This paper was peer-reviewed for scientific content.
Pages 676-681. In: D.E. Stott, R.H. Mohtar and G.C. Steinhardt (eds). 2001. Sustaining the Global Farm. Selected papers from the 10th International Soil Conservation Organization Meeting held May 24-29, 1999 at Purdue University and the USDA-ARS National Soil Erosion Research Laboratory.

Arbuscular Mycorrhizal Fungi (AMF) Spore Abundance is affected by Wastewater Pollution in Soils of Mezquital Valley in Central Mexico

*M.P. Ortega-Larrocea**

ABSTRACT

Long-term application of wastewater increase nutrients and toxic elements in agricultural soils such phosphorous and heavy metals. High inputs of these elements affect spore abundance of arbuscular mycorrhizal fungi (AMF) that was studied at Irrigation District 03 in Central México. Fields corresponding to two soil types (Leptosols and Vertisols) and which have been irrigated during 5 and 90 years were chosen for the study. The largest spore abundance was found in Leptosols, irrespective of the irrigation period. Both soil types showed significantly lower spore abundance in fields irrigated during 90 years, than in those irrigated for 5 years. This effect was more evident in Vertisols, where the distribution of spore abundance could not be attributed to any specific metal, but to the joint effect of all studied metals with the phosphorous contents. There where found less influence with the sporocarpic morphotypes with soil pollution categories. The abundance of *Glomus mosseae* sporocarps was more correlated with that of free spores, while the abundance of *Sclerocystis sinuosa* sporocarps behaved different. Results shown that wastewater irrigation produces changes in soil fabric that must be considered in future management practices in order to prevent an important decrement in the AMF native populations. Conservation of natural soil's properties can regulate the impact of pollution as it is observed in this two soil types.

INTRODUCTION

The use of residual water and sludge on irrigation of agricultural lands is practiced around the world at different scales, and having a different degree of soil contamination (British Geological Survey, BGS, 1998). Its re-utilization is less expensive than can due any treatment leading to prevent direct pollution of superficial aquatic bodies that are the direct receptors of the wastes when treatment is not a practical possibility. The use of wastewater instead expensive fertilizers in agricultural practices has been also a convenient solution in developing countries (Kelley et al., 1984; Bond, 1998). Irrigation District 03 was created in the semi-arid landscape of the Mezquital Valley in Central Mexico in order to solve Mexico City's problem of wastewater disposal and to make this zone agriculturally productive. Since the beginning of this century, wastewater reutilization has provided agronomic soils with nutrients and organic matter but pollutants have also been accumulating mainly in the topsoil (Siebe, 1994; Bond, 1998). General soil characteristics and heavy metal behavior as well as human health risks in relation to pathogens were well documented in this area (Siebe and Cifuentes, 1995; Siebe and Fischer, 1996; Siebe, 1998). High concentrations of phosphorous and also the increment in heavy metal concentrations introduced to the soils through wastewater irrigation are known to greatly influence the dynamics of soil microorganisms and in consequence, the soil's fertility (Dahlin et al., 1998). Arbuscular mycorrhizal fungi (AMF) are important soil's symbioses because of their improvement in plant mineral nutrition by a greater absorption of nutrients from the soils (Lambert et al., 1979). Mycorrhizal plants were shown to have greater tolerance to toxic metalas and other soil adverse conditions (Bagyaraj, 1995). In the same time, detrimental impacts of high nutrient inputs (mainly phosphorous) on AMF populations due to modern agricultural practices have been observed, as well as the occurrence of phosphorous tolerant fungal populations (Bagyaraj, 1995). Heavy metals act as strong selective agents and could be responsible for the development of resistant AM fungal strains (Griffioen, 1994; Leyval et al., 1995). The possible use of mycorrhizal fungi as bioindicators of polluted sites must be supported by consistent field studies. Leyval et al. (1997) reported a decline of AM propagule density in heavy metal polluted soils while Weissenhorn et al. (1995b) did not find any correlation between soil heavy metal contents and propagule distribution. In fact this impact seems to be controlled by soil buffering properties (Rathore & Singh, 1995). The source of pollutants as well as long periods of exposure is also two important factors regulating the stress and fungal adaptation (Griffioen, 1994). Most studies referring to these subjects focus on the impact of sewage-sludge depositions in a short time or on pollution sources like smelters and industrial activities (Miller et al., 1995; Weissenhorn et al., 1995a). Less information has been generated on the impact of long-term sewage effluent irrigation and soil buffering features. Research is needed to provide more knowledge about the effects of sewage effluent irrigation on AM fungal populations, especially since their importance for agricultural sustainability is widely recognized (Haselwandter et al., 1994).

Heavy metal and phosphorous inputs through long-term wastewater irrigation can have a negative impact on the abundance of indigenous AMF populations. The objective of this study was to analyze these effects on the spore abundance in two agriculturally important soil types in the Mezquital Valley in Central Mexico.

*M. P. Ortega-Larrocea, Instituto de Geología, Universidad Nacional Autónoma de México (UNAM). Apartado Postal 70-296, 04510, México D.F. *Corresponding author: mpol@hp.fciencias.unam.mx

MATERIALS AND METHODS

Soil sampling

The studies were done on two different soil units: a *eutric* Vertisol (V) by FAO (1988) or Typic Pellustert by USDA (1993), and a *mollic* Leptosol (L), FAO (1988) or Petrocalcic Calciustoll, USDA (1993). To emphasize the wastewater irrigation effects we selected fields that have been irrigated with wastewater for 90 years and soils recently incorporated to this system (5 years), previously cultivated only during the rainfall season.

The minimum number of cores and fields to be sampled was determined from a previous study. Eight fields recently cultivated with maize (*Zea mays* L.), one of the main crops cultivated in this valley, were sampled for each soil type and irrigation treatment (V05, V90, L05, L90) giving a total of 32 fields. At the end of autumn 1997, a composite sample of 10 cores from topsoil (0-20 cm) distributed evenly along a central zigzag line across the field was taken at each field. Fields with the same crop were chosen to diminish the crop effect. Soils were air dried at room temperature and stored at 4ºC until AMF spore abundance and soil analyses were made.

Spore and sporocarp abundance

Free soil spores and sporocarps of AM fungi were isolated by wet sieving and decanting from 300 g of mixed rhizosphere soil samples per field. The counting was done under a stereoscopic microscope. Permanent slides in polivinilic alcohol (PVLG) and PVLG-Melzer´s reagents were made for taxonomic determinations**.**

Soil chemical analyses

Total heavy metal contents were extracted with aqua regia ($HCl:HNO_3$ 2:1 v/v) and determined by atomic absorption spectrophotometry (Perkin-Elmer Model 3110) (Schlichting et al., 1995). Available P was extracted with 0.5 N $NaHCO_3$ and determined with ascorbic acid (Jackson, 1958).

Statistical analysis

Spore (SA) and sporocarp (GS and SS) abundances were analyzed by multivariate linear models (three-way ANOVA) to test the effect of treatment (years under irrigation), soil type (ST) and heavy metals and P soil contents (wastewater irrigation). Variables were transformed according to Box and Cox (1964) where the sum-of-squares error of variables is minimized to the significant value of confidence interval (95 %) of lambda as described by:

$$Y_{transf} = (Y^{lambda-1})(geometrical\ mean\ Y)^{-1}$$

Hierarchical clustering analysis was applied to correlate independently the spore abundance with soil types and irrigation time.

RESULTS

The abundances of intact free AMF spores (SA) and sporocarpic species *Glomus mosseae* (GS) and *Sclerocystis sinuosa* (SS) are shown in Table 1. Free-spore species were represented mainly by *Glomus mosseae* and *Glomus intraradices*. Other species such *Glomus geosporum* and *Glomus fasciculatum* were also found. Table 1 also shows mean values of total heavy metals and available phosphorous contents in studied soils.

The ANOVA for non-transformed data shows that soil type (ST) determined the mycorrhizae abundance (p=0.05). After data transformation and thus taking away the strong influence of the soil heterogeneity, the impact of wastewater irrigation time on morphotype abundances becomes evident in each of both analyzed soils (p=0.05). This was clearly demonstrated by clustering methods described below.

General spore abundance (SA) was distributed in three main groups: 1) low abundance with 20 to 1590 spores per soil kg^{-1}; 2) medium abundance with 1890 to 3020 spores per soil kg^{-1} and 3) large abundance with 3420 to 4820 spores per soil kg^{-1}. The lowest SA categories belong only to spores numbers founded in Vertisol soils and the largest to Leptosol soils. SA was also clustering among soil types, independently of irrigation time (pollution level) where Leptosols showed a larger variability with four abundance categories while in Vertisols only two groups were formed (Fig. 1).

For the contents of available phosphorous and total heavy metals in soils, there where also identified four pollution categories: a) the most polluted soils showing the largest contents of these elements (values corresponding to those of V90 and presented in Table 1); b) the less polluted ones with the lowest contents of both (values for L05 and

Table 1. AMF morphotype abundance (SA = free spores, SG = *Glomus mosseae* sporocarps, SS = *Sclerocystis sinuosa* sporocarps) and total soil heavy metals and available phosphorous ($mgkg^{-1}$) in Leptosols (L) and Vertisols (V) irrigated for 5 (05) and 90 (90) years with wastewater in Mezquital Valley of Central Mexico. Values are means (*n* =8) ± s.e.

	SA	SG	SS	Cr	Cu	Ni	Zn	Pb	P
L90	1736 ± 623	117 ± 50	36 ± 11	43.4 ± 2.3	44.4 ± 2.8	35.7 ± 1.6	166.1 ± 15.2	52.1 ± 2.8	64.6 ± 2.6
L05	2215 ± 608	132 ± 7	59 ± 13	20.2 ± 1.1	12.1 ± 0.9	23.0 ± 1.6	54.8 ± 4.0	29.3 ± 3.2	22.4 ± 1.9
V90	702 ± 165	64 ± 19	66 ± 12	45.3 ± 3.6	43.6 ± 3.9	42.0 ± 2.0	192.0 ± 15.7	46.5 ± 4.2	63.0 ± 4.2
V05	1552 ± 243	81 ± 14	59 ± 19	23.1 ± 0.4	16.0 ± 0.2	23.8 ± 0.2	78.0 ± 2.8	24.8 ± 2.5	37.0 ± 3.9

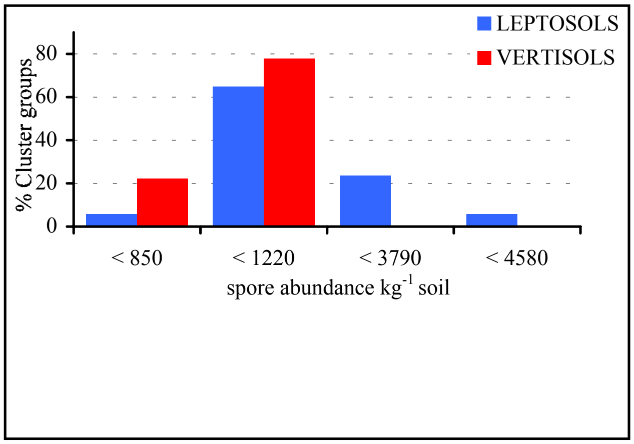

Figure 1. Categories of AMF spore abundance distribution in Mezquital Valley agricultural soils made by hierarchical cluster analysis.

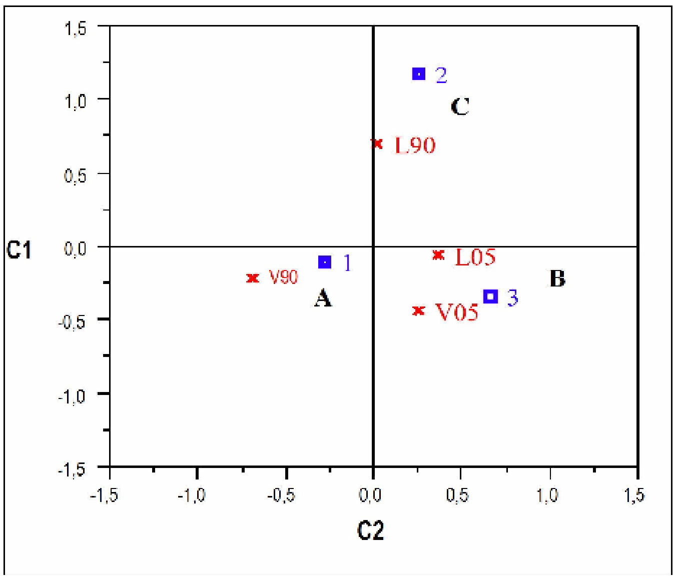

Figure 2. Association of AMF spore abundance groups (1-3) with soil pollution groups (A-C) made by clustering methods (C1, C2). 1 = lowest abundance (<1590 spores kg^{-1} soil), 2 = medium abundance (1890 and 3020 spores kg^{-1} soil), 3 = highest abundance (3420 and 4820 spores kg^{-1} soil). L05, V05 = Leptosols and Vertisols irrigated wit 5 years with wastewater, respectively; L90, V90 = Leptosols and Vertisols irrigated for 90 years.

V05 soils) and c) intermediate polluted soils with the medium values (L90 soils). The most polluted soils (a) and intermediate polluted soils (c) are those that have been irrigated with wastewater for the longest time.

Then associating SA groups with the soil pollution categories, the following groups resulted (Fig. 2):

Group A: Highly polluted soils and lowest SA.

Group B: Least polluted soils and medium SA.

Group C: Intermediately polluted soils and large SA.

The sporocarpic morphotype abundances were better associated with that of SA and cluster together as follows:

Group 1: low amounts of SA and of sporocarps of both species (GS and SS).

Group 2: large amounts of both morphotypes.

Group 3: low numbers of SA and GS but large numbers of SS.

Group 4: large amounts of SA, medium ones of GS but low numbers of SS.

Table 2 shows the determination coefficients, significance levels and the best fitted model made by regression analysis between morphotypes and soils content of heavy metals and phosphorous. In Vertisols for SA, significant and negative linear correlations resulted for all variables (p=0.01, 0.001). *Glomus* sporocarps abundance show no correlation. *Sclerocystis* sporocarps abundances were correlated linear and positively with Pb and P (p=0.05).

In Leptosols a linear negative correlation could be identified between SA and Ni and Zn contents in soils (p=0.05). To the correlations between spore abundances and Cr as well as Cu, 2nd order polynomials gave the best fits (p=0.05). *Glomus* sporocarps correlated significantly also in 2nd order polynomials with Cr, Cu, Ni and Zn. No model could explain satisfactorily the relation between *Sclerocystis* sporocarps and any metal or P soil contents.To determine the effect each of the metals or P could have in morphotype abundance, partial correlations were calculated independently of soil type or irrigation time. All correlations were week and they changed when P was either considered or not in the analysis (Table 3). Most of the correlations indicate a negative effect of individual metals and P on the morphotype abundances. The sporocarp abundances showed to be affected by more metals (mainly in Vertisols) than the free-spore abundances (SA). Instead, Cu was the only metal that shows a positive effect on sporocarp abundances.

DISCUSSION

The spore abundance, as well as the AMF species in the Mezquital Valley soils, were similar to those reported for agricultural soils and even for polluted soils (Weissenhorn et al., 1995a; Cuenca et al., 1998). The sporocarp morphotypes were well represented: *Glomus mosseae* was found as free spores as well as in form of sporocarps and abundant *Sclerocystis sinuosa* sporocarps were also present.

The largest spore abundance was found in long-term irrigated and recently irrigated Leptosols, while the lowest ones occur in Vertisols. This reflects that spore reduction is more severe in deep and clayey soils as Vertisols are. Leptosols although shallower, are bettering drained and aerated, and roots suffer less damage due to swelling and shrinking, a characteristic process of Vertisols (Wilding & Puentes, 1988). Vertisols are more homogeneous in their properties, and the amounts of contaminants and P they receive are larger, since more water is needed to achieve water contents near field capacity (Siebe, 1994). They also have a larger capacity to adsorb contaminants due to the larger organic matter and clay contents (Siebe & Fischer, 1995).

However, in both soil types, reduction of spore abundance due to long-term wastewater irrigation is probable, since high nutrient inputs to soils diminish the soil colonization potential and also the dependency of plants on

Table 2. Heavy metal determination coefficients (r^2) and best fitted model regressions for transformed arbuscular mycorrhizal spore abundance (SA), and *Glomus* and *Sclerocystis* sporocarps (GS and SS, respectively) in Vertisols (V) and Leptosols (L) in Mezquital Valley, México.

AMF-Soil type	Condition	Cr	Cu	Ni	Zn	Pb	P
SA-V	r^2	0.58**	0.67***	0.62***	0.63***	0.46**	0.62***
	Model	Linear -	Linear -	Linear -	Linear -	Linear -	Linear -
GS-V	r^2	--	--	--	--	--	--
	Model	--	--	--	--	--	--
SS-V	r^2	--	--	--	--	0.26*	0.30*
	Model	--	--	--	--	Linear +	Linear +
SA-L	r^2	0.37*	0.45*	0.24*	0.26*	--	--
	Model	2nd. Polinomial	2nd. Polinomial	Linear -	Linear -	--	--
GS-L	r^2	0.41*	0.51**	0.41*	0.56**	--	--
	Model	2nd. Polinomial	2nd. Polinomial	2nd. Polinomial	2nd. Polinomial	--	--
SS-L	r^2	--	--	--	--	--	--
	Model	--	--	--	--	--	--

* $p<0.05$, ** $p<0.01$, *** $p<0.001$, -- no correlation.

Table 3. Selected values of partial correlations between transformed mycorrhizal spore abundance (SA), *Glomus* sporocarp (GS) and *Sclerocystis* sporocarp (SS) abundances and heavy metals and phosphorous contents in mexican Mezquital soils.

Soil type	Metals strongly associated in soil with P influence	Metals strongly associated in soil withouth P influence	Related with SA with P influence	Related with SA without P influence	Related with GS with P influence	Related with GS without P influence	Related with SS with P influence	Related with SS withouth P influence
Vertisol	Cr-Cu (0.66)	Cr-Cu (0.44)		Cr (0.40)	Cr (-0.32)		Cr (-0.36)	Cr (-0.54)
	Cr-P (-0.58)			Cu (-0.39)	Cu (0.47)			
	Cu-P (0.68)				Ni (-0.37)			
		Cu-Zn (0.54)			Pb (-0.34)	Pb (-0.39)	Pb (0.48)	Pb (-0.51)
					P (-0.42)			
Leptosol	Cr-Cu (0.56)	Cr-Cu (0.68)				Cu (-0.32)	Cu (0.38)	Cu (0.31)
	Cu-Zn (0.67)	Cu-Zn (0.58)		Zn (-0.38)	Zn (-0.37)	Zn (-0.48)		
	Cu-P (0.59)	Zn-Ni (0.47)	P (0.37)				P (-0.38)	

mycorrhization (Höflich & Metz, 1997). This effect was observed by cluster analysis where the soils with large available P and total heavy metal concentrations had the lowest abundance and also when spore abundance was negatively correlated with the concentration of many of the studied metals. Most of these negative coefficients were present in long-term irrigated Vertisols indicating that all metals together have a negative influence on morphotype abundances. The lowest spore abundance (7 spore counts in 300 g of soil) was found in the soil with the largest heavy metal and available P. Cluster groups B and C, show that the highest SA was not present in the least polluted soils but in those with intermediate values of heavy metals and available P. Most of the data for these groups belonged to the less polluted Vertisols together with the most polluted Leptosols, resulting in medium values of metal pollution but the highest SA.

Groups 3 and 4 in cluster analysis of the morphotypes shows that *Sclerocystis* abundance behaves differently compared to that of free spores and sporocarps of *Glomus.* This might be due to the fact that *S. sinuosa* is exclusively sporocarpic, while *G. mosseae* can form sporocarps as well as free spores (Schenck & Pérez, 1990). This fact join with the minor impact founded by regression analysis suggest strongly that *Sclerocystis* sporocarp abundance could be more related to a host affinity than to a pollution effect. In the same way, *Glomus mosseae* sporocarp abundance (GS) seems to behave similarly as free spores (represented mainly also by *G. mosseae*), but they appear less sensitive to pollution effects. *Glomus* and *Sclerocystis* sporocarps did not correlate in general with most heavy metals or phosphorous, what can be attributed to the fact that by nature they are more resistant morphotypes (Table 3). Only in Leptosols, second order polynomials could be fit to some SA and *Glomus* sporocarp abundance and most of the different soil metal contents. This shows that low metal concentrations due to small irrigation periods had a positive effect on spore abundance until a reached threshold after which heavy metal and P soil concentrations started to be detrimental. This behavior was foreign in Vertisols where the heavy metal buffering capacities could be different (the pH values were not considered in this study, since they were very similar in all soils and varied in a neutral to slightly alkaline range, and thus not determinant for heavy metal availability). The adjusted models of the regressions could result from the simultaneous influence of all metals rather than from a detrimental effect of one particular. Negative linear or polynomial models could be adjusted to most of the regressions between free spores abundance and individual metals. However, partial correlation analyses did not reveal any specific effect of one particular metal on non-sporocarpic spores, while sporocarp abundance was correlated negatively with many of the studied metals. This indicates that free spores were affected to the combined effect of metals, while specific metals influenced the abundance of certain sporocarps.

Friedel et al. (2000) studied the effect of wastewater irrigation on soil organic matter, soil microbial biomass and microbial activities in the same study area. They found that soil organic matter increased in Vertisols due to a stabilization effect, while in Leptosols they observed a larger amount of more easily degradable organic matter. They concluded that after eighty years of wastewater irrigation no other severe negative impact on soil microbial biomass and activities could yet be observed and attributed this to the great buffer capacities of the soils. In the case of AM fungi, pollution impact will occur also on physiological level (symbiotic efficiency) since pollutants are added constantly and at low rates, allowing an adaptation of the organisms to pollution and therefore not reflecting only on spore abundance. Sambandan et al. (1992) found that AM colonization in Cu contaminated soils was established via mycelium and not by spore propagules.

CONCLUSIONS

The specific environmental condition prevalent in each of analyzed soil types was decisive for the development of AM fungi than management practices as log-term irrigation with wastewater. This investigation confirms the results of previous studies carried out in the Mezquital Valley determining the influence of the different soil types in regulating the pollution impact in this agro-ecosystem. The intensity of a wastewater negative effect varies with spore morphotype and depends on soil type. Surprisingly, P availability was not high enough yet to be a negative factor for decreasing AM fungi populations in soils, and its effect on spore abundance could be not always independent from that of heavy metals. Free spore abundance of AMF was a better indicator of pollution impact in these soils because sporocarps response to irrigation was less consistent. Although some pollutant concentrations in both soils are still not exceeding policy regulations (Gutierrez et al., 1995), heavy metal inputs should be diminished in order to prevent serious damages on AM spore abundance mainly in Vertisols, as already observed in the most polluted one. The results of this study allow to carry out physiological studies to evaluate the stress to which the AM symbioses are exposed, as a well as its role for the transference of heavy metals from the soil into the plant tissues and thus to the food chain.

ACKNOWLEDGMENTS

I´m grateful to Dr. I. Méndez for his help in the statistical analysis and Dr. C. Siebe and Dr. G. Bécard for the translation and content review of the manuscript. This investigation was carried out with the support of DGAPA (UNAM) doctoral scholarship.

REFERENCES

Bagyaraj, D.J. 1995. Influence of agricultural practices on vesicular arbuscular mycorrhizal fungi in soil. J. Soil Biol. Ecol. 15 (2):109-116.

Box, G. E.P. and D.R. Cox. 1964. Analysis of transformations. J. Royal Stat. Soc. B 26:211-243.

BGS (British Geological Survey). 1998. Protecting groundwater beneath wastewater recharge sites. http://www.hrwallingford.co.uk/projects/DFID-KAR/projects/files/R6231.html (verified 14 November 2001).

Bond, W.J. 1998. Effluent irrigation: an environmental challenge for soil science. Aust. J. Soil Res. 36:543-555.

Cuenca, G., Z. de Andrade and G. Escalante. 1998. Diversity of Glomalean spores from natural, disturbed and revegetated communities growing on nutrient-pour tropical soils. Soil Biol. Biochem. 30:711-719.

Dahlin, S., E. Witter, AM. Mårtensson, A. Turner and E. Bäth. 1998. Where's the limit? Changes in the microbiological properties of agricultural soils at low levels of metal contamination. Soil Biol. Biochem. 29:1405-1415.

FAO. 1988. Soil Map of the World. Revised Legend. World Soil Resources Report 60, Food and Agriculture Organization of the United Nations, Rome.

Friedel, J.K., T. Langer, C. Siebe and K. Stahr. 2000. Effect of long-term wastewater irrigation in Central Mexico on soil organic matter, soil microbial biomass and activity. Biol. Fert. Soils 31:414-421.

Griffioen, W.A.J. 1994. Characterization of a heavy metal-tolerant endomycorrhizal fungus from the sourroundings of a zinc refinery. Mycorrhiza 4: 197-200.

Gutierrez-Ruíz M.E., C. Siebe and I. Summer. 1995. Effects of land application of wastewater from Mexico City on soil fertility and heavy metal accumulation: a bibliographical review. Envir. Reviews 3:318-330.

Haselwandter, K., C. Leyval and F.E. Sanders. 1994. Impact of arbuscular mycorrhizal fungi upon plant uptake of heavy metals and radionucleides from soil. Pp. 179-196. In: S. Gianinnazi and H. Schuëpp (eds.). Impact of arbuscular mycorrhizas on sustainable agriculture and natural ecosystems. Birkhäuser, Basel.

Höflich, G. and R. Metz. 1997. Interaktionen ausgewählter Pflanzen-Mikroorganismen-Gesellschaften in schwermetallbelastete Rieselfeldbäden. Die Bodenkultur 48:239-247.

Jackson, M.L. 1958. Soil chemical analysis. Prentice Hall Englewood Cliffs. New Jersey. 490 p.

Kelley, W.D., T.W. Simpson, R.B. Reneau, G.D. McCart and D.C. Martens. 1984. Perception of technical personnel on research and education needs for land application of sewage sludge. Water, Air and Soil Pollution 22:181-185.

Lambert, D.H., D.E. Baker and H. Cole. 1979. The role of mycorrhizae in the interactions of phosphorous with zinc, copper and other elements. Soil Sci. Soc. Am. J. 43:976-980.

Leyval, C., B R. Singh and E.J. Joner. 1995. Occurence and infectivity of arbuscular mycorrhizal fungi in some Norwegian soils influenced by heavy metals and soil properties. Water, Air and Soil Poll. 84:203-216.

Leyval, C., K. Turnau and K. Haselwandler. 1997. Effect of heavy metal pollution on mycorrhiza colonization and function: physiological, ecological and applied aspects. Mycorrhiza 7:139-153.

Miller, R.W., A.S. Azzari and D.T. Gardiner. 1995. Heavy metals in crops as affected by soil types and sewage sludge rates. Commun. Soil Sci. Plant Anal. 26 (5&6):703-711.

Rathore, V.P. and H.P. Singh. 1995. Quantification and correlation of vesicular-arbuscular propagules with soil properties of some mollisosls of northern India. Mycorrhiza 5:201-203.

Sambandan, K., K. Kannan and N. Raman. 1992. Distribution of vesicular-arbuscular mycorrhizal fungi in heavy metal polluted soils of Tamil Nadu, India. J. Environ. Biol. 13:159-167.

Schenck, N.C. and Y. Pérez. 1990. Manual for the identification of VA mycorrhizal fungi (INVAM). Univ. of Florid, Gainesville. 280 p.

Schlichting, E., H.P. Blume and K. Stahr. 1995. Bodenkundliches praktikum. Blackwell Wissenschafts-Verlag, Berlin. 295 p.

Siebe, C. 1994. Accumulation and heavy metal availability in soils irrigated with wastewater in Irrigation District 03, Tula, Hidalgo, Mexico (In Spanish, with English abstract). Rev. Int. De Cont. Amb. 10:15-21.

Siebe, C. 1998. Nutrient inputs to soils and their uptake by alfalfa through long-term irrigation with untreated sewage effluent in Mexico. Soil Use and Management 14:119-122.

Siebe, C. and E. Cifuentes. 1995. Environmental impact of wastewater irrigation in Central Mexico: An overview. Int. J. of Environ. Health Res. 5 (2):161-173.

Siebe, C. and W.R. Fischer. 1996. Adsorption of Pb, Cd, Cu and Zn by two soils of volcanic origin under long term irrigation with untreated sewage effluent in central Mexico. Z. Pflanzenernach. Bodenk. 159:357-364.

USDA. 1993. Soil Survey Manual. Soil Survey Division Staff. United States Department of Agriculture, Washington, D.C., USA.

Weissenhorn, I., C. Leyval and J. Berthelin. 1995a. Bioavailability of heavy metals and abundance of arbuscular mycorrhizal in a soil polluted by atmospheric deposition from a smelter. Biol. Fertil. Soils 19:22-28.

Weissenhorn, I., M. Mench and C. Leyval. 1995b. Bioavailability of heavy metals and arbuscular mycorrhizal in a sewage-sludge amended sandy soil. Soil Biol. Biochem. 27 (3):287-296.

Wilding, L.P. and R. Puentes. 1988. Vertisols: their distribution, properties, classification and management. Texas A & M University System and Soil Management Support Services. 193 p