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Trace Elements in *Leccinum scabrum* Mushrooms and Topsoils from Kłodzka Dale in Sudety Mountains, Poland

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Abstract: In the current study, we determined concentrations and transfer rates of Ag, Al, Ba, Ca, Cd, Co, Cr, Cu, Fe, K, Mg, Mn, Na, Ni, Pb, P, Rb, Sr and Zn to Brown Birch Scaber Stalks (*Leccinum scabrum*) mushrooms emerged in the area of Sudety Mountains (Sudetes) in Poland. Fruiting bodies and topsoil samples beneath *L. scabrum* were collected form the Kłodzka Dale. The trace elements were determined using validated method and inductively coupled plasma - atomic emission spectroscopy (ICP-AES) for final measurement. Mushrooms contained Ag, Cr, Hg, Co, Ni and Sr at < 1.0 μg/g dry weight; Ba and Pb at ~1.0 μg/g dw; Cd at < 5 μg/g dw; Cu and Mn at > 10 μg/g dw; Al and Ca at ~100 μg/g dw; Fe, Na, Rb and Zn at 100 to 500 μg/g dw, Mg at ~1,000 μg/g dw; P at ~5,000 μg/g dw and K at ~30,000 μg/g dw. Ca, Mn and Ni were nearly equally distributed between stipes and caps; stipes compared to caps were enriched in Ba, Na and Sr, while caps were enriched in Ag, Al, Cd, Co, Cr, Cu, Fe, K, P, Pb, Rb and Zn. The values of bioconcentration factor (BCF) varied highly depending on chemical element and were >1 for Ag, Cd, Cu, K, Mg, Na, P, Rb and Zn, while <1 for Al, Ba, Ca, Co, Cr, Fe, Mn, Ni, Pb and Sr. Topsoil showed elevated content of lead and mean concentration was 99 ± 32 μg/g dw, while cadmium was at 0.41 ± 0.15 and those two highly toxic to human elements occurred in edible caps of *L. scabrum* at 4.5 ± 2.2 and 2.9 ± 2.0 μg/g dw, respectively.

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Introduction

Fungi and including mushrooms take important role in metallic elements and metalloids turnover in the ecosystems (Lepp et al. 1987; Falandysz and Borovička 2013). Numerous wild grown mushrooms are important as food resources to many local populations worldwide and they are also attractive and collected by fanciers and for commercial purchase in many countries (Aloupi et al. 2011; Liu et al. 2012; Nnorom et al. 2012, 2103; Yağız et al. 2008; Zhang et al. 2008, 2010). Edible wild grown mushrooms are relatively rich in essential trace elements such as K, Mg, Mn, Se, Cu, Zn (Chudzyński and Falandysz 2008; Kojta et al. 2011, 2012; Li et al. 2011; Szubstarska et al. 2012). The metals such as Hg, Cd and Pb are metallic elements, which are hazardous in diet. They, depending on species and/or site of collection can be found in mushrooms at elevated to high concentrations too (Chojnacka et al. 2012; Drewnowska et al. 2012a, b; Falandysz et al. 2002a-b, 2003a-d, 2007; Garcia et al. 2009; Gucia et al. 2012; Jarzyńska and Falandysz 2011; Melgar et al. 1998, 2009; Nasr and Arp 2011). These metallic elements are noted as problematic in the flesh of edible wild mushrooms – especially, when fruiting bodies had grown-up at the contaminated soils (Carvalho et al. 2005).

1 Materials and Methods

1.1 Mushrooms and soils

Fifteen mature specimens of Brown Birch Scaber Stalk called also Birch Bolete or Birch Scaber Stalk *(Leccinum scabrum)* (Bull.: Fr.) S. F. Gray synonyms *Boletus scaber* and *Krombholzia scabra* and surface soil layer (0-10 cm; 100 g) beneath the carpophores (fruiting bodies) were collected from forested areas of Kłodzka Dale in Sudety Mountains (southwestern Poland) in 2000 (Figure 1). The Sudety Mountains (Sudetes) form the mountain range in Central Europe and the highest peak is Śnieżka (1,602 m altitude; 50° 44'10"N and 15°44'24"E) in the Karkonosze mountains in Poland. Kłodzka Dale has an area of 500 km^2 and is situated between Middle and Eastern Sudetes. The dale is 400-1,000 m above sea level (mushrooms were collected from lower part of dale, e.g. from 400 to around 700 m) and its parent soil bedrock is highly diverse in rock types and comes from different geological ages (Bednarek and Prusinkiewicz 1980).

The samples were divided into caps and stipes, carefully hand- cleaned using a plastic knife to remove the attached soil particles and other debris (e.g. leaves), dried to constant weight at 65°C, crushed and pulverized in an agate mortar and kept in dry condition in brand new sealed polyethylene bags until chemical analysis.

The soil substrate samples were air dried at room temperature in clean condition for a few weeks and next sieved through a pore size 2 mm, and further dried in an electronic oven in 40°C for 48 hours.

1.2 Chemical analysis

The pulverized sub-samples (~0.5 g) of caps and stipes were weighted into polytetrafluoroethylene (PTFE) vessels, pre-digested for 24 hours with 7 mL concentrated nitric acid (65%, Suprapure, Merck) 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 diluted to 25 mL using deionized water and stored until instrumental analysis.

Metals from the soil samples of up 1.5 g were leached using 10 mL of 20% solution of nitric acid (Suprapur®, Merck) in open PTFE vessel, that were gently heated up 105°C by 2 h. After cooling, the leachates obtained were filtered through the Whatman No. 42 filter paper - directly into a volumetric flask, and volume was brought to 25 mL with deionized water (Brzostowski et al. 2009, 2011a-b).

Figure 1 Location of the sampling sites of *Leccinum scabrum* in Kłodzka Dale region (Sudety Mountains; outskrits of the Kamienica in the Bardo Śląskie Forest Inspectorate, Paczków and Złoty Stok; the left panel in rectangle) in Poland (the right panel).

Contents of 20 elements in mushrooms and soil were determined by inductively coupled plasma optical emission spectroscopy (ICP-AES; Optima 2000 DV, Perkin-Elmer, USA) and yttrium was used as an internal standard. Mercury was determined by cold-vapour atomic absorption spectroscopy (CV-AAS) using fully automated mercury monitor (Mercury monitor 3200, Thermo Separation Products, USA). Each spectroscopic measurement for each individual sample was repeated in triplicate. Detection limits were as follows: K, Mg, Na - 5 µg/g; Rb - 1 µg/g; Al, Ag, Ca, Cd, Co, Cr, Fe, Zn - 0.1 µg/g; Ba, Cu, Mn, Sr - 0.05 µg/g and Hg - 0.005 µg/g dry weight (dw).

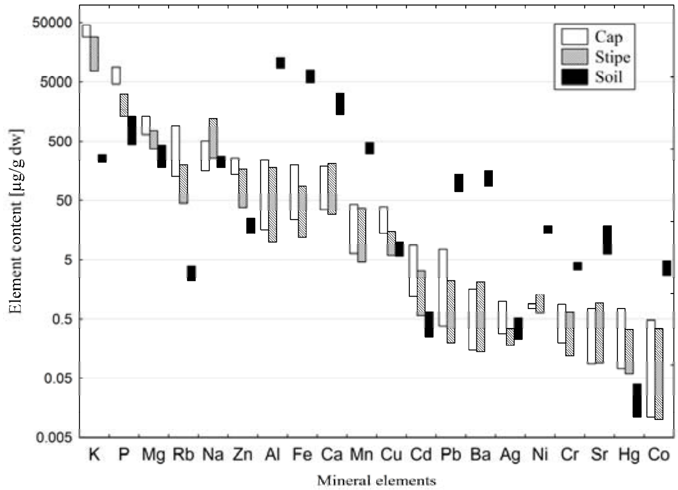

Figure 2 BCF value ranges of mineral elements of *L. scabrum*

1.3 Quality control/quality assurances

Both methods were well validated on a several occasions by participation in intercalibration trials and by periodic analysis of certified reference materials and detailed results were given to public in earlier articles (Falandysz 1990; Falandysz and Chwir 1997). Duplicates and blanks followed with every set of 10 mushroom or soil samples examined.

1.4 Soils pH and carbon

Soil pH has been determined according to method by Musgrove (1987). Briefly, to a 10 g aliquot of pooled air-dried soil sample (each made of 3-5 individual samples), 50 ml distilled water was added, and the mixture was left for 1 h at 25 °C. Thereafter, pH was determined using a pH meter (EC20 pH/ISE Meter, MODEL 50050, Hach Company, Ames, Iowa, USA). Soil organic carbon (matter) content was determined gravimetrically after combustion of soil organic matter (10 g air-dried sample; Loss-on-Ignition (LOI) method) at 800 °C in a furnace oven (type CNOL 8,2/1100, Lithuania). That method is one of basic methods used for determination of soil organic matter content and its accuracy and precision are to some degree limited (Qian et al. 2011).

2 Results and Discussion

The values of bioconcentration factor (BCF) varied highly depending on chemical element and were >1 for Ag, Cd, Cu, K, Mg, Na, P, Rb and Zn, while <1 for Al, Ba, Ca, Co, Cr, Fe, Mn, Ni, Pb and Sr (Figure 2). Topsoil showed elevated content of lead and mean concentration was 99 ± 32 µg/g dw, while cadmium was at 0.41 ± 0.15 and these two elements that are highly toxic to human occurred in edible caps of *L. scabrum* at 4.5 ± 2.2 and 2.9 ± 2.0 µg/g dw, respectively

2.1 Fruiting bodies

Table 1 (Supporting materials) summarizes data on 19 metallic elements and phosphorous in carpophores of *L. scabrum* and topsoils and related data such as elements distribution between caps and stipes ($Q_{C/S}$) values of elements bioconcentration factor (BCF) and basic statistics. Caps of *L. scabrum* are on average two-to-three fold richer in Ag, Al, Cd, Cu, Co, Cr, Cu, Fe, Hg, K, Mg, Pb,P, Rb and Zn compared to stipes and *vice versa* is for Na, while Ba, Ca, Mn, Ni and Sr are nearly equally distributed between those mushroom parts.

Among the mineral compounds determined, macroelement K had the greatest concentration (29,000-45,000 µg/g dw in caps, and followed by phosphorous (4,600-8,900 µg/g dw) and magnesium (650-1,300 µg/g dw) (Figure 3). The elements such as Rb, Na, Zn and Fe, occurred in caps at concentrations varying respectively between 130-920, 160-510, 140-260 and 24-200 µg/g dw. Next in their abundance were Ca (35-190

Table 1 Metallic elements content of Brown Birch Scaber Stalk (*Leccinum scabrum*) (μg/g dry weight; mean ± SD, range and median values) and their cap to stipe concentration quotient ($Q_{C/S}$) and bioconcentration (BCF) values

Element	Cap	Stipe	$Q_{C/S}$	Soil	BCF_C	BCF_S
Ag	0.69 ± 0.20 (0.28-1.0) 0.71	0.25 ± 0.05 (0.18-0.34) 0.25	2.7 ± 0.8 (1.3-4.3) 2.6	0.34 ± 0.10 (0.23-0.52) 0.30	2.0 ± 0.3 (1.2-2.5) 2.1	0.79 ± 0.21 (0.46-1.1) 0.82
Al	120 ± 77 (16-240) 140	46 ± 48 (10-180) 29	3.4 ± 2.4 (0.65-8.8) 2.9	11,000 ± 2,000 8,600-13,000 12,000	0.011 ± 0.007 (0.0015-0.022) 0.013	0.0041 ± 0.0043 (0.0009-0.0016) 0.0028
Ba	0.90 ± 0.48 (0.15-1.6) 0.89	1.1 ± 0.6 (0.14-2.1) 1.3	0.84 ± 0.32 (0.40-1.4) 0.77	130 ± 36 (87-160) 130	0.0059 ± 0.0037 (0.00011-0.0012) 0.0069	0.088 ± 0.045 (0.011-0.016) 0.011
Ca	120 ± 47 (35-190) 140	130 ± 47 (29-210) 130	0.98 ± 0.35 (0.34-1.7) 0.88	2,500 ± 800 (1,400-3,200) 2,600	0.050 ± 0.019 (0.014-0.077) 0.057	0.054 ± 0.019 (0.012-0.087) 0.050
Cd	4.5 ± 2.2 (1.2-8.8) 4.9	1.8 ± 0.8 (0.57-3.3) 1.6	2.5 ± 0.6 (1.6-3.5) 2.4	0.41 ± 0.15 (0.25-0.67) 0.34	11 ± 4 (4.0-21) 11	4.3 ± 1.0 (2.0-6.3) 4.2
Co	0.13 ± 0.14 (0.011-0.48) 0.092	0.089 ± 0.097 (0.010-0.34) 0.059	1.8 ± 1.6 (0.31-5.3) 1.2	3.5 ± 0.9 (2.7-4.8) 3.2	0.038 ± 0.039 (0.0031-0.14) 0.028	0.026 ± 0.028 (0.0029-0.098) 0.015
Cr	0.50 ± 0.27 (0.20-0.89) 0.39	0.34 ± 0.20 (0.12-0.66) 0.27	1.5 ± 0.3 (1.0-2.1) 1.6	4.0 ± 0.5 (3.4-4.5) 3.9	0.063 ± 0.034 (0.025-0.11) 0.058	0.043 ± 0.025 (0.015-0.084) 0.039
Cu	22 ± 7 (14-39) 20	10 ± 2 (6.0-15) 8.7	2.4 ± 0.8 (1.3-4.9) 2.2	7.9 ± 2.1 (5.8-10) 7.8	2.8 ± 0.9 (1.7-5.0) 2.7	1.2 ± 0.3 (0.76-1.9) 1.1
Fe	130±56 (24-200) 150	65 ± 26 (12-87) 76	2.0 ± 0.5 (1.1-2.8) 1.9	6,700 ± 1,400 4,800-8,000) 6,900	0.019 ± 0.008 (0.0036-0.029) 0.023	0.0092 ± 0.0038 (0.0019-0.013) 0.011
Hg*	0.38 ± 0.23 (0.072-0.76) 0.36	0.21 ± 0.08 (0.060-0.33) 0.23	1.8 ± 0.8 (1.1-3.5) 1.3	0.026 ± 0.010 (0.011-0.040) 0.026	13 ± 4 (6.0-21) 12	8.0 ± 1.7 (5.0-9.5) 8.3
K	36,000 ± 4,300 (29,000-45,000) 35,000	18,000 ± 5,100 (7,700-29,000) 21,000	2.1 ± 0.6 (1.2-3.8) 2.1	260 ± 40 220-300) 260	140 ± 16 (110-170) 130	68 ± 19 (29-110) 73
Mg	1,000 ± 100 (650-1,300) 1,000	510 ± 120 (370-750) 500	2.0 ± 0.4 (1.2-2.7) 1.9	290 ± 130 180-430 290	3.4 ± 0.4 (2.8-4.4) 3.4	1.8 ± 0.4 (1.3-2.6) 1.7
Mn	20 ± 11 (6.4-43) 16	20 ± 12 (4.6-37) 18	1.1 ± 0.3 (0.46-1.4) 1.2	400 ± 73 (310-480) 410	0.049 ± 0.028 (0.016-0.11) 0.047	0.049 ± 0.030 (0.011-0.092) 0.056
Na	310 ± 110 (160-510) 290	580 ± 230 (260-1,200) 560	0.59 ± 0.23 (0.21-0.95) 0.61	230 ± 47 (180-280) 220	1.4 ± 0.5 (0.71-2.3) 1.3	2.6 ± 1.0 (1.1-5.2) 2.4
Ni	0.84 ± 0.05 (0.76-0.91) 0.85	0.98 ± 0.19 (0.64-1.3) 1.0	0.90 ± 0.22 (0.67-1.4) 0.81	16 ± 2 (14-19) 16	0.052 ± 0.003 (0.047-0.056) 0.052	0.060 ± 0.012 (0.039-0.078) 0.064
Pb	2.9 ± 2.0 (0.38-7.5) 3.0	1.3 ± 0.6 (0.20-2.2) 1.2	2.3 ± 0.9 (1.3-4.3) 2.1	99 ± 32 (71-140) 95	0.029 ± 0.020 (0.0038-0.076) 0.032	0.013 ± 0.006 (0.0028-0.022) 0.013
P	6,100 ± 1,100 (4,600-8,900) 5,700	2,200 ± 500 (1,300-3,100} 2200	3.0 ± 1.3 (1.7-6.9) 2.5	930 ± 390 (440-1,300) 1,000	6.6 ± 1.2 (5.0-9.6) 6.3	2.4 ± 0.6 (1.4-3.3) 2.4
Rb	410 ± 260 (130-920) 320	110 ± 260 (45-200) 97	3.8 ± 1.2 (2.1-5.7) 3.8	2.8 ± 0.6 (2.2-3.9) 2.5	150±95 (47-330) 130	38 ± 18 (16-72) 52
Sr	0.47 ± 0.24 (0.087-0.76) 0.50	0.63 ± 0.35 (0.090-0.94) 0.83	0.82 ± 0.22 (0.51-1.3) 0.80	16 ± 6 (6.3-19) 19	0.030±0.015 (0.0055-0.048) 0.034	0.040 ± 0.022 (0.0058-0.062) 0.052
Zn	210 ± 34 (140-260) 220	98 ± 34 (38-170) 110	2.4 ± 1.0 (1.2-4.9) 2.0	20 ± 5 (14-25) 21	10±2 (6.9-13) 11	4.8 ± 1.7 (1.9-8.5) 5.4

Note: *Data after Falandysz and Bielawski (2007) μg/g dw), Al (16-240 μg/g dw) and followed with an order of magnitude less concentration by Cu (14-38 μg/g dw) and Mn (6.4-43 μg/g dw) (Figure 3). Element Cd was relatively high in caps but below the tolerance limit of 1.0 μg/g fresh product (10 μg/g dw; assuming 90% water content) set in the European Union (EU 2008) for cultivated mushrooms, and its content varied between 1.2-8.8 μg/g dw. Also toxic Pb, whose content in certain cultivated mushrooms is also regulated by the European law, in caps varied between 0.38 and 7.5 μg/g dw, and in a portion of caps exceeded tolerance limit of 0.2 μg/g fresh product (2 μg/g dw; assuming 90% water content) (EU 2008). Next in abundance was Ba with content in caps varying between 0.15 and 1.6 μg/g dw, while Ag, Cr, Hg, Co, Ni and Sr occurred at lesser concentration varying between 0.28-1.0, 0.20-0.89, 0.072-0.76, 0.011-0.48, 0.76-0.91 and 0.087-0.74 μg/g dw, respectively. Element Ag in caps of *L. scabrum* from background areas of northern part of Poland in earlier study was at 0.50 ± 0.32 (0.12-1.9; *n = 40*) μg/g dw and in stipes was 0.28 ± 0.16 (0.04-0.94; *n = 32*) μg/g dw (Falandysz et al. 1994), In available literature no data were found on occurrence of trace elements in morphological parts of *L. scabrum*. Stalk (stipe) of *L. scabrum* is also edible but fibrous especially when the specimen is getting older but the dried older ones are of culinary value also.

Compared to the other mushrooms of genus *Leccinum i.e.* the Hazel (Hard) Bolete *L. pseudoscabrum* (*L. griseum*) mushrooms that were collected from the forests in the northern part of Poland (Jarzyńska and Falandysz 2012), the *L. scabrum* was significantly richer (caps; median values; Mann-Whitney *U*-test) in Al, Ba, Cd, Co, Cr, Fe, Ni, Pb and Sr but less in Na, and quite close in Ca, Cu, Hg, K, Mg, Mn, Rb and Zn.

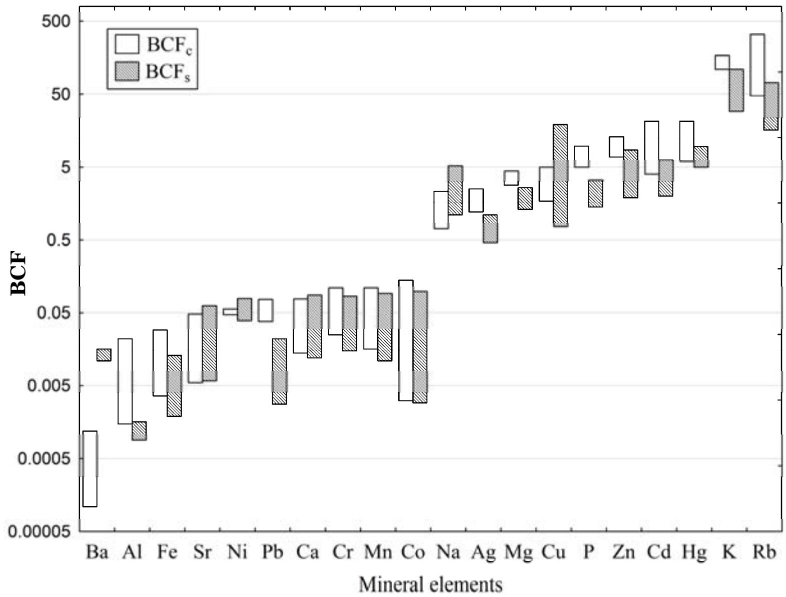

Figure 3 Mineral elements' concentration ranges of *L. scabrum*

2.2 Soil

The forest topsoil sampled in Kłodzka Dale was definitely acidic and its pH varied between 3.54 and 4.38, while organic carbon content was between 5.6 and 15.8 %. The Sudety Mountains is a region known as rich in mineral ores. In this study topsoil samples extracted with 20% solution of nitric acid were relatively rich in Pb (71-140 μg/g dw) and sodium (180-280 μg/g dw) and other elements were below a typical geochemical background for soils (Figure 3; Table 1, supporting materials). Available data on some metallic elements content of soils of Sudety Mountains relate to soils extracted by *aqua regia* (Kabata-Pendias and Pendias 2001).

2.3 Bioconcentration

The potential of fungi, plants or other vegetation or animals to accumulate chemical substances in body is estimated by calculating BCF values. Brown Birch Scaber Stalk was found to be a species that can efficiently bioconcentrate, in caps and stipes of fruiting bodies, the elements such as K (BCF_C: 110-170), Rb (47-330), Hg (6.0-21), Zn (6.9-13), Cd (4.2-21), P (5.0-9.6), Mg (2.8-4.4) and Cu (1.7-5.0). Also bioconcentrated in caps was Ag (BCF_C: 1.2-63), in stipes was Na (BCF_S: 1.1-5.2), while Al, Ca, Fe and Mn, which are relatively abundant in the fruiting bodies (Figure 2; Table 1, supporting materials) were excluded by the species (BCF much < 1), and excluded also were the minor trace elements such as Ba, Co, Cr, Ni, Pb and Sr

(BCF < 1).

The elements in this study that showed values of BCF > 1 in *L. scabrum* are also well bioaccumulated by other species of the genus *Leccinum*, e.g. by *L. pseudoscabrum* (*L. griseum*) and *L. duriusculum* (Jarzyńska and Falandysz 2012a,b). Also element Hg is well bioconcentraded by *L. rufum* and *L. scabrum* (Falandysz and Bielawski 2007). Mushrooms reveal variable and sometimes highly specific potential to take up various metallic and some other elements from soils and bioconcentrate them in their fruit-bodies to great concentration and some can hyperacumulate them (Borovička et al. 2007). No one of the mineral compounds determined could be considered as hyperacumulated by *L. scabrum*, while high values of BCF for K and Rb are rather typical for mushrooms and example can be *Boletus edulis* and *Xerocomus badius* (Falandysz et al. 2011; Kojta et al. 2012).

3 Conclusions

Certainly *L. scabrum* like many other mushrooms is able to sequester in carpophores various metallic elements and phosphorous and some mushrooms can absorb these elements from the soils very efficiently. Hence *L. scabrum* is a species playing important role in a biogeochemical turnover of many metals. A content of Pb in L. scabrum in this study was relatively high. This highlights the significance of certain species of edible mushrooms as source of essential but also hazardous metals such as Pb, Hg and Cd.

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