Executive Summary

Contract no.: FIGE-CT-2000-00014 — **MYRRH**
Title: **Use of Mycorrhizal Fungi for the Phytostabilisation of Radiocontaminated Environments Protection**

Introduction

Radionuclide pollution results from various activities such as mining and the provision of nuclear energy. Understanding the environmental redistribution of RN's is a prerequisite for the management and restoration of contaminated areas whether the pollution is diffuse (e.g. fallout radiocaesium in semi-natural ecosystems) or not (e.g. uranium in mining and milling sites). Because root uptake is one of the very first steps in RN dissemination, extensive research efforts have been devoted to the understanding of the mobility and bio-availability of radiocaesium and, to a lesser extent, to uranium.

Cs soil-to-plant-transfer patterns vary widely according to environmental conditions. In soils, trace Cs is retained specifically by sorption on a small number of highly selective sites associated with micaceous wedge zones born by vermiculitic minerals. Cs^+ binding characteristic in soil can be readily quantified. The uptake of radiocaesium by plant roots is essentially governed by the amount of highly selective frayed edge sites and concentrations of Cs^+, K^+ and NH_4^+ ions in the soil solution. Concerning uranium, both pH and chemical status of the substrate largely control U solubility and the formation of various complexes. Plant roots can readily adsorb both anionic and cationic U species. U speciation is thus essential to understand U mobility in soil and soil-to-plant transfer.

Up to date, most results were obtained without paying attention to the roots associated micro-organisms or in the absence of these micro-organisms. However, biological activities in soils are widely recognized as playing a vital part in nutrient cycling, element mobilization and availability to plant. Some micro-organisms are intimately associated with roots, therefore occupying an essential situation at the soil/plant interface. Among these soil micro-organisms, mycorrhizal fungi are of particular importance. These world-wide distributed symbionts concern nearly 80 % of the vascular land plants and are present in undisturbed ecosystems as well as in man-made agricultural and forestry systems and in heavy metals and RN contaminated areas. Their unique location at the interface between soil and root makes them key actors in the soil-to-plant continuum. Their major beneficial effects for plants concern improved nutrient uptake and increase tolerance/resistance to abiotic stress. Therefore various research programs were directed toward their potential use in agricultural and horticultural practices, re-forestation and bioremediation.

Unfortunately, the role of mycorrhizae in RN mobilization and transfer is largely neglected and poorly understood in a mechanistic way, though they can strongly influence the fate of these elements. Such poor apprehension remains a major obstacle in (1) the fuller understanding of the bio-availability of RN's, (2) the prediction of RN soil-to-plant transfer patterns, and (3) the use of these fungi in phytostabilisation/phytoremediation of RN-contaminated areas. Obviously, studying and quantifying the role of mycorrhizae in the fate

of radiocaesium and uranium in contaminated lands will fill a major gap in our understanding of the bio-availability of these RN's.

Objectives

The project aims to decipher and understand the role of mycorrhizal fungi in the mobilization and transfer of RN's to the plant. In other words, it is our objective to give a reply to the following question: do the mycorhizal fungi represent a potential tool in the phytostabilisation/phytoremediation strategy of RN-contaminated areas?

To achieve this objective, three activities were conducted at the start of the project: (1) the establishment of a mycorrhizal culture collection with information on isolates, (2) the establishment of a soil collection with information on soil characteristics and (3) the development of experimental systems for *in vivo* and *in vitro* plant/mycorrhizae/soil studies.

Based on these achievement, the bio-availability of RN in the rhizosphere (4) was investigated in the following years, with three sub-objectives (i) the contribution of mycorrhizae and roots to RN mobilization and transfer, (ii) the study of the processes involved in RN mobilization and transfer and (iii) the study of the influence of soil conditions on processes in RN mobilization and transfer in mycorrhizosphere.

Results

(1) Mycorrhizal culture collection: A collection of four AMF strains are maintained in the collection and used by the partners. Three of them (*Glomus intraradices* (2 strains) and *Glomus lamellosum*) are cultured *in vitro* and maintained in GINCO (http://www.mbla.ucl.ac.be/ginco-bel), with their main characteristics described. One strain (*Glomus mosseae*) is maintained *in vivo* in the collection. Two ECM isolates (*Laccaria bicolor* and *Pisolithus tinctorius*) are also maintained in the collection.

(2) Soil collection: A soil collection exhibiting a wide range of Cs and U retention and rhizospheric mobilization has been established. Five soils originated from Belgium and six from Germany were fully characterized (pH, CEC, % Organic matter, etc.). Root interception potential (RIP) and transfer factor (TF) were further determined for the Belgium soils and TF and U speciation and mobility for the German soils. These soils were added to a wider collection containing approximately 50 soils. The pH-dependent speciation of U in soil and solution has been illustrated. Different organic and inorganic ligands which interact with the oxy-hydroxide surface in particular were shown to strongly influence the U mobility.

(3) Experimental systems for *in vivo* and *in vitro* plant/mycorrhizae/soil study:

a. *In vivo*: Two experimental systems were developed for AMF and ECM. Both consist of a soil-filled main rooting compartment separated by a nylon mesh from a second soil-filled compartment labelled with the RN. This second compartment was placed either in the bottom of the main compartment (Experimental system I) or at the side of the main compartment. (Experimental system II). Experimental system I was used for AMF and ECM plants while the experimental system II was developed for AMF plants. The choice of mesh opening determined whether AMF/ECM hyphae only or both roots and hyphae could grow into the RN-labelled compartment. Both experimental systems were developed to study the contribution of AMF/ECM to the

uptake and transport of RN to plants. The experimental system II allowed also studying the dissipation of the RN in the side compartment in presence and absence of hyphae.

b. *In vitro*: Three experimental systems were developed for AMF. (1) The bi-compartmental fungi/host root system consists of a bi-compartmental Petri plate. The compartments are separated by a plastic barrier. The host root and the fungi were associated in one compartment whereas only the hyphae were allowed to grow in the other compartment labelled with the RN. This system was developed to study the effects of hyphae alone on transport of RN to the root. (2) The Tri-compartmental fungi/host root system consists of a tri-compartmental Petri plate. The three compartments are separated by a plastic barrier. The host root and the fungi were associated in one compartment whereas in a second compartment the fungi and the root were allowed to grow and in a third compartment only the fungi were allowed to grow. This system was developed to distinguish between Cs uptake by hyphae and roots by means of dual labelling with radiotracers. Hyphae grew into a compartment with ^{134}Cs-labelled MSR medium whilst both roots and hyphae grew into an identical compartment with ^{137}Cs-labelled MSR medium. The relative content of the two isotopes would reflect the Cs uptake from each of the labelling compartments. (3) The whole mycorrhizal plant *in vitro* culture system. This system consists in the *in vitro* mycorhization of whole plants, i.e. with roots and shoots, therefore providing a sink to the system, i.e. the shoot. This system allows investigating the transfer of RN from the mycorrhizal cell within the root to the shoot cell via the root cell.

(4) Bio-availability of RN in the mycorhizosphere: Results were obtained both for Cs and U.

For Cs, the results demonstrated:

1. the unambiguous role of extraradical mycelium of AMF to take up and translocate Cs to roots. This was shown under *in vitro* cultural conditions, i.e. in the absence of K and in solution phase. Under pot culture conditions, the uptake and translocation were not perceptible. These contrasting results are, however, not contradictory and could be related to the growth conditions, (e.g. the concentration of potassium) different in both systems and the culture system (liquid *in vitro* and solid *in vivo*). In any case, translocation was low
2. the higher affinity of the hyphae of AMF for Cs as compared to roots
3. the clear role of ECM in transport of Cs
4. the variability between ECM species, some being more efficient than other in transport of Cs.

For U, the results demonstrated:

1. the unambiguous U uptake and translocation ability of the extraradical mycelium of AMF
2. the influence of pH of the growth medium on uptake, translocation and transfer of U by AMF. In the rhizosphere, changes in soil characteristics also have a strong effect on U uptake by roots. An increase in rhizosphere pH coupled with a low P loading of the solution are both factors promoting the U mobility and absorption by roots of ryegrass

3. the higher flux rate in AMF hyphae than in roots and the role of the intraradical structures to the immobilization of U within mycorrhizal roots
4. the existence in AMF hyphal tissues of efficient mechanisms limiting the uptake and translocation of non-essential elements when compared to essential elements such as phosphorus
5. the higher sequestration potential of hyphae of AMF for U than translocation function and the converse for P
6. the reduction of U transport from root to shoot in presence of mycorrhizal *Medicago truncatula* plants versus non mycorrhizal plants.

Implications

The project MYRRH has strongly improved our understanding of the role of mycorrhizae in the uptake, translocation and transfer of two major RN, i.e. Cs and U, from soil to plant and, by the way, allows for a better insight of the potential use of mycorrhizae-plant associations for the phytostabilisation/phytoremediation of RN-contaminated areas.

From the results above, we can conclude that mycorrhizal fungi, AEM and ECM, have an impact on both Cs and U transport.

The impact of AMF on Cs uptake, translocation and transfer is limited (observable *in vitro* and not detectable *in vivo* – probably related to growth conditions and nutritional status) and by no way comparable to the impact of ECM on these three steps of transport.

Concerning U, the impact of AMF and ECM is evident both *in vitro* and *in vivo* and concern, at least, uptake and translocation. The transfer from fungal cells to plant cells is probably low (sequestration process) since the U concentration in shoots of mycorrhizal plants is markedly lower versus the control plants.

Both results obtained with AMF and ECM on Cs and U suggest a stronger potential of mycorrhizal fungi for phytostabilisation strategies than for phytoextraction. This is consistent with the potential of mycorrhizal fungi in protecting the plant from abiotic stresses and favouring land reclamation. Therefore in our case, the results obtained could be adapted to strategies oriented towards the control of run-off and land reclamation since numerous RN-contaminated sites are associated with industrial activities such as mining, presenting also heavy-metals problems for which the role of mycorrhizal fungi is well known. To improve the potential of mycorrhizal fungi, the selection of species exhibiting stronger CS and U phytostabilisation potentials should be conducted by a thorough screening for strains originating from difficult environmental sites.

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