

Original Research

Content of Pb, Hg, Zn, Mn, Cu, and Fe in Macrofungi Collected from Wkrzanska Forest in Northwestern Poland

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Abstract

The concentrations of 6 metallic elements (Pb, Hg, Zn, Mn, Cu, and Fe) were determined in fruiting bodies of 8 mushrooms species and underlying soil samples from Wkrzanska Forest in northwestern Poland. The study included the following species: *Russula rosea*, *Paxillus involutus*, *Amanita citrina*, *Boletus badius*, *Amanita excelsa*, *Amanita phalloides*, *Pluteus cervinus*, and *Hygrophoropsis aurantiaca*. The total content of Hg was determined using an AMA 254 Mercury Analyzer. The total content of other elements was determined using an F-AAS iCE 3000 atomic absorption spectrometer. The highest level of bioaccumulation in analyzed species was observed for Zn and Cu, especially in *Boletus badius* (170.7; 77.81 mg·kg⁻¹ dry weight). *Amanita citrina* accumulated Pb at the highest level (5.48±3.43 mg·kg⁻¹ dw. in caps). The maximum value of Hg was observed in *Boletus badius* caps (0.46 mg·kg⁻¹ dw.). Bioaccumulation factors revealed bio-exclusion of Mn, Pb, and Fe (BCFs<1.0). A few positive and statistically significant (p<0.05) Spearman correlation coefficients of metal-to-metal bioaccumulation in studied caps of macrofungi were observed.

Keywords: heavy metals, bioaccumulation, mushrooms, trace elements

Introduction

Heavy metals constitute one of the most important environmental problems. They can be transported in an environment in various forms: emitted into the atmosphere they are carried over long distances, accumulate in soil, sediments and organisms by bioaccumulation, and the biomagnification processes in the trophic chain [1]. Deposition of trace elements and heavy metals in vegetables [2] and mushrooms [3-6] to a large extent already has been proven, especially in areas potentially contaminated or located in the zone of pollutants, such as power plants, waste dumpsites, metal smelters, and chemical plants [7].

Due to the fact that overgrowing topsoil long-lived mycelium can retrieve with water significant amounts of trace elements and pass them to the fruiting bodies of macrofungi [8], there is a risk of bioaccumulation of detrimental elements in organisms of potential consumers – wild forest animals, such as roe deer, red deer, wild boars [9], as well as people. The international surveys revealed that macrofungi are able to accumulate in their fruiting bodies a large amounts of cadmium, lead, mercury, zinc, copper, manganese, iron, chromium, silver, and nickel absorbed from contaminated soil [3-6, 10-12].

The purpose of this study was to determine the level of heavy metals (Pb and Hg) and trace element (Zn, Mn, Cu, and Fe) concentrations in fruiting bodies of 8 species of macrofungi, and to indicate the bioaccumulation factors of elements from soil substrate to fruiting bodies.

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Materials and Methods

Mushroom and soil samples were collected in October 2010 from Wkrzanska Forest adjacent to Nowe Warpno in the West-Pomeranian voivodeship of north-western Poland (Fig. 1). The main branch of industry in the sampling area is fisheries, thus the forest environment is potentially unpolluted. Macrofungi and soil substrate samples were collected from dry coniferous forest, characteristic for this region. The forest was a monoculture of *Pinus sylvestris* with a few other species occurring only in undergrowth, like juniper and maple.

Generally, 25 samples (each comprising 2-5 fruiting bodies) of eight mushroom species with underneath fruiting bodies soil samples were collected. The examined species were edible *Boletus badius* (Fr.) Fr. (5 samples) and seven-species inedible for humans: *Russula rosea* Pers. (2 samples), *Paxillus involutus* (Batsch) Fr. (5 samples), *Amanita citrina* (Schaeff.) Pers. (9 samples) and 1 sample of each – *Amanita excelsa* (Fr.) P. Kumm., *Amanita phalloides* (Vaill. ex Fr.) Link, *Pluteus cervinus* P. Kumm., and *Hygrophoropsis aurantiaca* (Wulfen) Maire. 100 g of each soil sample was collected from a depth of 0-10 cm of the surface layer. The main soil type was typical podsol.

The fruiting bodies were cleaned from any debris, divided into cap and stalk, and then dried in a laboratory oven with a temperature up to 40°C. Dried samples were homogenized using a laboratory mill. Simultaneously, soil samples were air-dried for several days, sieved with 1 mm pore sieve, and then pulverized using a mortar.

Digestion of mushroom samples (0.5 g) was performed with a cold oxi-acid mixture of 65% HNO₃ and perhydrol (4 and 1 mL, respectively) for at least 12 hours and then put in a Milestone™ microwave oven. Digestion of soil samples (0.5 g) was conducted using 6 mL of a 5:1 concentrated HNO₃:HClO₄ (65:60%) mixture and 1 mL of perhydrol and also mineralized in the microwave oven. Mineralized samples were eventually diluted to 25 mL with deionized water. The purity of utilized acids was as follows (mg·kg⁻¹): Zn ≤ 0.02, Mn ≤ 0.01 Ni ≤ 0.02, Cu ≤ 0.01, Pb ≤ 0.01, Fe ≤ 0.1 for HNO₃, and Zn ≤ 0.1, Mn ≤ 0.02, Ni ≤ 0.1, Cu ≤ 0.1, Pb ≤ 0.1, and Fe ≤ 1.0 for HClO₄.

The Zn, Mn, Ni, Cu, Pb, and Fe concentrations in dry weight (dw.) of mushrooms and soil samples was determined using F-AAS iCE 3000 series atomic absorption spectrometer. The process was validated using certified reference materials: tea leaves (INCT-TL-1) produced by the Institute of Nuclear Chemistry and Technology Warsaw, Poland, and loamy sand (CRM 036-050) produced by Resource Technology Corporation (USA and UK). The limits of detection for determined elements were (mg·kg⁻¹): Zn – 0.003, Mn – 0.002, Ni – 0.008, Cu – 0.005, Pb – 0.013, and Fe – 0.004. The sensitivities of the element determination method were as follows (mg·kg⁻¹): Zn – 0.01, Mn – 0.02, Ni – 0.05, Cu – 0.033, Pb – 0.073, and Fe – 0.052.

Hg content was determined in dried and pulverized mushrooms and soil samples using an AMA 254 Mercury Analyzer. The limit of detection for mercury was 1 ng·kg⁻¹.

Obtained results were statistically analyzed with Statistica software (v. 10.0 Statsoft Inc. MR1). The data

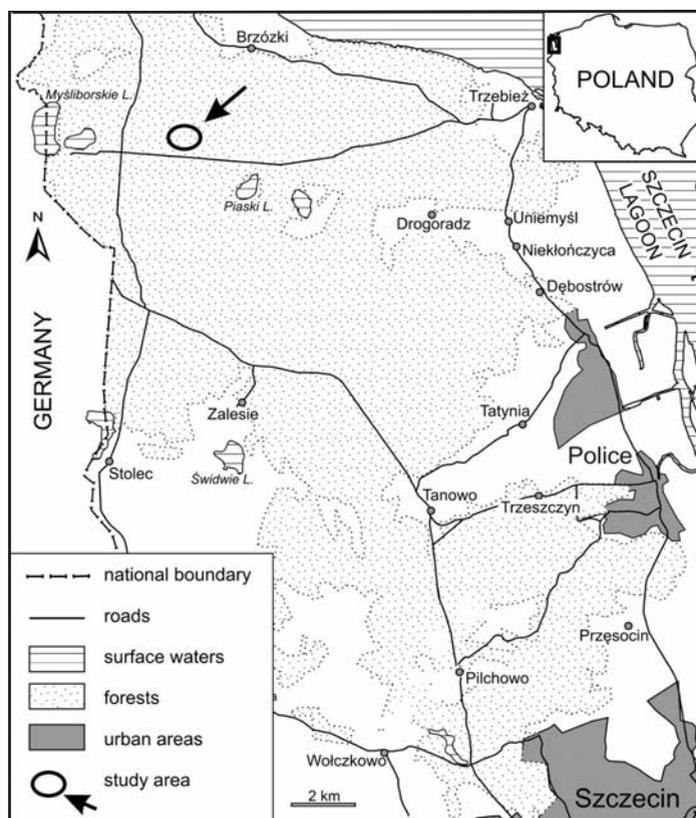


Fig. 1. Location of the sampling area.

were investigated to determine the distribution using Shapiro-Wilks' W-test. Then the element contents in fruiting bodies of analyzing species were compared with Kruskal-Wallis test ($p \leq 0.05$). Spearman's correlation coefficients ($p \leq 0.05$) between element concentrations in caps/stalks and soil were also determined.

Results and Discussion

The data of analyzed elements in dry weight of mushrooms and soil samples are given in Table 1. The contents of elements were characterized by arithmetical means value (AM), standard deviations (SD), minimum (Min), and maximum (Max) values. The bioaccumulation factors ($BCF_{\text{caps/soil}}$) of determined elements are presented in Table 2.

Maximum average concentration of Zn was observed in *Paxillus involutus* (205.2 mg·kg⁻¹ dw.). Nevertheless, the high level of this element was also determined in *Amanita excelsa* and *Boletus badius* caps, amounting to 176.0 and 170.7 mg·kg⁻¹ dw., respectively. *Boletus badius* caps accumulated also a large amount of Cu (77.81 mg·kg⁻¹ dw.). From all of the analyzed species, *Amanita citrina* distinguished by very high concentration of Pb amounting to 5.48 mg·kg⁻¹ dw. in caps and 3.58 mg·kg⁻¹ dw. in stalks, whereas the range of arithmetical mean in other species was 0.24–2.80 mg·kg⁻¹ dw.

There is currently no law about permissible maximum concentrations of heavy metals or trace elements in wild growing edible mushrooms. The European Union Regulation [13] concerns only cultivated mushrooms, as champignons or oyster mushrooms and it recommends the maximum content of Pb (0.30 mg·kg⁻¹ fresh weight = 2.7 mg·kg⁻¹ dry weight) and Cd (0.20 mg·kg⁻¹ fresh weight = 1.8 mg·kg⁻¹ dry weight). Nevertheless, currently ineffective Polish law Regulations [14, 15] used to dictate safe and permissible concentrations of Zn – 150.0 mg·kg⁻¹ dw. and Hg – 0.5 mg·kg⁻¹ dw. in wild growing edible mushrooms.

According to the presented limits, the average mean of Zn in caps of edible *Boletus badius* (170.7±26.9) was exceeded. The limit of 150.0 mg·kg⁻¹ dw. was also exceeded in caps of inedible species: *Paxillus involutus* (205.2±39.59 mg·kg⁻¹ dw.), *Amanita citrina* (190.3±28.81 mg·kg⁻¹ dw.), *Hygrophoropsis aurantiaca* (150.0 mg·kg⁻¹ dw.), and *Amanita excelsa* (176.0 mg·kg⁻¹ dw.). The excess of Hg limit wasn't observed in any of the examined macrofungi species. However, permissible concentrations of Pb were exceeded in some *Boletus badius* stalks (average mean = 1.76±2.27 mg·kg⁻¹ dw.; maximum = 5.11 mg·kg⁻¹ dw.).

There are also some WHO/FAO limits – PTWI (provisional tolerable weekly intake) for lead and mercury consumption, which equal 25 µg·kg⁻¹ body weight and 4 µg·kg⁻¹ body weight, respectively [16, 17]. Let's assume that mushroom moisture is approximately 90% and exemplar human weight is 65 kg, then a permissible amount of the analyzed *Boletus badius* consumed per week would be: 25 kg of caps and 9.0 kg of stalks for Pb limit and 18 kg of caps and 29 kg of stalks for Hg limit. Preceding calculations lead to the

conclusion that there is no significant risk of excessive intake of lead and mercury from collected *Boletus badius* fruiting bodies.

Bioaccumulation factor (BCF) between elements concentration in fruiting bodies (in this case – caps) and in soil substrate indicates the level of species ability to accumulate element. The highest average BCF of Zn (≈25.0) was observed in *Paxillus involutus*, *Boletus badius*, *Amanita citrina*, and *Amanita excelsa*. Mercury was accumulated in the highest level by *Pluteus cervinus* (BCF≈13.0). *Boletus badius* absorbed Cu the most (BCF≈60.0). Moreover, Pb, Mn, and Fe were excluded from bioaccumulation (BCF<1.0) in all of the examined species.

According to Bellion et al. [18], ectomycorrhizal fungi can exudate organic acids, which play a pivotal role in soil acidification. This species-dependent phenomenon is relevant to mobility of heavy metals and their bioavailability. Furthermore, some macrofungi are able to produce metals binding and neutralizing substances, which allows them to accumulate a significant amount of elements, even toxic ones.

Only one statistically significant difference ($p \leq 0.05$) in determined elements bioaccumulation by studied species of macrofungi was observed. Namely, between Hg concentration in *Paxillus involutus* caps (0.04±0.06 mg·kg⁻¹ dw.) and *Amanita citrina* caps (0.19±0.04 mg·kg⁻¹ dw.). Furthermore, a few positive and statistically significant ($p \leq 0.05$) Spearman correlation coefficients (r) of metal-to-metal bioaccumulation in studied caps of macrofungi were observed:

- *Paxillus involutus* Mn-Cu ($r = 0.9$)
- *Amanita citrina* Zn-Fe ($r = 0.8$) and Mn-Cu ($r = 0.7$)
- *Boletus badius* Hg-Fe ($r = 0.9$)

Presented above correlations conclude some elements that can assimilate and accumulate in fruiting bodies in a similar way.

Unfortunately, no statistically significant correlation ($p \leq 0.05$) between metal in fruiting bodies and soil samples was discovered, therefore the conclusion of using the mushrooms in soil pollution is impossible.

The concentration of Cu (22.7±0.76 mg·kg⁻¹ dw.), Fe (41.34±0.02 mg·kg⁻¹ dw.), Mn (13.41±2.26 mg·kg⁻¹ dw.), and Zn (56.25±7.73 mg·kg⁻¹ dw.) in *Russula rosea* caps in this study was lower than those obtained by Ayaz et al. [3] in mushrooms from the eastern Black Sea region of Turkey (39.40±0.17; 212.0±31.0; 62.2±2.0; 101.0±1.3 mg·kg⁻¹ dw., respectively). Borovička and Řanda [19] had investigated *Paxillus involutus* fruiting bodies collected from unpolluted sites of Czech and Slovak republics, and what is interesting, their findings of Fe (73.1–88.0 mg·kg⁻¹ dw.) and Zn (155.0–240.0 mg·kg⁻¹ dw.) concentrations in caps of mushrooms from the Slovak Republic were very similar to those obtained in this study (54.82–89.02; 156.1–246.1 mg·kg⁻¹ dw., respectively). The content of Hg in *Paxillus involutus* caps and stalks is generally very low, which was confirmed by the results of this (caps: 0.01–0.14 mg·kg⁻¹ dw.; stalks: 0.01–0.08 mg·kg⁻¹ dw.) and Falandysz and Brzostowski [20] research (0.01–0.11 mg·kg⁻¹ dw. in both – caps and stalks). Moreover, the Brzostowski et al. [21] results of bioaccumu-

Table 1. The contents of heavy metals and trace elements in macrofungi and topsoil samples (mg·kg⁻¹ dry weight).

Species	Zn			Mn			Cu			Pb			Hg			Fe		
	Cap	Stalk	Soil	Cap	Stalk	Soil	Cap	Stalk	Soil	Cap	Stalk	Soil	Cap	Stalk	Soil	Cap	Stalk	Soil
<i>Russula rosea</i> Pers.	AM	56.25	47.55	10.84	13.41	14.14	29.74	13.35	2.86	0.24	0.36	6.14	0.07	0.06	0.02	41.34	60.90	2117.1
	SD	7.73	8.66	2.06	2.26	2.24	16.94	1.40	1.61	0.04	0.45	4.50	0.01	0.01	0.01	0.02	20.90	162.8
	Min	50.78	41.42	9.38	11.81	12.55	17.76	12.36	1.72	0.21	0.04	2.95	0.06	0.05	0.01	41.32	46.12	2002.1
	Max	61.71	53.67	12.29	15.00	15.72	41.72	14.34	4.00	0.27	0.67	9.32	0.08	0.07	0.03	41.35	75.68	2232.2
<i>Paxillus involutus</i> (Batsch) Fr.	AM	205.2	181.8	8.13	19.13	46.02	32.39	59.64	1.65	0.69	0.78	8.47	0.04*	0.03	0.03	69.88	45.19	1747.1
	SD	39.59	52.89	1.57	16.36	33.24	22.42	38.43	0.24	0.87	0.63	3.78	0.06	0.03	0.01	13.24	18.23	331.6
	Min	156.1	109.4	5.91	4.48	13.18	18.47	35.03	1.28	0.08	0.20	4.88	0.01	0.01	0.02	54.82	20.89	1175.9
	Max	246.1	233.7	10.31	44.66	89.02	72.22	79.74	1.89	1.97	1.59	13.70	0.14	0.08	0.03	89.02	71.80	1967.5
<i>Amanita citrina</i> (Schaeff.) Pers.	AM	190.3	123.5	8.38	17.14	23.02	19.61	44.42	2.54	5.48	3.58	10.49	0.19*	0.11	0.03	110.1	115.1	1741.6
	SD	28.81	30.98	2.72	7.42	7.96	5.27	5.28	1.01	3.43	2.11	2.18	0.04	0.02	0.01	44.28	32.10	304.4
	Min	156.1	89.12	5.11	6.65	9.80	14.57	32.92	1.27	1.73	0.37	8.27	0.11	0.07	0.02	60.66	70.59	1110.9
	Max	231.9	184.7	13.43	31.73	34.72	31.92	67.88	4.06	12.9	7.15	13.75	0.23	0.14	0.05	195.1	154.7	2110.4
<i>Boletus badius</i> (Fr.) Fr.	AM	170.7	111.2	6.85	11.99	12.73	26.23	77.81	1.29	0.64	1.76	7.93	0.14	0.09	0.02	61.44	90.63	1596.6
	SD	26.90	20.13	1.56	10.96	6.98	9.67	17.27	0.04	0.93	2.27	3.24	0.04	0.02	0.00	12.49	48.73	273.5
	Min	150.6	83.79	5.11	5.99	6.10	15.10	52.39	1.27	0.05	0.12	4.88	0.08	0.06	0.02	52.06	30.59	1110.9
	Max	205.3	137.3	8.38	31.47	24.37	41.72	92.78	1.37	2.02	5.11	11.31	0.19	0.11	0.02	81.16	147.2	1738.6
<i>Hygrophoropsis aurantiaca</i> (Wulfen) Maire	150.0	93.98	7.81	37.25	52.91	25.40	12.23	3.46	1.66	<0.013			0.12	0.10	0.03	54.09	89.99	1914.5
<i>Pluteus cervinus</i> P. Kumm.	66.46	42.88	4.80	6.84	15.34	19.34	21.95	14.78	2.05	0.55	1.30	5.71	0.38	0.27	0.03	108.3	85.69	2073.8
<i>Amanita phalloides</i> (Vaill. ex Fr.) Link	50.75	15.93	19.64	8.10	20.65	67.48	51.48	31.62	12.21	0.30	1.25	4.10	0.46	0.25	0.07	159.8	94.28	195.8
<i>Amanita excelsa</i> (Fr.) P. Kumm.	176.0	115.5	7.18	7.80	13.71	18.50	54.97	28.34	3.43	2.80	4.26	8.01	0.18	0.13	0.02	213.0	193.7	2270.0

AM – arithmetical mean, SD – standard deviation, Min – minimum value, Max – maximum value,

*statistically significant difference (p≤0.05)

Table 2. The caps/soil bioaccumulation factors (BCF) of heavy metals and trace elements in macrofungi.

Species		BCF _{Caps/Soil}					
		Zn	Mn	Cu	Pb	Hg	Fe
<i>Russula rosea</i> Pers.	AM	5.19	0.45	7.94	0.04	3.50	0.02
	SD	3.76	0.13	0.47	0.01	1.00	0.00
	Min	5.41	0.66	12.88	0.07	6.00	0.02
	Max	5.02	0.36	5.81	0.03	2.67	0.02
<i>Paxillus involutus</i> (Batsch) Fr.	AM	25.24	0.59	35.30	0.08	1.54	0.04
	SD	25.22	0.73	69.02	0.23	10.25	0.04
	Min	26.41	0.24	27.37	0.02	0.50	0.05
	Max	23.87	0.62	42.19	0.14	4.67	0.05
<i>Amanita citrina</i> (Schaeff.) Pers.	AM	22.71	0.87	17.46	0.52	6.11	0.06
	SD	10.60	1.41	12.16	1.57	4.19	0.15
	Min	30.55	0.46	25.92	0.21	5.50	0.05
	Max	17.27	0.99	16.72	0.93	4.60	0.09
<i>Boletus badius</i> (Fr.) Fr.	AM	24.91	0.46	60.13	0.08	6.80	0.04
	SD	17.27	1.13	40.81	0.29	0.00	0.05
	Min	29.47	0.40	41.25	0.01	4.00	0.05
	Max	24.50	0.75	67.72	0.18	9.50	0.05
<i>Hygrophoropsis aurantiaca</i> (Wulfen) Maire		19.20	1.47	7.37	n. d.	4.00	0.03
<i>Pluteus cervinus</i> P. Kumm.		13.85	0.35	10.71	0.10	12.67	0.05
<i>Amanita phalloides</i> (Vaill. ex Fr.) Link		2.58	0.12	4.22	0.07	6.57	0.82
<i>Amanita excelsa</i> (Fr.) P. Kumm.		24.51	0.42	16.03	0.35	9.00	0.09

AM – arithmetical mean, SD – standard deviation, Min – minimum value, Max – maximum value, n. d. – no data

lation factors of some metallic elements in *Paxillus involutus* confirmed that Fe, Mn, and Pb are excluded from bioaccumulation by this species (BCF < 1.0).

The range of average mean of Pb content in *Boletus badius* (0.42-3.70 mg·kg⁻¹ dw. in caps) from different sites of Poland [4] is slightly higher than that obtained in this study (0.5-2.02 mg·kg⁻¹ dw.). However, the findings of Sembratowicz and Rusinek-Prystupa [22] in *Boletus badius* from Ożarów and Malastowska Magura (0.64 and 0.81 mg·kg⁻¹ dw. in caps) were comparable to our study (0.64±0.93 mg·kg⁻¹ dw.). The obtained results of Pb, Mn, and Fe bioaccumulation factors (Pb – 0.08, Mn – 0.46, Fe – 0.04) and Malinowska et al. [4] study (0.13, 0.23, 0.02, respectively) affirmed bio-exclusion of these elements by *Boletus badius*.

Conclusions

Analyzed macrofungi bioaccumulate large amounts of trace elements and heavy metals in their fruiting bodies, which may have a negative influence on potential con-

sumers. However, several elements, i.e. Mn, Pb, and Fe are excluded from bioaccumulation, despite their quite high concentration in soil. Usually, the concentration of bioaccumulated elements is higher in caps than in stalks of macrofungi. There are some differences in the bioaccumulation of some elements among individual species. The bioaccumulation of some metals in the macrofungi caps is significantly interrelated.

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