Eur Food Res Technol (2016) 242:1–10
DOI 10.1007/s00217-015-2512-0

ORIGINAL PAPER

Update on element content profiles in eleven wild edible mushrooms from family *Boletaceae*

Marija V. Dimitrijevic[1] · Violeta D. Mitic[1] · Jelena S. Cvetkovic[1] · Vesna P. Stankov Jovanovic[1] · Jelena J. Mutic[2] · Snezana D. Nikolic Mandic[2]

Received: 23 May 2015 / Revised: 13 July 2015 / Accepted: 18 July 2015 / Published online: 2 August 2015

Abstract The aim of this study was to determine and evaluate the amounts of major elements (Ca, Fe, K, Na and P), essential trace elements (Cu, Zn, Fe and Mn) and some other trace metals (Ag, Al, Co, Ni, Cr, Sr, Se, Bi, Rb) in eleven species of wild-grown common edible mushrooms from family *Boletaceae* (*Boletus appendiculatus, Boletus edulis, Boletus regius, Boletus fechtneri, Boletus impolitus, Boletus purpureus, Boletus rhodoxanthus, Leccinum crocipodium, Leccinum pseudoscaber, Xerocomellus chrysenteron, Xerocomus badius*) from Serbia. The measurements of major elements (Ca, Fe, K, Na and P) were carried out by inductively coupled plasma optical emission spectrometer (ICP-OES), while analytical measurements of the rest of studied elements were performed using an inductively coupled plasma mass spectrometer (ICP-MS), after microwave digestion. The results showed that the element concentrations were species-dependent. Potassium and phosphorous concentrations were found to be greater than those of the other mineral constituents in all tested species. Multivariate analysis included principal component analysis (PCA) and hierarchical cluster analyses (HCA). HCA grouped mushrooms in three statistically significant clusters, while PCA indicated connection between analyzed metals. Also, this paper highlights the importance of essential and nonessential elements of human health and their daily intake.

✉ Marija V. Dimitrijevic
marija.dimitrijevic@pmf.edu.rs

[1] Department of Chemistry, Faculty of Science and Mathematics, University of Nis, Visegradska 33, 18000 Nis, Serbia

[2] Faculty of Chemistry, University of Belgrade, Studentski trg 12-16, 11000 Belgrade, Serbia

Keywords Mushrooms · *Boletaceae* · ICP-OES · Principal component analysis · Hierarchical cluster analyses

Introduction

In recent time, the consumption of healthy food is increasing all over the world. A proper diet is one that involves a large intake of minerals, vitamins, antioxidants and low content of fat. Mushrooms in their composition correspond to this description, and for this reason they have been very popular in European and Asian cuisine. Mushroom is considered as healthy food containing considerable amounts of nutrients, such as water soluble vitamins, especially from the B group, macro- and microelements, carbohydrates and all essential amino acids. All these features contribute to their excellent taste, aroma and flavor and make them as a popular and favorite delicacy in many countries. Also, mushrooms have been reported as therapeutic food, useful in preventing diseases such as hypertension, hypercholesterolemia and cancer [44]. These functional characteristics are mainly due to their chemical composition [27].

Mushrooms are taking part in biodegradation of the substrate and play an important role in the constant changes that occur in nature, but that is poorly known. They contain large range of mineral, essential, nonessential trace elements and problematic heavy metals. Trace elements such as iron, copper, zinc and manganese are essential metals since they play an important role in biological systems [35]. In the same time, the bioavailability of iron in mushrooms is very large, so more of 90 % of the iron present can be absorbed by human body [22]. The essential metals can also produce toxic effects when the metal intake is excessively elevated [16]. Mushrooms possess effective mechanism to accumulate large amounts of heavy and toxic metals from the environment, so concentrations of heavy metals in mushrooms are higher than those in plants, fruits and vegetables. Due to this the feature of absorption metals from the environment and the possibility of entering into the food chain, it is of essential importance to determine the concentration and possible source of contamination in order to prevent risk to health. But, huge variability in the concentration of metals makes them unsuitable for precise determination of environmental pollution. Despite this, there is a difference in the concentration of metals in polluted and unpolluted regions. Mushrooms picked in uncontaminated areas are characterized by lower contents of heavy metals, when compared to mushrooms growing in contaminated soils [29]. Physiology of the species, accumulation of other metals, area of sample collection, distance from polluted areas, pH, and organic matter amount affect the concentration of metals in mushrooms. Based on this, it could be concluded that the accumulation of metals in mushrooms depends on environmental and fungal factors. Many wild edible mushroom species have been known to accumulate high concentrations of heavy metals such as lead, cadmium, iron, copper, manganese, zinc, chromium, nickel, aluminum and mercury [23].

Considering that interest for mushrooms rapidly increases, we have analyzed the levels of 21 macroelements, microelements and toxic elements (Ag, Al, As, Ca, Cd, Co, Cr, Cu, Fe, Hg, K, Mn, Na, Ni, Pb, Se, Sr, Zn, Bi, Rb, P), in the eleven species of mushrooms collected in Nis surrounding area (*Boletus appendiculatus, Boletus edulis, Boletus regius, Boletus fechtneri, Boletus impolitus, Boletus purpureus, Boletus rhodoxanthus, Leccinum crocipodium, Leccinum pseudoscaber, Xerocomellus chrysenteron, Xerocomus badius*). The *Boletaceae* family is most abundant in the mentioned area, containing over 100 species of mushrooms, very appreciated both among fungi collectors and locals. However, detailed chemical composition of many wild edible mushroom species from this family is not still elucidated although they constitute important place in healthy diet. *Boletus* has always been the most famous and most respected mushrooms for food and considered among the most delicious mushrooms that can be consumed in many ways. These edible mushrooms are very popular in Europe.

According to the authors' best knowledge; present study is the first one treating in comprehensive manner problem of mineral composition of the mushrooms of *Boletus* family.

Materials and methods

Instrumentation

The measurements of major elements (Ca, Fe, K, Na and P) were carried out in an Inductively Coupled Atomic Emission Spectrometer, ICP-OES (Thermo Scientific, UK), model 6500 Duo, equipped with a CID86 chip detector. This instrument operates sequentially with both radial and axial torch view. The entire system was controlled with iTEVA software. Instrument conditions and selected wavelengths are given in Table 1.

Table 1 Instrument operating conditions for determination

Spectrometer	iCAP 6500 Thermo Scientific
Nebulizer	Concentric
Spray chamber	Cyclonic
Radio frequency power (W)	1150
Principal argon flow rate (L/min)	12
Auxiliary argon flow rate (L/min)	0.5
Nebulizer flow rate (L/min)	0.5
Sample flow rate (ml/min)	1.0
Detector	CID86
Selected wavelengths (nm)	Fe (259.9); Na (589.5); Ca (373.6); Mg (279.5); K (766.4); P (177.4)

Analytical measurements of other elements were performed using an inductively coupled plasma mass spectrometer (ICP-MS, iCAP Q, Thermo Scientific Xseries 2, UK) equipped with flatapole collision reaction cell, a microconcentric nebulizer, nickel cones and a peristaltic sample delivery pump, running a quantitative analysis mode. The entire system was controlled with Qtegra Instrument Control Software. Each sample was analyzed in duplicate, and each analysis consisted of three replicates. Typical normal mode conditions and all measured isotopes are given in Table 2. The 18 elements were not all measured in the same analytical conditions (normal mode and collision cell mode). High-purity He (99.9999 % He, Messer, Serbia) was used, in order to minimize the potential problems caused by unidentified reactive contaminant species in the cell, when using the ICP-MS Thermo Xseries 2 in collision cell mode, typically using a collision cell gas flow of 3.5 mL/min of 7 % hydrogen in helium.

Microwave digestion was performed in microwave oven equipped with rotor holding 10 PTFE cuvettes (Ethos 1, Advanced Microwave Digestion System, Milestone, Italy).

Reagents and chemicals

All chemicals were of analytical grade and were supplied by Merck (Darmstadt, Germany). All glassware was soaked in 10 % HNO_3 for minimum 12 h and rinsed well with distilled water. Ultra-pure water was prepared by passing doubly de-ionized water from Milli-Q system (Millipore

Table 2 Measured isotopes and instrument operating conditions for determination

Rf power (W)	1548
Gas flows (L/min)	13.9: 1.09; 0.8
Acquisition time	3 × 50 s
Points per peak	3
Dwell time (ns)	10
Detector mode	Pulse
Replicates	3
Measured isotope	7Li, 27Al, 52Cr, 55Mn, 59Co, 60Ni, 65Cu, 66Zn, 71Ga, 75As, 78Se, 85Rb, 88Sr, 111Cd, 137Ba, 202Hg, 208Pb, 209Bi

Table 3 Determination accuracy of the analytical method

Elements	Certified values	Found values	Recovery (%)
As	0.042 ± 0.010	0.04 ± 0.010	95.24
Cd	0.12 ± 0.007	0.117 ± 0.031	97.50
Cr	24.8 ± 1.3	24.2 ± 1.5	97.58
Cu	10.2 ± 0.5	9.8 ± 0.8	96.08
Hg	0.0164 ± 0.0022	0.010 ± 0.005	60.98
Mn	82 ± 4	78 ± 8	95.12
Ni	15.2 ± 0.6	14.9 ± 0.7	98.03
Pb	1.67 ± 0.11	1.6 ± 0.10	95.81
Zn	30.5 ± 1.1	29.1 ± 0.5	95.41

Simplicity 185 System incorporating dual UV filters (185 and 254 nm) to remove carbon contamination).

Multi-element stock solution containing 1.000 g/L of major elements was used to prepare intermediate multi-element standard solutions for ICP-OES measurements and multi-element stock solution (VHG standards, Manchester, UK) containing 10 mg/L of 22 elements was used to prepare intermediate multi-element standard solutions for ICP-MS measurements. Internal Standard Mix (VHG standards, Manchester, UK) Li, Sc (50 µg/L) and Bi, Ga, Y, Tb, In (10 µg/L) was used.

Sample collection and preparation

The mushroom samples were collected during 2014, on the Mountain Jastrebac, in a rural unpolluted region near town Nis, Republic Serbia (43°23′44″N 21°27′32″E). To overcome variability, such as the stage of development, soil texture and environmental conditions, the mushroom samples were collected at five experimental points and from every experimental point was taken 300 g of each examined mushroom species (total 1500 g for each mushroom species). Mushrooms were cleaned of all surface contamination by little brush. After collection and taxonomic identification, the mushrooms were cut and dried at room temperature. They were stored at polyethylene bags until the analysis started.

Samples (0.25 g dried) were transferred into PTFE cuvettes, and 7 ml of 65 % HNO_3 and 1 ml 30 % H_2O_2 were added. Digestion was performed under following program: warm up for 10 min to 200° C and held for 15 min at that temperature. After cool off period, samples were quantitatively transferred into volumetric flask (25 ml) and diluted with distilled water.

The accuracy of our analytical method was determined using the European Reference Materials "ERM-CD281: As, Cd, Cr, Cu, Hg, Mn, Ni, Pb and Zn." The referenced value is reported as value ± SD (Table 3).

Statistical analysis

Multivariate analysis included principal component analysis (PCA), and hierarchical cluster analyses (HCA) were performed using a statistical package Statistica 8.0 (StatSoft, Tulsa, Oklahoma, USA). A probability level of $p < 0.05$ was considered statistically significant. Correlation between mushrooms was established using regression analysis at a 95 % significance level ($p \leq 0.05$).

Results

The obtained results revealed varying element concentrations, depending on the mushroom species. Contents of the individual elements are given in Table 4. All element concentrations were determined on a dry weight basis but for intake calculations, we used a 300-g portion of fresh mushrooms,. Considering that we cannot determine the recommended daily intake for heavy metals, we calculate provisional tolerable weekly intake (PTWI) and tolerable weekly intake (TWI); 60 kg of body weight was used for intake calculations as the weight of an average consumer.

Discussion

Macroelements (K, P, Ca and Na)

As shown in Table 4, total content of essential macroelements such as K, P, Ca and Na were in the range from 10091.98 to 21192.31 (K), 3857.59 to 10736.54 (P), 111.04 to 2327.72 (Ca) and 39.76 to 916.21 (Na) mg/kg, respectively. Large variations in concentrations that occur are a consequence of the affiliations to different genus of a family *Boletaceae*.

Table 4 Element contents (mg/kg dry weight) and dry matter of analyzed mushrooms (results are the means of triplicate analysis with good reproducibility)

Species	Dry matter (%)*	Ag	Al	As	Ca	Cd	Co	Cr	Cu	Fe	Hg
B. appendiculatus	10.93	3.06	83.21	1.66	122.69	1.25	0.33	0.24	34.25	59.90	1.07
B. edulis	10.85	2.11	67.08	0.73	112.86	1.83	0.72	1.04	15.47	55.21	2.05
B. fechtneri	9.03	0.74	34.63	0.33	202.62	1.01	0.49	0.17	5.29	24.40	1.18
B. impolitus	11.1	1.88	55.90	0.33	255.09	0.68	0.15	0.26	11.89	46.20	0.76
B. purpureus	7.65	2.33	27.74	0.32	111.04	2.41	0.12	0.17	5.61	29.07	0.39
B. regius	11.97	2.13	56.04	1.64	126.88	0.72	0.13	0.16	10.56	56.97	2.54
B. rhodoxanthus	8.58	6.08	163.65	0.40	212.22	0.87	0.07	0.30	15.96	74.02	0.98
L. crocipodium	9.2	0.22	33.88	0.08	275.25	1.09	0.41	0.44	17.34	26.28	4.03
L. pseudoscaber	10.18	3.04	607.79	0.51	2327.72	2.94	0.41	1.34	27.23	514.76	0.54
X. chrysenteron	9.85	0.14	67.78	0.17	315.58	1.52	0.15	0.53	7.91	45.83	0.14
X. badius	10.3	0.73	93.21	0.03	342.21	1.51	0.12	0.29	4.66	45.67	0.27

Species	Dry matter (%)*	K	Mn	Na	Ni	Pb	Se	Sr	Zn	Bi	Rb	P
B. appendiculatus	10.93	15322.99	12.99	98.79	0.82	0.48	0.84	0.72	112.92	0.04	23.74	7897.60
B. edulis	10.85	11781.20	5.05	68.66	1.17	10.58	2.32	2.11	69.94	0.44	57.83	4387.16
B. fechtneri	9.03	12033.63	9.94	854.74	0.57	0.39	1.41	0.58	92.61	0.22	377.63	5333.08
B. impolitus	11.1	15871.56	20.04	58.01	0.68	0.31	0.22	0.67	173.37	0.05	85.39	10077.88
B. purpureus	7.65	10091.98	2.96	916.21	0.68	0.61	0.33	0.98	301.57	0.03	91.04	6495.81
B. regius	11.97	13676.07	8.24	39.76	1.14	0.29	1.67	0.50	78.71	0.45	499.59	9134.35
B. rhodoxanthus	8.58	10518.39	13.93	63.76	0.40	0.49	0.48	0.51	83.69	1.40	117.70	3857.59
L. crocipodium	9.2	18856.85	11.54	155.97	0.44	4.38	0.62	1.62	68.46	0.18	82.54	6382.60
L. pseudoscaber	10.18	21192.31	14.74	435.79	1.69	1.61	0.38	4.71	130.33	0.03	185.81	10736.54
X. chrysenteron	9.85	14136.24	2.91	197.73	0.50	2.59	0.04	2.53	17.64	0.07	46.68	4386.92
X. badius	10.3	16588.82	23.79	78.80	1.20	1.75	0.13	1.50	53.69	0.02	145.95	4570.86

* Dry matter were presented based on air-dried weight

Potassium is third most common mineral in the human body, and it is a synonym for good health, playing an important role in many metabolic processes, including the proper functioning of the brain, heart and muscle. Potassium levels in many mushroom species are considerably higher than those in foods of plant origin [20]. Among wild edible mushroom species, the greatest concentrations of K was obtained in *L. pseudoscaber* (21192.31 mg/kg), while lowest was in *B. purpureus* (10091.98 mg/kg). According to earlier data, potassium contents in *L. pseudoscaber* have been reported to be 34000–43000 mg/kg [18] and 14000–41000 mg/kg in *B. edulis* [13]. Recommended daily dietary intake for potassium is 2000 mg. The highest concentration of K in analyzed mushrooms was 647.21 mg K/300 g fresh mushroom, which represents 32.36 % of recommended dietary intake. High blood pressure, stroke and weakened reflexes are just some of the consequences of the lack of potassium. Mushrooms are known as potassium-rich food, which is confirmed in our study. Compared with potassium, sodium content is significantly lower in studied mushrooms, which is very important since consummation of sodium-rich food may be related to high blood pressure. Among tested mushroom samples, *B. purpureus* showed the highest content of sodium (916.21 mg/kg), followed by *B. fechtneri* (854.74 mg/kg), while other mushrooms showed lower sodium content. *B. regius* showed the lowest content of sodium (39.76 mg/kg). Jarzyńska and Falandysz [18] analyzed mushrooms and reported that *L. pseudoscabrum* had average sodium concentration of 560 mg/kg, which is similar to the results of our study (435.79 mg/kg). Also, our result for sodium in *B. edulis* (68.66 mg/kg) is within the range which is earlier reported by Falandysz et al. [13] (17–550 mg/kg). Minimum daily requirement for Na is about 1500 mg, and the maximum daily intake is 2400 mg. The highest concentration of Na in analyzed mushrooms was only 21.03 mg Na/300 g fresh mushroom (which is 0.91 % of recommended dietary intake), which qualifies them as a food recommendable for consumption, without increase risk of hypertension.

Phosphorus is the second most abundant element in wild-grown edible mushrooms, and its concentration ranged between 3857.59 and 10736.54 mg/kg in *B. rhodoxanthus* and *L. pseudoscaber*, respectively.

Considering data of Quinche [32], usual contents in the popular mushrooms of the *Boletaceae* family were 5000–7000 µg/g dry matter. The values of *X. badius* phosphorus content obtained in this study (4570.86 mg/kg) are similar with values obtained by Kojta et al. [24] (3600–6000 mg/kg). Recommended dietary daily intake for phosphorus is 1000 mg. The highest concentration of P in analyzed mushrooms represents 327.89 mg P/300 g fresh mushroom, which is 32.79 % of recommended dietary intake.

Calcium occurs in very similar concentrations in the analyzed mushrooms. The average Ca content of all analyzed mushrooms was 207.65 mg/kg with an exception in *L. pseudoscaber* where content of Ca was extremely higher (2327.72 mg/kg).

According to Falandysz et al. (2008), total range of Ca in *B. edulis* was 5.6–420 mg/kg which is in agreement with our results (112.86 mg/kg). The fact that recommended daily dietary allowance for Ca is 900 mg, and the highest concentration of Ca registered in the present study was 69.83 mg/300 g of fresh mushroom (which is 7.76 % of daily average intake), might be considered from two points of view: from one, mushrooms represent small contribution in Ca intake, in case of Ca-deficient nutrition and from the other, mushrooms are safe food, in diet rich in Ca from other sources. Doses of Ca larger than 1500 mg/per day may cause stomach problems for sensitive individuals.

Considering the range of macroelements in analyzed mushroom species, a meal of 300-g portion will provide 231.61–647.21 mg of K; 99.29–335.59 mg of P; 1.43–23.15 mg of Na and 2.55–71.09 mg of Ca.

Essential trace elements (Cu, Zn, Fe and Mn)

Some mushroom species have ability to accumulate traces of transition metals, valuable as cofactors of numerous enzymes (Cu, Zn, Fe and Mn) and from this reason considered as essential, in its tissues at relatively high concentrations.

Results for copper are shown in Table 4. The highest content of Cu was found in mushroom *B. appendiculatus* (our value is 34.25 mg/kg) (18 mg/kg) [34], and the lowest was found in *X. badius* (our value is 4.66 mg/kg) (1.4–2.2 mg/kg) [23]. The highest content of Cu was found in mushroom *B. appendiculatus* (our value is 34.25 mg/kg) (18 mg/kg) [34] and the lowest in *X. badius* (our value is 4.66 mg/kg) (1.4–2.2 mg/kg) [23]. According to earlier data, copper contents of mushroom samples have been reported to be (11–77 mg/kg) [15], (51.99–85.76 mg/kg) [2], (109 mg/kg in polluted area) [23] and (18.3 mg/kg) [43], for *B. edulis*. Our value for *L. pseudoscabrum* (27.23 mg/kg) was similar to result recorded by [18], who analyzed different mushrooms and reported 19–48 mg/kg.

Zinc is an antagonist of some other metals (Cd, Pb, Ni), and its presence in certain mushrooms reduces the risks associated with the high concentrations of the other toxic metals [41]. Mushrooms are also recognized as zinc accumulators. In analyzed samples, range of zinc content was from 17.64 to 301.57 mg/kg for *X. chrysenteron* and *B. purpureus*, respectively. The permissible limit of zinc in food is 60 mg/kg [37]. More than half of samples showed higher content of Zn than it is allowed. However, concentrations of Zn in the literature have been reported to be in the ranges: *B. edulis*, (43–35 mg/kg) [15] and (63.7–133.4 mg/kg) [2]; *L. pseudoscabrum*, (93–420 mg/kg) [18]; *X. crysenteron* (111.9–162.3 mg/kg) [2] and *X. badius*, (162.3–225.7 mg/kg) [2]. Obtained zinc values are in agreement with the literature values.

Iron is very important for decreasing the incidence of anemia, and increased concentrations of iron can cause damage. The iron content in the mushrooms ranged from 24.39 mg/kg in *B. fechtneri* to 514.76 mg/kg in *L. pseudoscaber*. In the literature, the iron values in the dry weight of mushrooms were 1040 mg/kg [34] in *B. appendiculatis,* 664.6 mg/kg [43] in *B. edulis*, 44.9 mg/kg [28] in *L. crocipodium,* 72.1 mg/kg [28] in *X. badius*, enabling classification of analyzed samples as low to moderate in Fe content.

The highest manganese content was 23.79 mg/kg, for the species *X. badius*, whereas the lowest manganese content was 2.91 mg/kg, for the species *X. chrysenteron.* A similar study by Yağız [43] reported a mean Mn concentration of 27.9 mg/kg in *B. edulis*. Mleczek et al. [30] had reported Mn concentration of 19 mg/kg in *X. badius*. Results obtained in this study for manganese are in accordance with values reported in the literature.

A 300-g portion of analyzed mushroom species will provide 0.52–6.92 mg of Zn, 0.13–1.12 mg of Cu, 0.66–15.72 mg of Fe and 0.07–0.74 mg of Mn, rendering them as a good source of these metals.

Some other trace metals (Ag, Al, Co, Ni, Cr, Sr, Se, Bi, Rb)

Based on previous research, some species of the genus *Boletus* can also accumulate considerable amounts of selenium. Among tested mushroom samples, *B. edulis* showed the highest content of selenium (2.32 mg/kg), followed by *B. regius* (1.67 mg/kg) and the minimum selenium content showed *X. chrysenteron* (0.04 mg/kg). Recommended dietary daily intake for Se is 0.035 mg.

Despite low content of silver in the soils, the concentration in the mushrooms can be very high. The high Ag concentrations in hyperaccumulating macrofungal species might have some protective effect against pathogenic microfungi, bacteria, insect larvae or gastropoda, but this is yet to be tested [11]. In analyzed species, concentration of silver was different from one species to another, ranging between 0.14 mg/kg in *X. chrysenteron* and 6.08 mg/kg in *B. rhodoxanthus* species. In samples collected in west part of Russia, the Ag values were in range of 1.3–4.3 mg/kg for *B. edulis* and 1.4 mg/kg for *X. chrysenteron* [25]. The Ag concentration in examined *B. edulis* (2.11 mg/kg) agrees well with concentration reported in the literature for *B. edulis* by Falandysz et al. [10] (0.16–2.6 mg/kg). According to [28], contents of Ag in *B. edulis, B. appendiculatus, L. crocipodium* and *X. badius* were 2.74–6.5, 4.8, 2.63 and 2.65 mg/kg, respectively. These results are consistent with our research. The average daily intake of silver is 0.02–0.08 mg/day [42].

Trace amounts of nickel may be useful for organism, especially in activation of enzymes, but at higher levels it can be toxic. In all examined mushroom samples, the Ni content was observed to be in the range of 0.40–1.69 mg/kg, which is somewhat little lower than results reported by [28], 4.8–5.35 mg/kg (*B. edulis*), 15.7 mg/kg (*B. appendiculatus*) and 6.63 mg/kg (*L. crocipodium*). Since the recommended daily intake of nickel is between 0.10 and 0.30 mg/kg [40], consumption of reasonable amounts of the studied fungi provides necessary amounts of Ni.

Chromium is a metal, necessary in trace amounts for the normal metabolism, and deficiency can cause various disorders. Concentrations of Cr in examined samples were from 0.17 mg/kg to 1.34 mg/kg, decreasing in the following order: *L. pseudoscaber* > *B. edulis* > *X. chrysenteron* > *L. crocipodium* > *B. rhodoxanthus* > *X. badius* > *B. impolitus* > *B. appendiculatus* > *B. fechtneri* > *B. purpureus* > *B. regius*. Recommended daily intake of Cr is 50–200 μg (RDA, [33]), which could be provided through examined fungi consumption, though uncontrolled intake could be the source of elevated and risky amounts of Cr.

The average cobalt content in the mushrooms is about 0.50 mg/kg (EC 2008), and, in rare cases, the content can be as much as 1.0 mg/kg [9]. In our research, concentrations of Co were between 0.07 and 0.72 mg/kg, which are consistent with the previous studies. For mushrooms collected in Greece, Co concentration was found to be 0.17 mg/kg in *X. chrysenteron* [31]. Michelot et al. [28] reported Co concentrations at 1.1–1.94 mg/kg in *B. edulis,* 5.63 mg/kg in *B. appendiculatus* and 3.89 mg/kg in *L. crocipodium,* which is significantly higher than expected. Considering the average daily intake of cobalt (0.005–0.008 mg), studied fungi do not present health risk, if consumed in reasonable amounts.

Presence of strontium in the organism is associated with amount of calcium; therefore, supplementing larger amounts of Sr increases Ca. Bismuth is not classified as essential for humans, but it has synergists effect for iron, nickel, phosphorus and vitamin D. Results for Sr and Bi in studied species ranged from 0.50 to 4.71 and 0.03 to 1.40 mg/kg, respectively. Daily intake of strontium in many parts of the world is estimated to be up to 4 mg/day, and for bismuth 0.002–0.030 mg/day. Using the studied fungi in diet can provide enough amounts of mentioned metals.

In our study, maximum level of Al is 607.79 mg/kg in *L. pseudoscaber* and minimum is 27.74 mg/kg in *B. purpureus*. A similar study by [28] reported Al concentration of 68.7 mg/kg in *B. edulis*, which completely agrees with our result for *B. edulis* (67.08 mg/kg). The average aluminum intake for humans is 60 mg/day [38], which could be provided by consumption of studied fungi.

The average values of Rb concentration in tested mushrooms varied between 27.74 and 499.59 mg/kg. The concentrations of this element obtained in study by [15] from Italy ranged between 62.5 and 311.0 mg/kg in *B. edulis*, which is very similar to our result for the same mushroom (57.83 mg/kg). Recommended daily intake varies between 1 and 5 mg.

Based on the recommended daily intake of all mentioned metals, a meal consisting of 300-g portion fresh mushrooms will provide 0.004–0.10 mg of Ag, 0.64–18.56 mg of Al, 0.002–0.02 mg of Co, 0.004–0.04 mg of Cr, 0.01–0.05 mg of Ni, 0.001–0.08 mg of Se, 0.01–0.14 mg of Sr, 0.001–0.036 mg of Bi and 0.78–17.94 mg of Rb.

Toxic elements (As, Cd, Hg and Pb)

The general perception is that mushrooms are food unaffected by anthropogenic factor. However, it appeared that increased environmental pollution affects the chemical composition of mushrooms. The ability to accumulate metals is characteristic of each species, and accordingly, some mushrooms have this ability manifested in different degrees. In various macrofungi, growing on same substrate, concentrations of elements can vary over a wide range and can differ even a thousand times from species to species [26]. Among the hazardous elements which can be found in the mushrooms, the most common are Pb, Cd, Hg and As. These elements are considered as environmental contaminants due to diverse anthropogenic sources.

Cadmium is one of the most toxic metals accumulated mainly in kidneys, spleen and liver. The average Cd content in the studied species ranged from 0.68 mg/kg in *B. impolitus* to 2.94 mg/kg in *L. pseudoscaber*. Michelot et al. [28] reported a mean Cd content of 1.39–1.7 mg/kg in *B. edulis*, 3.34 mg/kg in *B. appendiculatus*, 1.78 mg/kg in *L. crocipodium* and 3.27 mg/kg in *X. badius*. Much similar content of Cd with ours (1.247 mg/kg) in *B. appendiculatus* was reported by [34], and it was 1.8 mg/kg. The provisional tolerable weekly intake (PTWI) for cadmium is 0.42 mg/week, and a tolerable weekly intake (TWI) is

0.15 mg/week [38]. Meal consisting of 300-g portion fresh mushrooms will provide 0.02–0.09 mg of Cd, which is on a weekly basis much lower than allowed.

As most heavy metals, lead can be accumulated in the body for a long time, and it is necessary to monitor its even very low concentration in potential sources. In this study, results for Pb ranged from 0.30 to 10.58 mg/kg for *B. regius* and *B. edulis*, respectively, and compared with the results of corresponding researches, obtained values are significantly lower, indicating that the environment where they harvested is unpolluted by lead. According [28] lead contents have been reported to be 34.2 mg/kg (*B. appendiculatus*), 20.3–21.2 mg/kg (*B. edulis*), 23.1 mg/kg (*L. crocipodium*) and 17.6 mg/kg (*X. badius*). These results are much higher than ours for the same samples, 0.48, 10.58, 4.38 and 1.75 mg/kg, respectively. In *L. pseudoscaber*, our result for Pb (1.61 mg/kg) is similar to the result of [18] (0.18–1.1 mg/kg). The acceptable daily intake of lead is 1.5–1.74 mg weekly for an individual of 60 kg bw [39]. Based on concentrations of Pb found in this research, consumption of 300-g mushrooms will provide 0.008–0.29 mg of lead. Only one mushroom shows higher levels of Pb (*B. edulis*), but within the allowed limits, examined mushrooms are considered safe and unpolluted.

Azevedo et al. [4] described in their paper that mercury exposure, even at low doses, affects endothelial and cardiovascular function. It can be efficiently accumulated by many mushrooms and especially when it is present in small concentrations in forest soils [12]. In this study, maximum level of Hg was found to be 4.03 mg/kg in *L. crocipodium* and minimum was 0.14 mg/kg in *X. chrysenteron.* Mushrooms *B. appendiculatus*, *B. edulis* and *X. chrysenteron* collected in Italy contain the following concentrations: 1.05, 2.67 and 0.23 mg/kg, respectively [7]. These values are very similar to our results for the same species, 1.07, 2.05 and 0.14 mg/kg, respectively. The PTWI for Hg is 0.30 mg (US EPA [36].

Arsenic has short half-life (several weeks), but its effects can be seen years after exposure and it is considered to be a carcinogen to humans (ATSDR) [1]. The normal levels of arsenic in wild mushrooms are usually less than 1 mg/kg dw [21]. In this study, total arsenic concentrations in different samples of mushrooms ranged between 0.03 and 1.66 mg/kg.

The Joint FAO/WHO Expert Committee on Food Additives (JEFCA) recommends that provisional tolerable intake of As is 0.90 mg for a person weighing 60 kg [14]. Excluding *B. appendiculatus* and *B. regius,* remaining analyzed species contained fewer milligrams than allowed provisional tolerable intakes. Cocchi et al. [7] investigated As contents of mushrooms and reported: *Boletus edulis* (0.10 mg/kg FW), *B. appendiculatus* (0.10 mg/kg FW) and *X. chrysenteron* (0.10 mg/kg FW).

Statistical analysis

All data were reported as mean ± standard deviation of three replicates. Variables were standardized before statistical analysis to compatible units from distribution with a mean of 0 and standard deviation of 1. At the beginning of statistical analysis, correlation matrix was calculated, giving the correlation coefficients between each pair of metals. Correlation between metal content was established using regression analysis at a 95 % significance level ($p \leq 0.05$). Each term of the matrix is a number ranging from −1 to +1: The + or − sign indicates a positive or negative interdependence between variables (direction) and the absolute value indicates the strength of the interdependence [5]. If correlation between two metals >0.75, then it is consider strong, between 0.75 and 0.5 medium and 0.5–0.3 weak. The strongest correlation was between iron and aluminum ($r = 0.99$, $p < 0.05$). Another groups represented by calcium and iron also displayed a significant strong correlation ($r = 0.98$, $p < 0.05$), calcium and aluminum ($r = 0.97$, $p < 0.05$).

Agglomerative hierarchical clustering (AHC)

In order to classify species of mushrooms based on the metals composition, Ward's method cluster analysis was used to produce cluster groups (Fig. 1). Ward's method minimizes the sum of squares of any two (hypothetical) clusters that can be formed at each step. HCA calculates the distances (or correlation) between all samples using a defined metric such as Euclidean distance and Manhattan distance [6]. We decided to use Euclidean distance because based on it we can see which pairs show the most similarity. The linkage distance is showed as

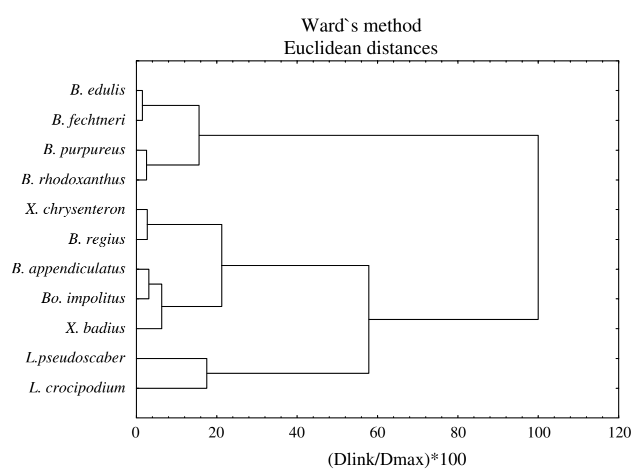

Fig. 1 Cluster analysis diagram of mushroom species based on chemical composition

Table 5 Significant components, eigenvalues, total variance and cumulative

Element	PC 1	PC 2	PC 3	PC 4
Al	**−0.93**	0.20	0.10	−0.04
As	−0.04	−0.02	−0.62	**0.65**
Ca	**−0.95**	0.21	0.16	−0.07
Co	−0.32	**−0.73**	0.16	0.31
Cr	**−0.85**	−0.42	0.25	0.00
Cu	**−0.57**	−0.10	−0.49	0.33
Fe	**−0.95**	0.19	0.11	0.05
Hg	0.10	**−0.57**	−0.41	0.21
K	**−0.74**	0.04	−0.33	−0.29
Mn	−0.23	0.35	−0.46	**−0.58**
Na	0.06	0.35	**0.74**	0.39
Ni	**−0.77**	−0.01	−0.06	0.10
Pb	−0.14	**−0.91**	0.19	0.01
Sr	**−0.88**	−0.17	0.33	−0.14
Zn	0.06	**0.57**	0.31	0.54
P	**−0.54**	0.45	−0.39	0.38
Eigenvalues	6.14	2.81	2.20	1.72
Total variance (%)	38.35	17.55	13.73	10.74
Cumulative (%)	38.35	55.90	69.64	80.38

Bold correlations are significant at $p < 0.05$

D_{link}/D_{max}, which represents the quotient between the linkage distances for a particular case divided by the maximal linkage distance.

In this study, we used only variables that are correlated with other metals and the aim of HCA is to investigate similarities between used mushrooms species. According to HCA, mushrooms are grouped in three statistically significant clusters at $(D_{link}/D_{max}) \times 100 < 50$. The strongest clustering is observed for *B. edulis* and *B. fechtneri* (272—Euclidean distances) which showed close association with *B. purpureus* and *B. rhodoxanthus* (461—Euclidean distances). These mushrooms belong to first cluster. Second cluster is divided in two sub-clusters: First contains *B. appendiculatus* and *B. impolitus* (565—Euclidean distances)/X. badius and second sub-cluster is observed for *X. chrysenteron* and *B. regius* (498—Euclidean distances).

Third significant cluster is observed among *L. crocipodium* and *L. pseudoscaber* (3199—Euclidean distances), and these mushrooms belong to the same genus, *Leccinellum*, and they differ the most.

Principal component analysis (PCA)

In order to understand the connection between analyzed metals, we have performed PCA. The PCA is a data reduction technique used in determining the number of variables to explain the observed variances in the data [3]. PCA was carried out on 16 metals and 11 mushroom species (*B. appendiculatus, B. edulis, B. regius, B. fechtneri, B. impolitus, B. purpureus, B. rhodoxanthus, L. crocipodium, L. pseudoscaber, X. chrysenteron, X. badius*) and applied to reduce the number of original variables. PCA with no rotation was applied to data set [17]. In this study, we extracted four principal components, with eigenvalues higher than 1.0, as suggested by the Kaiser criterion [19]. Table 5 presents the number of significant components, eigenvalues, total variance and cumulative.

PCA showed 80.38 % information regarding mineral composition of mushrooms, and using this statistical tool we have succeeded to transform the original variables into new uncorrelated variables, called components, which are linear combinations of the original variables. PC 1, with high loadings,

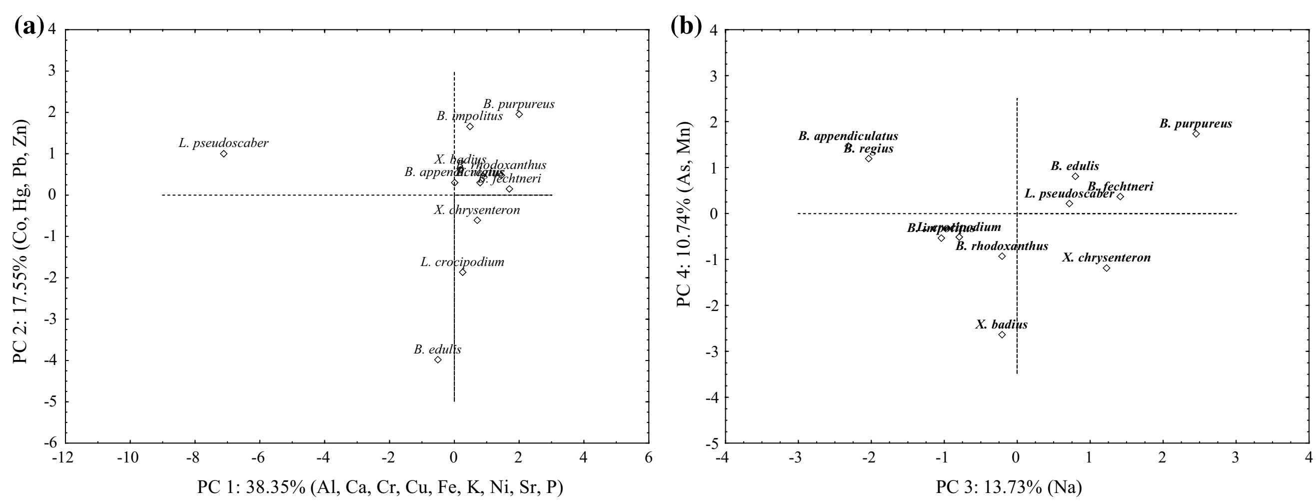

Fig. 2 Plots of loadings based on the chemical composition in mushroom species of **a** first and second and **b** third and fourth factors

>0.7 (except for *P* 0.54), explained 38.35 % of total variance, and it is strongly influenced by negatively correlated variables, describing Al, Ca, Cr, Cu, Fe, K, Ni, Sr and P (Fig. 1a).

PC 2 was loaded by negatively correlated Hg, Co and Pb (−0.57, −0.73 and −0.91, respectively) and positively correlated Zn (0.57) and explained 17.55 % of the total variance.

PC 3 component explained 13.73 % (Fig. 2b) of total variance, and it is strongly influenced by positively correlated variable, Na (0.74). PC 4 explained 10.74 % of the total variance, and it is made up of Mn (−0.58) and As (0.65).

Conclusion

Knowledge of heavy metal contents of wild-growing mushrooms is important for public health due the fact that some mushrooms are widely consumed in many countries. Our results confirm that mushrooms are very good source of minerals, providing a balanced nutrition. According to obtained, mushroom such as *L. pseudoscaber* is very good source of K, providing a natural source for people who were suffering from high blood pressure. Same mushroom has sufficient level of Fe, which is very important for anemic people. In conclusion, this study and its data: (1) increase the scientific knowledge about mineral composition of wild edible mushrooms, (2) indicate that mushrooms represent a major source of essential minerals for human health and (3) suggest that consumption of these mushrooms does not represent a toxicological risk for human.

Acknowledgments The research was supported by Ministry of Education, Science and Technological Development of the Republic of Serbia [172051]. Marija Dimitrijevic is grateful to Ministry of Education, Science and Technological Development of the Republic of Serbia for providing scholarships for researchers. The authors wish to acknowledge to Marjan Kustera and Mycological Society of Nis for the sampling and determining mushrooms samples.

Compliance with ethical standards

Conflict of interest All authors declare that they have no conflict of interest.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

Informed consent Informed consent was obtained from all individual participants included in the study.

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