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Metal content in fruit-bodies and mycorrhizas of *Pisolithus arrhizus* from zinc wastes in Poland

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Pisolithus arrhizus has been selected for investigation as one of the ectomycorrhizal species most resistant to stress factors. Metal content in fruit-bodies and mycorrhizas was estimated to evaluate their role as bioindicators and to check whether mycorrhizas have any special properties for heavy metal accumulation. Fruit-bodies and mycorrhizas were collected from zinc wastes in Katowice—Wełnowiec and analyzed using conventional atomic absorption spectroscopy and energy dispersive spectroscopy accompanying scanning electron microscopy. Differences in tendencies to accumulate metals within sporophores and mycorrhizas were found. The fruit-bodies accumulated Al (up to 640 $\mu g\ g^{-1}$), while high concentrations of Al, Zn, Fe, Ca and Si were noted in the outer mantle of the mycorrhizas, in the material secreted and in the mycelium wall. The content of elements varied depending on the age of mycorrhizas. The ability of extramatrical mycelium and hyphae forming mycorrhizal mantle to immobilize potentially toxic elements might indicate biofiltering properties though the next step should include investigations on ability of the fungus to prevent element uptake by the plant.

Key words: *Pisolithus arrhizus*, heavy metals, Al bioaccumulation, X-ray analysis (EDS), AAS, SEM.

INTRODUCTION

Pisolithus arrhizus (Pers.) Rausch. (= *Pisolithus tinctorius* (Pers.) Cocker et Couch) is one of the most intensively studied ectomycorrhizal fungi. It is considered to be a very effective species in the recultivation of heavily polluted or disturbed areas (L a n d i s et al. 1990). In natural communities it is a relatively rare species occurring on poor, sandy soils and forming mycorrhizas with a broad range of host species. It is noted quite frequently on mining wastes of brown and black coal, slate, kaolin and black wastes from anthracite mining (Schramm 1966; Lisiewska and Siedlaczek 1982; Derbsch and Schmitt 1987; Kreisel 1987). The fruit-bodies are known to accumulate high levels of Al and Cr (Medve and Sayre 1994; Cochrane 1978). The species improves the growth of *Pinus strobus* seedlings in substratum supplemented with Al (Schier and McQuattie 1995) also diminishing the foliar symptoms of Al toxicity. Although the authors suggested that the amelioration of Al toxicity by mycorrhizal colonization resulted from enhanced uptake of nutrients, especially P, rather than the reduced uptake of Al, the data presented show a significant decrease in the Al content of the needles of mycorrhizal seedlings. According to Tam (1995) the mycelium of *Pisolithus arrhizus* cultivated on agar medium was able to withstand high concentrations of Al, Fe, Cu or Zn and to a much lesser extent Ni, Cd, Cr and Hg. The energy dispersion X-ray spectroscopy showed that the slime produced on the surface of the mycelium and cell wall were responsible for the complexation of potentially toxic elements. A pigmented cell wall layer of *P. arrhizus* mycelium cultivated on media supplemented with cadmium dust was observed using electron energy loss spectroscopy, to contain Al and Cd (Turnau et al. 1994). At the same time the presence of cysteine-rich proteins in the same cell wall layer was demonstrated. Gruhn and Miller (1991) indicated that tyrosinase, the enzyme active in melanin formation, might also be stimulated by the presence of heavy metals. Further TEM investigations by Turnau et al. (1994) showed intracellular localization of the metals in *P. arrhizus* cultivated on agar containing heavy metals. Such elements as Cd, Ti, Ni, Cu, Al, Fe as well as P, S and N were found in phosphate-rich, vacuolar material giving a positive reaction to the Gomori-Swift test for cysteine-rich material. This suggested the possibility of metal-binding protein deposition within vacuoles as a resistance mechanism. The existence of proteins with thiolate clusters in *P. arrhizus* cultivated in heavy metal supplemented media were indicated cytochemically by Morselt et al. (1986). Metallothionein-like proteins have been widely implicated in the detoxification and storage of cadmium, zinc and copper ions (Kagi and Kojima 1987). Among ectomycorrhizal fungi, only in case of *Laccaria laccata* and *Paxillus involutus* copper binding proteins of this kind have been isolated and characterized (Howe et al. 1997). Strains of the same species differ in their ability to produce the substances.

The successful growth of the mycelium could also be attributed to the resistance of acid phosphatase to heavy metals and to an efficient system of dolipori cutting off parts of the mycelium which have taken up lethal levels of metals (T u r n a u and D e x h e i m e r 1995). Most of the above mentioned papers are concerned with mycelium grown in agar cultures.

The present paper is part of a study on metal tolerant ectomycorrhizal fungi, their selection, characterization and utilization for restoration of polluted forests. The metal content in fruit-bodies and mycorrhizas of *P. arrhizus* collected from zinc wastes in Katowice, were determined to evaluate their importance for bioindication of potentially toxic elements and to check whether mycorrhizal mantle has special properties for heavy metal accumulation.

MATERIAL AND METHODS

Fruit-bodies of *Pisolithus arrhizus* were found in summer 1996 in close vicinity to *Betula pendula* and *Populus tremula* on 20-year-old zinc wastes in Katowice – Welnowiec (N-W part of the wastes). The pH value of the spoil mound ranged from 6.4 to 8.2 but in the place where *P. arrhizus* occurred the value was 3.8 to 3.9. The wastes were characterized by low levels of organic matter, nitrogen, phosphorus and high levels of heavy metals concentrated mainly in the surface layer (T o k a r s k a-G u z i k et al. 1991). Composite soil samples (0 – 5 cm) for chemical analysis were collected from the place where *P. arrhizus* was localized and from several other areas of the wastes of similar age. The analysis of the total content of elements (extracted in 1N HCl) in soil, which were estimated with atomic absorption spectrophotometer (Varian 20BQ), showed that the surface layer of the wastes was an extremely heterogenous material regarding the element content (Tab. 1). The part of the wastes where *P. arrhizus* was found differed significantly from the rest of the wastes with respect to the element content and pH value. Only the levels of Al and Cu were similar as in the other parts of the wastes. The analysis of metals extracted in $Ca(NO_3)_2$ revealed much lower availability of such elements as Pb and Cd while a reverse case was observed for Cu and Zn (Tab. 1).

The content of elements in fruit-bodies was determined by AAS after wet digestion with a 4:1 mixture of nitric and perchloric acids.

Roots for ectomycorrhiza selection were collected from the locations where fruit-bodies were present. Mycorrhizas were selected under a stereomicroscope and identified according to A g e r e r (1987 – 1995) and W e i s s (1991, 1992). They were washed, air dried, mounted on carbon stubs and covered with carbon. The outer layer of the mycorrhizal mantles was subsequently analyzed with energy dispersive spectrometry (EDS) with a lithium-silicon detector (NORAN) connected to scanning microscope Jeol JSM 5410. The estimated depth of the electron beam penetration was 3 – 5 μm (Monte Carlo Simulation by David C. Joy, version Feb. 1995). Computer analysis was carried out using the Voyager 3.6 program.

Table 1

Total element content (extracted in 1N HCl) and $Ca(NO_3)_2$ extractable metals in the 25-year-old zinc waste substratum outside and in the place where *P. arrhizus* occurred (mg kg^{-1} dry weight)

	Zinc waste substratum extraction in HCl	Zinc waste substratum extraction in $Ca(NO_3)_2$	*P. arrhizus* stand extraction in HCl	*P. arrhizus* stand extraction in $Ca(NO_3)_2$
Pb	17 630	1.5	380	1.8
Zn	18 512	42.5	1 794	94.7
Cd	528	2.5	43	0.06
Cu	380	0.3	882	4.8
Fe	17 713	n.d.	850	n.d
Mn	36 333	n.d.	41	n.d
Ca	71 583	n.d.	3 312	n.d.
Al	3 599	n.d.	2 796	n.d.
Cr	n.d.	n.d.	15.8	n.d

n.d. — not determined

RESULTS AND DISCUSSION

Element content in fruit-bodies of *P. arrhizus*

Nearly 10 times less Ca (up to 400 $\mu g\ g^{-1}$), Zn (up to 150 $\mu g\ g^{-1}$), Fe (up to 90 $\mu g\ g^{-1}$) and about 4 times less Al (640 $\mu g\ g^{-1}$) were found in fruit-bodies of *P. arrhizus* (Tab. 2) than in the substratum collected from places where the fungus was growing. A similar situation was observed in the case of such elements as Cu, which however did not exceed 10 $\mu g\ g^{-1}$, while Cd and Pb levels were below 1 $\mu g\ g^{-1}$. Tyler (1980) defined the terms bioconcentration and bioexclusion as the concentration of a metal respectively ten times higher or lower than the mean value estimated for a wide range of fungal species. According to these definitions *P. arrhizus* from zinc wastes in Katowice–Welnowiec had the property of Al bioconcentration as its level in the fruit-bodies of this species was over 20 times higher than the mean content of Al in fungal sporophores analyzed by Tyler (1980). Considering data for 130 species of *Basidiomycetes* given by Tyler (1980), in *P. arrhizus* sporophores levels of Cu, Cd, Ni, Na and K are rather low while the values of Pb, Cr, Fe, Zn and Mg are close to the mean values obtained for other fungi.

Comparatively high (three times higher than the mean values for basidiomycete fruit-bodies) levels of Ca were found in *P. arrhizus* sporophores from Poland. The species was also analyzed by M e d v e and S a y r e (1994) from bituminous stripmine spoils whose substratum was characterized by lower Zn, Cd and Cu contents while Al exceeded the level of Al in zinc wastes in Katowice—Wełnowiec by more than three times. In this case the Al content in fruit-bodies of *P. arrhizus* was also 3—4 times higher than in the case of sporophores collected from Polish zinc wastes. Al bioconcentrators are rather rare. T y l e r (1980) found only one case of this phenomenon — *Hymenochaete* sp. in which a maximum of 427 $\mu g\ g^{-1}$ was measured. M e d v e and S a y r e (1994) also found bioconcentration of Cr in *P. arrhizus*, which was not indicated in fruit-bodies of fungi growing in Poland. The terms "bioconcentrators" and "bioexcluders" do not take into account any kind of relation to the metal content or their "bioavailability" in the substratum. Despite the high levels of total heavy metal content in the waste material in Poland the contents of Pb and Zn extractable in $Ca(NO_3)_2$ are more similar to the levels found in *P. arrhizus* sporophores (Tabs 1, 2). At the same time the content of Cd was 10 times higher and that of Cu twice as high as the respective Cd and Cu levels extracted in $Ca(NO_3)_2$ from the waste material.

T a b l e 2

Element content in fruit-bodies of *Pisolithus arrhizus* collected from zinc wastes in Katowice—Wełnowiec

	Mean value	SD
Pb	1.5	0.63
Zn	110.8	42.5
Cd	0.5	0.1
Cu	7.8	1.1
Fe	56.9	26.4
Cr	0.3	0.2
Ni	0.5	0.5
Mg	1007.0	256.0
Ca	382.0	230.0
Al	630.0	10.0
K	8961.0	563.0
Na	27.7	9.5

Explanation: data obtained with conventional AAS; data given in $\mu g\ g^{-1}$ dry weight

Metal content in the fungal mantle of *P. arrhizus* mycorrhizas

The analysis of metal content in *P. arrhizus* carried out by EDS connected to SEM revealed the accumulation of such elements as Fe, Zn, Al, Ca and Si within hyphae of the outer mantle (Tab. 3). The content of elements increased with the age of the mycorrhiza. Considerably high levels of Ca, Al, Ti and Mg were found in dead mycorrhizas. The accumulative role of the mantle is possible as it was already shown in the case of other mycorrhizas (T u r n a u et al. 1996). The mycorrhizas of *P. arrhizus* from zinc wastes in Katowice—Wełnowiec observed with SEM showed a very compact structure of the mantle. The hyphae were interconnected by the abundant extracellular material. D e n n y and R i d g e (1995) suggested that fungal slime was

Table 3
Element content in mycorrhizas of *Pisolithus arrhizus* collected from zinc wastes in Katowice–Wełnowiec

	Mean value	SD
Zn	0.23	0.15
Fe	1.09	0.53
Mg	0.16	0.03
Al	2.57	1.35
Ca	2.19	0.99
Si	3.10	1.77
P	0.11	0.08
S	0.23	0.05
Cl	0.09	0.03
K	0.46	0.05
Na	0.11	0.03
Ti	0.06	0.02

Explanation: data obtained with energy dispersive spectroscopy (EDS); data given in % total element weight

the principal metal binding site, which certainly took part in the detoxification mechanism in the present case. In addition the presence of polysaccharidous material (PATAg test) and cysteine rich proteins (Gomori-Swift reaction) within the cell wall of *P. arrhizus* mycelium was indicated by Turnau et al. (1994). It was demonstrated that such elements as Ca, Al and low levels of Cd were found within the outer wall layer in fixed, dehydrated and embedded in resin mycelium. The results presently obtained confirmed the presence of Ca and Al but also suggested that most of the Zn was removed during the preparation of the material for TEM. In this case the observation of the dried fungus with SEM is more reliable. In addition, SEM observations also showed the presence of Si and Al in the cell wall, which would suggest the presence of aluminosilicate complexes on the wall surface. Up to 3.1% of Si were found in fungal walls of the mantle of *Pisolithus* mycorrhizas while the Ca content reached 2.5%, in comparison to the Si content of diatoms ranging from 3.5 to 15% and Ca ranging from 10 to 35% dry weight depending on the species (unpublished data, obtained with the same EDS method). Generally mycorrhizas from industrial wastes contained higher amounts of Si than mycorrhizas from natural soils. Mycorrhizas of *P. arrhizus* differed from all the hitherto analyzed mycorrhizas in the content of Si, which was higher than that of Ca. The high Si and Ca content might be responsible for the biofiltering of Al in the cell wall of the mycelium of this species. The material which was excreted on the surface of the extramatrical mycelium and on the mycelium forming the fungal mantle of *P. arrhizus* mycorrhizas was also comparatively rich in such elements as sulphur and phosphorus.

There were no statistically significant differences between extramatrical hyphae and the hyphae forming the fungal mantle in respect to metal content. This is in contradiction with the data obtained by Denny and Wilkins (1987) for other species of ectomycorrhizal fungi forming mycorrhizas with *Betula* spp. where extramatrical mycelium was the main place of heavy metal sequestration.

In the case of *P. arrhizus* mycorrhizas from zinc wastes in Poland Cu, Cd, Pb, Ni, Cr were not found by the EDS method (probably below the detection level). When fruit-bodies and mycorrhizas are compared similar tendencies for accumulation of high levels of Al were observed. However, such elements as Zn and Fe reached higher values than in other mycorrhizas. The content of these two elements within fruit-bodies was on an average level as the levels given by T y l e r (1980). More exact comparison of data obtained with two different techniques could be misleading as EDS technique involves calculations based on standardless analysis or on virtual standards which are not sufficient for biological material. Increased levels of Fe and Zn in mycorrhizas suggested differences in heavy metal sequestration between sporocarps and mycorrhizas.

When analysing the heavy metal content within ectomycorrhizas it is very important to bear in mind that not only species and strains of fungi differ in element sequestration properties but also the presence of plant influences the fungal activity, which results in structural and metabolic modifications leading to differences in abilities to immobilize elements even within the same mycorrhiza (T u r n a u et al. 1996; L e y v a l et al. 1997). Generally much more substances which could take part in this phenomenon are localized within the outer fungal mantle.

The selection of species for recultivation of industrial wastes only on the basis of metal content within fruit-bodies has a limited value. The ability of extramatrical mycelium and hyphae forming mycorrhizal mantle to immobilize potentially toxic elements might indicate biofiltering properties though the next step should include investigations on the ability of the fungus to prevent element uptake by the plant. Further investigations should be carried out using, for example, the techniques designed by J e n t s c h k e et al. (1991).

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REFERENCES

A g e r e r R. 1987—1995. Colour Atlas of Ectomycorrhizae. Einhorn-Verlag, Schwabisch Gmund.

C o c h r a n e C. E. 1978. Aluminium and pH effects on growth and mycorrhizal relationships of *Pisolithus tinctorius*. (Master's thesis) Iowa State University.

D e n n y H. J., R i d g e I. 1995. Fungal slime and its role in the mycorrhizal amelioration of zinc toxicity to higher plants. New Phytol. 130: 251—257.

D e n n y H. J., W i l k i n s D. A. 1987. Zinc tolerance in *Betula* spp. IV. The mechanism of ectomycorrhizal amelioration of zinc toxicity. New Phytol. 106: 545—553.

Derbsch H., Schmitt J. A. 1987. Atlas der Pilze des Saarlandes 2: Nachweise, Okologie. Nat. Landsch. Sonderb. 3: 1–816.
Gruhn C. M., Miller O. K. 1991. Effect of copper on tyrosinase activity and polyamine content of some ectomycorrhizal fungi. Mycol. Res. 95 (3): 268–272.
Howe R., Evans R. L., Ketteridge S. W. 1997. Copper-binding proteins in ectomycorrhizal fungi. New Phytol. 135: 123–131.
Jentschke G., Godbold D. L., Huttermann A. 1991. Culture of mycorrhizal tree seedlings under controlled conditions: effects of nitrogen and aluminium. Physiol. Plant. 81: 408–416.
Kagi J. H. R., Kojima Y. 1987. Chemistry and biochemistry of metallothionein. In: J. H. R. Kagi, Y. Kojima (eds.) Metallothioneins II. Birkhauser Verl., Basel: 25–61.
Kreisel H. 1987. Pilzflora der Deutschen Demokratischen Republik. Fischer, Jena.
Landis T. D., Tinus R. W., McDonald S. E., Barnett J. P. 1990. The container tree Nursery manual. 5, USDA Forest Service, Public Affairs Off., Washington, DC.
Lisiewska M., Siedlaczek S. 1982. The initial investigations on the occurrence of macromycetes on the coal mine heap "Smolnica". Arch. Ochr. Środ. 1–4: 93–110.
Leyval C., Turnau K., Haselwandter K. 1997. Effect of heavy metal pollution on mycorrhizal colonization and function: physiological, ecological and applied aspects. Mycorrhiza 7: 139–153.
Medve R. J., Sayre W. G. 1994. Heavy metals in red pines, basidiomycete sporocarps and soils on bituminous stripmine spoils. J. Pensylv. Acad. Sc. 68 (3): 131–135.
Morselt A. F. W., Smits W. T. M., Limonard T. 1986. Histochemical demonstration of heavy metal tolerance in ectomycorrhizal fungi. Plant Soil 96: 417–420.
Schier G. A., McQuattie C. J. 1995. Effect of aluminum on the growth, anatomy, and nutrien content of ectomycorrhizal and nonmycorrhizal eastern white pine seedlings. Can. J. For. Res. 25: 1252–1262.
Schramm J. R. 1966. Plant colonization studies on block wastes from anthracite mining in Pennsylvania. Trans. Am. Philos. Soc. 56: 1–190.
Tam P. C. F. 1995. Heavy metal tolerance by ectomycorrhizal fungi and metal amelioration by *Pisolithus tinctorius*. Mycorrhiza 5 (3): 191–187.
Tokarska-Guzik B., Rostański A., Klotz S. 1991. Roślinność hałdy pocynkowej w Katowicach–Wełnowcu. Acta Biol. Siles. 19 (36): 94–102.
Turnau K., Dexheimer J. 1995. Acid phoshatase activity in *Pisolithus arrhizus* mycelium treated with cadmium dust. Mycorrhiza 5 (3): 205–211.
Turnau K., Kottke I., Dexheimer J., Botton B. 1994. Element distribution in *Pisolithus arrhizus* mycelium treated with cadmium dust. Bot. Ann. 74: 137–142.
Turnau K., Kottke I., Dexheimer J. 1996. Toxic element filtering in *Rhizopogon roseolus/Pinus sylvestris* mycorrhizas collected from calamine dumps. Mycol. Res. 100: 16–22.
Tyler G. 1980. Metals in sporophores of *Basidiomycetes*. Trans. Br. Mycol. Soc. 74 (1): 41–49.
Weiss M. 1991. *Pisolithus tinctorius*. In: R. Agerer (ed.) Colour Atlas of Ectomycorrhizae, plate 63. Einhorn-Verl., Schwabisch Gmünd.
Weiss M. 1992. Ectomycorrhizae formed by *Pisolithus tinctorius* (*Basidiomycetes*) on Norway spruce. Crypt. Bot. 2: 337–344.

Zawartość metali w owocnikach i mikoryzach *Pisolithus arrhizus* zebranych na hałdach cynkowych

Streszczenie

Pisolithus arrhizus wybrany został do badań ze względu na jego szczególną odporność na wysokie stężenia metali ciężkich. Zawartość metali w owocnikach i mikoryzach oznaczono w celu stwierdzenia przydatności ich jako wskaźników zanieczyszczenia oraz dla sprawdzenia czy mikoryzy mają zdolność akumulowania metali ciężkich w mufce. Owocniki i mikoryzy zebrane zostały na hałdach cynkowych w Katowicach – Welnowcu i przeanalizowane za pomocą spektrofotometru AAS oraz mikroskopu skaningowego z przystawką EDS. W analizowanym materiale stwierdzono istotne różnice w zawartości metali. Owocniki akumulowały glin (do 640 $\mu g\ g^{-1}$), podczas gdy w mufce obok glinu stwierdzono także duże ilości cynku i żelaza, którym towarzyszyły wapń i krzem. Zawartość metali rosła w mufkach mikoryz starych lub martwych. Zdolność grzybni ekstramatrykalnej oraz grzybni budującej mufkę do wiązania metali potencjalnie toksycznych wskazuje na przydatność mikoryzy w detoksyfikacji tych metali, ale ostateczna decyzja odnośnie np. wprowadzenia danego szczepu czy gatunku w ramach rekultywacji, powinna zostać podjęta po zbadaniu efektywności w utrzymaniu tych elementów w formie niedostępnej dla rośliny.