Diversity of Arbuscular Mycorrhizal Fungus Populations in Heavy-Metal-Contaminated Soils

C. DEL VAL,* J. M. BAREA, AND C. AZCÓN-AGUILAR
Departamento de Microbiología del Suelo y Sistemas Simbióticos, Estación Experimental del Zaidín,
CSIC, 18008 Granada, Spain

Received 17 August 1998/Accepted 22 November 1998

High concentrations of heavy metals have been shown to adversely affect the size, diversity, and activity of microbial populations in soil. The aim of this work was to determine how the diversity of arbuscular mycorrhizal (AM) fungi is affected by the addition of sewage-amended sludge containing heavy metals in a long-term experiment. Due to the reduced number of indigenous AM fungal (AMF) propagules in the experimental soils, several host plants with different life cycles were used to multiply indigenous fungi. Six AMF ecotypes were found in the experimental soils, showing consistent differences with regard to their tolerance to the presence of heavy metals. AMF ecotypes ranged from very sensitive to the presence of metals to relatively tolerant to high rates of heavy metals in soil. Total AMF spore numbers decreased with increasing amounts of heavy metals in the soil. However, species richness and diversity as measured by the Shannon-Wiener index increased in soils receiving intermediate rates of sludge contamination but decreased in soils receiving the highest rate of heavy-metal-contaminated sludge. Relative densities of most AMF species were also significantly influenced by soil treatments. Host plant species exerted a selective influence on AMF population size and diversity. We conclude based on the results of this study that size and diversity of AMF populations were modified in metal-polluted soils, even in those with metal concentrations that were below the upper limits accepted by the European Union for agricultural soils.

In recent years, several studies have shown the harmful effects of metals on microbial diversity and activity in soil (8, 10, 28). The accumulation of metals in soils at high concentrations can be due to anthropogenic activities such as the application of sewage sludge. This practice has been widely used for nutrient recycling and is accepted for waste disposal in agricultural soils (32). However, the addition of sludge considerably increases the amount of heavy metals in soil, causing changes in soil properties which could be toxic for soil microorganisms (10). The primary chemical change in soil is acidification, which increases the availability of metal in the soil solution to toxic levels which can persist for extremely long periods of time. In spite of this, rates of 50 to 100 kg of dry matter per hectare per year are currently applied to agricultural soils. Thus, the contribution of sewage sludge to the overall input of heavy metals in soils is considerable. In this context, there is increasing concern about the possible side effects on microbial populations, especially after long-term sludge applications to acidic soils.

Soil microorganisms are known to play a key role in the mobilization and immobilization of metal cations, thereby changing their availability to plants (6). Arbuscular mycorrhizal fungi (AMF) are soil microorganisms that establish mutual symbioses with the majority of higher plants, providing a direct physical link between soil and plant roots (3). AMF occur in almost all habitats and climates (4), including in disturbed soils such as those derived from mine activities (9), but soil degradation usually produces changes in the diversity and abundance of AMF populations (21, 23, 27). Mycorrhizal fungal populations are critical during and after soil disturbance because of their role in the establishment and survival of plants (18, 30). Thus, changes in the diversity of their population produced by the application of high amounts of metal are expected to interfere with the possible beneficial effects of this symbiotic association, since reestablishment of AMF populations is slow (12). However, only a few studies have been carried out involving interactions between AMF and metals as a source of soil disturbance. Most of the results already obtained derive from laboratory and pot experiments, with metal salts used as the source of heavy metals, which are not very representative of natural field conditions, under which metals usually accumulate in a less-available chemical form. Heavy metals can delay, reduce, and even completely eliminate AM colonization and AMF spore germination in the field (14), and a negative correlation between Zn concentrations and AM colonization has been reported in soil treated with urban-industrial sludge (7). In other studies, however, the addition of metal-containing sludge did not significantly affect AM development under field conditions (2), probably because different AMF ecotypes can exhibit different degrees of metal tolerance (26). Thus, a relatively high rate of mycorrhizal colonization can be found in plants growing in very polluted soils (31). A higher tolerance to Cu, Zn, Cd, and Pb of indigenous fungi from sludge-polluted sites in comparison to those of reference isolates from unpolluted soils has been described previously (13, 15). To our knowledge, no studies have been reported on the long-term effects of increasing concentrations of sewage sludge on the diversity of mycorrhizal propagules or on the influence of the host plant on AM fungal diversity in heavy-metal-polluted soils.

Our aim in this study was to determine how AM fungal diversity is affected by the addition of sewage-amended sludge in a long-term experiment compared with that of the appropriate noncontaminated control soils. Since the host plant can affect the structure and composition of the AMF population, four different plants with different life cycles and growth strategies were used.
at 180 kg of N ha⁻¹ treatments were applied to the experimental plots as follows: inorganic fertilizer and high-metal sludge at 100 m³ or 300 m³ ha⁻¹ year⁻¹. Each treatment was replicated four times. The sludges were applied annually from 1980 to 1990, and the changes induced in soil properties are recorded in the study of Chaudri et al. (10). However, the highest amounts of heavy metals in the experimental plots were still within the upper limits accepted by the European Union for metal concentrations in soils receiving sewage sludge. The main characteristics of the test soil, in which the range of heavy-metal concentrations was created by the addition of uncontaminated and contaminated sludge, as it was received from the treatment plant. Portions of the sludge were contaminated by adding water-soluble chlorides (10) of the heavy metal sludge, as it was received from the treatment plant. Portions of the sludge were contaminated by adding water-soluble chlorides (10) of the heavy metal sludge, as it was received from the treatment plant. Portions of the sludge were contaminated by adding water-soluble chlorides (10) of the heavy metal sludge, as it was received from the treatment plant. Portions of the sludge were contaminated by adding water-soluble chlorides (10) of the heavy metal sludge, as it was received from the treatment plant. Portions of the sludge were contaminated by adding water-soluble chlorides (10) of the heavy metal sludge, as it was received from the treatment plant.

### MATERIALS AND METHODS

**Experimental site.** A long-term sewage sludge field experiment site located at the Federal Research Centre for Agriculture in Braunschweig (Germany) was established in 1980. The experimental site was formerly (40 years ago) a woodland and was later converted into arable soil. The soil (silty loam) contains 5% clay, 50% silt, and 45% sand, with a pH ranging from 5.3 to 6.0 (10). The sewage sludge was obtained from a local sewage works, but was rather low in heavy metal (low-metal sludge), as it was received from the treatment plant. Portions of the sludge were contaminated by adding water-soluble chlorides (10) of the heavy metals Pb, Cd, Cr, Cu, Ni, Hg, and Zn in order to obtain a higher metal concentration (high-metal sludge) and were incubated for 6 weeks. Five different treatments were applied to the experimental plots as follows: inorganic fertilizer at 180 kg of N ha⁻¹ year⁻¹, low-metal sludge at 100 m³ or 300 m³ ha⁻¹ year⁻¹, and high-metal sludge at 100 m³ or 300 m³ ha⁻¹ year⁻¹. Each treatment was replicated four times. The sludges were applied annually from 1980 to 1990, and the changes induced in soil properties are recorded in the study of Chaudri et al. (10). However, the highest amounts of heavy metals in the experimental plots were still within the upper limits accepted by the European Union for metal concentrations in soils receiving sewage sludge. The main characteristics of the test soil, in which the range of heavy-metal concentrations was created by the combination of different doses and types of sludge, are shown in Table 1 (29).

**Soil sampling.** The study was carried out with soil from plots receiving the different treatments. At sampling (June, 1996), maize plants were growing at the experimental site. Spring rape had been grown before maize. Five cores of the arable-soil layer (0 to 20 cm) were randomly taken from maize rhizosphere in the center of each of replicates per soil treatment to avoid edge effects. Samples were then pooled, thoroughly mixed, sieved through 4-mm-pore-size mesh, and stored at 4°C until used.

**Multiplication of AMF: establishment of trap cultures.** The natural mycorrhizal potential in the soil samples in terms of both the number of AMF spores and mycorrhizal fungal diversity was calculated by using the Shannon-Wiener index, which combines two components of diversity, species richness and evenness of individuals among the species (24).

**Statistical analysis.** A two-way analysis of variance (ANOVA) was used to evaluate the effect of the different host species and soil treatments on total spore number, species richness, and diversity of the AM fungi. Relative densities were arcsine-square-root transformed before the two-way ANOVA was applied to evaluate the effects of the different treatments on the densities of the AM fungal species present in the plots. ANOVA was followed by Duncan’s test when appropriate.

### RESULTS

Six AM fungal species belonging to the genus *Glomus* were found in rhizosphere samples from the different experimental trap plants and soil treatments as follows: *G. claroideum*, *G. mosseae*, and four additional, unidentified species numbered III to VI. Total AMF spore number decreased significantly with increasing amounts of heavy metals in soil, from 550 spores (per 100 g of dry soil) in the control plot to 30 spores in the 300 m³ ha⁻¹ year⁻¹ contaminated sludge (Fig. 1). In the 100 m³ ha⁻¹ year⁻¹ uncontaminated and contaminated sludge plots, the number of spores averaged 330 and 230 per 100 g of soil, respectively. This value decreased to 110 and 30 spores in the soils added at a rate of 300 m³ ha⁻¹ year⁻¹. Host plant also had a significant effect on the total AMF spores produced in the rhizosphere, *Sorghum bicolor* being the trap plant that produced AMF spores most effectively (Fig. 1).

Both species richness and the Shannon-Wiener index rating increased at intermediate levels of soil contamination (100 m³ ha⁻¹ year⁻¹).

### TABLE 1. Chemical and physicochemical characteristics of the test soils

<table>
<thead>
<tr>
<th>Sludge treatment</th>
<th>Organic matter (%)</th>
<th>NH₄NO₃ P⁺ (mg/liter)</th>
<th>Total P (mg/kg)</th>
<th>pH</th>
<th>Free-cation concn in the soil solution (µg/liter⁻¹)</th>
<th>Total metal concn (mg/kg of soil)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Zn²⁺</td>
<td>Cd²⁺</td>
</tr>
<tr>
<td>Unsludged</td>
<td>1.5</td>
<td>0.9</td>
<td>462</td>
<td>6.0</td>
<td>105</td>
<td>1.5</td>
</tr>
<tr>
<td>100 m³ ha⁻¹ yr⁻¹ low metal</td>
<td>1.6</td>
<td>1.6</td>
<td>758</td>
<td>5.9</td>
<td>835</td>
<td>1.6</td>
</tr>
<tr>
<td>100 m³ ha⁻¹ yr⁻¹ high metal</td>
<td>1.8</td>
<td>1.7</td>
<td>822</td>
<td>6.0</td>
<td>1,940</td>
<td>9.3</td>
</tr>
<tr>
<td>300 m³ ha⁻¹ yr⁻¹ low metal</td>
<td>2.0</td>
<td>3.0</td>
<td>1,488</td>
<td>5.5</td>
<td>6,780</td>
<td>16</td>
</tr>
<tr>
<td>300 m³ ha⁻¹ yr⁻¹ high metal</td>
<td>2.3</td>
<td>3.4</td>
<td>1,762</td>
<td>5.3</td>
<td>22,700</td>
<td>90</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>Glomus species</th>
<th>Soil treatment</th>
<th>Host plant</th>
<th>Soil × plant interaction</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>F</td>
<td>P</td>
<td>F</td>
<td>P</td>
</tr>
<tr>
<td><em>Glomus</em> sp. III</td>
<td>20.53</td>
<td>0.0001</td>
<td>31.77</td>
<td>0.0001</td>
</tr>
<tr>
<td>G. mosseae</td>
<td>12.87</td>
<td>0.0001</td>
<td>4.30</td>
<td>0.0032</td>
</tr>
<tr>
<td>G. claroideum</td>
<td>3.94</td>
<td>0.0006</td>
<td>11.08</td>
<td>0.0001</td>
</tr>
<tr>
<td><em>Glomus</em> sp. IV</td>
<td>4.87</td>
<td>0.0018</td>
<td>0.91</td>
<td>0.44</td>
</tr>
<tr>
<td><em>Glomus</em> sp. V</td>
<td>9.94</td>
<td>0.0001</td>
<td>5.12</td>
<td>0.0032</td>
</tr>
<tr>
<td><em>Glomus</em> sp. VI</td>
<td>9.20</td>
<td>0.0001</td>
<td>6.97</td>
<td>0.0004</td>
</tr>
</tbody>
</table>

*F* is the between treatment variance/within treatment variance ratio and provides a value for the equality of treatment means. The *P* value obtained for each *F*, according to the corresponding degree of freedom, as follows (soil treatment: 4; host plant: 3; soil × plant interaction: 12), indicates the probability that differences between treatment means are due to sampling error.
ha\(^{-1}\) year\(^{-1}\) and 300 m\(^3\) of low-metal sludge), decreasing at the highest contamination level (300 m\(^3\) ha\(^{-1}\) year\(^{-1}\) contaminated sludge) (Fig. 1). Host plants also exerted a differential effect on AMF diversity, with *A. porrum* and *S. bicolor* promoting significantly higher levels of diversity in their rhizospheres than those produced by *T. vulgaris* and *T. repens* (Fig. 1).

Relative densities of all AMF species were significantly influenced by soil sludge treatments. Four species, mainly *Glomus* sp. III, were significantly influenced by the host plant, and all *Glomus* species except *Glomus* sp. IV were significantly influenced by the soil \times\ times plant interaction (Fig. 2 and Table 2). The composition of the AM fungal population in the various host plants’ rhizospheres, as affected by the different soil treatments, is recorded in Fig. 2. *Glomus* sp. III was the most common AMF species in the rhizospheres of *A. porrum* and *S. bicolor* in unpolluted soils, but its population decreased sharply with increasing metal content in soils. At higher rates of metal sludge contamination, *G. claroideum* seemed to be the AMF species with the best ability to sporulate, becoming the most common species in the rhizospheres of both plants. Both *T. repens* and *T. vulgaris* were colonized mainly by *G. claroideum*, even in the unpolluted soil. *G. claroideum* and *Glomus* sp. V were the most common fungi in the rhizospheres of all host plants growing in soils treated with 300 m\(^3\) ha\(^{-1}\) year\(^{-1}\) of contaminated sludge.

The effects of the interaction between soil treatment and host plant on the diversity of the AMF species and the richness of the AMF species, as measured by the Shannon-Wiener index, are recorded in Tables 3 and 4, respectively. Both response variables decreased significantly in the soil amended with the highest rate of contamination. Different host plants showed similar trends with regard to the changes they induced in species richness. The low diversity promoted by *T. repens* and *T. vulgaris* in their rhizospheres is remarkable, however.

The overall effects of soil contamination and host plant on the AMF population are illustrated in Fig. 2. As indicated above, *Glomus* sp. III was very sensitive to the presence of metals in soil, and its propagules practically disappeared in the most contaminated soil, while *G. claroideum* maintained a similar relative density in all soils independent of sludge treatment. *G. mosseae* showed another pattern, increasing its density at intermediate rates of contamination. It is noteworthy
that *Glomus* sp. III was abundant in *A. porrum* and *S. bicolor* rhizospheres, since it was almost absent from the rhizosphere of *T. repens*.

A negative correlation ($P \leq 0.001$) was shown between the total number of AMF spores and soil metal content (Table 5). For metals, both the total amount of Zn and the free Zn cation concentration determined in the soil solution gave the lowest correlation coefficients with total number of spores, which also correlated negatively with the P content of the soil (both total and available) and positively with soil pH. *Glomus* sp. III was negatively correlated with the total content of all the metals studied (Ni, Cu, Cd, Pb, and Zn), corroborating its sensitivity to the presence of heavy metals (Table 5). *Glomus* sp. V, however, did not show a significant correlation with the content of the metals studied, except for the free Zn cations in the soil solution (Table 5).

**DISCUSSION**

Long-term sludge application with increasing concentrations of heavy metals produced a significant decrease in both the size and diversity of AMF populations in soil.

The total number of AMF spores strongly decreased with the addition of increasing amounts of heavy metals, but the AMF propagules never disappeared completely in soils amended with the highest rates of sludge, suggesting a certain adaptation of these indigenous AMF to such environmental stress. Notably, the total number of AMF spores correlated

---

**TABLE 3. Diversity of AMF populations by the Shannon-Wiener index as affected by soil amendment and different types of host plants**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Mean for indicated host plant*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>A. porrum</em></td>
</tr>
<tr>
<td>Unsludged</td>
<td>2.13 bcd</td>
</tr>
<tr>
<td>Low metal, 100 m$^3$ ha$^{-1}$ yr$^{-1}$</td>
<td>4.28 ab</td>
</tr>
<tr>
<td>High metal, 100 m$^3$ ha$^{-1}$ yr$^{-1}$</td>
<td>4.05 ab</td>
</tr>
<tr>
<td>Low metal, 300 m$^3$ ha$^{-1}$ yr$^{-1}$</td>
<td>5.32 a</td>
</tr>
<tr>
<td>High metal, 300 m$^3$ ha$^{-1}$ yr$^{-1}$</td>
<td>0.99 d</td>
</tr>
</tbody>
</table>

* Means followed by the same letter did not differ significantly ($P \leq 0.01$) by Duncan's test.
negatively with the metal (Zn, Cu, Cd, and Ni) content of the soils, but the correlation coefficient was higher for the concentration of free cations (Zn$^{2+}$ and Cd$^{2+}$) in the soil solution. When present in excess, these ions are generally assumed to be the chemical species that are taken up by and are toxic to soil microbes (16). In previous studies on AMF and Cu (17) and Zn and Cd (39), no correlation was found between the concentration of these metals in sludge-amended agricultural soils and AMF populations. Despite their availability, methods to measure free-ion activity in the soil solution have rarely been used in studies relating to heavy metal and AMF. The use of such methods as a reference for comparison would probably help elucidate the reason for the discrepancies found between different studies. AMF population size also correlated negatively with the P content of the soil, a result that is well documented (35).

Species richness and diversity as measured by the Shannon-Wiener index increased at moderate levels of soil contamination. This increase in AM propagule diversity could be a fungal stress response whereby fungal ecotypes better adapted to unpolluted soil but affected at intermediate rates of contamination allow other fungi, possibly less competitive in nonstressed soils but better adapted to heavy metals, to colonize the roots and complete their life cycles. Thus, the number of fungal ecotypes in these soils can be increased. However, at the highest levels of soil pollution, both indices diminished sharply. This may have resulted from a fungitoxic effect of metals, causing certain AMF species’ inability to colonize the root system and/or to multiply in the rhizosphere. Only AMF species better adapted to the disturbance produced by the addition of metals would overcome the stress situation and complete their life cycles. A similar response model concerning diversity, suggested for other microbial groups (37), may hold true for *Rhizobium leguminosarum* bv. *trifolii* from the same experimental field in Braunschweig; the relationship between genetic diversity within populations and heavy-metal stress in soils may lead to an increase in diversity with a moderate metal loading, followed by a sharp decrease at higher levels of stress (16). These changes in genetic diversity may be crucial in determining the response of a population to changing conditions. However, genetic diversity studies have not yet been described for AM fungi; thus, it is not possible to relate the phenotypical changes found in the present study to changes in the genetic structure of the AMF population.

*Glomus* sp. III spore density was very much influenced by both soil treatments and host plant species. This fungal ecotype appears to be very sensitive to increasing concentrations of metals in soil, disappearing almost completely in the most polluted soil. At intermediate rates of contamination, *Glomus* sp. III was replaced by other species, such as *G. claroideum*. In fact, *G. claroideum* maintained similar relative density levels in all treatments, showing a higher degree of tolerance to heavy metals than the other fungal species present in the soil, as has been described for other AMF ecotypes (13, 15, 25, 38). *Glomus* sp. V was preferentially found in soils with the highest level of contamination, indicating a low competitiveness of this fungus in the absence of the stress situation to which it is well adapted. Major differences among the species in terms of both numbers of spores and tolerance to metals suggest that fungi follow different strategies to establish symbiosis and probably reveal differences in functioning.

The host plant-mediated effect on the composition and diversity of the AM fungal community is noteworthy. In particular, the high diversity promoted by *S. bicolor* and *A. porrum* contrasts with the poor levels induced by *T. repens* and *T. vulgaris*. *S. bicolor* appeared to be a good host for spore production, possibly because of the higher root growth rate of this plant species, which can facilitate further contact with most AM fungi present in the soil. In contrast, in the rhizosphere of *T. vulgaris*, a species with a very slow growth rate and a poorly developed root system, AMF population size and diversity were very low. These results corroborate the key role of the host plant as a selective force in maintaining specific populations of these ecologically obligate fungal symbionts (1, 5, 22). The root growth rate seems critical to allow colonization by certain AM fungi; thus, the present results provide new insights into the specificity concept in arbuscular mycorrhizas.

The reasons underlying stress-related changes in the diversity of AMF populations, particularly those due to the presence of heavy metals, are not completely understood. It is well known that heavy metals cannot be chemically degraded. Therefore, remediation of metal-polluted soils is limited mainly to immobilization, for example by phytostabilization,

<table>
<thead>
<tr>
<th>Variable</th>
<th>Mean for indicated host plant*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>A. porrum</em></td>
</tr>
<tr>
<td>Unsludged</td>
<td>3.0 abcd</td>
</tr>
<tr>
<td>Low metal, 100 m$^3$ ha$^{-1}$ yr$^{-1}$</td>
<td>4.0 a</td>
</tr>
<tr>
<td>High metal, 100 m$^3$ ha$^{-1}$ yr$^{-1}$</td>
<td>3.0 abcd</td>
</tr>
<tr>
<td>Low metal, 300 m$^3$ ha$^{-1}$ yr$^{-1}$</td>
<td>3.5 ab</td>
</tr>
<tr>
<td>High metal, 300 m$^3$ ha$^{-1}$ yr$^{-1}$</td>
<td>2.0 bedef</td>
</tr>
</tbody>
</table>

* Means followed by the same letter did not differ significantly ($P \leq 0.01$) by Duncan’s test.

TABLE 5. Correlation coefficients* for several parameters of the test soils and some characteristics of the AMF spore populations

<table>
<thead>
<tr>
<th>Variable</th>
<th>Total no. of AMF spores</th>
<th>Spore density</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>Glomus</em> sp. III</td>
<td><em>Glomus</em> sp. V</td>
</tr>
<tr>
<td>pH</td>
<td>0.939**</td>
<td>NS</td>
</tr>
<tr>
<td>$P_{\text{total a}}$</td>
<td>-0.971**</td>
<td>NS</td>
</tr>
<tr>
<td>$P_{\text{available b}}$</td>
<td>-0.959**</td>
<td>NS</td>
</tr>
<tr>
<td>NO$_3$-N</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Zn</td>
<td>-0.978***</td>
<td>-0.931**</td>
</tr>
<tr>
<td>Cd</td>
<td>-0.957***</td>
<td>-0.884*</td>
</tr>
<tr>
<td>Cu</td>
<td>-0.905*</td>
<td>-0.951**</td>
</tr>
<tr>
<td>$\text{Zn}^{2+}$</td>
<td>-0.922**</td>
<td>-0.894*</td>
</tr>
<tr>
<td>$\text{Cu}^{2+}$</td>
<td>-0.897*</td>
<td>-0.971**</td>
</tr>
<tr>
<td>$\text{Cu}^{2+}$</td>
<td>-0.941**</td>
<td>NS</td>
</tr>
<tr>
<td>$\text{Cu}^{2+}$</td>
<td>-0.952**</td>
<td>NS</td>
</tr>
</tbody>
</table>

* Extracted with aqua regia.
* Extracted with NH$_4$NO$_3$.
* Free in soil solution (29).
* “*” significant at $P \leq 0.05$; “**” significant at $P \leq 0.01$; “***” significant at $P \leq 0.001$; NS, not significant.
which consists of promoting plant growth to reduce or elimi-
nate the bioavailability of metals (11). In this context, AMF
constitute an important functional component of the soil-plant
system that is critical for sustainable productivity in stressed
soils (3). A better understanding of the mechanisms behind
these changes in AMF diversity, and particularly of those on
which AMF adaptation and tolerance to metals are based, is
important, since such an understanding could facilitate the
management of these soil microorganisms for a restoration
and/or bioremediation program.

ACKNOWLEDGMENTS

We thank the European Commission (project ENVIRONMENT
EV5V-0415) for supporting this research.

We also thank B. Knight and S. P. McGrath, from IACR-Rotham-
sted (Harpenden, United Kingdom) for supplying the soil chemical
analyses and speciation data and Custodia Cano for valuable technical
support.

REFERENCES

1995. Patterns and regulation of mycorrhizal plant and fungal diversity. Plant
spore populations in abandoned agricultural field after five years of sludge
structure, function, molecular biology and biotechnology, Springer-Verlag,
Heidelberg, Germany.
mycorrhizal fungi and rhizosphere microorganism within the context of sus-
Multitrophic interactions in terrestrial systems. Cambridge, United King-
dom.
dependent sporulation and species diversity of arbuscular mycorrhizal fungus in a
mown grassland. J. Ecol. 84:71–82.
environmental mobility of radionuclides. p. 483–527. In J. M. Bollang and
microbial biomass estimates in soils contaminated with metals. Soil Bio-
chem. 18:343–353.
Kadua region of tropical Australia. II. Propagules of mycorrhizal fungus in
1993. Enumeration of indigenous Rhizobium leguminosarum biivar trifolii
a strip mine in Illinois: fifteen years after establishment. Restoration Ecol.
mycorrhize on heavy metal (Zn and Pb) uptake and growth of Lygus
mycorrhizal infection and heavy metals in plants. The effects of heavy metals
microorganisms and microbial processes in agricultural soils: a review. Soil
infection of Agrostis capillaris population on a copper-contaminated soil.
Plant Soil 158:83–89.
19. Hewitt, E. J. 1966. Sand and water culture methods used in the studies of
plant nutrition, p. 430–434. Technical communications. vol. 22. Common-
wealth Agricultural Bureau, London, United Kingdom.
on vesicular arbuscular mycorrhizal fungi in soils from different vegetation
clover is delayed in soils contaminated with heavy metals from past sewage sludge
the experimental analysis of distribution and abundance, 3rd ed. Harper and
Row, New York, N.Y.
Influence of endomycorrhiza in maize uptake of Pb, Cu and Cd applied as
mineral salts and sewage sludge, p. 204–207. In J. G. Farmer (ed.), Heavy
metals in environment. CEP Consultants Ltd.
mycorrhizal fungi from heavy metal polluted soils. A summary of results, p.
452–454. In C. Azcon-Aguilar and J. M. Barea (ed.). Mycorrhize in inte-
grated systems: from genes to plant development. European Commission,
Brussels, Belgium.
27. Loth, C. 1996. Abundance of arbuscular mycorrhizal fungi spores at different
metals in sewage sludge on soils, microorganisms and plants. J. Ind. Micro-
29. McGrath, S. P., B. Knight, K. Kihlham, S. Preston, and G. L. Paton. Assess-
ment of the toxicity of metals in soils amended with sewage sludge using a
chemical speciation technique and a lux-based biosensor. Environ. Toxicol.
Chem., in press.
ecosystem restoration and reclamation, p. 488–517. In M. J. Allen (ed.),
Mycorrhizal functioning. Chapman and Hall, New York, N.Y.
31. Pawloska, T. E., J. Blaszkowski, and A. Rithling. 1996. The mycorrhizal status of
plants colonizing a calamine spoil mound in southern Poland. Mycorrhiza
6:499–505.
32. Sauerbeck, D. R. 1986. Effects of agricultural practices on the physical,
chemical and biological properties of soils: use of sewage sludge and agri-
bases for soil protection in the European Community. Elsevier, London,
England.
34. Sieverding, E. 1991. Vesicular-arbuscular mycorrhiza management in trop-
ical agrosystems. GTZ, Eichborn, Germany.
37. Wardle, D. A., and K. E. Giller. 1996. The quest for a contemporary eco-
mycorrhizal (AM) fungi from heavy-metal polluted soils. Plant Soil 157:247–
256.
arbuscular mycorrhizal fungal spores isolated from heavy metal-polluted and