

Heavy Metal Content in Wood-Decaying Fungi Collected in Prague and in the National Park Sumava in the Czech Republic

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Accumulation of metals by fungi has been known for a few decades and a number of works describing metal content in fruit bodies collected in different areas have been published (Mejstrik and Lepšová 1993). A key role in metal accumulation by fungi has been attached to cell wall polysaccharides, cysteine-rich proteins and pigments like melanin (Siegel *et al.* 1990). Some higher fungi are known to have the ability to accumulate toxic elements such as As, Cd or Pb from the environment (Stijve *et al.* 1990, Vetter 1994, Tyler 1982). Heavy metal content in many terrestrial fungi correlates with metal concentration in the soil in which they grow (Gast *et al.* 1988). In the case of edible fungi, toxic metals may be incorporated into food chains.

Fungal species growing on wood contain, in general, lower concentrations of heavy metals than fungi growing on soil (Mutsch *et al.* 1979), probably due to limited contact of mycelia with the soil. Nevertheless, wood-inhabiting fungi growing in polluted areas may contain higher amounts of toxic metals than fungi growing in unpolluted areas, as we demonstrated for beryllium (Gabriel *et al.* 1995) previously. Wood-decaying fungi take up heavy metals by deposition of particles from the atmosphere and absorption from the substrate. Literature data indicate that heavy metal content decreases from soil through roots to stems (Salt *et al.* 1995). Earlier experiments (Brunnett and Zadrazil 1981, Gabriel *et al.* 1996a) confirmed translocation of heavy metals from substrate into the fruiting bodies of lignocellulose decomposing fungi. Atmospheric dry or wet depositions represent another considerable source of metals in plants and plant related parasites or saprophytes (Hovmand *et al.* 1983).

The purpose of this work was to examine heavy metal content (Al, Cd, Cu, Pb and Zn) in six wood-decaying fungal species collected in polluted and unpolluted areas in the Czech Republic.

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MATERIALS AND METHODS

Fruit bodies of the basidiomycetes *Daedalea quercina*, *Stereum hirsutum*, *Schizophyllum commune*, *Ganoderma applanatum*, *Fomitopsis pinicola* and *Hirneola auricula-iudae* were collected in both the unpolluted (National Park Šumava) and polluted (Prague) areas in the Czech Republic between autumn 1994 and summer 1995. In all sites, fruit bodies of each studied species were collected together and subsequently processed as one sample. The data obtained from each sample thus represent an average for several individuals grown on one site.

Metal content was determined by atomic absorption spectrometry. Dried samples were homogenised, taken up in HNO_3 and after microwave digestion in MDS 2000 (CEM, Matthews, USA) analysed on Spectr AA 300A (Varian, Melbourne, Australia) at wavelengths 396.2 nm (Al), 228.8 nm (Cd), 327.4 nm (Cu), 283.3 nm (Pb), and 213.9 nm (Zn). Deuterium background correction was used for cadmium and lead.

RESULTS AND DISCUSSION

Sample sites in the National Park Šumava (southern Bohemia), area with negligible industrial pollution were designated as unpolluted. The area of Prague was designated as polluted, based on the data on atmospheric deposition; the average concentration of solid particles in atmospheric deposition is $60 \mu\text{g.m}^{-3}$ and locally exceeds $100 \mu\text{g.m}^{-3}$ per year in Prague. Emissions of SO_2 and NO_x were 83.9 tons per year and km^2 and 75.5 tons per year and km^2 in 1992, respectively. The same data reported for sulphur and nitrogen oxides in southern Bohemia were 4.4 tons per year and km^2 and 2.7 tons per year and km^2 (Bozó *et al.* 1992).

Wood-decaying fungi do not photosynthesise and therefore they are not particularly sensitive to elevated concentrations of sulphur and nitrogen oxides compared with lichens or mosses and they can grow and fructificate in heavily polluted areas. As they mainly take up heavy metals from the atmosphere, fruit bodies of wood-decaying fungi seem to be useful indicators of atmospheric pollution by heavy metals. The use of these fungi as bioindicators of environmental pollution by heavy metals is limited by nutrient requirements of the studied species. Some wood-decaying fungi grow and fructificate only on particular plant species: *e.g.* *D. quercina* prefers oaks (*Quercus* sp.), *H. auricula-iudae* prefers elder (*Sambucus nigra*). This disadvantage may be overcome by collection of several fungal species chosen on the base of their natural distribution.

Fruit bodies of six common species of wood-decaying fungi were collected. Total number of samples from polluted areas was 94 and from unpolluted was 46. Averages and standard deviations of Al, Cd, Cu, Pb and Zn content for all fungal species together are given in the table below. Statistical treatment of the data by

t-test confirmed significant differences in Al, Cd, Pb and Zn content between both groups at the probability level of 95 %.

Table 1. Heavy metal content in all fungal samples ($\mu\text{g.g}^{-1}$).

Metal	Polluted area (Prague)	Unpolluted area (NP Šumava)
	$\bar{x} \pm S_x$	$\bar{x} \pm S_x$
Al	417.3 ± 66.6	110.4 ± 12.3
Cd	1.36 ± 0.17	0.78 ± 0.12
Cu	18.7 ± 2.2	12.7 ± 2.1
Pb	4.97 ± 0.50	0.97 ± 0.18
Zn	51.16 ± 2.88	67.12 ± 5.70

Numerous published works describing heavy metal content in fungi are devoted to edible or market species (Stijve and Bourqui 1991, Byrne and Tušek-Znidaric 1990). Only some of them deal also with wood-decaying fungi and those are briefly discussed below.

Distribution of Al in samples is given in Fig. 1. Values ranged from 10.0 to 4086.6 $\mu\text{g.g}^{-1}$ (polluted area) and from 4.1 to 560.0 $\mu\text{g.g}^{-1}$ (unpolluted area). Aluminum content in some *Polyporaceae* grown in southern Sweden (Tyler 1982) ranged from 20 to 79 $\mu\text{g.g}^{-1}$, *Xylaria hypoxylon* contained 104 $\mu\text{g.g}^{-1}$ Al and *Hypholoma hygrophilum* 7 $\mu\text{g.g}^{-1}$.

Distribution of Cd in samples is given in Fig. 2. Cadmium content ranged from 0.03 to 8.93 $\mu\text{g.g}^{-1}$ in samples from polluted areas and from 0.01 to 3.14 $\mu\text{g.g}^{-1}$ in samples from unpolluted areas. Previously reported data for wood-inhabiting fungi are mostly lower than 1 $\mu\text{g.g}^{-1}$: Tyler (1982) reported for some *Polyporaceae* range from 0.15 to 0.92 $\mu\text{g.g}^{-1}$. Vetter (1994) found for the same family range from 0.23 to 1.9 $\mu\text{g.g}^{-1}$. Range 0.3 - 0.5 $\mu\text{g.g}^{-1}$ was reported for *Pleurotus* sp. (Bano *et al.* 1981). Maximum value 4.5 $\mu\text{g.g}^{-1}$ found Tyler in *Pluteus cervinus*.

Fig. 3 shows distribution of copper in collected samples. Content of Cu in fungi ranged from 3.3 to 158.4 $\mu\text{g.g}^{-1}$ (polluted areas) and from 2.0 to 56.0 $\mu\text{g.g}^{-1}$ (unpolluted areas). Average Cu concentration in wood-decaying fungi found by Mutsch *et al.* (1979) was 19 $\mu\text{g.g}^{-1}$, two or three times lower than that in soil inhabiting species. Contents ranging from 1.6 to 10 $\mu\text{g.g}^{-1}$ reported by Tyler (1982) for some *Polyporaceae*, but *Hypholoma sublateralitum* contained 23 and *Pluteus cervinus* 26 $\mu\text{g.g}^{-1}$. In *Pleurotus* sp. Cu content ranged from 12 to 22 $\mu\text{g.g}^{-1}$ (Bano *et al.* 1981).

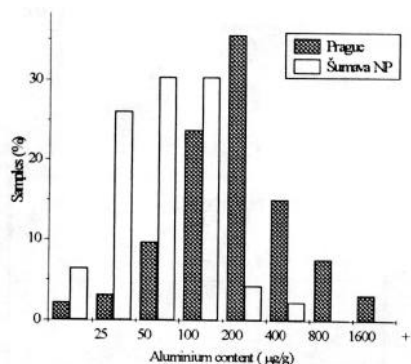


Figure 1. Distribution of Al in samples from Prague and from NP Šumava.

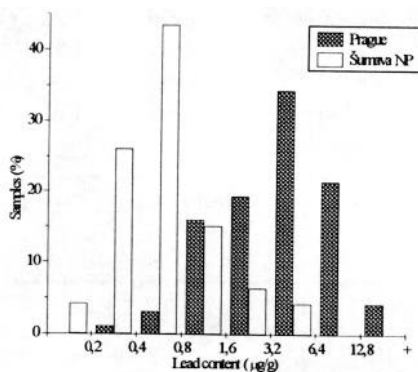


Figure 4. Distribution of Pb in samples from Prague and from NP Šumava.

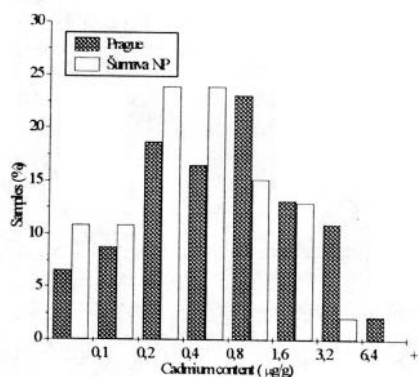


Figure 2. Distribution of Cd in samples from Prague and from NP Šumava.

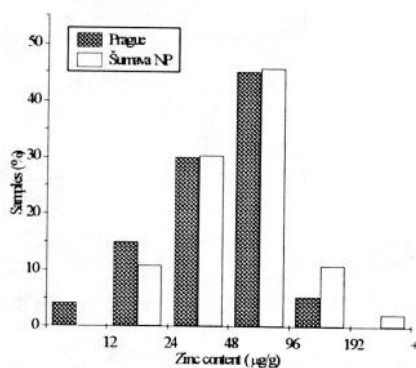


Figure 5. Distribution of Zn in samples from Prague and from NP Šumava.

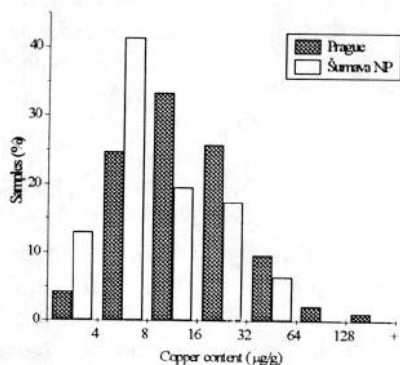


Figure 3. Distribution of Cu in samples from Prague and from NP Šumava.



Figure 6. Location of sample sites.

The distribution of lead content in samples is shown in Fig. 4. Minimum and maximum values were 0.4 and 35.2 $\mu\text{g.g}^{-1}$ for samples from polluted areas and 0.04 and 5.4 $\mu\text{g.g}^{-1}$ for samples from unpolluted areas. Tyler (1982) reported Pb concentrations from 0.7 to 6.4 $\mu\text{g.g}^{-1}$ for some *Polyporaceae*, with the maximum value in *Polyporus radiatus*. Strmisková *et al.* (1992) found only 0.019 $\mu\text{g.g}^{-1}$ lead in oyster mushroom (*Pleurotus ostreatus*). However, in this case the samples were obtained from the cultivated mushroom.

Contrary to all previously metals discussed, content of Zn in samples from unpolluted areas was significantly higher than that in samples from polluted areas. Distribution of zinc concentrations in samples is given in Fig. 5. Minimum and maximum values were 10 and 160 $\mu\text{g.g}^{-1}$ for samples from polluted areas and 15 and 241 $\mu\text{g.g}^{-1}$ for samples from unpolluted areas. Mutsch *et al.* (1979) analysed 12 species of wood-decaying fungi and reported average value 44 $\mu\text{g.g}^{-1}$. Three to four times higher contents were found in soil inhabiting species. Tyler (1982) reported for some *Polyporaceae* Zn content in the range from 9 to 116 $\mu\text{g.g}^{-1}$. Fungi are known as zinc accumulators - sporophore:substrate ratio for Zn ranges from 1 to 10. Bano *et al.* (1981) found from 59 to 130 $\mu\text{g.g}^{-1}$ Zn in *Pleurotus* sp.

The main sources of atmospheric pollution by toxic metals are mining and burning of oil or coal and industrial facilities, mainly smelters. Lepšova and Král (1988) measured Cd and Pb content in fruit bodies of mycorrhizal and edible fungi grown in the vicinity of a lead smelter. The authors found concentrations in samples decreasing with the increasing distance from the stack. The main source of Pb in the lower part of atmosphere are motor vehicles. Point sources of lead contamination are mainly smelters. Mosehlom *et al.* (1992) reported about 15.3 mg.m^{-2} as monthly deposition in the near vicinity of the car battery factory in Denmark; reference background for the area was 2.4 mg.m^{-2} . Our results are in order with Pb distribution pattern: maximum value was found in a sample grown near a railway station with a high density of bus traffic. In general, higher Pb contents among samples collected in unpolluted areas were always found in the vicinity of roads or settlements.

Zinc is - due to its biological significance - widespread among living organisms. The average Zn content in terrestrial fungi is about 100 $\mu\text{g.g}^{-1}$ d.w. which is little higher than that in most vascular plant tissues (Mejstřík and Lepšova 1993). In this case, zinc taken up from woody substrate may be an important part of the total Zn content in fungus. The main sources of Zn in the lower atmosphere over Eastern and Central Europe are smelters, power plants and other large industrial facilities. Concentrations of all measured metals in individual species with exception of zinc, were higher in polluted areas than in unpolluted areas.

Laboratory experiments with cultures of wood-rotting fungi cultivated in the presence of equimolar concentrations of various metals revealed that fungi differ in their affinities to metals. For example, cultures of *D. quercina*, *S. commune* and

Table 2 Metal content in individual fungal species ($\mu\text{g}\cdot\text{g}^{-1}$). Data are represented as means and ranges.

Species	n	Area [*]	Al	Cd	Cu	Pb	Zn
<i>Daedalea quercina</i>	2	U	360.0 160.0-560.0	0.22 0.18-0.26	9.19 5.12-7.04	1.27 1.04-1.49	128.0 15.0-241.0
	13	P	630.9 48.0-3700.0	0.30 0.03-1.43	19.99 4.65-47.81	4.53 0.69-11.40	27.7 10.0-160.0
<i>Fomitopsis pinicola</i>	20	U	84.5 21.7-178.8	0.43 0.02-1.22	7.68 2.04-25.00	0.47 0.21-0.86	71.1 40.1-119.0
	2	P	85.1 70.2-100.0	1.41 1.32-1.49	11.87 7.93-15.81	4.12 0.74-7.49	58.5 47.0-70.0
<i>Ganoderma applanatum</i>	14	U	71.7 4.1-246.7	1.03 0.22-2.35	20.45 4.22-56.01	0.50 0.04-1.00	66.6 17.0-171.0
	5	P	228.1 61.6-510.0	1.88 0.82-3.39	24.48 5.65-60.77	2.19 1.00-2.33	55.0 28.0-80.0
<i>Hirneola auricula-iudae</i>	3	U	131.0 110.0-170.0	0.18 0.09-0.27	5.48 3.37-6.91	3.47 2.56-4.55	25.0 15.0-3.0
	27	P	425.3 45.7-4086.6	1.09 0.18-3.20	13.26 4.11-38.47	4.94 0.90-15.31	52.0 19.0-120.0
<i>Schizophyllum commune</i>	2	U	190.2 153.3-227.1	0.22 0.18-0.25	4.66 4.36-4.95	4.56 3.68-5.44	67.7 46.7-88.3
	20	P	352.9 96.0-990.7	1.25 0.09-8.93	27.73 3.38-158.38	7.97 0.36-35.24	61.8 21.7-99.0
<i>Stereum hirsutum</i>	5	U	178.4 112.1-203.9	2.30 0.73-3.14	21.12 7.85-48.59	1.31 0.73-4.36	53.4 17.0-88.0
	27	P	413.7 10.0-2681.9	2.14 0.20-8.29	16.24 3.26-38.86	3.55 0.85-9.88	52.5 17.0-138.0

^{*}) U = unpolluted (the National Park Šumava), P = polluted (the area of Prague)

S. hirsutum accumulated preferably lead, while cultures of *G. applanatum* accumulated aluminium (Gabriel *et al.* 1994). This fact, together with previously mentioned different nutrient requirements of different fungi may limit the use of wood-rotting fungi as bioindicators. On the other hand, these fungi are tolerant to high concentrations of toxic metals. In our previous experiments mycelium of *D. quercina* survived successfully even in the presence of millimolar concentrations of cadmium (Gabriel *et al.* 1996b). Finally, the influence of different nutrient requirements and different affinities to metals may be suppressed by collection of

several fungal species, As they fructify in one stand for many years, wood-rotting fungi may bring useful information esp. on trends of atmospheric pollution by metals in studied regions.

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REFERENCES

- Bano Z, Nagaraja K, Vibhakar S, Kapur OP (1981) Mineral and the heavy metal contents in the sporophores of *Pleurotus* species. Mushroom Newsletter Tropics 2:3-7.
- Bozó I, Alcamo J, Bartnicki J, Olendrzynski K (1992) Total deposition and budgets of heavy metals over Eastern Europe. Quart J Hungarian Meteor Serv 96:61-80.
- Brunnert H, Zadrazil F (1981) Translocation of cadmium and mercury into the fruit bodies of *Agrocybe aegerita* in a model system using agar platelets as substrate. Eur J Appl Microbial Biotechnol 12: 179-182.
- Byrne AR, Tušek-Znidaric M (1990) Studies of the uptake and binding of trace metals in fungi. Part I. Accumulation and characterization of mercury and silver in the cultivated mushroom, *Agaricus bisporus*. Appl Organometal Chem 4:43-48.
- Gabriel J, Mokrejš M, Bilý J, Rychlovský P (1994) Accumulation of heavy metals by some wood-rotting fungi. Folia Microbiol 39: 115-118.
- Gabriel J, Rychlovský P, Krenzelok M (1995) Beryllium content in some wood-rotting fungi in Czech Republic. Toxicol Environ Chem 50:233-236.
- Gabriel J, Capelari M, Rychlovský P, Krenzelok M, Zadrazil F (1996a) Influence of cadmium on the growth of *Agrocybe perfratilis* and two *Pleurotus* spp and translocation from polluted substrate and soil to fruitbodies. Toxicol Environ Chem
- Gabriel J, Kofronová O, Rychlovský P, Krenzelok M (1996b) Accumulation and effect of cadmium in the wood-rotting basidiomycete *Daedalea quercina*. Bull Environ Contam. Toxicol 57:383-390.
- Gast CH, Jansen E, Bierling J, Haanstra L (1988) Heavy metals in mushrooms and their relationship with soil characteristics. Chemosphere 17:789-799.
- Hovmand MF, Tjell JC, Mosbaek H (1983) Plant uptake of airborne cadmium. Environ Pollut Ser A, 30:27-38.
- Lepšová A, Král R (1988) Lead and cadmium in fruiting bodies of macrofungi in the vicinity of a lead smelter. Sci Tot Environ 76: 129-138.
- Mejstřík V, Lepšová A (1993) Applicability of fungi to the monitoring of environmental pollution by heavy metals. In: Markert B (ed) Plants as biomonitors, VCH, Weinheim, pp 365-378.
- Moseholm L, Larsen EH, Andersen B, Nielsen MN (1992) Atmospheric deposition of trace elements around point sources and human health risk

- assessment. I.: Impact zones near a source of lead emission. *Sci Tot Environ* 126:243-262.
- Mutsch F, Horak O, Kinzel H (1979) Spurenelemente in höheren pilzen. *Z Pflanzenphysiol* 94:1-10.
- Salt DE, Blaylock M, Kumar NPBA, Dushenkov V, Ensley BD, Chet I, Raskin I (1995) Phytoremediation: a novel strategy for the removal of toxic metals from the environment using plants. *Biotechnology* 13:468-474.
- Siegel SM, Galun M, Siegel BZ (1990) Filamentous fungi as metal biosorbents: a review. *Water Air Soil Pollut* 53:335-344.
- Stijve T, Vellinga EC, Herrmann, A (1990) Arsenic accumulation in some higher fungi. *Persoonia* 14:161-166.
- Stijve T, Bourqui B (1991) Arsenic in edible mushrooms. *Deuts Lebensmit Rundschau* 87:307-310.
- Strmisková G, Strmiska F, Dubravický J (1992) Mineral composition of oyster mushroom. *Nahrung* 36:210-212.
- Tyler G (1982) Metal accumulation by wood-decaying fungi. *Chemosphere* 11:1141-1146.
- Vetter J (1994) Data on arsenic and cadmium contents of some common mushrooms. *Toxicon* 32:11-15