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Bioavailability of heavy metals and abundance of arbuscular mycorrhiza in a soil polluted by atmospheric deposition from a smelter

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Abstract The bioavailability of heavy metals (Cd, Zn, Pb, Cu) and the abundance of arbuscular mycorrhiza (AM) were studied in two agricultural fields close to a Pb-Zn smelter and three fields outside the pollution zone all cultivated with maize (*Zea mays* L.). Metal extractability with ethylenediaminetetraacetic acid (EDTA)-NH_4OAc and $Ca(NO_3)_2$, plant metal uptake, and mycorrhizal parameters (spore number, root colonization) were assessed at two growth stages (six-leaf and maturity). Despite regular liming, the availability of Cd, Zn, and Pb was markedly higher in the two metal-polluted fields than in the three uncontaminated fields. However, the AM abundance was not correlated with metal availability. Root colonization and spore numbers in the metal polluted fields were relatively high, though at plant maturity the former was significantly lower than in one of the uncontaminated fields. The very low AM abundance in the two other unpolluted fields was related to other factors, particular soil and plant P status and soil pH. AM root colonization did not substantially prevent plant metal accumulation, since the metal concentrations in maize grown on the polluted fields strongly exceeded normal values, and for Cd and Pb reached the limits of toxicity for animal feed.

Key words Arbuscular mycorrhiza · Limed silty loam Heavy metals · Pb-Zn smelter · Root colonization Spore numbers · Tolerance · *Zea mays*

Introduction

The Nord-Pas-de-Calais region in France is one of the most important in Europe for mining activities and non-ferrous metal production (Godin 1986). Though the area is highly urbanized and industrialized, agricultural activity remains important, although menaced by air pollution, mainly heavy-metal dust and sulfuric acid. The Pb-Zn smelter Métaleurope in Noyelles-Godault (Pas-de-Calais) produces all the French primary Pb and one-third of the Zn (Godin 1986). It has been operating for more than 60 years, and emits heavy metal into the air, in particular Pb, Zn, and Cd at 5.5, 40, and 0.3 kg ha^{-1} $year^{-1}$, respectively, measured 1 km from the emission source (Direction Régional de l'Industrie, de la Recherche et de l'Environnement 1992). Consequently, contents of these metals in the local agricultural soils are above normal, with average values of 542, 705, and 11 mg kg^{-1} soil dry matter for Pb, Zn, and Cd, respectively (A. Gomez, personal communication 1992). To avoid yield decreases these soils are regularly limed to reduce metal availability and hence toxicity. Thus, the soils contain appreciable quantities of heavy metals without any obvious sign of toxicity to higher plants. Soil microorganisms may, however, be more susceptible to these concentrations of metal in soil. Symbiotic N_2 fixation, for example, has been found to be particularly sensitive to heavy metals (McGrath et al. 1988).

The AM symbiosis between endogenaceous fungi and the roots of most agricultural plants is an important soil-plant intermediary, increasing the plant uptake of P and micronutrients (Tinker and Gildon 1983). AM spore germination and root colonization can be severely affected by elevated concentrations of heavy metals (Hepper and Smith 1976; Gildon and Tinker 1983; Koomen et al. 1990). However, most of this knowledge is derived from laboratory and pot experiments with added metal salts, which are not representative of the field conditions where metals are usually accumulated over long periods of time and in a less available chemical form. There are few reports of AM occurring on metal-polluted sites (Gildon and Tinker 1983; Ietswaart et al. 1992), and these mainly concern sewage sludge as a source of contamination (Angle and Heckman 1986; Koomen et al. 1990; Leyval et al. 1991).

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The objective of the present study was to assess the relation between soil metal availability and mycorrhizal abundance. Metal extractability, plant metal uptake, the number of mycorrhizal spores, and root colonization were assessed at two growth stages in two agricultural fields close to the Pb-Zn smelter Métaleurope and three fields outside the pollution zone, all cropped with maize.

Materials and methods

Sampling sites and soil characteristics

We sampled two agricultural fields, P1 (pollution level 1) and P2 (pollution level 2), 1600 and 750 m, respectively, from a Pb-Zn smelter (Noyelles-Godault, Pas-de-Calais, France). The soil is a silty loam which is regularly limed with sugar house foams and is cultivated in a 3-year rotation with maize, wheat, and sugarbeet. Three control fields, C1, C2, and C3, outside the pollution zone were also sampled. C2 is a calcareous soil, while C1 and C3 are acid soils. None of the five sites were experimental plots but were fields on commercial farms and were subjected to normal agricultural practices for intensively grown crops in north-west Europe. Table 1 summarizes the physicochemical soil characteristics, including trace metal concentrations.

Soil sampling and preparation

The soils were sampled in June and October 1991. Five subsamples of soil were collected from the surface horizon (0–20 cm) at random points in each field. The subsamples were bulked, sieved moist to <4 mm, and air-dried for soil extractions and mycorrhizal spore counts. Aliquots were oven-dried at 105 °C to determine dry matter weights.

Soil extractions

Single extractions with two different extractants, 1 *M* $CH_3CHOONH_4$ with 0.1 *M* EDTA and 0.1 *N* $Ca(NO_3)_2$, were performed on the soils sampled in October in order to estimate metal bioavailability. The EDTA-NH_4OAc extraction reflects the metal fraction that can be mobilized from the soil solid-phase by desorption and decomplexation. $Ca(NO_3)_2$, like other unbuffered neutral salt solutions, is a weak extractant, which does not alter the natural pH. It therefore reflects the concentration of trace metals in the soil solution and the readily exchangeable fraction, both strongly influenced by soil pH (Sauerbeck and Styperek 1985). Triplicate aliquots of 5 g (NH_4OAc-EDTA) or 10 g [$Ca(NO_3)_2$] of soil were shaken in plastic flasks with 50 ml extractant for 2 h at 20 °C. Then the extracts were filtered through ash-free paper and the $Ca(NO_3)_2$ extracts were acidified with 14 *N* nitric acid (1 ml) to prevent metal adsorption. All solutions were kept at 4 °C until analysis. Concentrations of metals (Cd, Zn, Pb, Cu, Ni, and Mn) were determined by either flame (Varian Spectra A 20) or graphite furnace (Varian Spectra A 300 with a deuterium background correction, A 400 with a Zeeman background correction) atomic absorption spectrometry, according to the metal concentration. Each solution was analyzed in triplicate using standards in a similar matrix. Blanks were analyzed in the same way.

Plant sampling and preparation

Five maize plants per field were randomly sampled at two growth stages, six-leaf (June 1991) and maturity (October 1991). Whole plants were excavated and divided into root and shoot parts, both carefully washed free of adhering soil particles using deionized water. To recover a maximum of the fine root system, the surrounding soil was subjected to a wet-sieving procedure. Washed plant material was dried at 80 °C, weighed, and chopped into small pieces using a coarse wood grinder. A subsample was ground in a stainless steel mill (Retsch) using a 0.12-mm mesh. Another subsample was oven-dried at 105 °C to determine the dry matter weight.

Table 1 Soil characteristics. Data refer to dry matter (105 °C) of surface soil (0–20 cm) sieved at <4 mm. Field names: P1, Trinquies; P2, Place Evin Gauche; C2 is a rendzina composed of 48.4% (w/w) fine soil (<4 mm) and 51.6% chalk (coarse fraction). The pH values correspond to the two soil samplings. *Olsen P* refers to extraction with 0.5 *N* $NaHCO_3$. *Total soil metals* refers to fusion with lithium metaborate followed by acid (HCl) dissolution. *CEC* cation exchange capacity

Soil	Polluted		Unpolluted		
	P1	P2	C1	C2	C3
Location	1600 and 750 m from smelter		Outside pollution zone		
Clay ($g\,kg^{-1}$)	174	162	101	327	207
Silt ($g\,kg^{-1}$)	386	598	288	544	750
Sand ($g\,kg^{-1}$)	440	240	611	129	43
$CaCO_3$ ($g\,kg^{-1}$)	0	16.3	0	536	0
pH (H_2O)	7.1–7.6	7.5–7.8	4.9–5.5	8.0	5.1
Organic matter ($g\,kg^{-1}$)	27	28.7	14.1	41.5	17.5
Organic C ($g\,kg^{-1}$)	15.7	16.7	8.2	24.1	10.2
Total N ($g\,kg^{-1}$)	1.1	1.0	0.6	2.1	0.9
Olsen P ($mg\,kg^{-1}$)	85	94	107	69	101
CEC ($cmol\,kg^{-1}$)	20.0	16.4	7.1	19.1	10.0
Exchangeable cations ($mg\,kg^{-1}$)					
Ca	3600	3000	1200	3400	1700
K	242	265	265	569	211
Mg	134	74	46	48	98
Na	69	32	5	55	41
Total soil metals ($mg\,kg^{-1}$)					
Cd	7.3	17.7	0.5	0.6	0.6
Zn	425	1220	48	66	67
Pb	385	895	27	44	32
Cu	34	45	16	55	22

Plant analysis

Finely ground plant material (500 mg samples) was wet-digested with 14 *N* HNO_3 (6 ml) in a microwave digesting system (CEM MDS-81 D) using special Teflon pressure vessels (CEM P/N 221000). Heating power was applied in four steps (5 and 10 min at 35% ≈ 220 W, 2×20 min at 60% ≈ 380 W), each followed by a release of nitrous gases accumulated in the vessels to avoid losses via the pressure security valve. The digest was made up to 50 ml with distilled water. A certified reference sample (ryegrass CRM 281, EC Community Bureau of References, BCR), was included in the analysis as well as blanks of reagents. The solutions were analyzed for metals in the same way as the soil extracts. The P concentration was determined by inductively-coupled plasma atomic emission spectrometry (Jobin Yvon 32).

Mycorrhizal parameters

The number of spores in the soil samples of June and October was assessed. The spores were extracted from three replicates of 50 g soil by wet-sieving (1-mm and 63-μm sieves) and centrifugation in 50% sucrose solution (Walker et al. 1982). They were then spread on filter paper with grid lines in a Petri dish and counted under a dissecting microscope. Only intact spores were counted.

For determination of mycorrhizal root colonization, a subsample of 1 g fresh lateral roots was taken randomly from 1-cm segments (whole root system) dispersed in water. The subsamples were cleared and stained with acid glycerol trypan blue according to Koske and Gemma (1989). Thirty stained root segment subsamples were mounted on slides in polyvinylalcohol-lacto-glycerol (Koske and Tessier 1983) and examined under a compound microscope (×150). The percentage of mycorrhizal root cortex was estimated by rating the density of infection using a five-class system according to Trouvelot et al. (1986).

Data analysis and presentation

All concentrations refer to soil or plant dry matter determined at 105 °C. Means and SEM were calculated for three (soil data: extractable metal concentrations, spore numbers) or five (plant data: metal concentrations, root colonization) replicate values. Mycorrhizal colonization data (%) were square-root transformed and spore numbers were log transformed prior to one-way analysis of variance ($P<0.01$). Means were compared by Tukey's multiple range test ($P<0.05$). Linear regression analysis was performed to describe the correlations between physicochemical soil factors, including soil metal contents, and plant metal contents, and mycorrhizal parameters (r^2 = adjusted coefficient of determination). To describe the relationship between P and mycorrhizal root colonization a non-linear regression curve was fitted using an exponential model (r^2 = corrected coefficient of determination).

Results and discussion

Metal extractability

The two fields in the close vicinity of the smelter, particularly P 2, exhibit total Cd, Zn, and Pb concentrations (Table 1) well above those of the fields outside the pollution zone and of the European Community (EC) limits for agricultural soils (Commission of the European Community 1986).

Despite regular liming, the Cd concentrations extracted by both EDTA-NH_4OAc and $Ca(NO_3)_2$ were much higher in the polluted soils than in the control soils, and higher in P 2 than in P 1 soils (Table 2). These differences were larger with EDTA-NH_4OAc than with $Ca(NO_3)_2$, which indicates a considerable fixation of Cd on organic matter or mineral particles in the contaminated soils. However, the proportion of Cd in the total metal extracted by $Ca(NO_3)_2$ was the largest compared to the other metals, suggesting higher solubility, i.e. bioavailability. The marked difference in $Ca(NO_3)_2$-extractable Cd between the three unpolluted soils (Table 2) with a similar total Cd background (Table 1), demonstrates the strong influence of pH on Cd availability.

For Zn, only the EDTA-NH_4OAc extraction clearly reflected the total quantity of this metal in the different fields (Table 2). With $Ca(NO_3)_2$, only the Zn concentration extracted from P 2 was elevated above the controls. The quantity of Zn extracted from the two control soils with a low pH (C 1 and C 3) was of the same order as that for P 1 despite their 10 times lower total Zn content. Thus the solubility of Zn also appeared to be very dependent on soil pH, though it was generally lower than for Cd (as a percentage of the total). This is in accordance with results from Mench et al. (1994), who studied the effect of mineral additives on metal mobility and plant availability in the highly contaminated P 2 soil. The EDTA-NH_4OAc-extractable Cd and Zn concentration in the P 2 soil exceeded EDTA-extractable concentrations reported to cause a 50% reduction in N_2 fixation in soil (5.3 and 165 mg kg^{-1}, respectively; McGrath et al. 1988).

Pb concentrations in both extracts were considerably higher for the two polluted soils compared to the controls. The $Ca(NO_3)_2$ extract, however, revealed no difference between P 1 and P 2. The extremely low Pb recovery

Table 2 Extractable fractions of metals of polluted (P1, P2) and unpolluted (C1, C2, C3) soils expressed as mg kg^{-1} dry matter and as a percentage of the total soil metal concentration (values in *parentheses*). *EDTA* ethylenediaminetetraacetic acid, *DL* detection limit

Soil	P1	P2	C1	C2	C3
EDTA-NH_4OAc-extractable metals (mg kg^{-1})					
Cd	4.2 (58)	13.6 (77)	0.2 (40)	0.3 (50)	0.2 (33)
Zn	95 (22)	350 (29)	5.9 (12)	12.2 (18)	4.8 (7)
Pb	278 (72)	567 (63)	10.6 (39)	14.6 (33)	9.4 (29)
Cu	7.3 (21)	10.7 (24)	3.6 (23)	3.5 (6)	3.8 (17)
$Ca(NO_3)_2$-extractable metals (mg kg^{-1})					
Cd	0.21 (2.9)	0.38 (2.1)	0.07(14)	0.01 (1.7)	0.10 (17)
Zn	1.07 (0.3)	3.97 (0.3)	1.21 (2.5)	0.05 (0.08)	1.19 (1.8)
Pb	0.16 (0.04)	0.17 (0.02)	<DL	<DL	<DL
Cu	0.04 (0.1)	0.07 (0.2)	<DL	0.05 (0.09)	<DL

(as a percentage of the total) by $Ca(NO_3)$ in contrast to its relatively high extraction ratio with EDTA-NH_4OAc demonstrates the low solubility and high affinity to organic matter of this element. Cu solubility ($Ca(NO_3)_2$ extract) was also low. A difference between P1 and P2 and the control plots was found only for the EDTA-NH_4OAc extractable fraction.

Plant metal uptake

Plant metal contents indicate the metal availability and hence potential toxicity in a soil, particularly in the rhizosphere (Sauerbeck 1982). The Cd, Zn, and Pb concentrations in the maize plants (Fig. 1) were markedly higher for the two polluted soils (P1 and P2) than for the control soils. This cannot be attributed to a concentration of metals in the plant tissue through a lower biomass production (Table 3).

The plant Cd concentration was well correlated with the Cd extractability from the different soils (Table 2). In the two polluted fields (P1 and P2), particularly at the six-leaf stage, it strongly exceeded normal values ($<0.5\ mg\ kg^{-1}$; Stoeppler 1991) and toxicity limits for animal feed ($0.5-1\ mg\ kg^{-1}$; Sauerbeck 1982). The Cd root concentrations were generally higher than the shoot concentrations, which is in accordance with a previous report (Jarvis et al. 1976).

Plant Zn concentrations in the polluted soils did not exceed the control values as much as Cd did. This corresponds with the lower solubility of Zn. Plant Zn was better related to EDTA-NH_4OAc- than to $Ca(NO_3)_2$-extractable Zn. The shoot Zn was generally higher than the root Zn, except on P2 soil. The phytotoxic threshold value of $300\ mg\ kg^{-1}$ for maize proposed by Hinesly et al. (1977) was only reached on P2 soil.

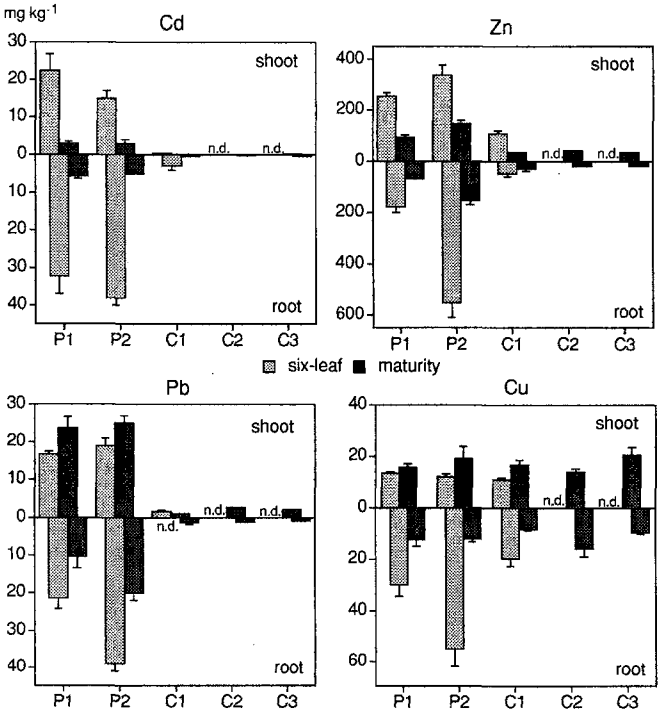

Fig. 1 Shoot and root metal concentrations ($mg\ kg^{-1}$ dry matter) of maize plants from two metal-polluted (P1 and P2) and three unpolluted control soils (C1, C2, C3) at two growth stages. Means ± SEM ($n = 5$); *n.d.* not determined

Table 3 Shoot and root dry weight (g) of maize plants (means ± SEM, $n = 5$)

Soil	Six-leaf stage		Maturity stage	
	Shoot	Root	Shoot	Root
P1	3.72 ± 0.24	0.46 ± 0.05	199.2 ± 20.7	6.9 ± 1.0
P2	2.08 ± 0.18	0.31 ± 0.04	254.0 ± 22.3	9.9 ± 1.1
C1	1.84 ± 0.13	0.22 ± 0.02	210.0 ± 12.7	8.1 ± 0.5
C2			160.0 ± 14.3	5.1 ± 0.8
C3			191.8 ± 13.0	6.3 ± 0.9

The higher Pb content of plants in the polluted fields also corresponds with the soil extractability data. However, the shoot Pb content did not differ between P1 and P2 and was higher at maturity than at the six-leaf stage, whereas plant metal concentrations are usually diluted towards the end of plant growth (Cd and Zn). This suggests that atmospheric Pb made a considerable contribution to the plant Pb accumulation, which is not surprising in the vicinity of the smelter where, despite the installation of filters, dust emission is still recorded (Direction Régional de l'Industrie, de la Recherche et de l'Environnement 1992). Plant Pb concentrations in the polluted plots reached the average phytotoxic ($10-20\ mg\ kg^{-1}$) and zootoxic ($10-30\ mg\ kg^{-1}$) threshold values recorded by Sauerbeck (1982). The atmospheric contribution to plant Pb levels may largely exceed that of the soil (Dalenberg and Van Driel 1990). For Cd and Zn this is usually less critical than for Pb, due to their relatively greater bioavailability in the soil (Dalenberg and Van Driel 1990). For Cu, only the root uptake at the six-leaf stage reflected the extractable soil fractions. There was no difference in shoot Cu concentrations between polluted and control soils.

Mycorrhizal abundance

Maize roots in the metal-contaminated fields (P1 and P2) were colonized by AM fungi for up to 40% of the root cortex and spore numbers were increased to the end of the season, suggesting that sporulation occurred (Fig. 2A, B). However, the percentage of mycorrhizal colonization at the maturity stage (Fig. 2A) was significantly lower in the two contaminated soils than in the calcareous control soil (C2). In contrast, the number of spores did not differ significantly between the polluted fields and C2 field (Fig. 2B). Mycorrhizal root colonization in the two acid control soils was significantly lower than in C2 and was at the same level (C3) or significantly lower (C1) than in the polluted soils (Fig. 2A). The number of spores was

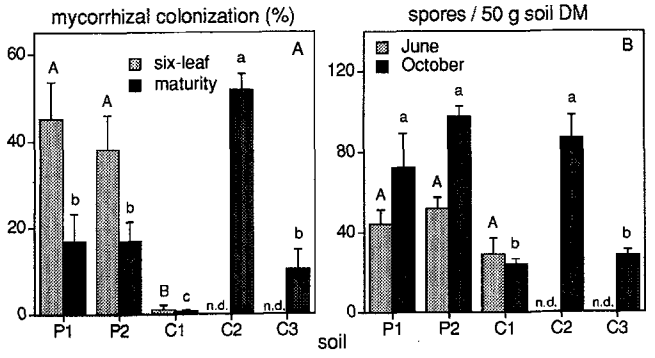

Fig. 2 Mycorrhizal root colonization (**A**) and spore counts (**B**) in two metal-polluted (P1 and P2) and three unpolluted control soils (C1, C2, C3). Means ± SEM (A: $n = 5$, B: $n = 3$). Different *letters* above *columns* indicate a significant difference at $P < 0.05$ (Tukey test). *n.d.* not determined, *DM* dry matter

also significantly lower in these two control fields than in the polluted fields (Fig. 2B). There was no significant difference between P1 and P2 in root colonization and spore number, despite the higher metal availability in P2 soil (Table 2, Fig. 1). No correlation between soil and plant metal concentrations and mycorrhizal parameters could be established. The mycorrhizal development in the two polluted soils suggests that the indigenous AM population is adapted to the elevated metal concentrations. Metal tolerance by AM fungi in polluted soils has been suggested before (Gildon and Tinker 1983; Koomen et al. 1990; Leyval et al. 1991). The dominant spore type isolated from P2 soil, *Glomus mosseae*, was more tolerant to Cd than a reference *G. mosseae*, when spore germination was tested in vitro (Weissenhorn et al. 1993). In contrast to the AM fungal population, the rhizobial population in the P2 soil was found to be suppressed by the high metal load, particularly Zn (A. Chaudri, personal communication 1993).

The extremely low mycorrhizal potential of the two acid control soils (C1 and C3) demonstrates the importance of other soil factors, which appear to influence mycorrhizal development more than elevated Cd, Zn, and Pb concentrations such as in the present study. Organic matter, cation exchange capacity, pH, and exchangeable Ca were positively correlated with mycorrhizal root colonization and spore numbers, whereas the correlation with available P (Olsen P) was inverse (Fig. 3). There was also a strong negative relationship between the plant P status and mycorrhizal root colonization at the six-leaf stage (Fig. 4A), following an exponential curve. It is widely accepted that the P availability in soils controls mycorrhizal establishment via the plant P concentration (Menge et al. 1978). At maturity, however, this relationship was much weaker (Fig. 4B).

High levels of mycorrhizal colonization have been observed over a wide range of soil pH values (Read et al. 1976), although different AM fungi seem to vary greatly in their preference for a particular pH range (Wang et al. 1985). A low pH can have a considerable indirect effect on mycorrhiza through the increase in toxic metals in the soil solution. Raju et al. (1988), for example, found reduced AM colonization of sorghum plants in an Al-rich acid soil. The Al concentration in a water saturation extract from the acid C1 soil was much higher than for the alkaline C2 and P2 soils (182.4 $\mu g\,l^{-1}$, <detection limit and 1.6 $\mu g\,l^{-1}$, respectively). $Ca(NO_3)_2$-extractable Mn fractions in the two acid soils C1 and C3 were also clearly higher than for the three soils with a neutral to alkaline pH (Table 4). Thus, in addition to high P availability, these indirect pH effects could help suppress mycorrhizal

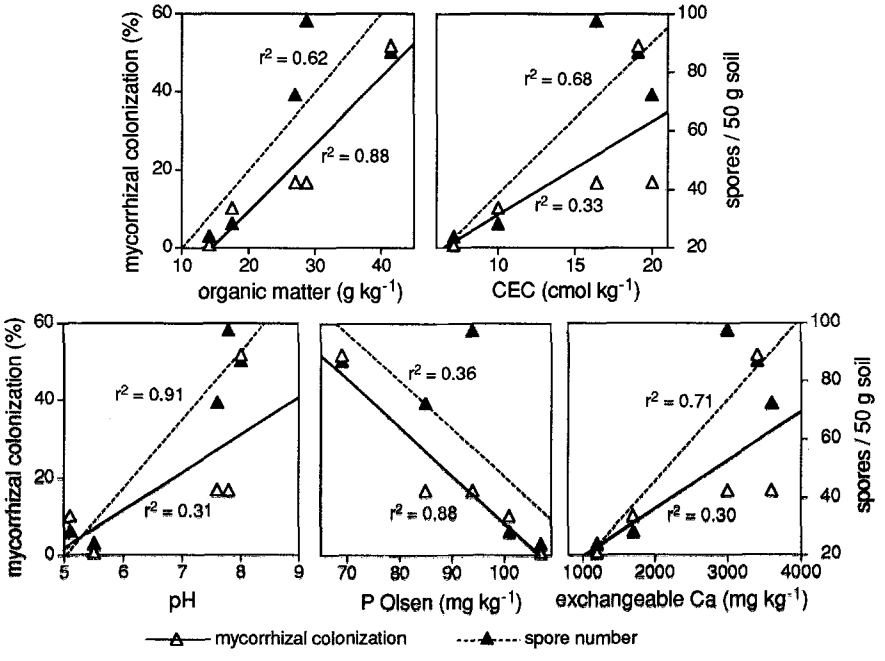

Fig. 3 Relationship between five soil factors and mycorrhizal colonization of maize plants or mycorrhizal spore numbers at maturity stage in the field plots ($n = 5$). *CEC* cation exchange capacity

Table 4 Ethylenediaminetetraacetic acid (EDTA) NH_4OAc-extractable and $Ca(NO_3)_2$-extractable soil Mn fractions (mg kg^{-1} dry matter); values in *parentheses* represent the percentage of the EDTA-NH_4OAc-extractable fraction

Soil	P1	P2	C1	C2	C3
EDTA-NH_4OAc	5.4	7.7	62.5	22.5	72.5
$Ca(NO_3)_2$	1.2 (23)	1.5 (20)	23.7 (38)	2.6 (12)	33.1 (46)

development in the two control soils C1 and C3. Furthermore, the metal availability in the two polluted fields, P1 and P2, which are regularly limed, would increase as soon as the soil pH decreased. In this case toxic effects on the AM population cannot be excluded, particularly if the changes are sudden (Killham and Firestone 1983; Wang et al. 1985; Angle and Heckman 1986).

The other three soil factors, organic matter, cation exchange capacity, and exchangeable Ca, are interdependent and together influence the composition of the soil solution. Ca ions, for example, compete with other divalent metals and precipitate phosphate at an alkaline pH, thus lowering their availability. Therefore these soil parameters might all contribute indirectly to favorable conditions for mycorrhizal development. Hepper and O'Shea (1984) also found that the Ca concentration in the nutrient solution had a direct positive influence on the mycorrhizal root colonization of lettuce.

Plant Cd, Zn, and Pb contents were considerably higher in the two contaminated fields than in the uncontaminated fields (Fig. 1), despite relatively high levels of AM root colonization in the polluted fields. Therefore, the results of this study do not indicate that mycorrhizal colonization had a substantial effect in preventing metal accumulation in the maize plants growing on the two polluted soils.

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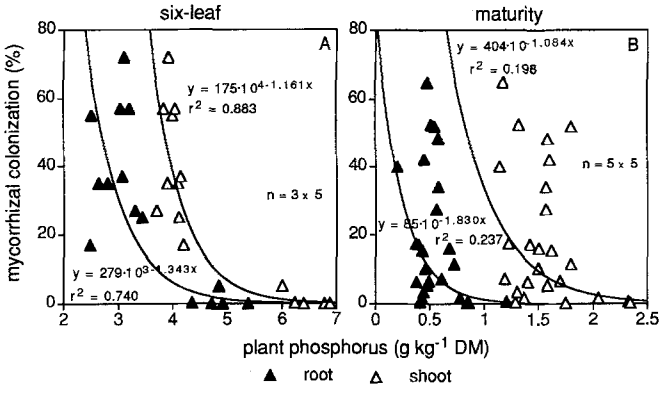

Fig. 4 Relationship between P root and shoot concentrations and mycorrhizal root colonization of maize at six-leaf (**A**) and maturity (**B**) stage. *DM* dry matter

References

Angle JS, Heckman JR (1986) Effect of soil pH and sewage sludge on VA mycorrhizal infection of soybeans. Plant Soil 93: 437–441

Commission of the European Community (1986) Council Directive on the protection of the environment and in particular of the soil, when sewage sludge is used in agriculture. CEC, Brussels, L 181:6–12

Dalenberg JW, Van Driel W (1990) Contribution of atmospheric deposition to heavy-metal concentrations in field crops. Neth J Agric Sci 38:369–379

Direction Régional de l'Industrie, de la Recherche et de l'Environnement (1992) La qualité de l'air dans la région Nord/Pas-de-Calais en 1991. DRIRE, Division Environnement Industriel, Douai

Gildon A, Tinker PB (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

Godin P (1986) Modèles pour une évaluation de la pollution des sols. Thèse Dr-Ing, Institut Nationale de la Recherche Agricole (INRA)/Paris-Grignon

Hepper CM, O'Shea J (1984) Vesicular-arbuscular mycorrhizal infection in lettuce (*Lactuca sativa*) in relation to calcium supply. Plant Soil 82:61–68

Hepper CM, Smith GA (1976) Observations on the germination of *Endogone* spores. Trans Br Mycol Soc 66:189–194

Hinesly TD, Jones RL, Ziegler EL, Tyler JJ (1977) Effects of annual and accumulative applications of sewage sludge on assimilation of zinc and cadmium by corn (*Zea mays* L.). Environ Sci Technol 11:182–188

Ietswaart JH, Griffioen WAJ, Ernst WHO (1992) Seasonality of VAM infection in three populations of *Agrostis capillaris* (Gramineae) on soil with or without heavy metal enrichment. Plant Soil 139:67–73

Jarvis SC, Jones LHP, Hooper MJ (1976) Cadmium uptake from solution by plants and its transport from roots to shoots. Plant Soil 44:179–191

Killham K, Firestone MK (1983) Vesicular arbuscular mycorrhizal mediation of grass response to acidic and heavy metal deposition. Plant Soil 72:39–48

Koomen I, McGrath SP, Giller I (1990) Mycorrhizal infection of clover is delayed in soils contaminated with heavy metals from past sewage sludge applications. Soil Biol Biochem 22:871–873

Koske RE, Gemma JN (1989) A modified procedure for staining roots to detect VA mycorrhizas. Mycol Res 92:486–505

Koske RE, Tessier B (1983) A convenient, permanent slide mounting medium. Mycol Soc Am Newslett 34:59

Leyval C, Berthelin J, Schontz D, Weissenhorn I, Morel JL (1991) Influence of endomycorrhizas on maize uptake of Pb, Cu, Zn and Cd applied as mineral salts or sewage sludges. In: Farmer JG (ed) Heavy metals in the environment. CEP Consultants Ltd, Edinburgh, pp 204–207

McGrath SP, Brookes PC, Giller KE (1988) Effects of potentially toxic elements in soil derived from past applications of sewage sludge on nitrogen fixation by *Trifolium repens* L. Soil Biol Biochem 20:415–424

Mench M, Vangronsveld J, Didier V, Clijsters H (1994) Evaluation of metal mobility, plant availability and immobilization by chemical agents in a limed silty soil. Environ Pollut 86:279–286

Menge JA, Steirle D, Bagyaraj DJ, Johnson ELV, Leonard RT (1978) Phosphorus concentrations in plants responsible for inhibition of mycorrhizal infection. New Phytol 80:575–578

Raju PS, Clark RB, Ellis JR, Maranville JW (1988) Effects of VA mycorrhizae on mineral uptake in sorghum genotypes grown on acid soil. Commun Soil Sci Plant Anal 19:909–918

Read DJ, Koucheki HK, Hodgson J (1976) Vesicular-arbuscular mycorrhiza in natural vegetation systems. I. Occurrence of infection. New Phytol 77:641–653

Sauerbeck D (1982) Welche Schwermetallgehalte in Pflanzen dürfen nicht überschritten werden, um Wachstumsbeeinträchtigungen zu vermeiden? Landwirtschaftl Forsch Sonderheft 39:105–129

Sauerbeck DR, Styperek P (1985) Significance of ion species and concentration for the determination of "available" cadmium in soil. FAO Eur Cooperative Network on Trace Elements Newslett 4:21–25

Stoeppler M (1991) Cadmium. In: Merian E (ed) Metals and their compounds in the environment. VCH, Weinheim, pp 803–851

Tinker PB, Gildon A (1983) Mycorrhizal fungi and ion uptake. In: Robb DA, Pierpoint SW (eds) Metals and micronutrients: uptake and utilization of metals by plants. Academic Press, London, pp 21–32

Trouvelot A, Kough JL, Gianinazzi-Pearson V (1986) Mesure du taux de mycorhization VA d'un système radiculaire. Recherche de méthodes d'estimation ayant une signification fonctionnelle. In: Gianinazzi-Pearson V, Gianinazzi S (eds) Aspects physiologiques et génétiques des mycorhizes. 1er Symp Eur sur les Mycorhizes. INRA, Paris, pp 217–221

Walker C, Mize CW, McNabb HS Jr (1982) Populations of endogenaceous fungi at two locations in central Iowa. Can J Bot 60:2518–2529

Wang GM, Stribley DP, Tinker PB, Walker C (1985) Soil pH and vesicular-arbuscular mycorrhizas. In: Fitter AH, Atkinson D, Read DJ, Usher MB (eds) Ecological interactions in soil: plants, microbes and animals. Blackwell, Oxford, pp 219–224

Weissenhorn I, Leyval C, Berthelin J (1993) Cd-tolerant arbuscular mycorrhizal (AM) fungi from heavy-metal polluted soils. Plant Soil 157:247–256