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# Elemental profile of edible mushrooms from a forest near a major Romanian city

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**Abstract.** We determined the elemental profile of 16 edible mushroom species from the Făget Forest, near Cluj-Napoca, and of 12 species from the Apuseni Mountains. One-way ANOVA showed no difference in the elemental content of mushrooms when the two regions were compared. Some species accumulated high amounts of trace elements, i.e. *Boletus edulis* (Ag, S, Zn), *Macrolepiota procera* (Cu), *Lactarius volemus* (Co), *Russula emetica* (Mn), *Armillariella mellea*, and *Chantarellus cibarius* (Cr). The cadmium content was the highest in the case of *Leccinum scabrum* and *Boletus edulis*. These two species presented elevated risk levels for all age-groups when they are consumed regularly.

**Keywords:** wild edible mushrooms, heavy metals, ICP-OES, trace elements

### 1. Introduction

The consumption of wild grown edible mushrooms is a worldwide custom. In Transylvania (Romania), there is a strong tradition of collecting wild edible fungi for eating and selling. Edibility assumes the absence of poisonous effects, a pleasant taste and aroma, and nevertheless the lack of concentrations of accumulated toxic elements dangerous to human health. However, this last aspect is often ignored by the collectors. Edible mushrooms constitute an important and widely appreciated part of the human nutrition because they are rich sources of

proteins, fibres, vitamins, antioxidants, and minerals. Some elements are essential for living organisms, including chromium, cobalt, copper, iron, manganese, and zinc. Wild edible mushrooms constitute one of the main sources of these elements [1]. In the last few decades, the mapping of elemental content of mushrooms was at the centre of many chemical researches. These studies revealed specific elemental preferences of some mushrooms. For example, *Macrolepiota procera* accumulates mainly copper, but it can contain high amounts of Mn, Zn, Cr, Ni, even Cd [2–5]; *Chantarellus cibarius* shows preference for Mn, Fe [6]; *Boletus edulis* is known for its high content of Se, Ag, Mn, and Zn [2, 5, 7] and *Armillariella mellea* for iron [2, 8]. There are also some species, which are known for their low mineral content (*Lactarius spp., Russula spp., Hypholoma fasciculare, Hydnum repandum*) [2, 4, 7, 9–14]. Relying on several studies, a review paper highlighted the uneven distribution of the elements in the fruiting body of the mushrooms: the highest concentrations of metals can be found in the sporophore, followed by the other parts of the cap. The smallest metal content is found in the stipe [7].

Today it is also known the ability of some mushroom species to accumulate considerable amounts of metals as well as toxic elements with regard to the human organism, such as mercury, cadmium, lead, arsenic, or radionuclides [7, 15–19]. Near highly contaminated sites (smelters, metal mining, thermal power plants, industrial areas) and main roads, high content of heavy metals was detected in mature fruiting bodies of macrofungi [8, 9, 20–22]. A recent study on mushrooms growing in urban areas showed unusually elevated concentrations of Cd and Pb in the fruiting bodies [23].

These findings led us to hypothesize that a large city as Cluj-Napoca may have polluting effect over the mushrooms growing in the forest lying in the close vicinity of it. The city is the economic and cultural centre of Transylvania; in the last few years it has developed rapidly. With its increased road and air traffic and its expanding trends, it may pollute the Făget Forest, which is situated on the western and southern side of the city. Many inhabitants of Cluj-Napoca have the habit of going mushroom picking in the Făget for consumption purposes. The most popular species are *Lactarius piperatus*, *Boletus edulis*, *Armillariella mellea*, *Macrolepiota procera*, *Russula spp.*, and *Agaricus spp*.

Our aim in this study was to determine the elemental composition of mushrooms growing in the Făget Forest, and thus to assess the health risk arisen from the long-term consumption of them. We also established the background levels of the elements in mushrooms by choosing an area considered unpolluted in the Apuseni Natural Park.

### 2. Materials and methods

### Description of the sampling sites

The Făget Forest is part of the Făgetul Clujului – Valea Morii Natura 2000 Network (ROSCI0074). It lies at an average altitude of 625 m and it consists dominantly of beech, less hornbeam, and spruce. The prevailing wind direction is from the west, the average annual temperature is 8.4°C, and the average annual precipitation is 663 mm [24]. The control site consisted of the surroundings of Padiş, at an average altitude of 1,127 m, situated in the Apuseni Natural Park, also part of the Natura 2000 Network (ROSCI 0002). The average annual temperature is 6.0°C and the average annual precipitation is 1,400 mm [24]. This site is covered with pinewoods; the dominant species is the *Picea abies*. In order to collect mushroom species whose habitat is the deciduous forest, we chose the beech forests at lower altitudes but close to Padis.

## Sampling and sample proceeding

A total number of 80 mushrooms of 16 species were collected from the Făget Forest and 60 mushrooms of 12 species from the Apuseni Natural Park in July-October 2014. At least three individuals were picked up of the large-size species and at most 7 individuals of the small-size species. The samples were stored in plastic bags. The mushrooms were cleaned with tap water, and rinsed with distilled water. Further on, the caps and stipes were processed separately. They were dried at room temperature, and air-dried at 105°C. The mashed and sieved powders, having the particle diameters less than 315 µm, were stored in plastic bottles until the digestion process. For the instrumental analysis, three samples were digested in parallel for each part (caps and stipes) of any mushroom species. The decomposition of organic matter was carried out at atmospheric pressure, using a digestion mixture of 5 mL 65% HNO<sub>3</sub> (Merck, suprapur) and 4 mL 30% H<sub>2</sub>O<sub>2</sub> (Merck, suprapur). The solution of nitric acid was added at room temperature to a mass of 0.2000 g sample, twelve hours prior to the heating process. The digestion was done at 90°C for 4 hours on an electric heating plate. The hydrogen-peroxide solution was added in two steps. The resulting digests were analysed with Agilent 5100 ICP-OES spectrometer, applying the simultaneous measuring technique. The calibration was performed with multi-elemental standards, and calibration curves were obtained for each element. A total of 20 elements (Ag, Al, Ba, Ca, Cd, Co, Cr, Cu, Fe, K, Li, Mg, Mn, Na, P, S, Si, Sr, Zn) were quantified.

### Method of the risk assessment

The risk assessment of the heavy metals to human health due to the consumption of the mushrooms can be evaluated by calculating the hazard quotient

(HQ) for each metal of interest and the hazard index (HI), which is the sum of the hazard quotients [25, 26]. The HQ is the ratio between the exposure and the reference dose ( $Eq.\ I$ ). One can assume the existence of a risk for mushroom consumption when the HQ > 1.

$$HQ_{metal} = \frac{DI \cdot C_{metal}}{R_f D \cdot BW} \tag{1}$$

where DI is the daily intake of mushrooms (kg day<sup>-1</sup>),  $C_{metal}$  is the concentration of the metal in the mushroom given for dry weight (mg kg<sup>-1</sup>),  $R_fD$  is the oral reference dose for the metal given for unit body weight (mg kg<sup>-1</sup> day<sup>-1</sup>), BW is the average human body weight (kg). We used the  $R_fD$  values published in the IRIS (Integrated Risk Information System) database, and they can be seen in *Table 1* [27]. We used average body weights for different age-groups as reported by the guidance of the European Food Safety Authority [28]. The age-groups and BW values are given in *Table 3*.

The HI provides information about the joint health risk caused by the heavy metals when a specific mushroom is consumed. We calculated the hazard index as given by Eq. 2.

$$HI = HQ_{Cd} + HQ_{Cr} + HQ_{Mn} + HQ_{Ni} + HQ_{Zn}$$
 (2)

Statistical analysis

The statistical analyses were performed with R software (version 3.2.1, R Foundation for Statistical Computing) and Past software. We created a data matrix where the rows represented the caps and stipes of the mushroom species collected from the Făget Forest, while the columns contained the concentrations of the 20 studied elements. Before the statistical analysis, the concentration data were standardized, that is transforming the distributions of the variables in order to achieve distributions with zero means and standard deviations of 1.0. Thus, the differences in the concentration of major and minor elements, which were as huge as 4–6 orders of magnitude, were equilibrated. We studied the correlation between the elements with the Spearman rank correlation test. In order to compare the elemental composition of mushrooms grown in the selected geographical regions and the elemental distribution in the caps and stipes, we performed one-way ANOVA. Prior to the analysis, outliers were identified by Dixon Q-test for each element, and excluded from the dataset.

### 3. Results and discussion

The concentration means (n = 3) for the elements quantified in the mushrooms from the Făget Forest are shown in *Table 1* and *Table 2*. The *Boletus edulis* 

accumulated high amounts of Ag, S, and Zn; the *Macrolepiota procera* accumulated Cu; *Lactarius volemus* had the most elevated concentration of Co; *Russula emetica* preferred Mn; *Armillariella mellea* and *Chantarellus cibarius* accumulated Cr. The cadmium content was the highest in the case of the *Leccinum scabrum* and *Boletus edulis*. The *Lactarius deliciosus*, *Lactarius volemus*, and *Amanita rubescens* species had the lowest mineral content.

The one-way ANOVA test revealed no significant differences between the elemental compositions of the mushrooms coming from the two selected areas. An exception was the Ba, which had significantly (p = 0.035, Fisher's F = 4.92, df = 28) higher concentrations in the mushrooms from Făget than in the Apuseni Mountains. We also compared the elemental composition of caps and stipes of the 16 mushroom species using one-way ANOVA. Relying on the Fisher's F values, the Ca, Cr, Li, Ni, and Zn concentrations were significantly (p < 0.05) higher in the caps than in the stipes of the fungi.

According to the Spearman rank correlation test (*Table 4*) carried out based on the concentrations of elements present in the mushroom caps and stipes from the Făget Forest, we found significant (p < 0.05) correlation between the alkalineearth metals (Ca-Sr, Ba-Sr, Ba-Ca), where rho was higher than 0.60. A strong correlation was also between the Mn with Ca, Ba, Sr, and P (rho > 0.70). Magnesium was not correlated with these elements, but it had rho values between 0.41 and 0.53 with transitional metals such as Ag, Cd, Cu, and Fe. An interesting finding was the significant inverse correlation of sulphur with Ca, Ba, Sr, and Mn, and stronger positive correlation with Ag, Cd, Zn, and P. We also found significant correlations between other pairs of elements such as Fe-Mn, Fe-Cr, Fe-Na, K-Na, K-Al, Fe-Al, Cd-Zn, Cd-Ag, and Cu-Mg. Other studies on mushrooms found the same correlations between elements [4, 29].

In order to assess the contribution of some heavy metals to the health risk of mushroom consumption, we calculated the hazard quotients. The highest hazard quotient values were found for Cd (0.034-2.216), followed by Zn (0.014-0.201), but Cr (0.00001-0.00026) showed the lowest values. We evaluated the health risk of mushroom consumption concerning different age-groups relying on the hazard index  $(Table\ 3)$ . Assuming that the mushroom season lasts 6 months (from May until October), in the calculus we reckoned with a weekly mushroom consumption of 1 kg (DI = 0.143 kg day<sup>-1</sup> fresh weight) for adults and adolescents beyond 14, and of 0.5 kg (DI = 0.071 kg day<sup>-1</sup> fresh weight) for children and teenagers. The average water content of the mushroom fruiting body was considered as 90% [2].

Table 1. The concentrations (mg  ${\rm kg}^{-1}$ , dry weight) of alkaline, alkaline-earth metals and non-metals in the mushrooms from the Făget Forest

Mushroom species	Parts	K	Na	Li	Ca	Mg	Ва	Sr	P	S	Si
Agaricus	cap	27049	83.32	0.21	68.85	1065.1	2.53	0.55	10107	4886	81.83
campestris	stipe	29924	102.28	0.25	158.96	645.5	1.04	0.62	5954	4418	121.60
Amanita	cap	60512	509.09	0.21	445.12	1311.6	2.20	0.75	10234	8612	25.87
rubescens	stipe	41674	580.04	0.25	277.59	492.3	1.08	0.50	5013	5998	15.36
Amillariella	cap	44370	441.56	0.21	836.68	1078.1	1.79	0.96	6506	1925	38.94
mellea	stipe	48270	428.33	0.02	703.69	699.8	6.36	1.50	1574	668	80.26
Boletus	cap	33814	163.19	0.05	334.83	819.4	0.42	0.33	7821	17378	30.88
edulis	stipe	25441	115.37	0.02	237.04	585.8	0.62	0.25	5052	16867	32.46
Chantarellus	cap	33815	151.63	0.17	481.59	968.4	1.87	1.08	3676	868	49.95
cibarius	stipe	34654	139.07	0.54	576.72	516.7	2.75	1.46	2060	488	35.97
Hydnum	cap	46990	404.80	0.02	235.09	1115.8	1.96	0.50	7808	1913	45.35
repandum	stipe	40230	380.31	0.38	481.65	788.3	5.24	0.96	5901	1426	24.28
Hypholoma	cap	29577	317.49	0.37	448.35	947.7	1.37	0.67	6716	1943	27.97
fasciculare	stipe	23506	194.70	0.17	328.75	421.4	2.04	0.79	2936	1023	59.97
Lactarius	cap	21312	77.30	0.02	231.48	889.7	0.58	0.62	4265	1587	45.56
deliciosus	stipe	15049	115.02	0.05	310.43	485.8	1.44	0.96	2407	1061	23.40
Lactarius	cap	33823	172.81	0.25	248.26	802.5	1.12	0.37	4833	5479	48.37
piperatus	stipe	43218	245.02	0.42	193.42	459.4	0.87	0.33	2529	3493	38.33
Lactarius	cap	25340	161.61	0.09	296.08	612.7	1.67	0.62	3626	1495	71.03
volemus	stipe	23430	209.87	0.26	294.78	373.8	1.25	0.58	1905	1136	61.65
Leccinum	cap	37567	295.93	0.26	387.18	825.2	1.83	0.62	5704	3891	32.09
scabrum	stipe	33132	202.61	0.14	417.59	313.1	5.11	0.87	2301	1717	59.23
Macrolepiota	cap	31953	137.69	0.42	137.18	1250.0	1.87	0.33	12461	5301	28.18
procera	stipe	38198	475.30	0.02	649.49	972.9	5.56	2.16	9545	5683	69.17
Russula	cap	36915	207.33	0.02	275.99	781.4	2.66	0.75	4819	2162	31.79
alutacea	stipe	27366	494.47	0.50	129.89	364.6	0.79	0.25	2146	1167	41.70
Russula	cap	37968	284.18	0.17	601.78	868.1	5.69	1.33	4324	1356	49.65
emetica	stipe	21798	248.88	0.53	793.20	810.9	6.68	1.47	6752	1698	49.97
Russula	cap	40459	155.27	0.14	200.09	1030.4	0.87	0.42	5956	4007	24.69
virescens	stipe	42346	210.27	0.66	327.66	547.2	1.33	0.66	3241	2001	36.86
Tricholoma	cap	32438	262.65	0.62	378.45	1104.7	1.25	0.58	12068	2993	37.12
portentosum	stipe	26396	377.16	0.25	963.06	531.41	3.57	1.79	2413	933	46.39

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Mushroom species	Parts	Ag	Al	Cd	Co	Cr	Cu	Fe	Mn	Ni	Zn
Agaricus	cap	2.82	23.74	0.76	0.24	0.439	64.01	88.47	8.2	0.33	115.56
campestris	stipe	11.76	52.13	0.61	0.18	0.548	73.79	80.22	10.1	0.34	60.61
Amanita	cap	0.50	115.76	0.91	0.25	0.166	41.48	101.77	26.5	0.75	137.89
rubescens	stipe	0.11	55.50	0.50	0.37	0.075	18.85	44.43	9.8	0.67	78.51
Amillariella	cap	0.54	112.92	2.96	0.12	1.915	14.95	102.27	26.5	0.50	57.38
mellea	stipe	0.37	198.61	0.71	0.12	0.416	7.89	208.28	63.1	0.25	20.15
Boletus	cap	14.61	14.69	4.62	0.25	0.075	30.58	50.06	8.7	0.62	163.21
edulis	stipe	7.86	10.39	2.58	0.25	0.075	17.71	38.48	6.6	0.33	89.11
Chantarellus	cap	0.37	70.44	0.50	0.12	1.704	45.92	94.75	16.1	0.33	69.15
cibarius	stipe	0.12	36.93	0.17	0.12	1.831	20.69	35.26	19.5	0.79	34.05
Hydnum	cap	0.58	133.72	0.62	0.12	0.375	35.32	218.26	23.7	0.25	52.43
repandum	stipe	1.71	53.99	0.29	0.12	0.191	31.03	86.95	54.0	5.23	39.80
Hypholoma	cap	5.54	26.14	1.00	0.12	0.075	32.59	128.13	19.9	0.25	64.15
fasciculare	stipe	3.71	35.69	0.37	0.12	0.075	18.45	84.71	29.1	0.25	38.94
Lactarius	cap	0.25	52.41	1.62	0.12	0.091	7.44	80.17	4.4	0.25	70.77
deliciosus	stipe	0.12	31.32	0.75	0.12	0.075	8.23	32.97	3.7	0.37	56.72
Lactarius	cap	6.03	34.01	2.70	0.29	0.091	64.47	52.55	8.3	0.29	116.44
piperatus	stipe	1.21	21.32	0.87	0.75	0.191	38.54	29.89	4.3	0.25	71.08
Lactarius	cap	0.42	25.35	0.87	8.78	0.125	65.15	37.43	22.8	2.29	61.12
volemus	stipe	0.22	53.67	0.33	5.32	0.150	40.21	58.16	15.0	1.00	42.79
Leccinum	cap	0.46	46.60	10.85	0.12	0.091	27.89	85.63	27.2	0.25	110.70
scabrum	stipe	0.12	60.23	4.36	0.12	0.075	11.59	46.27	44.0	0.37	47.85
Macrolepiota	cap	7.78	40.84	2.54	0.12	0.192	147.30	76.06	13.4	0.25	74.15
procera	stipe	6.64	137.71	1.41	0.29	0.091	148.04	96.40	24.2	0.25	67.67
Russula	cap	0.66	37.78	1.41	0.12	0.075	36.66	76.27	23.5	0.83	85.56
alutacea	stipe	0.11	24.30	0.37	0.50	0.075	22.38	92.94	10.0	0.75	295.22
Russula	cap	1.12	22.97	1.12	0.25	0.075	38.63	56.46	67.0	0.25	83.42

Table 2. The concentrations (mg kg<sup>-1</sup>, dry weight) of minor and trace elements in the mushrooms from the Făget Forest

For adults and adolescents, only two species, the *Boletus edulis* and *Leccinum scabrum*, pose a health risk when the mushroom is regularly consumed. Children under 10 years of age and toddlers are exposed to a greater degree with regard to several species. The mushrooms with the highest risk levels were the *Boletus edulis*, *Leccinum scabrum*, *Russulacea spp.*, *Lactarius spp.*, and *Armillariella mellea*. However, the mushrooms with the lowest HI values were: *Chantarellus cibarius*, *Hydnum repandum*, *Agaricus campestris*, and *Lactarius volemus*.

0.14 0.217

0.091

0.150

0.075

0.075

0.81

0.50

0.12

0.17

44.43

42.01

23.93

64.77

30.59

90.10

45.41

47.39

73.71

50.17

82.0 7.93

14.5 6.27

13.0 2.16

19.1 0.37

4.07

44.2

46.55

94.40

74.78

81.20

65.38

4.11

0.46

0.25

4.28

0.21

stipe

cap

stipe

cap

stipe

emetica

Russula

virescens

Tricholoma

portentosum

57.73

65.94

98.64

43.13

25.98

0.51

0.87

0.25

1.71

0.46

Table 3. The hazard index values for the caps of mushrooms grown in the Făget Forest

Hazard index (HI)									
Age-groups	1–3	3–10	10–14	14–18	adult				
Average body weight (kg) <sup>#</sup>	12	23	43	61	70				
Agaricus campestris	0.72	0.38	0.20	0.29	0.25				
Amanita rubescens	0.95	0.49	0.26	0.38	0.33				
Armillariella mellea	$1.99^{*}$	$1.04^{*}$	0.56	0.79	0.69				
Boletus edulis	$3.11^{*}$	1.62*	0.87	1.23*	$1.07^{*}$				
Chantarellus cibarius	0.51	0.27	0.14	0.20	0.18				
Hydnum repandum	0.58	0.30	0.16	0.23	0.20				
Hypholoma fasciculare	0.81	0.42	0.23	0.32	0.28				
Lactarius deliciosus	1.12*	0.59	0.31	0.45	0.39				
Lactarius piperatus	$1.87^{*}$	0.98	0.52	0.74	0.65				
Lactarius volemus	0.80	0.42	0.22	0.32	0.28				
Leccinum scabrum	$6.76^{*}$	3,53*	1.89*	$2.68^{*}$	$2.33^{*}$				
Macrolepiota procera	$1.71^*$	0.89	0.48	0.68	0.59				
Russula alutacea	1.13*	0.59	0.31	0.45	0.39				
Russula emetic	1.12*	0.58	0.31	0.44	0.39				
Russula virescens	0.95	0.50	0.26	0.38	0.33				
Tricholoma portentosum	1.26*	0.66	0.35	0.50	0.44				

\*Source: European Food Safety Authority Journal [24]

### 4. Conclusions

The comparative study of the wild edible mushrooms from the Făget Forest and the Apuseni Mountains showed no significant differences in the concentration of 19 macro-, micro-, and trace elements. Only barium was significantly higher in the mushrooms grown in the Făget. The hazard index values were relatively low. Two species (*Boletus edulis* and *Leccinum scabrum*) may be considered to have slightly elevated risk levels when they are consumed regularly. The element with the highest contribution to the hazard index was the Cd. However, other studies revealed the cadmium-accumulating capacity of these species [2, 6]. We can conclude that the polluting effect of Cluj-Napoca as a city with high traffic and continuous expanding trends was not detectable in the wild-growing mushrooms.

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