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Metals of King Bolete (*Boletus edulis*) Bull.: Fr. collected at the same site over two years

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The caps of King Bolete collected from the same site over two years had similar content of Cu, Fe, K, Mg, Mn, Na and Zn but different of Al, Ba, Ca, Cd, Hg and Sr ($p < 0.05$; U Mann-Whitney test). In the case of stipes, statistically significant difference was noted only for Hg ($p < 0.05$). These findings imply that under a real environmental condition and stable geochemical composition of the surface horizon of soil substrate, the biological factors related probably to mycelium and to year-to-year fluctuating weather conditions, can cause variation in some metallic elements content of wild grown mushrooms. Fluctuation in minerals content of mushrooms collected from the same region over time have to be considered as one of parameters impacting their nutritional status as wild food. This imply also, that a more intensive research is needed to confirm, if elevated content of certain metals in mushrooms from the background (unpolluted) areas of different geochemical bedrock composition is in fact a feature related to geochemistry of parent soil bedrock and/or to natural seasonal variability - that can take place over a period of several years of mycelium life.

Key words: Food, fungi, heavy metals, higher fungi, mineral composition, mushrooms, nutrition, wild food.

INTRODUCTION

The edible fruiting bodies of many wild-grown higher fungi (mushrooms, macromycetes) are delicacy worldwide and many are of high commercial value (Chang, 1990; Falandysz et al., 1994; Falandysz and Gucia, 2008; Læssøe et al., 1996; Zhang et al., 2008). There are also numerous edible mushroom species of low or any commercial value but traditionally popular as gourmet by many local nations. Nevertheless, credible evaluation of wild-grown mushrooms nutritional value has so far been limited. This is due to fragmentary knowledge of their composition due to the very limited information on the availability of their constituents (Garcia et al., 2009).

The wild grown mushrooms intake rates are largely unknown either for the countries' general populations or at the regional, national, local or the individual mushroom

dish fanciers' scale world-wide. For example, in Sweden, annual consumption rate of wild-grown mushrooms was assessed as 1 kg per capita and common (Yellow) chanterelle (*Cantharellus cibarius*) is most famous representative (Rangel-Castro et al., 2002). In rural areas of Sweden, and in the rural areas of many other European countries, wild-mushrooms pick-up is a common practice until now. For example in the Czech Republic, 72% of all families take part in wild-grown mushrooms pick-up with yield of 7 kg fresh weight per family annually, while their annual intake rate by some fanciers can be up to 10 kg fresh weight per capita (Šišák, 1996; Svoboda et al., 2006). In China, as reported for the individuals from the Liangshan Yi nationality, an annual rate of wild grown mushrooms consumption could locally even exceed 20-24 kg per capita (Zhang et al., 2008). Another question is the structure (species, origin, treatment, volume) of wild-grown mushrooms intake.

In Poland, wild-grown mushrooms pick-up is a very

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popular form of recreation among the citizens and in practice is a common manner among the villagers, and highly emphasized are the forest mushrooms. A meal made of 300 g of fresh mushrooms is considered as common intake rate per capita daily in a week period by the wild-grown mushroom dish fanciers or persons from a low budget in the rural households, which live near to the forested areas (Chodorowski et al., 2003). A single dish made maximum of 500 g fruiting bodies (fresh weight) collected in wild and eaten can be also a real scenario of daily intake rate of mushrooms per capita (Gerber, 1989). One of the interesting features of higher fungi chemical composition and properties is abundance of metallic elements and metalloids accumulated in fruiting bodies (Brzostowski et al., 2009; Chudzyński et al., 2008; Chudzyński and Falandysz, 2009; Doğan et al., 2006; Falandysz et al., 2001; Falandysz, 2008; Melgar et al., 1998, 2009; Stijve, 1992, 1993). A species-specific accumulation of certain elements in fruiting bodies is evident for many mushrooms, while impact of habitat site - as related to the parent soil bedrock geochemistry is also noted, and including a neighborhood to metal ore smelters, metal refineries, metal ore mines or deposits, and polluted urbanized areas etc. (Barcan et al., 1998; Bargagli and Baldi, 1984; Borovička et al., 2010; Collin-Hansen et al., 2005; Falandysz and Brzostowski, 2007; Falandysz et al., 2007a, c; Müller et al., 1997; Nikkarinen and Mertanen, 2004; Ohtonen, 1982; Řanda and Kučera, 2004).

Data published on the minerals composition of fruiting bodies of Fly Agaric, did indicate that metallic element content can vary considerably between the sites or between the reports (Falandysz, 2008; Falandysz et al., 2007d). But accuracy of data published also can be a problem (Borovička and Řanda, 2007; Falandysz, 2008).

Fruiting bodies of King Bolete are highly valued as gourmet, and this mushroom can be naturally abundant in essential to man trace-elements such as selenium (~ 20 µg/g dry weight in caps), zinc (~ 200 µg/g dw), copper (~ 45 µg/g dw), and also in essential macro-elements such as potassium (~ 25 000 µg/g dw), magnesium (~ 800 µg/g dw) or calcium (~ 55 µg/g dw) (Falandysz, 2008; Falandysz et al., 2008; Frankowska et al., 2010). King Bolete is mushroom, that bio-concentrates mercury and cadmium, while bio-excludes lead (Alonso et al., 2000; Falandysz et al., 2007e; Frankowska et al., 2010; Garcia et al., 1998; Melgar et al., 2009). Some variability has been noted on cadmium, copper and zinc content of caps of King Bolete, which were collected close to zinc smelter in Norway in 1998 and 2002 (Collin-Hansen et al., 2005).

The aim of this study was to examine, using a validated analytical method and a representative number of fruiting bodies of similar size, if there could be any variation in Al, Ba, Ca, Cd, Cu, Fe, Hg, K, Mg, Mn, Na, Sr and Zn composition of King Bolete collected from the same and unpolluted site over two years period. Outcome could

have an impact in assessment of the species nutritional status.

MATERIALS AND METHODS

Samples

Two sets of fifteen mature specimens of King Bolete (*Boletus edulis*) Bull.: Fr. and corresponding samples of humifying and mineral soil substratum layer (0-10 cm), which originated from the same area, were collected in 2000 and 2001. The stands of the fruiting bodies and soil collections were the same or nearly the same over two years. The sampling area (site) was localized nearby to the town of Kościerzyna in the Pomorskie Voivodeship, Poland.

The pulverized sub-samples (~ 400 mg) of dry caps and stipes (all dried at 65°C to constant weight), were weighted into a pressure resistant and analytical quality pro-digestive vessels made of polytetrafluoroethylene (PTFE), pre-digested for 24 h with concentrated nitric acid (65%; Suprapure®, Merck; 7 ml) at room temperature, and further digested under pressure in an automatic microwave digestion system type MARS 5 of CEM Corp., Matthews, NC, USA. The digest was diluted to 25 ml using deionized water and subjected to instrumental analysis.

Fresh fruiting bodies, after clean up with a plastic knife from any visible plant vegetation and soil substrate debris with the bottom part of stipe cut away, for several days were air-dried. Further, each specimen was separated to two parts - cap and stipe and the samples were air dried in clean condition for 3 days, and next an electric oven dried at 65°C to constant weight. Dried samples were subsequently pulverized in an agate mortar and kept in brand new sealed polyethylene bags in dry condition.

Soil substrate samples, after removal of any visible organisms, small stones, sticks and leaves were air dried in room temperature for several weeks in clean condition. Next, the soil samples were sieved through a pore size of 2 mm plastic sieve and sealed in brand new polyethylene bags and kept in dry and clean condition. To determine elements other than mercury, the soil sub-samples (~ 2.5 g) were cold treated with concentrated nitric acid (65%; 6 ml) and hydrofluoric acid (40%; 4 ml) for 24 h in quartz vessels and extracts obtained after filtering through Whatmann No. 42 filter paper into polyethylene bottle were subjected for chemical analysis.

Final elements determination was by using inductively coupled plasma-optical emission spectrometry (ICP-OES) using Optima 2000 DV spectrometer (Perkin Elmer, USA) with ultrasound cross-flow nebulizer, and external standard method with yttrium (20 mg/L) as internal standard. The acids were of analytical grade (Suprapur® Merck). Deionised water was used to prepare solutions.

The total mercury content of mushrooms and soil was determined using cold-vapour atomic absorption spectroscopy (CV-AAS), and details of the method have been given in other reports (Falandysz and Brzostowski, 2007; Falandysz et al., 2007c, e).

Quality control/quality assurance

The analytical methods used were verified through analysis of several certified reference materials and through the participation in inter-laboratory trials. The standard reference material used was such as Dogfish muscle (DORM2; National Institute of Standards, Ottawa, Canada). Recent intercalibration trials were related to the Polish reference materials program: Oriental tobacco leaves (CTA-OTL-1), tea leaves (INTC-TL-1) and polish herbal blend (INCT-MPH-2), as described in earlier reports (Falandysz et al., 2007a, b, d; Falandysz et al., 2008).

The method of mercury determination was validated and

controlled for several occasions by participation in international calibration activities like GESM/Food Euro proficiency testing exercise, IAEA trials, Aquacon Project 9 "Soil Analysis", and analysis of certified plant material (Table 1) and within-run reproducibility control (Falandysz and Brzostowski, 2007; Falandysz and Chwir, 1997; Falandysz et al., 2007c, e). Discrepancies between certified values and concentrations quantified were below 10%. In day-by-day runs, with every set of 10 mushroom samples was one blank sample digest, diluted and analyzed. For blank samples, no major interferences were found for the element quantified.

All data obtained on trace element concentrations were statistically treated and to find possible statistically significant difference between variables, a test of U Man-Whitney was applied. The computer software Statistica version 5.0 used was for data statistical analysis.

RESULTS AND DISCUSSION

The caps of King Bolete collected over two years showed similar content of Cu, Fe, K, Mg, Mn, Na and Zn ($p > 0.05$; Table 1). All these six metallic elements are essential to fungi (Gadd, 2007). Also essential to fungi is Ca but its concentration in caps of King Bolete in this study varied significantly between 2000 and 2001 ($p < 0.05$). Also Al, Ba, Cd, Hg and Sr content of caps varied over two years period ($p < 0.05$). Al, Ba, Cd, Hg and Sr are considered as non-essential to fungi. Al-Fe, Ba-Sr and Ba-Fe in caps of King Bolete were positively correlated. In the case of stipes, statistically significant difference over two years was noted only for Hg ($p < 0.05$; Table 1).

Several factors can be considered as causing variability in metallic elements content of wild-grown mushrooms. The geochemical factors such as a parent soil bedrock composition, and chemical forms and availability of metallic elements in soil volume impacted by mycelium seem to be more or less constant at least over a few years or decades of years, if a given forested site was undisturbed by man. Nevertheless, the upper forest soil horizon is under continuous pressure from micro biota, invertebrates, vegetation and decaying organic matter or airborne deposition of environmental contaminants. Soil characteristics such as acidity or organic matter (carbon) content also could be considered as possibly impacting metallic elements bioconcentration potential by fungi but usually no relationships could be found (Alonso et al., 2000; Falandysz et al., 2007d; Gast et al., 1988; Melgar et al., 1998, 2009). Biological and genetic factors are related to species-specific features including mushroom fruiting body chemical composition and presence of functional groups, production of chelating and binding agents and enzymes, phenomenon of mycorrhiza, and mycelium age (Gadd, 2007). Also, environmental factors such as topography or weather conditions can be considered (Collin-Hansen et al., 2005; Kwapuliński et al., 2008).

In a study published, some variation was found in Cu, Zn and Cd content of Kings' Bolete caps originating from the same or nearly the same site - contaminated, because of

emission from the Odda zinc smelter in Norway. In mentioned report 4 specimens collected in 1998 and 16 collected in 2002 contained in caps, respectively, 60 ± 29 (25-89) and 580 ± 139 $\mu\text{g Cu/g dw}$; 300 ± 88 (183-388) and 97 ± 23 $\mu\text{g Zn/g dw}$; and 55 ± 50 (8.7-126) and 148 ± 66 $\mu\text{g Cd/g dw}$ (Collin-Hansen et al., 2005). No data on Cu, Zn and Cd in surface soil horizon beneath to these King Bolete specimens were provided. These authors speculated that reason for the discrepancies in Cd, Cu and Zn content of King Bolete between 1998 and 2002, can be due to the local topographic differences; also variation in density (yield) of fruiting bodies was markedly higher in 2002 than in 1998; age of mycelium can play a role, and an older one can be more efficient than younger in metals translocation into fruiting bodies; higher ambient air temperature in 2002 than in 1998, and what can stimulate fruiting bodies production by an old mycelia silent earlier for a several years; higher temperature would be expected to increase the flow of water through fruiting bodies, thereby creating an increased potential of the metallic elements translocation; also "mycelium containing high concentration of toxic metals such as Cd and Hg (e.g. old mycelium) may require better conditions for fructification to be stimulated" (Collin-Hansen et al., 2005).

Table 1 contains also data on minerals of top soil layer, cap to stipe minerals concentration quotient ($Q_{C/S}$) and on cap/stipe to soil minerals concentration quotient (BCF; bioconcentration factor) values. K, Na, Ba, Cd, Cu, Fe, Sr and Zn occurred in top layer of soil in similar concentrations in 2000 and 2001 ($p > 0.05$), while for Al, Ca, Mg, Mn and Hg concentrations determined were greater in 2000 ($p < 0.05$) (Table 1). The mineral status of natural soil can be among the factors that can influence metallic elements composition of mushrooms but their availability matters, and the species ability (amount and quality of binding sites for metal ions) to sequestrate these elements.

A greater concentration of magnesium and manganese determined in soil in 2001 than in 2000 had no impact on their BCF values in caps. In case of calcium, its BCF value in caps was greater in 2000 (Table 1). The $Q_{C/S}$ values remained constant ($p > 0.05$) over two years for K, Mg, Na, Cd, Cu, Hg, Mn, and Zn, and varied for Al, Ba, Ca, Fe and Sr (greater in 2001 than 2000; $p < 0.05$). The specimens examined were mature and roughly similar in size. Hence a greater content of Al, Ba, Ca, Fe and Sr in 2000, when compared to 2001 can be related to variation in content of binding sites of fruiting bodies.

Bioconcentration or transfer factor (BCF, TF) values in caps and stipes remained constant over two years for K, Al, Ba, Fe, Hg, Mn, Sr and Zn ($p > 0.05$), while varied for Cd and Cu ($p < 0.05$; U Mann-Whitney test; Table 1).

Conclusion

Data obtained in this study imply that under a real

Table 1. Metallic elements in King Bolete and soil from the same site over 2000 and 2001, cap to stipe element content quotient and BCF values (mean, SD, median value and range).

Element	2001 n = 15						2000 n = 15					
	Cap	Stipe	Soil	Q _{C/S}	BCF _{Cap}	BCF _{Stipe}	Cap	Stipe	Soil	Q _{C/S}	BCF _{Cap}	BCF _{Stipe}
mg/g dry weight												
K	25±4	16±4	7.8±0.8	1.6	3.3±0.7	2.1±0.6	25±4	16±3	8.6±0.5	1.6	2.9±0.4	1.9±0.4
	24	16	7.9		3.1	2.0	25	17	8.5		3.0	2.0
	19-34	8.7-21	6.3-9.2		2.4-5.4	1.2-3.3	20-34	10-23	7.7-9.4		2.2-3.8	1.1-2.5
Mg	0.80±0.12	0.48±0.11	0.48±0.31	1.7	2.5±2.4	1.4±1.2	0.76±0.10	0.49±0.11	0.60±0.25	1.6	1.5±0.7	0.97±0.56
	0.79	0.47	0.38		2.1	1.2	0.79	0.48	0.61		1.3	0.85
	0.55-1	0.33-0.66	0.26-0.70		0.80-4.1	0.60-2.3	0.63-0.96	0.27-0.67	0.30-0.94		0.80-2.6	0.29-1.6
Na	0.19±0.07	0.40±0.24	4.3±1.7	0.48	0.08±0.04	0.14±0.01	0.16±0.07	0.28±0.14	4.3±0.5	0.57	0.04±0.02	0.06±0.04
	0.17	0.34	4.0		0.07	0.14	0.16	0.25	4.2		0.04	0.06
	0.090-0.33	0.11-0.99	3.1-5.5		0.05-0.10	0.13-0.15	0.032-0.32	0.11-0.54	3.6-4.9		0.01-0.07	0.03-0.15
µg/g dry weight												
Al	13±9	37±51	8100±720	0.35	0.002±0.001	0.004±0.005	59±57	39±25	13000±1300	1.5	0.003±0.003	0.003±0.002
	11	18	8200		0.003	0.003	22	23	13000		0.003	0.003
	6.0-41	10-190	7900-10000		0.001-0.004	0.001-0.020	10-200	12-88	11000-15000		0.002-0.008	0.001-0.005
Ba	0.23±0.15	0.53±0.24	260±56	0.43	0.001±0.001	0.002±0.002	0.62±0.41	0.60±0.41	290±74	1.0	0.002±0.001	0.002±0.002
	0.19	0.48	240		0.001	0.002	0.63	0.51	300		0.002	0.002
	0.06-0.62	0.13-0.95	210-350		0.001-0.002	0.001-0.006	0.13-1.6	0.24-1.7	220-400		0.001-0.005	0.001-0.006
Ca	55±54	100±59	570±130	0.55	0.10±0.06	0.17±0.08	140±60	99±35	1200±400	1.4	0.12±0.08	0.11±0.12
	53	89	580		0.10	0.15	150	96	1000		0.14	0.10
	15-200	42-260	480-670		0.03-0.40	0.09-0.53	52-240	31-180	560-1500		0.04-0.26	0.02-0.29
Cd	3.3±1.7	1.4±1.4	0.26±0.07	2.4	12±3	5.2±4.2	1.4±1.1	0.76±0.54	0.22±0.06	1.8	5.8±3.1	3.1±1.6
	2.6	0.86	0.25		10	4.6	1.2	0.68	0.21		5.5	2.9
	1.4-7.1	0.24-5.7	0.15-0.43		6.0-17	0.9-18	0.09-3.3	0.09-1.7	0.13-0.32		0.7-10	0.7-5.3
Cu	25±10	11±5	4.3±2.7	2.3	5.6±3.2	2.8±1.9	38±25	15±10	3.2±1.3	2.5	14±12	5.6±5.2
	24	11	3.8		6.3	2.9	42	17	2.9		10	6.1
	12-50	4.6-24	0.30-9.2		2.1-19	1.3-8.9	9.0-110	2.0-39	1.4-5.9		3.0-41	0.60-20

Table 1. conut'd

Fe	53±16	47±12	4600±1900	1.1	0.01±0.01	0.01±0.01	64±40	39±18	4700±530	1.6	0.01±0.01	0.009±0.000
	49	46	4400		0.01	0.01	44	31	4800		0.01	0.008
	32-93	29-72	3300-5900		0.01-0.02	0.01-0.02	27-150	17-75	4100-5500		0.01-0.02	0.004-0.012
Hg	2.8±1.0	1.5±0.6	0.03±0.01	1.9	81±10	44±10	5.2±1.5	2.1±0.6	0.05±0.01	2.5	110±9	45±12
	2.5	1.3	0.03		80	42	5.9	1.9	0.05		110	40
	1.6-4.5	0.84-2.7	0.02-0.06		63-99	29-61	3.1-7.5	1.3-3.2	0.03-0.06		92-120	26-78
Mn	14±8	20±8	150±41	0.70	0.18±0.18	0.10±0.03	16±9	17±7	330±260	0.94	0.10±0.15	0.11±0.15
	10	18	140		0.12	0.09	15	15	310		0.12	0.10
	7.7-38	10-35	120-180		0.05-0.31	0.07-0.12	6.0-39	9.0-35	50-730		0.02-0.43	0.02-0.44
Sr	0.10±0.10	0.25±0.15	20±6	0.40	0.01±0.02	0.01±0.01	0.21±0.10	0.30±0.10	21±5	0.70	0.011±0.009	0.01±0.01
	0.06	0.20	19		0.01	0.01	0.22	0.26	20		0.01	0.01
	0.02-0.38	0.08-0.69	16-24		0.003-0.02	0.007-0.014	0.05-0.44	0.13-0.57	13-25		0.002-0.021	0.01-0.03
Zn	120±27	59±23	20±10	2.0	7.4±3.6	3.6±2.4	130±33	68±23	23±8	1.9	6.3±1.9	3.2±1.3
	120	58	18		6.2	3.3	120	60	21		5.8	2.9
	72-160	23-110	8.0-36		2.7-12	1.1-11	85-19	29-100	13-36		4.1-12	1.4-7.1

environmental conditions and stable geochemical composition of soil substrate, biological factors, e.g. age of mycelium or more likely year-to-year fluctuating weather conditions, can be a cause of variation in some metallic elements content of fruiting bodies of wild-grown edible fungi collected from the same region over several years. These observed variations in minerals composition of King Bolete have to be considered when assessing a nutritional status of wild food, and publishing official tables of minerals composition of foods. They imply also, that a more intensive research is needed to confirm if elevated content of certain metals in mushrooms from the background (unpolluted) areas of different geochemical bedrock composition is in fact a

feature related to parent soil bedrock as noted in some reports, or is related to natural seasonal variability that could take place over a period of several years in mycelia live. Such studies could include several years periods and at least annual collections of a representative number of mushroom samples (≥ 15 specimens per species, site and season), and having a standardized size (e.g. mature).

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