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Assessing the tolerance to heavy metals of arbuscular mycorrhizal fungi isolated from sewage sludge-contaminated soils

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Abstract

Different fungal ecotypes were isolated from soils which had received long-term applications of metal-contaminated sewage sludge with the aim of studying the degree of tolerance and adaptation to heavy metals of arbuscular mycorrhizal (AM) fungi. The development and structural aspects of AM colonization produced by the different fungal isolates were studied using two host plants, *Allium porrum* and *Sorghum bicolor*, which were grown in either contaminated or non-contaminated soils. Four different AM fungi were successfully isolated from the experimental field plots: (i) *Glomus claroideum*, isolated from plots receiving only inorganic fertilizer; (ii) another apparently similar ecotype of *Glomus claroideum*, but isolated from plots with 300 m^3 ha^{-1} $year^{-1}$ of contaminated sludge added, (iii) an unidentified *Glomus sp*., present only in the less contaminated plots (100 m^3 ha^{-1} $year^{-1}$ of unamended sludge) and (iv) *Glomus mosseae*, isolated from plots receiving 100 or 300 m^3 ha^{-1} $year^{-1}$ of amended or unamended sludge (intermediate rates of contamination). There were consistent differences in behaviour among the four AM fungi tested with regard to the colonization levels they produced in non-contaminated and contaminated soils. Both total and arbuscular colonization were affected by heavy metal contamination. The main conclusions of this study are that *Glomus* sp. and *G. mosseae* isolates are strongly inhibited by heavy metals, which acted mainly by interfering with the growth of the external mycelium, and also by limiting the production of arbuscules. Our results suggest that *G. claroideum* isolates, particularly the ecotype which was isolated from the plots receiving the highest dose of metal-contaminated sludge, shows a potential adaptation to increased metal concentration in soil. © 1999 Elsevier Science B.V.

Keywords: Arbuscular mycorrhizal fungi; Heavy metals; Sewage sludge-contaminated soils; Stress tolerance

1. Introduction

Sewage sludge application to agricultural land has been a widely accepted practice for decades. It aids in the recycling of essential nutrients and also acts as a source of organic matter improving the structure and water-holding properties of the soil (Koomen et al., 1990). However, sludges often contain appreciable amounts of potentially toxic metals which can persist, long after the sludge addition stops, in the cultivated soil layer, thereby increasing their availability to toxic levels (McGrath, 1987). Some studies carried out in soils amended with metal-contaminated sludges, such as Woburn, UK (Brooks and McGrath, 1984; McGrath et al., 1988), Braunchweig, Germany (Fließbach et al., 1994) and Ultuna, Sweden (Witter et al., 1993), have shown deleterious effects of the metals on the activity

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and diversity of soil microbial populations (McGrath et al., 1995). None of these experimental sites had especially high metal concentration levels, and some were still below the maximum limits set by the EU for agricultural sludge applications (Brooks et al., 1986; McGrath et al., 1988, 1995; Chaudri et al., 1993).

In the context of microbial diversity studies, arbuscular mycorrhizal (AM) fungi, which are among the most common components of soil microbial populations, must be considered. These fungi belong to the order Glomales in the Zygomycetes, and establish, with the majority of higher plants, the most widespread mutualistic symbiosis on earth, called arbuscular mycorrhiza (Barea and Jeffries, 1995). Both the AM symbiosis and the AM fungi (AMF) occur in almost all habitats and climates (Barea et al., 1997), including disturbed soils (Bundrett et al., 1996; McGonigle and Miller, 1996). Soil degradation usually produces changes in the diversity and abundance of AM fungal populations (Koomen et al., 1990; Jasper et al., 1991; Loth, 1996; del Val et al., 1998). This is critical because of the role of mycorrhizal fungi in plant establishment and survival (Miller and Jastrow, 1992; Haselwandter and Dowen, 1996). High metal concentrations are consequently expected to interfere with possible beneficial effects of the mycorrhizal symbiosis. However, very few studies have been carried out involving the interactions between AMF and heavy metals. Most of the results already obtained derive from laboratory and pot experiments using metal salt-amended soils, which are not representative of field conditions where metals are often accumulated over long periods of time, usually in less available chemical forms. On the other hand, few and not very consistent results are currently available on the significance and behaviour of these fungi in soils contaminated with metals derived from sewage sludge applications. Gildon and Tinker (1981) reported that high amounts of heavy metals can delay, reduce or even completely eliminate AMF spore germination and AM colonization at concentrations at which phytotoxic effects were not observed. Similarly, Boyle and Paul (1988) reported a negative correlation between Zn concentrations in a soil treated with urban–industrial sludge and AM colonization in barley. In other studies, however, the addition of metal-containing sludge did not affect AM development under field conditions (Arnold and Kaputska, 1987). These contrasting results may be explained due to the fact that different AMF ecotypes can exhibit varying degrees of tolerance to metals (Haselwandter et al., 1994). A higher tolerance to Cu, Zn, Cd and Pb of indigenous fungi from sludge-polluted sites, in comparison to reference isolates from unpolluted soils, has been reported (Gildon and Tinker, 1983; Weissenhorn et al., 1993; Díaz et al., 1996).

With the aim of studying the degree of tolerance and adaptation to heavy metals of AMF populations, different fungi were isolated from soils which had undergone long-term field experiments with metal-contaminated sewage sludge applications. The formation of the mycorrhiza by the various endophytes isolated, as well as the structural aspect of the symbiosis in contaminated and non-contaminated soils, were studied using different host plants.

2. Materials and methods

2.1. Mycorrhizal fungi isolation and multiplication

2.1.1. Soil origin and characteristics

AM fungi were isolated from soils taken from a long-term field experiment on sewage sludge application located in the Federal Research Centre for Agriculture in Braunchweig (Germany). Two different sludges were used: one to which heavy metals were previously added (contaminated sludge) and another that did not receive extra addition of heavy metals (non-contaminated sludge). The experimental site was a woodland until 40 years ago, when it was converted into arable soil. The soil (silty loam) contains 5% clay, 50% silt and 45% sand, and its pH ranges between 5.3–6.0 (Chaudri et al., 1993). Five different treatments were applied annually from 1980 to 1990, to the experimental plots: inorganic fertilizer at 180 kg N ha^{-1} $year^{-1}$, uncontaminated sewage sludge at 100 and 300 m^3 ha^{-1} $year^{-1}$, and metal-contaminated sludge at the same two rates of application. The application of different sludges caused significant changes in soil properties (Chaudri et al., 1993). However, the highest amounts of heavy metals in the experimental site still remained within the range of the accepted EU upper limits for metal concentrations in agricultural soils amended with sewage sludges.

2.1.2. Isolation procedure

Isolation of AMF from the plots corresponding to the different sludge treatments was carried out by means of the standard wet-sieving and decanting technique. The spores were morphologically grouped under the microscope and groups of 10–20 apparently similar spores were employed as starting inoculum for multiplication, using *Sorghum bicolor* (L.) Moench as the host plant. The soil of provenance of each fungus, previously sterilized and diluted with sand (1 : 1, v : v) was the substrate used to multiply the corresponding AMF isolate. The fungi that successfully multiplied and sporulated in the soil were checked for purity, identified to genus and, where possible, to the species level.

2.2. AM fungal tolerance to heavy metals

2.2.1. Experimental design

To study the tolerance to heavy metals of the different AM fungi, a greenhouse experiment was carried out following a factorial design. It consisted of 2 soils × 4 mycorrhizal fungi × 2 host plants. Eight replicates of each of the 16 treatments were prepared.

2.2.2. Experimental soil

The study was carried out in soils from the long-term field experiment on sewage sludge application (Section 2.1.1), using only the inorganically fertilized (non-sludge amended) soil, and soil amended with 300 $m^3\ ha^{-1}\ year^{-1}$ contaminated sludge (contaminated soil) used. The main characteristics of the soils are recorded in Table 1.

2.2.3. Plant and growth conditions

Two host plants, *Sorghum bicolor* (L.) Moench and *Allium porrum* L. were used. Seeds were surface-sterilized, pre-germinated in Petri dishes and sown after emergence into 350 cm^3 pots (one seedling/pot) containing a sterile mixture of the appropriate soil with sand (1 : 1, v : v) to minimize soil compaction in the pots. At sowing, all plants were inoculated with one of the four AMF isolated from the experimental plots (see Section 3). Mycorrhizal inoculum consisted of crude soil (15 g/plant) from stock cultures of the corresponding AM fungus, containing spores, mycelium and colonized root fragments. Plants were grown in a greenhouse, with temperatures ranging from 18 to 25°C, and relative humidity from 60 to 80%. Plants were irrigated every 3 days with tap water and, once a week they were fertilized with Long Ashton nutrient solution lacking phosphorus (Hewitt, 1966). Plants were harvested after 4 and 8 weeks of growth.

2.2.4. Measurements

At harvest, roots were washed to remove soil particles and shoot and root weight was determined. Mycorrhizal colonization (percentage of root length) was estimated microscopically after staining the root system with trypan blue (Phillips and Hayman, 1970), by using the magnified intersection method (McGonigle et al., 1990). The presence of structural characteristics of arbuscular mycorrhiza, such as coils, arbuscules and vesicles, was also recorded and the percentage of total root length containing these structures calculated. The presence or absence of AMF external mycelium on non-mycorrhizal root fragments was also recorded.

Table 1
Soil characteristics and total heavy metal concentration of the test soils

Soil[a]	Organic matter (%)	NH_4NO_3 P[b] (mg/l)	Total P[c] (mg/l)	pH	Total metal concentration (mg/kg soil)[d]				
					Zn	Cd	Cu	Ni	Pb
NC	1.5	0.9	461.7	6.0	43.0	0.3	9.8	8.6	28.8
C	2.3	3.4	1761.9	5.3	294.6	2.8	91.6	29.0	111.4

[a]NC, non-contaminated (with the addition of inorganic fertilizer at 180 kg N $ha^{-1}\ year^{-1}$); C, contaminated (amended with 300 $m^3\ ha^{-1}\ year^{-1}$ of metal-contaminated sewage sludge).
[b]NH_4NO_3 extracted P.
[c]Aqua regia extracted P.
[d]Aqua regia extracted metals.
Analyses performed by Drs. B. Knight and S.P. McGrath, from IACR-Rothamsted (UK) within the context of an EU project.

2.2.5. Statistical analysis

Data were processed by two-way analysis of variance (ANOVA) and Fisher's protected least significant differences (LSD) when appropriate. All data expressed as a percentage were arcsin-square-root transformed prior to statistical analysis.

3. Results

3.1. Mycorrhizal fungi isolation

Four different AMF were successfully isolated from the experimental plots: (i) *Glomus claroideum* Schenck and Smith, isolated from the plots without sludge addition (Gcl 2); (ii) another apparently similar *Glomus claroideum* ecotype, but isolated from the plots amended with 300 m^3 ha^{-1} $year^{-1}$ contaminated sludge (Gcl 7); (iii) an unidentified *Glomus sp.* (referred to as *Glomus* sp. III in del Val et al., 1998) present only in the less contaminated plots (those without sludge addition or amended with 100 m^3 ha^{-1} $year^{-1}$ of uncontaminated sludge) (Gsp) and (iv) *Glomus mosseae* (Nicholson and Genderman) Genderman and Trappe, present in the plots amended with 100 or 300 m^3 ha^{-1} $year^{-1}$ uncontaminated sludge or 100 m^3 ha^{-1} $year^{-1}$ of the contaminated one (Gm).

3.2. AM fungal tolerance to heavy metals

There were consistent differences in behaviour among the four indigenous AM fungi tested with regard to the mycorrhizal colonization levels they produced in non-contaminated and contaminated soils. For both plant species, leek and sorghum, *Glomus sp.* and *Glomus mosseae* were the best colonizers in the non-contaminated soil but only significantly so in leek (Tables 2 and 3). Mycorrhizal colonization was significantly reduced in the contaminated soil for the four AMF isolates, this reduction being significantly higher for *Glomus* sp. and *G. mosseae*, at both harvest times (Tables 2 and 3). *Glomus mosseae* seemed to recover at the second harvest from the inhibition induced by the polluted soil, but this ecotype always produced lower colonization levels than the isolates of *Glomus claroideum* (Table 2). Both isolates of *G. claroideum*, 2 (isolated from the non-contaminated soil) and 7 (isolated from the most heavily contaminated plot), produced a significantly higher colonization level in the heavy metal amended soil, at both harvest times, than that produced by the other two isolates (Table 2). Both *G. claroideum* isolates differed in their degree of tolerance to heavy metals, *G. claroideum* 7 being the fungus that produced greater

Table 2
Mycorrhizal colonization of the root system of *Allium porrum* (leek) and development of external mycelium on the non-colonized roots after inoculation with several AM fungi at two harvest times, as affected by soil contamination with heavy metals

AM fungi[a]	4 Weeks		8 Weeks	
	Mycorrhizal colonization[b]	External mycelium[c]	Mycorrhizal colonization[b]	External mycelium[c]
Non-contaminated soil				
Gcl 2	26.3 b	4.4 bc	31.3 ab	0.9 c
Gcl 7	24.7 b	1.3 c	28.9 abc	0.4 c
Gsp	35.7 a	5.5 b	40.4 a	6.9 a
Gm	30.6 ab	11.6 a	36.2 a	5.7 ab
Contaminated soil				
Gcl 2	10.9 c	3.6 bc	16.5 cde	0.4 c
Gcl 7	14.5 c	1.1 c	20.0 bcd	1.1 c
Gsp	2.7 d	0.0 d	5.9 e	0.3 c
Gm	2.6 d	2.5 bc	10.3 de	1.4 bc

[a]Gcl 2, *Glomus claroideum* isolated from a non-contaminated soil; Gcl 7, *Glomus claroideum* isolated from a contaminated soil; Gsp, *Glomus* sp.; Gm, *Glomus mosseae*.
[b]Percentage of mycorrhizal root length.
[c]Percentage of non-colonized root length having attached external hyphae of AM fungi.
Numbers in each column followed by the same letter did not differ significantly ($p \leq 0.05$) by Fisher's Protected LSD test.

Table 3
Mycorrhizal colonization of the root system of *Sorghum bicolor* (sorghum) and development of external mycelium on the non-colonized roots after inoculation with several AM fungi at two harvest times, as affected by soil contamination with heavy metals

AM fungi[a]	4 Weeks		8 Weeks	
	Mycorrhizal colonization[b]	External mycelium[c]	Mycorrhizal colonization[b]	External mycelium[c]
Non-contaminated soil				
Gcl 2	8.5 ab	13.2 bc	21.6 abc	1.5 d
Gcl 7	7.9 ab	9.6 cd	14.7 bc	9.5 bc
Gsp	9.1 a	23.8 ab	31.8 a	24.6 a
Gm	13.6 a	35.4 a	25.6 ab	15.4 abc
Contaminated soil				
Gcl 2	1.1 c	14.5 bc	13.6 bc	8.8 c
Gcl 7	6.3 ab	19.7 abc	15.4 bc	18.6 ab
Gsp	0.3 c	2.9 de	3.2 d	0.5 d
Gm	2.5 bc	1.2 e	12.4 c	2.0 d

[a]Gcl 2, *Glomus claroideum* isolated from a non-contaminated soil; Gcl 7, *Glomus claroideum* isolated from a contaminated soil; Gsp, *Glomus* sp.; Gm, *Glomus mosseae*.
[b]Percentage of mycorrhizal root length.
[c]Percentage of non-colonized root length having attached external hyphae of AM fungi.
Numbers in each column followed by the same letter did not differ significantly ($p \leq 0.05$) by Fisher's Protected LSD test.

percentage of root colonization in the contaminated soil (Tables 2 and 3). The behaviour of the various AMF isolates was similar when in symbiosis with either leek or sorghum, although colonization values were higher for leek plants (Tables 2 and 3). The ability of the different fungal isolates to establish the mycorrhizal symbiosis was reflected in the growth response of the plants. In non-contaminated soils *Glomus* sp. and *G. mosseae* were the most effective isolates in promoting plant growth, while in contaminated soils *G. claroideum* 7 was the most effective fungus in improving growth although no significant differences were found (Table 6).

There was a strong inhibition of the external mycelium from *Glomus* sp. and *G. mosseae* attached to the root surface in the contaminated soil (Tables 2 and 3). This inhibition was not evidenced in the case of both *G. claroideum* isolates. The strong stimulation of external mycelium induced by sorghum roots is noteworthy.

Although a certain inhibition was evident, both of the *G. claroideum* isolates to some extent retained the ability to form arbuscules when associated with leek in the metal-contaminated soil (Table 4), especially in the case of the *G. claroideum* 7 isolate at both harvest times. In contrast, almost no arbuscules were found in leek plants colonized by either *Glomus* sp. or *G. mosseae* growing in contaminated soil.

The behaviour of the different isolates was similar for sorghum plants, although the amount of arbuscular colonization was much lower in most situations, and was not detected in *Glomus* sp. inoculated plants grown in contaminated soil, either after 4 or 8 weeks of growth (Tables 5 and 6).

Coil production by *Glomus* sp. and *G. mosseae* was also strongly inhibited in metal-contaminated soil, especially in mycorrhizal plants with *Glomus* sp. The colonization ability of *G. mosseae* seemed to recover from metal contamination after 8 weeks and both coils and arbuscules were detected at this time in the colonized root tissues (Tables 4 and 5).

In conclusion, it is clear that the addition of heavy metal-contaminated sludge produced a significant inhibition of all the mycorrhizal parameters determined. This general trend was shown for both host plants, although the colonization levels were higher for leek than for sorghum. This is probably due to the more extensive root system of the sorghum plants, thereby 'diluting' the extent of colonization. Total, and particularly arbuscular colonization, was the mycorrhizal parameter most affected by heavy metal contamination.

Table 4
Percentage of root length of *Allium porrum* (leek) containing coiled hyphae or arbuscules produced by several AM fungi at two harvest times, as affected by soil contamination with heavy metals

AM fungi[a]	4 Weeks		8 Weeks	
	AM colonization with coils[b]	AM colonization with arbuscules[c]	AM colonization with coils[a]	AM colonization with arbuscules[c]
Non-contaminated soil				
Gcl 2	1.7 a	20.1 b	12.1 a	15.1 abc
Gcl 7	0.1 bc	22.1 b	4.4 bc	19.4 abc
Gsp	1.5 a	31.5 a	8.3 ab	26.8 a
Gm	2.4 a	25.8 ab	5.5 ab	22.9 ab
Contaminated soil				
Gcl 2	0.8 ab	3.3 d	6.8 ab	9.5 cd
Gcl 7	1.5 a	9.3 c	3.2 bc	13.8 bc
Gsp	0.1 bc	1.3 de	0.0 d	0.3 e
Gm	0.0 c	0.5 e	1.2 cd	2.7 de

[a]Gcl 2, *Glomus claroideum* isolated from a non-contaminated soil; Gcl 7, *Glomus claroideum* isolated from a contaminated soil; Gsp, *Glomus* sp.; Gm, *Glomus mosseae*.
[b]Percentage of total root length containing coiled hyphae of AM fungi.
[c]Percentage of total root length containing arbuscules.
Numbers in each column followed by the same letter did not differ significantly ($p \leq 0.05$) by Fisher's Protected LSD test.

Table 5
Percentage of root length of *Sorghum bicolor* (sorghum) containing coiled hyphae or arbuscules produced by several AM fungi at two harvest times, as affected by soil contamination with heavy metals

AM fungi[a]	4 Weeks		8 Weeks	
	AM colonization with coils[b]	AM colonization with arbuscules[c]	AM colonization with coils[b]	AM colonization with arbuscules[c]
Non-contaminated soil				
Gcl 2	5.2 a	4.1 a	11.4 ab	7.74 ab
Gcl 7	3.5 ab	4.6 a	5.1 bc	3.24 bc
Gsp	4.1 a	3.1 ab	14.5 a	15.45 a
Gm	7.3a	6.7 a	10.6 ab	11.12 a
Contaminated soil				
Gcl 2	0.6 c	0.7 b	3.1 c	3.3 bc
Gcl 7	1.0 bc	0.2 b	11.5 ab	1.5 cd
Gsp	0.1 c	0.0 b	0.0 d	0.0 d
Gm	1.0 bc	0.2 b	6.1 abc	3.0 bc

[a]Gcl 2, *Glomus claroideum* isolated from a non-contaminated soil; Gcl 7, *Glomus claroideum* isolated from a contaminated soil; Gsp, *Glomus* sp.; Gm, *Glomus mosseae*.
[b]Percentage of total root length containing coiled hyphae of AM fungi.
[c]Percentage of total root length containing arbuscules.
Numbers in each column followed by the same letter did not differ significantly ($p \leq 0.05$) by Fisher's Protected LSD test.

4. Discussion

Few studies have been carried out on the effect of sludge application with increasing concentrations of heavy metals on mycorrhizal formation and development. In particular, as far as we know, this is the first report in which different indigenous AM fungi, isolated from a long-term sewage-amended field soil, have been re-inoculated into the same soil in order to analyse the effect of such heavy metal contamination

Table 6
Shoot and root biomass (g) of *Allium porrum* and *Sorghum bicolor* after 8 weeks growing in both experimental soils

AM fungi[a]	*Allium porrum*	*Sorghum bicolor*
Non-contaminated soil		
Gcl 2	0.28 d	4.34 d
Gcl 7	0.48 d	4.84 d
Gsp	1.33 bc	5.73 cd
Gm	1.53 ab	7.63 bc
Contaminated soil		
Gcl 2	1.52 ab	12.01 a
Gcl 7	1.73 a	12.40 a
Gsp	1.50 ab	11.51 a
Gm	1.02 bc	9.68 ab

[a]Gcl 2, *Glomus claroideum* isolated from a non-contaminated soil; Gcl 7, *Glomus claroideum* isolated from a contaminated soil; Gsp, *Glomus* sp.; Gm, *Glomus mosseae*.
Numbers in each column followed by the same letter did not differ significantly ($p \leq 0.05$) by Fisher's Protected LSD test.

treatment on mycorrhizal colonization and, particularly, on the development of the characteristic structures of the AM symbiosis. The data reported indicate that the levels of AM colonization produced by the AMF isolates decrease significantly with the application of heavy metal-amended sludge ($300\ m^3\ ha^{-1}\ year^{-1}$). This effect may have been more pronounced because of the high availability of metals, due to the low pH of the experimental soil, as has already been reported (Angle and Heckman, 1986; Leyval et al., 1994). Negative effects of large quantities of heavy metals on AMF endophytes have previously been described in relation to the inhibition of AMF spore germination and hyphal extension in vitro (Hepper, 1979; McGee, 1987), a decrease in spore population (del Val et al., 1998) and the reduction or delay of the root colonizing ability (Gildon and Tinker, 1981; Koomen et al., 1990; Griffioen et al., 1994; Leyval et al., 1994, 1996; Vidal et al., 1996).

Despite the general inhibition in the establishment of the mycorrhizal symbiosis, the four isolates displayed different degrees of tolerance to heavy metals, *Glomus* sp. (isolated from the non-polluted soil) being the most sensitive fungus, while *G. claroideum* 7 (isolated from the most contaminated soil) was the most tolerant. The effectiveness of the different AM fungal isolates in improving plant growth depended on the level of heavy metals in soil. In non-contaminated soils, *Glomus* sp. and *G. mosseae* were the most effective, whereas *G. claroideum*, specially the isolate number 7, were slightly more efficient in the polluted soil. The recovery of the colonization ability at 8 weeks by *G. mosseae* suggests that mycorrhizal colonization was only delayed by the metals. However, *Glomus* sp. did not show such recovery. This markedly different susceptibility to heavy metals among the four different AM fungi corroborate previous findings concerning other fungi and ecosystems (Gildon and Tinker, 1983; Leyval et al., 1991; Weissenhorn et al., 1993; Díaz et al., 1996).

The inhibition of mycorrhizal colonization in the contaminated soil could at least partially be, due to the inhibition of fungal spread in the soil, a key point because the spread of mycorrhizal colonization is caused mainly by the growth of the external mycelium in the soil and along the root system (Schubert et al., 1988). The colonization of the root surface by the external mycelium of *Glomus* sp. and *Glomus mosseae* was strongly inhibited in the contaminated soil. This indicates that hyphal growth in the soil and, consequently, the pre-infective phases of these fungi were very sensitive to the presence of heavy metals, as previously suggested (Vidal et al., 1996). In non-contaminated soil, however, both fungi colonized the root surface better than the *G. claroideum* isolates. Colonization of the root surface by the external mycelium of the *G. claroideum* isolates did not significantly decrease in the contaminated soil. This suggests that the external phase of these fungi is largely unaffected by the presence of metals. These differences in the extramatrical development of the fungi under metal stress could partially explain the differences in behaviour among the various isolates.

Reduction in the frequency of arbuscule formation in contaminated soil was a general pattern followed by all the isolates, but expressed in different degrees for each of them. In the case of *Glomus* sp. arbuscules were practically absent in the roots growing in the polluted soil. In contrast, *G. claroideum* isolates, mainly *G. claroideum* 7, retained their ability to form arbuscules to some extent. Since arbuscules are the key structures for the transference of nutrients between the fungus and the plant, it follows that these isolates are able to maintain to some extent a functional symbiosis in the metal-contaminated soil. All in all, plant growth was not significantly improved by

either of both *G. claroideum* isolates in contaminated soil. Probably this could be due to the increased fertility level induced by sludge application. In this regard, in both plants the behaviour of *G. mosseae* is noteworthy. This fungus promoted plant growth efficiently in non-contaminated soil. The presence of metals depressed its colonization ability initially, but the low level of mycorrhiza established appeared enough to reduce plant growth. Work is in progress trying to elucidate the differential functioning of the tolerant and sensitive AMF ecotypes in contaminated soils.

Different AMF followed the same trend when associated with either leek or sorghum. This contrasts with other studies suggesting that the effect of heavy metals on mycorrhizal colonization can vary according to the host plant (Gildon and Tinker, 1983), but agrees with those of Díaz et al. (1996), who found no significant differences between *G. mosseae* and *G. macrocarpum* when colonizing *Anthyllis cystisoides* or *Lygeum spartum* in soils with different levels of heavy metals.

It can be concluded that *Glomus* sp. and *G. mosseae* isolates are strongly inhibited by the presence of heavy metals limiting the growth of the external mycelium and the formation of arbuscules. However, our results suggest a potential adaptation of *G. claroideum* isolates, particularly *G. claroideum* 7, to high metal concentrations in soil.

Since AM fungi play important roles in the restoration of contaminated ecosystems, the development of tolerant AMF ecotypes to different stress situations is a subject of particular relevance, and it is thus important to screen indigenous isolates in order to guarantee the effectiveness of AM symbioses.

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