Impact of arbuscular mycorrhizal fungi on uranium accumulation by plants

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Abstract

Contamination by uranium (U) occurs principally at U mining and processing sites. Uranium can have tremendous environmental consequences, as it is highly toxic to a broad range of organisms and can be dispersed in both terrestrial and aquatic environments. Remediation strategies of U-contaminated soils have included physical and chemical procedures, which may be beneficial, but are costly and can lead to further environmental damage. Phytoremediation has been proposed as a promising alternative, which relies on the capacity of plants and their associated microorganisms to stabilize or extract contaminants from soils. In this paper, we review the role of a group of plant symbiotic fungi, i.e. arbuscular mycorrhizal fungi, which constitute an essential link between the soil and the roots. These fungi participate in U immobilization in soils and within plant roots and they can reduce root-to-shoot translocation of U. However, there is a need to evaluate these observations in terms of their importance for phytostabilization strategies.

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1. Introduction

Uranium (U), which is the most abundant of the naturally occurring actinides, is not as rare as once thought. Its concentration in the earth's crust may range from 1 to 4 mg kg^{−1} in sedimentary rocks to tens or even hundreds of mg kg^{−1} in phosphate rich deposits (Qureshi et al., 2001; Langmuir, 1997) or in U ore deposits (Plant et al., 2003).

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It is considered to be more abundant than mercury, antimony, silver, or cadmium, and is about as abundant as molybdenum or arsenic. Uranium participates, along with other radionuclides, in dramatic phenomena such as volcanism, earthquakes, and age-scale hydrothermal activity associated with ore deposition due to the dissipation of heat energy from the earth (Plant and Saunders, 1996). It is naturally present in both aquatic and terrestrial environments (Coward and Burnett, 1994). However, anthropogenic U contamination of the environment, by mining and milling operations, especially, can contribute to important environmental degradation.

Even before its formal discovery by the German chemist, Martin Klaproth, in 1789, U has been used for a wide array of purposes starting from colouring glass and ceramics to its actual use in arms, munitions and the nuclear power industry. However, if its economic importance cannot be overlooked, the centuries of mining and milling of U, and of other elements during its exploitation, have resulted in the production of considerable amounts of radioactive waste materials which pose a threat to ecosystems and public health. In a toxicological context, it is, however, not the radioactivity of U that poses the biggest problem, as the most abundant U isotopes are only very weakly radioactive. Instead, it is the strongly acute chemical toxicity of U that is of most concern (Sheppard et al., 2005). At numerous sites, the improper storage of U-rich wastes has led to U contamination of the surrounding environment, as U can be dispersed in soil by run-off and dispersed through the air by wind. Another possible source of U pollution is the direct application of U-rich phosphate rock as an alternative to commercial processed P fertilizers (Van Kauwenbergh, 1997; Andersson and Siman, 1991; Williams and David, 1976). Continuous application over years may therefore lead to large area contamination of arable soil in developing countries (Chen et al., 2005a).

Uranium has no biological function. Yet, a wide range of organisms in both terrestrial and aquatic environments take up U (Kovalsky et al., 1967). For instance, plants (Shahandeh et al., 2001; Huang et al., 1998; Ebbs et al., 1998), bacteria, algae, and fungi (Abdelouas et al., 1999; Suzuki and Banfield, 1999) were shown to accumulate U. It has also been shown that the biological activity of bacteria, algae, fungi and plants can affect U speciation and thus U bioavailability by modifying the pH, extracellular binding, transformation and formation of complexes or precipitates (Kalin et al., 2004; Sar et al., 2004; Anderson et al., 2003; Dushenkov, 2003). These organisms can thus participate in reducing or enhancing U entry into the food chain, but could also be used to develop bioremediation tools to decontaminate U-polluted environments.

Soil properties (Shahandeh and Hossner, 2002), including pH (Ebbs et al., 1998) and soil microorganisms (Gadd, 1996) are recognised to play an important role in the accumulation of U by plants. However, in most studies (e.g. Vandenhove and Van Hees, 2004; Ebbs et al., 1998; Huang et al., 1998; Saric et al., 1995; Ibrahim and Whicker, 1988) no considerations of the effects of soil microorganisms were taken into account.

Plant roots are naturally associated with microorganisms, and these associations can have direct or indirect effects on the mobility, availability and acquisition of elements by plants. Huang et al. (1998) and Shahandeh et al. (2001) therefore proposed that long-term rehabilitation of U-contaminated sites could be performed using phytoremediation techniques and that associated microbiota should be considered. Among these microorganisms, mycorrhizal fungi are of particular interest as they occupy a unique position between the soil and the plant roots and act as an extension of the roots that transport elements to the plant. They have thus been shown to enhance plant mineral uptake, but also to restrict the accumulation of certain trace elements by plants. The possibility that mycorrhizal fungi could transport or immobilize U is therefore of great interest (Declerck et al., 2002).

Within the mycorrhizal fungi, arbuscular mycorrhizal (AM) fungi are the most widely distributed (Smith and Read, 1997) and have been suspected to eventually play a role in U immobilization and plant U accumulation (Declerck et al., 2002). However, despite these potential roles of AM fungi, no studies were performed on AM transport of U until the beginning of the European project MYRRH (use of mycorrhizal fungi for the phytostabilisation of radio-contaminated environments – 2000–2004). Nevertheless, Weiersbye et al. (1999) observed higher concentrations of U in intraradical structures of an AM fungus as compared to host root tissues. Whether or not this possible accumulation of U by such AM intraradical structures could have altered plant U acquisition or accumulation was not determined. The purpose of this paper is to review the recent findings on the role of AM fungi on U immobilization and plant U accumulation.

2. Role of AM fungi in the uptake, translocation and immobilization of U

2.1. Effect of pH

The first study on the role of an AM fungus on plant U acquisition was performed by Rufyikiri et al. (2002). These authors investigated whether the extraradical mycelium of *Glomus intraradices* (MUCL 41833) could take up U and

translocate it towards its host. The experimental system selected for this first study was an in vitro bi-compartmented culture system (Dupré de Boulois et al., 2008; Declerck et al., 2005). Briefly, this system allows the culture of root organs in association with an AM fungus under monoxenic culture conditions (Declerck et al., 2005) and permits transport studies where only the fungal partner has access to a compartment containing a radio-tracer (Rufyikiri et al., 2005). This spatial separation is achieved by using bi-compartment Petri plates where a root and associated AM fungi are grown in one compartment (i.e. the root compartment, RC) with extraradical fungal mycelium developing into a neighbouring compartment (i.e. the hyphal compartment, HC) (Rufyikiri et al., 2005). This compartmented in vitro system was preferred by Rufyikiri et al. (2002) over bi-compartmented pot systems containing soil because pH of the medium where the extraradical mycelium was developing could be precisely controlled. Indeed, it is known that the pH can play a prevalent role on the process of U uptake because of its effects on the extracellular charges of fungal mycelium (Zhou, 1999; Gadd and White, 1990) and on dominant U species available to the fungal mycelium (Suzuki and Banfield, 1999; Grenthe et al., 1992). In the experiment of Rufyikiri et al. (2002), the pH of the medium in contact with the extraradical mycelium was adjusted to 4, 5.5 or 8 at the beginning of the experiment. The dominant U species present were uranyl cations and uranyl sulfate at pH 4, uranyl phosphate at pH 5.5, and anionic uranyl carbonate at pH 8 (Rufyikiri et al., 2002).

The results obtained in the study of Rufyikiri et al. (2002) showed that *G. intraradices* could take up, immobilize and translocate U towards its host. The influence of the pH on these processes, by modifying U speciation and therefore U species and U species composition in contact with the extraradical mycelium, was also demonstrated. The uptake and adsorption of U represented 2.2, 1.4 and 0.9% of the initial U supply at pH 4, 5.5 and 8, respectively, and 22, 12 and 25% of this U were subsequently translocated towards the roots. Thus, it appeared that uranyl cations and uranyl sulfate (pH 4) were taken up and translocated to a higher extent than uranyl phosphate (pH 5.5). Uranyl carbonate (pH 8) was translocated to a similar extent as uranyl cations and uranyl sulfate, but its uptake was very limited. Effects of U speciation on root uptake and root-to-shoot translocation of U were also observed in nutrient solution where *Pisum sativum* at pH 5 took up and translocated the uranyl cation more readily than the hydroxyl and carbonate U species present in the solution at pH 6 and 8, respectively (Ebbs et al., 1998). The results of Rufyikiri et al. (2002) finally showed that the immobilization of U in the fungal biomass depended on the U species present at different pH. If only the fungal biomass in the labelled compartment was considered, the immobilization of U represented 2.2, 59 and 59% of the U taken up and adsorbed by *G. intraradices* at pH 4, 5.5 and 8, respectively. Rufyikiri et al. (2002) suggested that this variability of immobilization could be the result of intracellular complexation of U by polyphosphates or the adsorption of U on negatively charged constituents of the fungal tissues due to high cation exchange capacity (CEC) (Joner et al., 2000). Sequestration of U by glycoproteins (including glomalin) produced by the extraradical hyphae could also be possible as these proteins have been shown to adsorb several metal cations (González-Chávez et al., 2004). In the study of Rufyikiri et al. (2002), the high H^+ activity in the medium probably led to a low adsorption of UO_2^{2+} onto the surface of the fungal mycelium at pH 4. This would explain the low total values for uranyl sorption (adsorption + absorption) at this pH. At pH 5.5 and 8, the same binding sites were probably more active in UO_2^{2+} adsorption and consequently, the positively charged cations present in the media could be adsorbed on the surface of the fungal hyphae and their glycoproteins. However, increasing the pH of the medium did not enhance U adsorption, even if more negative charges were to be expected. Indeed, at higher pH, and especially at pH 8, the major U species in solution were neutral or negatively charged. Nonetheless, at pH 5.5, Rufyikiri et al. (2003) observed that Cu-extractable U represented 15% of the U content of the fungal mycelium, and that further extraction with HCl desorbed 67% of U (200 mg fresh roots and 20 mg fresh extraradical mycelium were equilibrated for 3 h with 15 ml and 5 ml of a solution containing 10^{-2} M $CuSO_4$ and then with 10^{-1} M HCl). Finally, possible adsorption of U on intracellular membranes is also to be excluded as cytoplasmic pH was probably near neutral and vesicular or vacuolar pH is slightly acidic (Rasmussen et al., 2000; Saito et al., 2004). Consequently, Rufyikiri et al. (2002) suggested that following the uptake of U, the differences of U immobilization and translocation between different pH treatments were probably due to the complexation of U with polyphosphates and this especially at pH 5.5 where phosphate species are dominant.

Sequestration of U by polyphosphates has been observed in an isolate of a gram-positive bacteria closely related to *Arthrobacter ilicis* (Suzuki and Banfield, 2004). Rufyikiri et al. (2002) therefore suggested that polyphosphates could play a major role in the immobilization of U by *G. intraradices*. However, it should be pointed out that polyphosphates are not immobile, as they are involved in the translocation of P, and probably other elements from the extraradical hyphae to the intraradical structures of AM fungi (see Dupré de Boulois et al., 2008). Nonetheless, if we suppose

that polyphosphates participate in the immobilization of U in the extraradical mycelium, it should be noticed that the pH of the cytoplasm, and of organelles of AM fungal mycelium is probably stable due to close metabolic control as it can be observed for any organisms or cellular compartment (Ayling et al., 1997; Kurkdjian and Guern, 1989). Therefore, the complexation of U with polyphosphates should have been equivalent for different pH treatments. Consequently, if the uptake, translocation and immobilization of U have been affected by the pH of the medium, the role of U species presents in the medium probably had only an effect on extracellular adsorption and on the capacity of *G. intraradices* to take up these species. It could also be assumed that the pH of the medium might be responsible for the efficiency and activity of the transporters responsible for U uptake, but also of other elements that could be associated with U or compete with U. Furthermore, the pH of the medium could have influenced the overall physiology of the AM fungi.

2.2. Effect of phosphorus

In soils, mobile P occurs as negatively charged phosphate ion H_2PO_4^- in acid soils or HPO_4^{2-} in alkaline ones. These anions react with U to form poorly soluble uranyl phosphates or precipitation products that are stable under most geochemical conditions (Jerden and Sinha, 2003; Sandino and Bruno, 1992). Phosphorus fertilization may thus reduce U mobility and bioavailability. Reports on the uptake of U by roots confirmed this hypothesis and pointed out the important role of P in the chemical immobilization of U in soils as U–phosphate complexes (Rufyikiri et al., 2006; Ebbs et al., 1998). Accordingly, it has been suggested that U root-to-shoot translocation might be influenced by the improved P nutrition of mycorrhizal plants caused by P transport by AM fungi (Chen et al., 2005a,b).

Chen et al. (2005b) conducted an experiment to assess the effect of P fertilization on mycorrhizal and non-mycorrhizal wildtype (WT) and bald root barley mutant (Brb) plants. They observed increased shoot U contents for non-mycorrhizal plants, but unaffected shoot U concentration with increasing P levels, while P improved shoot biomass. They also observed that moderate P level (20 mg P kg⁻¹ soil) increased root U accumulation, but that high P level (60 mg P kg⁻¹ soil) decreased it. These observations corroborate those obtained by Chen et al. (2005a) with either no P added or 50 mg P kg⁻¹ soil. The first observation suggested that P, which participated in higher shoot biomass production, caused a dilution effect on U accumulation in shoots. For roots, the results suggested that U–P interactions are probably concentration dependent. However, these results contradict the results of Rufyikiri et al. (2006) and the finding of Ebbs et al. (1998) that U–phosphate complexes immobilize U. In addition, the predominant U species present at the pH of their substrate (8.3) were most probably uranyl carbonate species and not uranyl phosphate species. Chen et al. (2005b) did not see a clear pattern of increasing P fertilization of mycorrhizal plants on the accumulation of U in either roots or shoots. They explained that the U–P interactions became more complex, due to the response of mycorrhizal fungi to P. Measurement of the concentration of U in the soil solution with increasing P fertilization and U species determination would have been valuable in this study.

2.3. Contribution of AM fungal mycelium and roots to U uptake, translocation and immobilization

Although AM fungal mycelium was shown to take up, translocate but also immobilize U (Rufyikiri et al., 2002), the relative contribution of AM extraradical mycelium, and of mycorrhizal or non-mycorrhizal roots to take up, translocate and immobilize U was not investigated. Rufyikiri et al. (2003) therefore carried out an in vitro experiment to investigate whether an AM fungus contributed significantly to U acquisition by roots as compared to direct U uptake by the roots.

The AM fungus turned out to exert a major influence on root uptake of U. Indeed, while non-mycorrhizal roots took up and/or adsorbed 8% of the U supplied over a 2-week labelling period, for mycorrhizal roots it represented 26%. They also noticed that the biomass-specific U content of the fungal extraradical biomass was 5.5- and 9.7-fold higher than the one of the mycorrhizal and non-mycorrhizal root, respectively. However, it is worth noticing that the capacity of the AM fungus to translocate U was nonetheless higher than the one of mycorrhizal and non-mycorrhizal roots. Indeed, while mycorrhizal and non-mycorrhizal roots translocated about 50 and 15% of the U that they took up and adsorbed, respectively, AM hyphae translocated over 70% of the U that they took up or adsorbed.

The difference of U immobilization between the roots and the AM fungus could be due to the higher cation exchange capacity (CEC) of the fungal mycelium (Joner et al., 2000). However, although Rufyikiri et al. (2003) measured a higher CEC of the fungal mycelium, the Cu-extractable U represented only 15 and 6% of the U contents

of the mycelium and roots, respectively. Further extraction procedures using HCl showed that 67 and 47% of U could be desorbed, while most Ca and Mg were extracted. Rufyikiri et al. (2003) therefore suggested that other mechanisms of immobilization than adsorption were involved as Ca^{2+} and Mg^{2+} were released, probably as cations competing with UO_2^{2+} for the fungal and roots binding sites. Rufyikiri et al. (2003) consequently proposed that the immobilization of U by fungal hyphae and along the roots could be due to the formation of complexes or precipitates. González-Chávez et al. (2004) showed for Cu that some glomalin–Cu complexes were more stable than CuCl_2 . Therefore, it is possible that some U could remain in glomalin–U complexes even after HCl extraction. For the roots, complexation or precipitation of U with oxalate, phosphate, or polynuclear hydroxyl formation was suggested (Rufyikiri et al., 2003).

Rufyikiri et al. (2003) also observed a larger U concentration in mycorrhizal roots than in non-mycorrhizal roots developing in their labelled compartment. They suggested that it could be due to improved U uptake mechanisms of the mycorrhizal roots and/or the immobilization of U in intraradical fungal structures after its translocation from the extraradical to the intraradical mycelium of the AM fungus. The first hypothesis could be supported by the fact that the mycorrhizal association can modify the expression of several transporter genes (Burleigh and Bechmann, 2002). The second hypothesis, nevertheless, appears more plausible as the translocation of U to the roots by fungal mycelium was demonstrated (Rufyikiri et al., 2002, 2003), and as Weiersbye et al. (1999) observed high concentrations of U in the vesicles of an AM fungus.

Weiersbye et al. (1999) suggested that these intraradical structures could sequester metals and radionuclides and consequently reduce their (chemo-)toxicity. Indeed, as these structures serve as storage of lipids and have a low metabolic activity (van Aarle and Olsson, 2003; Graham et al., 1995; Gerdemann, 1968), compartmentalization of toxic elements within them would not greatly affect the AM fungi. This immobilization would also limit the transfer of these elements from the AM fungi to the plant. Finally, it has to be noticed that in heavy metal contaminated sites, vesicle abundance can increase (Chen et al., 2005c; Whitfield et al., 2004; Turnau et al., 1996).

2.4. Role of root hairs and AM fungi in the acquisition of U by plants

In terms of nutrient acquisition by roots, it appears that root hairs and AM fungal mycelium are functionally equivalent (Jakobsen et al., 2005). Plant species with fine and dense root systems, as well as plants with long root hairs, are for instance less dependent on mycorrhizal fungi for P acquisition (Schweiger et al., 1995; Baon et al., 1994; Baylis, 1975). These plants are therefore considered to have a lower mycorrhizal dependency than plants with short and thick roots or fewer or shorter root hairs (Declerck et al., 1995; Baon et al., 1994). This lower dependency is accompanied by a lower root colonisation, a reduced P acquisition through the AM fungi and, in general, a reduced plant growth promotion (Khaliq and Sanders, 2000; Fay et al., 1996; Plenchette and Morel, 1996).

Chen et al. (2005a) studied the influence of *G. intraradices* and of root hairs on the uptake of U from U-containing phosphate rocks by wildtype (WT) and root hairless mutant (Brb) of barley. As previously mentioned, rock phosphate is used as an alternative of commercial fertilizers in developing countries, principally, because of their low cost and strong plant responses (Rajan et al., 1996; Semoka et al., 1992). However, some rock phosphates possess relatively high concentration of U (310 mg kg^{-1} for the one used in the study of Chen et al., 2005a), and repetitive application in fields could represent a source of U pollution which might threaten plant yield, as well as animal and human health, due to the chemotoxicity of U. In the experimental system of Chen et al. (2005a), the working concentration of U was only 6.2 mg kg^{-1} and probably not toxic.

The uptake of U by non-mycorrhizal plants was much higher for WT barley than for Brb mutants. Interestingly, U uptake was higher in mycorrhizal than in non-mycorrhizal Brb mutants. The larger absorbing area provided by root hairs or by the mycorrhizal extraradical mycelium appeared therefore determinant in U accumulation by plants. However, this hypothesis should be taken cautiously as no difference in U accumulation of WT was observed between mycorrhizal and non-mycorrhizal plants. The fact that mycorrhizal hyphae can act as an alternative to root hairs' uptake (Schweiger et al., 1995) could explain this observation. Indeed, a reduction of the uptake of U, and possibly of other elements, by root hairs could be prevented by the mycorrhizal symbiosis. For instance, root colonisation by *G. intraradices* did not enhance P uptake and growth of WT barley. This could imply that WT barley was not dependent on a mycorrhizal association for nutrient acquisition. Therefore, the participation of mycorrhizal hyphae in the uptake of U could also be very limited in WT barley. In the case of the Brb mutants, the mycorrhizal dependency is obvious as P uptake by these plants was significantly increased. Chen et al. (2005a) concluded that AM fungi could be essential to

support the growth of plants having no or poorly developed root hairs. The higher U uptake in mycorrhizal Brb mutants probably represents the participation of the extraradical mycelium of the AM fungi in U acquisition by these plants. However, the mycorrhizal fungal mycelium could not entirely replace the uptake of P or U by the root hairs (excluding potential effects of the Brb mutation on other morphological or physiological characteristic of the plant and symbiosis). Therefore, in addition to the importance of the mycorrhizal status of plants having no or poorly developed root hairs in selecting plant species for phytoremediation (Chen et al., 2005a), the mycorrhizal dependency and biomass production of these plants should also be considered.

Chen et al. (2005a) also observed that less than 15% of plant U content was present in the shoots. This is in accordance with other studies showing that U accumulates predominantly in the roots (e.g. Saric et al., 1995; Zafrir et al., 1992; Sheppard and Thibault, 1984). They also noticed that the non-inoculated Brb plants had a markedly higher shoot-to-root ratio of total U uptake than the mycorrhizal Brb plants. This further confirms that in the presence of AM fungi, accumulation of U in shoots is reduced.

As previously mentioned, Chen et al. (2005a) observed a higher U concentration in mycorrhizal roots compared to non-mycorrhizal roots of the Brb mutant plants. This could be due to U immobilization in the fungal intraradical mycelium after U uptake and translocation, and would also explain the lower U accumulation in the mycorrhizal plants. In WT barley, the participation of *G. intraradices* in U immobilization was most likely limited. Thus it can be suggested that root uptake was the main mechanism responsible for U acquisition. Therefore, the main mechanisms of U accumulation in plants being root uptake, the translocation of U from root-to-shoot would have been influenced by the mycorrhizal status of the plant.

3. Speciation of U due to fungal and root activity and role of mineral cations in the transport of U by AM fungi

Rufyikiri et al. (2002) showed that the U species present at a given pH was of major importance for AM uptake, translocation and immobilization of U. However, several studies (e.g. Bago and Azcon-Aguilar, 1997; Bago et al., 1996) have shown that pH modifications occur in the rhizosphere and hyphosphere due to the ion uptake and efflux activities of roots and AM fungi. In order to know if such modifications were also recorded in their system, Rufyikiri et al. (2003) measured the impact of the extraradical mycelium and roots metabolic activities on the pH of the medium contained in the labelled compartment. When only the extraradical mycelium of the AM fungus was in the labelled compartment, a pH increase was observed (from 5.5 to 6–6.5). However, when roots, mycorrhizal or not were in the labelled compartment, a pH decrease was noted (from 5.5 to 5). In spite of this, Rufyikiri et al. (2003) did not discuss the effect of these modifications on the uptake, translocation or immobilization of U. It is nonetheless evident that U speciation would have differed due to the modification of the pH of the medium. Indeed, U speciation does not only depend on the pH, but also on the composition of the solution in which it is present (Grenthe et al., 1992). It should be noticed that during the course of the experiments of Rufyikiri et al. (2002, 2003), secretion or efflux of various compounds by AM fungus and roots could also have modified U bioavailability. For instance, glomalin could have participated in the immobilization of U in the medium even if it mainly remains tightly bound to the AM mycelium (Driver et al., 2005).

In the study of Rufyikiri et al. (2004a) a dual labelling of the medium for extraradical hyphal uptake with ^{233}U and ^{33}P was performed to compare U transport processes of U and P, the latter being a positive control for symbiotic functioning. The results showed that *G. intraradices* took up and translocated these elements, but that the efficiency of *G. intraradices* in these processes varied between the two elements. Indeed, P was taken up and translocated to a much higher extent than U. Furthermore, competition between U and other cations such as Ca^{2+} , Mg^{2+} or K^{+} for sites of adsorption (Rufyikiri et al., 2002), for uptake through cation transporters and for complexation with polyphosphates or immobilization in fungal structures such as vesicles could probably occur.

It should be pointed out that the transport of P by AM fungi can probably play a role in U uptake, as U can be associated with P in the medium (uranyl phosphate) and in AM intracellular structures (polyphosphates). As a consequence, the uptake and translocation of U by AM fungi in the systems used by Rufyikiri et al. (2002, 2003) could have been affected by the probable rapid depletion of P in the medium.

As already mentioned, speculation on the U species present within the fungal and plant biomass, including different organelles, tissues and organs should be taken cautiously. In the studies of Rufyikiri et al. (2002, 2003), but also of

Ebbs et al. (1998), no difference was mentioned between U species present in their medium and the U species that were actually contained within the AM fungi or plants. Intracellular pH of the cytoplasm, vesicle and vacuole of AM fungi is maintained within a close range to ensure a large number of cellular activities and is strictly controlled (Kurkdjian and Guern, 1989). For this reason, Jolicœur et al. (1998) did not observe any modification of the cytosolic pH of *Gigaspora margarita* at varying external pH. Therefore, U species that are taken up by the extraradical mycelium of an AM fungus or plant roots will be affected by both the pH and the mineral composition of the cells or organelles that they enter.

4. Influence of AM fungi on root-to-shoot U translocation

The results obtained by Rufyikiri et al. (2002, 2003, 2004a) using in vitro culture systems demonstrated the role of an AM fungus in the uptake, translocation and immobilization of U. However, these studies did not allow determining whether AM fungi can transfer U to their host or whether it is immobilized in the fungal intraradical structures. Therefore it was not possible to determine whether AM fungi can influence the accumulation of U in plant tissues. Furthermore, while addressing the accumulation of U in plants, the influence of AM fungi on the partition of U between roots and shoot is of great radioecological importance, both for the cycling of U in ecosystems and for remediation strategies.

The study presented by Rufyikiri et al. (2004b) aimed at determining the role of the AM fungus *G. intraradices* in these processes. Mycorrhizal and non-mycorrhizal plants were grown in a substrate containing U concentrations from 0 to 87 mg kg⁻¹ dry soil. The initial soil pH was 5.2, and the predominant U species was uranyl ions (added as uranyl acetate).

The first observations aimed at establishing whether, by increasing U concentrations in soil up to 90 times the natural U background, the plant biomass and mycorrhizal colonisation would be affected by U. The results presented by Rufyikiri et al. (2004b) showed that the biomass production of neither roots nor shoot was influenced by U levels. Rufyikiri et al. (2004b) suggested that at the U levels tested, the fraction of U in the soil solution was probably extremely low due principally to the pH of the soil and were therefore not threatening for plant development. This is similar to previous observations and is in accordance with U ecotoxicity thresholds of 250 mg kg⁻¹ dry soil proposed to be protective for terrestrial plants (Sheppard et al., 2005). The root colonisation of AM plants was high and typical intraradical structures were observed at all U levels and did not vary among them. Plant biomass enhancement by the AM fungus was also observed and was attributed to the improved mineral nutrition of the plants. This gain was especially due to a higher shoot biomass which is often observed as the result of improved P nutrition resulting from the active transport of this element by AM fungi (Smith and Read, 1997). Measurements of the extraradical mycelium biomass or fungal activity were not performed in this study, but it is possible to assume that U did not affect *G. intraradices*. Indeed, Sheppard et al. (1992) showed that phosphatase activity in soils was affected by U only when its concentration reached 100 mg kg⁻¹ dry soil.

Uranium root levels, for mycorrhizal or non-mycorrhizal roots were always higher (60–360-fold) than the one in the shoots and were consistent with previous results (Saric et al., 1995; Zafrir et al., 1992; Sheppard and Thibault, 1984). The novel information provided by this study was that *G. intraradices* decreased the concentration of U in the shoot of clover (*Trifolium subterraneum*) at the highest U concentration in soil (i.e. 87 mg kg⁻¹). The authors thus suggested that *G. intraradices* might decrease U concentration in shoots of plants grown at high levels of U by reducing U translocation from root-to-shoot. Chen et al. (2003) also demonstrated that the effect of AM fungi on Zn uptake changed with increasing Zn concentration in soil. Below a critical Zn soil concentration, Zn uptake was enhanced, while above this level Zn translocation to the shoot decreased. The results of Rufyikiri et al. (2004b) were corroborated by those obtained by Chen et al. (2005a,b,d, 2006) who observed an increased U immobilization in roots, a lower U accumulation in shoots and consequently, a lower translocation of U from root-to-shoot when plants were colonized by AM fungi. Chen et al. (2005d) also noticed, using a compartmented pot system, that more U was partitioned to the shoots of *Medicago truncatula* when roots and hyphae could take up U than when only hyphae could develop in the contaminated soil compartment. This indicated that U taken up through the root pathway could be more easily translocated to the shoots than when U was taken up by the fungal hyphae. This further showed that U taken up by AM fungi is, at least partly, immobilized within the intraradical structures of the fungal symbionts.

5. Conclusion

The results presented here on the participation of AM fungi in the biochemical cycling of U show that these symbiotic fungi have a potential for use in phytoremediation strategies aiming at stabilization of U in soils, with concurrent reduction of U accumulation within aerial plant parts. Our literature review revealed that AM fungi can immobilize U in soils by adsorption, and potentially by the formation of complexes with AM glycoproteins and intracellular polyphosphates. In addition, even though AM fungi can transfer U to their hosts, and thus participate directly in U accumulation by plants, it is also clear that most of the U translocated by AM fungi towards their intraradical mycelium remains within AM fungal structures, thereby restricting root-to-shoot translocation of U.

However, further research is required in order to develop phytostabilization strategies involving AM fungi. Until now, studies have been limited to in vitro or pot culture condition and field trials are necessary to evaluate the feasibility of adopting AM fungi in U phytostabilization strategies. It should also be interesting to study AM fungal strains from U-polluted sites. AM fungi do develop tolerance towards heavy metals (Gildon and Tinker, 1981) and other microorganisms than AM fungi, do develop such tolerance towards U (Joner et al., 2007). Such enhanced tolerance would be highly valuable as it could allow better protection of AM plant hosts and eventually increase the stabilization of U and other pollutants.

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