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Role of mycorrhiza-forming fungi in the bioremediation of agricultural soils contaminated with heavy metals: a systematic review

Papel de los hongos formadores de micorrizas en la biorremediación de suelos agrícolas contaminados con metales pesados: revisión sistemática

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ABSTRACT

INTRODUCTION: the objective this research was to describe the role of mycorrhizal fungi in bioremediation processes of soils contaminated with heavy metals through a systematic review of the scientific literature.

METHODS: a systematic review of the scientific literature published between 2003 and 2014 was conducted in the databases ScienceDirect, Springer Link and EBSCO. This search produced a total of 39 original articles, filtered by inclusion and exclusion criteria. Complementary literature obtained by the search tool Google Academic was also included.

RESULTS AND CONCLUSION: the study revealed that endomycorrhyzae and ectomycorrhizae are the mycorrhizal types most frequently described in soils contaminated by heavy metals and that they have mechanisms of tolerance to stress generated by these elements which include adsorption and absorption of heavy metals, promotion of plant growth, alteration of the biochemical and physiological properties of the plant and production of metabolites.

KEY WORDS: mycorrhizae, biodegradation, environmental health, phytoremediation.

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RESUMEN

Introducción: el objetivo de esta investigación fue describir el papel de los hongos micorrízicos en los procesos de biorremediación de suelos contaminados con metales pesados a partir de una revisión sistemática de la literatura científica.

Metódos: se realizó una revisión sistemática de la literatura científica publicada entre 2003 y 2014 en las bases de datos ScienceDirect, Springer Link y EBSCO. Esta búsqueda produjo un total de 39 artículos originales, filtrados por criterios de inclusión y exclusión. Adicionalmente, se incluyó literatura complementaria obtenida mediante la herramienta de búsqueda Google Academic.

Resultados y conlusión: este estudio reveló que las endomicorrizas y las ectomicorrizas son los tipos de micorrizas más frecuentemente descritos en suelos contaminados por metales pesados y que tienen mecanismos de tolerancia al estrés generado por estos elementos, los que incluyen la absorción y absorción de metales pesados, la promoción del crecimiento de las plantas, la alteración de propiedades bioquímicas y fisiológicas de la planta y producción de metabolitos.

Palabras clave: micorrizas, biodegradación, salud ambiental, fitorremediación.

INTRODUCTION

Heavy metals occur naturally in the environment and some, such as iron (Fe), manganese (Mn), copper (Cu), zinc (Zn) and nickel (Ni) are necessary for the growth of plants as catalysts of different enzymatic reactions and oxidoreduction.[1] However, they are toxic to most organisms when present in high environmental concentrations.[2]

As well as the heavy metals of lithogenic origin, there are anthropogenic sources related to industrial and agricultural activities such as mining, metallurgy, galvanoplasty, atmospheric deposition, combustion of fossil fuels, elimination of residues, long-term application of sewage treatments, application of fertilisers, commercial biocides and irrigation with residual water.[3]

Contamination of the soil resulting from these activities gives rise to local accumulation of these heavy metals. For example, mean concentrations of Zn, Cd and Pb in uncontaminated soils are 80, 0.1-0.5 and 15 ppm respectively; however, their concentrations in contaminated soils are considerably higher: Zn:>20,000ppm, Cd:>14.000ppm and Pb:>7000ppm.[4] This type of contamination may persist for hundreds or thousands of years in the soil, even after its incorporation has been arrested. This is the case for heavy metals such as Cd, Cu and Pb which may have half-lives in soil of 15-1100, 310-1.500 and 740-5.900 years, respectively.[5]

Heavy metals can be absorbed by the roots of crops or leached into aquifers, thus contaminating underground water.[5] They are potentially phytotoxic given that they have the capacity to influence the permeability and functioning of the plasma membrane, causing oxidative stress which negatively affects different cellular components and thus the plant tissues,[1] causing weak growth, lowered productivity, reduction of nutrient absorption, metabolic disorders and, in legumes, a diminished capacity to fix molecular nitrogen.[6]

Such effects entail great losses in agricultural productivity. It is estimated that some 12 million tons of cereals contaminated with heavy metals are produced each year in China, causing direct economic losses of over three thousand million dollars.[7] Furthermore, their incorporation into the food chain poses a grave threat to human health, since most of them have been shown to be cancerigenous, even at low concentrations.[8]

Various mitigation strategies have been implemented in response to this problem; one of which (phytoremediation) exploits the capacity of plants to assimilate or accumulate heavy metals in their tissues. This constitutes an economical and sustainable alternative for the detoxification of contaminated soils. Nevertheless, there are limitations to its application. Some examples of these are: the capacity of plants to withstand the stress generated by the presence of heavy metals at high concentrations, and the solubility, mobility or availability of these elements. Certain chelating agents are currently used to increase the solubility of metals and in this way improve the rate of phytoextraction. This may produce environmental risks since these chelating agents may themselves exert toxic effects on plants and soil microorganisms, resulting in a negative impact on the stability of the soil ecosystem and its function.[9] It is therefore important to develop environmentally friendly techniques that improve the phytoextraction rate of heavy metals.

Arbuscular mycorrhizal (AM) fungi are present in the soils of most ecosystems, including those that are contaminated. They interact with the roots of more than 80% of land plants and can be considered as functional extensions of the roots of these species, considerably amplifying the volume of soil from which nutrients can be absorbed. They are also widely recognised as being involved in the improvement of plant growth in severely disturbed sites, given that they capture micronutrients and water and deliver part to their hosts thus favouring the nutritional state of the latter.[10] In the same way, heavy metals are absorbed through the fungal hyphae and can be transported to the plant. Thus, in certain cases the mycorrhized plants show a greater recruitment of heavy metals in the root and transport these to other tissues, while in others AM fungi contribute to the immobilisation in soil of these heavy metals.

Despite the great potential that this fungus-plant association could represent for the improvement of phytoremedial processes, the role of these fungi in such processes has not been clearly established although it has been described in the scientific literature. The objective of the present study was thus to describe the role of mycorrhizae-forming fungi in the remediation of heavy metals in agricultural soils to provide references for development of new sustainable alternatives which could impact positively on food-producing systems.

MATERIALS & MÉTHODS

SEARCH STRATEGY

The study was carried out in conformity with the PRISMA.[11] A systematic search of the literature was carried out in September 2014 of the databases ScienceDirect, Springer Link and EBSCO, for sensitivity using DeCS describers, for completeness using non-DeCS describers and for specificity using the combination by Boolean operators of terms defined according to the research question.

The search was performed with the general route [((biodegradation OR bioremediation) AND mycorrhizae), crossed with the operator “AND” with the terms (zinc OR lead OR (mercury OR mercuric) OR cadmium) AND soils)]. The time limits “2003 to present”, “between 2003 and 2014” and “September 2003-September 2014” were used in the databases ScienceDirect, Springer Link and EBSCO respectively, to cover the scientific literature published between September 2003 and September 2014. The specific search routes used in the three databases are shown in Table 1.

Table 1. Search routes

ScienceDirect
((((biodegradation OR bioremediation) AND mycorrhizae) AND (zinc OR lead OR (mercury OR mercuric) OR cadmium))) and TITLE-ABSTR-KEY(soils)

Springer Link
(((biodegradation OR bioremediation) AND mycorrhizae) AND (zinc OR lead OR (mercury OR mercuric) OR cadmium)) and (soils)

EBSCO
(biodegradation OR bioremediation AND mycorrhizae) AND (zinc OR lead OR (mercury OR mercuric) OR cadmium) AND TI soils

Citations found together with their respective abstracts were imported to the EndNote software for management of references, which eliminated citations duplicated between the databases. The search protocol was applied independently by two reviewers, whose differences were analysed and resolved by mutual agreement.

INCLUSION AND EXCLUSION CRITERIA

Only original articles published in English in the scientific literature during the past 11 years (September 2003 to September 2014) and which reported the use of mycorrhizae to decontaminate soils with heavy metals or their effects on the growth of plants in soils contaminated with these elements were included. Articles which presented the interactions between heavy metals and mycorrhiza-forming fungi were also taken into account.

Articles which only considered plants as a mechanism for remediation of soils contaminated with heavy metals were excluded, as were those which investigated soil decontamination by chemical or enzymatic agents, unrelated to symbiosis. Articles in which mixed innocula of bacteria or saprophytic fungi and mycorrhizal fungi were used without individually evaluating the effect of these on plants and the concentration of heavy metals in the soil were also excluded.

Data from each publication were extracted and tabulated for subsequent analysis, using the data collection form shown in Table 2.

Table 2. Data collection form applied to each artícle

General data

1. Title 2. Journal 3. Year of publication

Identification of the mycorrhizae present in soils contaminated with heavy metals

4. Type of mycorrhizae 5. Genus 6. Species

Physiological processes of mycorrhizal fungi present in soils contaminated with heavy metals

7. Adsorption and absorption of metals 8. Effect of symbiosis on the productivity of the mycorrhized plant based on the dry biomass (roots and buds) 9. Alteration of the biochemical and physiological properties of the host plant (production of proteins or other molecules induced in response to stress generated by heavy metals). 10. Production of metabolites. Información adicional 11. Observaciones

RESULTS AND DISCUSSIÓN

Implementation of the search protocol by the routes described previously produced a total of 439 articles published between 2003 and 2014 (ScienceDirect 260, Springer Link 71 and EBSCO 108). Ten references duplicated between the databases (reference management software EndNote) were subsequently eliminated and 429 publications evaluated based on the title and abstract, of which 367 were rejected for not satisfying the inclusion criteria determined according to the research question. The complete text of 62 articles was analysed and as a result, 23 were eliminated based on the exclusion criteria. Implementation of the search protocol in the three databases included 39 articles in the systematic review (see Fig. 1).

To these 39 articles a further 22 original ones were added for completeness from journals not indexed in the databases, which satisfied both the inclusion and exclusion criteria. This “grey” literature was obtained from the Google Academic search tool.

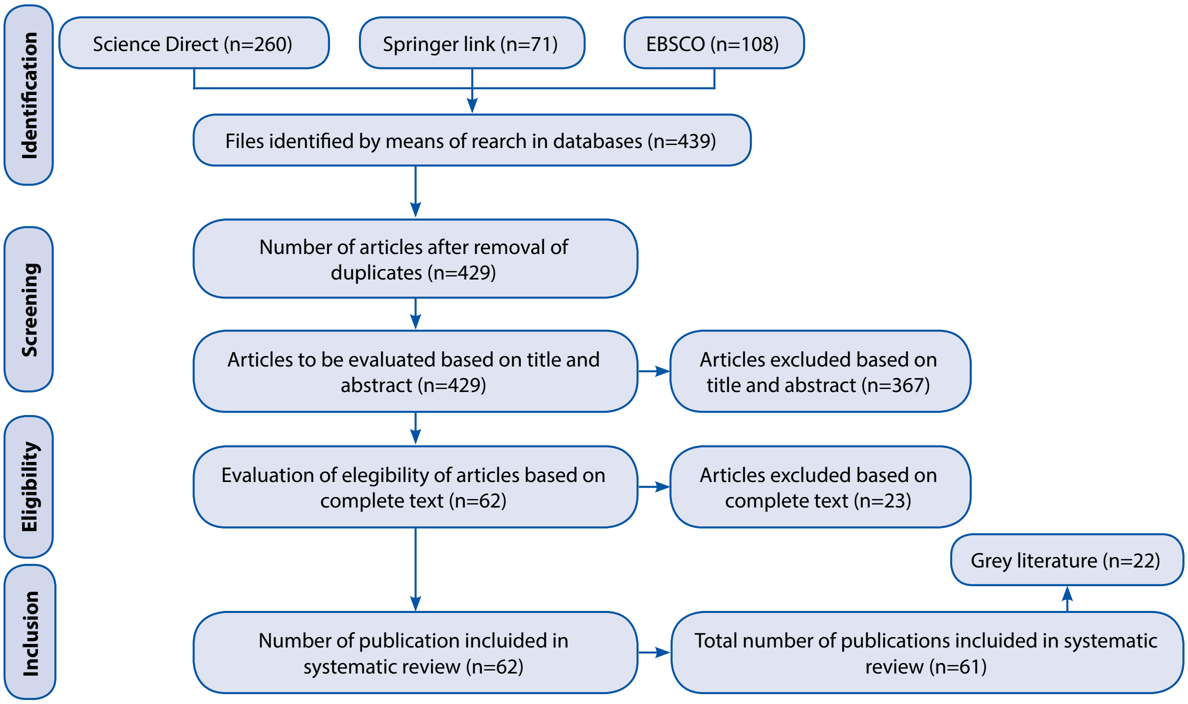

Figure 1. Flow chart of search strategy used (Urrútia & Bonfill, 2010)

DISTRIBUTION OF SCIENTIFIC ARTICLES OBTAINED IN THE SYSTEMATIC REVIEW, ACCORDING TO SCIENTIFIC JOURNAL, COUNTRY AND YEAR OF PUBLICATION

Systematic search of the scientific literature produced between 2003 and 2014 concerning the research topic produced 61 articles from studies carried out in 20 countries (Fig. 2).

The increased concentration of heavy metals due to productive activities is a problem common to most countries of the world. However according to information obtained during the systematic review, research into the role of mycorrhizae-forming fungi in bioremediation of soils contaminated with heavy metals is only just beginning to be considered in developed countries such as the US, Canada and Spain. It is totally absent in some developing nations such as Colombia, which despite the importance of both mining and agriculture to its economy has yet to demonstrate much interest in the study of bioremediation technologies.

Canada, China, Spain and India are the countries that recorded the greatest numbers of scientific articles, especially China with 32% of all publications (Fig. 3).

The interest of China in this topic is probably due to the fact that it is one of the major global producers and consumers of heavy metals, given that it possesses 12% of the world's total mineral reserve. Although mining is a key activity for the socioeconomic development of China it also causes grave damage to the environment. In a study carried out over 6 years by the Chinese government, it was determined that the country's soils were being severely contaminated by agricultural and industrial activities, especially mining which on its own generated 1.5 million hectares unsuitable for anything else; a figure which is increasing at a rate of 46,700 ha. per year.[12]

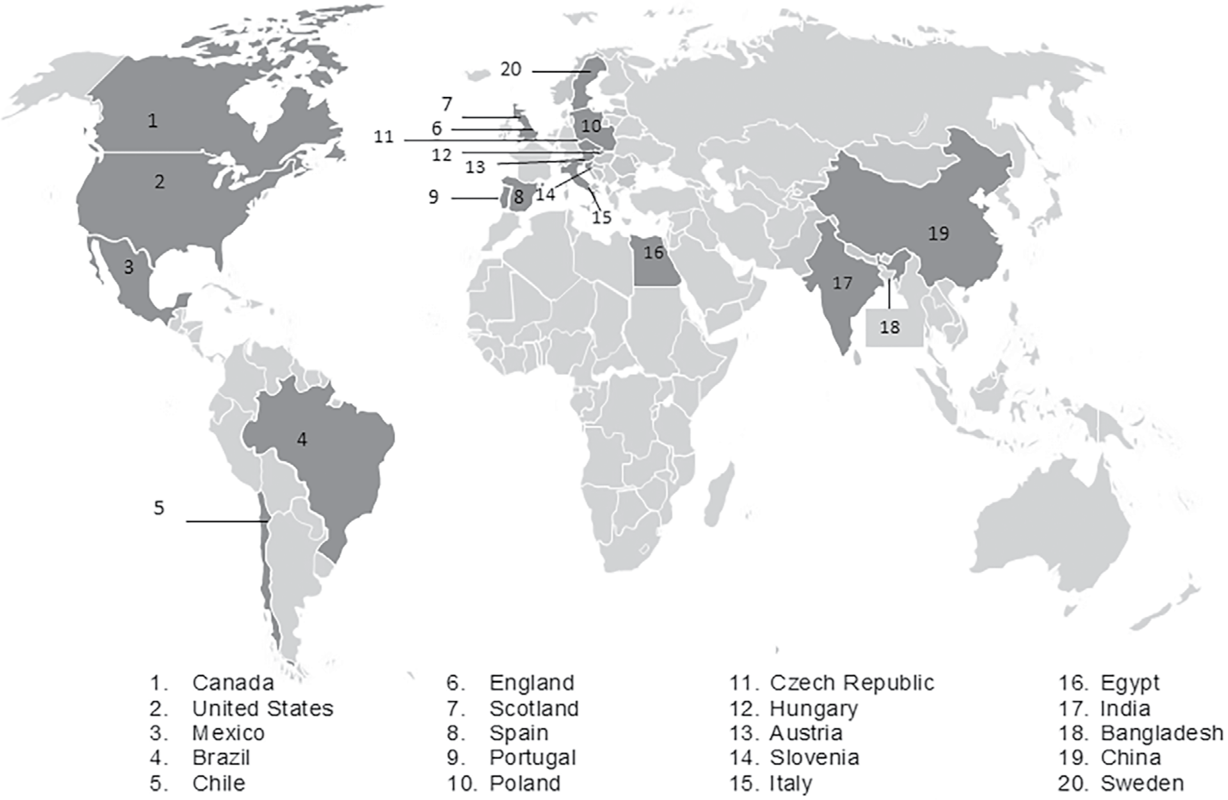

Figure 2. Countries of origin of the publications obtained during the study

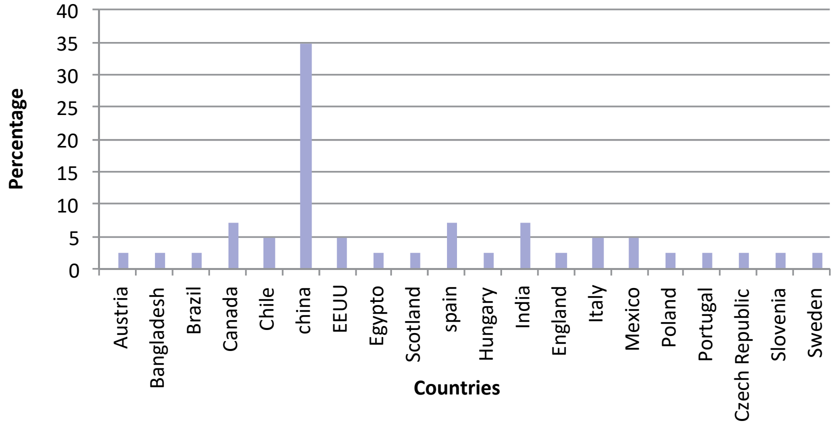

Figure 3. Percentage of publications by country

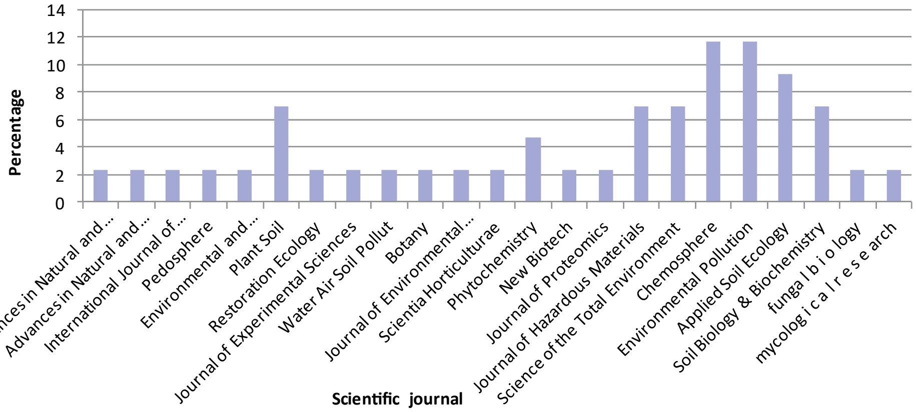

Figure 4. Percentage of publications by scientific journal

Systematic review also revealed that the scientific information obtained during the search was concentrated in certain journals specialising in soils and the environment. Those recording most publications were *Chemosphere, Environmental Pollution, Applied Soil Ecology, Journal of Hazardous Materials, Science of the Total Environment, Soil Biology & Biochemistry* and *Plant Soil*. The scientific journals in which the articles included in the systematic review were published and their publication frequency are shown below.

With regard to the year of publication, it was found that the scientific production of the past 10 years on the study topic did not present a clear growth tendency over time and reached its highest level in 2010. The percentages of publications per year are presented in the following graph.

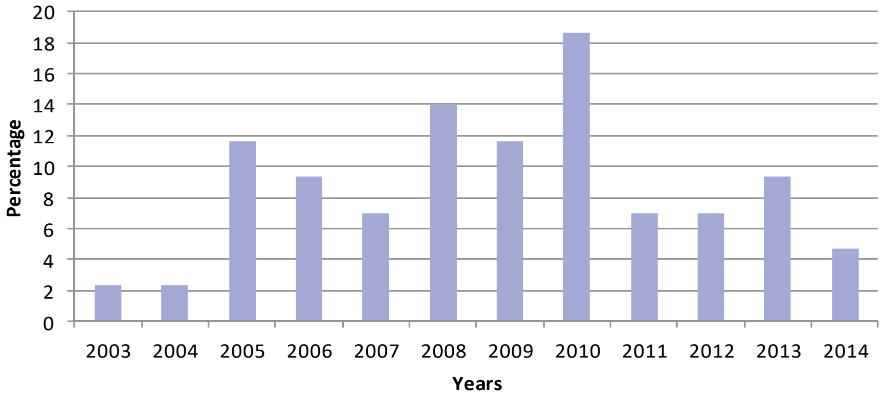

Figure 5. Percentage of scientific publications in the past 10 years

SPECIES OF MYCORRHIZAE PRESENT IN SOILS CONTAMINATED WITH HEAVY METALS

Mycorrhizal fungi are among the most important microorganisms of the soil system and play an important role in the successful establishment and survival of certain species of plants in disturbed environments. Some reports show that symbiosis established by these fungi helps accelerate the biological recuperation of zones affected by industrial activities, such as mines or soils contaminated with chemical elements.[4,13,14]

The mycorrhizae-forming fungi are obligate mutualist organisms that associate with the roots and rhizomes of plants. There are three types of mycorrhizae-forming fungi, classified according to their patterns of colonisation into: *endomycorrhizae* (Ericoid, Orquidoid and Arbuscular*)* which colonise the interior of the root cells; *ectomycorrhizae* which colonise the surface without penetrating the cells, to form a layer or mantle around the root and *ectendomycorrhizae* (Arbutoides and Monotropoides*)*, which combine the characteristics of the previous types of mycorrhizae, *i.e.,* they form a mantle around the segment of infected root and also penetrate between the cells, forming a network known as the Hartig net.[15]

In general, mycorrhizal fungi isolated from soils contaminated with heavy metals are able to tolerate higher concentrations of these elements than strains isolated from uncontaminated soils16 and thus may have a greater resistance and improved role against toxicity.

In soils contaminated with metals the presence of the two first types of mycorrhiza (endomycorrhizae and ectomycorrhizae) has been established. The arbuscular mycorrhizal fungi; a monophyletic fungal group belonging to the phylum Glomeromycota is the type most studied in these soils.[17] These fungi colonise 80% of vascular plant species and are found in almost all the world's soils.[18]

According to the literature, fungi of the genera *Gigaspora, Glomus*, *Scutellospora* and *Acaulospora* can develop in environments contaminated with heavy metals.[19] However, these environments show a loss of biodiversity due to the predominance of a single species, generally belonging to the genus *Glomus*, over genera such as *Acaulospora* and *Scutellospora*, which are abundant in undisturbed communities but scarce in environments that present high concentrations of heavy metals.[20]

The species *Gigaspora rosea, Glomus claroideum, Gl. desertícola, Gl. caledonium, Gl. intraradices* and *Gl. mosseae* are the commonest and most studied arbuscular mycorrhizal fungi from soils contaminated with heavy metals; the last of these being predominant in these environments.[20] The predominance of *Gl. mosseae* in habitats perturbed with heavy metals is well documented,[21] and it has been suggested that the high density of *Glomus* spores is related to the high organic material content of contaminated soils, which leads the propagules of arbuscular mycorrhizal fungi to become established in stages of mycotrophic succession.[22]

The ericoid mycorrhiza species *Hymenoscyphus ericae* and *Oidiodendron maius* have also been reported in soils contaminated with heavy metals, albeit less frequently (Table 3).

Soil basidiomycetes such as ectomycorrhizal fungi (ECM), are also able to grow in soils contaminated with heavy metals; among the genera reported in the literature were found: *Cenococcum, Hebeloma, Laccaria, Lactarius, Paxillus, Rhizopogon, Suillus, Thelephora* and *Tylospora* with predominance of the species *Suillus bovinus, Suillus luteus* and *Rhizopogon roseolus, Rhizopogon luteolus, Laccaria laccata, Cenococcum geophilum* and to a lesser extent *Suillus variegatus, Hebeloma velutipes* and *Paxillus involutus.*

Ectendomycorrhiza-forming fungi are not reported in the literature as species commonly present in these soils, probably due to the fact that their distribution and abundance there is so low that their isolation and further study is impossible. Some of the species of mycorrhizae most reported in the literature as being tolerant to stress by heavy metals are shown in Table 3.

Table 3. Mycorrhiza species present in soils contaminated with heavy metals

Type of mycorrhiza	Subtype	Species of mycorrhiza	Metal	Reference
Endomycorrhizae	**Mycorrhiza arbuscular**	*Gigaspora rosea*	Cu, Cd, Pb	(González et al., 2004) [23]; (González et al., 2009)[19]
		Glomus desertícola	Pb, Cu	(Arriagada et al., 2005) [24]; (Arriagada et al., 2009)[25]
		Glomus caledonium	Zn, Cd, U, Pb	(Chen et al., 2005) [26]; (Wang et al., 2005)[27]; (Chen et al.,2006)[28]
		Glomus intraradices	Cd, As, U, Pb, Zn, Cu	(Yan et al., 2009)[29]; (Leung et al., 2013)[30]; (Chen et al., 2006)[28]; (Orlowska et al., 2012)[31]; (Bissonnette et al., 2010)[32]; (Janoušková & Pavlíková, 2010)[33]; (López et al., 2008)[34]; (Meier et al., 2011)[16]; (Ching et al., 2007)[35]; (Wang et al., 2005)[27]; (Audet et al., 2009)[36]; (Hassan et al., 2013)[37]; (Elahi et al., 2010)[38]; (Xu et al., 2012)[39]; (Wang et al., 2006)[40]; (Chen et al., 2008)[41]; (Wei et al., 2014);[42] (Zhang et al.[43]
		Glomus mosseae	Cd, As, Cu, U, Zn, Pb	(Vivas et al., 2006)[44]; (Yu et al., 2009)[45]; (Chen et al.,2007)[21]; (Chen et al.,2006)[28]; (Yu et al., 2005)[46]; (Hassan et al., 2013)[37]; (Vivas et al., 2005)[47]; (Gar & Singla, 2012)[48]; (González et al., 2004)[23]; (Yan et al., 2009)[29]; (Citterio et al., 2005)[49], (Bona et al., 2010)[50]; (Ortega et al., 2010)[22]; (Leung et al., 2013)[30]; (Ching et al., 2007)[35]; (Chang et al., 2009)[51]; (Arriagada et al., 2005)[24]; (Medina et al., 2006)[52]; (Punamiya et al., 2010)[9]; (Xu, et al., 2012)[39]; (Wang et al, 2006)[40]
		Glomus claroideum	Cu	(Meier *et al.,* 2011)[16]; (Orlowska *et al.,* 2012)[31]
	Mycorrhiza ericoid	*Hymenoscyphus ericae*	Zn, Pb	(Krpata et al, 2008)[53]; (Fomina et al., 2005)[54]
		Oidiodendron maius	Cd, Cu, Pb, Zn	(Fomina et al., 2005)[54]
Ectomycorrhizae *Suillus luteus* *Suillus variegatus* *Rhizopogon roseolus* *Rhizopogon luteolus* *Laccaria laccata* *Cenococcum geophilum* *Hebeloma velutipes* *Paxillus involutus*		*Suillus bovinus*	Hg, Zn, Cd, Cu, Pb,	(Colpaert et al., 2005)[55]; (Crane et al., 2010)[56]; (Sousa et al., 2012)[57]; (Fomina et al., 2005)[54]; (Johansson et al., 2008)[58]
		Hg, Zn, Cd, Cu, Pb, As	(Colpaert et al., 2005)[55]; (Crane et al., 2010)[56]; (Fomina et al., 2005)[54]	
		Pb, Cd, As	(Johansson et al., 2008)[58]	
		Cd, Cu, Pb, As	(Crane et al., 2010)[56]; (Sousa et al., 2012)[57]; (Johansson et al., 2008)[58]	
		Hg, Cd, Cu, Pb, Zn	(Colpaert et al., 2005)[55]; (Crane et al., 2010)[56]; (Fomina et al., 2005)[54]	
		Hg, Zn, Pb, Cd, Cu	(Crane et al., 2010)[56]; (Krpata et al., 2008)[53]; (Fomina et al., 2005)[54]	
		Hg, Cd, Cu, Pb ,Zn	(Crane et al., 2010)[56]; (Fomina et al., 2005)[54]	
		Pb, Cd, As	(Johansson et al., 2008)[58]	
		Pb, Cd, As	(Johansson et al., 2008)[58]	

BIOLOGICAL PROCESSES CARRIED OUT BY MYCORRHIZA-FORMING FUNGI IN THE PRESENCE OF HEAVY METALS

Mycorrhizal fungi can mitigate the toxicity of heavy metals by various mechanisms, such as improved nutrient uptake, sequestration of heavy metals in fungal structures, production of metabolites and induction of proteins in plants.[34]

ADSORPTION AND ABSORPTION OF HEAVY METALS

Mycorrhizal fungi affect the bioavailability of heavy metals, so that they cannot be absorbed by plants by means of the external hyphae stabilise the structure of the soil and exploit the nutrients present. These fungi protect themselves and their hosts from contamination by heavy metals by binding these elements to the components of their cell walls or by storing large quantities of them in their cytosol.[53]

Some mycorrhizal fungi are able to accumulate a great variety of heavy metals, although the hyperaccumulation of a specific metal does not imply the capacity to hyperaccumulate another. This suggests, that there are several mechanisms which contribute to the accumulation of heavy metals by mycorrhizal fungi; it may be that the differences between these mechanisms are partly related to the fact that some metals such as zinc are essential micronutrients, while others such as cadmium and mercury do not have any known biological function in fungi.[56] For example, *Paxillus involutus* accumulates cadmium principally in the vacuoles and zinc in the cell walls and cytoplasm.[53]

Some mycorrhizal fungi take up arsenic and retain it in the mycelium or other tissues, which largely reduces the accumulation in the plant and thus confers an increased tolerance to this metal.[45] The species *Gl. moseae* shows a greater capacity to accumulate cadmium in the roots of the plant without translocating it to the buds[29,33] explain this difference as being due to several mechanisms including: (i) a high affinity for heavy metals in the hyphae of arbuscular mycorrhizal fungi that are tolerant to these elements (ii) chelation or precipitation of heavy metals in the plant mediated by symbiosis, and (iii) alterations caused by the innoculation of the arbuscular fungus in the expression of the cadmium stress response gene.

In this way heavy metals can be sequestered by the mycorrhizae through absorption mechanisms independently of their translocation or not toward the plant plant and through absorption mechanisms in which binding of these elements to the hyphal walls may occur, followed by preciptation as metal-binding complexes into the surrounding environment[36] via production of glycoproteins by the external mycelium.[47]

These two mechanisms of tolerance to heavy metals of mycorrhizae could improve bioremediation technologies mediated by plants, such as phytostabilisation and phytoextraction, since adsorption by the hyphae and fungal exudates help immobilise heavy metals in soil reducing their bioavailability, while the process of absorption improves the transport and accumulation rate of these heavy metals in the roots and aerial biomass of plants.

PROMOTION OF PLANT GROWTH

Mycorrhizal fungi can improve nutrition of plants by providing an extensive network of extraradicular hyphae, which explore the soil, absorb nutrients and transport them to the roots. Modifications in the radicular system of the plant generally result in greater length and increased ramification, allowing a more efficient absorption of nutrients.[49] This absorption mechanism used by fungi may represent an increase of 10-20% of the biomass of mycorrhized plants in compared to non-mycorrhized ones under conditions of stress by heavy metals, given the binding properties of fungal tissues, their exudation of binders and particularly the immobilisation of excess metallic ions.[36]

Studies show that mycorrhized plants present better growth characteristics than non-mycorrhized ones due to the retention of metal in the fungal mycelia by means of absorption.[59] The mycelium provides an increased surface area for the absorption of nutrients (N, P) and improves their acquisition, optimising growth of the host plant.[60] Mycorrhizae may also reestablish the capacity to retain water in the leaves of mycorrhized plants and avoid the loss of chlorophyll pigments under conditions of stress by heavy metals, promoting the accumulation of sugars and proteins rich in proline[48] All this has a beneficial effect given that sites contaminated with heavy metals generally have a scarcity of the mineral nutrients needed by plants, so that and these mycorrhizae help plants obtain more nutrients and resist contamination with these elements.[61]

Improved growth of plants present in soils contaminated with heavy metals due to symbiosis with mycorrhizal fungi could be a key factor in the implementation of phytoremediation technologies, since one of the greatest limitations to the application of these resides in the capacity of plants to withstand stress generated by the presence of these heavy metals at high concentrations.

ALTERATION OF BIOLOGICAL PROCESSES CARRIED OUT BY MYCORRHIZED PLANTS UNDER CONDITIONS OF STRESS BY HEAVY METALS

It has been well documented that mycorrhizae can improve tolerance to heavy metals of their host plants; some of the theories proposed to explain this tolerance are based on alterations of the latter's biochemical and physiological properties.[62,63]

Gar & Singla[48] established that colonisation of *Gl. mosseae* could increase the relative contents of water and chlorphyll pigments between 15 and 59% respectively in mycorrhized plants exposed to growing concentrations of arsenic in comparison with other non-mycorrhized plants. This effect was most notable at low concentrations of the metal.

Mycorrhizae can also increase urease and phosphatase activity; these being enzymes that play important roles in the availability of nutrients such as phosphorus and nitrogen for plants.[40] reported improved activity of urease and phospharate in soils contaminated with cadmium, uranium, zinc, lead and cadmium, after colonisation with mixed innocula of native mycorrhizae belonging to the genera *Glomus*, *Gigaspora*, *Acaulospora* and *Scutellospora*. The increase in activity of both hydrolytic enzymes in soil could be: (a) the result of their production by mycorrhizal fungi; (b) the liberation of greater quantities of radicular exudates by mycorrhized plants due to modifications induced in their radicular systems; or (c) improvement in their nutrition or the potentiation of a plant response mechanism to stress.[64]

Additionally, the presence of heavy metals in soil and mycorrhizal symbiosis can induce changes in the gene expression pattern in plants and modulate expression of proteins.[29] The overexpression of energy metabolism proteins such as the beta subunit ATP-syntethase, malate dehydrogenase, binding proteins for guanosine triphosphate (GTP) and glycolytic enzymes has been reported in mycorrhized plants in the presence of heavy metals such as cadmium and arsenic.[17]

Higher expression of malate dehydrogenase could signify an increase in the activity of the tricarboxylic acid cycle. This would support the findings of Lohse et al.,[65] who recorded a reduction of the metabolites fumarate and malate in the mycorrhized roots of *M. truncatula*. The expression of a GTP-binding protein could be implicated in the signalling of mediation processes of plant defence reactions.[17]

Glycolytic enzymes may form part of the detoxification mechanisms of heavy metals in mycorrhized plants. Bona et al.[50] reported the expression of an isoform of glyceraldehyde-3P-dehydrogenase (GAPDH) in the presence of arsenic, which increased when there was symbiosis of *P. vittata* with mycorrhizae-forming fungi. Glyceraldehyde-3P-dehydrogenase can function as an arseniate reductase in the tissues of *P. vittata*, since instead of catalysing the formation of 1,3-diphosphoglycerate deglyceraldehyde-3-phosphate it can use arseniate rather than phosphate to convert glyceraldehyde3-phosphate into 1-arseno-3-phosphoglycerate.[50] Arsenilated metabolites can subsequently be reduced to arsenite by all the enzymes that catalyse the arsenilytic and phosphorolytic processes.[17]

However, the reduction of arseniate to arsenite can also be regulated by mycorrhizal symbiosis.30 revealed that higher activity of arseniate reducrate (AR) was present in *P. vittata* treated with a mixed innoculate of *Glomus moseae* and native mycorrhizae. According to this study, the high activity of AR in *P. vittata* can be attributed to the fact that symbiosis improves soil conditions and favours the formation and synthesis of AR in plant roots to catalyse arseniate conversion to arsenite in the root at an optimal rate.

Arseniate competes with phosphate transporters of plants and is actively absorbed by them, thus interfering with the DNA phosphate groups and the energetic reactions. Production of the AR enzyme constitutes a plant defence strategy given that it impedes absorption of the arseniate and permits the normal mobilisation of phosphates in soils contaminated with arsenic.[30]

PRODUCTION OF METABOLITES

Mycorrhizal fungi can interact with toxic heavy metals and minerals that contain heavy metals in a variety of forms, depending on their tolerance and capacity to influence the mobility of these susbstances. Heavy metals can be mobilised by fungi through the formation of complexes and protonolysis by means of microbial and siderophore metabolites. In Gadd's study.[54,55] Among the metabolites most reported in the literature are glycoproteins, peptides and organic acids. Some of these are described below:

Glomalin

Glomalin is a soluble alkaline glycoprotein produced in abundance by mycorrhizae-forming fungi. The molecule is formed by a complex of reiterated monomeric structures bound by hydrophobic interactions; it contains tightly bound iron (0.04-8.8%) but lacks phenolic compounds like tannins.[18] This glycoprotein is transferred across the fungal hyphae, binds to soil particles and helps stabilise the aggregates.[66]

Glomalin can also bind with cations present in the soil in quantities that vary depending on the type of soil. It appears to be efficient at sequestering different heavy metals, especially copper, lead and cadmium,[23,67] as well as Fe, Mn and Zn[61]

González *et al.*[23] demonstrated that copper can be sequestered by glomalin produced by *Giaspora rosea* not only by electrostatic sorption, but also by formation of glomalin–copper complexes independently of the binding of tested copper ($Cu(NO_3)_2$, $CuCl_2$ and $Cu(CH_3COO)_2$). This sequestration does not only occur in the mucilaginous zone of the exterior wall of the hyphae and the cell wall, but also within the hyphal cytoplasm. In this study it was suggested that this accumulation is related to iron (Fe) present in the glomalin structures.[23] These results support the presumption that glomalin liberated into the soil by these mycorrhizae-forming fungi contributes to the reduced bioavailability of potentially toxic heavy metals in the soil.

Metalloproteins

The toxicity of heavy metals is related to inactivation of functional proteins including enzymes, through direct binding of the metallic ion or oxidative damage caused by the accelerated generation of reactive oxygen species.[68]

Metalloproteins are peptides bound to heavy metals that play important roles in detoxification of the latter and regulation of intracellular concentrations of the metals essential to eukaryotes, including higher plants, fungi and microalgae. They are composed of some 70-75 aminoacids with a high content of cysteine, an aminoacid able to form complexes with cations by the sulphydryl group.[68,69]

Pallara, et al[70] demonstrated that innoculation with *Gl.mosseae* contributes to more efficient control of heavy metals at the cellular level due to the production of metalloproteins. This result agrees with the findings of Zhang et al.,[71] who reported that heavy metals can be chelated by metalloproteins and that the resultant metal-binding complexes are generally compartmentalised within vacuoles.

Chelation of heavy metals by metalloproteins may constitute an important mitigation mechanism for stress generated by heavy metals, given that they form complexes with a wide range of elements such as cadmium, copper, zinc, mercury and arsenic, as well as the form in which this binding is presented at molecular level, synthesis of chains of various lengths generating molecular complexes that reduce the bioavailability of these elements.[68]

Low molecular weight organic acids

The production of organic acids by mycorrhizal fungi could have different effects on the mobilisation of heavy metals, since they provide both a source of protons for solubilisation of the metal and the anion to form a complex with the metal cation. [54] The formation or not of complexes depends on factors such as the relative concentrations of anions and heavy metals, pH and the stability constants of the various complexes.

An example of this is heterotrophic leaching, which occurs through acidification of the fungal environment by an efflux of protons and organic acids across the plasma membrane. This leads to competition between protons and heavy metals in the metal-anion complexes in soils, resulting in free metal anions.[58] This allows mobilisation of heavy metals through the soil and makes them more available and toxic to non-bioaccumulatory plants.

Fomina et al.[54] concluded that many endomycorrhizal, ericoid and ectomycorrhizal fungi are able to solubilise different toxic heavy metals (cadmium, copper, lead, zinc) contained in minerals. This dissolution frees toxic metallic components.

By contrast, anions of liberated organic acids can form complexes with metal. Oxalic acid esters and citric acid salts such as oxalate and citrate respectively are characterised by having 1-3 carboxyl groups that allow them to form stable complexes with metallic cations in solution and displace anions from soils.[54] The commonest form of oxalate complex in the soil environment is calcium oxalate. Crystals of calcium oxalate are formed by precipitation of solubilised calcium, which commonly associates, with hyphae of the mycorrhizae. However oxalate exudation by these fungi may also produce deoxalate complexes with other heavy metals such as cadmium, aluminium, lead and arsenic, the last of these at low concentrations.[58]

CONCLUSIONS

The systematic review revealed a predominance of genera and species of mycorrhizal fungi belonging to the endomycorrhizal and ectomycorrhizal types in soils contaminated by heavy metals. The reason for the low diversity reported has not been clearly described in the literature. A study is therefore needed that leads to understanding of the factors that influence mycotrophic succession patterns of these fungi in environments perturbed by contamination with heavy metals.

Mycorrhizae isolated from soils contaminated with heavy metals have greater tolerance to these elements and can thus perform better in bioremediation processes than those isolated from uncontaminated environments. However, the high concentrations of heavy metals in some soils are not only the result of human activities. Each soil has natural quantities of these elements depending on the composition of the rocky material from which it was derived. In such cases the natural presence of heavy metals such as copper, lead, cadmium, arsenic and uranium that persist in soil for long periods[13] could yield species of mycorrhizae better adapted to this contamination than those submitted to selection pressure resulting from occasional anthropogenic activity.

Immobilisation of heavy metals in soil by production of metabolites and their accumulation in fungal structures are perhaps some of the most significant contributions of mycorrhizae to bioremediation processes, since they avoid dispersal of contaminants through the soil, facilitating regeneration of areas that have been severely contaminated by human activities and impeding their leaching into in water destined for human consumption, or passage through trophic chains.

Mycorrhizal fungi constitute an economical and environmentally friendly alternative for improvement of bioremediation technologies such as phytostabilisation and phytoextraction. Phytostabilisation is favoured by metal adsorption by fungal hyphae, sequestration by chelating proteins and the formation of anion-metal complexes derived from the production of low molecular weight organic acids. On the other hand, phytoextraction is favoured by processes of absorption and translocation of heavy metals in plants mediated by mycorrhizae. However, it should be pointed out that although these mechanisms contribute to heavy metal extraction by plants that are bioaccumulators, they can also have a negative effect on the survival of non-bioaccumulatory species.

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