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Studies Concerning Heavy Metals Bioaccumulation of Wild Edible Mushrooms from Industrial Area by Using Spectrometric Techniques

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Abstract The aim of this work was to determine the heavy metal content of the fruiting bodies of four species of wild edible mushrooms and their respective substrates. The samples were collected from Dambovita County, Romania, at various distances from of a metal smelter, to asses the concentration of Cr, Mn, Fe, Ni, Cu, Zn, Se and Cd in the wild edible mushrooms and their substrate using Energy Dispersive X-ray Fluorescence (EDXRF) spectrometry together with Flame Atomic Absorption (FAAS) spectrometry. A quantitative evaluation of the relationship of element uptake by mushrooms from substrate was made by calculating the coefficient accumulation (K_a). A high accumulation of Zn (K_a range 1.01 to 2.01) was observed in mushrooms growing in the vicinity of the metal smelter.

Keywords Mushroom · Heavy metals · FAAS · EDXRF · Bioaccumulation · Pollution

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Heavy metal pollution is a major problem concerning the environment quality. One of the main sources of these pollutants in the atmosphere is industrial processes like metal smelter wich exist in Dambovita County, Romania.

Many studies (Kalac and Svoboda 2005; Kalac et al. 1991; Ita et al. 2006; Sesli and Tuzen 1999) revealed a high ability of mushrooms to accumulate common pollutants present in the biosphere at trace levels, mainly heavy metals and radionuclides. Compared to green plants, mushrooms can build up large concentrations of some heavy metals, particularly cadmium, mercury, copper and lead (Kalac and Svoboda 2004). In many research (Svoboda et al. 2006; Antonijevic and Maric 2008; Svoboda and Kalac 2003) the concentrations of heavy metals have been observed in the fruiting bodies (Courtecuisse 1999) of different mushrooms collected adjacent to heavy metal smelters, landfills of sewage sludge, emission area. Mushrooms are generally capable of accumulating heavy metals and then become their source in food chain (Kalac 2009).

Therefore, the determination of heavy metal concentration in the fruiting bodies of mushrooms is essential in dietary intake studies, because mushrooms form a non-negligible part of the diet in many countries, especially for certain population groups. Different heavy metals are toxic, such as As, Cd, Ni and Hg. On the other hand, many elements are essential for the human metabolism, such as Fe, Zn, Mn, Cu, Cr and Se, but only in low concentrations, because they are enzyme activators. These elements become toxic as the concentration increases (Turkekul et al. 2004; Yamaça et al. 2007). The minerals can be accumulated in mushrooms, and this accumulation is generally species metabolism-dependent and also strongly affected by the chemical composition of the substrate from which mushrooms get their nutrients.

The aim of this work was to determine the heavy metal content of the fruiting bodies of four species: *Lycoperdon perlatum*, *Pleurotus ostreatus*, *Fistulina hepatica, Armillariella mellea* and their substrate, collected at various distances from a metal smelter in Dambovita County, Romania. The concentrations of Mn, Fe, Ni, Cu and Zn in the samples were determined by Energy Dispersive X-Ray Fluorescence (EDXRF) spectrometry and the concentrations of Cd, Cr, Ni, Se and Pb were determined by Flame Atomic Absorption (FAA) spectrometry. All the samples, were collected at three distances (0.5, 4.5 and 10.5 km) from the metal smelter. From the same collecting point were taken $n = 5$ samples of young fruiting bodies of mushrooms and their substrate.

Materials and Methods

Young mushrooms species, *Lycoperdon perlatum*, *Pleurotus ostreatus, Fistulina hepatica* and *Armillariella mellea* were collected from metal smelter of Dambovita County, Romania, in the same direction of wind.

The samples prelevation of mushrooms were made at different times of the day: morning, afternoon and mid-day by uprooting their substrate, poplar bark, oak bark and soil, with the aid of a plastic scalpel. The fruiting bodies of *Lycoperdon perlatum* were white and had various cap diameters from 2.3 to 8 cm. The fruiting bodies of *Pleurotus ostreatus* were pale brown with a cap diameter ranged from 4 to 10 cm. The fruiting bodies of *Fistulina hepatica* were yellowish with a cap diameter ranged from 5 to 15 cm. The fruiting bodies of *Armillariella mellea* were honey yellow and cap diameter ranged from 3 to 10 cm.

Details concerning the families, the habitat and the edibility about the analyzed mushrooms species are presented in Table 1.

Freshly collected fruiting bodies of mushrooms were washed with deionised water and were cut with a plastic knife in small pieces. After that, the samples were dried at 60°C between 12 and 15 h, then grinded until to fine powder and finally weighed.

The substrate samples (poplar bark oak bark and soil from 0 to 5 cm depth) were dried at 70°C in 24 h. After drying the solid samples have been grinded until to fine powder and weighed.

The Mn, Fe, Cu and Zn concentration in samples was determined by Energy Dispersive X-Ray Fluorescence (Wagner 1998; Winefordner 1999; Arai 2004, Ene et al. 2009) technique, using the ElvaX spectrometer having a X-ray tube with Rh anode, operated at 50 kV and 100 μA. Two grams of each sample were pressed manually, without any chemical treatment, in a plastic vial with Mylar in the bottom and then analyzed for 300 s. The characteristic X-rays were detected by a multichannel spectrometer based on a solid state Si-pin-diode X-ray detector with a 140 μm Be window and a energy resolution of 200 eV at 5.9 keV. ElvaX software was used to interpret the EDXRF spectra. The accuracy and precision of the results as evaluated by measuring a certified reference sample (NIST SRM 1571-Orchard leaves). Good agreements were achieved between certified values and data obtained (EURACHEM/CITAC guide 2003), with recoveries ranging from 98 to 104%.

The Cr, Ni, Se, Cd and Pb concentrations were determined by Flame Atomic Absorption spectrometry using the AVANTA GBC spectrometer with hollow cathode lamps (HCL). For FAAS analyses powdered samples were digested in a Berghof MWS-2 microwave digestion system. Fungus samples (500 mg) were introduced into the digestion vessels together with 3 mL nitric acid and 5 mL hydrogen peroxide. After the digestion time (40 min) the vessels have cooled to room temperature (about 30 min.). The clear solution volume was made up to 50 mL for each sample using deionised water. Solid substrates (500 mg) were introduced into the digestion vessels together with 3 mL nitric acid and 9 mL hydrochloric acid (aqua regia). After digestion time (30 min) the vessels have cooled to room temperature and the clear solution volume is made up to 50 mL for each sample using deionised water.

Determination of Cr, Ni, Se, Cd and Pb concentrations in mushrooms and their substrate were performed using the method of calibration curve according to the absorber concentration (Wagner 1998; Sperling and Welz 1999). Several solutions of different known concentrations were prepared and the elemental concentration in unknown sample was determined by extrapolation from the calibration curve. All samples concentrations were reported as mg/kg dry weight of material.

Table 1 Families, habitat and edibility of analyzed mushrooms species

Mushrooms species	Family	Habitat	Edibility
Lycoperdon perlatum	Agaricaceae	Soil	Edible only young and white
Pleurotus ostreatus	Polyporaceae	Poplar bark	Edible
Fistulina hepatica	Fistulinaceae	Oak bark	Edible only young
Armillariella mellea	Tricholomataceae	Soil	Edible only young

Table 2 Mean concentration of heavy metals in *Pleurotus ostreatus* fruiting body and their substrate (mg/kg d.w)

Distance from metal smelter (km)	Sample (n = 5)	Cr*	Mn	Fe	Ni*	Cu	Zn	Se*	Cd*	Pb*
0.50	*Pleurotus ostreatus*	1.81	12.40	387.00	1.85	12.50	41.30	2.64	0.95	0.64
	Substrate poplar bark	3.41	29.20	406.00	3.56	15.30	40.70	4.27	2.25	5.52
4.5	*Pleurotus ostreatus*	1.74	11.50	321.00	1.33	10.90	39.50	2.79	0.87	nd
	Substrate poplar bark	3.28	27.80	352.00	2.84	12.20	38.20	5.61	2.14	2.13
10.50	*Pleurotus ostratus*	1.08	11.80	284.00	1.29	10.20	37.90	2.57	0.87	nd
	Substrate poplar bark	2.79	28.20	304.00	2.70	12.60	35.40	4.43	2.12	2.42
RDS %		1.1–7.5	2.5–6.4	4.8–11.2	1.1–3.7	2.8–7.5	1.3–4.5	1.8–5.3	1.1–12.6	3.2–4.6

*FAA spectrometry concentrations

Table 3 Mean concentration of heavy metals in *Lycoperdon perlatum* fruiting body and their substrate (mg/kg d.w)

Distance from metal smelter (km)	Sample (n = 5)	Cr*	Mn	Fe	Ni*	Cu	Zn	Se*	Cd*	Pb*
0.5	*Lycoperdon perlatum*	1.94	13.90	782.00	1.96	10.90	134.00	14.20	1.63	3.47
	Substrate soil	18.96	168.00	6470.00	9.63	24.70	75.20	19.30	5.27	12.50
4.5	*Lycoperdon perlatum*	1.87	13.30	656.00	1.83	10.20	136.00	15.40	1.58	0.87
	Substrate soil	11.93	145.00	5563.00	8.42	20.90	67.80	22.50	4.52	5.64
10.5	*Lycoperdon perlatum*	1.91	12.60	623.00	1.94	11.80	127.00	14.80	1.64	0.71
	Substrate soil	11.78	142.00	4470.00	8.75	21.40	72.90	19.70	4.49	5.23
RDS %		4.5–11.1	4.1–7.8	3.7–12.5	1.3–5.2	3.2–9.1	1.1–8.5	1.2–3.7	1.4–12.5	3.2–4.6

* FAA spectrometry concentrations

Table 4 Mean concentration of heavy metals in *Fistulina hepatica* fruiting body and their substrate (mg/kg d.w)

Distance from metal smelter (km)	Sample (n = 5)	Cr*	Mn	Fe	Ni*	Cu	Zn	Se*	Cd*	Pb*
0.50	*Fistulina hepatica*	1.65	11.00	352.00	1.32	10.45	45.60	2.15	0.70	0.80
	Substrate oak bark	3.23	26.90	421.00	3.12	12.70	42.85	3.85	2.31	3.25
4.5	*Fistulina hepatica*	1.34	10.75	305.00	1.21	9.24	43.50	2.45	0.56	nd
	Substrate oak bark	3.01	25.20	352.00	2.90	11.20	41.80	4.15	2.09	2.53
10.50	*Fistulina hepatica*	1.10	10.42	223.00	0.92	9.02	40.60	2.35	0.52	nd
	Substrate oak bark	2.83	26.20	267.00	2.05	10.60	38.50	4.07	2.02	2.02
RDS %		1.2–6.5	1.6–7.3	3.5–12.4	1.2–3.5	2.2–6.7	1.1–4.4	1.5–4.5	1.6–12.6	1.4–5.9

* FAA spectrometry concentrations

The pH of solid substrates was determined, according to ISO 10390:2005 method, with a pH meter Consort P501 at room temperature.

Results and Discussion

The heavy metal contents of the samples are given in Tables 2, 3, 4 and 5. All the metal concentrations were determined on a dry weight basis.

The content of heavy metals of the fruiting bodies of *Pleurotus ostreatus* ranged from 1.08 to 1.81, 11.8–12.4, 284–387, 1.29–1.85, 10.2–12.5, 37.9–41.3, 2.57–2.79 and 0.87–0.95 mg/kg d.w. for Cr, Mn, Fe, Ni, Cu, Zn, Se and Cd, respectively. The Pb concentration in the same samples was determined only in the sample collected in the vicinity of the metal smelter.

The content of heavy metals of the fruiting bodies of *Lycoperdon perlatum* ranged from 1.87 to 1.94, 12.6–13.9, 623–782, 1.83–1.96, 10.2–11.8. 127–134, 14.2–15.4, 1.58–1.64 and 0.71–3.47 mg/kg d.w. for Cr, Mn, Fe, Ni, Cu, Zn, Se, Cd and Pb, respectively.

The heavy metals levels in *Fistulina hepatica* fruiting bodies ranged from 1.10 to 1.65, 10.42–11.00, 223–352,

Table 5 Mean concentration of heavy metals in *Armillariella mellea* fruiting body and their substrate (mg/kg d.w)

Distance from metal smelter (km)	Sample (n = 5)	Cr*	Mn	Fe	Ni*	Cu	Zn	Se*	Cd*	Pb*
0.50	*Armillariella mellea*	1.80	10.91	745.00	1.85	10.43	158.20	12.08	1.10	7.50
	Substrate soil	13.43	130.6	6650.07	8.20	32.8	113.26	14.16	3.24	11.46
4.5	*Armillariella mellea*	1.53	9.20	539.00	1.26	8.95	124.00	12.79	0.60	2.50
	Substrate soil	12.80	126.75	5784.00	7.15	28.94	93.50	15.61	2.75	6.45
10.50	*Armillariella mellea*	0.93	7.85	432.00	1.02	6.43	80.50	12.42	0.82	1.65
	Substrate soil	11.55	123.45	4032.00	7.45	26.80	52.34	17.89	2.55	4.76
RDS %		3.1–5.8	2.0–4.8	5.7–13.9	1.0–2.6	2.2–6.7	3.6–8.2	2.4–5.8	1.2–8.5	2.7–5.3

* FAA spectrometry concentrations

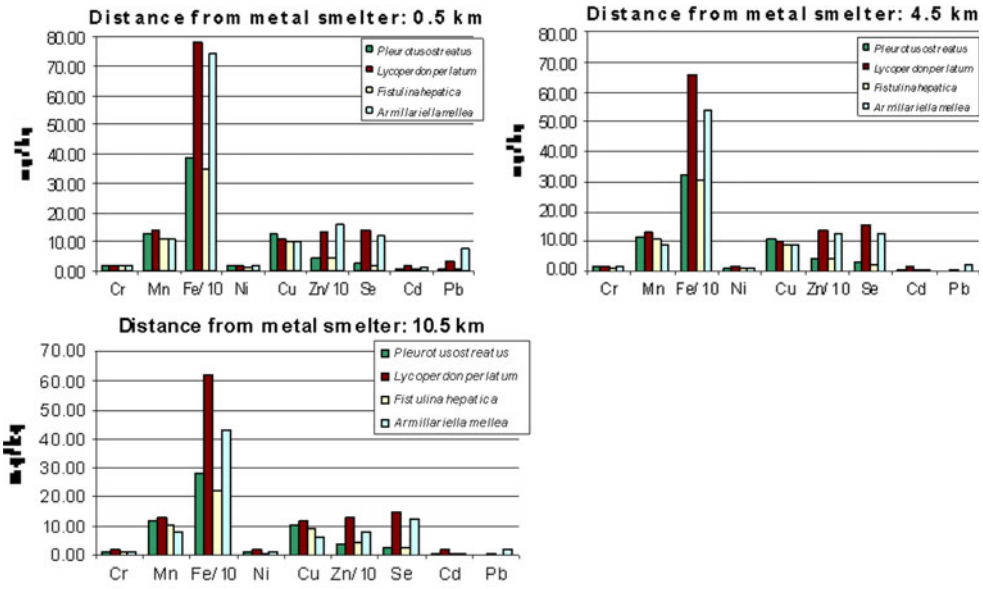

Fig. 1 The concentration of heavy metals in mushrooms species at 0.5, 4.5 and 10.5 km distance from metal smelter

0.92–1.32, 9.02–10.45, 40.60–45.60, 2.15–2.35 and 0.52–0.70 mg/kg d.w. for Cr, Mn, Fe, Ni, Cu, Zn, Se and Cd, respectively. The Pb level, 0.80 mg/kg d.w., in *Fistulina hepatica* fruiting body was determined only in the sample collected in the vicinity of the metal smelter.

In *Armillariella mellea* fruiting bodies the heavy metals level for Cr, Mn, Fe, Ni, Cu, Zn, Se, Cd and Pb, ranged from 0.93 to 1.80, 7.85–10.91, 432–745, 1.02–1.85, 6.43–10.43, 80.5–158.2, 12–08–12.42 and 1.65–7.5 mg/kg d.w.

Amongst the tested species, Zn was the most accumulated by *Armillariella mellea* (158.20 mg/kg d.w.) collected at 0.5 km from the metal smelter, and was the least accumulated by *Pleurotus ostreatus* (41.3 mg/kg d.w.) collected at the same distance from the metal smelter. A very low concentration of Mn were obtained in *Fistulina hepatica* (10.42 mg/kg d.w.) at 10.5 km from the metal smelter and in *Armillariella mellea* (7.85 mg/kg d.w.) collected samples at the same distance. A comparison between the heavy metals concentrations in mushrooms collected at various distances from the metal smelter is presented in Fig. 1.

By measuring the pH of the soil samples it were found to be a slyghtly acids (pH ranged from 6.35 to 6.90) which is the main reason of the high content in Zn and Fe of *Lycoperdon perlatum* and *Armillariella mellea* species.

The heavy metals concentrations obtained in this study in soil and in the fruiting body of the mushrooms were compared with the admitted maximum level of certain contaminants in soil,established by Romanian law 756/1997 (Table 6) and also in foodstuffs, established by the Commission of the European Communities (Commission Regulation [EC] No 1881/2006(Table 7). We can state that the values of some heavy metals such as Cd, Cu, Se, Cr and Fe in soil substrate of *Lycoperdon perlatum* and *Armillariella mellea* exceed the intervention levels stipulated in Romanian law. Furthermore, a high concentration of Pb, Cd and Cr was observed in all studied mushrooms species, especially in *Lycoperdon perlatum* and *Armillariella*

Table 6 Comparative values of heavy metals levels in soil with maximum levels from Romanian law 756/1997

Reference values for heavy metals from soil according with Romanian law 756/1997 (mg/kg d.w)				Mean concentration of heavy metals from soil (EDXRF and FAAS) (mg/kg d.w)	
Metals	Normal values	Maximum levels	Intervention levels	Substrate soil (n = 5) *Lycoperdon perlatum*	Substrate soil (n = 5) *Armillariella mellea*
Cadmium	1	3–5	5–10	5.27–4.49	3.24–2.55
Cooper	20	100–250	200–500	24.7–21.4	32.8–26.8
Manganese	900	1500–2000	2500–4000	168.0–142	130.6–123.45
Nickel	20	75–200	150–500	9.63–8.75	8.20–7.45
Lead	20	50–250	100–1000	12.5–5.23	11.46–4.76
Selenium	1	3–10	5–20	19.3–19.7	14.16–17.89
Zinc	100	300–700	600–1500	75.2–72.9	113.26–52.34
Chromium	1	4–10	10–20	18.96–11.78	13.43–11.55
Iron	3000	3000–4500	4500–7000	6470–4470	6650.07–4032.0

Table 7 Comparative values of toxic heavy metals in studied edible mushrooms with maximum levels of commission regulation

Commission Regulation (EC) No 1881/2006 setting maximum levels for certain contaminants in foodstuffs: section 3—Metals *Vegetables*		Mean concentration of heavy metals of mushrooms species fruiting body under analysis (mg/kg d.w)			
Metals	Maximum levels (mg/kg d.w)	*Pleurotus ostreatus*	*Lycoperdon perlatum*	*Fistulina hepatica*	*Armillariella mellea*
Lead (Pb)	0.10	0.64	3.47	0.80	7.50–1.65
Cadmium (Cd)	0.050–0.10	0.95–0.87	1.63–1.58	0.70–0.52	1.1–0.82
Chromium (Cr)	0.10	1.81–1.08	1.94–1.87	1.65–1.10	1.80–0.93

Table 8 Ka—accumulation coefficient of heavy metals in edible wild mushrooms fruiting body

Distance from metal smelter (km)	Cr	Mn	Fe	Ni	Cu	Zn	Se	Cd	Pb
Pleurotus ostreatus									
0.5	0.53	0.42	0.95	0.52	0.82	1.01	0.62	0.42	0.12
4.5	0.53	0.41	0.91	0.47	0.89	1.03	0.50	0.41	0.00
10.5	0.39	0.42	0.93	0.48	0.81	1.07	0.58	0.41	0.00
Lycoperdon perlatum									
0.5	0.10	0.08	0.12	0.20	0.44	1.78	0.74	0.31	0.28
4.5	0.16	0.09	0.12	0.22	0.49	2.01	0.68	0.35	0.15
10.5	0.16	0.09	0.14	0.22	0.55	1.74	0.75	0.37	0.14
Fistulina hepatica									
0.5	0.51	0.40	0.83	0.42	0.82	1.06	0.55	0.30	0.24
4.5	0.44	0.42	0.86	0.41	0.83	1.04	0.59	0.26	0.00
10.5	0.38	0.39	0.84	0.44	0.85	1.05	0.57	0.25	0.00
Armillariella mellea									
0.5	0.13	0.08	0.11	0.22	0.31	1.39	0.81	0.33	0.65
4.5	0.11	0.07	0.09	0.17	0.30	1.32	0.82	0.21	0.38
10.5	0.08	0.06	0.10	0.13	0.23	1.53	0.69	0.32	0.34

mellea (due the polluted soil habitat) compared with values recommended by Commission Regulation (EC) No 1881/2006.

A heavy metal accumulation takes place in the analysed mushrooms species. The coefficient of accumulation of heavy metals was calculated using relation:

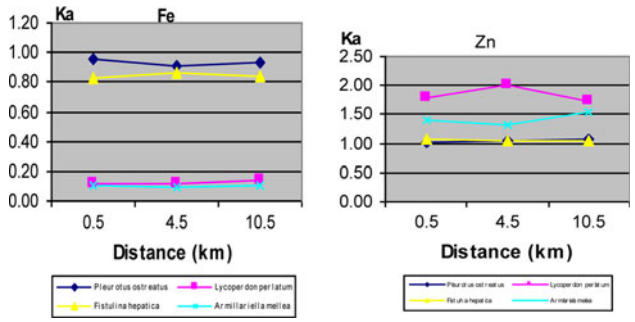

Fig. 2 The dependency of accumulation coefficients of Fe and Zn on distance from metal smelter

$$K_a = \frac{C_m}{C_s},$$

were: C_m is the concentration of heavy metal in mushroom and C_s is the concentration of heavy metal in mushroom substrate.

The coefficients of accumulation of samples are given in Table 8. The dependency of coefficients of accumulation of Fe and Zn on distance from metal smelter are given in the Fig. 2.

The coefficients of accumulation of Zn are higher for *Lycoperdon perlatum* and *Armillariella mellea* mushrooms species (trees bark substrate) and the coefficients of accumulation of Fe are higher only for *Pleurotus ostreatus* and *Fistulina hepatica* mushrooms species (soil substrate).

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