CONTENTS OF MERCURY, CADMIUM AND LEAD IN EDIBLE MUSHROOMS AND IN UNDERLYING SUBSTRATES FROM A RURAL AREA WITH AN OCCURRENCE OF SERPENTINES AND AMPHIBOLES

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Abstract

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Three harmful metals were determined using ICP-MS and AAS techniques in 51 samples of fruiting bodies of 9 edible mushroom species. The mushrooms were collected from four sites in a rural area in SW Moravia, Czech Republic, unpolluted by human activities but known by an increased occurrence of serpentines and amphiboles. The metals were determined also in 11 samples of three horizons of underlying substrates using an ICP-OES method. As compared to background levels from an unpolluted region of S Bohemia, mercury and cadmium contents in mushrooms were very comparable and lead contents were lower. Levels of cadmium 2.0 and 10.0 mg kg^{-1} dry matter were exceeded in 86.3 and 15.7% of samples, respectively. Mercury and lead contents above 5.0 and 10.0 mg kg^{-1} dry matter, respectively, were found only sporadically. Thus, high consumption of mushrooms from the observed area may contribute considerably to the body burden with cadmium. The metal contents in the underlying soils were very comparable with data for the C European forests. It seems from the results that presence of serpentines and amphiboles in soil substrate did not influence the metal contents in fruiting bodies of the analysed mushrooms.

Key words: edible mushrooms, heavy metals, mercury, cadmium, lead, underlying substrate

Introduction

Wild growing mushrooms have been a very popular delicacy in many countries of C and E Europe. For instance, 72% of families collected mushrooms with a mean yearly level of 7 kg per household during the first half of 1990s in the Czech Republic (Šišák, 1996). However, yearly consumption exceeds 10 kg in some individuals.

Many original papers reported a high accumulation of several trace elements by some mushroom species, including those widely consumed, as reviewed by Kalač and Svoboda (2000). Two considerably accumulated metals, cadmium and mercury have been of primary interest. As resulted from the review, contents of cadmium, mercury and lead in mushroom fruiting bodies increase in areas contaminated with the metals, such as in the vicinity of both operational and historical metal smelters, within cities or along highways. However, the rate of the metal contents does not correlate significantly with the level of environmental pollution. Mushroom fruiting bodies cannot therefore be used as a reliable bioindicator of the level of a site contamination with the metals (Wondratschek, Röder, 1993). Levels of harmful elements have therefore been investigated mainly in edible mushrooms during the last decade. Only sporadic information deals with an impact of geological origin on trace element composition and content in mushroom fruiting bodies (Jongmans et al., 1997; Nikkarinen, Mertanen, 2004).

The objective of the present work was to determine the highest risk metals in fruiting bodies of widely consumed mushroom species growing in an area known by an increased occurrence of serpentines and amphiboles.

Materials and methods

Sampling

The study was carried out at four sites over an area of 0.7–2.7 ha in a rural region near a small town Moravský Krumlov, SW Moravia, about 30 km south-west from the city of Brno. The sites were up to 15 km apart, around the Nature Reserve Serpentine steppe near Mohelno, at an altitude of 230–460 m a. s. l.. The nuclear power station Dukovany (4x 440 MW), in operation since 1985, lies near the sites. No roads with an extensive traffic have crossed the tested area.

Mushrooms were collected and underlying soils were sampled in mixed forests with prevailing oak, beech, acacia, spruce and pine during the period 2001–2003. One complete fruiting body was used as a sample. The bodies were cleaned of all surface contamination by a stainless steel knife. No washing or caps peeling was applied. The bodies were sliced and dried at an ambient temperature in a dust-free room in a manner typical for mushroom preparation for culinary purposes.

Eleven samples of underlying substrate for the determination of three observed metals were taken. Sampling procedure was the same as used for the testing of the nutritional status of forest soils. Three horizons were sampled: organic layer (O, in a range between 0–1 and 0–12 cm); upper mineral horizon enriched with humus (Ah, ranging between 2–6 and 13–25 cm) and mineral layer (B, ranging between 7–20 and 26–40 cm). The samples were air dried in a layer less than 15 mm in a laboratory until dryness, milled and particles below 1 and 2 mm for organic and mineral horizons, respectively, were separated by sieving and homogenised.

Analytical procedures

Mercury was determined in the homogenised dried mushroom samples (0.1–0.2 g) using a cold-vapour AAS analyser (AMA 254, Altec Prague, Czech Republic) with a detection limit of 1.5 ng kg^{-1} dry matter. Mean differences between duplicates were up to 5%.

For cadmium and lead determination, about 0.3 g of a dried mushroom sample was wet digested with 5 ml of suprapure concentrated nitric acid (Merck, Germany) in closed Teflon® vessel in a microwave oven MDS 2000 (CEM Corp., USA). The digest was diluted to 50 ml with deionized water (MILLI-Q Element, Millipore, France) and filtrated. The determination of both the metal concentrations was performed using a mass spectrometer with inductively coupled plasma (ICP-MS) PQExCell (VG Elemental, UK) under standard conditions with a Meinhard concentric nebulizer. For all measurements presented, quantification was performed using an aqueous multi-element standard solution Merck VI (CertiPUR, Merck, Germany). The calibration procedure consisted of ten measurement replicates of the instrumental blank and five measurement replicates of the differently diluted standard solutions. To eliminate non-spectral interferences, internal standardisation was used. The results were within determination limits for cadmium and lead (calculated as 10-fold of standard deviation from ten replicates of the instrumental blank solution) 0.02 and 0.1 mg kg^{-1} dry matter, respectively. A reference material CRM BCR No 60, Trace elements in an aquatic plant *Lagarosiphon major* (Commission of the European Communities, Community Bureau of Reference, Belgium) was used for the evaluation of the measurement precision and accuracy.

Differences between determined and certified contents were up to 3, 7 and 4% for mercury, cadmium and lead, respectively.

The underlying soil samples were dried at 105 °C to constant weight and ashed in a furnace at 450 °C. The metals were extracted from the ash with 2 M HNO_3. Cadmium and lead contents were determined by an ICP-OES method using an apparatus Iris Interpid (Thermo Jarrell Ash, USA), mercury was quantified using cold-vapour AAS as described above. Soil pH was measured in a suspension with 0.2 M KCl in relation 1:5 (w/v) using an ion-selective electrode.

Statistical method

Differences between the mean contents of three elements from the tested and background areas were tested by Student´s test. Several values below the detection limits were used for the calculations as halves of the detection limits.

Results and discussion

In total, 77 samples of 24 mushroom species were collected and analysed, however, data of only 51 samples of 9 species with at least four samples per species will be presented. Contents of the individual metals are given in Tables 1–3. Data from unpolluted rural areas of S Bohemia, published by Kalač et al. (1989) for the all observed metals and moreover by Kalač and Šlapetová (1997) for mercury, were used as the background levels of the metal contents in mushroom fruiting bodies. These background contents match well with data reported from unpolluted areas of several European countries (for review see Kalač, Svoboda, 2000).

Unfortunately, the numbers of samples within mushroom species from the individual four sites were not ample enough for statistical evaluation. However, no evident differences in the metal contents among the sites were observed.

T a b l e 1. Contents of mercury (mg kg^{-1} dry matter) in mushrooms from the tested area and from the background area. Two data sets of background levels were available for seven species – the first line is used from Kalač et al. (1989), the second one from Kalač and Šlapetová (1997).

Species	Southern Moravia				South Bohemia		
	n	x	S_x	Range	n	x	S_x
Boletus edulis	5	4.34**	1.72	2.0-6.1	19	2.25	0.93
					8	4.56	2.50
Boletus reticulatus	7	2.36	1.58	0.3-5.0	19	2.39	2.14
					10	2.97	3.02
Xerocomus subtomentosus	6	0.39	0.17	0.2-0.6	6	0.47	0.22
					15	0.57	0.53
Xerocomus chrysenteron					21	0.52	0.39
	7	0.43**	0.20	0.16-0.7	7	0.66	0.16
Xerocomus badius	6	1.06	1.07	0.4-3.2	25	0.38	0.28
					14	0.61	0.22
Russula cyanoxantha	7	1.18*	0.73	0.3-2.3	7	0.56	0.27
Macrolepiota procera					9	5.01	4.43
	6	2.52**	0.50	1.8-3.0	8	5.03	2.36
Amanita rubescens	4	0.90*	0.16	0.7-1.1	12	0.61	0.33
					21	1.20	0.94
Lycoperdon perlatum	8	1.45	0.64	0.15-2.4	-	-	-

Significance level of differences: * $p < 0.1$; ** $p < 0.05$
Notes: n – number of samples, x – mean value, S_x – standard deviation

T a b l e 2. Contents of cadmium (mg kg^{-1} dry matter) in mushrooms from the tested area and from the background area.

Species	Southern Moravia				South Bohemia		
	n	x	S_x	Range	n	x	S_x
Boletus edulis	4	5.53	7.41	1.1-18.5	19	2.29	1.33
Boletus reticulatus	5	4.17	4.48	0.5-11.9	19	2.50	2.23
Xerocomus subtomentosus	5	4.41	4.67	1.7-12.7	6	1.13	1.79
Xerocomus chrysenteron	6	4.18	3.70	1.0-10.2	23	1.35	3.37
Xerocomus badius	6	3.26	4.17	0.3-11.3	25	0.89	0.59
Russula cyanoxantha	7	1.93	2.24	0.6-6.7	7	2.73	3.52
Macrolepiota procera	6	2.90	3.64	0.2-10.1	10	1.82	1.88
Amanita rubescens	4	2.10	1.11	0.6-3.0	12	1.25	0.88
Lycoperdon perlatum	8	3.37	2.26	0.5-7.2	-	-	-

Notes: n, x, Sx – see Table 1

High values of standard deviation, even higher than mean values (Tables 1–3), were caused by an abnormal metal content distribution. A very wide range of metal contents within a species is quite common in mushrooms (Kalač, Svoboda, 2000). Such situation is different from that in plants.

T a b l e 3. Contents of lead (mg kg^{-1} dry matter) in mushrooms from the tested area and from the background area.

Species	Southern Moravia				South Bohemia		
	n	x	S_x	Range	n	x	S_x
Boletus edulis	4	1.29	0.76	0.7-2.6	20	1.21	1.05
Boletus reticulatus	5	1.10	1.40	ND-3.5	17	1.48	1.01
Xerocomus subtomentosus	5	1.19	1.07	0.2-2.8	5	0.42	0.34
Xerocomus chrysenteron	6	0.91	0.82	ND-2.0	22	1.12	0.58
Xerocomus badius	6	0.74	0.75	ND-1.8	25	1.26	0.91
Russula cyanoxantha	7	3.57	7.45	ND-20	7	0.92	0.23
Macrolepiota procera	6	2.52*	1.62	0.5-4.7	10	4.87	2.68
Amanita rubescens	4	0.57***	0.49	ND-1.2	12	2.06	0.94
Lycoperdon perlatum	8	2.51	3.43	ND-9.8	-	-	-

Significance level of differences: * $p < 0.1$; *** $p < 0.01$
Notes: n, x, Sx – see Table 1

Some mushroom species are known to be heavy cadmium and/or mercury accumulators. The reported bioaccumulation factors are 50–300 and 30–500 for cadmium and mercury, respectively (Seeger, 1982). On the contrary, both bioaccumulation and bioexclusion were observed in lead. While Dolischka and Wagner (1981) reported bioaccumulation factors usually below 0.1, Rudawska and Leski (2005a) observed values both below and above 1.0. The ability seems to be species-dependent. The most of mushroom species take their nutrients preferably from the organic horizon. Thus, metal contents in the surface substrate layer should be preferentially considered. The highest levels of mercury, cadmium and lead were determined in the top organic layer of forest soils, the lowest ones in deeper mineral horizons (Eriksson, 2002; Probst et al., 2003; Sauve et al., 2003). The same situation was observed in the sampled soils (Table 4). Central Institute for Supervising and Testing in Agriculture in Brno found median values 0.29, 0.06 and 0.04 mg kg^{-1} dry matter for cadmium

T a b l e 4. Acidity and metal contents (mg kg^{-1} dry soil) in three horizons of forest soils (n = 11) from the tested sites.

	pH/KCl			Mercury			Cadmium			Lead		
	O	Ah	B	O	Ah	B	O	Ah	B	O	Ah	B
x	4.33	4.44	4.55	0.22	0.10	0.04	0.29	-	-	34.3	22.7	9.4
S_x	0.45	0.66	0.72	0.09	0.05	0.02	0.10	-	-	15.9	11.8	3.6
x_{min}	3.7	3.8	3.9	0.07	0.03	0.02	0.03	<0.10	<0.10	9.7	9.9	4.2
x_{max}	5.0	5.9	6.2	0.37	0.24	0.11	0.41	0.70	0.24	60.2	49.3	16.9

Horizons: O – organic layer, Ah – upper mineral layer, B – lower mineral layer
Notes: x, S_x – see Table 1. Mean and standard deviation of cadmium content in mineral horizons are not given due to numerous values below the limit of detection 0.10 mg kg^{-1} dry soil.

and 62.8, 36.0 and 20.0 mg kg^{-1} dry matter for lead in O, Ah and B horizons, respectively, in 741 forest soil samples from the Českomoravská vysočina highlands to which the tested area had been adjacent (unpublished data).

According to Malinowska et al. (2004), heavy metal bioavailability from soil substrate by mushroom mycelium is affected by numerous factors, such as pH value, redox potential, organic matter content, mineralogy of clay, cation exchange capacity of the solid phase and composition of the soil solution. However, Gast et al. (1988) did not find any relationship between cadmium and lead contents in fruiting bodies and pH value and organic matter content of the underlaying top soil layer 0–5 cm. High soil acidity enhances heavy metals mobility and availability. Nevertheless, pH values determined in our samples (Table 4) are within levels usual for forest soils and a possible effect of basic serpentines and amphiboles is not apparent.

Some countries established statutory limits for the metal contents in edible mushrooms. The limits 5.0, 2.0 and 10.0 mg kg^{-1} dry matter for mercury, cadmium and lead, respectively, in wild growing mushrooms were valid in the Czech Republic until its entry to the EU on 1 May 2004. Considerably lower limits, 1.0 and 2.0 mg kg^{-1} dry matter for cadmium and lead, respectively, have been valid in Poland since 2001 (Rudawska, Leski, 2005b). In the EU, the limits 2.0 and 3.0 mg kg^{-1} dry matter for cadmium and lead, respectively, are valid for cultivated mushrooms (EEC Directive 221/2002/EC). For the calculation of dietary intake, usually 300 g of fresh mushrooms per meal and mushroom mean dry matter content 10%, are assumed.

No apparent considerable trend in the differences was observed between mercury contents in mushrooms from the tested and background areas (Table 1) despite of statistical significance in several species. *Boletus edulis, B. aestivalis, Macrolepiota procera* and *Lycoperdon perlatum* have been known to be medium mercury accumulators (Kalač, Svoboda, 2000). However, the Czech statutory limit was somewhat exceeded only in two samples of *Boletus edulis.* Mercury contents in organic horizons (Table 4) varied between 0.07 and 0.37 mg kg^{-1} dry soil. Such levels are very comparable with data from Poland and Bavaria (Falandysz et al., 1996; Schwesig et al., 1999).

No significant differences in mushroom cadmium contents were found between the tested and background areas (Table 2) despite the increased mean values in the most species from the southern Moravia. However, cadmium contents varied very widely. None of the tested species has been known as cadmium accumulator. Usual levels for the observed species have been between 0.5–5 mg kg^{-1} dry matter within Europe (Kalač, Svoboda, 2000). The observed cadmium contents of 0.03–0.41 mg kg^{-1} dry organic layer are somewhat lower than mean and median values 0.57 and 0.35 mg kg^{-1} dry matter, respectively, reported for organic horizon 0–10 cm of unpolluted Polish forests (Andersen et al., 1994). Unfortunately, data on cadmium partitioning in underlying substrate and on its bioavailability for mushrooms are lacking in any literature. The increased contents of cadmium as compared to the mentioned background levels from the adjacent Českomoravská vysočina highlands were observed in the samples of mineral horizons from the testing site near the Nature Reserve Serpentine steppe, however, not in organic horizon of the same samples. As organic horizon absorbs most of the metals contained in depositions, the observed increased cadmium content in

mineral horizons should be of geological origin. However, basic serpentines and amphiboles are assessed as the rocks with low levels of available cadmium. We do not know and do not suppose any source of anthropogenic load of the tested area with cadmium.

The former Czech statutory limit for cadmium 2.0 mg kg^{-1} dry matter was exceeded in 86.3% of samples. Moreover, 15.7% of samples exceeded level of 10.0 mg kg^{-1} dry matter. Information on cadmium bioavailability from mushroom meals in humans has been limited. However, a comparable and higher absorption from mushrooms than from inorganic cadmium salts was reported (Seeger et al., 1986; Lind et al., 1995). Thus, consumption of mushrooms from the observed area should be restricted in the individuals with their very high intake.

Lead contents in fruiting bodies were very low, in two species even significantly lower than in mushrooms from the background area. The observed lead contents in the substrate are lower than mean and median levels of 101 and 82 mg kg^{-1} dry matter, respectively, reported for unpolluted organic horizons from Poland (Andersen et al., 1994) and also lower than the above mentioned data for the Českomoravská vysočina highlands. It can be therefore concluded that lead contents in mushrooms from the observed area do not represent any health risk.

Similar situation in the metal contents was observed also in 26 analysed mushroom samples of 15 species having low number of samples per a species.

Conclusion

An information on factual levels of detrimental metals has been required for countries with traditionally high consumption of wild growing edible mushrooms. While numerous works dealt with the metal contents in mushrooms growing in polluted areas, data on geological impact have been scarce. It seems from the presented results that occurrence of serpentines and amphiboles in soil substrate did not influence the metal contents in fruiting bodies of the analysed mushrooms.

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