HEAVY METAL BINDING PROPERTIES OF *PINUS SYLVESTRIS* MYCORRHIZAS FROM INDUSTRIAL WASTES

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ABSTRACT

Mycorrhizas of *Pinus sylvestris*, collected from zinc wastes in Poland and France were investigated using transmission electron microscope (TEM) and scanning electron microscope (SEM) equipped with energy dispersion spectroscopy (EDS) and electron energy loss spectroscopy (EELS). At both sites, mycorrhizas of *Hebeloma* were the most frequent, however, they were often characterised by a sparse or only locally developed fungal mantle. Mycorrhizas formed by suilloid fungi were much less frequent, and usually produced a clearly defined fungal mantle characterised by abundant formation of pigments and crystals covering the hyphae of the outer mantle. These two groups of mycorrhizas differed in their heavy metal binding properties. A biofiltering effect of Pb and Zn by the fungal mantle was observed only in the case of suilloid mycorrhizas, which represented up to 10% of the total number of mycorrhizas. No statistical differences between the mantle, the cortical cell walls and the vascular tissue were demonstrated in mycorrhizas formed by other fungi dominating on industrial wastes. In the case of *Hebeloma* and *Inocybe*, however, elements such as Cu and Cd were present in higher amounts in the extramatrical mycelium, whereas no or only low amounts of these elements were detected within fungal mantles, mainly in mycorrhizas from the French waste. Analysis of the root systems has shown relatively high percentage of nonmycorrhizal short roots, suggesting the inhibition of mycorrhiza formation or a decreased number of mycorrhizal propagules. The role of dead roots and mycorrhizas in biosorption and immobilization of heavy metals was discussed.

KEY WORDS: ectomycorrhizas, heavy metal distribution, biofiltering effect, industrial wastes, EDS, EELS microanalysis.

INTRODUCTION

Heavy industry produces considerable quantities of industrial wastes, which are open for revegetation processes. However, because of the low fertility and high toxicity of the substratum, plant colonisation of such places is slow. A phyto-microbial approach, including the matching of tolerant ecotypes of plants and microbes has great potential. Mycorrhizal fungi have been shown to have a positive effect on nutrient uptake, plant growth and fitness (Smith and Read 1997). Their presence may also reduce the toxic effects of heavy metals (reviewed by Leyval et al. 1997). The species composition of mycorrhizas has been shown to be affected by soil pollution (Rühling and Söderström 1990; Kowalski et al. 1989). However, some mycorrhizal species may be extremely resistant to heavy metals and may form fruitbodies even in forests treated with up to 5000 tons per square km of industrial dusts containing high concentrations of Cd, Pb, Zn and other heavy metals (Turnau and Kozłowska 1991). On the other hand, the correlation between relative numbers of fruitbodies and belowground ectomycorrhizal community structure is generally low (Arnolds 1991; Gardes and Bruns 1996; Dahlberg et al. 1997), thus estimation made on the basis of sporocarp production could be very risky.

Scots pine (*Pinus sylvestris* L.) is one of the plants introduced into industrial wastes. In some cases growth and survival of pine growing on strip mine soils with high levels of metals and extremely low pH values was improved by inoculation with *Pisolithus tinctorius* (Marx and Altman 1979; Berry 1982). In laboratory conditions, an increase of metal tolerance of the host seedlings by inoculation with mycorrhizal fungi has been shown in the case of *Paxillus involutus* and certain strains of *Suillus bovinus* and *S. lu-*

teus, while in other fungal strains/species no effect was found (Colpaert and Van Assche 1992a, 1993). Little is known about specific detoxification mechanisms in mycorrhizal fungi (Gadd 1993). The differences in response may be attributed to the different level of prevention of heavy metal entry into the mycorrhizas or the sequestration of potentially toxic elements within mycelium, thus restricting their transfer towards the plant cells (Godbold et al. 1998; Hartley et al. 1997). The ability of the fungal mantle to bind effectively heavy metals has been demonstrated in the case of suilloid fungi from Polish wastes, using microanalytical tools such as energy dispersion system (EDS), electron energy loss spectroscopy (EELS) (Turnau et al. 1996) and PIXE microanalysis (Turnau et al. 2001). Ectomycorrhizas can not be considered to be universal ameliorators of metal toxicity in trees (Godbold et al. 1998). Data demonstrating the lack of biofiltering effect, obtained by similar techniques, are usually considered unsatisfactory and often remain unpublished, or the comparison between the morphotypes is impossible due to the use of different methods. The main aims of the present research were to characterise the most common mycorrhizal morphotypes on Polish industrial wastes and to evaluate their role in heavy metal binding properties of fungal mantles using the same microanalytical methods and similar conditions. As the sensitivity of microanalytical techniques allowing for metal detection on the micro scale is above the level of heavy metal content within mycorrhizas from nonpolluted places, the comparison between the same mycorrhizas from polluted and nonpolluted places was not possible. The mycorrhizas from Polish wastes were therefore compared to those collected from French wastes, which also contained high total levels of heavy metals, but where the availability of the individual potentially toxic elements was much lower.

MATERIALS AND METHODS

Fruitbodies of mycorrhizal fungi and root samples of *Pinus sylvestris* were collected from industrial wastes located in Chrzanow (Poland) and Villerupt (France). Both sites were characterised by low organic matter (about 1.5%), N (less than 0.05%) and P (about 50 mg kg^{-1} of P_2O_5) content and high heavy metal and Ca (2500 mg kg^{-1}) content (Table 1). The mean pH value of the substratum was 7.4 and 8.2 in Polish and French wastes, respectively. The structure of the mycorrhizal community was studied in detail on the Polish site. The substratum deposited there was obtained after removal of Zn and Pb from the metalliferous dolomite. The material had been deposited there 40 years ago and was subjected to chemical weathering, which resulted in formation of clay material poorly permeable to water. The root sampling and fruitbody collection was carried out on the area of 200 square m where pines appeared spontaneously about 20 years ago and presently reached the height of about 5m indicating a slow growth rate. The humus layer (1.5-4 cm) was composed of dead roots and other plant remains characterised by slow decomposition rate. Ten standard cores (20×5 cm) of soil were collected three times during the period of one year. The cores were divided into three layers, organic (mean depth of 2.7 cm), mineral sandy clay (mean 8.9 cm) and mineral clay layer (12.6 cm). Roots were washed with water, the mycorrhizas were grouped into morphotypes, counted and sampled for morphological observations. Nonmycorrhizal root length, number of individual mycorrhizal types, number of alive and dead mycorrhizal and nonmycorrhizal short roots were determined (Harvey et al. 1976). Mantle and rhizomorph preparations were observed with Nomarski's interference contrast in plan view and characterised according to Agerer (1991). Non-mycorrhizal short roots and mycorrhizas of individual types were counted and the percentage of each morphotype was calculated.

Mycorrhizas of the most frequently observed types from both sites were selected for further investigations carried out with light and electron microscopes.

SEM studies

After washing and removing soil particles, the roots were embedded in isopentan cooled in liquid nitrogen and lyophilised with a tissue dryer (EDWARDS ETD4). The mycorrhizas were subsequently cut transversally with a razor blade, mounted on carbon stubs and covered with carbon. They were observed with a scanning electron microscope (JEOL JSM-5400) and analysed with an energy dispersion spectroscope (EDS) equipped with a silicon/lithium detector (NORAN). The microscope was operated at 20 kV and at a magnification of 50-100 thousands. The estimated depth of the electron beam penetration was 2-4 µm (Monte Carlo Simulation by David C. Joy, version Feb. 1995). Computer analysis was carried out with the Voyager 3.6 program. At least twenty mycorrhizas of each morphotype were analysed. Point analysis was performed in five randomly selected places of the mantle, the cortical cell layer and the vascular tissue. If extramatrical mycelium was present it was also included in the analysis. Statistical analysis was carried out using a non-parametric test on net count data and on element % dry weight data obtained on the basis of reference standard calculation. Least significance differences were calculated at $p<0.05$.

TEM studies

Selected mycorrhizas were fixed in 2% glutaraldehyde/0.2 M Hepes buffer (1 : 1), post-fixed in osmium tetroxide, and after dehydration embedded in Spurr's resin as recommended by Kottke (1991). Semi-thin sections (0.5-

TABLE 1. Total and extractable (in 0.1 M Ca $(NO_3)_2$) heavy metal content in zinc wastes localised in Chrzanow (Poland) and in Villerupt (France); data in µg g^{-1}.

	Zn		Pb		Cd		Cu		Fe	
	total	extr. in Ca $(NO_3)_2$	total	extr. in Ca $(NO_3)_2$	total	extr. in Ca $(NO_3)_2$	total	extr. in Ca $(NO_3)_2$	total	extr. in Ca $(NO_3)_2$
Chrzanow	53303	78.59	15976	1.35	384.7	1.76	37.36	0.24	17 700	n.d.
Villerupt	1648	0.062	1196	0.009	2.88	0.00004	117.1	0.068	22 600	n.d.

-1.5 µm thickness) were stained with crystal violet for light microscopy. Ultra-thin sections of 80-120 nm thickness were stained with lead citrate and uranyl acetate and then observed with a Zeiss 902 TEM. Sections analysed with EDAX or EELS connected to CM 20 Philips TEM (operated at 120 kV) with spectrometer Gatan Model 666 (USA) were not stained.

RESULTS

Mycorrhizal fungi and mycorrhizal morphotypes of Pinus sylvestris on zinc wastes

The most abundant fruitbodies among mycorrhizal fungi on both zinc wastes were produced by *Suillus luteus* (Fr.) S.F. Gray, *Hebeloma mesophaeum* (Pers.) Quél. and *Tricholoma scalpturatum* (Fr.) Quél. Extremely common on Polish wastes were also fruitbodies of *Rhizopogon roseolus* (Corda in Sturm) Th. Fr. Additionally *Chroogomphus rutilus* (Schaeff.:Fr.) O.K. Miller, *Cortinarius* cfr. *decipiens* (Pers.:Fr.) Fr., *Hebeloma antracophilum* R. Mre., *H. fastibile* (Pers.:Fr.) Kummer, *Inocybe lacera* (Fr.:Fr.) Kummer, *Russula consobrina* (Fr.:Fr) Fr., *Thelephora terrestris* Ehr. ex Willd.:Fr and *Helvella sulcata* Afz. ex Fr. were found. Additionally, on French wastes, *Inocybe agardhii* (Lund) Orton, *Paxillus involutus* (Batsch) Fr. and *Hebeloma crustuliniforme* (Pers.) Quél. were found.

33 000 short roots were analysed. 20% were found to be alive mycorrhizas. Nonmycorrhizal live short roots represented up to 43%, while the remaining short roots were considered as dead mycorrhizas or dead nonmycorrhizal short roots (Fig. 1). The highest percentage of dead short roots was observed in the mineral layer (layer 3) of the substratum. The mean number of mycorrhizas per each 100 cm of the host roots was 45 with the maximum mycorrhizas in the uppermost 5 cm of the substratum layer.

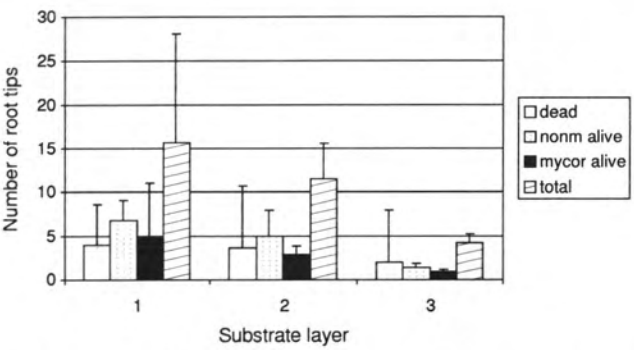

Fig. 1. Number of dead short roots (dead), alive nonmycorrhizal short roots (nonm alive), alive mycorrhizas (mycor alive) and total number of mycorrhizas per 1 cm of long *Pinus sylvestris* roots in organic layer (1), upper mineral layer (2) and lower mineral layer (3).

Among the most common mycorrhizas on Polish wastes (Fig. 2) were those formed by an unidentified species of *Hebeloma* (74%), characterised by a loose fungal mantle (Fig. 3C) often rich in encrusting sand grains. This morphotype differed from relatively uncommon mycorrhizas of *H. mesophaeum* by its yellowish mantle, slightly different mantle structure (transient between plectenchyma and

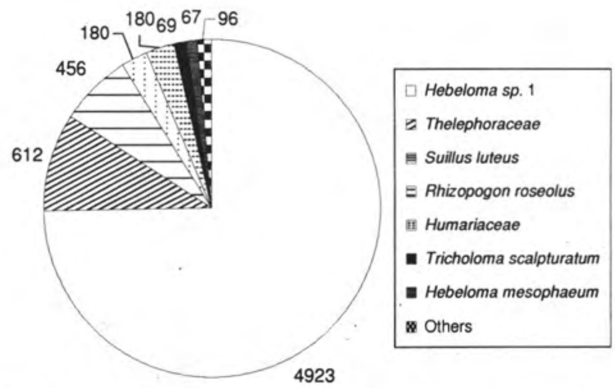

Fig. 2. Contribution of individual morphotypes in mycorrhizal community on Polish zinc wastes (numbers of each mycorrhizal type are given beside the graph).

pseudoparenchyma), thinner extramatrical hyphae and thicker mycelium walls.

Suillus luteus and *Rhizopogon roseolus*, which produced very abundant sporocarps, represented only up to 7 and 3%, respectively, of the total number of mycorrhizas. Still less abundant were *Hebeloma mesophaeum* (1%) and *Tricholoma scalpturatum* (2%). Darkly stained mycorrhizas, most probably produced by members of *Thelephorales*, contributed in up to 9%, whereas ascomycete mycorrhizas represented only up to 3% of the total number of mycorrhizas.

Mycorrhizal roots collected from French wastes were characterised by a similar high domination of *Hebeloma* mycorrhizas, a low frequency of suilloid and a high frequency of ascomycete mycorrhizas.

Nearly 40% of mycorrhizas from both sites formed a poorly developed or no mycorrhizal mantle (Fig. 3A, B). In most cases the tightly packed mycelium filled dead cells of surface layers of the roots. With the increasing volume of the fungus, root cell walls were torn apart and the mycelium was released. In such cases remnants of root cell walls were visible among fungal hyphae.

All mycorrhizas collected from French and Polish zinc wastes formed abundant vacuolar bodies, which were found in mycelium observed with light microscope after trypan blue staining or using Nomarski contrast (DIC). These bodies were present in the extramatrical mycelium, the fungal mantle and within the Hartig net.

TEM observations

By means of TEM equipped with micro-analytical systems (EDS and EELS), heavy metals localised in cell walls of the fungal mycelium were detected only in the case of suilloid mycorrhizas. Elements such as Zn, Pb, Fe and Cd were found within the electron dense material covering the mycelium (Figs 3D-F) of the outer fungal mantle or the extramatrical mycelium. It consisted mainly of amorphic material, whose element composition (as determined by means of EDS or EELS) was similar to vacuolar depositions (Fig. 4F). These depositions contained high level of N, S and much less P and Ca than polyphosphate granules from reference mycelium of *Pisolithus arrhizus* cultivated on agar without or with heavy metals or in mycelium of the same species collected from natural stands. At the same time the ratio of the mentioned elements was similar to vacuolar bodies observed in mycelia of *Suillus luteus* cultivated with or without heavy metals.

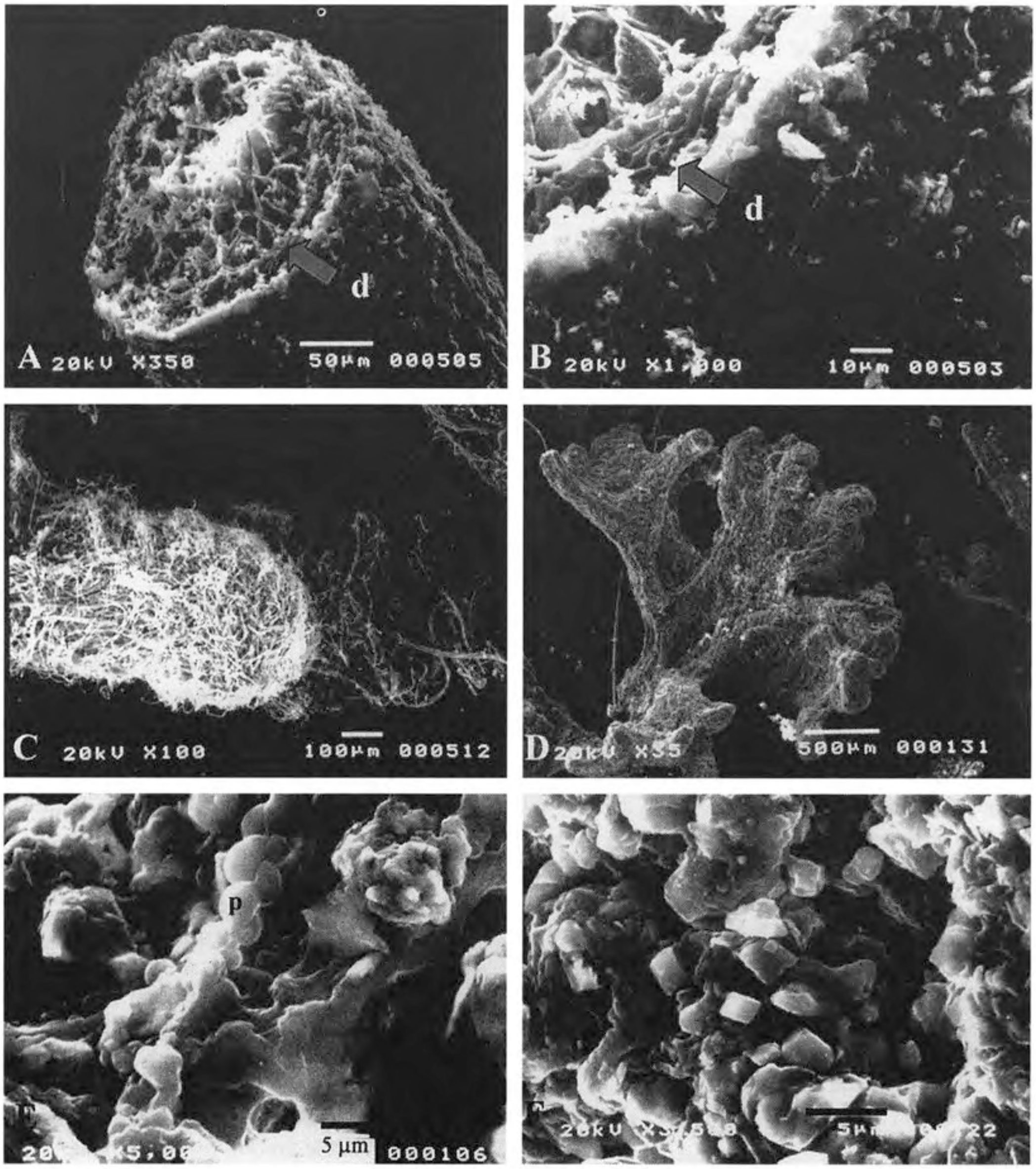

Fig. 3. SEM micrographs of *Pinus sylvestris* mycorrhizas from zinc wastes in Poland: A-B – mycorrhizas without fungal mantle but the mycelium (d) is visible in the outer layer of root cells; C – mycorrhiza with loose fungal mantle and abundant extramatrical mycelium; D-F – *Rhizopogon roseolus*/*Pinus sylvestris*: D – general view of the mycorrhiza; E – mycelium producing pigment droplets (p); F – crystals and crystalloids on mantle surface.

Moreover, vacuolar depositions within the mycelium of mycorrhizas from industrial wastes were in most cases a few times bigger than polyphosphate granules and often less regular in shape (Fig. 4). In the case of suilloid fungi they were also present in dead hyphae (no cytoplasm visible).

Besides, heavy metals were also found within crystalline or crystalloid depositions (Fig. 3F) present between hyphae or embedded within amorphic material. The crystals, disappearing during treatment with uranyl acetate for conventional observations (Fig. 4C), were identified as carbonates as they showed characteristic peaks for C=O bonds at 288 eV and C-O at 299 eV detected additionally to Ca peaks. The composition of the crystalloids was similar to amorphic and vacuolar depositions (Fig. 4F). Intercellular heavy metal sequestering material was not present in deeper layers of the mantle, or in the Hartig net. Less often and in lower levels, heavy metals were detected in mycorrhizas from French wastes.

SEM observations

Non-mycorrhizal short roots. This group of roots showed the highest mortality and the highest heavy metal content. Alive short roots contained up to 1% dry weight of heavy metals, while their content could be even higher in dead short roots (Fig. 5). Living short mycorrhizal roots accumulated heavy metals mainly within outer root layers, similarly to long roots. In such cases up to 2.5% dry weight of Pb and up to 3% dry weight of Zn were detected in outer periclinal cell walls of rhizodermis.

Extramatrical mycelium. According to EDS analysis accompanied by SEM, heavy metals were often detected within extramatrical hyphae. Zn, Pb, Fe (Fig. 6) and Ti were the most frequently detected elements. The highest concentration of Zn was found in *Rhizopogon*, of Pb in suilloid fungi, of Ti in ascomycete mycelium from Polish wastes, while the extramatrical mycelium of French mycorrhizas contained the highest concentration of Fe and rarely other potentially toxic elements. Elements such as Cd and Cu

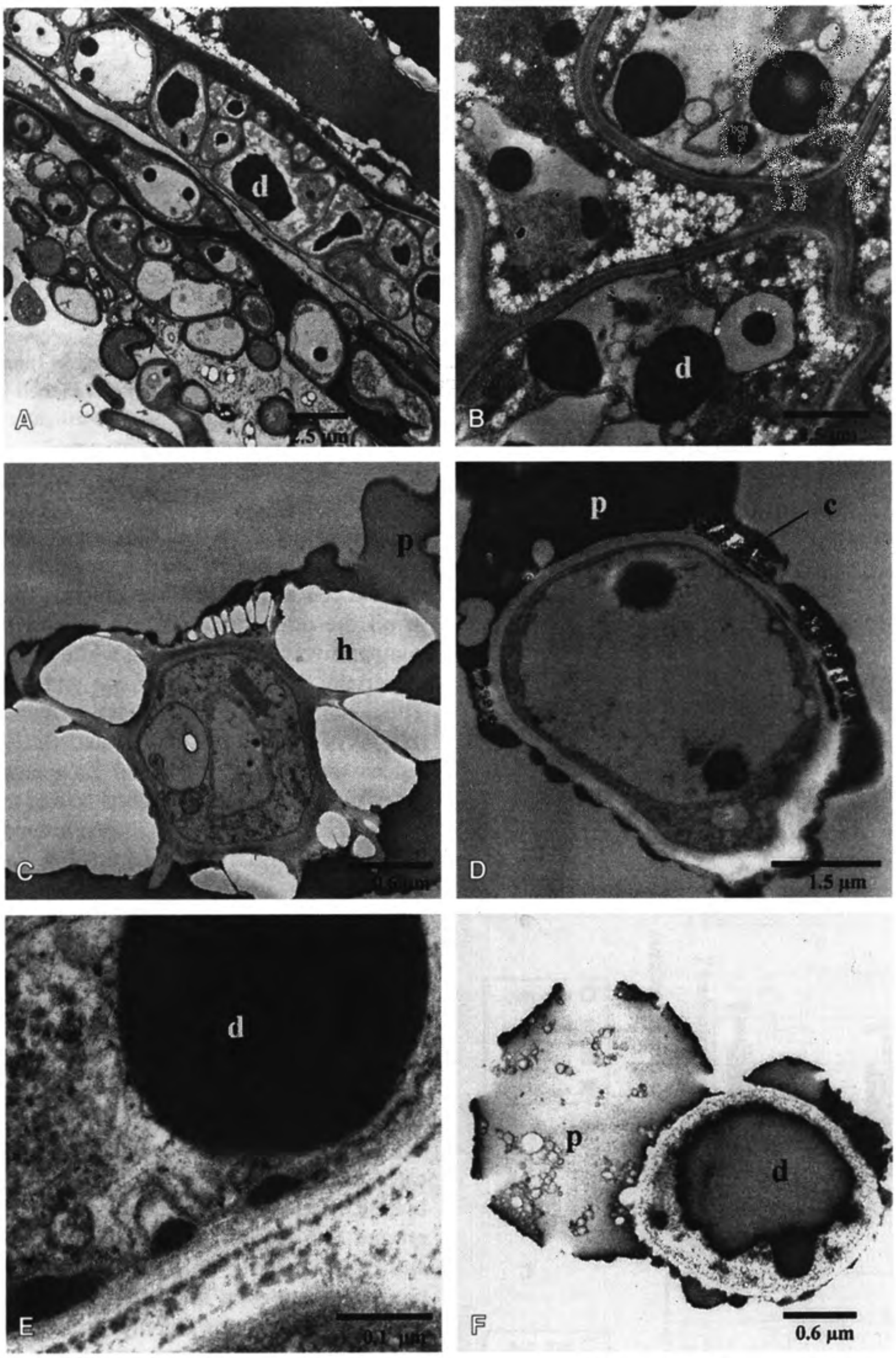

Fig. 4. TEM micrographs of *Pinus sylvestris* mycorrhizas from zinc wastes: A-B – *Hebeloma sp.* 1 mycorrhiza with granular and amorphic depositions (d) within vacuoles; C-E – *Suillus luteus* mycorrhizas: C – holes (h) appearing after treatment with uranyl acetate in places where carbonate crystals were present; D – crystals (c) on the mycelium cell wall, covered by pigment (p); E – vacuolar depositions (d) filling vacuoles; F – *Rhizopogon roseolus* extramatrical mycelium with pigment (p) on the surface and similar material within mycelial vacuoles.

were found only in extramatrical hyphae of *Hebeloma* and *Inocybe* spp. in mycorrhizas from both sites, where Mn content was usually also very high.

Fungal mantle. In most mycorrhizas from Polish wastes Zn, Fe and Pb were detected within the fungal mantles (Fig. 6). The highest amounts of Fe were found within *Hebeloma*, *Tricholoma*, ascomycete and brown mycorrhiza mantles. Zn concentration was usually similar between the fungi, except for *Tricholoma* and members of *Thelephoraceae* where much lower levels were found (Fig. 6B). Suilloid mycorrhizas contained statistically more Zn and Pb (Fig. 6A, B), especially if only data for extracellular depositions were taken into account. Mantles containing high levels of heavy metals were also characterised by high Mg and Mn concentrations. Low amounts of Zn, no Pb and high concentrations of Fe were found within the mantles of mycorrhizas from French wastes. Cu was detected in low concentrations in *Hebeloma* with white rhizomorphs, ascomycete and *Suillus* mycorrhizas from French wastes, and on Polish wastes only in the case of *Tricholoma*. Similar levels of Ca, Al, and S were found in mantles from French and Polish *Suillus*, while Mg, Mn, Zn and Pb concentration

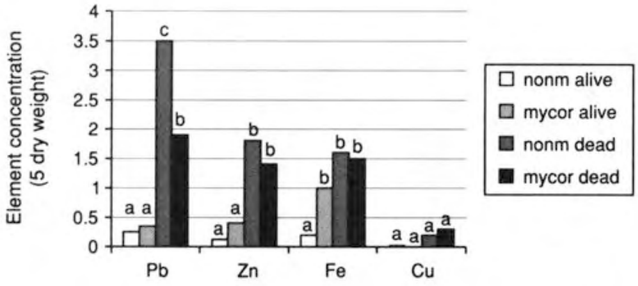

Fig. 5. Mean element concentration in alive nonmycorrhizal short roots (nonm alive) and dead nonmycorrhizal short roots (nonm dead), alive (mycor alive) and dead (mycor dead) mycorrhizas. Different letters above columns indicate a statistically significant difference at $p<0.05$.

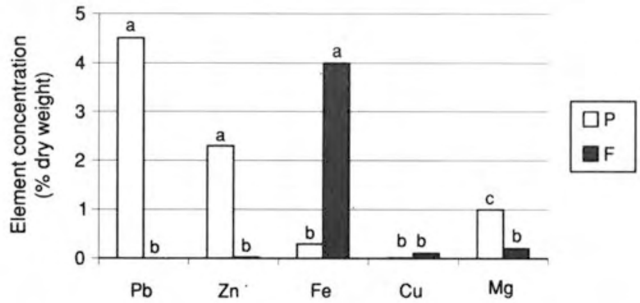

Fig. 7. Mean element concentrations in fungal mantle of *Suillus luteus* collected from Polish (P) and French (F) wastes. Different letters above columns indicate a statistically significant difference at $p<0.05$.

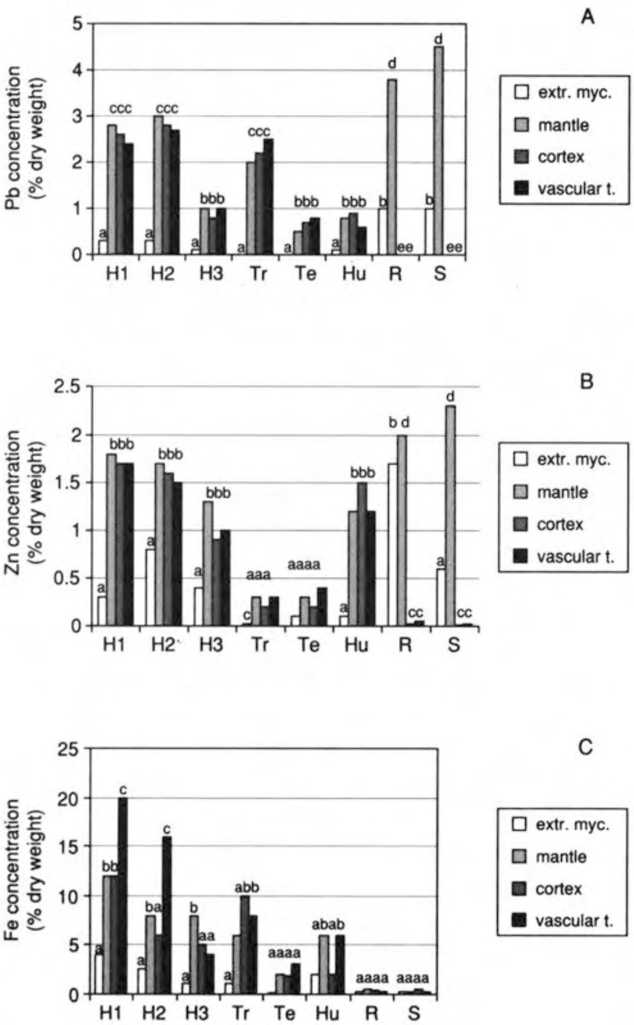

Fig. 6. Mean Pb (A), Zn (B) and Fe (C) content in fungal mantle, cortex and vascular tissues of mycorrhizas from Polish wastes: H1, H2, H3 – *Hebeloma* spp.; Tr – *Tricholoma* sp; Te – *Thelephoraceae* member; Hu – *Humariaceae* member; R – *Rhizopogon roseolus*; S – *Suillus luteus*. Different letters above columns indicate a statistically significant difference at $p<0.05$.

were lower and Cu, Fe and P contents were higher in *Suillus* from French than from Polish wastes (Fig. 7).

Cortical cell walls and vascular tissues. Low concentrations or no Zn and Pb were detected in vascular tissues and cortical cell walls of suilloid mycorrhizas. On the contrary, in all the other mycorrhizas, Zn was regularly found in comparatively high amounts, while Pb was especially high in certain species of *Hebeloma* and *Tricholoma* (Fig. 6). These elements were usually accompanied by high concentrations of Mn, Ca and Fe. No statistically significant differences were found in Pb and Zn concentrations between outer and inner parts of mycorrhizas of the last group. Such differences could be easily shown in the first group (the suilloid mycorrhizas). Except for a few cases, Fe concentration was significantly higher in vascular tissues than in the mantle.

DISCUSSION

Heavy metal pollution is known as a factor affecting aboveground and underground ecosystems (Greszta et al. 1979). Although little is known about ectomycorrhizal community structure in polluted soils, several studies indicated that pollution also inhibits mycorrhizal development both in experimental conditions and in field studies (Colpaert and Van Asche 1992a, b; Schneider et al. 1989; Perrin and Estivalet 1990; Chappelka et al. 1991). The results obtained in the present investigation indicate that also on Polish industrial wastes ectomycorrhizas were developed to a lesser extent, as the percentage of nonmycorrhizal roots was high compared to results of other studies on unpolluted places and even on other heavy metal containing industrial wastes (Danielson 1991; Golldack 1999). Also the percentage of dead mycorrhizal roots was high, what may suggest the decrease of the life-span of the mycorrhiza or the decrease of the decomposition rate. Faster replacement of mycelium under heavy metal stress has already been shown by Colpaert and Van Assche (1993). The increase in mycelial/mycorrhizal turn over may be responsible for the decreased heavy metal supply to the host. Two different phenomena may be involved. Extraradical mycelium is active in nutrient transfer towards the plant only if the hyphae are not affected by high metal uptake. The mycelium of higher fungi is protected against transferring heavy metals to the remaining mycelium by the system of septal aparatus which cuts off degenerating parts (Turnau and Dexheimer 1995). The second phenomenon involves the passive metal sequestration ability of the cells or mycelia termed as biosorption. It can be responsible for metal binding and accumulation by hyphae, which are no longer metabolically active. Due to lack of barriers created by active plasma membranes the biomass can be much more effective dead than alive (Volesky 1990). Dead roots, extramatrical mycelia and mycorrhiza may therefore play a role as biosorbent material. As pointed out in the present paper dead roots and dead mycorrhizas indeed contained much higher levels of heavy metals.

The majority of research on heavy metal uptake and accumulation by ectomycorrhizal fungi were based on experiments where the mycelium was exposed to different heavy metal concentrations (Poitou and Olivier 1989; Blaudez et al. 2000). Other studies pointed out the specific accumulation of certain metals within fruitbodies formed in nonpolluted and polluted areas (Tyler 1980; Turnau and Kozłowska 1991). Analyses of mycorrhizas were much less common. Using atomic absorption spectroscopy, Berthelsen et al. (1995) found the highest uptake of Zn and Cu in *Cortinarius semisanguineus* and of Cd in *Suillus* spp. while the uptake of Pb was very low. The results, based on EDS analyses, showed a diversity among heavy metal accumulation properties of the extramatrical mycelium and mantles as well as the role of the mantles in reducing the metal transfer towards the plant vascular tissue. Three groups of mycorrhizal morphotypes were distinguished. The first one, including mycorrhizas of *Hebeloma* spp, *Thelephora* and *Tricholoma* was characterized by a relatively well developed fungal mantle, which, however, did not inhibit the metal transfer into the plant cells, resulting in high accumulation of elements such as Pb, Zn and Fe in vascular tissues. This may be due to the absence of efficient heavy metal sequestering substances such as pigments or crystals within or on the surface of the mantle. In some cases chelating of Cu and Cd within extramatrical mycelium was observed and its role in decreasing metal toxicity to the plants and mycorrhizas should not be overlooked.

The second group of mycorrhizas, mostly formed by ascomycete members, produced poorly developed or no fungal mantle on the root surface. Also in this case the role of the extraradical mycelium could be of importance but except for this the mycorrhizas did not differ in metal uptake from nonmycorrhizal roots.

On the contrary, mycorrhizas of suilloid fungi, in addition to the role of rhizomorphs and mycelium, also developed the barrier in the form of the mantle sequestering heavy metals. This results in a statistically important decrease of metal transfer into the plant tissues. The phenomenon has been described previously in the case of *Rhizopogon roseolus* collected from galmanic wastes in Poland (Turnau et al. 1996, 1999), and metals such as Cd and Zn were shown to be accumulated mostly within pigment crystalloids, droplet-like substances or crystals of organic acids formed in the outer fungal mantle or on the mantle surface. In the present investigation a similar situation was not only found in *Rhizopogon roseolus,* but also in *Suillus luteus* mycorrhizas. Additionally to pigments, carbonic acids have been observed to play a role in heavy metal sequestration as shown by TEM microanalysis. This method, however, may only indicate the presence of the elements within the ultrastructurally recognised substances. Further studies should be carried out to isolate chemically characterised substances or functional groups, which are directly involved in metal binding and complexation. Other mechanisms, which are usually mentioned as involved in protection against heavy metals, are binding by extracellular slime polysaccharides within the cell wall (Denny and Wilkins 1987) and intracellular compartmentation by metallothionein-like substances and phosphate rich vacuolar depositions (Morselt et al. 1986; Turnau et al. 1993, 1994). Both mycorrhizas are also hydrophobic what certainly is of importance in metal filtering within the mycorrhiza.

The suilloid mycorrhiza type was represented in up to 10% of the total number of mycorrhizas, whereas the fungi involved formed abundant fruitbodies on both sites studied. According to Gardes and Bruns (1996) this may be a common pattern for *Suillus* species, which invest less in vegetative growth and persistence and more in fruitbody production than other fungi. They might obtain more carbon either from the symbiotic plant or these fungi may have access to biotrophically and saprobically obtained carbon (Bonello et al. 1998). It is possible that these fungi were more efficient in producing mycorrhiza in early successional stages of pine growth (Danielson and Visser 1989) and/or they might have been outcompeted by other mycorrhizal species. Preliminary research on the introduction of different strains of these fungi into industrial wastes together with young seedlings has shown that some strains may be more effective in competition, while others are simply inhibited. High pH value of the substratum may be responsible for such a situation. Successful introduction of mycorrhizal fungi usually concerns areas characterised by very low pH values which is the reason for a much higher toxicity and a much lower competition in such places. Further research is needed to follow up this line towards the practical application of the results.

Mycorrhizas collected from Polish wastes accumulated much more heavy metals than those from French wastes. This was clearly due to much higher content and availability of heavy metals within the Polish wastes.

In all investigated mycorrhizas, abundant vacuolar bodies were observed. However, they differed from typical polyphosphate granules in appearance and element content. The modification of the vacuolar bodies due to the presence of potentially toxic metals (Turnau et al. 1994), the increase in N level (Kottke et al. 1995) and the increase in CO_2 level (Turnau et al. 2001) have already been described. In some fungi, pigments can also be deposited in these vacuoles. Vacuolar depositions rich in P, N accompanied by several other elements are certainly not an artifact resulting from chemical preparation, as they have been demonstrated in alive fungi. This supports data obtained by Bücking et al. (1998). The composition of vacuolar depositions differs strongly depending on growth conditions; there is also a strong diversity between fungal species. Vacuolar depositions are a storage compartment for nutrition or detrimental elements (Klionsky et al. 1990). In our material, especially in large, amorphic depositions, the level of P was comparatively low and it would be inappropriate to call these depositions polyphosphate granules, as fungal pigments can also be deposited in such vacuoles. This subject certainly needs further research.

Several microscopic techniques were used in the present paper as complementary methods. As in all micro-analytical systems coupled to electron microscopy, the EDS and

EELS data are regarded only as no more than semi-quantitative. It is not possible to produce accurate standards for the evaluation of local heavy metal concentration within the extremely heterogenic fungal and plant tissues. There are also differences in the depth of the electron beam penetration between these tissues and between different cell parts. Therefore, in the present paper the statistical analysis was carried out on net counts and on element concentrations calculated by the computer. This method allows the comparison of the samples, at least when similar structures are analysed. The analysis of the mycorrhizas from non-polluted sites gave no results concerning the heavy metals concentration due to low sensitivity of the detection system. This was the reason why material from French wastes characterised by lower toxicity was used. In addition, a few selected samples were studied with a proton microscope equipped with a X-ray microprobe (unpublished data) which gave much more adequate data concerning quantification and the results were comparable to those shown above.

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WIĄZANIE METALI CIĘŻKICH W OBRĘBIE MIKORYZ SOSNY Z HAŁD PRZEMYSŁOWYCH

STRESZCZENIE

Mikoryzy sosny (*Pinus sylvestris*), zebrane z hałd cynkowych w Polsce i Francji, poddano analizie za pomocą mikroskopów transmisyjnego i skaningowego wyposażonych w mikroanalizatory EDS i EELS. Najpowszechniejszym morfotypem były mikoryzy tworzone przez grzyby z rodzaju *Hebeloma*, charakteryzujące się słabo rozwiniętą mufką grzybniową. Rzadsze (do 10%) były mikoryzy grzybów suilloidalnych, które cechowały się mufką znacznie lepiej wykształconą i obficie inkrustowaną krystaloidami pigmentu. Mikoryzy suilloidalne i mikoryzy *Hebeloma* różniły się pod względem efektywności wiązania metali w obrębie mufki. Akumulację Pb i Zn stwierdzono jedynie w przypadku mikoryz suilloidalnych. W pozostałych morfotypach nie stwierdzono różnic w zawartości metali ciężkich w obrębie mufki oraz tkankach korzenia. W przypadku *Hebeloma* i *Inocybe* zanotowano jednak wyższą zawartość Cu i Cd w grzybni ekstramatrykalnej niż w mufce. Analiza systemu korzeniowego wykazała większy udział korzeni niemikoryzowych niż mikoryzowych, co sugeruje zahamowanie procesu tworzenia mikoryz lub obniżoną liczebność propagul. W pracy przedyskutowano rolę martwych korzeni i mikoryz w biosorpcji i unieruchamianiu metali ciężkich.

SŁOWA KLUCZOWE: ektomikoryzy, metale ciężkie, efekt biofiltracji, hałdy przemysłowe, mikroanaliza.