Effect of Arbuscular Mycorrhizal Fungi on Heavy Metal Toxicity to *Trifolium pratense* in Soils Contaminated with Cd, Zn and Ni Salts

I. VÖRÖS, B. BIRÓ, T. TAKÁCS, K. KÖVES-PÉCHY and K. BUJTÁS

Research Institute for Soil Science and Agricultural Chemistry (RISSAC) of the Hungarian Academy of Sciences, Budapest

Introduction

The majority of species in natural, semi-natural and agricultural plant communities are susceptible to infection by arbuscular endomycorrhizal fungi (AMF) (FRANCIS & READ, 1994). AM fungi belong to the most common mycorrhizal type (GERDEMANN, 1968) and are accepted as mutualistic symbionts of about 90% of the higher plants (MILLER et al., 1994).

Symbioses between arbuscular endomycorrhizal fungi and plant roots play an important role in enhancing plant uptake of phosphorus, macronutrients and micronutrients in most agricultural soils. The exact mechanisms of improved nutrition in mycorrhizal plants are not established clearly and there may be a combination of effects. There is probably a direct effect via increased hyphal uptake as COOPER & TINKER (1978) demonstrated for Zn. Changes in hormonal balance – as secondary effects – can influence nutrient mobilization (TARAFDAR & MARSCHNER, 1994a,b) and uptake (SINGH et al., 1986), increased root exudates might affect chelation of soil nutrients (SCHWAB et al., 1983), and alterations of root:shoot ratios might also affect rooting density (HUNT et al., 1975).

In addition to macro- and micronutrient uptake, mycorrhiza may potentially affect the uptake of heavy metals, as a function of several factors, such as: the physico-chemical properties of the soil (WANG & CHAO, 1992), soil fertility (LAMBERT et al., 1979; THOMPSON, 1990), pH (EL-KHERBAWY et al., 1989; KILLHAM & FIRESTONE, 1983), the host plants (GRIFFIOEN & ERNST, 1989; KUCEY & JANZEN, 1987), the fungi involved (GILDON & TINKER, 1981) and, above all, the concentration of the metals in the soil.

GILDON & TINKER (1981, 1983) have shown that AMF may affect heavy metal uptake and some AM fungi are tolerant to high soil concentrations of metals. Colonization with metal tolerant isolates of AM fungi was found to protect plants against the toxic effects of excessive concentration of heavy metals. AMF colonization reduced the toxic effects of Zn on the growth of two grass species (DUECK et al., 1986). Not all data, however, have confirmed that AMF reduce plant uptake of heavy metals (SCHÜEPP et al., 1987; HEGGO et al., 1990; WEISSENHORN et al., 1991).

KILLHAM & FIRESTONE (1983) reported that infection by AMF led to enhanced metal toxicity, especially from deficient soils.

The effects of metals on soil microbial biomass and on other microbial parameters are often studied in soils with a history of previous sewage sludge application (KOOMEN et al., 1990; LEYVAL et al., 1991). However, sewage sludge contains a mixture of various metals, thus the possibility of interactions among the metals cannot be excluded and it is difficult to establish the real effect of each metal separately.

To study the basic interactions between heavy metals, plants, and microbes, the use of soils containing single metals may be more suitable (BIRÓ et al., 1994). In such experiments soil samples with elevated metal concentrations are often produced by adding various amounts of metal salts to the soil. However, addition of metal salts creates a situation where the bound and free metal forms may not be in equilibrium for a time. Thus, long-term field experiments are very useful to study the effects of various metals on the interaction between soil and rhizosphere microorganisms and higher plants (KÖVES-PÉCHY et al., 1994).

We have conducted a pot experiment with red clover (*Trifolium pratense* L.) as test plant, under controlled conditions in a clima chamber, on soil samples of a long-term field experiment; with the aim of determining effects of Zn, Ni, and Cd on the growth, metal uptake, and stress tolerance of red clover and their interaction with the indigenous population of endomycorrhizal fungi in the element uptake of the host plants.

Materials and Methods

Soil sampling and preparation. – The soil originated from selected plots of a long-term field experiment carried out at Nagyhörcsök Experimental Station (Hungary) and is classified as a calcareous loamy chernozem. The main soil characteristics were: $pH_{(H2O)}$ 7.5; $pH_{(KCl)}$ 7.2; $CaCO_3$ content: 5–6.5%; humus content: 3%; clay fraction (< 0.002 mm) 20%; silt (0.02-0.05 mm): 40%.

In 1991 13 trace elements were mixed into the ploughed layer on the experimental site. The elements were applied as solutions of their salts, each on 3 levels in a split plot design. After this single massive contamination, various crops were grown at the site, with only N, P and K given yearly (100-100 kg N, P_2O_5 and K_2O/ ha). The experimental set-up is described in detail by KÁDÁR (1995).

In the fifth year of the experiment soil samples were taken from plots treated in 1991 with Zn, Ni, and Cd sulphates at rates of 90, 270 and 810 kg element/ ha (i. e. 30, 90, and 270 mg element/kg dry soil), and from the control plots.

From samples of each plot gamma-irradiated (with 15 kGy kg^{-1}) sterile, and similarly sterilized, but re-inoculated and mixed soil samples (10 ml AM-free soil suspension per pot) were prepared (500 ml distilled water kg^{-1} soil was adjusted through a 5 mm pore size sieve to exclude AM fungi). Soil samples from the long-term field experiments without any preceding treatment were also included in the pot experiment.

Plant growth. – Red clover was grown for five months in pots containing the original soil samples with indigenous AMF (*mycorrhiza and other soil microbes*), or the gamma-irradiated sterilized soil samples (*no microbes*), or the sterilized and re-inoculated soil samples (*only bacteria*) in a light room under controlled conditions (between 22 and 25 °C, with a 18/6 light/dark period).

The plants were cut after 3 and 5 months of growing. Prior to the second cut, parameters of photosynthesis (CO_2 release and activity of the photosynthetic electron-transport chain) were also measured. Fresh biomass and dry matter accumulation of shoots and roots were determined, as well as plant metal concentrations, and parameters of the mycorrhizal infection.

Chemical analyses. – Total metal contents of soil were determined in original and sterilized soil samples digested with cc. HNO_3 at 80 °C in a microwave oven. Plant available metal concentrations in the soil were estimated by the amounts extractable by acid ammonium acetate+EDTA, according to the method of LAKANEN & ERVIÖ (1971). Plant metal contents were assessed after wet digestion of the air-dried plant samples with HNO_3+H_2O_2. Metal contents were measured by inductively-coupled plasma atomic emission spectrometry (ICP-AES).

Mycorrhizal parameters. – A subsample of 1 g fresh lateral roots was randomly taken from 1 cm segments dispersed in water. They were cleared and stained with acid glycerol trypan blue according to PHILLIPS & HAYMAN (1970). The frequency (F) and intensity (M) of the mycorrhizal infection and the quantity of the arbuscules (A) were estimated by rating the density of infection on 30 one cm root segments using a five class system (TROUVELOT et al., 1985).

Statistical analyses. – All chemical measurements are given as the means of triplicate analyses of soil or plant samples. Standard errors of the means and least significant difference ($LSD_{5\%}$) were calculated and are represented as numerical values.

Results and Discussion

Content and availability of the metals in the soil

The total Zn, Ni, and Cd concentrations in the soil at the beginning of the experiment were similar to or somewhat lower than the originally applied concentrations, taking also into account the soil's native element content (especially for Zn). The plant available amounts of these metals also increased steadily with increasing metal application rates (Table 1).

Table 1

Total and available concentrations of contaminating elements in the soil prior to the pot experiment (mg/kg dry matter)

	Originally applied metal rates (mg/kg dry soil)			
	0	**30**	**90**	**270**
Zn – total	52.8	72.9	134	275
available	8.42	18.1	41.6	107
Ni – total	29.5	46.1	91.3	258
available	3.47	11.7	27.6	65.8
Cd – total	0.32	38.2	91.4	241
available	0.10	28.0	64.3	170

The total Zn concentrations in the plots treated with Ni or Cd were 52.9 ± 1.8 mg/kg, total Ni concentrations in the soil treated with Zn or Cd were 28.0 ± 0.7 mg/kg, and Cd concentrations in the plots treated with Zn or Ni were 0.38 ± 0.17 mg/kg. These values did not differ significantly from the values measured in the uncontaminated control treatment.

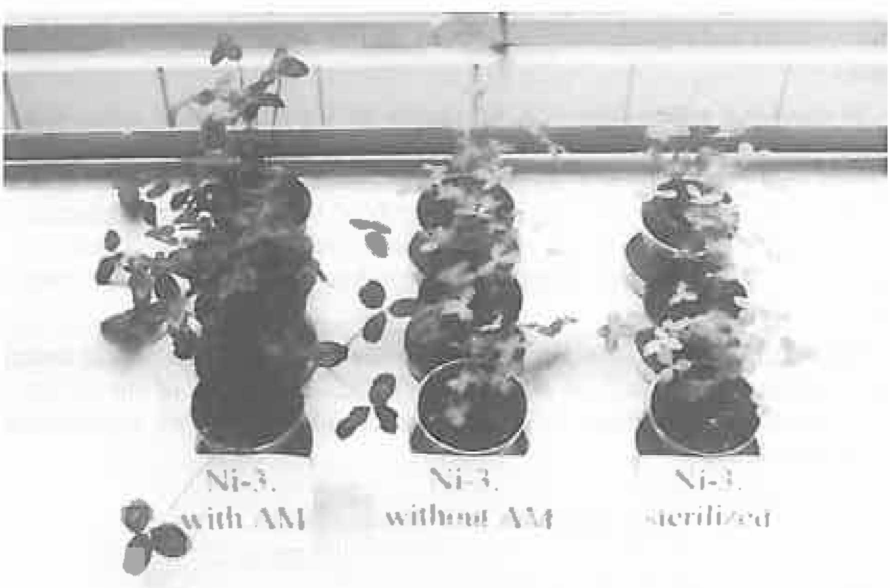

Figure 1

Effect of the highest Ni dose (270 mg/kg) on the growth of clover. (Note: Plants with the indigenous microbes (*original microflora, including AMF*) were greener and more healthy. Plants in the resuspended (*only microbes without AMF*) and in the sterilized (*no microbes*) treatments, were more pale and stressed)

Biomass of clover

The dry weight of clover shoots was not only affected by soil heavy metal content but also by the presence of AMF. For all three metals, the biomass of red clover shoots was significantly (P <0.1%) greater in pots containing original soil infected with AMF than in the gamma-irradiation sterilized soil (Figure 1, Table 2). The role of AMF in improving plant growth is widely recognized. AMF are associated with the majority of agricultural crops and can improve plant growth (MOSSE & HAYMAN, 1980) by increasing the root surface area and thus enhancing the uptake of nutrients, especially of P (SMITH, 1980). Addition of AMF-free soil suspension slightly increased the biomass as compared to the sterilized soil, but the differences were not significant.

Increasing rates of Zn and Ni had no significant effect on the biomass of the shoots. In contrast, increasing Cd rates significantly decreased the dry matter accumulation of clover in all three soil treatments, especially at the two higher rates. However, in the original soil this inhibitory effect of Cd was smaller, production decreased to the level of the sterilized soil variants only at the highest applied Cd rate.

Both cuts of clover showed the above features. Table 2 presents the total biomass (first + second cut) of the shoots. No significant differences were found in the dry matter accumulation of the roots in any of the treatments.

Table 2

Dry matter accumulation of red clover shoots (first and second cut, g/pot)

Soil samples	Originally applied Zn rates (mg/kg dry soil)				
	0	30	90	270	$LSD_{5\%}$
Sterilized	1.18	1.16	1.24	1.27	
Sterilized + suspension	1.23	1.32	1.65	1.40	0.40
Original	2.23	2.58	2.84	2.58	

Soil samples	Originally applied Ni rates (mg/kg dry soil)				
	0	30	90	270	$LSD_{5\%}$
Sterilized	1.18	1.12	1.17	1.28	
Sterilized + suspension	1.23	1.32	1.15	1.22	0.35
Original	2.32	2.56	2.49	2.44	

Soil samples	Originally applied Cd rates (mg/kg dry soil)				
	0	30	90	270	$LSD_{5\%}$
Sterilized	1.18	0.90	0.43	0.46	
Sterilized + suspension	1.23	1.00	0.76	0.32	0.40
Original	2.32	2.44	1.76	0.37	

Metal uptake of the host plant

Plant concentrations of the three metals in the shoots differed greatly: the highest concentrations were found for Zn and the lowest for Cd (Table 3). The Zn, Ni, and Cd concentrations of the shoots increased substantially when the metal levels were higher, both in the sterilized and sterilized+inoculated soil variants. At the highest applied metal rates the shoot Zn concentrations were 4- to 6-fold, the Ni concentrations 5 to 9-fold higher than in the control treatment. Cd concentrations were somewhat higher in the sterilized+reinoculated soil than in the sterilized soil.

The presence of AM fungi significantly ($P < 0.1\%$) affected the uptake of all three metals. In the plants grown on the original AMF-containing soil the increase of the shoot metal concentrations with elevating soil metal levels was slighter than in the plants grown on both sterilized soil variants. Zn, Ni and Cd concentrations in the presence of AMF were only between one-half and one-third of the values measured in plants grown on soils without AMF.

Concentrations of the metals in the roots also increased at the higher metal application rates, and were substantially smaller in the presence of AM fungi than in the case of sterilized soils.

Metal concentrations were generally higher in roots than in shoots, and were more similar to each other, i. e. they followed the original application rates more closely (Table 4). In consequence, the distribution of the metals taken up

Table 3

Metal concentrations in clover shoots (mg/kg dry weight)

Soil samples	Originally applied Zn rates (mg/kg dry soil)				
	0	30	90	270	$LSD_{5\%}$
Sterilized	22.8	56.9	128.0	197.3	31.1
Sterilized + suspension	29.6	81.6	125.8	162.0	
Original	26.3	31.9	51.6	63.3	

Soil samples	Originally applied Ni rates (mg/kg dry soil)				
	0	30	90	270	$LSD_{5\%}$
Sterilized	2.1	5.7	14.6	20.4	2.2
Sterilized + suspension	2.2	6.4	13.7	17.2	
Original	0.9	2.5	4.9	9.5	

Soil samples	Originally applied Cd rates (mg/kg dry soil)				
	0	30	90	270	$LSD_{5\%}$
Sterilized	0.04	2.69	4.86	12.9	1.9
Sterilized + suspension	0.08	6.12	6.29	10.5	
Original	0.05	3.19	3.73	6.8	

Table 4

Metal concentrations in clover roots (mg/kg dry weight)

Soil samples	Originally applied Zn rates (mg/kg dry soil)			
	0	30	90	270
Sterilized	44.2	61.4	124.6	305.0
Sterilized + suspension	39.4	43.4	164.0	198.0
Original	60.5	57.1	68.4	86.9

Soil samples	Originally applied Ni rates (mg/kg dry soil)			
	0	30	90	270
Sterilized	9.8	23.9	59.4	101.4
Sterilized + suspension	10.1	24.4	66.0	187.0
Original	6.3	16.7	30.9	157.5

Soil samples	Originally applied Cd rates (mg/kg dry soil)			
	0	30	90	270
Sterilized	1.4	90.2	146.6	325.0
Sterilized + suspension	1.1	72.8	143.1	302.0
Original	0.3	43.8	54.6	139.9

by the plants varied greatly for the three elements: while Zn was fairly uniformly distributed between the shoot and roots, the concentrations of Ni and especially of Cd were much higher in roots than in shoots.

The differences in the behaviour of the three metals indicate that in addition to the non-specific growth effects, the essentiality of the elements may have an impact on their uptake and transport in the soil–AMF–plant sytem. The uniform distribution of Zn is in agreement with its well-known physiological role as a micronutrient. The essentiality of Ni is debated (PAIS, 1992), and the plants may not have adequate transport mechanisms for this element.

Transport of Cd was exceptionally low as compared to most literature data. However, greatly differing Cd transport ratios are reported in the literature even for similar experiments and identical plant species (e. g. data of WEISSENHORN et al. (1995) for maize), and the variability of the transport data is further increased by comparing different soil and climatic conditions and different plant species. The Cd transport ratios calculated for our experiment are within the ranges reported by WEISSENHORN et al. (1995).

Mycorrhizal infection

The microscopic examination of the stained root samples revealed no root colonization by AM fungi neither in the irradiation-sterilized nor in the sterilized + AMF-free soil suspension reinoculated soil treatments. This means that no AMF infection occurred during the experimental period in any of the sterilized treatments. In contrast, high AMF infection frequency (F) was observed in the treatments with the original soil (Table 5), which suggests that the 4-year metal contamination period prior to the initiation of the pot experiment possibly allowed the selection of some appropriately infective metal tolerant AM fungi in the field experiment.

Table 5

Frequency of the AMF infection (F%) in red clover roots

	Originally applied metal rates (mg/kg dry soil)				
	0	30	90	270	$LSD_{5\%}$
Zn	95.6	95.6	96.7	86.7	5.9
Ni	95.6	90.0	100.0	92.2	6.0
Cd	95.6	97.8	82.2	81.1	10.1

Various parameters of the mycorrhizal root colonization in the treatments containing the original, non-sterilized soil are presented in Tables 5, 6 and 7. The frequency of the infection was universally very high, near to 100%. The highest intensity of mycorrhization was observed at the 90 mg/kg applied metal level in the Zn and Ni treatments, while in the Cd treatments the maximal intensity occurred at the 30 mg/kg metal rate. The quantity of the arbuscules was significantly higher in the two higher rates of Zn and Ni than in the control plants. In the Cd-treated soil the maximum number of arbuscules occurred at

Table 6

Intensity of AMF mycorrhization (M) in red clover roots

	Originally applied metal rates (mg/kg dry soil)				
	0	30	90	270	$LSD_{5\%}$
Zn	47.9	53.0	57.4	37.9	13.6
Ni	47.9	52.7	63.7	46.9	10.9
Cd	47.9	67.7	35.6	37.2	12.3

Table 7

Extent of AMF arbuscularity (a %) in red clover roots

	Originally applied metal rates (mg/kg dry soil)				
	0	30	90	270	$LSD_{5\%}$
Zn	9.91	3.89	23.56	28.39	16.86
Ni	9.91	7.23	12.75	19.88	8.04
Cd	9.91	32.21	26.59	6.03	11.54

the 30 mg/kg metal application rate, while at the highest metal rate the quantity of the arbuscules decreased below the value measured in the control treatment.

Comparing the AMF root colonization and the growth of the host plant, it can be stated that due to the presence of mycorrhizal symbiosis, generally there was a two-fold increase in the dry matter accumulation of the shoots as compared to the dry matter accumulation of the non-mycorrhizal plants, independent of the quality and quantity of the heavy metals present in the soil. In this respect Cd represented an exception: in the Cd treatments the biomass increasing effect of the mycorrhiza decreased at the higher metal rates, reaching the level of the non-mycorrhizal variant at the highest rate (270 mg/kg). In the Cd treatments a positive correlation was also found between the dry matter accumulation of the shoot and the quantity of the arbuscules in the root. Concentrations of all three investigated metals in the root and, especially in the shoot, were decreased by the symbiosis of the plant with the arbuscular mycorrhizal fungi.

Soil–plant transfer of the metals

Accumulation of the metals in the clover shoots depended on the level of soil contamination. Generally, actual plant metal concentrations increased to a lesser extent with increasing contamination levels than the available soil concentrations estimated by the AAAc+EDTA extraction method. Thus, the transfer of the elements between the soil and the plant shoots decreased when the metal application rates increased. A similar decrease in the relative uptake of Ni was described for species of the *Alyssum* genus when the soil was more contaminated (DE VARENNES et al., 1996).

The relative uptake of the metals by the plants in the original AMF-containing soil, as compared to the available soil concentrations, are shown in Table 8. Our calculated values are generally in good agreement with literature data of soil–plant concentration factors (CF).

In the AMF-free treatments the transfer of the metals from the soil to the herbage was greater than in the mycorrhizal plants, because all three metal concentrations in the shoots were two to three times higher in most treatments in

Table 8

Soil–plant transfer of the metals in the original soil ($c_{shoot}/c_{soil\ available}$)

	Originally applied metal rates (mg/kg dry soil)				CF data for uncontaminated pasture herbage*
	0	30	90	270	
Zn	3.1	1.76	1.24	0.59	0.08 - 1.7
Ni	0.26	0.21	0.18	0.14	0.07 - 0.9
Cd	0.50	0.11	0.06	0.04	<0.17 - 5.3

* COUGHTREY et al. (1985)

the absence of AMF than in its presence (Table 3), while the available amounts of the metals in the soil were not altered by the sterilization process.

This difference between the AMF-free and AMF-infected treatments became more pronounced as the metal application rates increased. These results support the idea that the endomycorrhiza have a plant protective role in heavy metal contaminated soils.

Summary

Root colonization with indigenous arbuscular mycorrhizal fungi and their influence on plant growth and metal uptake were studied in a pot experiment with red clover (*Trifolium pratense* L.). Soil samples of pots originated from a long-term field experiment, where the calcareous chernozem soil was contaminated 4 years prior to the pot experiment by a single massive application of Cd, or Ni, or Zn sulphates at rates of 0, 30, 90 and 270 mg metal per kg soil. Red clover was grown for five months in the original metal-spiked soil, in gamma-irradiated sterilized, and in similarly sterilized but with mycorrhiza-free soil suspension re-inoculated soil samples. Fresh weight, biomass production, elemental content of the roots and shoots, and parameters of mycorrhizal infection were measured.

There was an approx. two-fold increase in biomass of red clover shoots in the presence of AMF symbioses, while root dry matter accumulation was similar in all treatments. In the presence of AMF metal concentrations in roots and shoots were smaller (the latter to a greater extent) than metal concentrations in the non-mycorrhizal plants on sterilized soil. The results support the hypothesis that the presence of mycorrhizal symbiosis generally decreases metal transport into above ground plant parts. The infection frequency of AM fungi was only slightly influenced by the various metal rates, arbuscularity tended to be a more reliable indicator of harmful metal effects on higher plants.

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References

Biró, B. et al., 1994. Endomycorrhizal infection of maize affected by heavy metals. In: Conference of COST Action 8.21. 21-24 Oct. München. Abstracts. 32.

Cooper, K. M. & Tinker, P. B., 1978. Translocation and transfer of nutrients in vesicular arbuscular mycorrhiza. II. Uptake and translocation of phosphorus, zinc and sulphur. New Phytol. 81. 43-52.

Coughtrey, P. J., Jackson. D. & Thorne, M. C., 1985. Radionuclide Distribution and Transport in Terrestrial and Aquatic Ecosystems. A. A.Balkema. Rotterdam.

DUECK, T. A. et al., 1986. Vesicular arbuscular mycorrhizae decrease Zn toxicity to grasses growing in zinc-polluted soil. Soil Biol. Biochem. 18. 331-337.

EL-KHERBAWY, M. et al., 1989. Soil pH, rhizobia, and vesicular-arbuscular mycorrhizae inoculation effects on growth and heavy metals uptake of alfalfa (*Medicago sativa* L.). Biol. Fertil. Soils. 8. 61-65.

FRANCIS, R. & READ, D. J., 1994. The contribution of mycorrhizal fungi to the determination of plant. Plant and Soil. 159. 11-25.

GERDEMANN, J. W., 1968. Vesicular-arbuscular mycorrhiza and plant growth. Annuv. Rev. Phytopathol. 6. 396-418.

GILDON, A. & TINKER, P. B., 1981. A heavy metal tolerant strain of mycorrhizal fungus. Transaction of British Mycol. Soc. 77. 648-649.

GILDON, A. & TINKER, P. B., 1983. Interactions of vesicular arbuscular mycorrhizal infection and heavy metals in plants. I. The effect of heavy metals on the development of vesicular arbuscular mycorrhizas. New Phytol. 95. 247-261.

GRIFFIOEN, W. A. J. & ERNST, E. H. O., 1989: The role of VA mycorrhiza in the heavy metal tolerance of *Agrostis capillaris* L. Agric. Econyst. Environ. 29. 173-177.

HEGGO, A., ANGLE, J. S. & CHANEY, R. L., 1990: Effects of mycorrhizal fungi on heavy metal uptake by soybeans. Soil Biol. Biochem. 22. 865-869.

HUNT, R., STRIBLEY, D. P. & READ, D. J., 1975. Root/shoot equilibria in cranberry. Ann. Bot. 39. 807-810.

KÁDÁR, I., 1995. Contamination of the Soil–Plant–Animal–Man Foodchain by Chemical Elements in Hungary. (In Hungarian) Ministry of Environmental Protection and Land Management. Budapest.

KILLHAM, K. & FIRESTONE, M. K., 1983. Vesicular arbuscular mycorrhizal mediation of grass response to acidic and heavy metal deposition. Plant and Soil. 72. 39-48.

KOOMEN, I., MCGRATH, S. P. & GILLER, K. E., 1990. Mycorrhizal infection of clover is delayed in soils contaminated with heavy metals from past sewage sludge applications. Soil Biol. Biochem. 22. 871-873.

KÖVES-PÉCHY, K. et al., 1994. Nodulation and N_2 fixation of various *Rhizobium*–legume systems affected by field applied heavy metal salts. In: Transactions of 9th Nitrogen Workshop. 165-169. Technische Universität. Braunschweig.

KUCEY, R. M. N. & JANZEN, H. H., 1987. Effects of VAM and reduced nutrient availability on growth and phosphorus and micronutrient uptake of wheat and field beans under greenhouse conditions. Plant and Soil. 104. 71-78.

LAKANEN, E. & ERVIÖ, R., 1971. A comparison of eight extractants for the determination of plant available micronutrients on soil. Acta Agr. Fenn. 123. 223-232.

LAMBERT, D. H., BAKER, D. E. & COLE, H., 1979. The role of mycorrhizae in the interactions of phosphorus with zinc, copper and other elements. Soil Sci. Soc. Am. J. 43. 976-980.

LEYVAL, C. et al., 1991. Influence of endomycorrhizas on maize uptake of Pb, Cu, Zn and Cd applied as mineral salts or sewage sludges. In: Heavy Metals in the Environment. (Ed.: FARMER, J. G.) 204-207. CEP Consultants. Edinburgh.

MILLER, M., MCGONIGLE, T. & ADDY, H., 1994. An economic approach to evaluate the role of mycorrhizas in managed ecosystems. Plant and Soil. 159. 27-35.

MOSSE, B. & HAYMAN, D. S., 1980. Mycorrhiza in agricultural plants. In: Tropical Mycorrhiza Research. (Ed.: MIKOLA, P.) 213-230. University Press. Oxford.

PAIS, I., 1992. Criteria of essentiality, beneficiality and toxicity. What is too little and too much? In: IGBP Symposium of the Hungarian Academy of Sciences „Cycling of Nutritive Elements in Geo- and Biosphere" (Ed.: PAIS, I.) 59-77. University of Horticulture and Food Industry. Budapest,

PHILLIPS, J. M. & HAYMAN, D. S., 1970. Improved procedures for clearing roots and staining parasitic and VAM fungi for rapid assessment of infection. Trans. Brit. Mycol. Soc. 55. 158-161.

SCHÜEPP, H., DEHN, B. & STICHER, H., 1987. Interaktionen zwischen VA-Mycorrhizen und Swermetallbelastungen. In: Mycorrhiza and Plant Stress. Angew. Bot. **61.** 85-95.

SCHWAB, S. M., MENGE, J. A. & LEONARD, R. T., 1983. Quantitative and qualitative effects of phosphorus on extracts and exudates of sudangrass in relation to vesicular-arbuscular mycorrhiza formation. Plant Physiol. **73.** 761-769.

SINGH, J. P., KARAMONAOS, R. E. & STEWART, J. W. B., 1986: Phosphorus induced Zn deficiency in wheat on residual phosphorus plots. Agron. J. **78.** 668-675.

SMITH, S. S. E., 1980. Mycorrhizas of autotrophic higher plants. Biol. Rev. of Cambridge Philosophical Soc. 55. 475-510.

TARAFDAR, J. C. & MARSCHNER, H., 1994a. Phosphatase activity in the rhizosphere and hyposphere of VA mycorrhizal wheat supplied with inorganic and organic phosphorus. Soil Biol. Biochem. **26.** 387-395.

TARAFDAR, J. C. & MARSCHNER, H., 1994b. Efficiency of VAM hyphae in utilization of organic phosphorus by wheat plants. Soil Sci. Plant Nutr. **40.** 593-600.

THOMPSON, J. P., 1980. Soil sterilization methods to show VA-mycorrhizal aid P and Zn nutrition of wheat in vertisols. Soil Biol. Biochem. **22.** 229-240.

TROUVELOT, A., KOUGH, J. L. & GIANINAZZI-PEARSON, V., 1985. Mesure du Taux de mycorrhization VA d'un systeme radiculaire. In: Symposium Europeen sur les Mycorrhizes. 217-221. INRA Paris.

DE VARENNES, A. et al., 1996. Effects of heavy metals on the growth and mineral composition of a nickel hyperaccumulator. J. Plant Nutr. **19.** 669-676.

WANG, Y. P. & CHAO, C. C., 1992. Effects of vesicular-arbuscular mycorrhizae and heavy metals on the growth of soybean in major soil groups of Taiwan. J. Agric. Assoc. China New Ser. **157.** 6-20.

WEISSENHORN, I. et al., 1991. Va-mycorrhizal colonisation of maize in industrially polluted soils and heavy metal transfer to the plant. In: 3rd European Symposium on Mycorrhizas. Mycorrhizas in Ecosystems – Structure and Function, Sheffield, 19-23 August, 1991. 83.

WEISSENHORN, I. et al., 1995. Arbuscular mycorrhizal contribution to heavy metal uptake by maize (*Zea mays* L.) in pot culture with contaminated soil. Mycorrhiza **5.** 245-251.