

Contents of cadmium, mercury and lead in edible mushrooms growing in a historical silver-mining area

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Abstract

Three harmful metals were determined using AAS techniques in 285 samples of fruiting bodies of 15 wild-growing edible mushroom species. The mushrooms were collected from a forest on the fringe of a historical area of silver mining. The metals were also determined in a topsoil organic layer sampled from nine sites within the observed area. As compared to background levels from unpolluted sites from several European countries, cadmium contents were considerably elevated in nearly all the tested species; lead contents were increased in most of the species, while mercury contents were elevated only in certain species. Thus, many species from the observed area may contribute considerably to the body burden of the metals. *Agaricus silvaticus* accumulated cadmium extremely and *Lepista nuda* accumulated mercury. There were no obvious simple positive relationships between the contents of the observed metals in fruiting bodies and the contents of total metals in the soil organic layer.

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1. Introduction

Picking and consumption of wild-growing mushrooms has been very popular in many countries of Central and East Europe. For instance, in the Czech Republic, 72% of families collected mushrooms, with a mean yearly level 7 kg per household in the first half of the 1990s (Šišák, 1996). However, yearly consumption exceeds 10 kg in some individuals.

Tens of papers have reported a high accumulation of several trace elements by some mushroom species, including edible and widely-consumed ones, as reviewed by Kalač and Svoboda (2000). Two considerably accumulated metals, cadmium and mercury, have been of primary interest. Contents of those elements increase

in polluted areas, mainly within cities and in emission areas of contemporary operated metal smelters (Alonso, Salgado, García, & Melgar, 2000; Colpaert, Van den Koornhuyse, Adriaensen, & Van Gronsveld, 2000; Kalač, Nižnanská, Bevilaqua, & Stašková, 1996; Melgar, Alonso, Pérez-López, & García, 1998; Thomet, Vogel, & Krähenbühl, 1999; Wondratschek & Röder, 1993). However, there exist several findings indicating that mushroom contamination can also be elevated in areas of historical mining and processing of metal ores, mainly those of mercury (Bargagli & Baldi, 1984; Fischer, Rapsomanikis, Andraea, & Baldi, 1995; Svoboda, Zimmermannová, & Kalač, 2000) or silver (Řanda & Kučera, 2004).

The aim of the present work was to determine the contents of three toxicologically important metals, cadmium, mercury and lead, in fruiting bodies of commonly consumed mushrooms growing in a forest adjacent to an historical silver-mining area.

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2. Materials and methods

2.1. Study area

The study was carried out in a forest near a village called Borek, situated 6 km north–east of the city of České Budějovice, South Bohemia, Czech Republic (Fig. 1), during the period 1997–2000. The observed area (0.5×0.5 km) is on the fringe of a historical Rudolfov silver-mining area. Silver ores argentite and pyrargyrite and silver-yielding galena and sphalerite, with relatively high cadmium contents were here mined and processed mainly during the second half of the 16th century. Residues of mining activities, mainly eroded tailings, have remained. Current contamination with heavy metals from the nearby city of České Budějovice with about 100,000 inhabitants can be assessed as low due to a relatively low level of emissions and prevailing westerly and north-westerly winds.

The observed area has an altitude of 410–425 m above sea level. Acidic cambisol has been covered with a full-grown coniferous forest with prevailing spruce and dispersed groups of oaks and birches. A stream, Kyselá voda (“Acidic Water”), forming an eastern boundary of the observed area, runs from the residues of the former mining. The suburban forest has been widely exploited for edible mushroom picking.

2.2. Mushroom and substrate sampling

One complete fruiting body of a mushroom was taken as a sample. The fruiting bodies were cleaned of all surface contamination by a stainless steel knife. No washing or caps peeling was used. Fruiting bodies were sliced and dried at an ambient temperature in the usual

manner for mushroom preservation for culinary purposes.

In total, 436 mushroom samples of 48 species were analysed. However, data for only 285 samples of 15 edible species belonging to seven families with at least five samples per species are presented. Their list is given in Table 1.

Underlying substrate for the determination of three investigated metals was sampled in nine sites systematically covering (in a chessboard manner) the observed area (Fig. 2). The distances between neighbouring sampling sites were 200 m. Top litter layers of needles, leaves and sprigs were removed. The organic layer (horizon A_0), from which most saprophytic mushrooms primarily take their nutrients, of thickness ranging between 0–3 and 0–6.5 cm, without little stones and rootlets, was sampled. The samples were air-dried in a laboratory for one month and then sieved using a vibrating screen with a 2 mm mesh.

2.3. Analytical procedures

Mercury was determined in the homogenised dried samples of mushrooms (0.1–0.2 g) and of organic layer (0.4–0.5 g) using a cold-vapour AAS analyser (AMA 254, Altec Prague, Czech Republic) with a detection limit of 1.5 ng kg^{-1} . Mean differences between duplicates were up to 5%.

Approximately 0.3–0.4 g of dried samples were used for cadmium and lead determination. A sample was wet-digested with 5 ml of concentrated nitric acid in closed polytetrafluoroethylene (PTFE) vessels in a microwave oven MDS 2000 (CEM Corp., USA). The digest was diluted to 25 ml with re-distilled water and filtered. A Spectra AA 640 apparatus (Varian Techtron, Australia), with electrothermic atomisation, was used for atomic absorption spectrometry measurements at wavelengths 228.8 and 217.0 nm for cadmium and lead, respectively. The sensitivities were 0.1 and $1.0 \mu\text{g dm}^{-3}$ for cadmium and lead, respectively. Detection limits were 0.04 and 0.4 mg kg^{-1} dry matter for cadmium and lead, respectively. The analyses were carried out in duplicate with differences between the measurements of up to 10% and 15% for cadmium and lead, respectively.

Blank background levels were below the detection limits for all three elements. An epiphytic lichen *Evernia prunastri*, IAEA-336 and light sandy soil, RM 7002 were used as the reference materials. Differences between experimentally determined and certified contents were up to 10%, 3% and 5% for cadmium, mercury and lead, respectively.

2.4. Statistical method

Differences between the mean contents of three elements from the tested and the background areas were tested by the *t*-test.

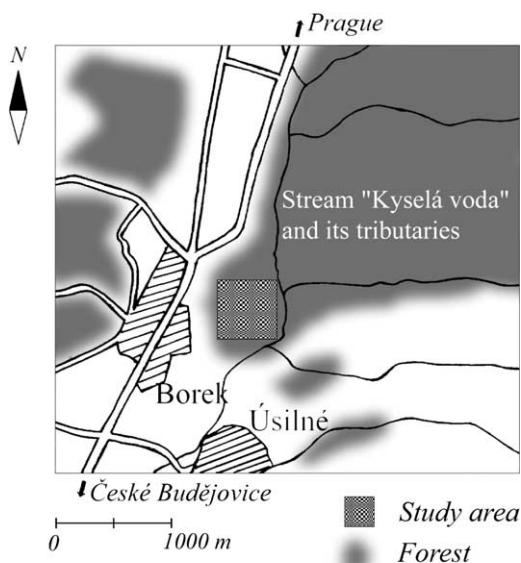


Fig. 1. A map of the study area.

Table 1
List of analysed mushroom species

Family	Species	Number of samples
<i>Cantharellaceae</i>	<i>Cantharellus cibarius</i> Fr.	6
<i>Tricholomataceae</i>	<i>Lepista nuda</i> (Bull. ex Fr.) W.G.Smith	11
	<i>Armillaria mellea</i> (Vahl ex Fr.) Kumm.	10
	<i>Collybia dryophila</i> (Bull. ex Fr.) Kumm.	18
	<i>Marasmius oreades</i> (Bolt. ex Fr.) Fr.	19
	<i>Amanita rubescens</i> (Pers. ex Fr.) S.F.Gray	15
<i>Amanitaceae</i>		
<i>Agaricaceae</i>	<i>Macrolepiota rhacodes</i> (Vitt.) Sing.	31
	<i>Agaricus silvaticus</i> Schaeff. ex Fr.	5
<i>Boletaceae</i>	<i>Suillus bovinus</i> (L. ex Fr.) O. Kuntze	30
	<i>Leccinum scabrum</i> (Bull. ex Fr.) S.F.Gray	10
	<i>Xerocomus badius</i> Fr. Gilb.	28
	<i>Xerocomus chrysenteron</i> (Bull. ex St.A.) Quéf.	67
<i>Russulaceae</i>	<i>Lactarius piperatus</i> (Fr.) S.F.Gray	5
	<i>Russula cyanoxantha</i> (Schaeff. ex Schw.) Fr.	9
	<i>Lycoperdon perlatum</i> Pers. ex Pers.	21
<i>Sclerodermataceae</i>		

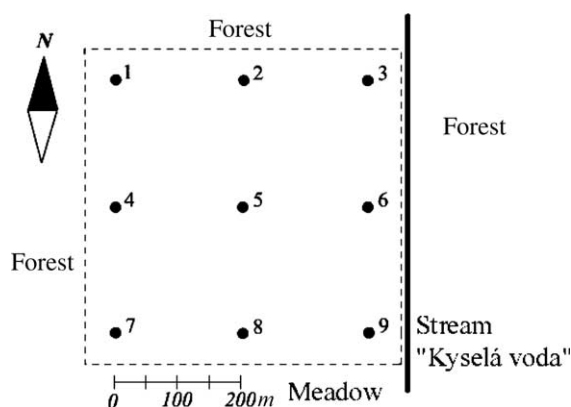


Fig. 2. A sketch of organic layer sampling within the observed area.

3. Results and discussion

Contents of the individual metals are given in Tables 2–4. Usual background metal levels for unpolluted areas, reported from several European countries (Kalač & Svoboda, 2000), are available for 11 of the observed species. Comparison of our results with the literature data had thus to be limited to those species. Statistical testing of differences between the mean metal contents in mushrooms from the tested area and from unpolluted rural areas of South Bohemia (Kalač, Wittingerová, Stašková, Šimák, & Bastl, 1989) were limited to only eight species with available background data (Table 5).

The determined cadmium contents (Table 2) were elevated, usually several times, as compared to the literature background values in eight out of 11 species, while they were comparable only in *Cantharellus cibarius* and *Russula cyanoxantha*. Similarly, six out of eight compared species from the tested area had significantly increased cadmium contents (Table 5). *Agaricus silvaticus*, with extreme mean cadmium content of 149 mg kg⁻¹ dry matter, has been known as a highly accu-

Table 2

Contents of cadmium (mg kg⁻¹ dry matter) in mushroom fruiting bodies

Species	\bar{x}	s_x	x_{\min}	x_{\max}	Usual background ^a
<i>Cantharellus cibarius</i>	0.5	0.5	0.04	1.4	<1
<i>Lepista nuda</i>	5.1	2.1	2.6	8.9	0.5–2
<i>Armillaria mellea</i>	9.2	3.2	3.8	15.4	2–5
<i>Collybia dryophilla</i>	1.8	0.6	0.7	2.9	
<i>Marasmius oreades</i>	4.65	3.8	0.25	17.5	
<i>Amanita rubescens</i>	10.0	4.6	2.6	17.5	1–2
<i>Macrolepiota rhacodes</i>	4.0	4.9	1.3	27.2	0.5–2
<i>Agaricus silvaticus</i>	149	15.3	135	166	5–50
<i>Suillus bovinus</i>	2.8	5.0	0.25	21.0	
<i>Leccinum scabrum</i>	2.45	1.5	0.15	5.6	2–5
<i>Xerocomus badius</i>	2.7	2.1	0.6	8.6	0.5–2
<i>Xerocomus chrysenteron</i>	3.4	5.6	0.15	39.8	1–2
<i>Lactarius piperatus</i>	1.2	0.6	0.45	2.2	
<i>Russula cyanoxantha</i>	1.7	1.0	0.6	4.1	1–5
<i>Lycoperdon perlatum</i>	3.15	1.5	1.0	7.8	0.5–1

^a Usual background contents for unpolluted areas in Tables 2–4 are taken from several European countries, as reviewed by Kalač and Svoboda (2000).

mulating species (Kalač & Stašková, 1994; Vetter, 1994). Thus, cadmium contents were commonly considerably elevated as compared to mushrooms from the European background areas.

Among edible species not given in Table 2, mean cadmium contents of 13.3 and 4.6 mg kg⁻¹ dry matter were found in *Clitocybe nebularis* ($n = 4$) and in *Russula decolorans* ($n = 3$), respectively. High mean cadmium content 48.8 mg kg⁻¹ dry matter was determined in mildly toxic *Mycena pura* ($n = 10$). The species known as an accumulator of cadmium was recommended by Dietl (1987) as a bioindicator of substrate pollution with the metal. Relative standard deviation of 15.6% in our group of samples was low compared to the other tested species, thus supporting such an opinion.

Table 3
Contents of mercury (mg kg⁻¹ dry matter) in mushroom fruiting bodies

Species	\bar{x}	s_x	x_{\min}	x_{\max}	Usual background
<i>Cantharellus cibarius</i>	0.25	0.2	0.03	0.6	<0.5
<i>Lepista nuda</i>	12.9	6.4	2.1	22.4	2–20
<i>Armillaria mellea</i>	4.2	2.7	1.6	9.6	<0.5
<i>Collybia dryophilla</i>	0.3	0.1	0.24	0.45	
<i>Marasmius oreades</i>	1.35	0.7	0.3	3.3	
<i>Amanita rubescens</i>	1.55	1.2	0.25	4.0	0.5–2
<i>Macrolepiota rhacodes</i>	2.95	1.5	0.9	7.9	2–10
<i>Agaricus silvaticus</i>	2.85	1.4	1.3	4.9	
<i>Suillus bovinus</i>	0.55	0.4	0.15	1.55	
<i>Leccinum scabrum</i>	0.5	0.3	0.1	1.0	0.3 for caps and 0.2 for stalks ^a
<i>Xerocomus badius</i>	1.3	1.1	0.1	3.0	<1
<i>Xerocomus chrysenteron</i>	0.6	0.4	0.03	1.9	<1
<i>Lactarius piperatus</i>	1.55	1.5	0.6	4.2	
<i>Russula cyanoxantha</i>	0.8	0.9	0.04	2.6	0.5–1
<i>Lycoperdon perlatum</i>	2.0	0.6	0.3	3.2	1–5

^a Data from numerous Polish sites gathered by Falandysz and Bielawski (2001).

Table 4
Contents of lead (mg kg⁻¹ dry matter) in mushroom fruiting bodies

Species	\bar{x}	s_x	x_{\min}	x_{\max}	Usual background
<i>Cantharellus cibarius</i>	3.1	2.0	1.0	6.2	1–2
<i>Lepista nuda</i>	10.5	5.7	2.6	21.6	5–10
<i>Armillaria mellea</i>	8.9	3.5	3.2	15.9	1–2
<i>Collybia dryophilla</i>	1.7	1.3	0.1	3.15	
<i>Marasmius oreades</i>	5.7	4.4	1.0	15.8	
<i>Amanita rubescens</i>	8.7	4.4	2.0	18.3	1–5
<i>Macrolepiota rhacodes</i>	13.6	7.2	6.1	31.2	5–20
<i>Agaricus silvaticus</i>	12.6	7.8	6.0	25.9	
<i>Suillus bovinus</i>	1.95	1.6	0.14	6.9	
<i>Leccinum scabrum</i>	7.7	9.6	0.9	29.2	1–2
<i>Xerocomus badius</i>	2.15	1.5	0.35	6.2	2–5
<i>Xerocomus chrysenteron</i>	4.6	3.9	1.0	21.5	1–2
<i>Lactarius piperatus</i>	1.8	1.2	0.25	3.2	
<i>Russula cyanoxantha</i>	4.0	2.1	2.1	8.0	1–2
<i>Lycoperdon perlatum</i>	16.2	6.4	6.0	37.6	5–20

In contrary to cadmium, mercury contents (Tables 3 and 5) were elevated only in certain species. A surprising level was observed in wood-decaying *Armillaria mellea*. On the contrary, mercury content in *Macrolepiota rhacodes* from the tested area was significantly lower than in fruiting bodies from the background areas. *Lepista nuda* has been known as an accumulator of mercury. In addition to the species given in Table 3, relatively high mercury contents were observed in two other edible species, namely 7.6 and 2.6 mg kg⁻¹ dry matter, in popular *Boletus aestivalis* ($n = 4$) and in *Clitocybe nebularis* ($n = 3$), respectively. The reported background levels for *B. aestivalis* are 1–5 mg kg⁻¹ dry matter (Kalač & Svoboda, 2000).

The elevated mean lead contents (Tables 4 and 5), usually about twofold or threefold the background values, were observed mainly in *Armillaria mellea*, *Amanita rubescens*, *Xerocomus chrysenteron* and *Russula cyanoxantha*. Relatively high mean lead contents were found in two species with a low number of samples. Both *Clitocybe nebularis* ($n = 3$) and *Lactarius seriffuus* ($n = 3$) contained 6.6 mg of lead per kg of dry matter. The observed area is at least 250 m away from a road with intense traffic. Mushroom contamination with lead from leaded fuel is not likely at such a distance (Isiloglu, Merdivan, & Yilmaz, 2001; Kuthan, 1979).

Contents of the metals in fruiting bodies are generally species-dependent; some species are now known as accumulators of the individual metals. Cadmium and mercury are accumulated in fruiting bodies, while lead contents are lower in fruiting bodies than in underlying substrate. The reported bioaccumulation factors (as reviewed by Kalač & Svoboda, 2000) are 50–300, 30–500 and 0.1–0.2 for cadmium, mercury and lead, respectively. In our opinion, metal levels in fruiting bodies of wild-growing mushrooms are considerably affected by the age of mycelium and by the interval between the waves of fruiting body formation (fructifications). According to Malinowska, Szefer, and Falandysz (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, Jansen, Bierling, and Haanstra (1988) did not find any relationship between cadmium and lead contents in fruiting bodies and pH value and organic matter content of the underlying top soil layer 0–5 cm. Moreover, Nikkarinen and Mertanen (2004) reported the effect of natural geochemistry on trace element contents in the tested mushrooms, *Boletus edulis* and *Lactarius trivialis*.

The proportion of the metal contents in fruiting bodies originating from atmospheric depositions has been of less importance due to the short lifetime of a fruiting body, which is usually only 10–14 days.

Contents of the observed metals in the organic substrates sampled within the observed area are given in Table 6.

Total cadmium contents, varying between 0.10 and 0.21 mg kg⁻¹ dry matter, can be assessed as low. Usual cadmium contents, observed in forest organic horizon from unpolluted sites within the Czech Republic, have varied between 0.20 and 0.40 mg kg⁻¹ dry matter. Mean and median values 0.57 and 0.35 mg kg⁻¹ dry matter were reported for 14 forest sites throughout Poland with a wide range 0.1–2.9 mg kg⁻¹ dry matter in an organic horizon of 0–10 cm (Andersen, Ødegård, Vogt, & Seip, 1994). Higher concentrations of cadmium, lead and mercury were determined in the organic layer of forest soils

Table 5

Statistical evaluation of cadmium, mercury and lead contents (mg kg^{-1} dry matter) in mushrooms from the tested area (TA) and from the background areas (BAs) of South Bohemia (Kalač et al., 1989)

Species	Number of samples TA/BAs	Cadmium				Mercury				Lead			
		Tested areas		Background areas		Tested areas		Background areas		Tested areas		Background areas	
		\bar{x}	s_x	\bar{x}	s_x	\bar{x}	s_x	\bar{x}	s_x	\bar{x}	s_x	\bar{x}	s_x
<i>Cantharellus cibarius</i>	6/10	0.5	0.5	0.29	0.26	0.25*	0.2	0.07	0.03	3.1	2.0	1.58	2.90
<i>Lepista nuda</i>	11/5	5.1***	2.1	0.92	0.39	12.9	6.4	11.0	9.42	10.5**	5.7	5.12	3.20
<i>Armillaria mellea</i>	10/7	9.2***	3.2	2.96	1.17	4.2***	2.7	0.16	0.05	8.9***	3.5	1.63	1.41
<i>Amanita rubescens</i>	15/12	10.0***	4.6	1.25	0.88	1.55**	1.2	0.61	0.33	8.7***	4.4	2.06	0.94
<i>Macrolepiota rhacodes</i>	31/10	4.0***	4.9	0.95	0.58	2.95*	1.5	4.38	2.36	13.6	7.2	8.34	10.3
<i>Xerocomus badius</i>	28/25	2.7***	2.1	0.89	0.59	1.3***	1.1	0.38	0.28	2.15**	1.5	1.26	0.91
<i>Xerocomus chrysenteron</i>	67/22	3.4**	5.6	1.35	3.37	0.6	0.4	0.52	0.39	4.6***	3.9	1.12	0.58
<i>Russula cyanoxantha</i>	9/7	1.7	1.0	2.73	3.52	0.8	0.9	0.56	1.00	4.0***	2.1	0.92	0.23

Significance level of differences. * $P < 0.1$; ** $P < 0.05$; *** $P < 0.01$.

Table 6

Contents of cadmium, mercury and lead (mg.kg^{-1} dry matter) in soil organic layer within the observed area

Sampling site (see Fig. 2)	Cadmium	Mercury	Lead
1	0.21	0.34	80.0
2	0.11	0.50	45.4
3	0.18	0.73	123
4	0.17	0.60	50.2
5	0.11	0.31	49.2
6	0.20	0.37	71.4
7	0.10	0.34	55.4
8	0.10	0.65	38.9
9	0.19	0.35	66.4
\bar{x}	0.15	0.47	64.4
s_x	0.05	0.16	25.6

than in mineral soil horizons (Eriksson, 2002; Probst, Hernandez, Probst, & Ulrich, 2003; Sauve, Manna, Turmel, Roy, & Courchesne, 2003).

Thus, good bioavailability of substrate cadmium can be supposed in our study area due to the elevated contents in most of the tested mushrooms and to relatively low levels in the organic layer.

Conversely to cadmium, total mercury contents in organic substrate, ranging between 0.31 and 0.65 mg kg^{-1} dry matter (Table 6), are relatively high. The reported levels in forest organic horizon vary between 0.05 and 0.35, around 0.1 and >0.4 mg kg^{-1} dry matter, in the Czech Republic, Poland and Bavaria (Falandsz et al., 1996; Schwesig, Ilgen, & Matzner, 1999). As mercury contents in mushrooms, given in Table 3, are in accordance with the usual background levels, usual or somewhat decreased bioavailability of mercury by mushrooms from the substrate within the study area can be assumed.

The total lead contents varied mostly between 40 and 80 mg kg^{-1} dry matter (Table 6). The reported levels in forest topsoils are 10–60 mg kg^{-1} dry matter in the

Czech Republic, and mean and median values 101 and 82 mg kg^{-1} dry matter, respectively, in Poland (Andersen et al., 1994). Because six species had increased lead contents as compared to the background levels (Table 5), usual or increased bioavailability of lead from the underlying substrate can be supposed.

Current Czech statutory limits for the metal contents in wild-growing edible mushrooms are 2.0, 5.0 and 10.0 mg kg^{-1} dry matter for cadmium, mercury and lead, respectively. In the EU, the limits of 2.0 and 3.0 mg kg^{-1} dry matter for cadmium and lead, respectively, are valid for cultivated mushroom (EEC Directive 2001/22/EC). A lot of species from the observed area exceeded the limits, the worst situation being in cadmium. Another consideration from the health risk of view, from mushroom consumption is the FAO/WHO provisional tolerable weekly intake. There are limits of 7, 4.3 and 25 μg per kg of bodyweight for cadmium, mercury and lead, respectively. For intake calculations, usually a 300 g portion of fresh mushrooms per meal is assumed, which contains 30 g of dry matter. A tolerable weekly intake for a person with a bodyweight of 60 kg is thus reached by a single portion of 300 g of fresh mushrooms containing 14, 10 or 50 mg kg^{-1} dry matter of cadmium, mercury or lead, respectively. Mean metal contents of only two observed species exceeded such limits, *Agaricus silvaticus* in cadmium and *Lepista nuda* in mercury. However, the mushrooms are not the only source of dietary heavy metals, and moreover, wild-growing mushrooms are usually consumed repeatedly during the relatively short growing periods.

Limited literature data on the metal bioavailability from consumed mushrooms were reviewed by Kalač and Svoboda (2000). Cadmium absorption was observed to be comparable and higher from mushrooms than from inorganic cadmium salts. Cadmium level in blood serum increased considerably following mush-

room consumption. The proportion of highly toxic methylmercury seems to be low, usually only a few per cent of the total mercury content. Data on chemical forms of lead compounds in mushrooms and their bioavailability in man have been lacking.

In conclusion, many mushroom species from the observed area of the historical silver mining and processing activities have elevated cadmium and lead contents and their consumption should be restricted. It may be supposed that a similar situation can exist in other areas of former metallic ore mining and processing. There are no obvious simple positive relationships between the contents of the observed metals in fruiting bodies and the contents of total metals in soil organic layer.

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