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Accumulation and Effect of Cadmium in the Wood-Rotting Basidiomycete *Daedalea quercina*

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The ability of fungi to accumulate metals is a known phenomenon that is studied from both the industrial and ecological point of views. The biosorption and removal of various cations could be useful in recovery of precious or strategic metals (e.g. Nakajima and Sakaguchi 1993) as well as in the removal of toxic heavy metals from contaminated water (Siegel *et al.* 1990). The most frequently used group of organisms are filamentous fungi (e.g. Siegel *et al.* 1990, Pümpel and Schinner 1993) which are widely used in fermentation industries to produce varied metabolites. The application of mycelial wastes as adsorbents or ion-exchangers for the removal of heavy metals represents a possibility for further utilization of these biotechnological by-products (Voleský 1994). So far, little attention has been paid to interactions of heavy metals with higher fungi. Most of the work deals with metal translocation and uptake from various substrates or with the heavy metal content in fruiting bodies collected in different areas. In several cases this content seems to reflect the concentrations of atmospheric heavy metal.

Most wood-rotting basidiomycetes can be found in high yields and are easily cultivated allowing various model experiments such as the investigation of metal translocation and uptake or the study of heavy metal induced changes in fungal morphology and biochemistry. The effect of Cd, Zn and some other metals on the growth of some ectomycorrhizal fungi (Darlington and Rauser 1988, Colpaert and Van Assche 1992) and hyphomycetes (Rózycki 1993, Failla and Niehaus 1986) have been investigated. Recently, element distribution in mycelium of *Pisolithus arrhizus* (PERS.) RAUSCH. treated with Cd dust has been described (Turnau *et al.* 1994). Some alterations in mycelial morphology were reported by Lilly *et al.* (1992) who found the loss of hyphae orientation and decrease of clamp connections in mycelium of wood-rotting basidiomycete *Schizophyllum commune* FR.:FR. treated with milimolar concentrations of Cd. When submerged mycelial pellets of this fungus where cultivated in the presence of lead, color change was documented (Gabriel *et al.* 1994).

The aim of this work was to study both the biosorption of Al, Cd, Cu, Pb, and Zn in native fungal pellets of the wood-rotting basidiomycete *Daedalea*

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quercina and the investigation of its stress response to high concentrations of Cd ions.

MATERIALS AND METHODS

The basidiomycete *Daedalea quercina* (L) PERS. was a strain deposited in the Culture Collection of the Basidiomycetes, Institute of Microbiology, Academy of Sciences of Czech Republic (CCBAS 558) and was grown and maintained in solid or liquid GC medium containing glucose 20 (g/L), corn-steep (7 g/L), and agar (2 g/L). The 250 mL cultivation flasks containing 70 mL of medium supplemented with the appropriate amount of metal salt were shaken (reciprocal frequency, 2 Hz) at 28°C. Heavy metal stock solutions were prepared from $Cd(NO_3)_2 \cdot 4H_2O$, $CuSO_4 \cdot 5H_2O$, $KAl(SO_4)_2 \cdot 12H_2O$, $Pb(OAc)_2$ and $ZnSO_4 \cdot 7H_2O$ in concentrations of 1 mol/L or 0.1 mol/L and the appropriate volume was added to each cultivation flask before sterilization. The flasks were inoculated with equal amounts of the inoculum prepared by 5-days cultivation in liquid GC medium. Cultures were harvested as follows: flasks were collected, filtered, and mycelia were washed on a filter with 250 mL of distilled water. Mycelia were then re-suspended in 1 L of distilled water and decanted using 2 L of distilled water. Mycelial dry weight, pH of cultivation liquid, and content of heavy metals in mycelia were determined in each experiment.

The effect of pH on cadmium sorption was studied using 4-day old mycelium, grown on standard liquid GC medium. Mycelium was spread into cultivation flasks containing 1 mM solution of cadmium(II) nitrate in citrate buffers of pH 3.00, 4.00, 5.00, 6.00, and 7.00, respectively. After 12 hr of incubation on reciprocal shaker at 28°C the mycelia were harvested and washed as described above and the Cd contents were measured.

Fruiting bodies of *D. quercina* were collected in both heavily polluted areas (the center of Prague) and in other localities far from the industrial sources of air pollution (in the National Park Šumava in Southern Bohemia). The selection of these two areas was based on the data on average concentrations of SO_2, NO_x, C_xH_y, and solid deposition (in $kg.yr^{-1}.km^{-2}$) reported (Héniková and Beneš 1994) for years 1993-1994.

Heavy metal content was determined by atomic absorption spectrometry. Dried samples were combusted by gradual temperature increase from 150°C to 350°C (50°C/h) and then twice dissolved in concentrated HNO_3 and evaporated to dryness. Dry samples were again taken up in HNO_3 and analyzed on Spectr AA 300A (Varian, Melbourne, Australia) at wavelengths 213.9 nm (Zn), 228.8 nm (Cd), 283.3 nm (Pb), 327.4 nm (Cu), and 396.2 nm (Al). Deuterium background correction was used for Cd and Pb.

Mycelial pellets were fixed in 3% glutaraldehyde prepared in cacodylate buffer (pH 7.2) for scanning electron microscopy. Fixation time was 1 hr at room temperature. After washing in cacodylate buffer, the pellets were dehydrated through an ethanol/water series. The samples were then critical-point dried using liquid carbon dioxide as the transitional fluid, mounted on aluminium stubs by means of conductive silver paint, sputter-coated with gold

in Polaron coating unit, and viewed in a Tesla BS 300 scanning electron microscope.

RESULTS AND DISCUSSION

Biosorption of heavy metals in submerged cultures of the basidiomycete *D. quercina* was studied with Al, Cd, Cu, Pb and Zn. Similar to previously reported experiments, metal content in mycelia increased with increasing metal content in culture media (Table 1). The growth of the fungus was strongly inhibited in metal concentrations higher than 5.10^{-3} mol/L.

Table 1. Metal content in mycelium of *D. quercina* (ppm) after a 7-d cultivation on liquid medium containing different concentrations of metals. Means and standard deviations from three cultivations.

	Concentrations in culture liquid (mol/L)				
Metal	10^{-7}	10^{-6}	10^{-5}	10^{-4}	10^{-3}
Al	9 ± 0.5	22 ± 1.2	70 ± 3.0	130 ± 10.1	1910 ± 312.0
Cu	21.7 ± 1.5	27.7 ± 4.2	36.5 ± 4.5	101.7 ± 6.8	4997.0 ± 350.2
Pb	8.6 ± 1.1	22.0 ± 1.5	122.3 ± 8.9	3065.5 ± 240.0	3632.5 ± 420.0
Zn	50 ± 4	158 ± 12	263 ± 35	530 ± 48	13556 ± 850

The Cd uptake was also studied in detail and the relationship between concentration of Cd in cultivation broth and Cd content in dried mycelium is shown in Fig. 1. Concentrations below 0.1 mmol/L Cd in the fermentation broth showed only negligible content of the metal in dried mycelia. In the fermentation broth containing 5 or 10 mmol/L Cd, no growth was observed; after 7 days only dead inoculum was presented in the flasks.

The effects of pH on Cd biosorption was studied using 1 mmol/L Cd in citrate buffers. The relationship between pH and Cd uptake exhibits a maximum at pH 6.0. At pH values 3, 4, 5, and 7, 22%, 54%, 81%, and 98%, respectively until the maximum value was achieved. Optimum pH found for Cd biosorption lies around 6.0. The same pH value was reported by Nakajima and Sakaguchi (1993) for uranium biosorption in basidiomycete *Tricholoma conglobatum*. The optimum pH for silver biosorption in *Penicillium* sp. has been reported at about 7 (Pümpel and Schinner 1993), as well as for Zn and Ni in *Rhizopus arrhizus*, while the optimum pH value for Pb biosorption in the same fungus was about 5.0 (Fourest and Roux 1992).

Wood-rotting fungi, contrary to lichens, grow and fructificate in industrial areas with high levels of atmospheric SO_2 and NO_x pollution. We collected fruiting bodies of *D. quercina* in both polluted and unpolluted areas. Average Cd content (10 samples in each group) was found to be 0.56 ± 0.53 ppm in samples from the polluted areas and 0.30 ± 0.25 ppm in samples from the unpolluted areas. For a recent model of atmospheric Cd deposition in Europe see Stanners and Bordeau (1995).

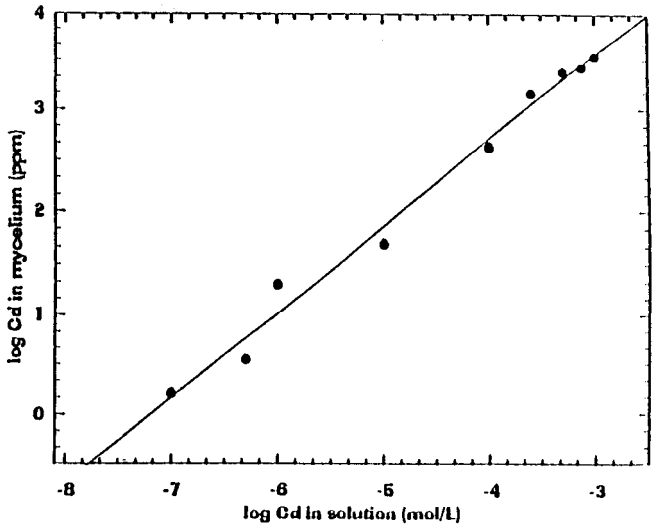

Figure 1. Cadmium uptake by submerged cultures of *D. quercina.* Parameters of linear regression equation Y = A + B*X: A = 6.12309 (SD, 0.18247), B = 0.85058 (SD, 0.03793), r^2 = 0.98627

Submerged cultivation of *D. quercina* in the presence of 1 mM Cd led to the mycelial content of about 400 ppm (Fig. 1). This value is many times higher that the Cd content of our samples collected in heavy polluted areas. The effect of 0.1 milimolar concentrations of Cd on the growth of fungus was investigated with liquid medium. Differences in the growth curves on the GC medium are shown in Fig. 2. The cultures maximum growth curve with Cd was reached two days latter than in the control experiment. The absolute mycelial weights were about two times lower than in the blank.

Milimolar concentration of Cd influenced not only the growth rate of the mycelial culture but also caused significant differences in the appearance of fungal pellets; pellets grown on Cd were brownish-yellow while the control mycelium was white. Variations in the morphology of the mycelial pellets induced by the presence of Cd salts were determined by means of scanning electron microscopy. This study confirmed that control mycelium is formed by branching septated hyphae. Numerous clamp connections were found on the mycelial hyphae (Fig. 3). The presence of Cd in the growth medium reduced the quantity of clamp connections on the mycelial hyphae and limited their branching. Hyphae were also shorter and thicker than in the control experiment (Fig. 4).

Previously we described color change in the basidiomycete *Schizophyllum commune* grown on lead nitrate; controls were white and Pb-treated samples were dark (Gabriel *et al.* 1994). Mycelial pellets of *D. quercina* harvested from 1 mmol/L Cd had a brownish-yellow color while the control samples remained white. The nature of these changes cannot be discussed without a detailed chemical analysis. Formation of melanins or other organic pigments or biosorption of precipitated inorganic salts should be considered.

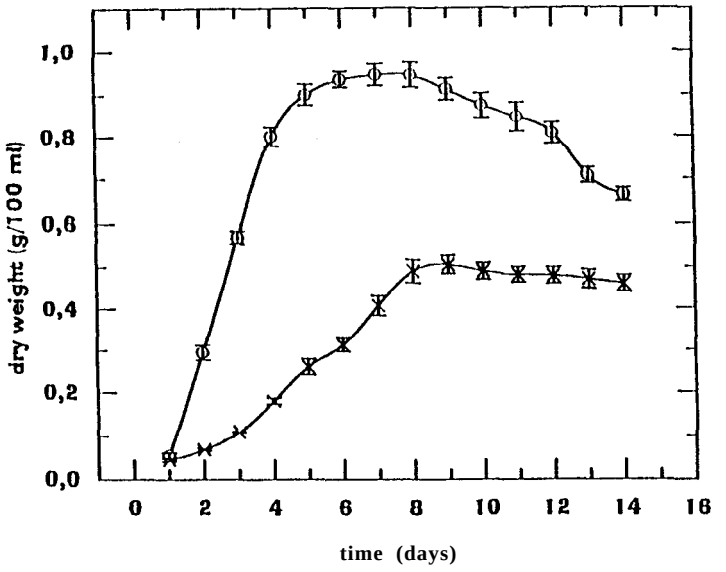

Figure 2. Growth curve of *D. quercina* grown on 0.1 mM cadmium nitrate (bottom curve) and control (top).

The changes in mycelial morphology caused by Cd have been recently studied with the basidiomycete *S. comnune* (Lilly *et al.* 1992) grown on agar plates. The loss of hyphal growth orientation was observed. Darlington and Rauser (1988) have reported increased branching frequency in Cd-treated mycelium of the basidiomycete *Paxillus involutus* (BATSCH.:FR.) FR. Increasing density of the mycelium with increasing concentration of Cd also has been reported (Colpaert and Van Assche 1992) for the basidiomycete *Suillus bovinus;* the authors explained this phenomenon by local exposure of hyphae to different concentrations of Cd in a dense mycelium and in a sparse mycelium. On the contrary in our experiments with submerged cultivations, hyphae orientation of Cd-treated pellets exhibited higher uniformity than control cultivations and Cd-treated hyphae were shorter and thicker (Fig. 4B). Nevertheless, it may result in the same decrease in local Cd concentration, as it was suggested for solid state cultivation. Moreover, thickness of the cell wall can play a role in Cd tolerance of fun i grown in polluted areas. In the comparative experiment with two strains of *S. bovinus* isolated from polluted and unpolluted soil, Colpaert and Van Assche (1992) found that the latter isolate accumulated much more Cd from the medium than the isolate from polluted soil. The authors considered that fungal strains growing in strongly polluted soils are better equipped to withstand elevated concentrations of heavy metals. So far it is not clear whether it can be extended to fungi growing on wood.

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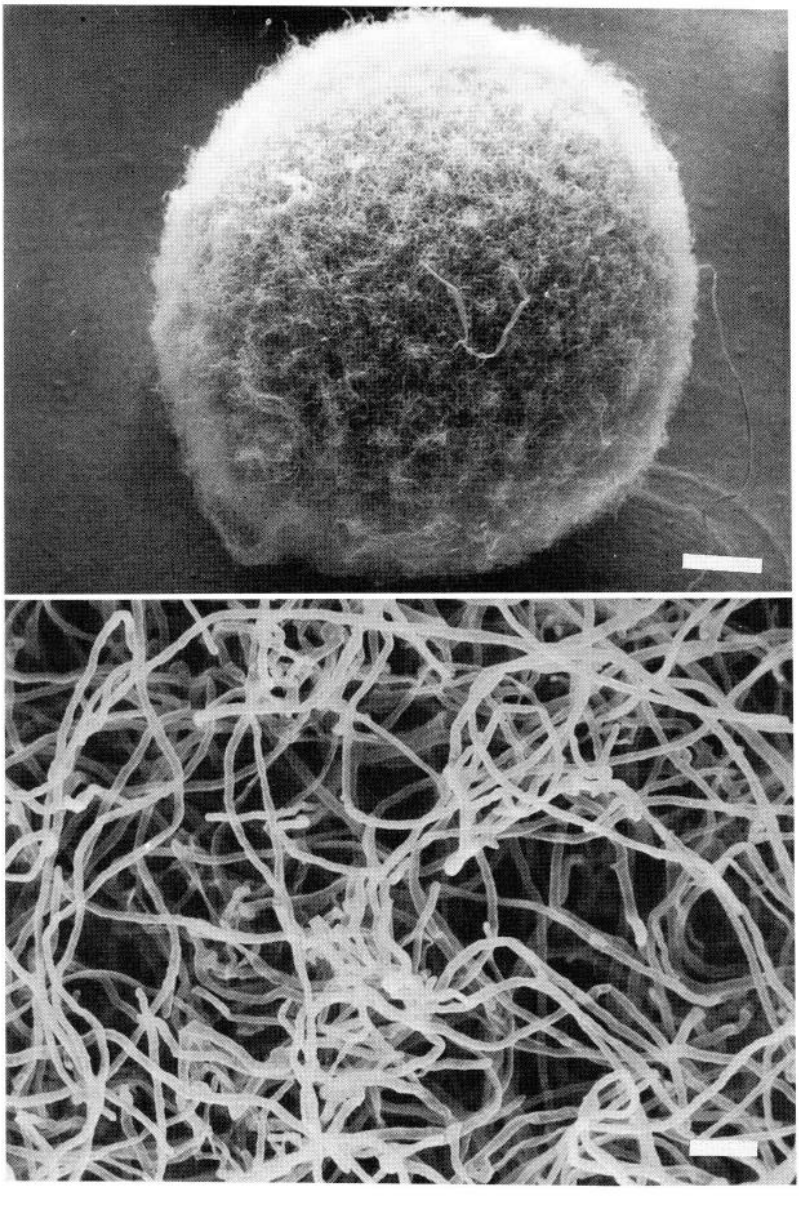

Figure 3. SEM micrographs of control *D. quercina* pellets. Bar represent 200 µm (A /upper/, magnif. 60x) and 10 µm (B /lower/, magnif. 1000x).

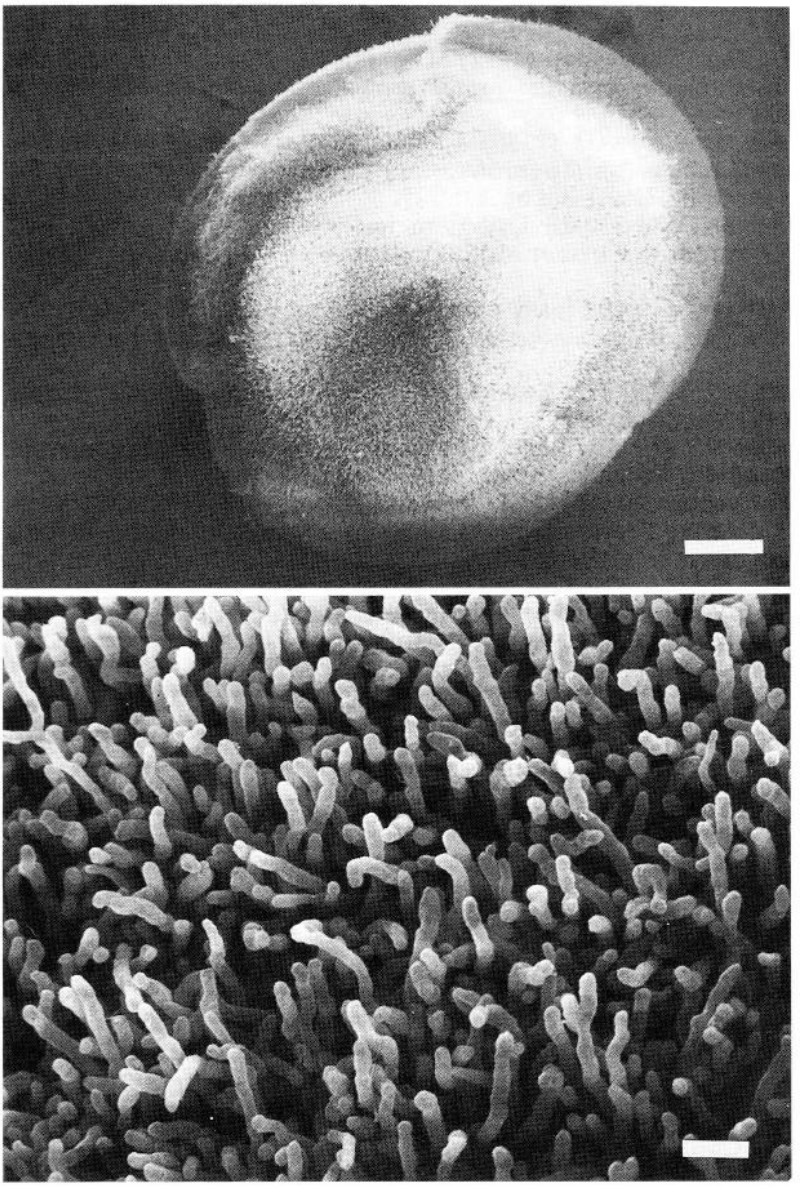

Figure 4. SEM micrographs of *D. quercina* pellets from cultivation on 1.10^{-4} mol/L $Cd(NO_3)_2$. Bar represent 200 µm (A /upper/, magnif. 60x) and 10 µm (B /lower/, magnif. 1000x).

REFERENCES

Colpaert JV, Van Assche JA (1992) The effects of cadmium and the cadmium-zinc interaction on the axenic growth of ectomycorrhizal fungi. Plant and Soil 145:237-243.

Darlington AB, Rauser WE (1988) Cadmium alters the growth of the ectomycorrhizal fungus *Paxillus involutus:* a new growth model accounts for changes in branching. Can J Bot 66:225-229.

Failla LJ, Niehaus WG (1986) Cadmium ion stimulation of growth and versicolorin synthesis in a mutant strain of *Aspergilus parasiticus.* Exp Mycol 10:144-149.

Fourest E, Roux J-C (1992) Heavy metal biosorption by fungal mycelial by-products: mechanisms and influence of pH. Appl Microbiol Biotechnol 37:399-403.

Gabriel J, Mokrejš M, Bílý J, Rychlovský P (1994) Accumulation of heavy metals by some wood-rotting fungi. Folia Microbiol 39: 115-118.

Héniková S, Beneš J (1994) Annual Report on the State of Environment in the Czech Republic. Czech Institute of Ecology (in Czech).

Lilly WW, Wallweber GJ, Lukefahr TA (1992) Cadmium absorption and its effects on growth and mycelial morphology of the basidiomycete fungus, *Schizophyllum commune.* Microbios 72:227-237.

Nakajima A, Sakaguchi T (1993) Accumulation of uranium by basidiomycetes. Appl Microbiol Biotechnol 38:574-578.

Pümpel T, Schinner F (1993) Native fungal pellets as biosorbents for heavy metals. FEMS Microbiol Rev 11: 159-164.

Rózycki H (1993) Effect of heavy metals (Pb, Zn, Cu and Cd) on mycelial growth of *Cylindrocarpon destructans* (ZINSSM.) SCHOLTEN. Zentralbl Mikrobiol 148:265-275.

Siegel SM, Galun M, Siegel BZ (1980) Filamentous fungi as metal biosorbents: a review. Water Air and Soil Pollut 53:335-344.

Stanners D, Bordeau P (1995) Europe's Environment. EEA Copenhagen, Denmark.

Turnau K, Kottke I, Dexheimer J, Botton B (1994) Element distribution in mycelium of *Pisolitlhus arrhizus* treated with cadmium dust. Ann Bot 74: 137-142.

Voleský B (1994) Advances in biosorption of metals: Selection of biomass type. FEMS Microbiol Rev 14:291-302.