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CONCENTRATION OF SELECTED TRACE ELEMENTS IN *XEROCOMUS BADIUS* MUSHROOM BODIES – A HEALTH RISK FOR HUMANS?

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ABSTRACT

Introduction. As regards a significant intake of wild growing edible mushrooms, especially in East and Central Europe, concentrations of toxic elements should be periodically analysed. The aim of the study was to assess changes in concentrations of selected trace elements (Ba, Cd, Co, Cu, Fe, Hg, Mn, Ni, Pb, Sr and Zn) in a mushroom species, *Xerocomus badius*.
Material and methods. *Xerocomus badius* fruiting bodies were collected from five regions of Poland within the last 20 years (selected years when these mushrooms were growing). Flame atomic absorption spectrometry (FAAS) was used for determination of 10 elements while for Hg cold vapour atomic absorption spectrometry (CVAAS) was used.
Results. Generally the results show no significant differences in the accumulation efficiency of individual elements by mushrooms collected from different regions of Poland, but significant differences were observed in the accumulation efficiency of these elements by mushrooms collected in particular years of their harvest. The highest accumulation indicated by bioconcentration factors (BCFs) was observed for Cu (10.03), Hg (148.15) and Zn (4.88).
Conclusion. Concentrations of Cu, Fe, Mn, Zn in the tested mushrooms were found to be lower than the values of the recommended dietary allowances (RDA), therefore the levels of these elements are not toxic for people. In our opinion, occasional consumption of these mushroom fruiting bodies within the last 20 years in Poland did not provide significant amounts of analysed trace elements (no more than other foods).

Key words: accumulation, mushroom, trace elements, *Xerocomus badius*

INTRODUCTION

Edible mushrooms are homely food for people in eastern and central Europe, while in western and northern European countries wild edible fungi are less popular [Druzhinina and Palma-Oliveira 2004]. Many studies have confirmed the high and balanced nutritional value of mushrooms [Dadáková et al. 2009], since they are rich sources of digestible proteins, vitamins B, D and K and in some cases vitamins

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A and C [Sanmee et al. 2003]. Carpophores are a good source of minerals, particularly K, P, Ca, Mg and Na [Mattila et al. 2001]. Mushrooms are considered not only as spice and taste ingredients, but also as a nutritional supplement in the human diet and can also play a role as functional foods [Cheung 2008]. It is worth stressing here that many studies have focused on the medicinal properties of mushrooms [Mantovani et al. 2008, Wasser 2011]. They have also been reported to show anti-inflammatory, antibacterial, antiviral and antioxidant potential [Robles-Hernandez et al. 2008].

Nevertheless, it is also necessary to consider safety and precautions in consumption of wild edible fungi [Drewnowska et al. 2012]. Mushrooms are known to accumulate high amounts of heavy metals, both in ecologically pure [Kojta et al. 2011] and in contaminated areas [Carvalho et al. 2005]. There are mushroom species with a particular capacity for bioaccumulation of certain trace elements [Falandysz et al. 2008]. It was reported that *Boletus edulis*, *Suillus luteus* and *Macrolepiota procera* contained high amounts of Hg even in samples collected in unpolluted areas [Falandysz et al. 2007, Chudzyński et al. 2011, Gucia et al. 2012].

It was observed that the bioconcentration factor (BCF) of heavy metals, especially Hg [Falandysz and Bielawski 2007, Falandysz and Gucia 2008], Cd and Pb [Falandysz et al. 2011], in mushrooms was much higher than in such crop plants as tomato, pepper and corn [Tuzen 2003]. Trace element concentrations in edible mushrooms have been studied in Poland [Kowalewska et al. 2007] and other European countries, e.g. the Czech Republic, Turkey, Spain and Italy [Cocchi et al. 2006, Svoboda et al. 2006, Kalac 2010] and knowledge on the toxicological risk of heavy metals for humans in significantly greater than it was assumed some years ago [Melgar et al. 2009, Falandysz et al. 2012].

Xerocomus badius (*X. b.*) is a popular wild edible mushroom in many regions of Poland and selected European countries and is readily gathered and consumed by mushroom pickers. Many authors have demonstrated high contents of trace elements in carpophores of *X. b.* collected in different regions of Poland [Falandysz and Bielawski 2001, Malinowska et al. 2004, Rudawska and Leski 2005].

The purpose of our study was to determine selected trace elements' presence (Ba, Cd, Co, Cu, Fe, Hg, Mn, Ni, Pb, Sr and Zn) in this mushroom species as a food by estimation of changes in selected trace element concentrations in fruiting bodies of *X.b.* collected within the last 20 years from five regions of Poland.

MATERIAL AND METHODS

Mushroom sample collection

Fruiting bodies of *X. b.* were collected within the last 20 years (selected years when the mushrooms were grown in the same locations) from 5 regions of Poland comprising the following provinces: the Pomeranian Voivodeship (P_{2009}(n = 17), P_{2008}(n = 9), P_{2007}(n = 16), P_{2005}(n = 14), P_{1995}(n = 23) and P_{1991} (n = 6)), the Greater Poland Voivodeship (G_{2009} (n = 8), G_{2008}(n = 17), G_{2007}(n = 19), G_{2005}(n = 16), G_{2003}(n = 15), G_{2001}(n = 10), G_{1998}(n = 22) and G_{1995} (n = 14)), the Łódź Voivodeship (C_{2008}(n = 18), C_{2007} (n = 25), C_{2003}(n = 8) and C_{2001}(n = 11)), the Opole and Silesian Voivodeship (S_{2005}(n = 9), S_{2003}(n = 15), S_{2001} (n = 12), S_{1998}(n = 10), S_{1995}(n = 5) and S_{1991}(n = 14)) and the Lower Silesian Voivodeship (L_{2008}(n = 12), L_{2007} (n = 11), L_{2001}(n = 7), L_{1998}(n = 16), L_{1995}(n = 11) and L_{1991}(n = 7)). The numbers presented in round brackets (n) provide information on the number of mushroom samples (specimens) collected per year and per location. For precise geographical information concerning the tested territory the ranges of latitude and longitude are provided: Pomeranian Voivodeship 53°34′22.22″--54°39′20.66″ N, 16°33′46.48″-18°15′03.91″ E; Greater Poland Voivodeship 51°53′19.58″-52°56′27.74″ N, 15°09′14.73″-17°44′39.12″ E; Łódź Voivodeship 51°47′10.08″-51°50′40.72″ N, 19°15′20.97″-19°56′ 27.31″ E; Silesian Voivodeship 49°58′14.12″-50°35′ 55.47″ N, 17°37′04.61″-19°38′02.91″ E; and Lower Silesian Voivodeship 51°18′47.44″-51°47′19.70″ N, 14°58′48.06″-16°24′15.41″ E.

Soil sample collection

Soil samples from each location where mushrooms were collected from a depth of 5 cm below the ground surface around the mushroom fruiting bodies. Everywhere where greenery (grass, moss) was found it was removed and soil was collected from the same depth. Soil samples (about 0.5 kg) were collected using a soil auger (5 cm outside diameter × 1.5 m tube length), and transferred to air-tight polypropylene containers filled

to the brim (to prevent changes in the chemical composition). Then materials (mushrooms and soils) were transported to the laboratory within 24 h.

Mushroom material preparation

The mushrooms after collection were carefully washed with distilled water (Milli-Q Advantage A10 Water Purification Systems, Merck Millipore) to remove soil particles, they were dried in an electric drier at 105 ±2°C for 48 h (analysis of mushroom dry weight), then dry samples (a whole mushroom: cap and stipe jointly) were ground to powder for 2 min in a laboratory Cutting Boll Mill 200 by RETSCH. The material (3 representative samples, 1 g each) was mineralized in a CEM Mars 5 Xpress microwave mineralization system (CEM Corp., Matthews, NC, USA) in a closed system (55 mL vessels) using 6 mL of concentrated HNO_3 (Suprapoor®, Merck) and 1 mL of 30% H_2O_2 (Fluka). Digestion of the mushroom materials was performed according to a microwave program composed of three stages: the first stage – power 600 W, time 3 min, temperature 100°C; the second stage – power 600 W, time 3 min, temperature 120°C; and the third stage – power 1200 W, time 8 min, temperature 200°C. Materials after digestion were filtered through 45-mm filters (Sartorius Stedim Biotech, Grade 1288), quantitatively transferred and diluted with deionized water to 50 mL final volume.

Soil analysis

The soil samples for trace elements analysis were prepared in the same way as mushroom samples (drying, grinding and mineralization), and analyses of total trace element contents in soils were performed according to the Polish Standard PN-ISO 11047 (2001). The content of Hg was analysed by cold vapour atomic absorption spectrometry (CVAAS). The other analysed soil parameters were total organic carbon, total nitrogen, redox potential, electrolytic conduction, dry matter and pH. The analysed soil parameters included:

1. Total organic carbon content [PN-ISO 14235]
2. Total nitrogen content (Kjeldahl method)
3. Redox potential [ISO 11271:2002]
4. Electrolytic conduction [PN-ISO 1265+AC1: 1997]
5. pH [PN-ISO 10390:1997]
6. Water content [PN-ISO 11465: 1999].

Characteristics of soils analysed from the studied regions of Poland are presented as ranges of the lowest and highest values in Table 1.

Analytical method and calculation

Concentrations of selected trace elements (Ba, Cd, Co, Cu, Fe, Mn, Ni, Pb, Sr and Zn) in mushrooms were analyzed with flame atomic absorption spectrometry (FAAS) using an Agilent Technologies AA Duo – AA280FS/AA280Z spectrometer (Agilent Technologies, Mulgrave, Victoria, Australia) equipped with a Varian hollow-cathode lamp (HCL). Total Hg concentration was analysed by cold vapour atomic absorption spectrometry (CVAAS) by the same spectrometer with the VGA-77 (lamp current – 4 mA, wave length – 253.7 nm; slit width – 0.5R nm) and 1000 mg/L mercury standard (SpectraPure, Arlington, Texas). Calibration curves were prepared before the analysis with four replicates per trace element concentration. Experimental conditions of the applied analysical method (techniques) and statistical parameters of the calibration lines are presented in Table 2.

According to Gast et al. [1988], the efficiency of trace element accumulation may be characterised by bioconcentration factor (BCF) values calculated as the ratio of trace element concentration in mushrooms to the concentration of this trace element in soil.

Verification of recorded results

Results were validated on the basis of a certified reference material, Mushroom powder – Trace elements IC-CS-M-1, analysed in every twentieth determination set and simultaneous analyses of randomly selected samples using an interlaboratory comparison (another laboratory using atomic absorption spectrometry). The use of interlaboratory comparison was necessary because no universal mushroom material with certified values for all tested trace elements was available (Table 3).

Statistical methods

To determine similarities in accumulation efficiency for 11 trace elements taken up by fruiting bodies of *X. b.* collected from five regions of Poland, the clustering trait algorithm was used with cluster analysis by the Ward method for graphical presentation of results and determination of Euclidean distances. Single-factor

Table 1. Characteristics of the samples described by ranges of particular parameter values or trace element concentrations on a dry-weight basis within last 20 years

Parameter/ element	Unit	Provinces (regions) of Poland				
		Pomeranian Voivodeship	Greater Poland Voivodeship	Łódź Voivodeship	Silesian Voivodeship	Lower Silesian Voivodeship
pH	–	4.28-5.71	5.70-5.53	4.85-5.97	3.97-5.04	5.20-6.34
Conductivity	$mS \cdot m^{-1}$	35-89	29-95	88-101	101-131	42-57
Redox potential	mV	198-402	219-331	255-309	209-472	253-326
Ba	$mg \cdot kg^{-1}$	15.84-21.85	11.42-15.74	15.39-17.33	16.22-25.35	9.06-14.58
C	%	1.14-1.87	1.22-2.02	1.95-2.45	1.44-1.85	1.73-2.06
Cd	$mg \cdot kg^{-1}$	0.24-0.66	0.59-0.92	0.89-1.87	1.08-1.45	0.16-3.52
Co	$mg \cdot kg^{-1}$	1.53-2.95	1.21-1.27	1.90-3.04	2.20-3.72	2.38-4.92
Cu	$mg \cdot kg^{-1}$	1.47-3.14	1.64-3.03	2.01-3.59	2.54-4.02	2.36-3.91
Fe	%	0.27-0.51	0.47-1.29	0.49-0.92	0.64-1.94	0.63-1.23
Hg	$mg \cdot kg^{-1}$	0.03-0.09	0.04-0.08	0.04-0.06	0.14-0.27	0.02-0.07
Mn	%	0.02-0.03	0.01-0.03	0.08-0.14	0.05-0.09	0.06-0.11
N	%	0.06-0.11	0.08-0.15	0.07-0.10	0.09-0.22	0.04-0.09
Ni	$mg \cdot kg^{-1}$	1.94-2.48	2.05-4.92	3.05-7.29	3.49-5.92	3.89-5.96
Pb	$mg \cdot kg^{-1}$	3.02-6.57	4.72-6.86	5.34-7.38	5.08-9.24	6.42-9.07
Sr	$mg \cdot kg^{-1}$	0.21-2.48	0.79-2.15	1.64-2.77	2.82-4.26	1.42-2.28
Zn	$mg \cdot kg^{-1}$	10.39-18.46	9.50-18.61	8.02-14.39	13.34-21.84	13.7-19.94

Table 2. Experimental conditions of used methods and statistical parameters of the calibration lines

Element		Ba	Cd	Co	Cu	Fe	Mn	Ni	Pb	Sr	Zn
Technique		FAAS	FAAS	FAAS	FAAS	FAAS	FAAS	FAAS	FAAS	FAAS	FAAS
Wavelength	nm	553.6	228.8	240.7	324.8	248.3	279.5	232.0	217	460.7	213.9
Slit width	nm	0.5	0.5	0.5	0.5	0.2	0.2	0.2	1.0	0.5	1.0
Lamp current	mA	20	4	7	4	5	5	4	9	10	5
Model		Quadratic – provides a second order least squares line forced through zero									
Sensitivity	B(x)	0.005	0.16	0.08	0.01	0.002	0.004	0.05	0.04	0.06	0.09
LOD	$mg \cdot kg^{-1}$	0.015	0.02	0.21	0.16	0.003	0.005	0.16	0.15	0.12	0.02
Minimum concentration	$C_{min}/mg \cdot kg^{-1}$	0.214	0.25	0.22	2.61	43.26	7.004	0.19	0.41	0.13	1.55
Maximum concentration	$C_{max}/mg \cdot kg^{-1}$	2.288	2.49	2.27	18.23	206.54	31.771	1.64	3.72	1.25	47.96
Correlation coefficient	r	0.9993	0.9991	0.9993	0.9995	0.9993	0.9990	0.9989	0.9997	0.9994	0.9992

Table 3. Comparison of trace element concentrations (mg·kg^{-1}) on the basis of standard curve and after corrections by certified reference material of mushroom IC-CS-M-1 and results of interlaboratory comparison

Trace element	CRM		Interlaboratory comparison	
	certified value	authors' results	other laboratory result	authors' results
Ba	–	–	0.48 ±0.05	0.42 ±0.03
Cd	0.273 ±0.093	0.269 ±0.085	1.21 ±0.11	1.24 ±0.08
Co	–	–	0.82 ±0.07	0.86 ±0.09
Cu	9.12 ±0.83	9.17 ±0.92	8.61 ±0.61	8.48 ±0.46
Fe	–	–	110.12 ±8.34	107.12 ±7.70
Hg	0.174 ±0.018	0.177 ±0.016	1.15 ±0.09	1.23 ±0.06
Mn	–	–	21.47 ±1.57	21.28 ±1.19
Ni	–	–	1.09 ±0.08	0.98 ±0.04
Pb	0.476 ±0.041	0.482 ±0.039	0.47 ±0.05	0.45 ±0.03
Sr	–	–	0.24 ±0.02	0.28 ±0.02
Zn	60.94 ±4.62	61.03 ±4.85	18.33 ±1.63	18.51 ±1.32

Table 4. Trace elements concentration (mg·kg^{-1} d.w.) in mushrooms (mean, SD) collected from different regions of Poland and BCF values ranges in round brackets

Trace elements	Voivodeships (region) of Poland				
	Pomeranian Voivodeship	Greater Poland Voivodeship	Łódź Voivodeship	Silesian Voivodeship	Lower Silesian Voivodeship
1	2	3	4	5	6
Ba	1.28 ±0.37^{b}	1.52 ±0.57^{bc}	1.42 ±0.15^{a}	1.08 ±0.24^{a}	1.71 ±0.31^{ab}
	(0.03-0.11)	(0.02-0.22)	(0.07-0.11)	(0.03-0.09)	(0.09-0.24)
Cd	1.47 ±0.36^{a}	1.59 ±0.45^{a}	1.89 ±0.60^{a}	1.67 ±0.52^{a}	1.69 ±0.34^{a}
	(1.56-9.33)	(0.93-4.16)	(0.50-2.98)	(0.58-2.34)	(0.35-14.61)
Co	1.07 ±0.28^{a}	1.06 ±0.32^{a}	1.19 ±0.42^{ab}	1.16 ±0.33^{a}	1.57 ±0.56^{b}
	(0.24-0.98)	(0.46-1.21)	(0.17-0.89)	(0.20-0.74)	(0.18-1.03)
Cu	9.70 ±2.79^{a}	10.49 ±2.54^{a}	10.50 ±3.78^{ab}	11.04 ±3.69^{ab}	13.59 ±4.40^{b}
	(1.91-9.60)	(2.58-10.03)	(1.27-7.12)	(1.55-6.97)	(1.47-8.38)
Fe	180.17 ±77.61^{a}	154.97 ±55.03^{a}	158.95 ±56.19^{a}	183.26 ±59.30^{a}	147.09 ±43.76^{a}
	(0.02-0.12)	(0.01-0.06)	(0.01-0.05)	(0.01-0.04)	(0.01-0.03)
Hg	1.72 ±0.38^{a}	1.43 ±0.31^{a}	1.50 ±0.36^{a}	1.71 ±0.36^{ab}	2.08 ±0.54^{b}
	(15.14-78.90)	(10.61-47.40)	(14.98-45.08)	(5.00-17.73)	(19.99-148.15)

Table 4 – cont.

1	2	3	4	5	6
Mn	20.07 ±3.56[a]	20.79 ±4.27[a]	20.01 ±2.17[b]	25.26 ±6.00[a]	20.87 ±4.20[a]
	(0.05-0.13)	(0.04-0.30)	(0.01-0.03)	(0.02-0.07)	(0.01-0.05)
Ni	1.03 ±0.14[ab]	0.92 ±0.19[a]	0.89 ±0.28[a]	1.14 ±0.21[b]	0.87 ±0.23[a]
	(0.31-0.63)	(0.14-0.67)	(0.08-0.42)	(0.14-0.40)	(0.09-0.31)
Pb	0.51 ±0.19[a]	0.73 ±0.13[a]	0.82 ±0.18[a]	1.10 ±0.30[ab]	0.98 ±0.40[b]
	(0.04-0.27)	(0.08-0.21)	(0.08-0.19)	(0.09-0.34)	(0.04-0.26)
Sr	0.51 ±0.13[a]	0.51 ±0.12[a]	0.49 ±0.11[a]	0.53 ±0.18[a]	0.51 ±0.17[a]
	(0.12-3.49)	(0.15-0.97)	(0.11-0.40)	(0.06-0.28)	(0.13-0.60)
Zn	29.13 ±10.90[b]	30.76 ±6.09[a]	30.34 ±6.67[a]	31.21 ±10.40[ab]	38.81 ±10.28[ab]
	(1.01-4.88)	(1.19-4.34)	(1.39-4.88)	(0.92-3.67)	(1.36-4.19)

[a,b] Means within rows, with different letters, differ significantly at $P \leq 0.05$ (Tukey test).

multivariate analysis of variance (MANOVA) [Morrison 1976] was applied to examine the differences in mean values of all the tested trace elements between all years of sample collection from particular regions of Poland. Rejection of the null hypothesis of no differences between years of sample collection from all regions justified testing of particular hypotheses concerning individual comparisons of regions with respect to all trace elements using the proper F-statistic [Mahalanobis 1936]. For configuration of regions (years of sample collection) with regard to all 11 trace elements, canonical variate analysis was performed.

RESULTS

Analysis of accumulation efficiency of individual trace elements by fruiting bodies of *X. b.* showed in general no significant differences in their accumulation efficiency by mushrooms collected from different regions of Poland (Table 4), whereas significant differences in accumulation efficiency of elements were observed between years of sample collection.

The accumulation results presented in Table 4 for particular regions of Poland are average values calculated based on mushroom samples collected in all years when mushrooms were growing. Generally no differences in Cd, Fe and Sr accumulation were observed for all the examined regions of Poland; however, for the other analysed trace elements significant differences were mainly observed between mushrooms collected from the Silesia region and other regions. Additionally, the differences between the highest and the lowest accumulation ($mg \cdot kg^{-1}$ d.w.) were region-dependent and were the highest for mushrooms collected from the Pomeranian Voivodeship for Ba (1.06), Fe (224.04) and Zn (32.10); the Silesia Voivodeship for (Cd (1.68), Hg (1.13) and Mn (17.89) and the Lower Silesia Voivodeship for Co (1.60), Cu (14.05), Ni (0.68), Pb (1.28) and Sr (0.55).

Efficiency of trace element accumulation by fruiting bodies of *X. b.* was also the basis for the analysis of calculated bioconcentration factors (BCFs). Considering the large number of results and significant differences in trace element concentrations in analysed fruiting bodies of mushroom species and in soil samples, BCF values are presented as ranges of values (Table 4). For the majority of trace elements in mushrooms collected from regions of Poland, the BCF values were similar. BCF values > 1 were observed only for Cu, Hg and Zn accumulation and significant differences between the lowest and the highest BCF values for these elements in all tested mushrooms were 1.3-10.1, 5.0-148.1 and 1.01-4.88, respectively. For the other heavy metals, BCF values were < 1, which indicates no accumulation of these elements.

To determine similarities in trace element accumulation by *X.b.* fruiting bodies collected from all

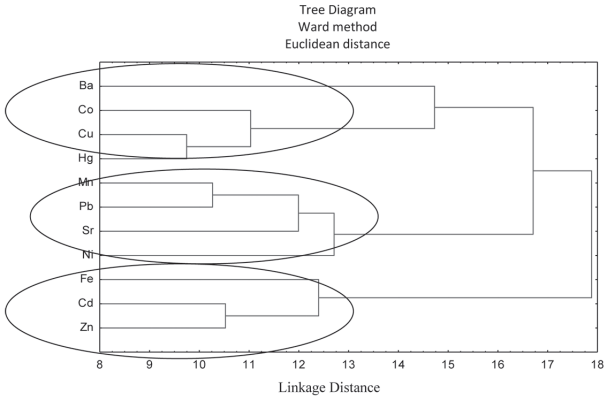

Fig. 1. Horizontal Hierarchical Tree Plot for studied elements

the studied regions of Poland, the clustering "objects" algorithm was used. The graphical presentation of results by the Ward method allowed 3 areas of similarities to be determined: Ba, Co, Cu, Hg, and/or Mn, Pb, Sr, Ni, and/or Cd, Fe, Zn. The similar values of Euclidean distances in the case of those groups allows us to conclude that accumulation of tested trace elements in particular groups is similar (Fig. 1).

Additionally, the hypothesis of no differences between mushrooms collected in a particular year and from a particular region of Poland was tested. Based on hypothesis testing at $F_{0.01}$, significant differences in accumulation efficiency of all tested trace elements from different years and locations of sample collection were found. Based on canonical analysis, relationships between particular observations are presented in Figure 2, which shows mean values of all results (all years

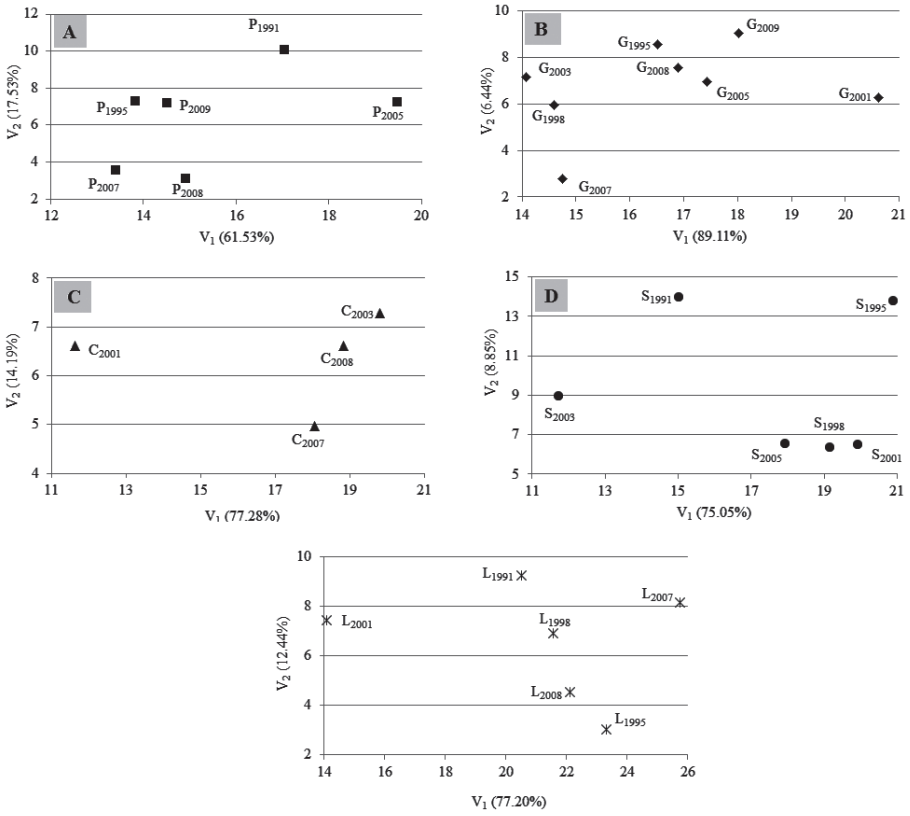

Fig. 2. Configuration of observations in the system of the first two canonical variables (V_1 and V_2) as regards accumulation efficiency for all trace elements depending on all years of mushroom sample collection

of fruiting body collection) for individual regions of Poland.

Such a presentation of relationships between observations was necessary due to the lack of possibility to draw a dendrite only for an individual region. Canonical variables V_1 and V_2 were used as coordinates in a 2-dimensional system (on a plane) obtained as a result of transformation of an 11-dimensional space with information loss of about 8% [100% – (31.53% + 14.88%)].

DISCUSSION

For a long time mushrooms have played an important role in the environment, because they comprise over 1.5 million species and they have medicinal and nutritional values [Wasser 2002, Guillamón et al. 2010]. They have been used as substrates for the production of clinically important drugs, e.g. steroids, hormones, penicillin and painkillers [Smith et al. 2002]. The results presented here indicate diverse accumulation levels of trace elements by fruiting bodies of *X.b.* It is necessary to determine whether mushrooms are sources of such high amounts of trace elements that they should be eliminated from people's diet or their intake be reduced.

The most significant aspect is determining the actual amounts of trace element intake by humans and the influence of these doses on their life and health. According to the data presented here, the highest concentrations (mg·kg^{-1} d.w.) of studied trace elements (all regions of Poland) were as follows: 2.56 (Ba), 2.65 (Cd), 2.46 (Co), 337.29 (Fe), 2.96 (Hg), 35.14 (Mn), 1.39 (Ni), 1.72 (Pb), 0.85 (Sr) and 57.34 (Zn). There are insufficient statistical data on mushroom intake; therefore for theoretical considerations we suggest a medium intake of 300 g (wet mass) of a "mushroom" dish, which is equivalent to 30 g of dry mass of mushrooms (assuming 90% water content). According to the determined concentrations of trace elements their intake would be as follows: 0.08 (Ba), 0.08 (Cd), 0.08 (Co), 10.12 (Fe), 0.09 (Hg), 1.05 (Mn), 0.04 (Ni), 0.05 (Pb), 0.03 (Sr) and 1.73 (Zn) mg·day^{-1}.

To determine the health risk for people posed by concentrations (doses) of trace elements tested by us, we used the recommended intakes, such as recommended dietary allowances (RDAs) according to the dietary reference intakes (DRIs) presented in 2001 by the National Academy of Sciences, Food and Nutrition Board, USA. RDA values for some described elements (for males aged 31-50 years) are 8 (Fe), 2.3 (Mn), 0.9 (Cu) and 11 mg (Zn) a day; therefore the account for 21.97% (Cu), 42.16% (Fe), 15.28% (Mn) and 5.21% (Zn) of RDA. The above allows us to conclude that fruiting bodies of *X. b.* tested in our studies did not constitute a direct health risk for humans [Gençcelep et al. 2009] with reference to these elements only. Unfortunately, mushrooms also contain some other toxic elements, especially Hg [Melgar et al. 2009] and post-Chernobyl radiocaesium [Kalac 2001, Giovani et al. 2004]. In the Polish law, limited concentrations (mg·kg^{-1}) are determined only for As (0.2), Cd (0.15), Hg (0.05) and Pb (0.5) [Ordinance of Ministry... 2003]. All of the above trace elements are highly toxic and accumulate in selected human organs leading to a gradual destruction of the organism [WHO 1996, Sharma et al. 2009, Yanagisawa et al. 2009]. Therefore it is necessary to determine whether the intake of mushrooms should be limited. In our opinion, it is so when mushrooms constitute a significant part of people's diet, and it is not necessary when mushroom intake is occasional.

The content of metals, including heavy metals, in vegetables and fruits has been the object of numerous investigations in recent years and some of them demonstrate that widely consumed vegetables may be characterised by a similar or higher accumulation of these elements in edible parts in comparison with mushrooms. In our experiments, the content of Pb in *X. b.* ranged from 0.51 ±0.19 to 1.10 ±0.30 mg·kg^{-1} d.w. In the case of carrot, which is a frequently consumed vegetable, the concentration of this metal can be very high, amounting to 20.35 mg·kg^{-1} d.w. [Sharma and Chettri 2005]. According to earlier studies of Bosiacki [2007], in Poland the content of Pb in this vegetable was determined at 7.0 mg·kg^{-1} d.w., and in India at 5.21 mg·kg^{-1} d.w. [Haloi et al. 2010]. Significant amounts of Pb were determined in lettuce, 4.11--11.59 mg·kg^{-1} d.w. [Bosiacki 2007], and radish, 30.93 mg·kg^{-1} d.w. [Sharma and Chettri 2005]. Zahir et al. [2009] determined Pb in very frequently and commonly consumed bananas, at the level of 3.15 ±0.67 mg·kg^{-1} d.w. Sharma and Chettri [2005] found presence of Ni in carrot cultivated in the region of the

Kathmandu Valley (Nepal) at levels ranging from 0.25 to 3.50 mg·kg^{-1} d.w., whereas its levels in leaves of spinach ranged between 2.5 and 7.25 mg·kg^{-1} d.w. High Ni concentrations were also determined in widely consumed fruits. Mahdavian and Somashekar [2010] reported concentrations of this metal in oranges and bananas in India at 33.2 and 8.9 mg·kg^{-1} d.w., respectively. In our investigations, levels of Ni in *X. b.* collected in various parts of Poland ranged between 0.87 ±0.23 and 1.14 ±0.21 mg·kg^{-1} d.w.

Bosiacki [2007] found that carrot in the area of the city of Poznań (Poland) contained between 0.82 and 3.26 mg·kg^{-1} d.w. Cd, whereas celery contained 0.80--4.73 mg·kg^{-1} d.w. of this element. On the other hand, lettuce contained 1.10-3.98 mg·kg^{-1} d.w. of this metal, while parsley leaves contained 0.80-3.58 mg·kg^{-1} d.w. and tomato fruits 0.55-3.22 mg·kg^{-1} d.w. Levels of Cd in cauliflowers collected in urban areas in India ranged between 0.60 and 4.30 mg·kg^{-1} d.w. [Sharma et al. 2009], in orange fruits it was up to 15.39 mg·kg^{-1} d.w. and in bananas 13.36 mg·kg^{-1} d.w. [Mahdavian and Somashekar 2010]. In our experiments, the concentration of Cd in mushrooms ranged between 1.47 ±0.36 and 1.89 ±0.60 mg·kg^{-1} d.w.

The average content of Ba determined by Smoleń et al. [2010 a, b] in carrot was 28.0, in lettuce 7.93 and in spinach 6.10 mg·kg^{-1} d.w. The concentration of barium found in the fruiting bodies of *X.b.* ranged from 1.08 ±0.24 to 1.71 ±0.31 mg·kg^{-1} d.w. The same researchers found presence of Sr in carrot, lettuce and spinach at levels of 13.84, 25.21 and 40.86 mg·kg^{-1} d.w., respectively. In our experiments, the concentration of this metal in mushrooms ranged from 0.49 ±0.11 to 0.53 ±0.18 mg·kg^{-1} d.w.

The above review of results indicates that the threat to human health associated with the contents of Pb, Ni, Cd, Sr and Ba concentrations in *X. b.* mushrooms collected in Poland is not higher than that caused by the consumption of vegetables in different regions in the world and, in some cases, it can even be considerably lower.

The content of Hg can pose a problem, as in *X. b.* carpophores it was determined at the level ranging from 1.50 ±0.36 to 2.08 ±0.54 mg·kg^{-1} d.w. In different species of vegetables, Hg concentrations can fluctuate within a wide range, but they usually do not exceed 1 mg·kg^{-1} d.w. [Reis et al. 2009]. The content in *X. b.* carpophores of the other examined metals is not significantly different from the results for many vegetables in other studies [Singh and Kumar 2006, Haloi et al. 2010]. Their concentrations in the examined mushrooms can be presented as follows in a series of decreasing concentration: Fe > Zn > Mn > Cu > Co, which was also observed in our studies.

Tüzen [2003] compared the accumulation of heavy metals by some selected species of vegetables, as well as wild growing mushrooms and found that the bioconcentration factor (BCF) was many times higher in mushrooms in comparison with vegetables. This referred in particular to Cd, Zn and Cu. This fact was also corroborated in the case of *X. b.* by our experiments, because BCFs for the above-mentioned metals assumed values higher than for the other metals. However, the highest BCF values were recorded for Hg. Falandysz et al. [2004] determined Hg in *X. b.* and reported Hg BCFs in the caps of this species at 1.8 to 33 and in stems from 0.7 to 11. These values were lower than those determined in our investigations for whole fruiting bodies as they ranged from 5.00 to 148.15. Malinowska et al. [2004] determined BCFs in caps and stems of *X. b.* at the following levels: 2.78 and 1.43 for Cd, 9.45 and 6.54 for Zn, 23.9 and 10.2 for Cu, and 0.13 and 0.19 for Pb, respectively, whereas Rudawska and Leski [2005] reported BCFs of 2.3 and 1.5 for Cd, 157 and 136 for Zn, and 6.1 and 4.6 for Pb. The values quoted above were significantly different from our results: 0.35-14.61, 0.92-4.88, 1.47-10.03 and 0.04-0.34, respectively, for Cd, Zn, Cu and Pb. Campos et al. [2009] determined BCFs for 15 mushroom species from central Spain and the obtained values were as follows: for Zn 3.10-8.31, for Cu 0.64-16.72, for Ni 0.13-1.27, and for Co 0.41-1.02. In our trials the determined BCF values in *X. b.* were the following: for Ni from 0.08 to 0.67 and for Co from 0.17 to 1.21. The considerable variability recorded in bioaccumulation in the case of some metals may indicate that this feature is characteristic not only of a particular fungal species, but also environmental conditions. This fact was also confirmed by Kowalewska et al. [2007] and by Shin et al. [2007]. In addition, differences in metal accumulation may further be influenced by substrate composition, content of organic matter, and pH [Kalac and Svoboda 2000, Kalac 2010].

The results of our investigations indicate variation with respect to the effectiveness of metal accumulation by *X. b.* growing in different regions of Poland. Pacner [2005], who investigated Pb, Cd, Cu, Zn and Ni concentrations in several species of edible mushrooms growing in two different sites in the Czech Republic, reported significant differences in the accumulation of the above-listed metals associated with the place of harvesting, alsoin the case of *X. b*. A significant influence of the region of harvest on the content of Hg in *Leccinium scabrum* carpophores collected from 12 spatially distant sites in Poland in 1998-2001 was reported by Falandysz and Bielawski [2007]. Those authors pointed at differences in Hg accumulation by this mushroom species in caps and stipes.

In other studies, Zhang et al. [2008] investigated Cu, Zn, Pb, Cd, and As concentrations in 17 mushroom species collected from an area situated close to an iron ore mine and from a clean mountain area and reported that the accumulation of the examined metals depended, primarily, on the mushroom species, but their concentrations in carpophores were also strongly influenced by the place of collection. According to a number of studies, the highest trace element accumulation in mushrooms is recorded in industrial and urban areas, mainly due to environmental pollution [Svoboda et al. 2006, Zhang et al. 2008, Chen et al. 2009, Melgar et al. 2009, Kalac 2010]. In our investigations relatively high concentrations of a majority of the examined elements, in comparison with the other regions, were observed in the fruiting bodies of *X. b.* harvested in the Lower Silesia. This region is heavily industrialized and there are also coal mines which have been exploited for many decades. Also Pacner [2005] reported many-fold higher contents of elements in the carpophores of *X. b.* picked in the area of the Moravian-Silesian Region in comparison with the fruiting bodies of these mushrooms collected in the Protected Landscape Area Moravskoslezeské Beskydy. In our experiments we also found that the concentrations of the examined elements depended on the year of carpophore collection during the period of 20 years of harvesting from natural sites. It is also possible that the recorded variations in the content of the examined metals may be additionally attributed to differences in precipitation and its distribution, as this could influence the intensity of element accumulation from the soil solution. These variations were also observed in our study and in that of Falandysz et al. [2001], who investigated 38 elements in 18 species of wild edible mushrooms collected from selected areas in the Pomeranian Province. A similar accumulation in *X. b.* fruiting bodies in both studies was observed only for Mn, whereas accumulation of Cu and Zn was higher and Fe lower than in our studies. Also differences in temperatures in consecutive years of harvest could exert a similar effect as temperature affects the development of mycelium and the rate of carpophore growth. The impact of environmental conditions on the accumulation of trace elements was also stressed by Kalač [2010] and Malinowska et al. [2004].

CONCLUSION

Accumulation efficiency of tested elements in studied material by *X. b.* fruiting bodies was similar in different regions of Poland, with year to year diversity. The highest, but still not significant, accumulation levels were observed for copper, mercury and zinc, but other toxic elements were also present. On the basis of achieved results we assume that in Poland, during last 20 years occasional intake of *X. b.* fruiting bodies has no negative effect on human health (comparable to other foods).

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ZAWARTOŚĆ WYBRANYCH PIERWIASTKÓW ŚLADOWYCH W OWOCNIKACH PODGRZYBKA BRUNATNEGO – RYZYKO DLA ZDROWIA LUDZI?

STRESZCZENIE

Wstęp. Ze względu na istotne spożycie dziko rosnących grzybów jadalnych we wschodniej i centralnej Europie powinna być okresowo analizowana zawartość w nich pierwiastków toksycznych. Celem pracy była ocena zmian w zawartości wybranych pierwiastków śladowych (Ba, Cd, Co, Cu, Fe, Hg, Mn, Ni, Pb, Sr i Zn) w owocnikach podgrzybka brunatnego.

Materiały i metody. Owocniki podgrzybka brunatnego pobierano z pięciu regionów Polski w ciągu ostatnich 20 lat (wybrane lata, w których zaobserwowano wzrost owocników na tym samym terenie). Zawartość 10 pierwiastków śladowych analizowano metodą atomowej spektrometrii absorpcyjnej z atomizacją w płomieniu (FAAS), natomiast Hg – metodą atomowej spektrometrii absorpcyjnej z generowaniem zimnych par (CVAAS).

Wyniki. Wyniki wskazały na brak istotnych różnic w efektywności akumulacji poszczególnych pierwiastków pobieranych z różnych regionów Polski oraz istotne różnice w akumulacji pierwiastków przez grzyby pobierane w poszczególnych latach ich zbioru. Największą akumulację określoną wartościami współczynnika biokoncentracji stwierdzono dla Cu (10,03), Hg (148,15) oraz Zn (4,88).

Wnioski. Stężenie Cu, Fe, Mn, Zn w badanych grzybach było mniejsze niż wartości dziennego zalecanego spożycia (RGA), dlatego poziomy tych metali nie są toksyczne dla ludzi. Naszym zdaniem, sporadyczne jedzenie owocników podgrzybka brunatnego w ciągu ostatnich 20 lat w Polsce nie dostarczało istotnych ilości analizowanych pierwiastków śladowych (nie więcej niż inne produkty żywnościowe).

Słowa kluczowe: akumulacja, grzyby, pierwiastki śladowe, podgrzybek brunatny

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