OCCURRENCE AND INFECTIVITY OF ARBUSCULAR MYCORRHIZAL FUNGI IN SOME NORWEGIAN SOILS INFLUENCED BY HEAVY METALS AND SOIL PROPERTIES

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Abstract. Mycorrhizae are ubiquitous symbiosis which can mediate uptake of some plant nutrients. In polluted soils they could be of great importance in heavy metal availability and toxicity to plants. Mycorrhizae have also been reported to protect plants against toxic metals. We investigated the occurrence and infectivity of arbuscular mycorrhizal (AM) spores as affected by heavy metal levels and other soil properties in Norwegian soils collected from heavy metal polluted, high natural background and non-polluted areas. Spore numbers, mycorrhizal infectivity and spore germination of indigenous mycorrhizal fungi and of a reference strain (*Glomus mosseae*) in soils showed lower values in two soils with high metal concentrations and in one soil with a low pH. Mycorrhizal infectivity was negatively correlated with extractable metals. Spore number and mycorrhizal infectivity in a soil with naturally high heavy metal content were not different to in non-polluted soils, and indigenous AM fungi appeared more tolerant to metals than those in non-polluted soils. Mycorrhizal infectivity, expressed as MSI_{50} values, was significantly correlated ($r' = 0.89$, $p < 0.05$) with the percentage of germinating *G. mosseae* spores in the soils. However, the number of spores per volume of soil was not significantly correlated with infectivity or spore germination of the reference strain. The spore germination method is discussed as a bioassay of heavy metal toxicity in soil.

Introduction

Heavy metals exert an adverse effect on microorganisms and microbial processes (Chang and Broadbent, 1981; Doelman and Haanstra, 1984; McGrath *et al.*, 1988). High concentrations of metals can be added to soils through anthropogenic sources such as application of sewage sludge and fertilizers or from atmospheric deposition. Natural occurrence of metals in bedrock can also contribute to high levels of metals in soil. For example, soils overlying alum shale bedrock in Norway contain relatively high amounts of total and easily extractable Cd, Zn, Cu and Mn (Jeng and Bergseth, 1992).

Arbuscular mycorrhizal (AM) fungi occur in almost all soils (Mosse *et al.* 1981). However, only a few studies have been conducted on interactions between arbuscular mycorrhizae and heavy metals of different origins in soils. Mycorrhizal colonization has been shown to be delayed, reduced and even eliminated by high concentrations of Zn, Cu, Ni and Cd (Gildon and Tinker, 1983; Graham *et al.*

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TABLE I
Location and identification of the soils

Soil	Location	Heavy metal source	Vegetation
NS1	Sundløkka, Østfold County	zinc smelter (250 m)	wheat field
NS2	Sundløkka, Østfold County	zinc smelter (50 m)	shrubs and trees
NS3	Moen Rakkestad, Østfold County	non polluted	oat field
NS4	Hedemark County	natural (alum shales)	potato field
NS5	Long-term experiment, University of Aas	non polluted	wheat field
NS6	Long-term experiment, University of Aas	non polluted	wheat field
NS8	Røsholt, Vestfold county	non polluted	oat field
NS9	Stavern, Vestfold county	non polluted	oat field

1986; McGee, 1987; Koomen *et al.*, 1990; Chao and Wang, 1991; Leyval *et al.*, 1991). Gildon and Tinker (1981) isolated a mycorrhizal strain which tolerated 100 mg kg^{-1} of Zn in soil. Similarly, Weissenhorn *et al.* (1993) isolated mycorrhizal fungi from two heavy metal polluted soils which were found to be more resistant to Cd than a reference strain (*Glomus mosseae*). It has been suggested that some mycorrhizal fungi could protect plants against the harmful effects of excessive heavy metals (Schüepp *et al.*, 1987; Sylvia and Williams, 1992).

Germination of mycorrhizal spores is also affected by soil properties especially pH and concentration of inorganic ions (Mosse and Hepper, 1975; Green *et al.*, 1976, Siqueira *et al.*, 1985). Some soil properties can favour specific fungi and change the species composition as well as the size of the fungal population. Indigenous mycorrhizal populations are often very sensitive to soil amendments (Hayman, 1982). The influence of heavy metals on endomycorrhizal fungi occurrence and mycorrhizal infectivity (number of infective propagules, Plenchette *et al.*, 1989) in soils will therefore depend on the amount of available metals coupled with the effect of other physical, chemical and biological properties of the soils. Mycorrhizae are additional factors of potential importance in heavy metal availability and toxicity to plants. Their occurrence and infectivity could also be used as a bioassay of heavy metal availability in soils.

We investigated the occurrence and infectivity of mycorrhizal spores in connection with heavy metal concentrations and other soil properties in Norwegian soils collected from heavy metal polluted sites, areas with high natural background and non-polluted sites. The abundance of AM fungi in soils was estimated by spore numbers. Mycorrhizal infectivity of soils was determined using soil dilutions with leeks as host-plant. Spore germination of indigenous mycorrhizal fungi was evalu-

TABLE II

Physico-chemical characteristics of the soils

Soil	Texture (%)			Bulk density kg L^{-1}	pH	Org. C g kg^{-1}	Total N g kg^{-1}	Olsen-P mg kg^{-1}	CEC[a] (me 100 g $soil^{-1}$)
	Sand	Silt	Clay						
NS1	22	51	27	0.82	5.3	2.4	0.29	70	22.8
NS2	n.d.	n.d.	n.d.	0.25	6.7	22.8	1.21	22	48.1
NS3	31	54	15	0.52	4.8	8.5	0.56	18	43.0
NS4	31	38	31	0.77	6.2	6.3	0.68	35	45.8
NS5	21	40	39	0.7	4.8	2.4	0.3	94	22.4
NS6	21	40	39	0.79	6.5	1.9	0.26	52	21.6
NS8	5	43	52	1.05	6.2	0.9	0.14	17	9.6
NS9	2	8	89	1.01	5.7	2.4	0.19	18	16.8

[a] Cation exchange capacity $Na^+ + Mg^{2+} + Ca^{2+} + K^+ + H^+$.

TABLE III

Heavy metal concentrations (mg kg^{-1}) in soils

Soil	Total contents						NH_4NO_3 extractable	
	Cd	Pb	Zn	Cu	Mn	Ni	Cd	Zn
NS1	2.8	103	575	30	307	18.8	0.228	150.5
NS2	131	6060	24410	1630	693	23.2	0.305	3767.5
NS3	0.68	58.2	184	23	192	12.7	n.d.	3.30
NS4	3.3	49.1	215	91	709	92.4	0.015	2.51
NS5	0.19	39.4	77	13	468	20.8	0.038	2.75
NS6	0.21	34.8	70	13	416	20.4	0.004	0.62
NS8	0.21	28.0	60	18	325	6.5	n.d.	1.51
NS9	0.11	18.5	23	9	209	3.2	n.d.	1.62

n.d.: not determined.

ated in the different soils. Since indigenous mycorrhizal fungi from different soils may have different germination rates, spore germination in the different soils was also tested with a reference strain (*Glomus mosseae*), which could be considered as a bioassay for toxicity of heavy metals in soils.

Material and Methods

Sampling and analysis of soil

Soils were collected from six different locations in south-eastern Norway in October 1992 (Table I) and contained different amounts of metals (Table III). NS1 and NS2 soils were collected from contaminated sites close to a zinc smelter, whereas NS4, developed on alum shales, is naturally high in metals (Jeng and Bergseth, 1992). Two soils, designated NS5 and NS6, were collected from a long-term experiment with different pH levels at the Experimental Station of the Department of Soil and Water Sciences, Agricultural University of Norway (Table II). NS3, NS8 and NS9 soils, were not contaminated but represented different pH and textural properties (Tables I–III). All soils were cultivated except NS2 which was an organic matter-rich forest soil with a vegetation of shrubs and trees, several of which were expected to be endomycorrhizal. Soil samples were taken from the upper 0–20 cm and stored at 4 °C until use.

A portion of each soil sample was air-dried, passed through a 2 mm sieve and stored for analyses of physical and chemical characteristics. Soil analyses were performed by the Laboratory of Agricultural Advisory Services, Aas, Norway. Soil texture was determined by the pipette method (Elonen, 1971). Soil pH was measured in water (1:2.5) after equilibration overnight. Organic carbon was measured by combustion in a EC–12 LECO-carbon analyser. Exchangeable cations were determined by extraction with 1M CH_3COONH_4 (pH 7.0) (Page *et al.*, 1982) and available P according to Olsen *et al.* (1954). Total heavy metal concentration in soil was determined by ICP-AES after digestion in *aqua regia* (Van Loon and Lichwa, 1973) (Table III). Extractable Cd and Zn were estimated by 1M NH_4NO_3 extraction (Symeonides and McRae, 1977). Metals in the extracts were determined by graphite furnace AAS with background correction.

AM fungi spore counts

Spores were extracted by wet-sieving (Walker *et al.*, 1982) from three 25 g aliquots of dry soil and counted. The aliquots were sieved through 1 mm and 45 μm sieves. The fraction collected between the two sieves was centrifuged for 5 min at 1000 g. The pellet was then resuspended in 50% sucrose and centrifuged for 1 min at 1000 g. Mycorrhizal spores were then recovered from the supernatant, which was poured through a 45 μm sieve, thoroughly rinsed with tap water and transferred to filter paper for counting and storage at 4 °C. No attempt was made to identify spores to taxonomic level.

Assessment of mycorrhizal infectivity

Measurement of mycorrhizal infectivity in soils (number of infective propagules) was carried out according to Plenchette *et al.* (1989) using 4-fold dilution series.

Leek seeds (*Allium porrum* L.) were surface disinfected (30% H_2O_2, 20 min, 3 rinses in sterile water) and pre-germinated. Seedlings were transplanted to 50 mL pots containing a total of 50 g of the original soils diluted with corresponding gamma-irradiated (10 kGy) soils. Two seedlings were transplanted to each pot and thinned to one after 7 days. Three dilutions (1:4, 1:16, 1:64) and two controls (only non-irradiated soil or only irradiated soil) were replicated five times. Irradiated soil was reinoculated with the original soil microflora except mycorrhizal fungi using 2 mL per pot of soil suspension (10 g of the corresponding soil in 200 mL water) sieved through a 5 μm filter (polycarbonate membranes, Bio-Rad, USA). The pots were covered with plastic beads, watered as necessary and kept in a growth chamber (21/16 °C, 16-h day, 240 μE m^{-2} s^{-1}, 60% relative humidity). Plants were harvested after 28 days. The soil was removed from the roots by gentle shaking and washing, and the whole root system was cleared and stained by the Koske and Gemma technique (1989). Each root system was mounted on microscope slides and observed at a × 150 magnification. Mycorrhizal colonization was recorded as negative or positive. Mycorrhizal infectivity was expressed as MSI_{50} units 100 g^{-1} soil [MSI_{50} = minimum soil dry wt required to colonize 50% of the plants] (Plenchette *et al.*, 1989).

TRAP CULTURES AND ISOLATION OF SPORES

Four pots of each soil, containing 600 cm^3 of non irradiated soil, were planted with leek as trap cultures for mycorrhizal fungi, and kept in the growth chamber under the same conditions as described above, except for light intensity that was raised to 320 μE m^{-2} s^{-1}. The plants were watered regularly to about 60% of water holding capacity. After 4 months, spores were extracted by wet sieving for the germination tests (bioassay). Roots were stained and mycorrhizal colonization measured as described above.

BIOASSAY OF SOIL TOXICITY TO MYCORRHIZAL FUNGI

Spore germination was used as a bioassay of heavy metal toxicity in the selected soils. *Glomus mosseae* (Nicol. & Gerd.) Gerd. & Trappe spores (obtained from V. Gianinazzi, INRA Dijon), kept in irradiated non-contaminated soil with leek, and spores isolated from NS4 and NS6 soils were used. Twenty to thirty spores were placed between two filter membranes (0.45 μm, 47 mm, HT Tuffryn for *G. mosseae* and cellulose nitrate membranes, MFS, USA for the indigenous fungi) held together by a slide frame as described by Weissenhorn *et al.* (1993). Petri dishes (15 cm diameter) were filled with 100 mL dry soil (< 2 mm). A filter paper, lining the bottom of the dish and extending out into a water-filled tray, moistened the soil by capillarity. The membrane sandwiches with spores were placed on the wet soil and covered with 100 mL of the same soil. The soil was allowed to moisten again up to water holding capacity. The plates were sealed and kept for 18 days

TABLE IV
Arbuscular mycorrhizal spore numbers and infectivity (MSI_{50}) in soils

Soils[a]	Spore number		MSI_{50}
	Spores 100 g^{-1} soil	Spores 100 mL^{-1} soil	Units 100 g^{-1} soil
NS1	13.3 ± 1.7 a[b]	10.7 ± 1.3 ab	0.4
NS2	34.7 ± 4.8 ac	8.7 ± 1.2 a	0
NS3	21.3 ± 6.7 ac	11.3 ± 3.7 a	0
NS4	25.3 ± 1.3 ac	19.3 ± 1.3 bc	14.4
NS5	3.3 ± 3.3 b	2.3 ± 2.3 a	0.4
NS6	44.0 ± 6.1 c	34.7 ± 4.9 b	35.5
NS8	46.7 ± 4.8 c	49.0 ± 5.1 d	12.3
NS9	26.7 ± 3.5 c	26.7 ± 3.5 bc	1.9

[a] Soil identification in Table I.
[b] Means (± SE) followed by different letters in the same column are significantly different at the 95% level (F-test).

at ambient temperature. The spore sandwiches were then removed from the soils and stained for one hour (Weissenhorn *et al.*, 1993). The percentage of germinated spores was counted at × 32 magnification. Three replicates were made for each soil.

DATA ANALYSIS

Spore counts and germination data were transformed (log (x + 1) and arcsine, respectively) according to St John and Koske (1988) and Sokal and Rohlf (1981) before variance analysis using one-way and two-way ANOVA with the Statview 1.03 computer program (Abacus Concept, California, USA). Spearman rank correlation analysis was used to relate mycorrhizal parameters to metal concentrations and other soil properties. Partial correlation analysis was used to examine the relationship between mycorrhizal infectivity and extractable Cd and Zn. Correlation analysis were performed using Statgraphics (STSC, Inc., Maryland, USA).

Results

SPORE COUNTS

No significant difference in the number of spores g^{-1} soil was observed between the non-polluted soils NS3, NS6, NS8 and NS9 (Table IV). However, it was significantly smaller in the non-polluted soil NS5, which had the lowest pH and the highest P content (Table II). The number of spores was also smaller in the polluted

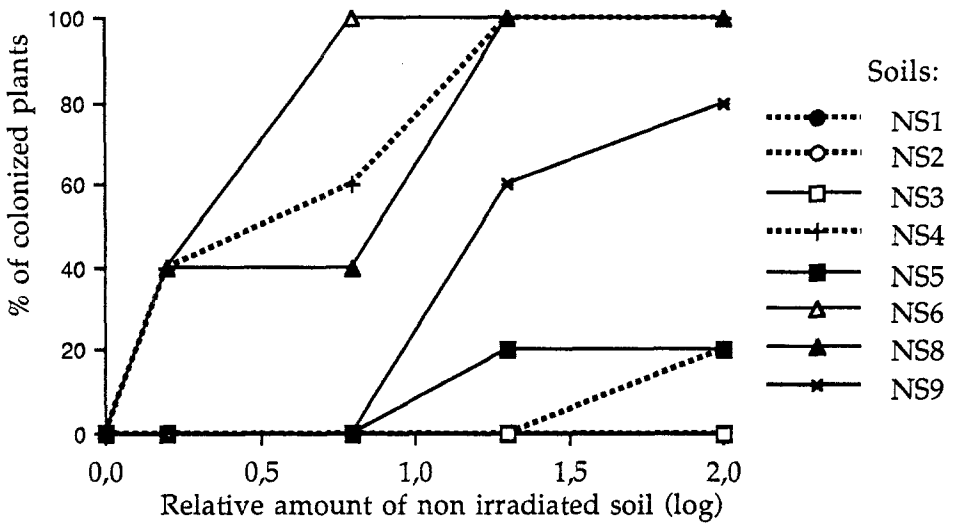

Fig. 1. Arbuscular-mycorrhizal infectivity of the soils: mycorrhizal colonization of leek plantlets grown for 4 weeks on increasing concentrations of non-irradiated soil.

NS1 soil than in the non-polluted NS6, NS8 and NS9 soils but not significantly different to the non-polluted NS3 soil with a pH below 5.0. The number of spores g^{-1} soil was high in the NS2 soil which contained much greater concentrations of heavy metals than in NS1. This was, however, due to differences in bulk density between these soils. When expressed on a volume basis, the spore counts did not differ much between these two polluted soils (Table IV) and were smaller than in the non-polluted NS6 to NS9 soils. Spore counts also decreased in the non-polluted NS3 and NS5 soils with low pH. The number of spores in NS4 soil, naturally enriched in heavy metals, tended to be higher than in the polluted soils and lower than in the non polluted soils, but the differences were not significant.

MYCORRHIZAL INFECTIVITY OF SOILS

The assessment of mycorrhizal infectivity showed that the selected soils can be divided in three groups (Figure 1): NS4, NS6 and NS8 with the highest mycorrhizal infectivity, NS9 showing a lag phase and NS1, NS2, NS3 and NS5 with a very low infectivity. The relationship between the logarithm of soil concentration and the percentage of mycorrhizal plants must be linear to quantify soil AM infectivity (Bouhot, 1980, Plenchette *et al.*, 1989). Regression curve characteristics were then calculated on the linear part of the curves. The soils could therefore be arranged in the following order according to their mycorrhizal infectivity expressed as MSI_{50} units 100 g^{-1} dry soil (Table IV):

$$NS6 > NS4 > NS8 > NS9 > NS5 = NS1 > NS2 = NS3 .$$

TABLE V
Spore germination of *Glomus mosseae* in different soils

Soils	% germination	
NS1	20.7 ± 4.7[a]	ab
NS2	3.7 ± 3.7	b
NS3	n.d.	
NS4	83.0 ± 6.3	c
NS5	9.7 ± 6.3	b
NS6	70.9 ± 4.2	c
NS8	25.2 ± 9.1	a
NS9	30.9 ± 12.1	a

[a] Means ± SE. Means followed by different letters are significantly different at the 95% level (F-test).

No mycorrhizal colonization was observed in the plants growing on NS2 and NS3 soils. However, leek plants grew very poorly on these two soils.

TRAP CULTURES AND ISOLATION OF SPORES

After 4 months of growth leek plants were well colonized by mycorrhizal fungi (30 to 50% of roots were colonized), except in NS2 soil where plants failed to grow. The number of spores collected from these trap cultures was very low except in NS4 and NS6 soils where a larger number of morphologically similar spores was observed. The most abundant type of spores from these two trap cultures was isolated.

BIOASSAY OF SOIL TOXICITY TO MYCORRHIZAL FUNGI

The highest percentage of spore germination for the reference fungus *Glomus mosseae* was observed in NS4 and NS6 soils. Compared to these soils, spore germination significantly decreased in the non-polluted NS8 and NS9 soils, and even more in the polluted NS1 and NS2 soils and in the acid NS5 soil (Table V).

Indigenous spores from NS4 soil had a higher germination rate than those from NS6 soil, when placed in polluted (NS1 and NS4) and non-polluted soil with high pH (NS6) (Figure 2). Germination rates were identical in the non-polluted, low pH soil (NS5). Germination of both kinds of spores was highest in the non-polluted NS6 soil and lowest in the polluted NS1 soil and in the low pH soil (NS5). However, germination of spores from NS4 soil in the NS1 soil was far less reduced than spores from NS6 soil.

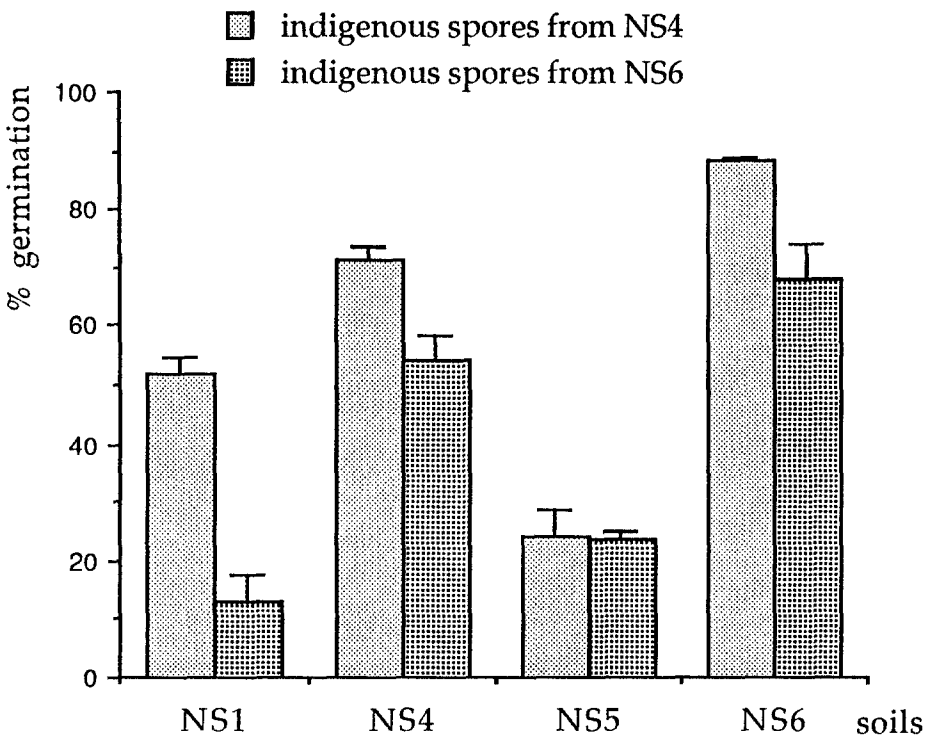

Fig. 2. Influence of soils with high (NS1 and NS4) and low (NS5 and NS6) heavy metal concentrations on the germination of indigenous arbuscular-mycorrhizal spores from NS4 and NS6 (mean and SE).

Discussion and Conclusion

Several methods can be used to assess the distribution and abundance of AM fungi in soils (Abbott and Robson, 1991). Two of them were used in the present study to compare the mycorrhizal potential of different soils. The number of spores in soil is relatively easy to measure for many species of AM fungi, but is not always related to mycorrhizal abundance. This is due to spore dormancy and the non-viability of some spores and the existence of non sporulating species (Abbott and Robson, 1991). In addition, not all spores are removed from soil by the wet-sieving/centrifugation procedure. It has also been suggested that the number of spores in soil may be poorly related to mycorrhizal colonization of roots, which can be initiated from other propagules such as colonized roots and viable mycelia (Abbott and Robson, 1991). Therefore, mycorrhizal infectivity, i.e. the number of infective propagules, which includes the previous parameters (viable spores and other propagules) was also assessed. Soil properties can also indirectly affect mycorrhizal colonization by influencing the growth of the host plant, as observed in the NS9 soil where the partial inhibition may be due to the effect of the high clay content of the soil restricting root growth. Spore germination of introduced fungi

in soils is another approach to soil receptivity to AM fungi, which has not often been used.

The number of mycorrhizal spores in the soils ranged from 3 to 46 100 g^{-1} soil which is in accordance with the results of Schenck and Kinloch (1980) and those of Bajwa *et al.* (1991) in a heavily polluted industrial area in Pakistan. Bohn and Liberta (1982) found 11 spores g^{-1} soil in surface-mined soils amended with sludge supernatants. Arnold and Kapustka (1987) found no difference in AM spore numbers per kg of dry soil between sludge-amended and unamended plots. However, our results suggest that spore density should be expressed on a volume basis in order to compare soils. When expressed per volume of soil, the heavy metal contaminated soils (NS1 and NS2) contained significantly lower numbers of spores than the non-contaminated soils.

In the present study, the number of spores, mycorrhizal infectivity, and *G. mosseae* spore germination generally decreased in the polluted (NS1 and NS2) and acid (NS3 and NS5) soils compared to the non-polluted soils with neutral pH. MSI_{50} values were significantly correlated ($r' = 0.89, p < 0.05$) with the percentage of reference spore germination in the soils. However, the number of spores per volume of soil was not significantly correlated with MSI_{50} values ($r' = 0.69$) or reference spore germination ($r' = 0.64$). Abbott and Robson (1982) attributed the poor correlation between total spore number and rate of colonization to possible colonization of roots by fine endophytes, producing small spores which are difficult to count, and to different rates of colonization by different fungal species. Johnson *et al.* (1991) also observed that spore number was not correlated with infectivity since dead spores may accumulate in the soil and since spore formation may be unrelated to the total fungal biomass.

Mycorrhizal parameters tended to be negatively related to total heavy metal concentrations and to P and N contents and CEC, and positively related to pH (Table VI). However significant correlations were only observed between MSI or spore numbers and extractable metals that were analysed. MSI_{50} values were negatively correlated with extractable cadmium and zinc, while spore numbers were negatively correlated with extractable zinc (Table VI). Since these parameters were highly autocorrelated, we used partial correlation analysis to examine their relationship with mycorrhizal parameters. Using this approach, extractable Cd and Zn were no longer significantly correlated with infectivity ($r = -0.71$ and $r = 0.47$) suggesting that they interact in their effects. Extractable amounts of Pb, Cu and Ni were not analysed, but may also have interacted with Cd and Zn. Concentrations of Cd, Zn, Cu, Mn and Ni were high in NS4 soil, where mycorrhizal parameters were comparable to those in uncontaminated soils. However, availability of the metals seemed to be very low in NS4 soil, as suggested by the amounts of Cd and Zn extracted with NH_4NO_3, in comparison to NS1 and NS2. The effect of heavy metals on mycorrhizal occurrence and infectivity, therefore, seems to be more a function of the available rather than total content, similar to the influence of AM fungi on metal uptake by plants (El-Kherbawy *et al.*, 1989).

TABLE VI

Spearman rank correlation between mycorrhizal parameters and soil parameters for the 8 sites

	pH	CEC	P	N	C org	Total					Extractable	
						Cd	Pb	Zn	Cu	Ni	Cd	Zn
Spore number/100 mL soil	0.36	–0.64	–0.60	–0.69	–0.63	–0.30	–0.69	–0.67	–0.36	–0.50	–0.70	–0.83[a]
% spore germination	0.13	–0.28	–0.18	–0.32	–0.26	–0.14	–0.46	–0.46	–0.25	–0.02	–0.80	–0.71
MSI_{50}	0.36	–0.50	0.05	–0.48	–0.67	–0.32	–0.67	–0.54	–0.39	0.05	–1.0[b]	–0.90[b]

[a] $p < 0.05$; [b] $p < 0.01$.

Koomen *et al.* (1990) showed that heavy metals delayed mycorrhizal development rather than completely suppressing it. This could explain why leek plants were heavily colonized after four months in most of the soils, while low to high root colonizations were observed after four weeks. Angle and Heckman (1986) also observed no effect of metal containing sludge or of low pH on mycorrhizal colonization of roots after 3 months.

The relationship between mycorrhizal colonization and soil chemical and physical properties vary markedly (Abbott and Robson, 1991). High levels of mycorrhizal colonization were observed over a wide range of soil pH, although some species are restricted to either acid or alkaline soils (Sieverding, 1991). In the present study there was a decrease in numbers of spores and mycorrhizal infectivity in the acid soils. Further, spore germination of *G. mosseae*, which does not tolerate low pH (Sieverding, 1991), and of indigenous fungi from NS4 and NS6 soils was reduced in the acid soils. Low pH increases the availability of most heavy metals and thereby increases their adverse effects. Angle and Heckman (1986) reported that the increased heavy metal solubility associated with a low pH soil inhibited mycorrhizal colonization. The present results confirm that low pH primarily affect AM-fungi spore numbers and mycorrhizal colonization.

The number of spores and reference spore germination also tended to be negatively related with the concentration of phosphorous in the soils, while MSI values were not. However, none of the soils had a very high available P concentration (Table II), which could mask or interact with other factors such as heavy metal content on mycorrhizal colonization (Lambert and Weidensaul, 1991).

Soil texture, organic matter and clay content also influence the bioavailability of pollutant heavy metals (Killham and Firestone, 1983). The relatively low concentrations of extractable Cd and Zn compared to their extremely high total contents in NS2 soil may be due to the high organic matter content.

The size of indigenous mycorrhizal populations was compared in different soils but not species composition, which might also have been affected by soil properties and plant species as reported by Johnson *et al.* (1992). Arines and Vilarino (1991) reported an experiment where only one species (*G. deserticola*) overcame the adverse effects of low pH and high Al and Mn levels and colonized the plants. There may be different responses to soil properties at spore germination, hyphal growth and root colonization level between AM fungi, as observed by Weissenhorn *et al.* (1993).

The higher germination rate of spores from NS4 soil compared to NS6 in the polluted NS1 soil supports the hypothesis of tolerance to heavy metals of indigenous mycorrhizal fungi from soils with high metal contents, as reported previously (Gildon and Tinker, 1981; Weissenhorn *et al.*, 1993). However, *G. mosseae* spore germination was not different in these two soils, suggesting that the availability of metals in the soil with naturally high metal content (NS4) was below the toxic level under the experimental conditions of the bioassay.

These results indicate that the naturally occurring metals in the soil overlying alum shale bedrock (NS4) were not toxic to AM. However, even at this non-toxic metal level, the AM fungi from the soil seemed to have developed a tolerance to heavy metals since they germinated better in NS1 soil than spores from the non-polluted NS6 soil.

The three methods used to assess AM fungal occurrence and infectivity in selected soils showed a decline associated with low pH and high heavy metal concentrations. Such rhizospheric microorganisms could therefore be used as indicators of soil toxicity and pH. Under moderately acidic to neutral conditions, the spore germination assay with the reference AM fungus could therefore be used as a bioindicator of heavy metal toxicity in soils and as a supplement to chemical extraction procedures routinely used to assess availability of metals. To be useful in acid soils, this method would necessitate the isolation and use of acid-tolerant fungi. Comparison of spores isolated from soils with the reference strain using the spore germination assay has a potential in evaluating adaptation to metals and other adverse conditions.

These results hold a great potential to expand the practical use of mycorrhizal fungi, but the relationship between tolerant fungi and plant uptake of toxic metals requires further elucidation.

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