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Adaptation of Mycorrhizal Fungi Isolated from Various Environments to Increased Levels of Selected Heavy Metal Concentrations

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Abstract

The selection of mycorrhizal fungi forming symbiotic association with trees growing in areas contaminated with heavy metals was observed. The aim of this study was to discover how ectomycorrhizal fungi, growing in habitats with high levels of heavy metal contamination, are adapted to this contamination in comparison with fungi that grow in nonpolluted environments. The experiments were performed using a vegetative mycelium isolated from fruiting bodies in clean cultures on agar. Changing concentrations of zinc, cadmium and lead in the growth medium, the growth of mycelium cultures of *Xerocomus badius* and *Amanita muscaria* was observed and compared. It was found that strains of *Amanita muscaria* and *Xerocomus badius* growing in contaminated areas were adapted to high concentrations of Zn, Cd and Pb. The degree of adaptation was related to the level of contamination of the area where this mycorrhizal fungus survived.

Keywords: ectomycorrhizae, mycorrhizal fungi, heavy metals, tolerance, *Xerocomus badius, Amanita muscaria*

Introduction

Ectomycorrhizal symbiosis results in more effective uptake of water and nutrients by trees, and increases their resistance to different diseases. Several papers suggest that it may also play a protective role in ecosystems exposed to high concentrations of heavy metals. Accummulating on the one hand and blocking the translocation of metal ions such as Ni^{2+}, Zn^{2+}, Cd^{2+}, Pb^{2+} and Al^{3+} on the other hand, mycorrhizal fungi may play the role of protective filters for plants [1, 2, 4, 5, 6, 10, 11].

Preliminary observations performed in regions exposed to high pollution by heavy metals indicate the selective appearance of some species of mycorrhizal fungi. A question was raised if selection as well as adaptation of mycorrhizal fungi developed in habitats with adverse characteristics.

The aim of this study was to determine whether ectomycorrhizal fungi appearing in contaminated environments with high amounts of heavy metal concentrations adapt to this contamination and survive. It is noteworthy whether their sensitivity was analogous to fungi growing in unpolluted environments.

Materials and Methods

Selection of Experimental Areas

Fungi isolated to determinine the sensitivity to a heavy metal pollution were taken from two areas located near industrial centers and from two sites outside emission regions that were classified as unpolluted. The area called Miasteczko Śląskie is located in the immediate vicinity of zinc and lead smelters. The area called Huta Katowice is located in a fresh coniferous forest to the north-west of the iron smelter "Huta Katowice". The areas classified as unpoluted are located in Długie [a town in Radom province], and in Potworów commune, 33 km the north-west of Radom. There are no large towns or industrial centers. The second area of low soil contamination is Sądów, a town in the Cybinka district of Zielona Góra province. It is about 65 km north-west of Zielona Góra, and within a radius of 25 km there are no large towns or industrial plants. All the areas are overgrown with fresh, thin coniferous forest.

Selection of Mycorrhizal Fungi Species

The appearance of fruiting bodies of cap fungi in the areas studied was observed in late autumn 1994. The species most frequently found in the vicinity of pines were: *Xerocomus badius (Fr.)Kuhn., Paxillus involutus (Batsch.) Fr., Xerocomus submentosus (Fr.) Kuhn ex Gilb.* and *Russula xerampelina (Shaeff.) Fr.* In the vicinity of birches, the appearance of fruiting bodies of *Amanita muscaria* and *Amanita citrina* was observed in all the areas. The species found in all the areas both contaminated and uncontaminated were *Xerocomus badius* and *Amanita muscaria.* They were selected for further experiments. Healthy fruiting bodies of *Xerocomus badius* and *Amanita muscaria* sized from 10 -14 cm in height and 5 cm of cap in diameter were picked from all the areas and the clean culture of vegetative mycelium was sampled using the tissue method proposed by Pachlewski [9]. It is based on the isolation under of plectenchyma pieces sized 0.5×0.5×0.5 cm from the center of the cap under sterile conditions and then cultivated in tubes using Pachlewski (Pp) growth medium [7] containing agar-15g, glucose-20 g, maltose-5g, $(NH_4)C_4H_4O_6$ - 0.5g, KH_2PO_4 - 1g, Tiaminum 100 μg/l.

The isolates were transfered onto fresh agar in tubes until some clean cultures were obtained.

Determination of Strains Sensitive to Chosen Heavy Metals

To determine the sensitivity of selected strains to heavy metals, increased doses of zinc, cadmium and lead ions were added to the agar growth medium. Isolated cultures of vegetative fungi were inoculated on Petri dishes with this medium. The doses of the metal ions were gradually increased to determine growth characteristics, adaptation levels and the moment of the mycelium necrosis. In order to determine the growth rate, a comparative scale of two-week-old cultures were used in relation to the control based on the Pachlewski method [8].

Growth Rate Scale Used in Work

from 0.0 to 0.5 -very slow growth
from 0.5 to 1.5 - slow growth
from 1.5 to 1.5 - intermediate growth , slower than control
from 2.5 to 3.0 - fast growth
from 3.0 to 4.0 - very fast growth

Results

Table 1 illustrates chemical analysis results of chosen research area soils.

Table 1 . Total concentration of: zinc, cadmium and lead ions in the surface soil (0-5) of selected research areas.

Spot	Content of Zn [μg/g]	Content of Cd [μg/g]	Content of Pb [μg/g]
	Places with high level of contamination		
Miasteczko Śląskie	2776.56	57.75	1551.55
Dąbrowa Górnicza [Huta Katowice]	1259.28	15.07	978.13
	Control places with low level of contamination		
Długie	81.51	2.44	23.19
Sądów	11.24	3.171	15.83

Figures 1-6 show the growth rate of vegetative mycelium of *X. badius* and *A. muscaria* related to the location of the strains' origins and concentrations of heavy metals in the growth medium. Zinc as a constituent element of many enzymes showed adverse effects on the growth rate of microorganisms only at high concentrations. A small addition of zinc had a stimulating effect on some fungi cultures (*A. muscaria* from Miasteczko Śląskie and from Długie, *X. badius* from M. Śląskie and Huta Katowice -Figs.1 and 4).

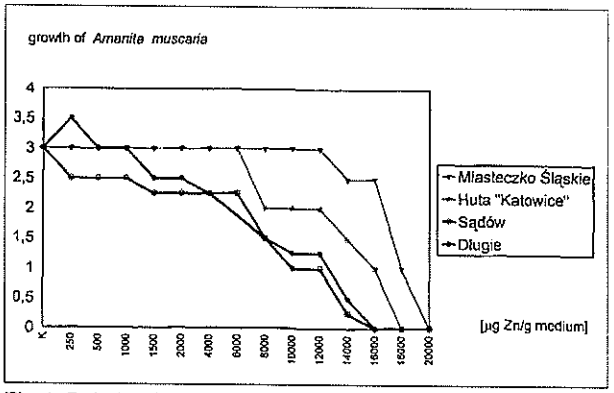


Fig. 1. Relationship between the growth of two week's old cultures of *A. Muscaria* and zinc concentration in the medium.

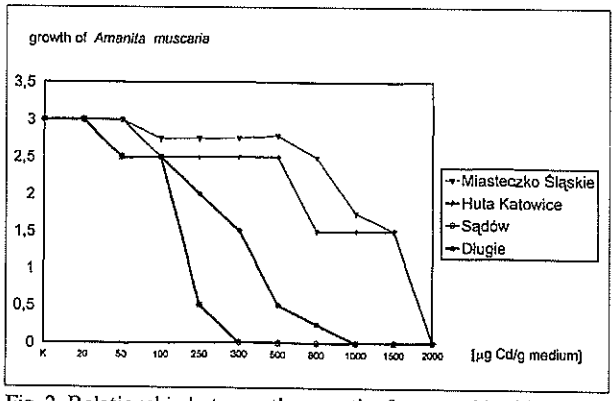


Fig. 2. Relationship between the growth of two week's old cultures of *A. Muscaria* and cadmium concentration in the medium.

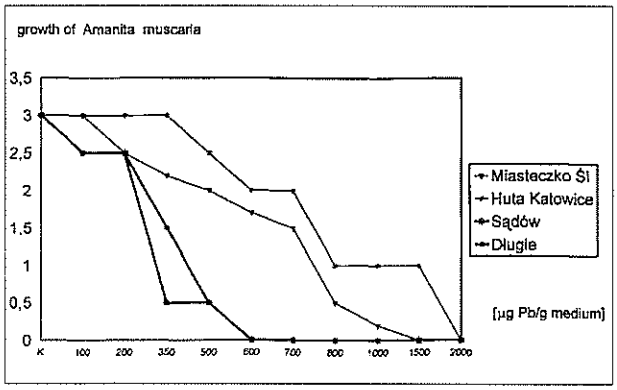


Fig. 3. Relationship between the growth of two week's old cultures of *A. Muscaria* and lead concentration in the medium.

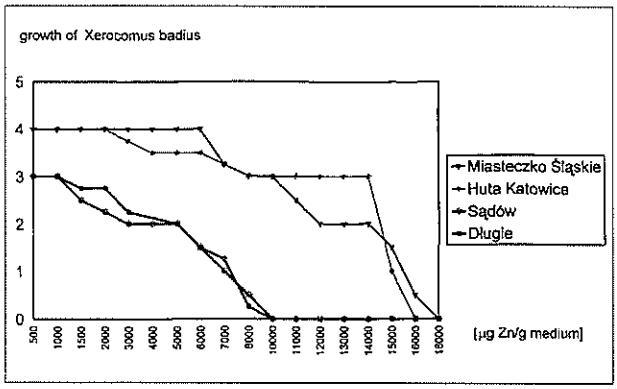


Fig. 4. Relationship between the growth of two week's old cultures of *X. Badius* and zinc concentration in the medium.

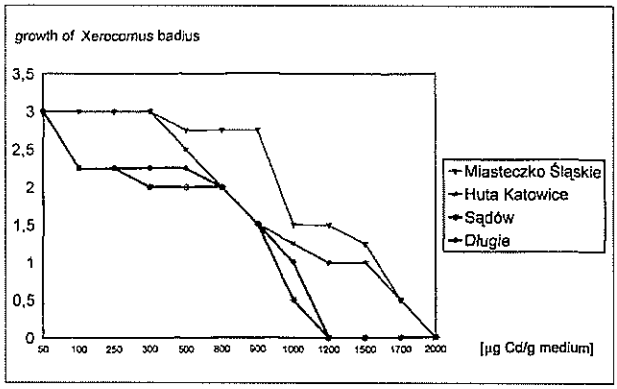


Fig. 5. Relationship between the growth of two week's old cultures of *X. Badius* and cadmium concentration in the medium.

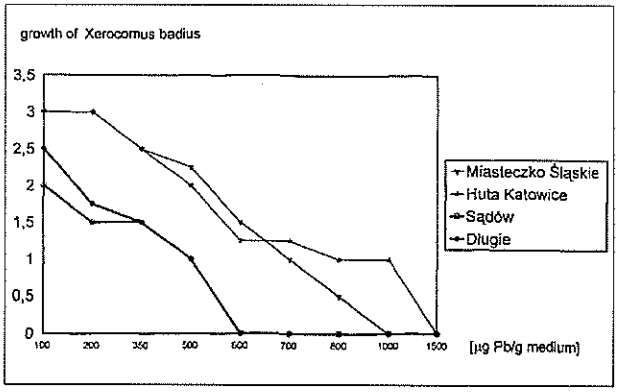


Fig. 6. Relationship between the growth of two week's old cultures of *X. Badius* and lead concentration in the medium.

Comparing tolerance levels to high concentrations of zinc, cadmium and lead by vegetative mycelium of *X. badius* and *A. muscaria*, significant differences were found between the strains. The most tolerant were the strains from the areas highly contaminated by heavy metals (e.g. Miasteczko Śląskie, Huta Katowice), which showed slow growth rate at concentrations of 1700 µgCd/g and 1500 µgPb/g in the growth medium (Figs. 2 and 5). Significantly lower tolerance was shown by the strain from Sądów that died at concentrations of 10,000 to 12,000 µg Zn/g, 1000 to 1200 µg Cd/g and 600 µg Pb/g in the growth medium.

Discussion of Results

The technique of isolation and cultivation of ectomycorrhizal mycelium in clean cultures, introduced by Melin at the turn of the century, forms the general model for physiological research of fungi growth and proliferation. It is also the method of verification under laboratory conditions of observation taken in the field. It is generally used as the method in the research on the influence of heavy metals on fungi. For example, Colpaert and Van Asche [3] studying the influence of cadmium on strains of *Suillus bovinus* and *Suillus luteus*, concluded that strains originating from contaminated soil were more tolerant to cadmium than those from clean soil. However, the isolates obtained from contaminated soils better protected the host in the presence of zinc. The results obtained in this study are similiar to those from some works performed with *Amanita muscaria* and *Xerocomus badius*.

The conclusion must not, however, be reached without deeper consideration because laboratory conditions eliminate many factors that may be of significance in natural habitats and also in vitro tests as proved by Jones and Hutchinson [6]. They indicated that in vitro research on ectomycorrhizal fungi have little in common with their behaviour in natural symbiosis.

They isolated fungi from contaminated regions in the vicinity of Sudbury and from nonpolluted areas. However, in further studies on isolates of *Laccaria laccata*, *Lactarius rufus* and *Scleroderma flavidum* in artificial cultures they found no correlation between the growth and tolerance of these fungi and their place of origin. The fungus *Scleroderma flavidum* was unable to proliferate in vitro as vegetative mycelium even low nickel concentrations, although it did so in regions with very high soil contamination. Contradictions between the results of various authors may be superficial as the adaptation ability of fungi may be directly linked to the species and even the strain of mycorrhizal fungi. It is suggested to adopt a rule that the result of negative adaptation does not exlude a species from being suitable for inoculation in regions contaminated by heavy metals, and the positive result may suggest a species being suitable. It is important to determine if the in-

Table 2. Analysis of Variance (new.sta); marked effects are significant at $p<0.05$

Stat. Basic	Analysis of Variance p<0.05		
	df Error	F	p
A. muscaria and zinc concentration	15*	6.3601*	0.00049*
A. muscaria and cadmium concentration	20*	4.4271*	0.00086*
A. muscaria and lead concentration	20*	6.3513*	0.00067*
X. badius and zinc concentration	20*	5.7838*	0.00013*
X. badius and cadmium concentration	20*	2.2047*	0.04354*
X. badius and lead concentration	20*	4.4686*	0.00086*

creased tolerance is of fenotypic or genotypic origin. Of importance is also the form in which the metal was used in vitro and in which it exists in the soil. Although certain questions still remain unanswered, it seems that detailed in vitro analysis of the survival of various fungi in contaminated regions allow to use certain fungi species as vaccines improving the resistance mechanisms of plants in response to increased metal concentration in soil.

Statistica

Statistically significant differences between means was confirmed by analysis of variance at $p<0.005$. Results are shown in Tab. 2.

Conclusions

Strains of mycorrhizal fungi isolated from regions highly contaminated with heavy metals are more resistant to industrial contamination than the strains from uncontaminated areas. We may conclude that strains of *Amanita muscaria* and *Xerocomus badius* obtained from contaminated regions were adapted to high levels of Zn, Cd and Pb. The degree of adaptation is in direct proportion to the level of contamination of the area from which the strain of mycorrhizal fungi originated.

References

1. BRADLEY R. Mycorrhizal infection and resistance to heavy metal toxicity in Calluna vulgaris. Nature, **292**, 335-337, **1981.**
2. BROWN M.T. Zinc tolerance of mycorrhizal Betula. New Phytol. **99**, 101-106, **1985.**
3. COLPERT J.V., VAN ASSCHE J.A. The effects of cadmium on ectomycorrhizal Pinus sylvestris L.. New Phytol **123**, 325-333, **1993.**
4. CZAJKOWSKA J. Mikoryza roślin użytkowych. PWN, Warszawa, **1988.**
5. JONES M.D. The effect of mycorrhizal infection on the response of *Betula papyrifera* to nickel and copper. New Phytol. **102**, 429-442, **1986.**
6. JONES M.D. The effects of nickel and copper on the axenic growth of ectomycorrhizal fungi. Can.J.Bot. **66**, 119-124, **1988.**
7. PACHLEWSKI R. Rhizopogon luteolus FR. w syntezie mikoryzowej z sosną /Pinus silvestris L./ w czystych kulturach na agarze. Warsaw, For Res. Inst. **346**,78-95, **1968.**
8. PACHLEWSKI R., CHRUŚCIAK E. Oddziaływanie ołowiu i cynku na wzrost niektórych grzybów mikoryzowych w kulturach in vitro. Acta Mycologica. **XXII (1)**, 73-80, **1986.**
9. PACHLEWSKI R., PACHLEWSKA J. Studies on symbiotic properties of mycorrhizal fungi of pine /Pinus silvestris L./ with the aid of the method of mycorrhizal synthesis in pure cultures on agar. Warsaw, For Res. Inst. **1974.**
10. RUDAWSKA M. Mikoryza in: Biologia sosny zwyczajnej. PWN Kórnik **1993.**
11. TURNAU K. Mikoryza w siedliskach skażonych metalami toksycznymi. Wiadomości Botaniczne. **37 (1/2)**, 43-58, **1993.**