



## THE INFLUENCE OF MYCORRHIZA AND EDTA APPLICATION ON HEAVY METAL UPTAKE BY DIFFERENT MAIZE VARIETIES

ANNA JURKIEWICZ<sup>1</sup>, ELŻBIETA ORŁOWSKA<sup>1</sup>, TERESA ANIELSKA<sup>1</sup>, BARBARA GODZIK<sup>2</sup>,  
AND KATARZYNA TURNAU<sup>1\*</sup>

<sup>1</sup>*Institute of Botany, Jagiellonian University, ul. Lubicz 46, 31-512 Cracow, Poland*

<sup>2</sup>*Institute of Botany, Polish Academy of Sciences, ul. Lubicz 46, 31-512 Cracow, Poland*

Received September 18, 2003; revision accepted February 6, 2004

This study investigated whether mycorrhizal colonization influences heavy metal uptake by maize. Two experiments were carried out. In the first, 15 commercially available maize varieties cultured on industrial waste substratum and inoculated or not with *Glomus intraradices*, were treated one week before harvest with EDTA, a chelating agent known to mobilize heavy metals in soil. Estimation of mycorrhizal parameters indicated differences between varieties, but differences between treatments of the same variety generally were not statistically significant. Although EDTA treatment strongly decreased the activity of fungal alkaline phosphatase (indicator of fungal viability), the treatment did not totally eliminate arbuscular mycorrhizal fungi (AMF) from the soil. The appearance of AMF structures within roots was modified in plants cultivated in EDTA-treated soil. Among the heavy metals studied, the highest impact of EDTA treatment on heavy metal uptake in shoots was found in the case of Pb. In most cases, EDTA treatment significantly increased the Pb level in shoots of mycorrhizal plants. Among the samples treated with EDTA, mycorrhizal plants of 6 cultivars showed higher Pb content in shoots than did nonmycorrhizal plants. Significant differences in heavy metal content in plant material were demonstrated between the varieties tested. In the second experiment, one selected cultivar was subjected to high soil Pb concentrations and to EDTA for one week, following cultivation in nonpolluted substratum. In this case, EDTA treatment more strongly influenced Pb uptake by nonmycorrhizal than by mycorrhizal plants. The results indicate the need to carefully screen cultivars as well as microorganism strains to be used in phytoextraction procedures.

**Key words:** Maize varieties, mycorrhiza, EDTA, heavy metals, phytoremediation, metal uptake.

### INTRODUCTION

Decontamination of industrial soils, usually containing high levels of pollutants such as heavy metals, is a major concern of today's environmental sciences. Usually the best solution involves immobilization of heavy metals by establishing an appropriate pH value, and stabilization of waste deposits to reduce erosion and further contamination of the surroundings (Adriano, 1992). Stabilization may involve the introduction of plants that can adapt to such conditions (phytostabilization). In other cases, however, the heavy metal content in the soil must be reduced, especially if the contaminated area is to

be used by the local community and may involve crop production. Unfortunately, traditional approaches relying on chemical extraction are usually costly, inefficient, disruptive to the soil structure, and destructive to biota (Adriano, 1992). Phytoextraction, aimed at removing contaminants from polluted soil by means of plants accumulating high concentrations of harmful compounds in their above-ground parts (Chaney et al., 2000), is a promising option. Certain plant species grow naturally on contaminated sites and accumulate high levels of, for example, heavy metals in shoots; these are called metal hyperaccumulators. However, these plants are usually small (Cunningham and Ow, 1996; Cha-

\*e-mail: ubturnau@cyf-kr.edu.pl

TABLE 1. Total and extractable [in 1 M NH<sub>4</sub>NO<sub>3</sub> and 0.1 M Ca(NO<sub>3</sub>)<sub>2</sub>] heavy metal content in the substratum; data in  $\mu\text{g g}^{-1} \pm \text{SD}$ 

Pb			Cd			Zn		
total	extr. in NH <sub>4</sub> NO <sub>3</sub>	extr. in Ca(NO <sub>3</sub> ) <sub>2</sub>	total	extr. in NH <sub>4</sub> NO <sub>3</sub>	extr. in Ca(NO <sub>3</sub> ) <sub>2</sub>	total	extr. in NH <sub>4</sub> NO <sub>3</sub>	extr. in Ca(NO <sub>3</sub> ) <sub>2</sub>
2790 ± 35	0.9 ± 0.2	3.03 ± 0.23	225 ± 20	1.9 ± 0.1	1.2 ± 0.5	15524 ± 60	37.2 ± 0.8	2.4 ± 0.6

ney et al., 1997) and their hyperaccumulation ability is usually specific to one element only, reducing their usefulness in phytoextraction procedures. High-biomass crops are of increasing interest as potential phytoextraction models. Three possibilities to improve phytoextraction are proposed: (1) transgenic plants; (2) hyperaccumulators or high-biomass crops such as maize, especially for soils relatively less polluted (Huang and Cunningham, 1996; Huang et al., 1997), treated with chemical chelating substances such as EDTA or sulphur; and (3) introducing or stimulating the development of rhizospheric organisms that will increase the uptake of metals by the plants.

The biotechnological approach aims at producing genetically modified plants characterized by increased tolerance to toxic compounds, higher biomass, and high uptake of heavy metals. A number of transgenic plants have already been obtained, either by transferring appropriate genes from bacteria or yeasts (e.g., Rensing et al., 1998; Karenlampi et al., 2000; Riba and Chupeau, 2001; Rugh, 2001) or by generated somatic hybridization between plants such as *Brassica napus* and *Thlaspi caerulescens* (Brewer et al., 1999). Most transgenic plants have been tested only under artificial conditions so far (Krämer and Chardonens, 2001), and they need further research before starting the application phase.

The use of synthetic chelates has been proposed because the amount of metals that plants extract from the soil depends largely on the metals' availability. In most soils, even highly polluted ones, only a relatively small percentage of the total metal pool is available to plants. These compounds mobilize metal ions and displace them into soil solution. Among a variety of chelates tested by Huang et al. (1997), EDTA was demonstrated to be most effective in mobilizing Pb. Blaylock et al. (1997) showed that it also increased the availability of other metals such as Cd, Cu, Ni and Zn. Experiments carried out on maize show that this common crop can take up as much as 3000 mg Pb kg<sup>-1</sup> in shoots when grown in laboratory conditions with EDTA (0.5 g kg<sup>-1</sup> soil) (Huang et al., 1997).

The possibility of using soil microorganisms to alter the substratum has been largely neglected so

far. However, new experimental data on their role in phytoremediation are emerging (Barea et al., 1997; Khan et al., 2000; Pawłowska et al., 2000; Carlot et al., 2002; Jeffries et al., 2003); arbuscular mycorrhizal fungi (AMF), which become symbiotic with the majority of herbaceous plant species (Smith and Read, 1997), are of special interest. AMF can influence plant community development, nutrient uptake, water relations and above-ground productivity (Jeffries et al., 2003), and they confer resistance to drought, pathogens and other kinds of environmental stressors. They may also influence the uptake of contaminants from the soil by their plant partners (Galli et al., 1994; Weissenhorn et al., 1995a; Hildebrandt et al., 1999; Kaldorf et al., 1999; Liu et al., 2000; Turnau and Mesjasz-Przybyłowicz, 2003).

The present research combined the use of chelates and mycorrhizal fungi. A range of maize varieties were cultivated on substratum rich in heavy metals to find out (i) whether there are differences between plant varieties in heavy metal uptake in the presence and absence of mycorrhizal colonization; (ii) whether the application of EDTA can modify the uptake of heavy metals by mycorrhizal plants; and (iii) whether increased toxicity (availability of heavy metals) resulting from the application of EDTA affects the vitality and the development of symbiosis by the fungus.

## MATERIALS AND METHODS

### CULTURE CONDITIONS

Two experiments were carried out. In both cases a strain of the AM fungus *Glomus intraradices*, selected previously as an effective colonizer of maize roots under these particular conditions (unpublished data), was used for inoculation. In the first experiment, 15 varieties of maize were inoculated or not with the mycorrhizal fungus, and after 7 weeks of cultivation they were treated with EDTA and harvested 7 days later. The second experiment was carried out on one selected cultivar following the

protocol by Huang et al. (1997), where the plants were subjected to high soil Pb concentrations and to EDTA for one week following a longer period of cultivation in nonpolluted substratum.

### Experiment I

Two sets of plants of 15 commercially available varieties of *Zea mays* (Bzura, Cedro, Dobosz, Fido, Grom, Kasia, Nimba, Proсна, Reduta, San, Tenet, Tytan, Waza, Wiarus, Wilga) were cultured in pots in sterile substratum composed of a 1:1:1 mixture of soil from industrial spoils in Chrzanów, sand and expanded clay (Tab. 1). The total metal content of the substratum was analyzed by AAS (Varian 220FS) after extraction in HClO<sub>4</sub> (MERCK). The exchangeable fraction of metal content was measured in 1 M NH<sub>4</sub>NO<sub>3</sub> (Carter, 1993). The pH of substratum measured in H<sub>2</sub>O was ~7.4. The mean total P content was 90 mg and the mean content of readily available P was 11.4 mg per 100 g substratum. The mean total N content was 0.09% of dry soil and the C content was approximately 6.05%. Maize seeds were soaked in redistilled water overnight and germinated on moist filter paper. Uniform seedlings were transplanted into pots containing the soil mixture, 4 seedlings per treatment. The plants were cultured in growth-room conditions under a 12 h photoperiod (light intensity 78 μmol s<sup>-1</sup> m<sup>-2</sup>).

Each set consisted of one noninoculated group of plants (three plants of each variety) and one group inoculated with *G. intraradices* (BEG E-1-99). One set of plants (inoculated or not) was cultured for 8 weeks and harvested. The second set was cultured for 7 weeks and each pot was treated with 500 mg EDTA kg<sup>-1</sup>. The plants were harvested 7 days later. Small root samples from all varieties were taken for aniline blue staining, and root samples from 4 varieties (Cedro, Nimba, Wilga, Wiarus) were used for ALP staining. The shoots and remaining roots were air-dried and weighed.

Additional plants of the selected maize varieties (Cedro, Nimba, Wilga and Wiarus) were cultivated under the same conditions in substratum containing *G. intraradices* for observation of mycorrhizal colonization and vitality after EDTA treatment. Samples of roots were collected a week and 6 weeks after EDTA treatment.

### Experiment II

Seedlings of the Nimba variety (selected as the variety most promising for heavy metal uptake) were

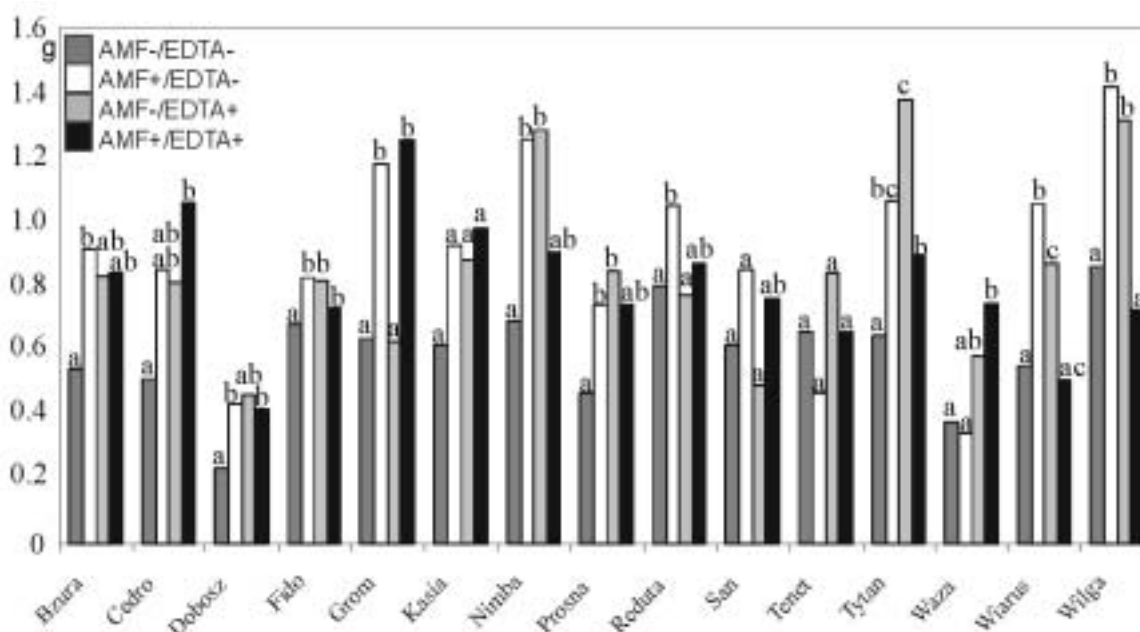
precultured in uncontaminated soil with or without the addition of inoculum (*G. intraradices*) for 4 weeks. Pb-treated soil (final lead concentration: 2500 mg kg<sup>-1</sup> soil in the form of lead acetate solution) was fertilized with N [150 mg kg<sup>-1</sup>, as (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>] and K (150 mg kg<sup>-1</sup>, as K<sub>2</sub>SO<sub>4</sub>), saturated with the lead acetate solution and allowed to stabilize for two weeks. The experiment was conducted at two pH levels: 5.5 and 6.5. EDTA was added to one set of pots (0.5 g kg<sup>-1</sup>, trisodium salt, SIGMA) and the soil allowed to stabilize for one week before the seedlings were planted. Inoculated seedlings were checked for mycorrhization and more inoculum was added to the inoculated seedlings during planting. The plants were grown in the control and lead-treated soil with or without EDTA for one week (4 replicates of each treatment). Water leaking out of the pots was collected. The plants were harvested and washed; the roots and shoots were air-dried and weighed, and their heavy metal content was determined by AAS (see below). Small samples of roots were taken to check for the presence of mycorrhiza.

#### ASSESSMENT OF MYCORRHIZAL PARAMETERS

The roots for estimation of mycorrhizal parameters were stained according to a modification of the method given by Phillips and Hayman (1970). Briefly, roots were rinsed in tap water, cleared in 10% KOH for 24 h, acidified in 5% lactic acid for 1–24 h at room temperature and stained in 0.01% aniline blue in pure lactic acid for 24 h. After staining the roots were stored in pure lactic acid. The roots were cut into 1 cm pieces, mounted on slides in glycerol and analyzed in order to assess the mycorrhizal parameters: frequency of mycorrhiza (F), relative mycorrhizal root length (M), and relative arbuscular richness (A) (Trouvelot et al., 1986; <http://www.dijon.inra.fr.bbceipm/Mychintec/Mycocalc-prg/download.html>).

#### ASSESSMENT OF ALKALINE PHOSPHATASE (ALP) ACTIVITY

The roots of four selected maize varieties (Cedro and Wiarus showing the highest, and Wilga and Nimba showing the lowest frequencies of colonization) were stained according to van Aarle et al. (2001) for estimation of alkaline phosphatase activity. Briefly, the roots were rinsed in tap water, cut into 1 cm pieces, mounted on slides and stained for 15 min with the ELF 97 Endogenous Phosphatase Detection Kit (Molecular Probes). After staining the roots were



**Fig. 1.** Shoot dry weight of 15 maize varieties, inoculated (AMF+) or not (AMF-) with *Glomus intraradices*, with and without EDTA treatment. Different letters above bars indicate statistically significant differences between treatments within variety.

incubated in the rinse buffer (30 mM Tris, 1.5 M NaCl and 0.05% Triton X-100, pH 8) for 15 min before the mounting medium from the kit was applied and the slides were closed with coverslips. The slides were stored at 5°C overnight before analysis. The roots were analyzed with a Nikon Eclipse 800C fluorescence microscope equipped with a mercury fluorescence lamp, and the mycorrhizal parameters were assessed as described above.

#### HEAVY METAL CONTENT IN PLANT MATERIAL

Plant material was rinsed in redistilled water, divided into underground and above-ground parts, dried to constant weight at 85°C and weighed. The dried and milled plant material was subsequently mineralized in a 4:1 mixture of Ultrapur concentrated HNO<sub>3</sub> (Merck) and HClO<sub>4</sub> (Merck) (Pinta, 1977; Grodzińska, 1978). Pb, Zn and Cd content in plant roots and shoots was analyzed by AAS (Varian 220FS).

#### HEAVY METAL CONTENT IN SOIL SOLUTION

The concentrations of Pb, Zn and Cd were determined by AAS in the solution leaking out of the pots collected during the first week of culture after the addition of EDTA.

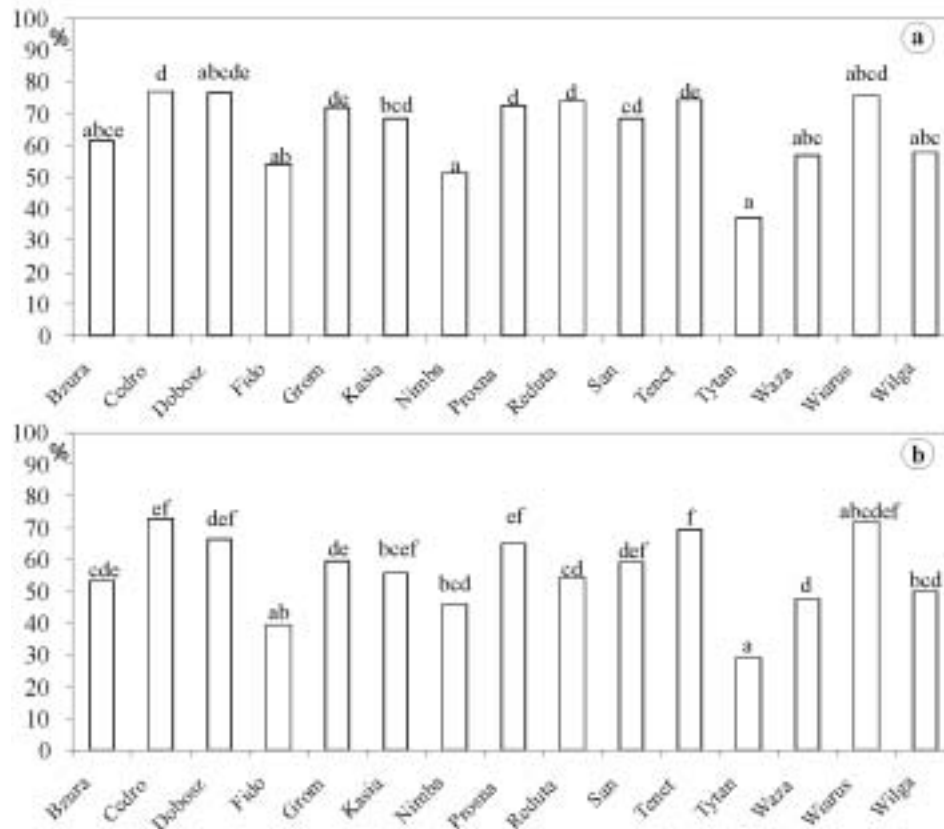
#### STATISTICAL ANALYSIS

All the data were analyzed using the U Mann-Whitney test with STATISTICA ver. 5.0 software (Sokal and Rohlf, 1981). The level of significance was taken as  $p < 0.05$ .

## RESULTS

#### ESTIMATION OF BIOMASS

In 80% of the varieties, mycorrhizal plants grown on substratum rich in heavy metals were characterized by higher shoot dry weight than the nonmycorrhizal controls (Fig. 1). In 53% of the varieties these differences were statistically significant. The highest dependence on mycorrhiza was observed in the case of Wiarus, Nimba, Grom and Dobosz. EDTA treatment applied one week before the end of the experiment had no significant effect on shoot biomass in over half of the varieties, while in a third of the nonmycorrhizal plants it resulted in a statistically significant increase of shoot biomass. Among all treatments, including mycorrhizal/nonmycorrhizal plants and the presence/absence of EDTA, the highest values of mean biomass per plant were obtained in the case of mycorrhizal plants of cv. Wilga without EDTA treatment. Lower mean biomass, though not



**Fig. 2.** Intensity of (a) mycorrhization (M%) and (b) arbuscule richness (A%) in roots of 15 maize varieties inoculated with *Glomus intraradices*, stained with aniline blue, without EDTA treatment. Different letters above bars indicate statistically significant differences within variety.

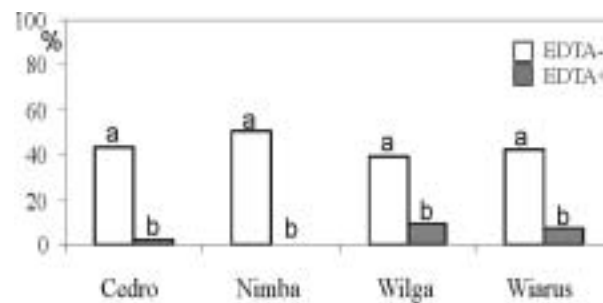
statistically significant, was found in nonmycorrhizal cv. Tytan and Nimba treated with EDTA.

#### MYCORRHIZAL COLONIZATION

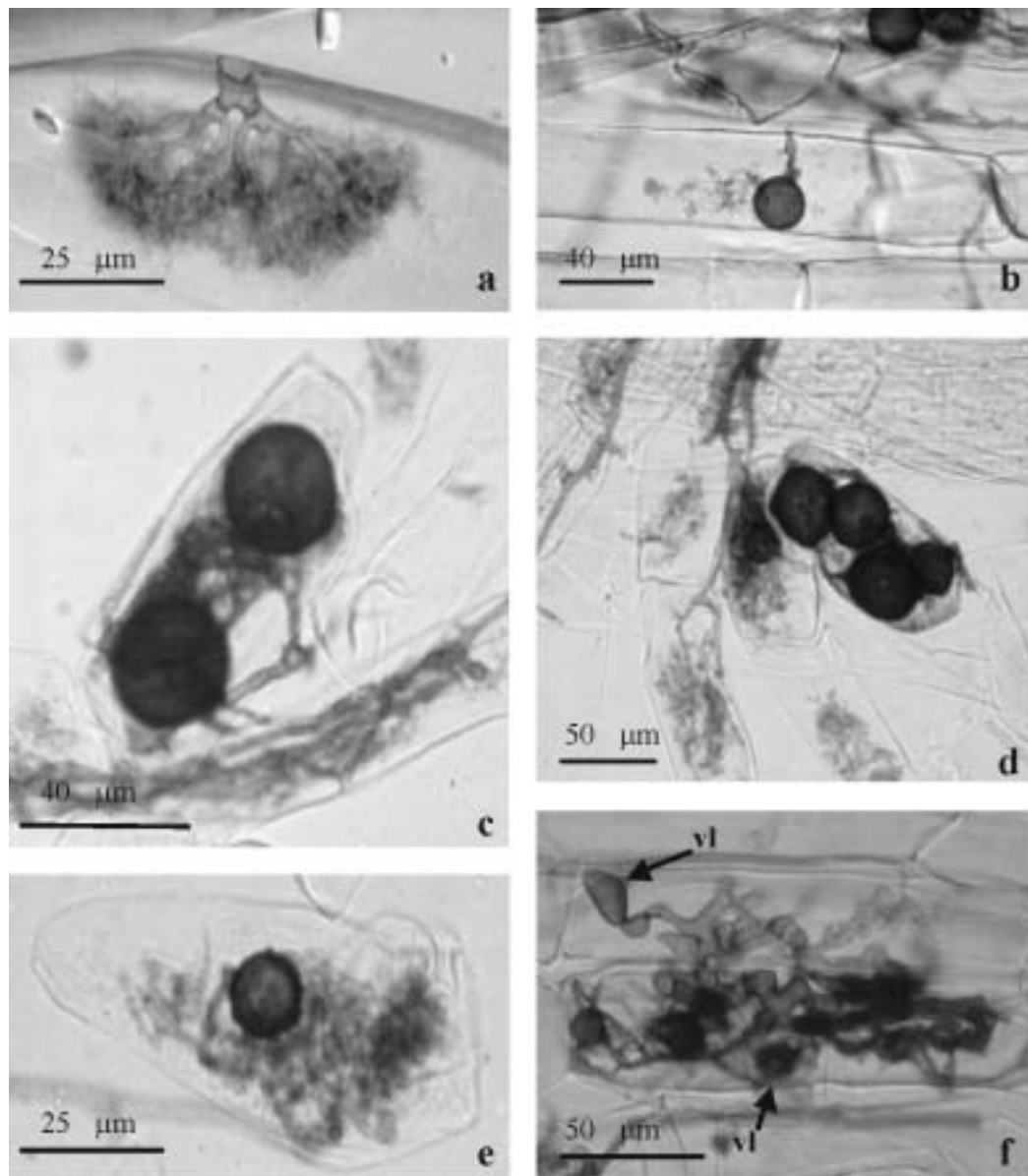
The frequency of mycorrhizal colonization in samples was high, ranging from 80 to 100%. Differences between EDTA-treated and untreated samples were statistically significant only in the case of Wiarus (not shown). The differences in mycorrhizal colonization and arbuscular richness were larger between cultivars (Fig. 2). In the case of M%, the lowest score was noted for cv. Tytan; in 5 other varieties this parameter ranged from 50 to 70%, and in 9 it ranged from 70 to 80%. The mean arbuscular richness (A%) ranged from 40 to 75% in the case of 13 cultivars, while the remaining 2 were below 40%.

Staining for alkaline phosphatase activity, providing information on the viability of the fungal partner within the roots, showed that EDTA treatment was not lethal to the fungus (Fig. 3). The most drastic differences were noted in arbuscule richness.

The values of vital A% were 2 to 85 times lower than total A%. In the case of cv. Nimba almost no viable fungal structures were found, and the decrease in the vitality of the intraradical mycorrhizal mycelium was greatest. The least decrease in viability was observed in the case of cv. Wilga and Wiarus. When viability results were compared between



**Fig. 3.** Arbuscule richness in roots of 4 selected maize varieties stained for alkaline phosphatase activity. Different letters above bars indicate statistically significant differences within variety at  $p < 0.05$ .



**Fig. 4.** Morphological changes in maize (cv. Cedro)/*Glomus intraradices* mycorrhizas grown on industrial waste substratum after EDTA treatment. (a) Arbuscule developed in roots not treated with EDTA, (b–f) Mycorrhizal structures developed in roots treated with EDTA. (b–e) Spores/vesicles developed in the same cortical cells, (f) Formation of thick, strongly septated mycelium accompanied by numerous vesicles (vl) within cortical cells of maize root.

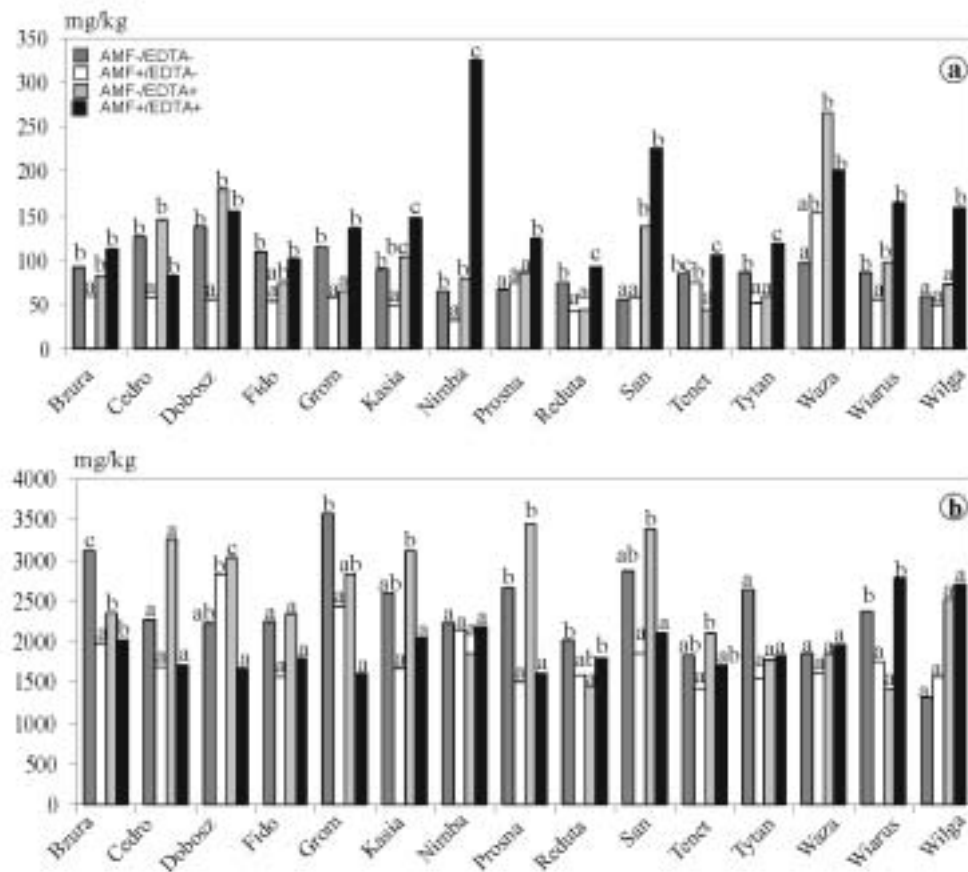
EDTA-treated and untreated plants, a statistically significant decrease in viability after EDTA treatment was demonstrated in all varieties tested (Fig. 3). In samples collected six weeks after EDTA treatment the vitality of the arbuscules was very low (~7%), although more abundant vesicles and spores were found in them than in samples of plants not treated with EDTA. They were commonly present in the same cells as arbuscules (Fig. 4), unlike in the control material. In addition, septated, strongly

branched mycelia often forming numerous vesicle-like structures were found in many cortical cells of roots treated with the EDTA (Fig. 4f).

#### HEAVY METAL CONTENT IN PLANT MATERIAL

##### Pb content

In most maize cultivars not treated with EDTA, Pb content in shoots of nonmycorrhizal plants was significantly higher (up to 140 mg kg<sup>-1</sup>) than in mycor-



**Fig. 5.** Shoot (a) and root (b) Pb content in 15 maize varieties, inoculated (AMF+) or not (AMF-) with *Glomus intraradices*, with and without EDTA treatment. Different letters above bars indicate statistically significant differences between treatments within variety.

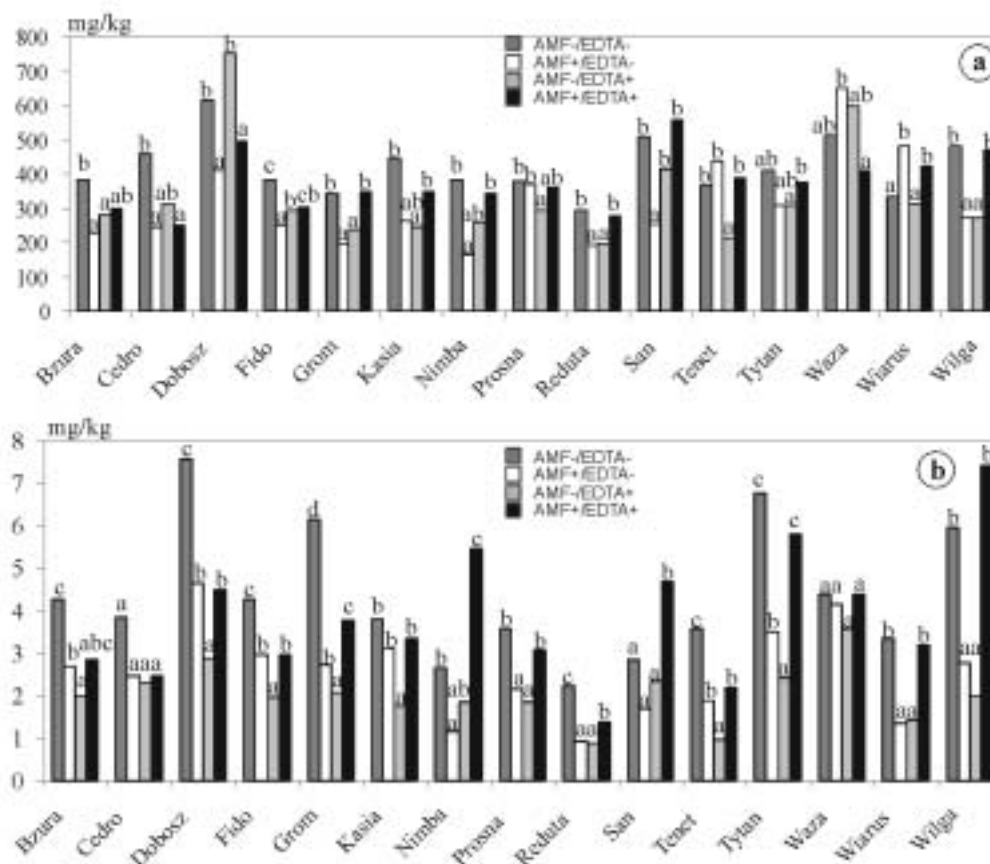
rhizal samples. EDTA treatment of nonmycorrhizal plants had no significant effect on shoot Pb content in 9 cultivars, significantly decreased shoot Pb content in 4 cultivars, and increased it in 2 cultivars where shoot Pb content reached  $260 \text{ mg kg}^{-1}$ . In most cases the Pb level in shoots of mycorrhizal plants treated with EDTA was significantly higher than in those not treated. Among samples treated with EDTA, 6 cultivars had higher shoot Pb in mycorrhizal plants than in nonmycorrhizal ones. In other cases the difference was not significant. The highest mean Pb content, reaching over  $300 \text{ mg kg}^{-1}$ , was in the Nimba variety (Fig. 5a). This cultivar also had significantly higher Pb content in shoots than most of the other cultivars.

The content of Pb in roots was much higher, reaching over  $3500 \text{ mg kg}^{-1}$  for nonmycorrhizal and  $2800 \text{ mg kg}^{-1}$  for mycorrhizal samples (Fig. 5b). The mean Pb content in nonmycorrhizal roots was higher than in mycorrhizal ones, but the differences

were statistically significant only in the case of 4 cultivars. In most cases, Pb content in nonmycorrhizal roots did not differ statistically between samples treated or not with EDTA. In both treatments, the level of Pb was significantly lower in 2 cultivars and significantly higher only in the case of the Dobosz cultivar. Root Pb content in EDTA-treated samples was usually lower in mycorrhizal than in nonmycorrhizal samples. This difference was statistically significant in 5 varieties. In cultivars Reduta and Wiarus the Pb level was significantly higher in EDTA-treated mycorrhizal plants.

#### Zn content

The highest Zn content ( $750 \text{ mg kg}^{-1}$ ) in shoots was observed in nonmycorrhizal plants of cv. Dobosz treated with EDTA (Fig. 6a). Mycorrhizal plants without EDTA treatment had lower Zn content in shoots, except for cv. Wiarus, Waza and Tenet, where mycorrhizal samples



**Fig. 6.** Shoot Zn (a) and Cd (b) content in 15 maize varieties, inoculated (AMF+) or not (AMF-) with *Glomus intraradices*, with and without EDTA treatment. Different letters above bars indicate statistically significant differences between treatments within variety.

had higher Zn content; however, the difference was statistically significant only for Wiarus. It is noteworthy that the values obtained for mycorrhizal plants of the Waza cultivar did not differ significantly from nonmycorrhizal cv. Dobosz treated with EDTA, mentioned above as the sample with the highest Zn content. In most cases, mycorrhizal samples treated with EDTA had shoot Zn levels similar to those of nonmycorrhizal samples without EDTA, and higher shoot Zn levels than in mycorrhizal samples without EDTA and nonmycorrhizal samples with EDTA. Nonmycorrhizal cv. Bzura and Grom without EDTA treatment had the highest Zn content per plant, significantly higher than other varieties in the same treatment group. Other treatments usually resulted in lower Zn content than the nonmycorrhizal samples without EDTA treatment.

#### Cd content

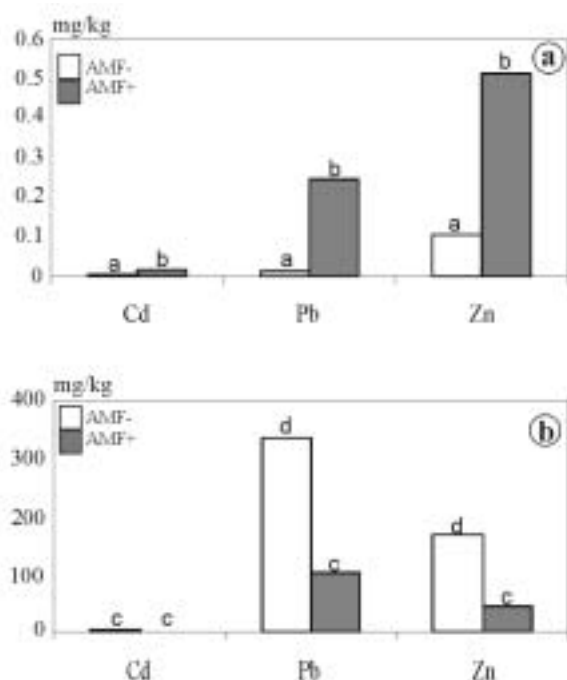
Mean shoot Cd content ranged from 0.9 to 7.8 mg kg<sup>-1</sup>, much lower than Pb and Zn content (Fig. 6b).

Among shoot samples collected from plants not treated with EDTA, those from mycorrhizal plants had significantly lower Cd content, with levels similar to those in nonmycorrhizal plants treated with EDTA. Lower Cd was also noted in mycorrhizal plants of 5 cultivars treated with EDTA. In two cases, Nimba and San, mycorrhizal EDTA-treated plants had significantly higher shoot Cd content than in the other treatments. The highest Cd content in shoots was in nonmycorrhizal cv. Dobosz not treated with EDTA, which, however, produced low biomass.

#### HEAVY METAL CONTENT IN SOIL SOLUTION

Analysis of heavy metal (HM) content in water flowing out of the pots containing the substratum in which the maize plants were cultivated indicated statistically significant differences between samples inoculated and not inoculated with arbuscular mycorrhizal fungi (AMF) (Fig. 7). When mycorrhizal fungi were absent, Pb, Cd and Zn release was 20, 4





**Fig. 7.** Heavy metal (HM) release into soil solution (a) without EDTA treatment, (b) after EDTA treatment in maize cv. Nimba. Different letters above bars indicate statistically significant differences within variety.

and 5 times higher, respectively. Addition of EDTA to the substratum was followed by a significant increase of heavy metals in the water from all experimental containers. The relation for HM release from mycorrhizal and nonmycorrhizal samples was the reverse – significantly less Pb and Zn in water from pots of the mycorrhizal group. For all maize varieties, the concentrations of Pb, Zn and Cd in soil solution were significantly higher after EDTA treatment in both the mycorrhizal and nonmycorrhizal groups. There were significant differences in the content of all three heavy metals between mycorrhizal and nonmycorrhizal plants in both the EDTA-treated and control groups, except for Cd in the EDTA-treated group.

#### HEAVY METAL UPTAKE BY MYCORRHIZAL AND NONMYCORRHIZAL PLANTS IN EXPERIMENT II

The plants in the second experiment, cultivated for a much shorter time, were several times smaller than those from the first experiment, and the mycorrhizal structures were much less developed (especially at the lower pH value), with arbuscules seldom found. In experiment II there were no statistically significant differences in biomass between mycorrhizal and nonmycorrhizal plants or between

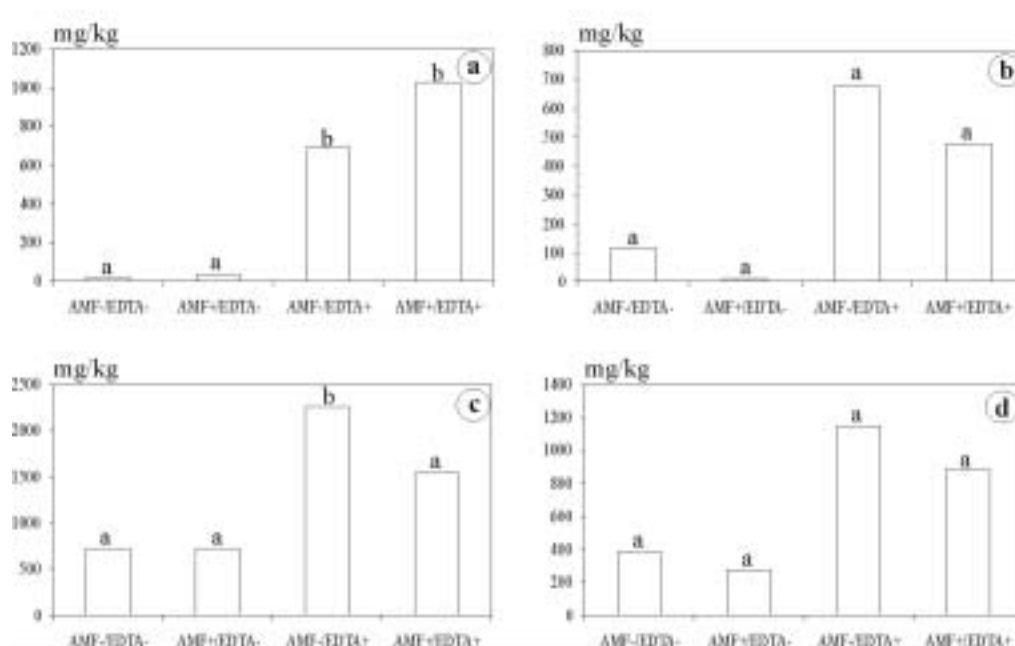
EDTA treatment and no EDTA treatment (data not shown).

There was a statistically significant difference in Pb concentrations between EDTA-treated and untreated plants, both in roots and in shoots (Fig. 8). More Pb accumulated in roots than in shoots, and in nonmycorrhizal than in mycorrhizal plants. The highest shoot Pb levels were noted in the EDTA-treated nonmycorrhizal group, although this difference was statistically significant only at pH 5.5. The highest Pb concentration was measured in roots of EDTA-treated noninoculated plants grown at pH 5.5. At pH 5.5, inoculation was associated with higher shoot and lower root Pb content; at pH 6.5, shoot and root Pb content were both lower in inoculated plants. In plants without EDTA treatment, inoculation had no statistically significant effect on root Pb concentration at both pH levels, but significantly reduced shoot Pb content.

#### DISCUSSION

Among other benefits of mycorrhizal colonization, many mycorrhizal plants show significantly higher shoot biomass than noninoculated controls (Smith and Read, 1997). This was also observed in the present study in most maize varieties cultivated on metal-rich substratum, but there were large differences between varieties. In the literature, not much attention has been paid to the possibility of large diversity in the effectiveness of phytoextraction between varieties of the same species, as was found in the present study. These findings stress the need to screen a large number of cultivars in order to select the best ones for further use. Moreover, the response of individual varieties to the use of chelators such as EDTA might differ.

The above-presented differences in the biomass of mycorrhizal and nonmycorrhizal plants grown on HM-rich substratum in the presence or absence of EDTA clearly confirm the role of mycorrhizal fungi in phytoremediation practices. In the present research, *Glomus intraradices* originating from non-polluted soils was applied. This species colonizes abundantly under natural and stressed conditions. Stimulation of growth and alleviation of metal toxicity of maize was also shown by Hildebrandt et al. (1999), who used the same species but an isolate from roots of *Viola calaminaria*, a heavy metal-tolerant plant. This would suggest that the species is constitutively tolerant to heavy metals; it would be interesting to compare the strains. A similar pattern



**Fig. 8.** Experiment II: shoot and root Pb content in maize cv. Nimba, inoculated (AMF+) or not (AMF-) with *Glomus intraradices*, with and without EDTA treatment, at two pH levels. (a) Shoots, pH 5.5, (b) Shoots, pH 6.5, (c) Roots, pH 5.5, (d) Roots, pH 6.5. Different letters above bars indicate statistically significant differences.

was found in *Zea mays* inoculated with *G. mosseae* (Weissenhorn et al., 1995a). Research carried out by Weissenhorn et al. (1995b) demonstrated that fungal strains isolated from industrial areas show better germination and colonization levels when cultured on contaminated substratum than strains from nonpolluted sites. It is advisable to test plants and fungi not only separately but in different combinations before optimal sets are selected.

Our results clearly indicate that mycorrhization usually decreases heavy metal uptake by plants, which could be of importance for food production in polluted areas. However, a few mycorrhizal maize varieties reacted with increased Pb uptake into shoots after EDTA treatment. The most efficient in this regard was cv. Nimba, with Pb content in shoots of mycorrhizal EDTA-treated plants about three times higher than in other treatments. The tendency was similar for Cd in this variety, while for Zn the presence of mycorrhiza seemed of less importance.

In some cultivars, mycorrhizal plants contained more heavy metals than EDTA-treated nonmycorrhizal plants. This would make the use of the toxic substances such as EDTA unnecessary and environmentally unprofitable. In other cases EDTA might be useful only with mycorrhizal plants (e.g., cv. Nimba). The main disadvantage of chelator-assisted phytoremediation is the risk of Pb-EDTA complexes leaching from the soil into the groundwater and

altering soil microbiota. In the present study, the mycorrhizal fungi survived the treatment although their condition was negatively affected. In previous work, the formation of spores and vesicles within the same cells where arbuscules were present was noted occasionally in samples originating from heavily polluted soils (unpublished data). In the present study this phenomenon was commonly observed in roots about three months old; this seems to be a good indicator of stress, suggesting acceleration of the propagule formation phase. So far relatively few studies have addressed the influence of chelators on soil microbial communities. Pawłowska et al. (2000) studied the influence of sulphur, used to increase metal availability in phytoextraction, on mycorrhizal fungi. The results suggested that both plant species and soil alteration affect AM fungi and mycorrhiza functioning. In that study, sulphur supplementation increased the number of vesicles in roots, while it decreased the spore population in the maize rhizosphere.

Metal sorption by mycorrhizal mycelia in artificial substrates has been well documented (Joner et al., 2000), but the significance of this phenomenon under field conditions has not yet been investigated. In our study we did not evaluate the role of extraradical mycelia, and it awaits further research. HM release into water from the pots with EDTA-treated soil was substantially lower with mycorrhizal plants

than with nonmycorrhizal plants. This would suggest that mycorrhizal fungi increase the availability of metals to the plants and may decrease pollutant runoff into the ground water.

In the present study, HM levels were higher in roots than in shoots, confirming previous reports (Weissenhorn et al., 1995a,b; Małkowski et al., 2002). This phenomenon is undesirable for phytoextraction, as metals remain in the unharvestable underground fraction. In most cultivars, however, HM content was lower in mycorrhizal roots than in nonmycorrhizal roots.

In general, HM content in shoots and roots was lower in the present study than as reported by Huang et al. (1997), who found shoot Pb above 3000 mg kg<sup>-1</sup> in EDTA-treated maize (under 350 mg kg<sup>-1</sup> in our study). These differences might be the result of differences in experimental conditions (e.g., soil composition, pH value linked to HM availability, etc.). The obtained results roughly match data reported by Weissenhorn et al. (1995a). Experiment II was an attempt to determine whether inoculation of maize with a mycorrhizal fungus (*Glomus intraradices*) would influence Pb accumulation in maize shoots under culture conditions similar to those described by Huang et al. (1997); our results were still lower than the values Huang et al. reported, and in addition the mycorrhizal plants had lower metal content than the nonmycorrhizal ones. The differences between results may be due to different soil conditions (our protocol used Pb added as a salt solution to uncontaminated soil to obtain a soil Pb level comparable to that of contaminated soil) and the different maize cultivar. Comparison of our two experiments underlines the importance of extramatrical mycelium in phytoremediation processes. The improved translocation of metal into shoots in the first experiment was not achieved in the second one, where the plants were removed from the nonpolluted substratum, destroying the network of soil mycelium. Moreover, the period of culture was too short to establish beneficial symbiosis, judging by the lack of differences in plant biomass between treatments and the poor development of mycorrhiza. Future research should take this into account.

In conclusion, the presented data show the potential for application of mycorrhiza in phytoremediation processes, but care should be taken to select the most effective cultivar and fungal isolate, and to design the experiment suitably. As with other crops, the use of maize is limited due to inappropriate partitioning of heavy metals between roots and shoots. To exploit fully the advantages of mycorrhiza

we still need plants such as *Berkheya coddii*, a nickel hyperaccumulator from South Africa producing economically advantageous biomass. With it, Turneau and Mesjasz-Przybyłowicz (2003) demonstrated increased Ni uptake in mycorrhizal plants as compared with nonmycorrhizal ones. They also reported three other Ni-hyperaccumulating plant species of the Asteraceae family to be mycorrhizal. Among them, Ni-accumulating and nonaccumulating ecotypes of *Senecio coronatus* were listed. This species is present in the European flora and might be of interest for further research. Mycorrhiza could also prove important in establishment and increasing the effectiveness of phytoextraction by transgenic plants.

Arbuscular mycorrhizal fungi are one component of the rhizospheric microbiota. Under natural conditions, plants and AMF are accompanied by other microorganisms such as plant growth promoting bacteria, which can further influence the availability of the metals, the partitioning of these elements within the system, and biomass production (e.g., Carlot et al., 2002). Improved understanding of the interactions within such systems may be crucial to the development of successful phytoremediation technologies.

#### ACKNOWLEDGEMENTS

The results in this paper were presented at the 'Biodiversity and ecotoxicology of industrial areas in reference to their bio-reclamation' International Conference held in Katowice on June 5–6, 2003. This publication was supported by the Provincial Fund for Environmental Protection and Water Management in Katowice.

The present work was supported by the European Community MYCOREM project (QLK-3-1999-00097). Maize seeds were kindly donated by Hodowla Roślin Smolice Sp. z o.o., Kobylin. AMF inoculum was provided by Biorize, France. The equipment used was financed by the Foundation for Polish Science (FNP) REGLE 25/97 and SUBIN 2000.

#### REFERENCES

- ADRIANO DC. 1992. *Biogeochemistry of trace metals. Advances in trace substances research*. CRC Press, Inc., Boca Raton, FL, USA.
- BAREA JM, AZCON-AGUILAR C, and AZCON R. 1997. Interactions between mycorrhizal fungi and rhizosphere microorganisms within the context of sustainable soil-plant systems. In: Gange AC, Brown VK [eds.], *Multitrophic interactions in terrestrial systems*, 65–77. United Kingdom, Cambridge.

- BLAYLOCK MJ, SALT DE, DUSHENKOV S, ZAKHAROVA O, GUSSMAN C, KAPULNIK Y, ENSLEY BD, and RASKIN I. 1997. Enhanced accumulation of Pb in indian mustard by soil-applied chelating agents. *Environmental Science and Technology* 31: 860–865.
- BREWER EP, SAUDERS JA, ANGLE JS, CHANEY RL, and MCINTOSH MS. 1999. Somatic hybridization between the zinc accumulator *Thlaspi caerulescens* and *Brassica napus*. *Theoretical and Applied Genetics* 99: 761–771.
- CARLOT M, GIACOMINI A, and CASELLA S. 2002. Aspects of plant-microbe interactions in heavy metal polluted soil. *Acta Biotechnologica* 22: 13–20.
- CARTER MR. 1993. *Soil sampling and methods of analysis*. Lewis Publishers, Canadian Society of Soil Sciences, Toronto, Canada.
- CHANEY RL, LEE YM, BROWN SL, HOMER FA, MALIK M, ANGLE JS, BAKER AJM, REEVES RD, and CHIN M. 2000. Improving metal hyperaccumulator wild plants to develop commercial phytoextraction systems: approaches and progress. In: Terry N, Banuelos G [eds.], *Phytoremediation of contaminated soil and water*, 129–158. CRC Press, Boca Raton, FL, USA.
- CHANEY RL, MALIK M, LI YM, BROWN SL, BREWER EP, ANGLE JS, and BAKER AJM. 1997. Phytoremediation of soil metals. *Current Opinion in Biotechnology* 8: 279–284.
- CUNNINGHAM S, and OW DW. 1996. Promises and prospects of phytoremediation. *Plant Physiology* 110: 715–719.
- GALLI U, SCHÜEPP H, and BRUNOLD C. 1994. Heavy metal binding by mycorrhizal fungi. *Physiologia Plantarum* 92: 364–368.
- GRODZIŃSKA K. 1978. Mosses as bioindicators of heavy metal pollution in Polish national parks. *Water, Air and Soil Pollution* 9: 83–97.
- HILDEBRANDT U, KALDORF M, and BOTHE H. 1999. The zinc violet and its colonization by arbuscular mycorrhizal fungi. *Journal of Plant Physiology* 154: 709–717.
- HUANG JW, and CUNNINGHAM SD. 1996. Lead phytoextraction: species variation in lead uptake and translocation. *The New Phytologist* 134: 75–84.
- HUANG JW, CHEN J, BERTI WR, and CUNNINGHAM SD. 1997. Phytoremediation of lead-contaminated soils: role of synthetic chelates in lead phytoextraction. *Environmental Science and Technology* 31: 800–805.
- JEFFRIES P, GIANINAZZI S, PEROTTO S, TURNAU K, and BAREA JM. 2003. The contribution of arbuscular mycorrhizal fungi in sustainable maintenance of plant health and soil fertility. *Biology and Fertility of Soils* 37: 1–16.
- JONER EJ, BRIONES R, and LEYVAL C. 2000. Metal-binding capacity of arbuscular mycorrhizal mycelium. *Plant and Soil* 226: 227–234.
- KALDORF M, KUHN AJ, SCHRÖDER WH, HILDEBRANDT U, and BOTHE H. 1999. Selective element deposits in maize colonized by a heavy metal tolerance conferring arbuscular mycorrhizal fungus. *Journal of Plant Physiology* 154: 718–728.
- KARENlampi S, SCHAT H, VANGRONSVELD J, VERKLEIJ JAC, VAN DER LELIE D, MERGEAY M, and TERVAHUTA AI. 2000. Genetic engineering in the improvement of plants for phytoremediation of metal polluted soils. *Environmental Pollution* 107: 225–231.
- KHAN AG, KUEK C, CHAUDHRY TM, KHOO CS, and HAYES WJ. 2000. Role of plants, mycorrhizae and phytochelators in heavy metal contaminated land remediation. *Chemosphere* 41: 197–207.
- KRÄMER U, and CHARDONNENS AN. 2001. The use of transgenic plants in the bioremediation of soils contaminated with trace elements. *Applied Microbiology and Biotechnology* 55: 661–672.
- LIU A, HAMEL C, HAMILTON RI, MA BL, and SMITH DL. 2000. Acquisition of Cu, Zn, Mn and Fe by mycorrhizal maize (*Zea mays* L.) grown in soil at different P and micronutrient levels. *Mycorrhiza* 9: 331–336.
- MALKOWSKI E, KITA A, GALAS W, KARZC W, and KUPERBERG M. 2002. Lead distribution in corn seedlings (*Zea mays* L.) and its effect on growth and the concentrations of potassium and calcium. *Plant Growth Regulation* 37: 69–76.
- PAWLOWSKA TE, CHANEY RL, CHIN M, and CHARVAT I. 2000. Effects of metal phytoextraction practices on the indigenous community of arbuscular mycorrhizal fungi at a metal contaminated landfill. *Applied and Environmental Microbiology* 66: 2526–2530.
- PHILLIPS JM, and HAYMAN DS. 1970. Improved procedures for clearing and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. *Transactions of the British Mycological Society* 55: 158–161.
- PINTA M. 1977. *Absorpcyjna spektrometria atomowa. Zastosowania w analizie chemicznej*. Państwowe Wydawnictwo Naukowe, Warszawa.
- RENSING C, SUN Y, MITRA B, and ROSEN BP. 1998. Pb(II)-translocating P-type ATPases. *Journal of Biological Chemistry* 273: 32614–32617.
- RIBA G, and CHUPEAU Y. 2001. Genetically modified plants. *Cellular and Molecular Biology* 47: 1319–1328.
- RUGH CL. 2001. Mercury detoxification with transgenic plants and other biotechnological breakthroughs. *In Vitro Cellular and Developmental Biology – Plant* 37: 321–325.
- SMITH SE, and READ DJ. 1997. *Mycorrhizal symbiosis*. Academic Press, London.
- SOKAL RR, and ROHLF FJ. 1981. *Biometry. The principles and practice of statistics in biological research*. W.H. Freeman and Company, New York.
- TURNAU K, and MESJASZ-PRZYBYLOWICZ J. 2003. Arbuscular mycorrhiza of *Berkheya coddii* and other Ni-hyperaccumulating members of Asteraceae from ultramafic soils in South Africa. *Mycorrhiza* 13: 185–90.
- TROUVELOT A, KOUGH JL, and GIANINAZZI-PEARSON V. 1986. Mesure du taux de mycorrhization VA d'un système racinaire. Recherche de méthodes d'estimation ayant une signification fonctionnelle. In: Gianinazzi-Pearson V, Gianinazzi S. [eds.], *Physiological and genetical aspects of mycorrhizae*, 217–221. INRA, Paris.
- VAN AARLE IM, OLSSON PA, and SÖDERSTRÖM B. 2001. Microscopic detection of phosphatase activity of saprophytic and arbuscular mycorrhizal fungi using a fluorogenic substrate. *Mycologia* 93: 17–24.
- WEISSENHORN I, LEYVAL C, BELGY G, and BERTHELIN J. 1995a. Arbuscular mycorrhizal contribution to heavy metal uptake by maize (*Zea mays* L.) in pot cultures with soil contaminated by atmospheric deposition. *Mycorrhiza* 5: 245–251.
- WEISSENHORN I, LEYVAL C, and BERTHELIN J. 1995b. Cd-tolerant arbuscular mycorrhizal (AM) fungi from heavy metal polluted soils. *Plant and Soil* 157: 247–256.