

## HEAVY METAL CONTENT AND LOCALIZATION IN MYCORRHIZAL *EUPHORBIA CYPARISSIAS* FROM ZINC WASTES IN SOUTHERN POLAND

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(Received: January 30, 1998. Accepted: February 20, 1998)

### ABSTRACT

Arbuscular mycorrhiza (AM) development, heavy metal uptake by the plant and localization of heavy metals within plant and AM fungal structures, has been investigated in *Euphorbia cyparissias* collected from zinc wastes in Poland and from two other stands differing in soil parameters. The plant was selected on the basis of preliminary research on mycorrhiza of plants colonizing zinc wastes. *E. cyparissias* was strongly mycorrhizal and arbuscules were abundant except at the very beginning of the vegetation period. Light microscopy after rhodizonate staining and SEM equipped with an EDS system have been used to localize heavy metals within the fungal mycelium and mycorrhizal roots. About 80% of the total intraradical mycelium show an increased content of heavy metals in comparison to the remaining 20% where heavy metals were under detection limit. At the same time the number of arbuscules formed by mycelium stained by rhodizonate is slightly lower than in mycelium containing a low level of these elements. Using the rhodizonate staining lead was found in epidermal cell walls, root hairs, in mycorrhizal mycelium and in crystalloids deposited within latex and inside cortical cells, around fungal hyphae. SEM observations with EDS system confirmed that these crystalloid depositions contained higher concentrations of Zn than root cell wall and fungal structures.

**KEY WORDS:** arbuscular mycorrhiza, element uptake, *Euphorbia cyparissias* L., heavy metals, industrial wastes.

### INTRODUCTION

Metal bearing industrial wastes pose a serious environmental threat. The zinc industry, which is especially active in southern region of Poland, produces tons of wastes every year which are often deposited close to areas inhabited by people. The wastes are usually a very poor plant-growth medium. It is devoid of adequate levels of organic matter, nitrogen and phosphorus, as well as having a very low water-holding capacity. Due to high wind erosion of the substratum there is an urgent need for plant introduction into such areas. Though mycorrhizas are known as a potentially important agent in the improvement of plant growth and in heavy metal detoxification, available data on the mycorrhizal status of plants appearing in highly polluted areas are conflicting (Leyval et al. 1997). In some cases the delay, reduction or even elimination of mycorrhizal colonization has been found (Gildon and Tinker 1983; Graham et al. 1986; McGee 1987; Koomen et al. 1990; Chao and Wang 1991; Leyval et al. 1991) while in others, high levels (sometimes even greater than in nonpolluted areas) of mycorrhizal colonization were observed (Weissenhorn et al. 1995 a, b; Turnau et al. 1996). The lack of information on the bioavailability of heavy metals has been suggested as the main impediment to comparing the data (Leyval et al. 1997).

The effect of arbuscular mycorrhizal symbiosis on heavy metal uptake by plants has been studied in laboratory and natural conditions. Some reports show a higher uptake of metals by mycorrhizal plants at high metal concentrations of the soil (Gildon and Tinker 1983; Killham and Firestone 1983; Weissenhorn and Leyval 1995), while others show a decreased heavy metal content attributed to mycorrhizal symbiosis (Schüepp et al. 1987; El-Kherbawy et al. 1989; Weissenhorn et al. 1995 a). In most of these studies, however, usually only the percentage of root colonization by mycorrhizal fungi is evaluated. Not much attention has been paid to the functional aspect of mycorrhizas, vitality, arbuscule development, nor is much known about the response of plants to fungal colonization in stressed conditions.

Investigation of revegetation processes on 25-year-old zinc wastes in Chrzanow near Cracow has shown that both non-mycorrhizal and mycorrhizal plants appear there. Within the second group, an important distinction was noted. Mycorrhizal development was much better (higher root colonization and much more abundant arbuscule formation) in case of *E. cyparissias*, which appeared naturally on zinc wastes, than in plant species as *Hippophae rhamnoides* and *Arrhenatherum elatius* which were artificially introduced as part of a program of recultivation. In the case of the last two species, which roots were analyzed monthly during two seasons, fungal

colonization occasionally reached up to 50% of the root system, but the arbuscule formation was an exceptional case (unpublished data). Because mycorrhizal colonization of *E. cyparissias* roots was much higher than *H. rhamnoides* and *A. elatius* roots, it was selected for research devoted to understanding fungal and plant strategies on zinc wastes. For comparison, its mycorrhizal status was analyzed at two other sites differing in environmental parameters.

Localization and estimation of heavy metal content in mycorrhizal roots of *E. cyparissias* were the main aims of this paper. Two complementary methods were used: electron microscopy accompanied by energy dispersion system (localizing elements on the ultrastructural level) and conventional atomic absorption spectroscopy.

The rhodizonate staining, a cytochemical indicator of lead at light microscopy level, was used here for the first time in mycorrhizal research. The main reason to employ this method was to select material for further research with light and electron microscope. The test has been applied before to localize lead in *Stigeoclonium tenue* (Silverberg 1975) in *Cladophora* (Theiss 1987) in lichens (Garty and Theiss 1990) and also in studies on lead translocation and localization in *Alium cepa* roots (Wierzbicka 1987 a, b). The test relies on the specific reaction of sodium rhodizonate with lead ions at pH 2.8 resulting in precipitation of a scarlet lead rhodizonates ( $2 \text{PbC}_6\text{O}_6 \cdot \text{Pb}(\text{OH})_2 \cdot \text{H}_2\text{O}$ ) (Feigl 1954, Glatzer and Hernandez 1972). With an increase in pH, rhodizonate forms a violet complex but at the same time the specificity for lead decreases. At pH over 5 also Zn and Cd salts give similar reaction while the change of colour in presence of Cu was noticed only at pH over 10. Presence of any other elements so far tested did not lead to formation of scarlet precipitation.

## MATERIALS AND METHODS

The investigations were carried out on 25-year-old industrial wastes located in Chrzanow (southern Poland, 30 km west of Cracow). The solid fraction of the wastes from the Trzebieńka zinc factory is deposited as sedimentation tanks

(Lekan and Piotrowska 1988). *E. cyparissias* was collected 25 years later from slopes of the sedimentation tanks where the mean pH value in  $\text{H}_2\text{O}$  was 7.4. (Table 2). For comparison, two other locations (stand A – Kostrze and stand B – Wierzbanowka Valley; both in close vicinity to Cracow) of the species were selected (Table 1, 2). Total and extractable (in 1 N HCl and 0.1 N  $\text{Ca}(\text{NO}_3)_2$ ) elements in soils were estimated with atomic absorption spectrometry (Nowosielski 1968; Weissenhorn et al. 1995 b), N by the Kjeldahl method and organic matter by the Tiurin method. The shoots of *E. cyparissias* were collected every two weeks during 1995 and once a month during 1996. A part of the material was washed, dried, ground, and following mineralization (Grodzinska 1978), the element content of the plants was estimated using AAS (spectrophotometer Phillips PU-9100x). In case of roots and rhizomes only one composite sample for each stand was analyzed after careful rinsing.

For estimation of mycorrhizal colonization, the roots were prepared according to the modified method of Philips and Hayman (1970). *E. cyparissias* roots, following careful washing with tap water, were softened using 7% KOH in water bath at 60°C, washed again with water, bleached in  $\text{H}_2\text{O}_2$  containing  $\text{NH}_3$  (10:1) for a few minutes, washed again in water, acidified in 5% lactic acid in water for 12-24 h, stained with 0.01% cotton blue in lactic acid (either for 1 h at 60°C or for 24 h at room temperature) and finally stored in lactic acid. Mycorrhizal frequency (F%), mycorrhizal root length (M%) and arbuscular richness (A%) were assessed (Trouvelot et al. 1986). Analysis of variance (ANOVA,  $\alpha = 0,05$ ) and an *a posteriori* Duncan's test were used to compare data, after arcsin transformation.

Soil samples were also taken from the same locations and the spores of Glomales were selected by wet sieving and centrifugation in a sucrose gradient (Tommerup 1992). Spores were examined and morphotypes were isolated using a stereomicroscope. No further attempt were made to identify the species. A few spores of each morphotype were mounted in PVLG and some were used for a rhodizonate test, described below, or for analysis by SEM equipped with EDS.

TABLE 1. Total and extractable (in 1N HCl and in  $\text{Ca}(\text{NO}_3)_2$ ) heavy metal content in zinc wastes, stand A and B as analyzed from samples collected underneath *E. cyparissias*.

	Zn			Pb			Cd			Cu		
	total	extr. in HCl	extr. in $\text{Ca}(\text{NO}_3)_2$	total	extr. in HCl	extr. in $\text{Ca}(\text{NO}_3)_2$	total	extr. in HCl	extr. in $\text{Ca}(\text{NO}_3)_2$	total	extr. in HCl	extr. in $\text{Ca}(\text{NO}_3)_2$
zinc waste	10409.1	4223.0	13.95	1666.7	1093.7	0.3	23.7	19.3	0.43	31.1	26.2	0.08
stand A	7.1	6.1	0.35	22.6	19.7	0.1	0.94	0.92	0.03	7.1	6.1	0.065
stand B	11.5	6.9	8.2	30.3	13.8	0.0	1.84	0.59	0.36	11.5	6.9	0.225

TABLE 2. Soil chemical characteristics of zinc wastes, stand A and B.

	type of soil	organic matter content [%]	N [%]	$\text{P}_2\text{O}_5$ [ $\text{mg kg}^{-1}$ ]	$\text{K}_2\text{O}$ [ $\text{mg kg}^{-1}$ ]	CaO [ $\text{mg kg}^{-1}$ ]	pH in $\text{H}_2\text{O}$
zinc waste	sandy soil	1.12	0.044	74	69	2509	7.4
stand A	sandy soil	4.99	0.301	134	137	4960	7.2
stand B	silty soil	3.50	0.151	45	135	1053	4.6

The procedure for the rhodizonate test modified after Garty and Theiss (1990) was as follows: 50 mg of the rhodizonian was dissolved in 25 ml of a buffer (pH 2.8) containing 1.5 g of tartaric acid and 1.9 g of sodium bitartrate in 100 ml of distilled water. These roots, washed in water, often sectioned longitudinally, were soaked in this solution for 5 hours, transferred into a buffer as described above but without rhodizonate and observed under a stereomicroscope. Roots were selected according to intensity of reaction and 1cm-long root pieces were placed in lactic acid on a slide, covered and observed under a light microscope.

In addition, roots selected under the stereomicroscope according to their reaction to rhodizonate were stained with cotton blue and analysed according to Trouvelot et al. (1986).

SEM Jeol JSM 5410 with an energy dispersion system (EDS) (NORAN system) was used for analysis of spores (crushed and dried) and roots after standard preparation (fixed for 1h in 3% glutaraldehyde in 0.2 M Hepes buffer, dehydrated, critical-point dried and coated with carbon) and longitudinal sectioning. The samples were fixed on carbon stubs and covered with carbon prior to analysis. At least ten analyses were carried out for each kind of plant and fungal structure described.

TEM 902 Zeiss observations were carried out on samples prepared as described by Turnau et al (1993).

## RESULTS

### Heavy metal content of *E. cyparissias*

Despite a high heavy-metal content of zinc wastes, the concentration of potentially toxic elements in *E. cyparissias* shoots was rather low (Fig. 1). In comparison to the heavy metal content in shoots of specimens collected from stand A and B (natural stands) only Pb concentration had increased significantly. On the contrary, in shoots collected from stand B, where the pH value was much lower, Cu, Zn and Cd values were even higher than in shoots from the zinc wastes. No significant differences were found in the As content of all samples analyzed. Though there were important differences in the P, N and Ca contents of the analyzed stands no significant differences were found among these element contents in shoots. The heavy metal concentrations in roots from zinc wastes were 75  $\mu\text{g g}^{-1}$  for Zn, 20  $\mu\text{g g}^{-1}$  for Pb, 2  $\mu\text{g g}^{-1}$  for Cd, 6.5  $\mu\text{g g}^{-1}$  for Cu, in roots from stand A: 67  $\mu\text{g g}^{-1}$  for Zn, 12  $\mu\text{g g}^{-1}$  for Pb, 2  $\mu\text{g g}^{-1}$  for Cd and 4.5  $\mu\text{g g}^{-1}$  for Cu and from stand B: 50  $\mu\text{g g}^{-1}$  for Zn, 3  $\mu\text{g g}^{-1}$  for Pb, 0.1  $\mu\text{g g}^{-1}$  for Cd and 2.5  $\mu\text{g g}^{-1}$  for Cu. Roots from area of low pH contained 2 to 3 times less of Pb and Cu, 10 times less Cd and similar level of Zn than in roots from zinc wastes. On the contrary, Ca level in roots from stand B was nearly four times lower (7 150  $\text{g g}^{-1}$ ). The rhizomes from zinc wastes accumulated higher heavy metal concentrations up to 110  $\text{g g}^{-1}$  of Pb and 300  $\text{g g}^{-1}$  of Cu.

### Mycorrhizal status of *E. cyparissias* from zinc wastes and comparative areas

Mycorrhizal colonization seemed to be correlated more to the pH level than to the heavy-metal content (Fig. 2). No significant differences in F, M and A % were found between roots from zinc wastes and stand A, both characterized by a pH value of around 7. At the same time, mean values of F, M and A estimated in roots from stand B (pH 4.5) were at least

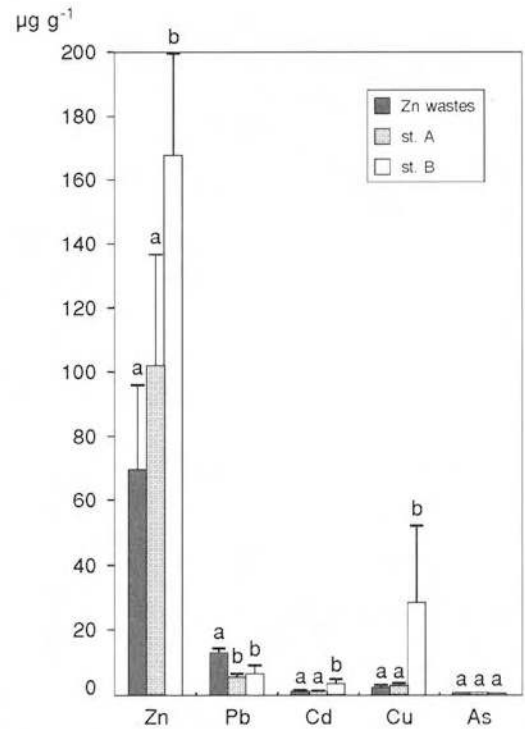


Fig. 1. Metal concentrations ( $\mu\text{g g}^{-1}$  d. wt) in *Euphorbia cyparissias* shoots collected from zinc wastes, stand A (Kostrze) and stand B (Wierzbanowka Valley). Means  $\pm$  SE (n = 5). Different letters above columns indicate a significant difference at  $p < 0.05$ .

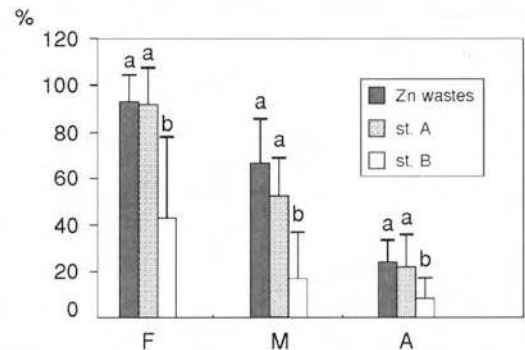
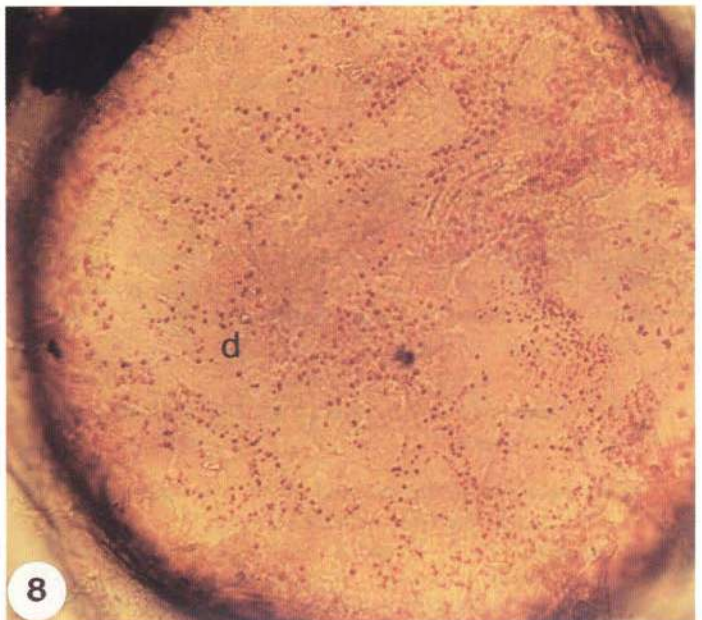
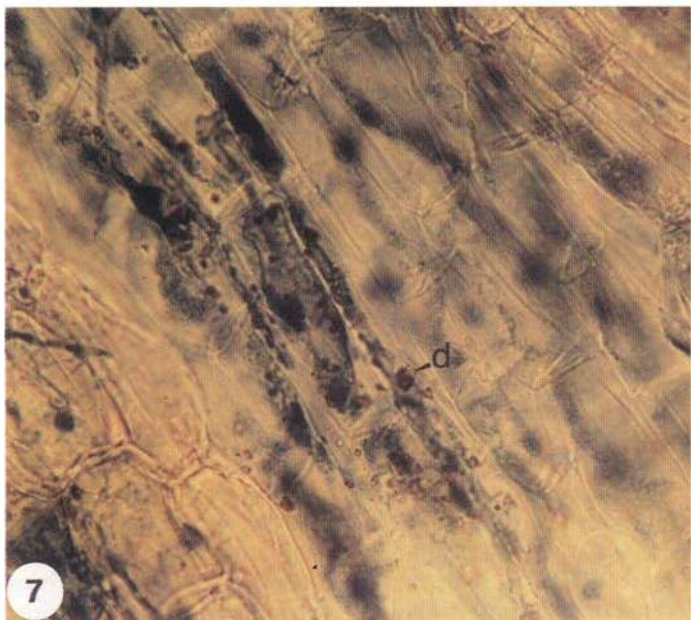
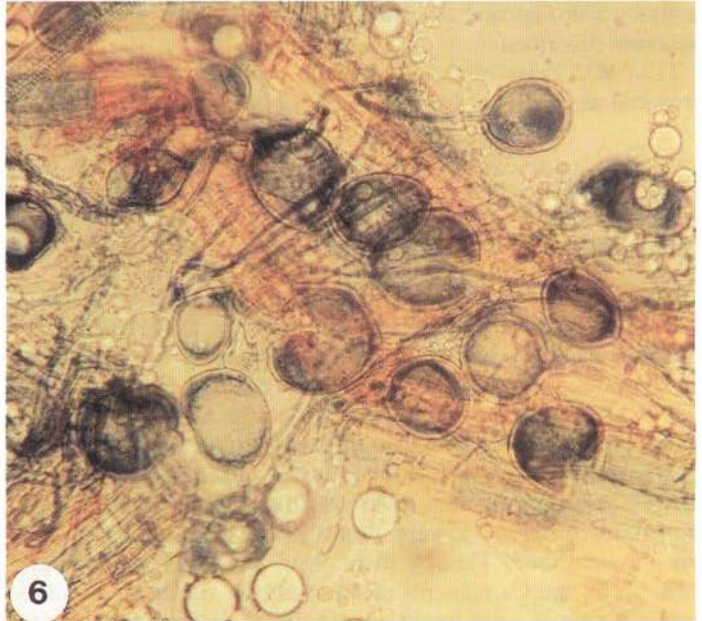
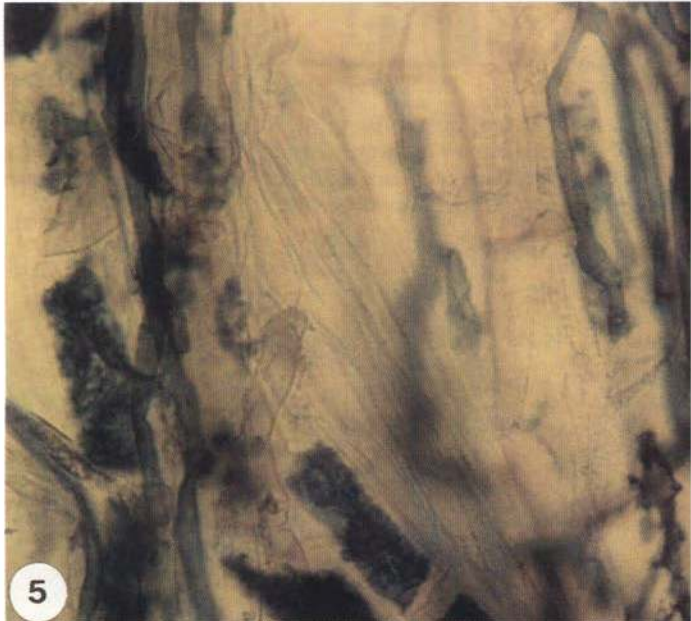
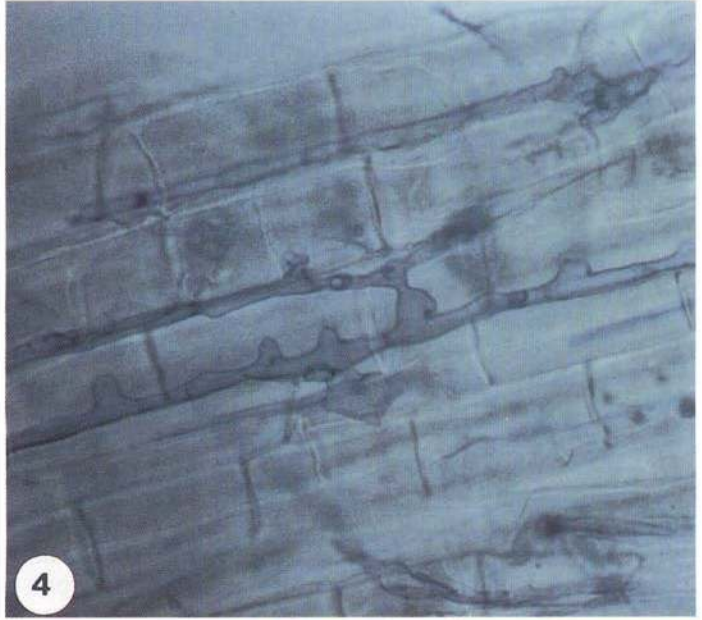
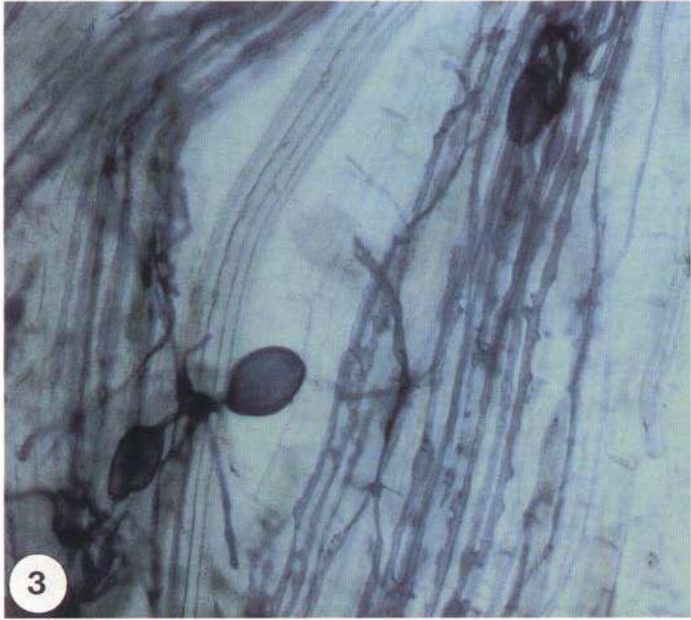


Fig. 2. Arbuscular mycorrhizal colonization of *Euphorbia cyparissias* collected from zinc wastes, stand A (Kostrze) and stand B (Wierzbanowka Valley). Frequency of infected root segments (F%), total mycorrhizal intensity (M%) and relative arbuscular richness in whole root system (A%). Means  $\pm$  SE (n = 5). Different letters above columns indicate a significant difference at  $p < 0.05$ .

two times lower. In the latter case, a high frequency of parasitic fungi and nematodes (eggs) within the roots was observed. On the basis of the above described data, stand A was selected as a control site for further work on the influence of pollution on mycorrhizal colonization. At the beginning of the vegetation season, parallelly growing hyphae (Fig. 3) without arbuscule formation or with so-called "aborted arbuscules" were abundant within the roots of *E. cyparissias* from zinc wastes (Fig. 4). Most of these roots, while observed with TEM, appeared to be dead though the hyphae were clearly vital (not shown). Later in the vegetation season this phenomenon was not observed. Abundant arbuscules were locally present (Fig. 5), sometimes with poorly-developed, fine branching or sometimes with a net- rather than a tree-like structure. The analysis of root material from unpolluted stand A has



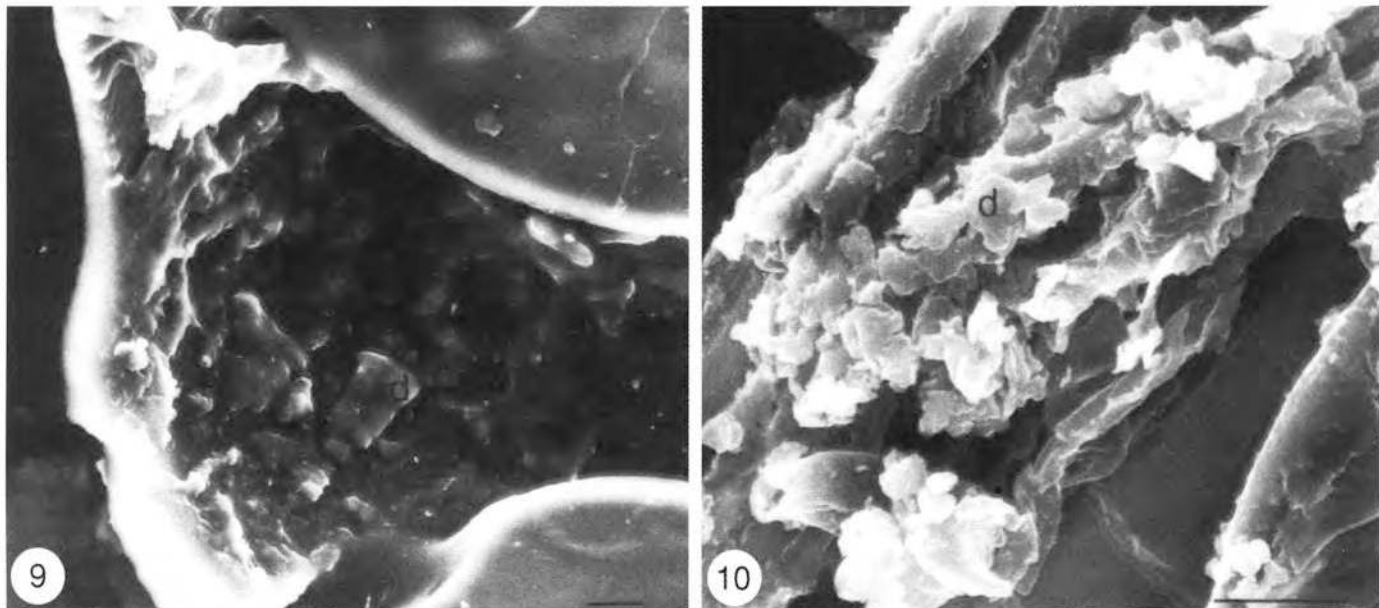


Fig. 9. SEM micrographs of the inner layer of a spore wall with small depositions (d) characterized by a high content of potentially toxic elements; bar = 10  $\mu$ m.

Fig. 10. SEM micrograph of a longitudinal section through a *Euphorbia cyparissias* root showing abundant depositions (d) around the hyphae; heavy metals have been detected in these depositions; bar = 10  $\mu$ m.

shown typical arbuscules, with well-developed, fine branching, no cases of mycelium growth completely devoid of arbuscule formation, as described from the zinc wastes.

#### Heavy metal localization according to the rhodizonate test

The rhodizonate staining within *E. cyparissias* roots from zinc wastes is much higher than in roots from control sites. The change of colour was visible in the cell walls of the epidermis, including the walls of root hairs. In up to 80% of roots, the intraradical mycelium was also stained. The stained intracellular mycelium was usually accompanied by distinctly stained depositions (crystaloids) localized within the plant cells (Fig. 7, 10). They were especially common around stained mycelium, often appearing as crystalline crust over fungal hyphae. In the remaining 20% of the roots no reaction of the mycelium to rhodizonate was observed despite of similar abundance of mycorrhiza (M%). The depositions were only occasionally observed and uniformly distributed. Similar depositions were localized within lactifers, within root hairs and to a lower extent within cortical and epidermal cells devoid of mycelium. The roots were divided into two classes according to intensity of rhodizonate staining. After clearing them with KOH and staining with cotton blue, F, M and A% were estimated. This method revealed no significant differences in F and M but showed clearly that in roots where the rhodizonate staining was poor, arbuscule development is more abundant than in stained ones (Fig. 11).

#### Heavy metal localization according to the energy dispersion system connected to SEM

Roots stained with rhodizonate or unstained (for comparison) from the zinc waste were observed with SEM equipped with an EDS system (Figs 12, 13, Table 3). In the epidermis cell walls a maximum 5% of heavy metals (Cu – up to 1.4%, Pb – 1.5% and Zn – 1.5% dry wt) was found. As in all biological samples, C and O were the main components of the cell walls. In addition Ca (3.8-5.0% wt), S (0.5-0.6%) and P (0.1-0.5%) were found. Much lower levels of heavy metals were found in the cell walls of root hairs (up to 2%).

Despite large differences in the heavy metal content of fungal cell walls, in mycelium in which heavy metals were found, the levels of Cu and Pb were significantly lower than in epidermal cell walls, while the Zn level was similar. On the surface of the intraradical mycelium, amorphous or crystalloid depositions were distinguished. In the amorphous ones a high level of S, P and As was found, while in crystalloids more Ca and Fe and slightly less As were detected. In both of them Si, Pb, Cu and Zn were more or less on a similar level (Tab. 3, Fig. 13). The depositions were characterized by a significantly higher level of Zn, Fe and Al than the epidermal cell wall, the fungal mycelium wall or any other structures analyzed. They were also very rich in Ca and Si.

Analysis of unstained roots has shown the presence of heavy metal only in outer cell wall of epidermis and in root hairs. The heavy metals were under detection limit within walls of fungal hyphae.

Fig. 3-4. Mycorrhizal mycelium growing parallelly within *Euphorbia cyparissias* roots, forming only "aborted arbuscules" – a common case at the beginning of the vegetation period on zinc wastes; magn.: 3 – 160x, 4 – 400x.

Fig. 5. Arbuscules within a *E. cyparissias* cortical cell from zinc wastes; magn. 400x.

Fig. 6. Spores of *Glomus sp.* formed intraradically; orange staining of cortical walls results from a positive reaction to the rhodizonate test, which is weak or negative in spore walls; magn. 160x.

Fig. 7. Depositions (d) formed within plant cells close to the fungal hyphae, giving a positive reaction to the rhodizonate test; magn. 400x.

Fig. 8. Depositions (d) stained by rhodizonate localized within spores of *Glomus sp.* isolated from nearby *E. cyparissias* roots in zinc wastes; magn. 400x.

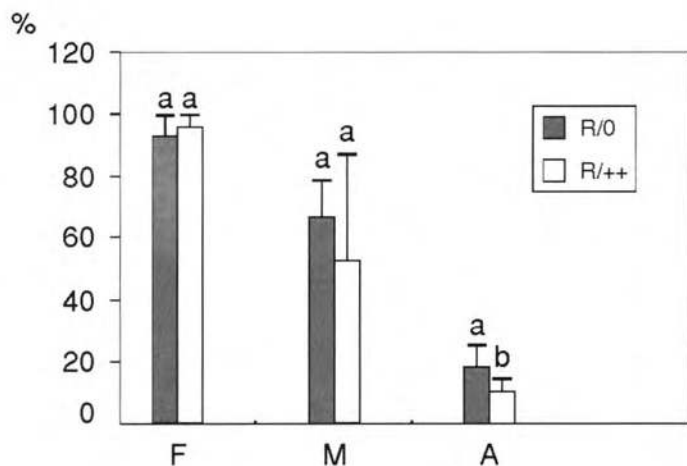


Fig. 11. Comparison of arbuscular mycorrhizal infection in *Euphorbia cyparissias* roots, collected from zinc wastes, showing a positive (R/++) or negative (R/0) reaction to rhodizonate test. The frequency of infected root segments (F%), total mycorrhizal intensity (M%), and relative arbuscular richness in the whole root system analyzed (A%) are given. Means  $\pm$  SE (n = 25). Different letters above columns indicate a significant difference at  $p < 0.05$ .

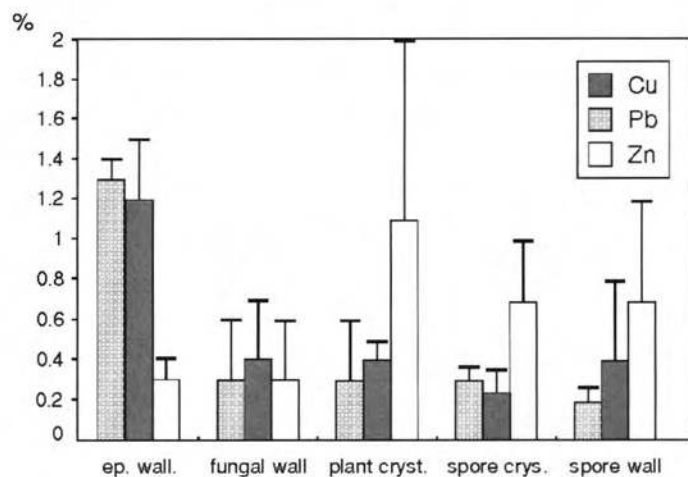


Fig. 12. Copper, lead and zinc content of epidermal cell wall (ep. wall), surface layer of the intraradical mycelium, plant crystaloids deposited around the mycelium (plant cryst.) spore depositions (spore cryst.) and spore walls as estimated by EDS analysis; mean values in % dry weight  $\pm$  SE (n = 10).

#### Heavy metal localization within glomalean spores

Further differences between the zinc wastes and control sites concerned the presence of a high number of intraradical spores within the roots from industrial wastes (Fig. 6). Six spore morphotypes were isolated from the zinc waste substratum surrounding the roots of *E. cyparissias*. They were much less frequent than spores within roots. In some cases, extraradical spores were found within dead spores of a different morphotype or within unidentified plant debris.

The rhodizonate test used for spores selected from zinc wastes revealed differences between morphotypes. Intraradical spores were usually devoid of heavy metals. Among the spores formed outside roots, two groups of morphotypes were distinguished. The first one (three morphotypes) was characterized by uniform, positive rhodizonate staining of the spore wall. The spore walls of the second group (two morphotypes) gave no reaction to rhodizonate and zero or low levels of heavy metals were found using a EDS analyzer. Instead, small depositions containing heavy metals were found on the inner surface of the spore wall (Figs 8-9). Pb and Cu had similar levels there as in plant crystaloids and in fungal cell walls, and the Zn content was only a bit lower than in plant crystaloids. Ca, Si and Al were also found in the spore depositions.

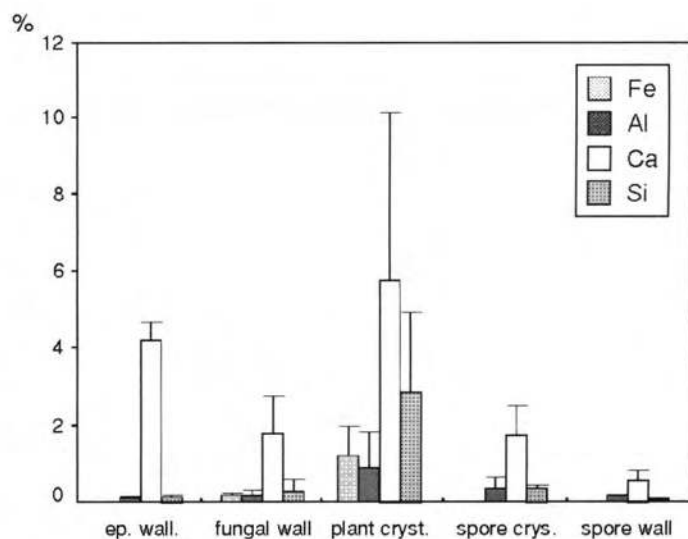


Fig. 13. Ferrous, aluminium, copper and silicon content of epidermal cell wall (ep. wall), surface layer of the intraradical mycelium, plant crystaloids deposited around the mycelium (plant cryst.) spore depositions (spore cryst.) and spore walls as estimated by EDS analysis; mean values in % dry weight  $\pm$  SE (n = 10).

TABLE 3. Element content in the epidermal cell wall (ep. wall), surface layer of the intraradical mycelium, plant crystaloids deposited around mycelium (plant cryst.) spore depositions (spore cryst.) and spore walls as estimated by EDS analysis; mean values in % dry weight ( $\pm$  SD; n = 10) different letters beside indicate a significant difference at  $p < 0.05$ .

	Cu	Pb	Zn	Ni	Fe	Al	Ca	Si
ep. wall	1.3 a	1.2 a	0.3 a	0 a	0 a	0.1 a	4.2 a	0.1 a
fungal wall	0.3 b	0.4 b	0.3 a	0 a	0.2 b	0.2 a	1.8 b	0.3 a
plant cryst.	0.3 b	0.4 b	1.1 b	0 a	1.2 c	0.9 b	5.8 a	2.9 b
spore cryst.	0.3 b	0.2 b	0.7 a	0.2 b	0 a	0.4 a	1.8 b	0.4 a
spore wall	0.2 b	0.4 b	0.6 a	0 a	0 a	0.1 a	0.6 c	0.1 a

The depositions or staining of cell walls were never observed within spores collected from control plots.

## DISCUSSION

*E. cyparissias* is a typical stress-tolerant plant, long-lived, spreading extensively by vegetative propagation and having a low maximum growth rate. Adapted for long-term survival, but with a poorly-developed root system, it apparently benefits from AM, by which it maximises the capture of water and mineral nutrients (Grime 1977). Mycorrhiza development however may lead to an increase or decrease in heavy metal uptake by the plant (see review by Leyval et al 1997). Metal bioavailability is a function not of only their total concentration, but also of such factors as pH, organic matter and biological factors (Berthelin et al. 1995). Despite the higher heavy-metal content on the zinc wastes Zn and Cd uptake by plant was lower than in stand B where the heavy metal concentration was very low. This can be attributed to the high availability of the metals, as shown by  $\text{Ca}(\text{NO}_3)_2$  extraction, in stand B due to lower pH and CaO content. In comparison to zinc wastes soil of stand B was characterized by higher Cu availability and nearly equal content of Cd. High pollution of this stand could be explained by the influence of aluminium smelter located nearby, operating for thirty years and emitting fluorine compounds into the air. The activity of this smelter resulted in strong decrease of soil pH and Ca content (Table 1). In such cases not only the toxicity of heavy metals is increased due to low pH but also the lack of Ca which is the antagonist towards heavy metals as Cd, Zn, Pb and Cu could be responsible for the situation (Kabata-Pendias and Pendias 1993).

In the zinc waste, although the total heavy metal concentrations were very high the availability was very low. However it was still higher than in stand A and it was of the same order of magnitude as in polluted soils studied by Weissenhorn et al. (1993, 1995 c). When comparing the heavy-metal content of plants and mycorrhizal development one must also remember that particularly at differing pH values and organic matter content of the substratum, not only the bioavailability of metals differ but the mycorrhizal colonization or functioning could also be altered. At minimum arbuscule richness if not vitality should be evaluated in the samples compared. In case of *E. cyparissias* growing on the zinc wastes, arbuscule modifications were observed, sometimes resulting in so-called "aborted" arbuscules or in weakly branched arbuscules. Preliminary observations on vitality of *E. cyparissias* roots suggest that these kind of structures are mainly formed within dead roots (unpublished results). Weak development of arbuscules and a slight decrease in arbuscule richness which was found in *E. cyparissias* exhibiting positively rhodizonate stained mycelium, might be a sign of mycelium disfunction or a result of plant influence (inhibition) on the fungus, but may also result from differences between species or strains regarding their ability to form arbuscules.

Differences in heavy metal content (shown by rhodizonate test and confirmed by EDS method) between mycorrhizal mycelia of *E. cyparissias* roots were probably due to different ability of fungal strains or species to sequester heavy metals within cell walls. Around intracellular mycelium characterized by the presence of heavy metals in hyphal walls, within the plant cytoplasm, depositions containing high levels of potentially toxic elements were found. Similar deposition were found within latices. The latex of some members of Euphorbiaceae

has previously been shown (Baier et al. 1992; Baier and Heinrich 1993) to contain Ca, K, Mg, Na and also is rich in several organic acids which could potentially bind heavy metals (Murphy and Levy 1983; Sieghardt 1985; Macaskie and Dean 1990). As the production of latex is now considered a detoxification mechanism (Baier and Heinrich 1993) it was not surprising that in *E. cyparissias* from zinc wastes heavy metals were also found in the latex. The depositions within cortical cells and within latex could function as a filter for elements transferred from the wastes and might play similar role as a barrier in a form of epidermis walls chelating heavy metals (Wierzbicka 1987b).

The ability of a plant symbiont to detoxify heavy metals transferred via the fungus has been shown in the case of *Pteridium aquilinum* (Turnau et al. 1993). This is an extremely resistant species which was one of the few ones able to colonize forest experimental plots treated with high level of cadmium and zinc dust in Poland (Turnau et al. 1993). When the mycorrhizal root sections were observed in TEM equipped with EELS/ESI the heavy-metal content was higher within the fungal cytoplasm than in the plant cytoplasm.

Also mycorrhizal fungi of *E. cyparissias* from zinc wastes have shown several strategies against heavy metals. The spores were more abundant within the roots, and because of this heavy metal access was diminished. The mycelium germinating from such spores was often growing intraradically towards freshly formed roots, using dead roots as tunnels of much lower toxicity. This phenomenon was most clearly visible at the beginning of the vegetation period. In spores formed in the soil, the cell wall or the depositions on the inner layer of the wall probably served as deposition sites for potentially toxic elements.

The accuracy of the rhodizonate test was confirmed in the present work by the EDS analyzer. In the future, the use of this test could lower the costs of SEM and EDS system application, as it is a good method for selecting material from polluted sites. The comparative investigation of rhodizonate treated and untreated material did not show any significant differences in the localization of elements. A comparison between chemically-fixed and dried material has shown that during fixation the level of some elements either decreases or increases. However, there was no significant differences in case of heavy metals. Though drying the samples is sufficient for analyzing cell walls of the epidermis, mycelium growing on the root surface or outer spore walls, visualization and recognition of plant or fungal crystalloids was much easier in fixed material.

## ACKNOWLEDGEMENTS

Special thanks are due to Dr Corinne Leyval (Centre de Pédologie Biologique, Nancy, France) and to Professor Paola Bonfante (Universita di Torino, Italy) for advice and critical reading of the manuscript. The author also wishes to thank Ms. Ewa Slusarczyk for the help in collecting material and slide preparation, Ms. Jadwiga Faber for excellent technical assistance with EDS analysis carried out at the Department of Zoology of Jagiellonian University (Electron Microscopy Unit) and to Dr Barbara Godzik (Polish Academy of Sciences, Krakow, Poland) for the help in estimation of heavy metal availability. The SEM and EDS equipment is the donation of the Foundation for Polish Science (SUBIN 94). The research was supported by the Committee of Scientific Research in Poland, grant no. PB 0860/P04/96/11.

## LITERATURE CITED

- BAIER W. R., HEINRICH G., 1993. Gas-liquid chromatographic analyses of the water-soluble compounds of plant latices. *Phyton* 33 (1): 77-85.
- BAIER W. R., KOSMUS W., HEINRICH G., 1992. Sodium, magnesium, potassium and calcium in plant latices. *Phyton* 31: 227-232.
- BERTHELIN J., MUNIER-LAMY C., LEYVAL C., 1995. Effect of microorganisms on mobility of heavy metals in soils. In: Huang PM, Berthelin J, Bollag JM, McGill WB, Page AL (eds) *Environmental Impacts of Soil Component Interactions: Vol. 2 – Metals, Other Inorganics and Microbial Activities*. II. Lewis Publishers, Boca Raton, pp. 3-17.
- CHAO C. C., WANG Y. P., 1991. Effects of heavy metals on vesicular-arbuscular mycorrhizae and nitrogen fixation of soybean in major soil groups of Taiwan. *J Chin Agric Chem* 29: 290-300.
- EL KHERBAWY M., ANGLE J. S., HEGGO A., CHANEY R. L., 1989. Soil pH, rhizobia and vesicular-arbuscular mycorrhizae inoculation effects on growth and heavy metal uptake of alfalfa (*Medicago sativa* L.). *Biol Fertil Soils* 8: 61-65.
- FEIGL F., 1954. *Spot Tests, V. 1. Inorganic Applications*. Elsevier Publ. Co. Amsterdam.
- GARTY J., THEISS H. B., 1990. The localization of lead in the lichen *Ramalina duriaei* (De Not.) Bagl. *Bot. Acta* 103: 311-314.
- GILDON A., TINKER P. B., 1983. Interactions of vesicular-arbuscular mycorrhizal infection and heavy metals in plants. 1. The effects of heavy metals on the development of vesicular-arbuscular mycorrhizas. *New Phytol* 95: 247-261.
- GLATEER F. A. B., HERNANDEZ L., 1972. Lead detection in living plant tissue using a new histochemical method. *J Air Pollut Control Assoc* 22: 463-467.
- GRAHAM J. H., TIMMER L. W., FARDELMANN D., 1986. Toxicity of fungicidal copper in soil to *Citrus* seedlings and vesicular-arbuscular mycorrhizal fungi. *Phytopathol* 76: 66-70.
- GRODZIŃSKA K., 1978. Mosses as bioindicators of heavy metal pollution in Polish national parks. *Water, Air and Soil Pollution* 9: 83-97.
- GRIME J. P., 1977. Evidence for the existence of three primary strategies in plants and its relevance to ecological and evolutionary theory. *An Nat* 111: 1169-1194.
- KABATA-PENDIAS A., PENDIAS H., 1993. *Biogeochemia pierwiastków śladowych*. PWN, Warszawa.
- KILLHAM K., FIRESTONE M. K., 1983. Vesicular arbuscular mycorrhizal mediation of grass response to acidic and heavy metal depositions. *Plant Soil* 72: 39-48.
- KOOMEN I., MCGRATH S. P., GILLER K., 1990. Mycorrhizal infection of clover is delayed in soils contaminated with heavy metals from past sewage sludge applications. *Soil Biol Biochem* 22: 871-873.
- LEKAN S., PIOTROWSKA M., 1988. Ocena stanu środowiska rolniczego wokół osadników odpadów poflotacyjnych i Zakładu Górniczego "Szyb Włodzimierz", Raport no. 413-34/87.
- LEYVAL C., BERTHELIN J., SCHONTZ D., WEISSENHORN I., MOREL J. L., 1991. Influence of endomycorrhizas on maize uptake of Pb, Cu and Cd applied as mineral salts or sewage sludge. In: J. G. Farmer (ed.), *Heavy metals in the environment*. CEP Consultants, Ltd., Edinburgh, pp. 204-207.
- LEYVAL C., TURNAU K., HASELWANDTER K., 1997. Effect of heavy metal pollution on mycorrhizal colonization and function: physiological, ecological and applied aspects. *Mycorrhiza* (in press).
- MACASKIE L. E., DEAN A. C. R., 1990. Metal-Sequestering biochemicals. In: B Volesky (ed.), *Biosorption of heavy metals*. CRC Press, Boca Raton, pp. 199-248.
- MC GEE P. A., 1987. Alteration of growth of *Solanum opacum* and *Plantago drummondii* and inhibition of regrowth of hyphae of vesicular-arbuscular fungi from dried pieces by manganese. *Plant Soil* 101: 227-233.
- MURPHY R. J., LEVY J. F., 1983. Production of copper oxalate by some copper tolerant fungi. *Trans Br Mycol Soc* 81: 165-168.
- NOWOSIELSKI O., 1968. *Metody oznaczania potrzeb nawożenia*. PWRiL, Warszawa, pp. 1-668.
- PHILLIPS J., HAYMAN D. S., 1970. Improved procedures for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. *Trans Br. Mycol. Soc.* 55: 158-161.
- SCHÜEPP H., DEHN B., STICHER H., 1987. Interaktionen zwischen VA-mycorrhizen und schwermetallbelastungen. *Angew Bot* 61: 85-95.
- SIEGHARDT H., 1985. Zur Frage der Besiedelung schwermetallhaltiger Abraumbalden in Bleiberg/Karnten. Teil I: *Silene vulgaris* ssp. *galerosa* (Jord.) Marsden-Jones & Turill. *Carinthia* II 175: 27-45.
- SILVERBERG B. A., 1975. Ultrastructural localization of lead in *Stigeoclonium tenue* (Chlorophyceae, Ulotrichales) as demonstrated by cytochemical and X-ray microanalysis. *Phycologia* 14: 265-274.
- THEISS H. B., 1987. Histochemischer Nachweis von Blei in *Cladophora*. *Mikrokosmos* 76: 108-109.
- TOMMERUP I. C., 1992. Methods for the study of the population biology of vesicular-arbuscular mycorrhizal fungi. In: JR Norris, DJ Read & AK Varma (eds) *Methods in Microbiology*. Vol. 24. Techniques for the study of mycorrhiza. Academic Press, London, pp. 23-51.
- TROUVELOT A., KOUGH J. L., GIANINAZZI-PEARSON V., 1986. Mesure du taux de mycorrhization VA dun systeme racinaire. Recherche de methodes destination ayant une signification fonctionnelle. In: V. Gianinazzi-Pearson & S. Gianinazzi (eds) *Physiological and Genetical Aspects of Mycorrhizae*. INRA, Paris, pp. 217-221.
- TURNAU K., KOTTKE I., OBERWINKLER F., 1993. Element localization in mycorrhizal roots of *Pteridium aquilinum* (L.) Kuhn collected from experimental plots treated with cadmium dust. *New Phytol* 123: 313-324.
- TURNAU K., MISZALSKI Z., TROUVELOT A., BONFANTE P., GIANINAZZI S., 1996. *Oxalis acetosella* as a monitoring plant on highly polluted soils. In: C. Azcon-Aguilar, J. M. Barea (eds) *Mycorrhizas in integrated systems from genes to plant development*. Proceedings of the fourth European Symposium on Mycorrhizas., pp. 483-486.
- WEISSENHORN I., LEYVAL C., BERTHELIN J., 1993. Cd-tolerant arbuscular mycorrhizal (AM) fungi from heavy-metal polluted soils. *Plant Soil* 157: 247-256.
- WEISSENHORN I., LEYVAL C., 1995. Root colonization of maize by a Cd-sensitive and a Cd-tolerant *Glomus mossae* and cadmium uptake in sand culture. *Plant Soil* 175: 233-238.
- WEISSENHORN I., LEYVAL C., BELGY G., BERTHELIN J., 1995a. Arbuscular mycorrhizal contribution to heavy metal uptake by maize (*Zea mays* L.) in pot culture with contaminated soil. *Mycorrhiza* 5: 245-251.
- WEISSENHORN I., LEYVAL C., BERTHELIN J., 1995b. Bioavailability of heavy metals and abundance of arbuscular mycorrhiza in a soil polluted by atmospheric deposition from a smelter. *Biol. Fert Soils* 19: 22-28.
- WEISSENHORN I., MENCH M., LEYVAL C., 1995c. Bioavailability of heavy metals and arbuscular mycorrhiza in a sewage-sludge-amended sandy soil. *Soil Biol. Biochem.* 27: 287-296.
- WIERZBICKA M., 1987a. Lead translocation and localization in *Allium cepa* roots. *Can. J. Bot.* 65: 1851-1860.
- WIERZBICKA M., 1987b. Lead accumulation and its translocation barriers in roots of *Allium cepa* L. – autoradiographic and ultrastructural studies. *Plant, Cell and Environment* 10: 17-26.



ZAWARTOŚĆ METALI CIĘŻKICH ORAZ ICH LOKALIZACJA W MIKORYZACH  
*EUPHORBIA CYPARISSIAS* L. Z HAŁDY HUTY CYNKU W POLSCE POŁUDNIOWEJ

STRESZCZENIE

Rozwój mikoryzy arbuskularnej, zawartość metali ciężkich w roślinie oraz lokalizacja metali w komórkach *E. cyparissias* oraz w strzępkach grzybni przebadana została w materiale zebrany na hałdzie huty cynku w Chrzanowie oraz z dwóch innych stanowisk o różnej zawartości metali ciężkich w podłożu. Badania nad lokalizacją metali ciężkich prowadzone były wstępnie za pomocą mikroskopu świetlnego, przy zastosowaniu testu rodizonianowego. Uzyskane wyniki pozwoliły na selekcję materiału do badań przy zastosowaniu mikroskopii skaningowej i technikę EDS. Zastosowanie obu metod pozwoliło na stwierdzenie wyraźnych różnic w akumulacji metali ciężkich w ścianach grzybni. Około 80% grzybni wewnątrz korzeni cechowała wysoka zawartość metali ciężkich podczas gdy w pozostałej części nie stwierdzono ich występowania. Grzybnia pierwszego typu tworzyła nieco mniej arbuskul. Występowanie metali ciężkich stwierdzono również w ścianach komórkowych epidermy, we włosnikach oraz w krystaloidach zdeponowanych w laktiferach i komórkach korykalnych (w bezpośrednim sąsiedztwie grzybni mikoryzowej). Badania za pomocą EDS wykazały, że krystaloidy zawierały znacznie wyższe stężenia Zn aniżeli pozostałe struktury w obrębie badanych mikoryz.

SŁOWA KLUCZOWE: mikoryza arbuskularna, pobór pierwiastków, *Euphorbia cyparissias* L., metale ciężkie, hałdy przemysłowe.