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Mineral composition of hypogeous fungi in Hungary

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Introduction

In the course of the work, 93 samples from 17 hypogeous fungus species belonging to 6 genera were taken from various habitats in Hungary and were analysed for the concentrations of 22 elements using the inductively coupled plasma spectroscopy ICP method. All the measurements were made in three independent replications.
The data were compared with the element contents of 625 epigeous fungi, previously determined using the same method. For all the genera, the elements present in the highest concentrations on a dry matter basis were potassium (6990-29590 ppm) and phosphorus (3400-9140 ppm). These were followed by the macroelements calcium (330-2190 ppm), magnesium (810-1000 ppm) and sodium (110-2990), and the microelements aluminium (30-450 ppm), zinc (60-340 ppm), iron (30-120 ppm) and copper (25-75 ppm), in different orders for each genus.
Until now the element contents of fungi have mostly been analysed to determine the nutritional value of edible fungi, and the data on other elements for instance total minerals are insufficient for further comparisons (Mattila et al., 2001).
Very little work has been published on the mineral contents of hypogeous large fungi, despite the fact that these include commercially important species such as *Tuber aestivum* and *T. melanosporum* (Ian et al., 2003). Most of the previous papers exhibited the following characteristics: (1) some species (e.g. *Terfezia* species, *Tuber melanosporum*) were investigated more frequently, and others rarely, if at all; (2) the analyses concentrated chiefly on toxicological and/or environmental aspects; (3) measurements were only made on a few elements (important from the nutritional point of view); (4) only cultivated fungi were included in the studies. The aim of the present work was to determine the element contents of various species of hypogeous fungi in order to answer the following questions: (1) Which characteristic differences can be observed between the element contents of hypogeous and epigeous fungi? (2) Which differences characterise the element contents of various genera of hypogeous fungi? (3) Is there any significant difference between the element contents of hypogeous Ascomycota and Basidiomycota genera? (4) Can any significant difference be observed between the element contents of edible and non-edible hypogeous fungi?

Materials and methods

A total of 93 hypogeous fungus samples were collected from habitats covering the whole area of Hungary, and were shown by macro- and microscopic analysis to represent 17 species belonging to 6 genera. The species names and number of samples are listed in Tab. 1.
After cleaning and drying, the fruit bodies were ground. The samples (200 mg of fungi powder) were digested in closed Teflon bombs in triplicate (2 cm^3 HNO_3 + 2 cm^3 H_2O_2 /30%/) at 1.56×10^5 Pa pressure for 20 min. The digested materials were filtered and diluted to 10 cm^3, after which the mineral element contents were determined by inductively coupled plasma spectroscopy. The data were evaluated using the GraphPad InStat program (version 3.00, 32 bit for Win 95/NT, GraphPad Software, San Diego, California, USA, www.graphpad.com) and the Kolmogorov-Smirnov test was used to determine the normality of the data. For data with normal distribution, one-way ANOVA was carried out using the Bonferroni post hoc test and the D (Welch) test, while in the case of non-normal distribution the Kruskall-Wallis test was applied, using Dunnett's post test (Dunn, 1964) to identify significant differences.

Tab. 1: Species names and number of the samples

Species name:	**Number of samples:**
Elaphomyces aculeatus Vittad.	6
Elaphomyces granulatus Fries	2
Elaphomyces muricatus Fries	33
Elaphomyces persoonii Vittad.	1
Elaphomyces reticulatus Vittad.	2
Elaphomyces virgatosporus Hollós	6
Gautieria borealis States, Fogel & Hosford	3
Mattirolomyces terfezioides (Mattir.), E. Fisch	2
Melanogaster ambiguus (Vittad.) Tulasne & C. Tulasne	2
Rhizopogon roseolus (Corda) Th. M. Fries	3
Rhizopogon vulgaris var. *intermedius* Svrcek	10
Tuber aestivum Vittad.	11
Tuber brumale Vittad.	1
Tuber excavatum Vittad.	4
Tuber ferrugineum Vittad.	1
Tuber mesentericum Vittad.	3
Tuber rapaeodorum Tul.	3

Results and discussion

The results obtained for the ICP analysis of 22 elements are presented in Tab. 2 for the six hypogeous fungus genera (*Elaphomyces*, *Gautieria*, *Mattirolomyces*, *Melanogaster*, *Rhizopogon*, *Tuber*) in comparison with a database containing data on 625 samples of epigeous fungi (Vetter, 2003).
By comparing the mean quantities of each element, three groups of elements could be distinguished, as illustrated in Tab. 3.
As can be seen in the Tab. 3, the fruit bodies of the two fungal groups had very similar mean contents of aluminium, boron, copper, strontium and titanium, while hypogeous fungi generally contained larger quantities of barium, calcium, molybdenum, sodium and zinc. On the other hand, the fruit bodies of hypogeous species had lower mean contents of arsenic, cadmium, cobalt, chromium, iron, potassium, magnesium, manganese, nickel, phosphorus, selenium and vanadium. Three of the macroelements (potassium, magnesium and phosphorus) were thus found in this group. The higher mean contents of arsenic, cadmium, selenium and vanadium in epigeous

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Tab. 2: Average element concentration of hypogeous and epigeous fungi with standard deviation

Elements	Average of 97 hypogeous materials (ppm)		Average of 625 epigeous materials (ppm)	
Al	150 ±	140	150 ±	217
As	4 ±	12	9 ±	15
B	13 ±	18	16 ±	45
Ba	8 ±	8	5 ±	5
Ca	1770 ±	1900	1460 ±	1270
Cd	2 ±	4	3 ±	7
Co	1 ±	1	1 ±	1
Cr	2 ±	1	3, ±	9
Cu	60 ±	40	60 ±	50
Fe	100 ±	80	240 ±	300
K	14800±	10500	34900 ±	14800
Mg	870 ±	300	1440 ±	640
Mn	17 ±	57	43 ±	45
Mo	2 ±	12	0,7 ±	0,6
Na	1880 ±	1600	325 ±	380
Ni	2 ±	2	4 ±	6
P	5625 ±	2625	7800 ±	4300
Se	0,3 ±	0,8	5 ±	4
Sr	5 ±	3	5 ±	5
Ti	2 ±	5	3 ±	3
V	0,4 ±	0,4	2 ±	14
Zn	260 ±	160	115 ±	110
Total	**25600**		**46558,00**	

Tab. 3: Grouping of elements based on their mean concentrations in hypogeous and epigeous fungi

Mean element concentration	Elements
Nearly identical	Al, B, Cu, Sr, Ti
Hypogeous species > epigeous species	Ba, Ca, Mo, Na, Zn
Hypogeous species < epigeous species	As, Cd, Co, Cr, Fe, K, Mg, Mn, Ni, P, Se, V

fungi can be explained by the fact that many fungal taxa, such as the *Amanita* genus, are known to accumulate these elements (Vetter, 2005). Giaccio et al., 1992 detected that copper and cadmium might be accumulated in the 7 studied truffle species, zinc appeared to be accumulated only in a certain range, while other examined elements did not seem to be accumulated. Granetti et al., 2005 also detected copper, cadmium and zinc accumulation in *T. melanosporum*. Maser et al., 2008 found high phosphorus and potassium, while low sodium and calcium content in fruit bodies, especially in comparison with eggs, milk and vegetables. Potassium, sodium and calcium as well as other micronutrients, however, could vary markedly among and within species, but in general, fruit bodies were particularly rich in manganese, copper and zinc.

Based on the complete database of the present studies in case of hypogeous species the difference between the minimum and maximum concentration of each element was smallest for phosphorus (8x) and magnesium (9x) and greatest for molybdenum (1780x), arsenic (835x) and boron (940x). In case of epigeous fungi the ranges were wider. The smallest difference was found for magnesium (13x) and the greatest for boron (3300x). It could also be seen that the most important elements (potassium, phosphorus, calcium, magnesium) were among those with the smallest coefficient of variation in the fruit bodies of both fungal groups.

When the deviation values were compared between genera it was observed that sodium was generally among the elements exhibiting the greatest deviation, with the exception of the *Elaphomyces* genus, where it had the smallest deviation in both relative and absolute terms. Of the four major elements, the deviation for calcium was the greatest in all the genera except *Rhizopogon*, where it was almost the smallest.

Averaged data groups are presented in Tab. 4 for the six hypogeous fungus genera to which most of the samples belonged (*Elaphomyces*, *Tuber*, *Rhizopogon*, *Gautieria*, *Mattirolomyces*, *Melanogaster*), together with the databases of the epigeous fungi used for comparative purposes.

The potassium contents of all the hypogeous fungal genera proved to be lower than that in epigeous fungi that tend to concentrate potassium 20-40-fold in fruit bodies (Seeger, 1978), but were the highest amounts in all hypogeous genera. Granetti et al., 2005 also showed potassium to be the highest content element in *T. melanosporum*. The lowest value of potassium (6994 ppm) was recorded for the *Elaphomyces* genus, which was very low even compared with the other hypogeous genera.

The opposite tendency was observed for sodium, an element of great importance from the biological point of view, as the sodium contents of the hypogeous fungi were higher than those measured in epigeous fungi, particularly in the case of *Elaphomyces*. The only exception was the *Tuber* genus, where the sodium content was approximately the same as in epigeous fungi, and *Mattirolomyces*, which exhibited a value of only 111 ppm, far lower than the mean Na content of epigeous fungi and even lower than the aluminium content of this genus.

In the case of calcium the average content was higher in hypogeous fungi, but this was principally due to the high values for *Tuber* and *Melanogaster*, as the calcium contents of the other genera were similar to the average for epigeous fungi. In fact, that of *Gautieria* was extremely low (330 ppm). The mean phosphorus content of the hypogeous fungi (5625 ppm) was well below the level recorded for epigeous fungi (7800 ppm). The phosphorus content of the Tuber genus hardly surpassed the epigeous mean (7810 ppm), while that of *Mattirolomyces* was far higher (9140 ppm). In the case of magnesium, which content depends on species and genus (Seeger and Manfred, 1979), none of the hypogeous genera had contents as high as the mean for the epigeous fungi (1440 ppm), with an average level of 870 ppm. It is interesting to note that the calcium: phosphorus and potassium:sodium ratios were also very different for the various genera.

Among the biologically important microelements, no significant differences could be detected in the contents of copper and boron. When the data series for the hypogeous fungi were analysed at the genus level (Tab. 4), it could be seen that, despite the similarity of the means, samples from the *Gautieria*, *Mattirolomyces* and *Melanogaster* genera had very low, hardly detectable contents of boron. The average quantity of manganese (17 ppm) in the hypogeous fungi was considerably lower than the mean value for the comparative database (40 ppm), yet the two data available for the Melanogaster genus averaged 300 ppm! A comparison of the mean contents of zinc indicated that the hypogeous fungi contained considerably more of this element (260 ppm) than their epigeous counterparts (115 ppm). When the genera were evaluated separately it could be clearly seen that the *Elaphomyces* genus, which was

Tab. 4: Average element concentration of six hypogeous fungi taxa and epigeous fungi with standard deviation

Elements	Average of 50 *Elaphomyces* materials (ppm)	Average of 3 *Gautieria* materials (ppm)	Average of 2 *Mattirolomyces* materials (ppm)	Average of 2 *Melanogaster* materials (ppm)	Average of 13 *Rhizopogon* materials (ppm)	Average of 23 *Tuber* materials (ppm)	Average of 625 epigeous fungi materials (ppm)
Al	175 ± 120	30 ± 3	450 ± 480	250 ± 210	70 ± 40	130 ± 160	150 ± 210
As	3 ± 10	20 ± 5	0,1 ± 0,0	4 ± 5	7 ± 25	0,1 ± 0,0	9 ± 15
B	12 ± 15	0,1 ± 0,0	0,1 ± 0,0	6 ± 8	13 ± 25	15 ± 20	15 ± 45
Ba	9 ± 75	2 ± 0,0	3 ± 0,5	15 ± 1	9 ± 10	5 ± 3	4 ± 5
Ca	1455 ± 2030	330 ± 130	1260 ± 330	2190 ± 1300	1570 ± 730	2170 ± 1250	1460 ± 1270
Cd	1 ± 45	1 ± 0,2	0,6 ± 0,0	0,1 ± 0,0	3 ± 6	3 ± 2	3 ± 7
Co	0,5 ± 0,65	4 ± 2	0,1 ± 0,0	0,3 ± 0,2	1 ± 1	0,2 ± 0,4	1 ± 1
Cr	1,3 ± 1	4 ± 0,3	2 ± 0	0,6 ± 0,6	1 ± 1	1 ± 0,5	3 ± 9
Cu	50 ± 20	25 ± 3	80 ± 60	35 ± 15	50 ± 70	65 ± 35	60 ± 50
Fe	100 ± 65	32,20 ± 3	55 ± 6	75 ± 5	120 ± 95	110 ± 110	240 ± 305
K	6990 ± 4590	18400 ± 1400	29585 ± 210	17295 ± 8000	23395 ± 13750	24110 ± 4245	34865 ± 14760
Mg	820 ± 335	890 ± 80	1000 ± 50	810 ± 320	830 ± 390	955 ± 210	1440 ± 640
Mn	8 ± 7	20 ± 5	10 ± 0	305 ± 365	13 ± 15	10 ± 7	45 ± 45
Mo	0,2 ± 0,1	0,1 ± 0,0	0,2 ± 0,1	0,5 ± 0,5	10 ± 35	0,2 ± 0,2	0,7 ± 0,6
Na	2990 ± 995	420 ± 170	110 ± 2,76	570 ± 505	870 ± 440	330 ± 280	325 ± 385
Ni	1 ± 1	15 ± 1	1 ± 0,4	1 ± 0,2	2 ± 2	1 ± 0,5	3 ± 6
P	4515 ± 2090	4970 ± 455	9140 ± 460	3405 ± 150	6030 ± 3410	7810 ± 2015	7800 ± 4320
Se	0,2 ± 0,6	0,1 ± 0,0	0,1 ± 0,0	0,1 ± 0,0	1 ± 1	0,1 ± 0,0	5 ± 4
Sr	3 ± 2	2, ± 1	5 ± 3,30	7 ± 4	4 ± 2	7 ± 4	5 ± 5
Ti	2 ± 1	0,2 ± 0,2	0,4 ± 0,1	1 ± 0,2	0,8 ± 0,5	4 ± 10	3 ± 3
V	1 ± 1	0,1 ± 0,0	0,2 ± 0,1	0,5 ± 0,5	0,2 ± 0,1	0,3 ± 0,2	2 ± 14
Zn	340 ± 130	60 ± 10	90 ± 1	55 ± 15	135 ± 105	190 ± 65	115 ± 110
Total	**17480**	**25225**	**41800**	**25015**	**33145**	**35915**	**46560**

represented by a large number of samples, had the highest zinc concentration (340 ppm), while that of *Gautieria* and *Melanogaster* was much lower (around 60 ppm).

Among the elements of interest from the toxicological point of view, the arsenic content was below the mean value recorded for the epigeous fungi, with the exception of the *Gautieria* genus, though it must not be forgotten that the relatively high average value (9 ppm) for epigeous fruit bodies was influenced by the presence of several accumulator genera (particularly species belonging to the *Agaricus* and *Macrolepiota* genera). This was also true of the concentrations of cadmium and chromium. Seeger (1978a) found highly scattered values of cadmium content in 402 epigeous fungi, and demonstrated that it was clearly species-dependent, and to a lesser extent genus-dependent.

The quantity of nickel was also lower in the hypogeous fungus group (1,9-3,6 ppm), though samples from the *Gautieria* genus had a surprisingly high content (14,7 ppm). The concentrations of selenium and vanadium must be viewed with similar reservations. In both cases the hypogeous fungi had very low values (in many cases below the detection limit) compared with most of the epigeous fungi, but here again the very high values recorded for a number of accumulating taxa (*Boletus* species in the case of selenium and *Amanita muscaria* for vanadium) were responsible for the higher average values for this fungal group.

In terms of the total mineral elements (Tab. 2 and 5), considerable differences were observed, as the total element content of the hypogeous fungi (25 ppm) was substantially less than that of fungi with epigeous fruit bodies (45 ppm). The analysis of individual genera revealed that the mineral contents of *Tuber* and *Mattirolomyces* were closest to those of the group used for comparison.

In Tab. 5 the hypogeous fungi were divided according to their taxonomic groupings into Ascomycota (76 samples) and Basidiomycota species (21 samples). When the data for these groups were compared the following differences were found:

a. Both taxonomic groups of hypogeous fungi contained approximately equal quantities of the elements B, Ba, Ca, Fe, Cr, Cu, Mg, P and Sr;
b. The element content of the Ascomycota group was greater than that of the Basidiomycota group in the case of Al, Na, Ti, V and Zn;
c. The Basidiomycota had higher concentrations of As, Cd, Co, K, Mn, Mo, Ni and Se.

A comparison of the total element quantities showed that hypogeous fungi belonging to the Basidiomycota had considerably higher element contents than the Ascomycota, mainly due to the great difference in the potassium concentrations. It should be noted, however, that the Ascomycota contained more zinc.

The hypogeous fungi were also grouped on the basis of edibility (Tab. 6), with 14 samples in the edible group and the majority (83) being inedible. The two groups exhibited a number of substantial differences, with higher quantities of boron, calcium, copper and magnesium, much higher contents of potassium and phosphorus, and lower quantities of zinc, manganese and sodium in the edible species. All in all, these fungi contained an average of 38580 ppm mineral elements, as compared with 23370 ppm in the inedible group.

Tab. 5: Average element concentration of hypogeous ascomycetes and basidiomycetes and epigeous fungi with standard deviation

Elements	Average of 76 ascomycetes materials (ppm)		Average of 21 basidiomycetes materials (ppm)		Average of 625 epigeous fungi materials (ppm)	
Al	170 ±	150	85 ±	80	150 ±	220
As	2 ±	10	7±	20	9 ±	15
B	13 ±	18	12 ±	20	16 ±	45
Ba	8 ±	6	10 ±	10	5 ±	5
Ca	1770 ±	2000	1760 ±	1490	1470 ±	1270
Cd	2 ±	3	3 ±	5	3 ±	7
Co	0,4 ±	0,5	1 ±	1	1 ±	2
Cr	1 ±	0,8	2 ±	2	3 ±	9
Fe	100 ±	80	100 ±	85	200 ±	300
Cu	60 ±	30	50 ±	60	60 ±	50
K	13000 ±	9400	21600 ±	12000	34900 ±	14800
Mg	900 ±	300	845 ±	320	1400 ±	600
Mn	9 ±	7	50 ±	120	40 ±	45
Mo	0,2 ±	0,2	6 ±	30	0,7 ±	0,6
Na	2100 ±	1500	1100 ±	1750	325 ±	380
Ni	1,0 ±	0,7	4 ±	4	4 ±	6
P	5680 ±	2600	5400 ±	2800	7800 ±	4320
Se	0,1 ±	0,5	0,7 ±	1	5 ±	5
Sr	5 ±	3	4, ±	2	5 ±	5
Ti	2 ±	6	0,8 ±	0,5	3 ±	4
V	0,4 ±	0,4	0,2 ±	0,2	2 ±	14
Zn	300 ±	150	120 ±	90	115 ±	110
Total	**24000**		**31200**		**45700**	

Tab. 6: Average element concentration of edible (*Tuber aestivum*, *Tuber brumale*, *Mattirolomyces terfezioides*) and non-edible hypogeous and epigeous fungi with standard deviation

Elements	Average of 14 edible hypogeous fungi materials (ppm)		Average of 83 non-edible hypogeous fungi materials (ppm)		Average of 625 epigeous fungi materials (ppm)	
Al	225 ±	250	140 ±	110	150 ±	220
As	0,1 ±	0,0	4 ±	13	9 ±	15
B	15 ±	18	10 ±	18	15 ±	45
Ba	6 ±	3	9 ±	8	5 ±	5
Ca	2535 ±	1200	1600 ±	1970	1500 ±	1270
Cd	3 ±	2	2 ±	4	3 ±	7
Co	0,1 ±	0,0	1 ±	1	1 ±	1
Cr	1 ±	0,4	2 ±	1	3 ±	9
Cu	75 ±	40	50 ±	40	60 ±	50
Fe	125 ±	120	100 ±	70	240 ±	300
K	25800 ±	2700	13000 ±	10200	34865 ±	14760
Mg	1000 ±	200	840 ±	310	1440 ±	640
Mn	10 ±	7	20 ±	60	40 ±	45
Mo	0,2 ±	0,2	2 ±	10	0,7 ±	0,6
Na	240 ±	200	2200 ±	1600	325 ±	380
Ni	1 ±	0,5	2 ±	2	4 ±	6
P	8300 ±	1700	5200 ±	2480	7800 ±	4320
Se	0,1 ±	0,0	0,3 ±	1	5 ±	5
Sr	8 ±	3	4 ±	3	5 ±	5
Ti	2 ±	3	2 ±	6	3 ±	3
V	0,1 ±	0,1	0,5 ±	0,4	2 ±	14
Zn	170 ±	60	270 ±	165	115 ±	110
Total	**38582,27**		**23460**		**46590**	

Conclusion

In our work we got a comprehensive view of the element content of several hypogeous fungi genera. Compared to the epigeous fungi we found higher element content in the hypogeous fungi. The main general difference was in the potassium level, however that was the highest of all the hypogeous genera and the epigeous fungi of the investigated elements. The most important elements (potassium, phosphorus, calcium, magnesium) in both groups belong to elements with the smallest coefficient of variation. We found significant difference in the studied questions, although the content of elements is strongly dependent on genera and species.

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