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RESTORATION OF MINE SOILS CONTAMINATED BY HEAVY METALS THROUGH IMPROVED FOREST SPECIES

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ABSTRACT

This work shows the option of restoration of contaminated environment by heavy metals reintroducing plants and soil microbes in a long-term schedule. Restoration of soils contaminated by heavy metals can involve three processes: immobilization, concentration and extraction. Phytorestoration is the most common system in use today.

We assume in this work that the presence of heavy metals, in the soils belonging to the mining zone in Valle del Alto Guadiato, affect directly to plants and to the corresponding inoculum of arbuscular mycorrhizal fungi. It is known that almost all the plants need the mycorrhizal symbiosis to survive in unaltered environment, in the case analyzed they can inclusive be more important.

The following options have been assessed: a) population of arbuscular mycorrhizal fungi associated to slag heap and to the existing vegetation; b) development of plant-model to study the susceptibility to different fungi. In this case Sorghum bicolor has been selected.

INTRODUCTION

The soils can be contaminated by heavy metals in different ways such as urban residuals, mining, fertilizers or pesticides, etc. Mining activity acquires great environmental importance. This activity originates deep modifications in the landscape producing not only visual modifications but also alterations over biotic elements such as plant populations and soil microorganisms. In this way the biological activity will be affected. Up to this moment the restoration of contaminated soils by heavy metals is limited to the immobilization processes, extraction and concentration. Among the options of solution phytorestoration has great interest since it incorporates plants with resistance to heavy metals.

The presence of appropriate microorganisms is fundamental for the maintenance of the plant populations (Harley and Smith, 1983). The microorganisms of soil are important in plant recovery because they can contribute to the readiness of nutrients and immobilization of heavy metals. At the same time the microorganisms can act as soil binders improving the soil structure. Arbuscular mycorrhizal (AM) fungi are an important group, which play an important role in symbiosis with the roots of almost all plants. These AM fungi provide a great resistance to plants in front to negative both biotic and abiotic elements (Ocampo, 1993). On the other hand the saprophyte fungi can be big degrader of toxic substances (Madrid *et al.*, 1996) and at the same time produce AM fungi growth-stimulating substances (Mc-Allister *et al.*, 1995).

In this way the physical-chemical properties of the soil, the host plant, the AM fungi, the concentration of metals in soil and the interaction of other microorganisms such as saprophyte fungi, can improve the soil restoration incorporating these soils to the productive system.

HEAVY METALS AS TOXIC ELEMENTS

The term "heavy metals" is used in general form and is accepted in environmental studies. However, this term refers strictly to metallic elements with a specific mass bigger than 5 $g \cdot cm^{-3}$ able to form sulphurs. Minor and trace elements would be the most correct term, since these last ones are only based on concentration. However, some of these metals are necessary micronutrients for the growth of the plants, such as B, Fe, Zn, Ni, Cu, Mn and Mo while others do not have well-known biological function, as the Cd, Pb and Hg (Marschner, 1995).

Minor and trace elements can be present in the soil in form of free metallic ions, complex of soluble metals, interchangeable metallic ions, metals together to compound organic, compound insoluble as oxides, carbonates and hydroxides or they can be part of the structure of metals silicates. The toxicity in the soil by these elements depends on its bioavailability, defined as the capacity of being transferred from the soil to an alive organism (Jouste, 1988). In accordance with Berthelin *et al.* (1995), the bioavailability does not only depend on the total concentration of the metal, but rather also of physical-chemical factors (pH, organic matter, clay content) and biological factors (bioabsorption, bioaccumulation and solubility).

Higher concentration of heavy metals in soils, act as selection power producing a plant population with a high resistance to these elements but with a low diversity of species in the different trophic levels (Ernst, 1990).

MICROORGANISMS

The tolerance of microorganisms to the soil heavy metals has been studied with object of eliminating metals of contaminated soils. At the same time it is interesting to know the adaptation from organisms to these conditions. It is known that higher concentrations of heavy metals in soils are toxic for bacteria and fungi.

The contamination of the soil by heavy metals restricts the establishment of plants at the same time the number and the activity of soil microorganisms is also reduced. The high toxicity of heavy metals to soil microbes and to microbiological processes, associated to the long term effects in the soil, are recognized as important facts at the moment although there is little information (Tyler et al., 1989). As example it can be reported the contamination problems for arsenic in the India and Chile where the human health has been affected. Soil microorganism may directly promote the establishment of plant species on waste sites by immobilizing the heavy metals in the soil; thereby the plant has a reduction of availability of heavy metals (Chanmugathas and Bollag, 1987).

The arbuscular mycorrhizal (AM) fungi benefit the growth and establishment of plants by different ways, increasing the efficiency of water use, through the efficiency in the uptake and assimilation of the nitrogen and phosphorus (Harley and Smith, 1983; Pearson and Jakobsen, 1993; Brundrett et al., 1996). The AM fungi are obligated symbionts of host plants that can serve as pioneer species in soils of mining areas (Schramm, 1966). There are evidences in relation that the arbuscular colonization can decreased the accumulation of heavy metals in plants grown in contaminated soils. It has been observed that plants inoculated reduces concentrations of Cu, Zn, Cd and Mn (George et al., 1994; Bürkert and Robson, 1994; Weissenhorn et al., 1995). In the same way, it has been reported that woody plants inoculated with AM fungi, increases the resistance to high concentrations of heavy metals (Pereira and Herrera, 1997).

The presence of AM fungi in contaminated soils with heavy metals, indicates an adaptation of that population. Nevertheless, in soils where is very high the concentration of metals, the quantities of AM fungi able to colonize plants is low. According the above the multiplication and inoculation with selected AM fungi could reduce the afforestation problems associated with these soils.

On the another hand the saprophyte fungi can increase the formation of AM fungi, segregate substances of industrial interest and degrade toxic substances (Madrid *et al.*, 1996) being interesting in the recovery of environment contaminated by heavy metals.

RESTORATION

As it has been pointed out, in the soils contaminated by heavy metals these elements can not be degraded chemically. Restoration of those soils is limited to processes of immobilization, extraction and concentration. The phytorestoration option has appeared in these last years as a valid tool. The phytoextraction uses plants with the power of accumulating metals, producing enough biomass in the field reducing the concentration of metals in the soil. Many of the accumulative plants belong to the family *Brassicaceae* (*Thlaspic aerulescens* non-mycotrophic). However, these plants produce little biomass being more interesting another with a higher productivity, such as woody species of *Salix* or *Eucalyptus* genus. Pereira and Herrera (1997) indicate that *Eucalyptus globulus* and *E. camaldulensis*, colonized by AM fungi increases both the production of biomass and the survival of plants growing in soils highly contaminated by heavy metals.

Another option of phyrestoration is the phytostabilization method. It consists on promoting the growth of plants to reduce or eliminate the metal availability, to minimize the erosion by water or wind, improving the characteristics of the soil (organic matter content) and reducing the leaching of metals. The phytostabilization is, however, a temporary solution, because metals are not eliminated and there is a risk, increased with the time, of mobilization in the rhizosphere and of transferring from the plants to the animals. For this reason, the plants for phytostabilization should immobilize the metals in the roots with a minor percentage of accumulation in the air part. In this way, the AM fungi are of great interest, since; they could bind the metals limiting their translocation to the air part.

POTENTIAL FOREST SPECIES

Different plants can be used in experimental assays as "Model" for the phytorestoration and phytostabilization. In this case the genus *Eucalyptus* has been chosen. This genus has different fast-growing species being one of them *Eucalyptus globulus* Labill. *E. globulus,* possesses a wide plasticity growing in impoverished or marginal soils, previously to establish the herbaceous *Sorghum bicolor* (L.) Moench, to incorporate AM fungi. During a long time it has been thought that *Eucalyptus* species drains the water resources, since it has been used to reduce the humidity of flooded areas. However when the soil water content diminishes the plants of *Eucalyptus* spread to grow more slowly reducing the water uptake (Montoya, 1995).

When the *Eucalyptus* plants are not subject to intensive production, the soil is not degraded but in many cases it has seen that the soil fertility has been improved. Montoya (1995), have reported that in very poor soils, as the sandbanks of Huelva (Spain) the *Eucalyptus* plantations have improved the soils. These kinds of soils at present time have a higher content of nutrients and organic matter, in relation to similar lands in deforested conditions.

The objectives in a forest restoration in these contaminated soils suggest that structure, functioning, diversity and dynamics of the ecosystem previous to the disturbed have to be emulated. An alternation of plant communities can be planned establishing mixed populations as a previous step to the establishment of the original species. In summary, the restoration concept try to create and generate a model whose final objective is the establishment of a similar native mixed mass.

RESEARCH

In order to develop the above concepts it has been done an integrated analysis of the contaminated sectors of the *Valle del Alto Guadiato*, in the north of the County of Córdoba (Spain). At the moment the research that is carrying out is in regard the association among soil microorganisms and plants and the recovering of contaminated soils by heavy metals. One specific topic is the study of the behavior and development of the interaction among *Sorghum bicolor* - Arbuscular Mycorrhizal – *Eucalyptus globulus* in contaminated soils by heavy metals.

Objectives

Determine the best strain of AM fungi to establish symbiosis wit the herbaceous *Sorghum bicolor.* A second objective is to test the value of the above symbiosis as a way to improve the condition of the soil. The last objective is to assess the responses of *Eucalyptus globulus* growing in the improved soils under inoculation of AM fungi in order to reintroduce these lands to productivity.

Methodology

The studied soils come from the County of Córdoba, belonging to the mining zone in "Valle del Alto Guadiato". The soils are contaminated by heavy metals generated by the mining activities. The samples were extracted directly of mining waste (slagheap).

The different soil mixtures will be sterilized to fluent vapour (1 hour for 3 days) in order to eliminate any native microorganism. The corresponding natural soil samples will be used like contrast (agricultural common soil). The seeds of *S. bicolor* have been obtained from the ETSIAM's collection. These seeds were sowed directly in gavels of 220 ml and 2 by alveolus.

The fungal species is *Glomus intrarradices* Schenk et Smith and *Glomus mosseae* (Nicol and Gerd) Gerd and Trappe, belonging to the collection of the "Estación Experimental del Zaidín" (C.S.I.C.), Granada. At the same time a natural fungus strain *Glomus sp.* isolated species of a slagheap was used. The sampling place was the mining zone in "Valle del Alto Guadiato".

Experiment

The effect of different types of AM fungi over plants of *S. bicolor* was studied. The plants were grown in a crowd-pearl (3:1) mixture. The treatments are the following: Control (non-inoculation) and inoculation by *Glomus mosseae*, *Glomus intrarradices* and *Glomus sp.* The plants are cultivated in a greenhouse (Cooling System) with natural cycle of light. The watering is daily and every 15 days a nutritive solution containing one fourth of phosphorus is applied (Hewitt, 1952).

The last step do not described in this work establishes the preparation of a substrate with 3 levels of contamination. The substrate is prepared by adding to the contaminated soil quartz sand in the proportion of 0% (0:1 v/v), 50% (1/1 v/v) and 100% (1:0 v/v). As a control the same design will be structured using in this case undisturbed natural soil instead of quartz sand. After growing period the height and the basal diameter of the plants will be assessed. At the same time nutrients in leaves and soil, fresh and dry weight belonging to both air and root zones will be determined. The results will be subjected to variance analysis (ANOVA) being compared the stockings by means of the test of Duncan (Duncan, 1955). According the results showing significant differences among treatments the test of Tukey for multiple comparisons will be used.

RESULTS

The results show that the degree of mycorrhization of the plants of *Sorghum bicolor* has a direct relation with the plant growth. The plants inoculated with propagule of *Glomus mosseae, Glomus intrarradices* and *Glomus* sp. have different level of colonization. No mycorrhization was observed in non-inoculated control.

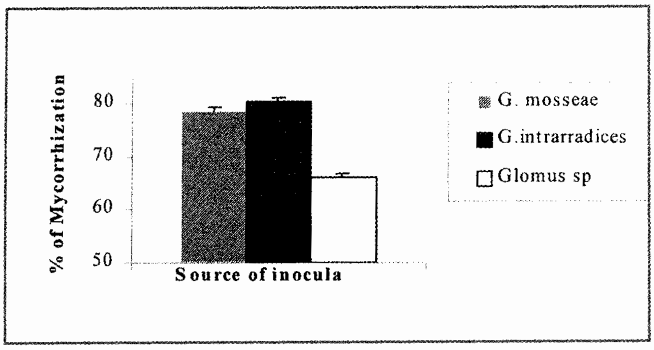

Figure 1. Mycorrhization reached by plants of *S. bicolor* inoculated at sowing time.

In the Figure 1 it is observed that plants of S. *bicolor* inoculated in the sowing time reach very high percentages of mycorrhization (78,7; 80,3 and 66,01%). These differences become significant at a level of 95% of confidence explained by the short time required by plants to reach the inoculate source because their high rooting power. The biggest differences are regarding the native endophyte isolated from contaminated lands. In that treatment the mycorrhization (66,01%) produce a bad response in plant growth showing that *S. bicolor* inoculated with *Glomus* sp. are smaller in absolute value that plants inoculated with *G. mosseae* and *G. intrarradices*.

The Figure 2 shows that the inoculated plants achieve bigger growths that the non-inoculated treatment being these differences significant for all the treatments. This express that the AM fungi affect positively the height growth in plants of S. *bicolor*. However there are some differences in efficiency being more effective the species *Glomus mosseae* and *Glomus intraradices* than the native one.

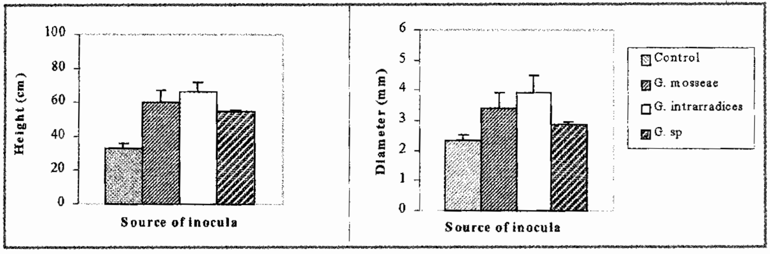

Figure 2. Height and diameter of *S. bicolor* after two months of inoculation.

At the same time, the Figure 2 shows that in all treatments, the inoculation affects positively the development of plants achieving bigger diameters averages regarding to non-inoculated plants. These differences have statistically significance showing once again that the inoculation with selected ecotypes of AM fungi can reduce the reforestation problems associated to soils contaminated by heavy metals. In the same way, the native AM fungi do not overcome in effectiveness to those selected, being *G. intrarradices* the one that highlights statistically of *G. mosseae* and *Glomus sp.*

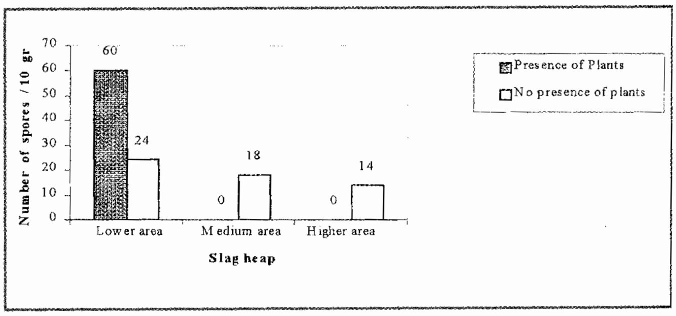

Figure 3. Number of spores in a Slag heap of the mining zone of the "Valle del Alto Guadiato".

In the Figure 3 it can be observed that the spores number in a slagheap decreased when the height of this increase. On the other hand, the percentage of colonization for pioneer species in the Slagheap did not overcome the 5% of covering (see Table 1). In Table 1 we can observe the species-colonizing Slagheap. From the above list Chenopodium is the unique non-mycotrophic genus. According to that it is possible to assume that many species develop the symbiosis with AM fungi since the first phases of slagheap colonization. This can explain why the number of spores increased considerably in the rhizosphere of pioneer plants (Figure 3).

Epecie	Cobertura	Tipo de Micorriza	Epecie	Cobertura	Tipo de Micorriza
Hirschfeldia incana	Mínima	No conocido	*Phalaris paradoxa*	Mínima	No conocido
Avena sterilis	Minima	MVA	*Cynodon dactylon*	Mínima	MVA
Lactuca serriola	1 - 5%	No conocido	*Rumex induratus*	1 - 5%	MVA
Atriplex hastata	Mínima	Posible MVA	*Avena barbata*	Mínima	MVA
Sonchus asper	Mínima	No conocido	*Bromus madritensis*	1 - 5%	MVA
Picris echioides	Mínima	No conocido	*Cnicus benedictus*	Mínima	No conocido
Bromus hordeaceus	1 - 5%	MVA	*Chenopodium album*	1 - 5%	No es Micorrizable
Trifolium angustifolium	Mínima	MVA	*Trifolium tomentosum*	Mínima	MVA
Phalaris brachystachys	Mínima	No conocido	*Hypochaeris glabra*	Mínima	No conocido
Bromus rubens	1 - 5%	No conocido	*Vicia benghalensis*	Mínima	MVA
Amaranthus albus	Mínima	MVA	*Leontodon taraxacoide*	Mínima	No conocido
Lolium rigidum	Mínima	No conocido	*Senecio vulgaris*	1 - 5%	No conocido

Table 1. Feasible pioneer vegetation of finding in an slagheap.

The Table 2 shows the main plant species growing in the zone of "Valle del Alto Guadiato". These species are forming part of forests or meadows dominating the encina (*Quercus ilex* var ballota). In this Table we can observed that almost 100% of the species establish some type of mycorrhizal symbiosis. Overlapping Table 1 and Table 2 it is observed that there are no species in common to both situations. However it is probably that AM fungi share both areas. Up to this moment there are no enough knowledge about the microsymbiont. Further studies will allow understanding the mechanism involved in the AM fungi resistance to heavy metals stress. A deep knowledge of mycorrhizal symbiosis could facilitate the selection of optimal strains to specific situations.

Finally the positive consequences of this symbiosis will depend on the degree of ecological adaptation of the AM fungi to the presence to the different heavy metals. For that reason isolation and selection of endophytes specially adapted to spe-

Especie	Nom Común	Ubicación	Tipo de Micorriza
Querqus ilex sbsp ballota	Encina	Dehesas	ECM
Querqus suber	Alcornoque	Bosque de Encinas	ECM
Pinus pinaster	Pino negral	Antiguo encinar	ECM, ETM
Arbutus unedo	Madroño	Escaso en el encinar	ARM
Crataegus monogyna	Espino blanco	Acompaña a la encina	MVA, ECM
Querqus coccifera	Coscoja	Acompaña a la encina	ECM
Salix salvifolia	Mimbrera	Lugares húmedos	MVA, ECM
Nerium oleander	Adelfa	Lugares húmedos	MVA
Myrtus communis	Arrayán	Acompaña a la encina	No conocida
Phillyrea angustifolia	Labiérnago	En el encinar	No conocida
Pistacea lentiscus	Lentisco	Muy frecuente en encina	MVA
Pistacea terebinthus	Cornicabra	En el encinar	MVA
Viburnum tinus	Durillo	Acompaña a la encina	MVA
Cistus albidus	Estepa blanca	Zona sur de la Cuenca	ECM, ETM
Cistus ladanifer	Jara	Matorral de encina	ECM, ETM
Cistus monspelliensis	Estepa negra	Sotobosque del encinar	ECM, ETM
Daphne gnidium	Torvisco	Acompaña a la encina	No conocida
Chamerops humilis	Palmito	Acompaña a la encina	MVA
Ceratonia siliqua	Algarrobo	Acompaña a la encina	MVA
Erica arborea	brezo	-----	ERM
Osyris alba	retama loca	----	No conocida
Genista hirsuta	Aulaga, Aliaga	Abundante en el matorra	MVA
Rosmarinus officinalis	Romero	Sotobosque del encinar	MVA

MVA: arbuscular mycorrhizal and arbuscular mycorrhizal with vesicles.
ECM: ectomycorrhiza ; **ETM**: ectendomycorrhiza
ARM: arbutoid mycorrhiza ; **ERM**: ericoid mycorrhiza
Source: Honrubia *et al*., 1995

Table 2. Main species of the natural vegetation of the "Valle del Alto Guadiato".

cific stress conditions is fundamental. The aspects to consider have to be done under a scientific and technical point of view joining theoretical to practical aspects. However it is interesting to point out that woody species because their long life can be consider as a source of permanent inocula in the soil.

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