIAO *ET AL.*, 2003). AMF is reported to enhance nutrient supply and improve water use efficiency and photosynthesis in salt marsh plant species, but the rate of AMF colonization drastically decreases at moderate salinities (CARAVACA *ET AL.*, 2005).

Root exudates (organic chemicals) enhance the mobility of metals and nutrients by (PÉREZ-ESTEBAN *ET AL.*, 2013) (i) acidification due to proton (H^+) release or by forming organic/amino acid-metal/mineral complexes(ii) intracellular binding compounds (*e.g.*, phytochelatins, organic acids, and amino acids) (iii) electron transfer by enzymes in the rhizosphere (*e.g.*, redox reactions), therefore enhancing phytoremediation efficiency (SESSITSCH *ET AL.*, 2013). The low molecular weight organic acids (LMWOAs) *e.g.*, oxalate, released by both mycorrhizal and non-mycorrhizal seedlings contributed to metal immobilization by stable metal complexes in soil (JOHANSSON *ET AL.*, 2008).

ESTABLISHMENT OF MUTUALISTIC SYMBIOSIS B/W PHYTOACCUMULATOR-AMF

a) Phyto Chemotaxis

Root exudates are chemo attractant signals for AMF (CHAPARRO *ET AL.*, 2013) in unique environment of rhizosphere and sources of organic and inorganic elements for fungi (COMPANT *ET AL.*, 2010) therefore creating crosstalk in plants and fungi (COMPANT *ET AL.*, 2011).

b) AMF Association

Zn violets enhance the rhizospheric phenomenon in polluted soil (TONIN *ET AL.*, 2001). Plant-fungal association is a species specific and competitive process (REINHOLD-HUREK AND HUREK, 2011). Rate of colonization of roots by mycorrhizal fungi increases with increasing heavy metal content in serpentine soil (AUDET AND CHAREST, 2006). AMF needed maximum nutrients during reproductive stage so they are suitable to flourish in HMs (reproductive period (VOGEL-MIKUS *ET AL.*, 2006) HMs from AMF (cell wall, cytoplasm, Vesicles, arbuscules, intraradical hyphae) stores in inner root parenchyma cells. Literature supported the vesicle as a storage compartment of HMs (TURNAU, 1998; WEIERSBYE *ET AL.*, 1999. AMF makes macro and micro colonies inside and outside tissues of the plants (MA *ET AL.*, 2015; WANG *ET AL.*, 2016).

PERFORMANCE OF MUTUALISTIC SYMBIOSIS

Plant–Fungal crosstalk is a significant and beneficial process to distinguish the underground communities. Metal resistant activity of AMF enhances membrane permeability and metabolic functions of phytohyperaccumulators in serpentine soil.

Nutrient-absorbing AMF facilitate the hyperaccumulators to absorb maximum minerals and nutrients from the contaminated soil as well as proficient in increasing the fertility of heavy metals contaminated soil (NAVARRO-NOYA *ET AL.*, 2012). This association prevents the plant from production of ethylene in soil stress conditions. AMF actors act as a biomodifiers and modify the original appearance of roots in prosperous style (Ahemad and Kibret, 2014). They act as biopesticides that kill phyto pathogens by releasing antifungal agents (Fig 1).

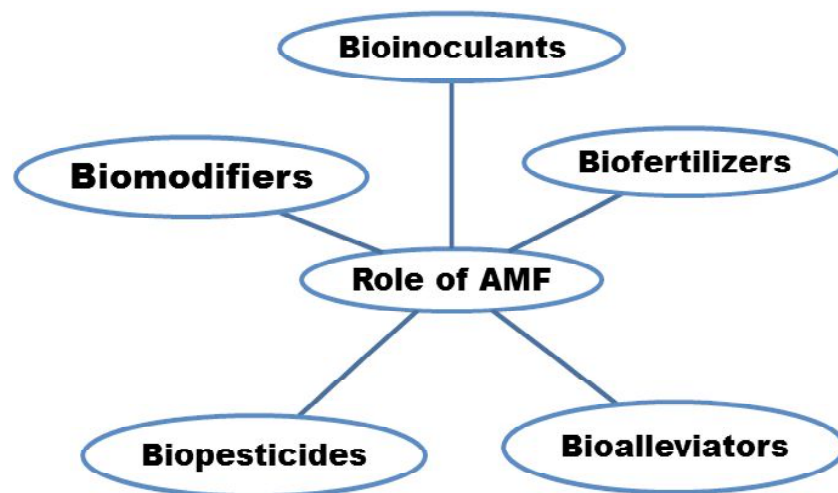


Fig. 1. Action of AMF for phytohyperaccumulators.

Phosphorous is major macronutrients unavailable for plant growth in soil (HARRIS AND LOTTERMOSER, 2006). The insoluble P compounds in soil can be solubilized by enzymes, organic acids and/or chelating agents excreted by both plants and fungi (JEONG *ET AL.*, 2013). AMF *Glomus* spp. is benefited for plant growth and nutrient (N, P, and K) uptake by leguminous trees grown on Pb/Zn mine tailings. Arbuscular mycorrhizal fungi (AMF) are capable of boosting plant growth (ORLOWSKA *ET AL.*, 2013) and nutrients uptake (GUO *ET AL.*, 2013), reduce metal induced toxicity (MEIER *ET AL.*, 2011), change metal availability through alteration of soil pH (RAJKUMAR *ET AL.*, 2012) and affect metal translocation (HUA *ET AL.*, 2009). Exudates from plant roots create intricate communication systems (nutrient and energy) with fungi of metalliferous sites called allelopathic actions, which attracts AMF. AMF induces the defense

mechanisms against phytopathogens directly through the solubilization of mineral nutrients (nitrogen, phosphate, potassium, iron, etc.), production of plant growth promoting substances (*e.g.*, phytohormones- indole-3-acetic acid (IAA), gibberellic acid, abscisic acid, and secretion of specific enzymes (*e.g.*, 1-aminocyclopropane-1-carboxylate deaminase) under stress conditions of soil (ULLAH *ET AL.*, 2015). Insoluble specific glycoprotein glomalin produced by hyphae of AMF helps in sequestering heavy metals outside the mycelium (GONZALES-CHAVEZ *ET AL.*, 2004). Accumulated HMs by AMF enters to plants and excreted from there by different organs.

AM fungi releases Myc factors lipochitooligosaccharide (MAILLET *ET AL.*, 2011) that lead to root and AM symbiosis. Genes encoding proteins (*e.g.*, metallothionein and glutathione) efficiently help in tolerance of AMF in contaminated soil. Expression of genes is done in intra and extraradical mycelium. Seven genes (SYM genes) have been identified that are required for fungal-root symbioses (Table 1).

ROLE OF PHYTOACCUMULATORS

Phytoaccumulators associated with AMF store and release the heavy metals in the following organs.

1: Trichome excretions

Cadmium is excreted by trichomes in a glycophyte species (tobacco) (CHOI *ET AL.*, 2004). SARRET *ET AL.* (2006) reported that Zn exposure increased trichome density in young leaves but not in mature leaves of tobacco. Similarly, Cu induced the expression of genes coding for sequestering metallothionein (MT) in *Arabidopsis* trichomes (GUO *ET AL.*, 2003). Glandular trichomes of the hyperaccumulating species *A. halleri* accumulate heavy metals but do not excrete them at the leaf surface.

2: Salt glands

Heavy metals especially Zn excretion takes place through glandular trichomes or salt glands (WEIS AND WEIS (2004).

3: Leaf succulence

In some phtohyperaccumulators, leaf lusciousness help in dilution of concentrated heavy metal contents and toxic impacts in photosynthetic leaves (WANG *ET AL.*, 2012). Absorbed salts increases leaf succulence (KATSCHNIG *ET AL.*, 2013) called reservoir due to sufficient space where water contaminated with toxic ions and heavy metals stores (THOMAS AND BOHNERT, 1993).

4: Histological distribution

Heavy metal accumulated compartments exhibit less metabolic activity. Vacuoles and Cell walls can tolerate HMs concentration (CARRIER *ET AL.*, 2003).

Table 1. Genes of the common symbiosis pathway.

| Signaling component-receptor Gene | Function | References |
|-----------------------------------|--|----------------------------------|
| kinase <i>SymRK</i> | Leucine-rich receptor-like kinase that plays an essential role for root endosymbioses with <i>Rhizobia</i> bacteria, AM fungi and <i>Frankia</i> bacteria, and is involved in the signal transduction to the cytoplasm after the perception of Nod or Myc factors. | (GHERBI ET AL., 2008) |
| <i>NUP85/</i> <i>NUP133</i> | Putative components of the nuclear pore complex that are involved in the transport of macromolecules through the nuclear envelope. | (PARNISKE 2008) |
| <i>CASTOR/</i> <i>POLLUX</i> | Cation channels in the nuclear envelope that are essential for the perinuclear calcium spiking after the perception of Nod or Myc factors. | (CHARPENTIER 2008). |
| <i>CCaMK</i> | Calcium and calmodulin-dependent protein kinase with three calcium binding motifs that acts as sensor of the nuclear calcium signatures and is involved in the phosphorylation of <i>CYCLOPS</i> . | (KISTNER ET AL., 2005) |
| <i>CYCLOPS</i> | Protein with unknown function that acts as phosphorylation target of <i>CCaMK</i> downstream of the nuclear calcium spiking and is presumably the branchpoint of the common SYM pathway. | (KISTNER ET AL., 2005) |
| <i>PAM1</i> | intracellular hyphae in mycorrhizal roots | (KISTNER ET AL., 2005) |
| <i>MtCbf1</i> and <i>MtCbf2</i> | Re-programming of root tissues during the establishment of an AM symbiosis. | (Hogekamp et al., 2011). |
| (BEG 34)/ (<i>GintZnT1</i>) | Increased transcript levels of a putative Zn transporter gene and protecting against Zn stress | (GONZALEZ-GUERRERO ET AL., 2005) |
| <i>GintABC1</i> | Cd and Cu detoxification in the ERMof <i>G. intraradices</i> . | (GONZALEZ-GUERRERO ET AL., 2006) |
| <i>Sy167</i> | Alleviating the HM-induced oxidative stress | (ULRICH ET AL., 2007) |

5: Mucilage

Polysaccharides are mixed in mucilage and found in various plant organs *e.g.* rhizomes, roots and seed endosperm of several plant species. Na^+ as a divalent cations stores in halophytes (GHANEM *ET AL.*, 2010) and in heavy metal form in shoots and root tissues (JAVED *ET AL.*, 2013). Arsenic (FOX *ET AL.*, 2012), chromium (LAKSHMANRAJ *ET AL.*, 2009) and aluminium (MIYASAKA AND HAWES, 2001) gather and stores in pectic polysaccharides.

MECHANISMS OF ALLEVIATION

Acidification, precipitation, chelation, complexation, and redox reactions, phytoremediation, detoxification, mobilization, immobilization, transformation, transport and distribution in hyperaccumulating plants helps to clean up metal contaminated soils through extraction (phytoextraction), stabilization (phytostabilization), and transformation (phytovolatilization) process (LEBEAU *ET AL.*, 2008; GLICK, 2010).

PHYTOACCUMULATOR-AMF SIGNALING

Plant-released signals

Flavonoids from roots are signaling components in plant–mycorrhizal formation (STEINKELLNER *ET AL.*, 2007). Flavonoids help in AMF spore germination, hyphal growth, differentiation, and root colonization in AMF-plant interactions (BADRI *ET AL.*, 2009; MANDAL *ET AL.*, 2010).

AMF-roots possessed intermediate levels of flavonoids pattern in early establishment, whereas, high levels of flavonoids (such as phytoalexin and medicarpin) in later stages of colonized roots (LAROSE *ET AL.*, 2002; BADRI *ET AL.*, 2009). Flavonoid exerts a negative or neutral effect on different fungi due to its specificity involved in mycorrhizal symbiosis formation (SCERVINO *ET AL.*, 2005).

Microbial Signals:

Myc factor of AMF (MAILLET *ET AL.*, 2011) modulates root system architecture (*e.g.*, lateral root branching, formation of new organs), facilitating symbiotic infections (MAILLET *ET AL.*, 2011), alter the chemical composition of root exudates, plant physiology and plant defense via releasing of various signaling molecules, such as volatile organic compounds (VOCs) are enabling host plants to colonize nutrient (*e.g.*, sulfur and iron) in poor soils (BAILLY AND WEISSKOPF, 2012).

METAL DETOXIFICATION

AMF are capable of metal translocation and distribution in inner root parenchyma cells (KALDORF *ET AL.*, 1999). The mechanisms involved (1) cell surface biosorption/precipitation of metals; (2) active efflux pumping of metals out of the cell via transporter system; (3) sequestration of

metals in intracellular compartments (mainly cell vacuole); (4) exclusion of metal chelates into the extracellular space; and (5) enzymatic redox reaction through conversion of metal ion into a non-toxic or less toxic state (Fig 2)

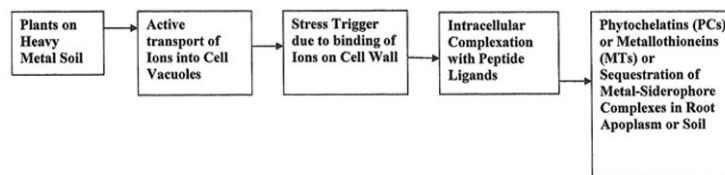


Fig.2 Mechanism of heavy metals detoxification.

Roots release exudates called LMWOAs (succinic acid, citric, oxalic, malic) (MEIER *ET AL.*, 2012) that encourage AMF growth. Chelators in rhizosphere dissolves sparingly soluble mineral, nutrients (Zn, Fe, P), and detoxify metals and metalloids (Pb, As, Cd, Cu) (LI *ET AL.*, 2013) and facilitate apoplast/symplastic movement of metal ions (MAGDZIAK *ET AL.*, 2011).

a) Bioaccumulation

Intracellular accumulation of HMs called Bioaccumulation, a complex process. Bioaccumulation process concentrates the toxic metals in non-living (biosorption) (MA *ET AL.*, 2013) or living (bioaccumulation) cells (RAJKUMAR *ET AL.*, 2012).

Physical and chemical Bonding of metals with waste materials of fungi called passive biosorption. It is not dependent on metabolism of fungi. But the metabolism-dependent active bioaccumulation involved carrier mediated ion pumps, complex permeation and endocytosis (CHOJNACKA, 2010).

S-layer proteins entrap metal ions either in bio or non bio cells called biosorption (GADD, 2004), while facilitator or carrier proteins are involved in bioaccumulation of P and S essential nutrients (SUAREZ AND REYES, 2002; ELANGO VAN *ET AL.*, 2005).

b) Bioleaching

Penicillium, *Aspergillus*, *Trichoderma* and *Fusarium* (MULLIGAN AND GALVEZ-CLOUTIER, 2003) uses their metabolic machineries in alleviating metal phytotoxicity by dissolution, complexation, adsorption and Redox reactions (PATHAK *ET AL.*, 2009). This reaction helps in bioleaching of heavy metals (Cd, Zn, Cu, Cr, Fe, Pb) from sediments, soils and sludge.

Metal Mobilization

Strong binding of metals to soil particles accounts for the insolubilization of metals in soil and contributes to their unavailability for plant uptake phytoextraction (MA *ET AL.*, 2013). Metal mobilizing fungi modify the rhizosphere and mobility in soil through biogeochemical cycling processes of heavy metals by acidification, protonation and chelation (RAJKUMAR *ET AL.*, 2012; SESSITSCH *ET AL.*, 2013).

c) Biogeochemical cycling of heavy metals

The influence of fungal activity on metal mobilization/immobilization, translocation and transformation improve metal bioremediation processes (SESSITSCH *ET AL.*, 2013; AHEMAD AND KIBRET, 2014). Acidification, chelation and protonation lead to mobilization of metals, whereas precipitation, alkalization, and complexation cause metal immobilization whereas chemical transformation can cause metal mobilization or immobilization of heavy metals.

d) Acidification

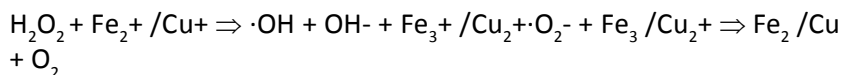
Mobility of metals decreases with increasing soil pH (RICHARDS *ET AL.*, 2000) has influenced on the activities of both plants and fungi. The hydrogen ions excreted by plant roots can displace heavy metal cations adsorbed on soil particles, leading to acidification of the rhizosphere. Root exudates can lower the pH of rhizosphere (SHEORAN *ET AL.*, 2011), which enhancing soil metal mobility and plant metal bioavailability in soil solution (ALFORD *ET AL.*, 2010).

e) Chelation

Natural organic chelators are known as metal-binding compounds, organic acid anions, siderophores, biosurfactants, and metallophores (SESSITSCH *ET AL.*, 2013) are released from both plants and fungi which scavenge metal ions (GADD, 2004) and reactive oxygen species (LEITENMAIER AND KÜPPER, 2013).

Reactive oxygen species (ROS- O_2^- , H_2O_2 , and $-OH$) are generated under stress conditions and bear strong oxidizing activities that can attack all types of biomolecules and represent intermediates emerging during the successive reduction of O_2 to H_2O_2 (Mittler 2002; Panda 2008). Plants experience certain heavy metal ions during the conversion plant accumulation of H_2O_2 into highly reactive $-OH$ molecule in a metal-catalyzed reaction via the Fenton reaction. The oxidized metal ions undergo are-reduction in subsequent reaction with superoxide radicals (O_2^-).

Fenton reaction



Haber Weiss reaction



The $\cdot\text{OH}$ molecule is one of the most reactive species known to initiate radical chain reactions leads to irreversible chemical modifications of various cellular components. protonated form of $\cdot\text{O}_2^-$ in lipid peroxidation Heavy metals (HM) like Cu, Zn, Cd, Cr, Pb, Hg, As, Fe etc under toxic concentration inactivates enzymic antioxidant defense system in plants resulting into increased ROS signaling generally leading to death of a plants including membrane permeability, chlorosis, growth retardation, browning of roots, effects on both photosystems, cell cycle arrest and others can be observed (MITTLER, 2002; TIWARI *ET AL.*, 2008). Plants and AM fungi have evolved several mechanisms to maintain ion homeostasis under elevated HM concentrations (CLEMENS 2001; HALL 2002). The basic principles of detoxification mechanisms include the extracellular HM chelation by root exudates and/or binding of HM to the rhizodermal cell walls uptake of HM avoiding. Active plant efflux systems control cytosolic concentrations of HMs.

Intracellularly the plant cell produces chelating agents such as phytochelatins and metallothioneins, which have high-affinity HM binding properties. The resulting complex can finally be exported from the cytoplasm across the tonoplast and become sequestered inside the vacuole (HALL, 2002). Proteins are involved in linkages with metals, thereby forming complex biochemical compounds called metal-proteins, metallothionein and peptide like phytochelatins. Besides these, organic acids and amino acids take active part in detoxification of heavy metals in plants (PAL AND RAI, 2009).

Metal-binding peptides (MTs-cysteine-rich) can eradicate the phytotoxic effect of free metal ions and allow metal uptake, sequestration, compartmentation, xylem loading, and transport within the plants (CAI AND MA, 2002). Phytochelatins (PCs) are synthesized from the tripeptide glutathione under PCs synthase (SOLANKI AND DHANKHAR, 2011). These PCs are immediately induced by heavy metal exposure in plant tissues (PAL AND RAI, 2010).

MTs also occur in AMF and those genes encoding several enzymes for PCs synthesis in mycorrhizal roots exposed to metal stress and enhancing photosynthetic activity (RIVERA-BECERRIL *ET AL.*, 2005).

Siderophore is produced in fungi due to Fe acquisition by different plant species (GAONKAR AND BHOSLE, 2013). Chelation through binding toxic metals to siderophores triggers the enhancement of plant Fe uptake capacity and the decrease of free metal ion concentration (DIMKPA *ET AL.*, 2008). Fe is a micronutrient and its low concentration in soil is

necessary to support robust plant and fungal life due to its low solubility under metal stress. Plants acquire sufficient Fe through three mechanisms, 1: (Fe solubilization by all dicots and monocots via rhizosphere acidification) 2: Secretion of phytosiderophores (PSs) and uptake Fe³⁺-PS, 3: uptake Fe³⁺-microbial siderophores by plants). Many studies have shown that PSs are able to solubilize and transport metals by chelation, and thus being secreted into the rhizosphere through a potassium-mugenic acid symporter (SAKAGUCHI *ET AL.*, 1999).

METAL IMMOBILIZATION

Some AM fungi also reduce plant metal uptake or translocation to aerial plant parts by decreasing metal bioavailability in soil via precipitation, alkalization, and complexation processes.

a) Alkalinization

Some AMF exhibit the ability to absorb metals through substratum alkalization activity by release of OH⁻, therefore affecting the metal stability in soil (Büdel *et al.*, 2004) and a reduction in metal phytoavailability in the rhizosphere by secreting glomalin (GIASSON *ET AL.*, 2008). AMF can act as metal sinks to reduce the mobile and available metal cations in soil suitable environment for plants growth in metal contaminated soils (Göhre and PASZKOWSKI, 2006) (Fig 3).

b) Complexation

The excretion of EPSs by plant associated (HOU *ET AL.*, 2013) metal biosorption onto to EPS include metal ion exchange, complexation with negatively charged functional groups, adsorption and precipitation (ZHANG *ET AL.*, 2006). AMF can reduce metal mobility in soil by excreting glomalin (insoluble metal-sorbing glycoprotein) that reduces metal mobility or sequesters metals and metal biostabilization in soil (VODNIK *ET AL.*, 2008) (Fig 3).

Glomalin-related soil proteins (GRSP) are sequestering Pb (0.21–1.78%) and Cd (0.38–0.98%) in an *in situ* field experiment consisted humic and fulvic acids in soil (WU *ET AL.*, 2014).

c) *Klebsiella planticola* precipitated cadmium (SHARMA *ET AL.*, 2000).

METAL TRANSPORT AND DISTRIBUTION

Different metals are differently mobile rate within a plant, *e.g.*, Cd and Zn are more mobile than Cu and Pb. The metal chelation with ligands (*e.g.*, organic acids, amino acids, and thiols) facilitates the metal movement from roots to shoots (ZACCHINI *ET AL.*, 2009). Due to the high cation exchange capability of the xylem cell, the metal movement is severely retarded when the metals are not chelated by ligands (SHENG *ET AL.*, 2008)

The future research should be focused on: (1) the mechanism of plant-microbe-metal interactions under stressful environmental conditions; (2) the effectiveness of co-inoculation of PGPB and AMF response to multiple biotic and/or abiotic stress; (3) the identification of functional genes of beneficial microbes for growth enhancement and metal metabolism; (4) the optimization of techniques for application in large scale polluted fields; and (5) the exploration of commercial production of bioinoculants for use in metal decontamination.

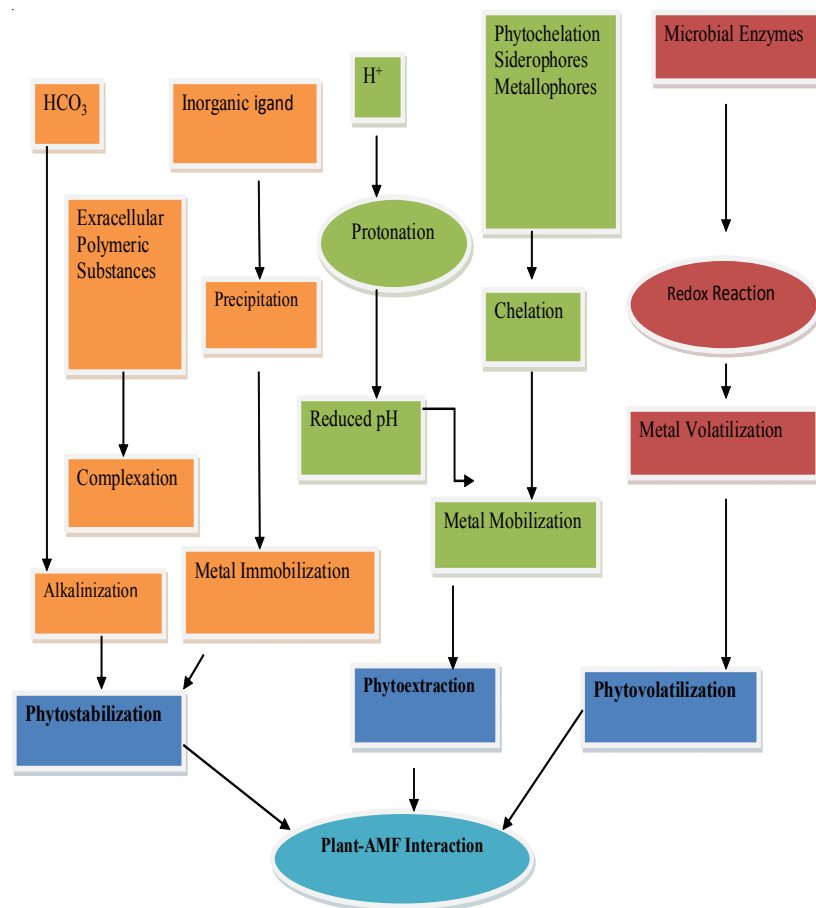


Fig. 3. Mechanism of heavy metals detoxification.

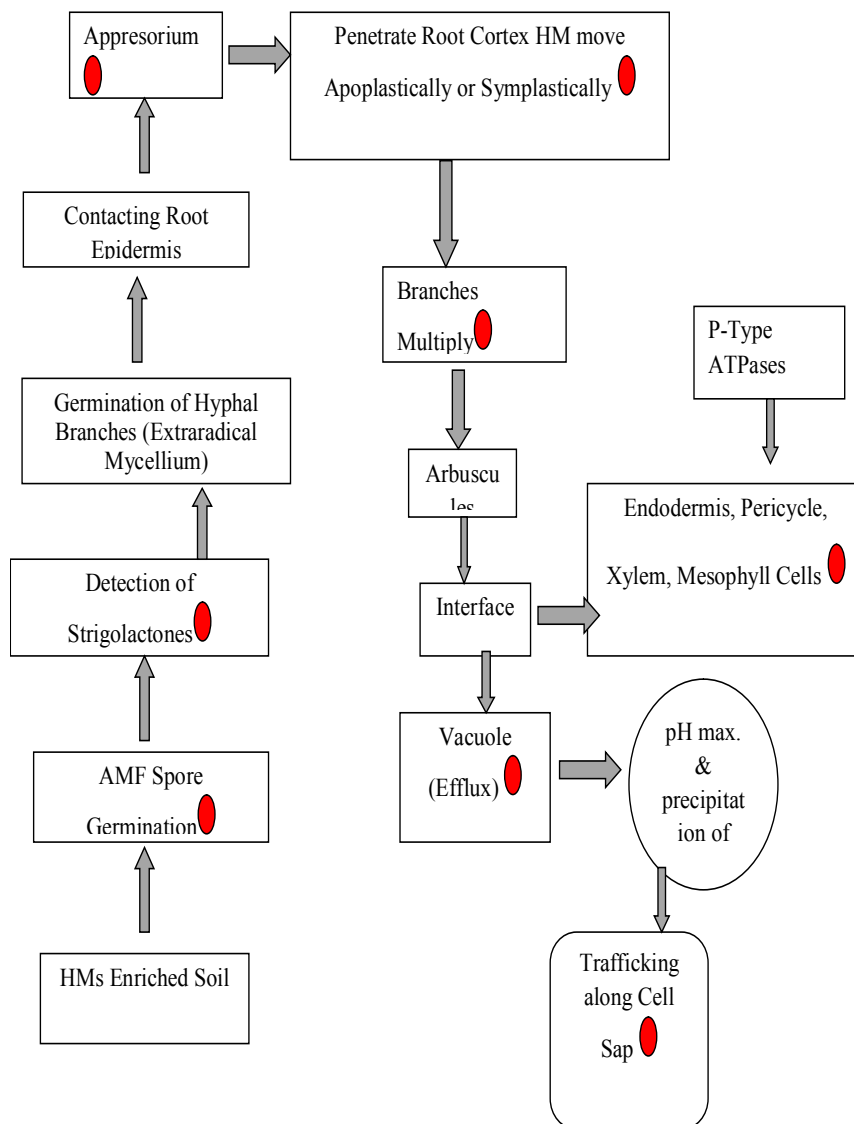


Fig. 4. Pathway for movement of heavy metals from soil to phytohyperaccumulators.

Table 2. Examples of AMF in HM's detoxification (literature citation).

| Host Plant | Colonizing AMF | Soil Condition | Mechanism | Experimental Area | Reference |
|--|---|---|---|----------------------------------|--------------------------|
| Sunflower | <i>Rhizophagus irregularis</i> metal mobilization or immobilization | Soils contaminated with three different Cd concentrations | Phytoextraction of Cd | Greenhouse | (MA ET AL., 2011). |
| Sunflower | <i>Funneliformis mosseae</i> | HM Contaminated Soil | Phytostabilization of Cd and Zn | In Field | (MA ET AL., 2011). |
| <i>Litchi chinensis</i> | <i>Glomus intraradices</i> and <i>Gigaspora margarita</i> | HM Contaminated Soil | Increased endogenous phytohormones level (IAA and isopentenyl adenosines), Plant Growth | In Field | (YAO ET AL., 2005) |
| | <i>Aspergillus</i> and <i>Rhizopus</i> | HM Contaminated Soil | Biosorption of Cr and Cd | In Field | (ZAFAR ET AL., 2007) |
| Yeast | AMF <i>Tuber melanosporum</i> | HM Contaminated Soil | Polypeptides MTs (TmelMT) and PC synthase (TmelPCS) enhanced tolerance to essential (Cu and Zn) and non-essential (Cd, As, and Hg), thiophilic metal ions | In Field | (BOLCHI ET AL., 2011) |
| <i>S. alfredii</i> , <i>Lolium perenne</i> | AMF <i>Glomus caledonium</i> and <i>G. mosseae</i> | Cd-contaminated acidic soil | Decreased soil DTPA-extractable Cd by 21–38% via alkalization process | In Field | (HU ET AL., 2013) |
| <i>Salix viminalis</i> <i>S. caprea</i> | <i>Hebeloma crustuliniforme</i> ectomycorrhiza associated bacteria <i>Micrococcus luteus</i> and <i>Sphingomonas</i> sp | | Cd 53 and 62%, respectively and Zn | In Field | (ZIMMER ET AL., 2009) |
| <i>Salix caprea</i> | fungus <i>Cadophora finlandica</i> inoculation with the rhizobacteria <i>Streptomyces</i> sp. and <i>Agromyces</i> sp. | Cd and Zn | Higher translocation of metals from roots to shoots | In Field | (SUSANNA ET AL., 2011) |
| <i>Sorghum bicolor</i> | <i>Glomus intraradices</i> and <i>G. spurgum</i> (AMF) | Cu- and Zn-contaminated soil | (Phytoremediation) reduced Cu and Zn under elevated Cu-Zn conditions, increased Cu and Zn uptake and translocation under normal Cu-Zn conditions | In Field | (TOLER ET AL., 2005) |
| <i>Thlaspi</i> ssp. <i>Medicago truncatula</i> | Gene- Sy167 (Intra and extraradical mycelia of <i>Glomus intraradices</i>) | Cd, Cu or Zn contaminated soil | Metallothionein (90 kD) and glutathione S-transferase- alleviation of damage caused by reactive oxygen species | In Field/ Breinigerberg soil | (ULRICH ET AL., 2007) |
| Tomato | <i>Glomus intraradices</i> | "Breinigerberg" soil-Zn | Reduction in Zn uptake | In Field | (FOUAD ET AL., 2005) |
| <i>Thlaspi praecox</i> | | Highly Cd, Zn and Pb contaminated substrate | Elevated nutrient demands on heavy metal uptake and tolerance | Greenhouse experiment | (KATARINA ET AL., 2006) |
| <i>Thymus polytrichus</i> | (AM) fungi | Zn contamination | Enhancing plant uptake of P and Zn | River South Tyne, United Kingdom | (WHITFIELD ET AL., 2004) |

Table 2 (cont.)

Table 2 (conclusion)

| | | | | | |
|--|---|-------------------------------------|--|------------------------------|---------------------------|
| <i>Deschampsia flexuosa</i> (C ₃ grass) | AMF soil biota | Zn contamination | Reduced Zn concentrations in shoots of <i>D. flexuosa</i> | In Field | (SYDNEY AND BRENDA, 2012) |
| <i>Sorghastrum nutans</i> (C ₄ grass) | AMF + Soil microbes | low-contaminated (LC) soil | Increased the efficacy of AMF from LC soils but decreased the efficacy of AMF from HC soils in promoting plant growth. | In Field | (SYDNEY AND BRENDA, 2012) |
| <i>Phytolacca mericana</i> , <i>Rehmannia glutinosa</i> <i>Perilla frutescens</i> <i>Litsea cubeba</i> <i>Dysphania ambrosioides</i> | AMF communities | Heavily polluted rhizospheric soils | (<i>Glomus</i> show nil effect on <i>D. ambrosioides</i>), <i>Kuklospora</i> and <i>Ambispora</i> show <i>D. ambrosioides</i> and the rhizosphere of <i>P. americana</i> . | Dabaoshan Mine region, China | (LIANG ET AL., 2010) |
| Spore germination, presymbiotic hyphal extension, symbiotic extraradical mycelium expansion and sporulation | <i>Glomus etunicatum</i> and <i>Glomus intraradices</i> | Cd, Pb, and Zn | <i>G. etunicatum</i> more sensitive than <i>G. intraradices</i> | in vitro | (TERESA AND IRIS, 2004) |
| <i>Aster tripolium</i> | <i>Glomus geosporum</i> | Cd and Cu concentrations | AMF enhance metal accumulation in the root system of <i>A. tripolium</i> | In Lab | (LUIS ET AL., 2006) |
| <i>Aster tripolium</i> | Contaminated Soil | AMF | AMF clearly increased Cd and Cu accumulation | In Field | (CARVALHO ET AL., 2006) |

SUMMARY

Plants and fungi coexist in serpentine soil (enrich with heavy metals) and their cohesive interactions play a vital role in metalliferous soil. Metallophytes colonized by AMF helps to alleviate the toxicity of metalliferous environments. In this work phytohyperaccumulator-AMF interaction heavy metals (HMs) stress called biology-based technology is examined. Phytohyperaccumulator includes phytovolatilization, phytostabilization, phytoextraction, chelate-enhancement strategy. AMF enhance the efficiency of hyperaccumulator by different mechanism. Acidification, precipitation, chelation, complexation, redox reactions, phytoremediation, detoxification, mobilization, immobilization, transformation, transport and distribution in hyperaccumulating plants helps to clean up metal contaminated soil through extraction (phytoextraction), stabilization (phytostabilization), and transformation (phytoextraction) processes.

KEYWORDS: Arbuscular; mycorrhizal fungi; glomalin; detoxification

SUMÁRIO

Plantas e fungos coexistem em solos serpentinos (ricos em metais pesados) e suas interações têm um papel fundamental nesses solos metalíferos. Metalófitos colonizados por fungos arbusculares micorrizais auxiliam a aliviar a toxicidade dos ambientes metalíferos. Neste trabalho, as interações de hiperacumuladores, FAM (fungos arbusculares micorrizais), que se desenrolam em condições de estresse por metais pesados, e que constituem uma parte da biologia tecnológica, são discutidas. Essas interações incluem estratégias de: fitovolatilização, fito-estabilização, fito-extração, *aprimoramento* quelático. FAM confere eficiência aos hiperacumuladores por meio de diferentes mecanismos. *E. g.*, acidificação, precipitação, quelação, complexificação, reações-redox, fitoremediação, detoxicação, mobilização, imobilização, transformação, transporte, distribuição, *i.e.*, processos que em plantas acumulantes ajudam a limpar a contaminação metálica mediante mecanismos de extração e estabilização.

PALAVRAS-CHAVE: Arbuscular; fungos micorrizais; glomalina; detoxificação

BIBLIOGRAPHY

- AHEMAD, M.; M. KIBRET. 2014. Mechanisms and applications of plant growth promoting rhizobacteria: current perspective. *J. King Saud Univ. Sci* 26: 1–20. 10.1016/j.jksus.2013.05.001.
- ALFORD, E. A.; E. A. H. PILON-SMITS; M. PASCHKE. 2010. Metallophytes – a view from the rhizosphere. *Plant Soil* 337: 33–50. 10.1007/s11104-010-0482-3.
- AUDET, P.; C. CHAREST. 2006. Effects of AM colonization on “wild tobacco” plants grown in zinc-contaminated soil. *Mycorrhiza* 16: 277–283.
- BADRI, D. V.; T. L. WEIR; D. VAN DER LELIE; J. M. VIVANCO. 2009. Rhizosphere chemical dialogues: plant-microbe interactions. *Curr. Opin. Biotechnol* 20: 642–650. 10.1016/j.copbio.2009.09.014.

- BAILLY, A.; L. WEISSKOPF. 2012. The modulating effect of bacterial volatiles on plant growth: current knowledge and future challenges. *Plant Signal Behavior* 7: 79–85. 10.4161/psb.7.1.18418.
- BARCELO, J.; C. POSCHENRIEDER. 1990. Plant water relations as affected by heavy metal stress: a review. *Journal of Plant Nutrition* 13: 1–37.
- BUEDEL, B.; B. WEBER; M. KUHL; H. PFANZ; D. SULTEMEYER; D. WESSELS. 2004. Reshaping of sandstone surfaces by cryptoendolithic cyanobacteria: bioalkalization causes chemical weathering in arid landscapes. *Geobiology* 2: 261–268. 10.1111/j.1472-4677.2004.00040.x.
- BUCKING, H. 2011. *Ectomycoremediation: An Eco-friendly Technique for the Remediation of Polluted Sites*. In: Rai M, Varma A (eds.) Diversity and Biotechnology of Ectomycorrhizae. *Soil Biology*. Berlin, Heidelberg: Springer. pp 209-29.
- CAI, Y.; L. Q. MA (eds). 2002. *Metal tolerance, accumulation, and detoxification in plants with emphasis on arsenic in terrestrial plants*, in *Biogeochemistry of Environmentally Important Trace Elements* (Washington, DC: American Chemical Society). 95–114.
- CARRIER, P.; A. BARYLA; M. HAVAUX. 2003. Cadmium distribution and microlocalization in oilseed rape (*Brassica napus*) after long-term growth on cadmium-contaminated soil. *Planta*. 216: 939–950.
- CARVALHO, S. M.; I. CAÇADOR; M. A. MARTINS-LOUÇÃO. 2006. Arbuscular mycorrhizal fungi enhance root cadmium and copper accumulation in the roots of the salt marsh plant *Aster tripolium* L. *Plant and Soil*. 285: 161–169.
- CARAVACA, F.; A. M. DEL MAR; P. TORRES; A. ROLDAN. 2005. Microbial activities and arbuscular mycorrhizal fungi colonization in the rhizosphere of the salt marsh plant *Inula crithmoides* L. Along a spatial salinity gradient. *Wetlands* 25: 350–355.
- CHARPENTIER, M.; R. BREDEMEIER; G. WANNER; N. TAKEDE; E. SCHLEIFF; M. PARNISKE. 2008. *Lotus japonicus* CASTOR and POLLUX are Ion Channels Essential for Perinuclear Calcium Spiking in Legume Root Endosymbiosis. *Plant Cell* 20: 3467-79.
- CHAPARRO, J. M.; D. V. BADRI; M. G. BAKKER; A. SUGIYAMA; D. K. MANTER; J. M. VIVANCO. 2013. *Root exudation of phytochemicals in Arabidopsis follows specific patterns that are developmentally programmed and correlate with soil microbial functions*. *PLoS ONE* 8:e55731 10.1371/journal.pone.0055731.

- CHOI, Y. E.; E. HARADA; G. H. KIM; E. S. YOON; H. SANO. 2004. Distribution of elements on tobacco trichomes and leaves under cadmium and sodium stresses. *Journal of Plant Biology* 47: 75–82.
- CHOJMACKA, K. 2010. Biosorption and bioaccumulation – the prospects for practical applications. *Environ. Int* 36: 299–307. 10.1016/j.envint.2009.12.001.
- CLEMENS, S. 2001. Molecular mechanism of plant metal tolerance and homeostasis. *Planta* 212: 475 – 486.
- COBBETT, C. 2003. Heavy metals and plants – model systems and hyperaccumulators. *New Phytologist* 159: 289–293.
- COMPANT, S; C. CLEMENT; A. SESSITSCH. 2010. Plant growth-promoting bacteria in the rhizo- and endosphere of plants: their role, colonization, mechanisms involved and prospects for utilization. *Soil Biol. Biochem* 42: 669–678. 10.1016/j.soilbio.2009.11.024.
- COMPANT, S; B. MITTER; J. G. COLLII-MULLL; H. GANGLE; A. SESSITSCH. 2011. Endophytes of grapevine flowers, berries, and seeds: identification of cultivable bacteria, comparison with other plant parts, and visualization of niches of colonization. *Microb. Ecol* 62: 188–197. 10.1007/s00248-011-9883-y.
- DIMKPA, C. O; A. SVATOS; P. DABROWSKA; A. SCHMIDT; W. BOLAND; E. KOTHE. 2008. Involvement of siderophores in the reduction of metal-induced inhibition of auxin synthesis in *Streptomyces* spp. *Chemosphere* 74: 19–25. 10.1016/j.chemosphere.2008.09.079.
- ELANGOVAN, R; S. AVISPA; B. ROHIT; P. LIGY; K. CHANDRARAJ. 2005. Reduction of Cr (VI) by a *Bacillus* sp. *Biotechnology Letter* 28: 247–252. 10.1007/s10529-005-5526-z.
- FINLEY, R. D. 2008. Ecological Aspects of Mycorrhizal Symbiosis: With Special Emphasis on the Functional Diversity of Interactions Involving the Extraradial Mycelium. *Journal of Experimental Botany* 59 (5): 1115-26.
- FOUAD, O; H. ULRICH; S. EMON; B. HERMANN. 2005. Differential gene expressions in arbuscular mycorrhizal-colonized tomato grown under HEAVY METAL STRESS. *JOURNAL OF PLANT PHYSIOLOGY* 162 (6): 634-649.
- FOX, D. I; T. PICHLER; D. H. YEH; N. A. ALCANTAR. 2012. Removing heavy metal in water: the interactions of cactus mucilage and arsenate (As (V)). *Environmental Science and Technology* 46: 4553–4559.
- GAONKAR, T; S. BHOSLE. 2013. Effect of metals on a siderophore producing bacterial isolate and its implications on microbial assisted bioremediation of metal contaminated soils. *Chemosphere* 93: 1835–1843. 10.1016/j.chemosphere.2013.06.036.

- GADD, G. M. 2004. Microbial influence on metal mobility and application for bioremediation. *Geoderma* 122: 109–119. 10.1016/j.geoderma.2004.01.002.
- GHANEM, M. E.; R. M. HAN; B. CLASSEN. 2010. Mucilage and polysaccharides in the halophyte plant species *Kosteletzkya virginica*: localization and composition in relation to salt stress. *Journal of Plant Physiology* 167: 382–392.
- GERBI, H.; K. MARKMANN; S. SVISTONOFF; J. ESTEVAN; D. AUTRAN; G. GICZEU; F. AUGUY; B. PERET; L. LAPLAZE; C. FRANCHE; M. PARNISKE; D. BOGUSE. 2008. SymRK Defines a Common Genetic Basis for Plant Root Endosymbioses with Arbuscular Mycorrhizal Fungi, Rhizobia, and Frankia Bacteria. *Proceedings of the National Academy of Sciences, U.S.A.* 105: 4928-32.
- GIASSON, P.; A. KARAM; A. JAOUICH. 2008. Arbuscular mycorrhizae and alleviation of soil stresses on plant growth, in *Mycorrhizae: sustainable agriculture and forestry*, eds Siddiqui Z. A., Akhtar M. S., Futai K., editors. (Dordrecht: Springer). 99–134. 10.1007/978-1-4020-8770-74.
- GLICK, B. R. 2010. Using soil bacteria to facilitate phytoremediation. *Biotechnology Advancement*: 367–374. 10.1016/j.biotechadv.2010.02.001.
- GONZÁLEZ-GUERRERO, M.; C. AZCO; N. AGUILAR; N. FERRO. 2006. *GintABCI* and *GintMTI* are involved in Cu and Cd homeostasis in *Glomus intraradices*. In: Abstracts of the 5th International Conference on Mycorrhiza, Granada, Spain.
- GONZÁLEZ-GUERRERO, M.; C. AZCO; N. AGUILAR; M. MOONEY; A. VALDERAS; C. W. MAC DIARMID; D. J. EIDE; N. FERRO. 2005. Characterization of a *Glomus intraradices* gene encoding a putative Zn transporter of the cation diffusion facilitator family. 42: 130–140.
- GONZÁLEZ-GUERRERO, M.; C. AZCO; N. AGUILAR; S. F. WRIGHT; K. A. NICOLS. 2004. The role of glomalin, a protein produced by arbuscular mycorrhizal fungi, in sequestering potentially toxic elements. *Environmental Pollution* 130: 317–323. 10.1016/j.envpol.2004.01.004.
- GOHRE, V.; U. PASZKOWSKI. 2006. Contribution of the arbuscular mycorrhizal symbiosis to heavy metal phytoremediation. *Planta* 223: 1115–1122. 10.1007/s00425-006-0225-0.
- GUO, Y.; W. BUNDITHYA; P. B. GOLDSBROUGH. 2003. Characterization of the metallothionein gene family: tissue-specific expression and induction

- during senescence and in response to copper. *New Phytologist* 159: 369–381.
- GUO, W.; R. X. ZHAO; W. J. ZHAO; R. Y. FU; J. Y. N. GUO BI. 2013. Effects of arbuscular mycorrhizal fungi on maize (*Zea mays* L.) and sorghum (*Sorghum bicolor* L. Moench) grown in rare earth elements of mine tailings. *Appl. Soil Ecol.* 72: 85–92. 10.1016/j.apsoil.2013.06.001.
- HALL, J. L. 2002. Cellular mechanism of heavy metal detoxification and tolerance. *Journal of Experimental Botany* 53(366): 1 – 11.
- HARRIS D. L.; B. G. LOTTERMOSE. 2006. Evaluation of phosphate fertilizers for ameliorating acid mine waste. *Applied Geochemistry* 21: 1216–1225. 10.1016/j.apgeochem.2006.03.009.
- HOGKAMP, C.; D. ARNDT; P. A. PEREIRA; J. D. BECKER; N. HOHNJEC; H. KUSTER. 2011. Laser Microdissection Unravels Cell-type-specific Transcription in Arbuscular Mycorrhizal Roots, Including CAAT-Box Transcription Factor Gene Expression Correlating with Fungal Contact and Spread. *Plant Physiology* 157 (4): 2023-43.
- JAVED, M. T.; E. STOLTZ; S. LINDBERG; M. GREGER. 2013. Changes in pH and organic acids in mucilage of *Eriophorum augustifolium* roots after exposure to elevated concentrations of toxic elements. *Environmental Science and Pollution Research* 20: 1876–1880.
- JEONG, S.; M. H. SUN; D. SHIN; K. NAM. 2013. Survival of introduced phosphate-solubilizing bacteria (PSB) and their impact on microbial community structure during the phytoextraction of Cd-contaminated soil. *Journal of Hazardous Material* 263: 441–449. 10.1016/j.jhazmat.2013.09.062.
- JOHANSSON, E. M.; P. M. A. FRANSSON; R. D. FINLAY VAN; P. A. W. HEES. 2008. Quantitative analysis of exudates from soil-living basidiomycetes in pure culture as a response to lead, cadmium and arsenic stress. *Soil Biology and Biochemistry* 40: 2225–2236. 10.1016/j.soilbio.2008.04.016.
- KALDORF, M.; A. KUHN; W. H. SCHRODER; U. HILDEBRANDT; H. BOTHE. 1999. Selective element deposits in maize colonized by a heavy metal tolerance conferring arbuscular mycorrhizal fungus. *Journal of Plant Physiology* 154: 718–728. 10.1016/S0176-1617(99)80250-8.
- KATARINA, V. M.; P. PAULA; K. PETER; N. MARIJANA; R. MARIJANA. 2006. Colonisation of a Zn, Cd and Pb hyperaccumulator *Thlaspi praecox* Wulfen with indigenous arbuscular mycorrhizal fungal mixture induces changes in heavy metal and nutrient uptake. *Environmental Pollution* 139 (2): 362-371.

- KATSCHNIG, D.; R. BROEKMAN; J. ROZEMA. 2013. Salt tolerance in the halophyte *Salicornia dolichostachya* Moss: growth, morphology and physiology. *Environmental and Experimental Botany* 92: 32–42.
- KISTNER, C.; T. WINZER; A. PITZCHKE; L. MULDER; S. SATO; T. KANEKO; S. TABATA; N. SANDAL; J. STOUGAARD; K. J. WEBB; K. SZCZYGLOWSKI; M. PARNISKE. 2005. Seven *Lotus japonicus* Genes Required for Transcriptional Reprogramming of the Root during Fungal and Bacterial Symbiosis. *Plant Cell* 17 (8): 2217-29.
- LAROSE, G.; R. CHENEVERT; P. MOUTOGLIS; S. GAGNE; Y. PICHE; H. VIERHEILING. 2002. Flavonoid levels in roots of *Medicago sativa* are modulated by the developmental stage of the symbiosis and the root colonizing arbuscular mycorrhizal fungus. *Journal of Plant Physiology* 159:1329–1339. 10.1078/0176-1617-00896.
- LSKSHMANRAJ, L.; A. GURUSAMY; M. B. GOBINATH; R. CHANDRAMOHAN. 2009. Studies on biosorption of hexavalent chromium from aqueous solutions by using boiled mucilaginous seeds of *Ocimum americanum*. *Journal of Hazardous Materials*. 169: 1141–1145.
- LEBEAU, T.; A. BRAUD; K. JEZEQUEL. 2008. Performance of bioaugmentation-assisted phytoextraction applied to metal contaminated soils: a review. *Environmental Pollution* 153: 497–522. 10.1016/j.envpol.2007.09.015.
- LEITENMAIER, B.; H. KUPPER. 2013. Compartmentation and complexation of metals in hyperaccumulator plants. *Frontier Plant Science* 4: 374 10.3389/fpls.2013.00374.
- LIANG, K.; Q. LONGA; J. YAQB; R. GUOA; Y. HENG; H. YONG; H. HUANGA; Z. HUI. 2010. Molecular community analysis of arbuscular mycorrhizal fungi associated with five selected plant species from heavy metal polluted soils. *European Journal of Soil Biology*. 46 (5):288-294.
- LIAO, J. P.; X. G. LIN; Z. H. CAO; Y. Q. SHI; M. H. WONG. 2003. Interactions between arbuscular mycorrhizae and heavy metals under sand culture experiment. *Chemosphere* 50: 847–853.
- LI, T.; Q. TAO; C. LIANG; M. J. SHOHAG; X. YANG; D. L. SPARKS. 2013. Complexation with dissolved organic matter and mobility control of heavy metals in the rhizosphere of hyperaccumulator *Sedum alfredii*. *Environmental Pollution* 182: 248–255. 10.1016/j.envpol.2013.07.025.
- LOMBI, E.; W. W. WENZEL; G. R. GOBRAN; D. C. ADRIANOD. 2001. Dependency of phytoavailability of metals on indigenous and induced rhizosphere processes: a review. In: Gobran GR, Wenzel WW, Lombi E, editors. , eds. Trace elements in the rhizosphere. Boca Raton, FL: CRC Press, 3–24.

- LUIS, M. C.; C. ISABEL; M. L. AMELIA; M. C. LUIS. 2006. Arbuscular mycorrhizal fungi enhance root cadmium and copper accumulation in the roots of the salt marsh plant *Aster tripolium* L. *Plant and Soil* 285: (1-2): 161-169.
- MA, Y.; M. RAJKUMAR; I. ROCHA; R. S. OLIVEIRA; H. FRREITAS. 2015. Serpentine bacteria influence metal translocation and bioconcentration of *Brassica juncea* and *Ricinus communis* grown in multi-metal polluted soils. *Frontier Plant Science* 5: 757 10.3389/fpls.2014.00757.
- MA, Y.; M. RAJKUMAR; I. ROCHA; R. S. OLIVEIRA; H. FREITAS. 2013. Phytoextraction of heavy metal polluted soils using *Sedum plumbizincicola* inoculated with metal mobilizing *Phyllobacterium myrsinacearum* RC6b. *Chemosphere* 93:1386-1392. 10.1016/j.chemosphere.2013.06.077.
- MAGDZIAK, Z.; M. KOZLOWSKA; Z. KACZMAREK; M. MLECZEK; T. CHADZINIKOLAU; K. DRZEWIECKA ET AL. 2011. Influence of Ca/Mg ratio on phytoextraction properties of *Salix viminalis*. II. Secretion of low molecular weight organic acids to the rhizosphere. *Ecotoxicology Environment Safe* 74: 33-40. 10.1007/s00468-012-0821-5.
- MANDAL, S. M.; D. CHAKRABORTY; S. DEY. 2010. Phenolic acids act as signaling molecules in plant-microbe symbioses. *Plant Signal Behavior* 5: 359-368. 10.4161/psb.5.4.10871.
- MAILLET, F.; V. POINSOT; O. ANDRE; V. PUECH-PAGES; A. HAOUY; M. GUEUNIER; L. CROMER; D. GIRAUDET; D. FORMEY; A. NIEBEL; E. A. MARTINEZ; H. DIRGUEZ; G. BECARD; J. DENARIE. 2011. Fungal Lipochitooligosaccharide Symbiotic Signals in Arbuscular Mycorrhiza. *Nature* 469: 58-63.
- MEIR, S.; R. AZCON; P. CARTES; F. BORIE; P. CORNEJO. 2011. Alleviation of Cu toxicity in *Oenothera picensis* by copper-adapted arbuscular mycorrhizal fungi and treated agro wasted residue. *Applied Soil Ecology* 48: 117-124. 10.1016/j.apsoil.2011.04.005.
- MILTTLER, R. 2002. Oxidative stress, antioxidants and stress tolerance. *Trends Plant Sciences* 7: 405-410.
- MIYASAKA, S. C.; M. C. HAWES. 2001. Possible role of root border cells in detection and avoidance of aluminium toxicity. *Plant Physiology*. 125: 1978-1987.
- MULLIGAN, C. N.; R. GALVEZ-CLOTIER. 2003. Bioremediation of metal contamination. *Environment Monitoring Assess.* 84: 45-60. 10.1023/A:1022874727526.
- NAVARRO-NOVA, Y. E.; E. HERNANDEZ-MENDOZA; J. MORALES-JIMENEZ; J. JAN-ROBLERO; E. MARTINEZ-ROMERO; C. HERNANDEZ-RODRIGUEZ. 2012. Isolation and characterization of nitrogen fixing heterotrophic bacteria

- from the rhizosphere of pioneer plants growing on mine tailings. *Applied Soil Ecology* 62: 52–60. 10.1016/j.apsoil.2012.07.011.
- PATHAK, A.; M. G. DASTIDAR; T. R. SREEKRISHNAN. 2009. Bioleaching of heavy metals from sewage sludge: a review. *Journal of Environmental Management* 90: 2343–2353. 10.1016/j.jenvman.2008.11.005.
- ORLOWSKA, E.; W. PRZYBYLOWICZ; D. ORLOWSKI; N. P. MONGWAKETSI; K. TURNAU; J. MESJASZ-PRZYBYLOWICZ. 2013. Mycorrhizal colonization affects the elemental distribution in roots of Ni-hyperaccumulator *Berkheya coddii* Roessler. *Environmental Pollution* 175: 100–109. 10.1016/j.envpol.2012.12.028.
- PAL, R.; J. P. N. RAI. 2009. Phytochelatins: Peptides Involved in Heavy Metal Detoxification. *Appl Biochem Biotech*. DOI. 10.1007/s12010-009-8565-4.
- PANDA, S. K. 2008. Impact of copper on reactive oxygen species, lipid peroxidation and antioxidants in *Lemna minor*. *Biol Plant* 52 (3): 561-564.
- PARNIZKE, M. 2008. Arbuscular Mycorrhiza: The Mother of Plant Root Endosymbioses. *Nature Reviews Microbiology* 6: 763-75.
- PEREZ-ESTEBAN, J.; C. ESCOLASTICO; A. MOLINER; A. MASAGUER. 2013. Chemical speciation and mobilization of copper and zinc in naturally contaminated mine soils with citric and tartaric acids. *Chemosphere* 90 276–283. 10.1016/j.chemosphere.2012.06.065.
- RAJKUMAR, M.; S. SANDHYA; M. N. V. PRASAD; H. FREITAS. 2012. Perspectives of plant-associated microbes in heavy metal phytoremediation. *Biotechnology Advancement* 30: 1562–1574. 10.1016/j.biotechadv.2012.04.011.
- RICHARDS, B. K.; T. S. STEENHUIS; J. H. PEVELY; M. B. McBRIDE. 2000. Effect of sludge-processing mode, soil texture and soil pH on metal mobility in undisturbed soil columns under accelerated loading. *Environmental Pollution* 109: 327–346. 10.1016/S0269-7491(99)00249-3.
- RIVERA-BECERRIL, F.; D. VAN TUINEN; F. MARTIN-LAURENT; A. METWALLY; K. J. DIETZ; S. GIANINAZZI *et al.* (2005). Molecular changes in *Pisum sativum* L. roots during arbuscular mycorrhiza buffering of cadmium stress. *Mycorrhiza* 16: 51–60. 10.1007/s00572-005-0016-7.
- REINHOLD-HUREK, B.; T. HUREK. 2011. Living inside plants: bacterial endophytes. *Curr. Opin. Plant Biol.* 14: 435–443. 10.1016/j.pbi.2011.04.004.
- SAKAGUCHI, T.; N. K. NISHIZAWA; H. NAKANISHI; E. YOSHIMURA; S. MORI. 1999. The role of potassium in the secretion of mugineic acids family

- phytosiderophores from iron-deficient barley roots. *Plant Soil* 215: 221–227. 10.1023/A:1004546112140.
- SESSITSCH, A.; M. KUFFNER; P. KIDD; J. VANGRONSVELD; W. W. WENZEL; K. FALLMANN *et al.* (2013). The role of plant-associated bacteria in the mobilization and phytoextraction of trace elements in contaminated soils. *Soil Biology and Biochemistry* 60: 182–194. 10.1016/j.soilbio.2013.01.012.
- SARRET, G.; E. HARADA; Y. E. CHOI *et al.* 2006. Trichomes of tobacco excrete zinc as zinc-substituted calcium carbonate and other zinc-containing compounds. *Plant Physiology* 141: 1021–1034.
- SCERVINO, J. M.; M. A. PONCE; R. ERRA-BASELLS; H. VIERHEILING; J. A. OCAMPO; A. GODEAS. 2005. Arbuscular mycorrhizal colonization of tomato by *Gigaspora* and *Glomus* species in the presence of root flavonoids. *Journal of Plant Physiology* 162: 625–633. 10.1016/j.jplph.2004.08.010.
- SHARMA, P. K.; D. L. BALVILL; A. FRENKEL; M. A. VAIRAVAMURTHY. 2000. A new *Klebsiella* planticola strain (Cd-1) grows anaerobically at high cadmium concentrations and precipitates cadmium sulfide. *Appl. Environ. Microbiology* 66: 3083–3087. 10.1128/AEM.66.7.3083-3087.2000.
- SHENG, X. F.; L. Y. HE; Q. Y. WANG; H. S. YE; C. JIANG. 2008. Effects of inoculation of biosurfactant producing *Bacillus* sp. J119 on plant growth and cadmium uptake in a cadmium amended soil. *Journal of Hazardous Mater* 155: 17–22. 10.1016/j.jhazmat.2007.10.107.
- SHEORAN, V.; A. S. SHEORAN; P. POONIA. 2011. Role of hyperaccumulators in phytoextraction of metals from contaminated mining sites, A review. *Critical Review Environmental, Science and Technology* 41: 168–214. 10.1080/10643380902718418.
- SIEVERDING, E. 1991. *Vesicular-arbuscular mycorrhiza management intropical agrosystems*. Technical Cooperation, Federal Republic of Germany Eschborn. ISBN 3-88085-462.
- SOLANKI, R.; R. DHANKHAR. 2011. Biochemical changes and adaptive strategies of plants under heavy metal stress. *Biologia* 66: 195–204. 10.2478/s11756-011-0005-6.
- STANLEY, L.; L. ISABELLE. 2015. How can we take advantage of halophyte properties to cope with heavy metal toxicity in salt-affected areas. *Annals Botany* 115 (3): 509–528. doi: 10.1093/aob/mcu264.
- STEINKELLNER, S.; V. LENDZEMO; I. LANGER; P. SCHWEIGER; T. KHAOSAAD; J. P. TOUSSAINT *et al.* 2007. Flavonoids and strigolactones in root exudates as signals in symbiotic and pathogenic plant–fungus interactions. *Molecules* 12: 1290–1306. 10.3390/12071290.

- SUAREZ, P.; R. REYES. 2002. Heavy metal incorporation in bacteria and its environmental significance. *Interciencia* 27: 160–172.
- SYDNEY, I. G.; B. C. BRENDA. 2012. Biotic contexts alter metal sequestration and AMF effects on plant growth in soils polluted with heavy metals. *Ecological Society of America* 93 (7): 1550–1559.
- TAYLOR, A. F. S.; I. ALEXANDER. 2005. The Ectomycorrhizal Symbiosis: Life in the Real World. *Mycologist* 19: 102-12.
- TERESA, E. P.; C. IRIS. 2004. Heavy-Metal Stress and Developmental Patterns of Arbuscular Mycorrhizal Fungi. *Environ. Microbiology* 70 (11): 6643-6649.
- TEWARI, R.; K. KUMAR; P. N. SHARMA. 2008. Morphology and physiology of zinc stressed mulberry plants. *Journal of Plant Nutrition and Soil Sciences* 171: 286–294.
- THOMAS, J. C.; H. J. BOHNERT. 1993. Salt stress perception and plant growth regulators in the halophyte *Mesembryanthemum crystallinum*. *Plant Physiology* 103: 1291–1304.
- TOLER, H. D.; J. B. MORTON; J. R. CUMMING. 2005. Growth and Metal Accumulation of Mycorrhizal Sorghum Exposed to Elevated Copper and Zinc. *Water, Air, and Soil Pollution* 164 (1-4): 155–172.
- TONIN, C.; P. VANDENKOORNHUYSE; E. J. JONER; J. STRACZEK; C. LEYVAL. 2001. Assessment of arbuscular mycorrhizal fungi diversity in the rhizosphere of *Viola calaminaria* and effect of these fungi on heavy metal uptake by clover. *Mycorrhiza* 10: 161–168.
- TURNAU, K. 1998. Heavy metal content and localization in mycorrhizal *Euphorbia cyparissias* from zinc wastes in southern Poland. *Acta Societate Botanica Polonia* 67: 105–113.
- ULLAH, A.; S. HENG; M. F. H. MUNIS; S. FAHAD; X. YANG. 2015. Phytoremediation of heavy metals assisted by plant growth promoting (PGP) bacteria: a review. *Environmental and Experimental Botany* 117: 28–40. 10.1016/j.envexpbot.2015.05.001.
- ULRICH, H.; R. MARJANA; B. HERMANN. 2007. Arbuscular mycorrhiza and heavy metal tolerance. *Phytochemistry* 68 (1): 139-146.
- ¹GRUPE DE RECHERCHE EN PHYSIOLOGIE VÉGÉTALE (GRPV) — Earth and Life Institute — Agronomy (ELI-A), Université catholique de Louvain, 4–5 (Bte 7.07.13) Place Croix du Sud, 1348 Louvain-la-Neuve, France and ²Institute of Plant Molecular Biology, Biology Centre CAS, Branišovská 31, 37005 eské Bud jovice, Czech Republic. ¹For correspondence. E-mail: eb.niavuolcu@sttul.yelnats
- VAZQUEZ, M. D.; C. POSCHENRIEDER; J. BARCELO; A. J. M. BAKER; P. HATTON; G. H. COPE. 1994. Compartmentation of zinc in roots and

- leaves of the zinc hyperaccumulator *Thlaspi caerulescens* J and C Presl. *Botanica Acta* 107: 243–250.
- VODNIK, D.; H. GRČMAN; I. MACEK; J. VAN; J. T. ELTEREN; M. KOVACEVIC. 2008. The contribution of glomalin-related soil protein to Pb and Zn sequestration in polluted soil. *Sci. Total Environ.* 392: 130–136. 10.1016/j.scitotenv.2007.11.016.
- VOGEL-MIKUS, K.; P. PONGRAC; P. KUMP; M. NECEMER; M. REGVAR. 2006. Colonisation of a Zn, Cd and Pb hyperaccumulator *Thlaspi praecox* Wulfen with indigenous arbuscular mycorrhizal fungal mixture induces changes in heavy metal and nutrient uptake. *Environmental Pollution* 139: 362–371.
- WANG, W. F.; Y. Y. ZHAI; L. X. CAO; H. M. TAN; R. D. ZHANG. 2016. Endophytic bacterial and fungal microbiota in sprouts, roots and stems of rice (*Oryza sativa* L.). *Microbiology Research* 188: 1–8. 10.1016/j.micres.2016.04.009.
- WANG, D.; H. WANG; B. HAN *et al.* 2012. Sodium instead of potassium and chloride is an important macronutrient to improve leaf succulence and shoot development for halophyte *Sesuvium portulacastrum*. *Plant Physiology and Biochemistry* 51: 53–62.
- WANG, B.; Y. L. QIU. 2006. Phylogenetic Distribution and Evolution of Mycorrhizas in Land Plants. *Mycorrhiza* 16(5): 299-363.
- WEIS, J.; P. WEIS. 2004. Metal uptake, transport and release by wetland plants: implications for phytoremediation and restoration. *Environment International* 169: 737–745.
- WEIERSBYE, I. M.; C. J. STRAKER; W. J. PRZYBYLOWICZ. 1999. MicroPIXE mapping of elemental distribution in arbuscular mycorrhizal roots of the grass, *Cynodon dactylon*, from gold and uranium mine tailings. *Nucl. Instrum. Methods Phys. Res., Sect. B* 158: 335–343.
- Whitfield, L.; A. J. Richards; D. L. Rimmer. 2004. Effects of mycorrhizal colonisation on *Thymus polytrichus* from heavy-metal-contaminated sites in northern England. *Mycorrhiza* 14 (1): 47–54.
- WU, Z. P.; K. MCGROUTHER; J. D. HUANG; P. B. WU; W. D. WU; H. L. WANG. 2014. Decomposition and the contribution of glomalin-related soil protein (GRSP) in heavy metal sequestration: field experiment. *Soil Biology and Biochemistry* 68: 283–290. 10.1016/j.soilbio.2013.10.010.
- ZACCHINI, M.; F. PIETRINI; G. S. MUGNOZZA; V. IORI; L. PIETROSANTI; A. MASSACCI. 2009. Metal tolerance, accumulation and translocation in poplar and willow clones treated with cadmium in hydroponics. *Water Air Soil Pollution* 197: 23–34. 10.1007/s11270-008-9788-7.

ZHANG, D. Y.; J. L. WANG; X. L. PAN. 2006. Cadmium sorption by EPSs produced by anaerobic sludge under sulfate-reducing conditions. *Journal of Hazardous Material* 138: 589–593. 10.1016/j.jhazmat.2006.05.092.

Recebido em 10 de janeiro de 2017.