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Morchella esculenta (L.) growing in Bulgaria: chemical profile and hazard index

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Morchella esculenta (L.) Pers. (morel) is one of the most widely appreciated wild edible mushrooms. The mushroom *Morchella esculenta* has long been known for its medicinal properties, especially its health-promoting effects, including antioxidant activity, hepatoprotective activity, antimicrobial properties and antitumor effect. *Morchella esculenta* is rich in carbohydrates (79.06 g 100 g^{-1} dw), followed by proteins (11.19 g 100 g^{-1} dw), ash (7.32 g 100 g^{-1} dw) and fat (2.43 g 100 g^{-1} dw). Also moisture (89.41%), and total energy (1624.14 kJ 100 g^{-1} dw) were calculated. The concentrations of heavy metals: Pb (0.28 mg kg^{-1}), Cd (0.10 mg kg^{-1}), Cu (0.18 mg kg^{-1}), Co (0.28 mg kg^{-1}), Zn (0.82 mg kg^{-1}), Ni (0.46 mg kg^{-1}), Cr (0.28 mg kg^{-1}), Fe (17.01 mg kg^{-1}), and Mn (0.96 mg kg^{-1}), were assayed in *Morchella esculenta*. Hazard index values were calculated for children: 1-3 years old (0.665), 3-10 years old (0.347), 10-14 years old (0.186), 14-18 years old (0.131) and for adults > 18 years (0.230). According to this study, the edible wild mushroom *Morchella esculenta* could be used in human nutrition due to its good parameters. Heavy metal content of samples indicated that the Batak mountain is an ecologically pure region in Bulgaria, and therefore the mushrooms collected from this location could be consumed without any risk for human health.

Keywords: *Morchella esculenta*, Chemical profile, Hazard index

INTRODUCTION

Mushrooms have a long and strong relationship with human beings and can have vital economic impact. For a long time mushrooms have been consumed and used by men for their taste and pleasant aroma [1]. Mushrooms have a high nutritional value with high amount of vitamins, proteins, minerals, fibers, trace elements but have low calories and cholesterol [2-7]. Many of them are used in preparing medicines for many years and have potential in maintaining sound health and active immune system of the human body. Mushrooms are consumed as medicines in the form of capsules but not as food [8, 9]. Mushrooms are also rich sources of various bioactive substances like antibacterial, antifungal, antiviral, anti-parasitic, antioxidant, anti-inflammatory, antiproliferative, anticancer, antitumor, cytotoxic, anti-hiv, hypocholesterolemic, antidiabetic, anticoagulant, hepatoprotective compounds, etc. [10-14]. From 14 000 species which are known, 2 000 species are safe for human use and among them 650 possess medicinal properties [15, 16].

Morchella esculenta (L.) Pers. (morel) is a well known and extraordinary mushroom species. The head is distinctly conical in shape. The head surface comprises a honeycomb of sharp ridges and deep pits and is rich-brown in colour. The texture is sponge-like. The head and stem are generally hollow. It grows generally on chalky soil in grassy woodlands, field margins ad roadside verges. *Morchella esculenta* is picked up every year if the weather condition is suitable for growth in Bulgaria. It is collected especially in April and May, and marketed abroad either fresh or dried.

In the literature no data are available regarding *Morchella esculenta* in Bulgaria: Therefore, the aim of this study was to investigate the chemical profile and hazard index of *Morchella esculenta* from the Batak Mountain.

EXPERIMENTAL

Samples

Fifteen mushroom samples were collected in 2016 and 2017 from the Batak Mountain by the authors themselves.

The Batak Mountain is located in the western Rhodopes. Its western border is defined by the Chepinska river, the southern border – by Dospatska river and Dospat dam, the eastern border – by Vacha river and the northern border – by the Thracian Plane (GPS41°46'02.6"N 24°08'48.4"E). The region is industry-free and is characterised with forests, land and low buildings.

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Reagents are qualified as "AR" (p.a. Merck & Fluka). The stock standard solutions for ICP determination of Fe, Ni, Cr, Cu, Co, Zn, Mn, Pb, and Cd at concentrations of 1000 mg L^{-1} were supplied by Merck, Darmstadt, Germany. Water was deionized in a Milli Q system (Millipore, Bedford, MA, USA) to a resistivity of 18.2 MΩ cm. All plastic and glassware was cleaned by soaking in dilute HNO_3 (1/9, v/v) and was rinsed with distilled water prior to use.

Sample preparation for nutritional analysis

The whole macrofungal samples were used in this study. Fresh samples, after the removal of extraneous material such as mud, bush, soil, plant, etc. by washing with demineralized water, were air-dried between Whatman filter papers. Approximately 5 g of each sample was taken immediately for the determination of moisture. Remaining samples were stored in a deep-freezer until use [17]. While examining the nutritional composition of mushroom samples, the maturation stage of them was not considered.

Proximate composition analysis

For determination of proximate value, the following parameters were studied by using the mushroom material.

Determination of crude protein: Protein content was determined using folin phenol reagent. 0.5 g of the powdered mushroom sample was extracted with 50 mL of 2% NaCl in a water bath at 60˚C for 1 h. The extract was filtered out and 50 mL of 3% copper acetate monohydrate was added to the filtrate to precipitate the protein. The precipitated protein was then centrifuged out and dissolves in 50 mL [18].

Determination of carbohydrates: Total carbohydrate was determined by adding 2 g of each sample in 50 mL distilled water, 0.2 mL of which was ten fold diluted. To 1 mL of the resulting solution and serial dilutions of glucose stock (10 mg 100 mL^{-1}) solution, 4 mL of anthrone reagent was added and the absorbance of the solutions was measured by a spectrophotometer at 620 nm against a reagent blank [19].

Determination of crude fat: Crude fat was determined by extracting 2 g moisture-free samples with petroleum ether in a soxhlet extractor, heating the flask on a sand bath for about 6 h till a drop taken from the drippings left no greasy stain on the filter paper. After boiling with petroleum ether, the residual petroleum ether was filtered using Whatman No 40 filter paper and the filtrate was evaporated in a preweighed beaker. Increase in weight of beaker gave the crude fat [20].

Determination of ash content: The powdered mushroom sample (3 g) was ashed in a previously ignited and cooled crucible of known weight in a Gallenkamp furnace at 55˚C for 6 h. The fairly cooled crucibles were put in desiccators and weighed [21].

Energy: Total energy was calculated according to the following equations: Total energy (kJ/100g) = 17 (g protein + g carbohydrate) + 37 (g lipid). The weight of individual nutrients is g/100 g dw sample [22].

Moisture content: The fresh weight of each mushroom sample was taken using a chemical balance. These samples were then oven-dried separately at 105˚C for 24 h. The loss in weight obtained after drying was regarded as the moisture content [23].

Digestion procedures

Multiwave 3000 closed vessel microwave system (maximum power was 1400 W, and maximum pressure in the Teflon vessels - 40 bar) was used in this study. The mushroom samples (0.25 g) were digested with 6 mL of HNO_3 (65%) and 1 mL of H_2O_2 (30%) in the microwave digestion system for 23 min and diluted to 25 mL with deionized water. A blank digest was carried out in the same way. All sample solutions were clear. Digestion conditions for the microwave system are given in Table 1.

Analytical procedure

Quantitative determination of the concentration of the studied trace elements (Fe, Ni, Cr, Cu, Co, Zn, Mn, Pb, Cd) was carried out in the mineralized samples by ICP Optima model 7000 DV spectrometer.

Accuracy and precision

In order to validate the method for accuracy and precision the certified reference material (CRM) - Virginia Tobacco Leaves (CTA-VTL-2) was analysed for the corresponding elements. The results are shown in Table 3. For evaluation of the correctness of the results, three generally accepted criteria were used as follows:

(1) $D = X - X_{CRM}$, where X is the measured value and X_{CRM} is the certified value. When D is within the limits of $\pm 2\sigma$, where σ is the standard deviation of the certified value, the result is considered to be good; when it is $-3\sigma \leq D \leq 3\sigma$ - satisfactory, and beyond these limits the result is unsatisfactory.

(2) $D\% = D / X_{CRM} \times 100$ - percentage difference. When the values of D% are in the limits $\pm 200\sigma / X_{CRM}$, the result is considered to be good;

when the value is in the limits ± 200σ / X_{CRM} and ± 300σ / X_{CRM} - satisfactory; and when it is out of the limits ± 300σ / X_{CRM}, the result is unsatisfactory.

(3) $Z = X - X_{CRM} / \sigma$. When $Z \leq 2$, the result is considered to be good; when $2 \leq Z \leq 3$ - satisfactory; when $Z > 3$ - unsatisfactory.

For evaluation of the accuracy of the digestion and measuring procedures, we have used the R criterion showing the extent of extraction of the element in percentage from the certified value. When the measured value X is within the limits of $X_{CRM} \pm U_{CRM}$, where U_{CRM} is the indefiniteness of the certified value, we accept the extent of extraction to be 100%. In all remaining cases, the extent of extraction is equal to X / $X_{CRM}\cdot 100$. As can be seen from the tables, the results obtained for all certified materials yield a recovery of 100% for all elements.

Method of risk assessment

The risk assessment of the heavy metals to human health due to the consumption of the mushrooms can be performed by calculating the hazard quotient (HQ) for each metal of interest and the hazard index (HI), which is the sum of the hazard quotients. The HQ is the ratio between the exposure and the reference dose (Eq. 1). One can assume the existence of a risk for mushroom consumption when the HQ > 1.

$$HQ_{metal} = \frac{DI.C_{metal}}{R_f D.BW} \quad (1)$$

where DI is the daily intake of mushrooms (kg day^{-1}), C_{metal} is the concentration of the metal in the mushroom given for dry weight (mg kg^{-1}), R_fD is the oral reference dose for the metal given for unit body weight (mg kg^{-1} day^{-1}), BW is the average human body weight (kg). The value of R_fD for Pb (0.004 mg kg^{-1} day^{-1}), for Cd (0.001 mg kg^{-1} day^{-1}), for Co (0.043 mg kg^{-1} day^{-1}), for Cu (0.04 mg kg^{-1} day^{-1}), for Mn (0.14 mg kg^{-1} day^{-1}), for Cr (0.003 mg kg^{-1} day^{-1}), for Ni (0.02 mg kg^{-1} day^{-1}) and for Zn (0.001 mg kg^{-1} day^{-1}), were taken from the Integrated Risk Information System. The average BW was taken as 70 kg for adults and 61, 43, 23, 12 kg for children: (14-18), (10-14), (3-10), (1-3) years old, respectively.

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:

$$HI = \Sigma HQ = HQ_{Cd} + HQ_{Pb} + HQ_{Ni} + HQ_{Cr} + HQ_{Cu} + HQ_{Co} + HQ_{Zn} + HQ_{Mn} \quad (2)$$

Statistical data processing

SPSS (Statistical Package for Social Science) program for Windows was used for statistical data processing.

RESULTS AND DISCUSSION

Chemical composition of Morchella esculenta

The main components of the chemical composition of *Morchella esculenta* are presented in Table 2. *Morchella esculenta* showed to be rich in carbohydrates (79.06 g 100 g^{-1} dw), which were the most abundant macronutrients. Proteins were present at 11.19 g 100 g^{-1} dw. Total energy value was established to be 1624.14 kJ 100 g^{-1} dw. A sample of *Morchella esculenta* growing in Portugal was also reported to have carbohydrates as the most abundant macronutrient [24]. The carbohydrates in mushrooms comprise various compounds: sugars (monosaccharides, their derivatives and oligosaccharides) and both reserve and structural polysaccharides [25].

Each value is expressed as mean ± SD (n = 15). Means with different letters within a row are significantly different ($p < 0.05$)

The results for the efficiency of microwave mineralization for Fe, Ni, Cr, Cu, Co, Zn, Mn, Pb and Cd determination in the certified reference material - Virginia tobacco CTA- VTA-2 are displayed in Table 3. The results from the descriptive analysis of the concentration of Fe, Ni, Cr, Cu, Co, Zn, Mn, Pb and Cd in *Morchella esculenta* samples are presented in Table 4.

Concentrations of nine metals (Fe, Ni, Cr, Cu, Co, Zn, Mn, Pb and Cd) were determined in this study. The trace element contents of the species depend on the ability of the species to extract elements from the substrate, and on the selective uptake and deposition of elements in tissues [26 - 32].

Table 1. Microwave acid digestion programme.

Step	Ramp time (min)	Hold time (min)	Cooling period (min)	Pressure (MPa)	Temperature (°C)
1	10	10	5	0.758	110
2	10	10	5	1.023	150
3	20	10	5	0.758	190

Table 2. Moisture (g 100 g^{-1} of fresh weight), macronutrients (g 100 g^{-1} of dry weight) and total energy (kJ 100 g^{-1} of dry weight) in the wild edible mushrooms.

Components	$\overline{X}$	SD	-95% Confid.	+95% Confid.
Moisture	89.41	± 1.73	88.45	90.37
Ash	7.32	± 0.76	6.90	7.74
Crude protein	11.19	± 0.76	10.77	11.61
Crude fat	2.43	± 0.29	2.27	2.59
Total carbohydrates	79.06	± 1.78	78.07	80.04
Total energy	1624.14	± 7.36	1620.07	1628.21

Table 3. Effectiveness of microwave mineralization in the determination of Fe, Ni, Cr, Cu, Co, Zn, Mn, Pb and Cd in Virginia Tobacco-CTA-VTA-2 certified reference material (n = 15).

Element	Certified value, mg kg^{-1}	Found value, mg kg^{-1} Microwave digestion	Recovery (%)
Fe	1083 ± 33	1160 ± 45	103
Ni	1.98 ± 0.21	1.92 ± 0.17	98.1
Cr	1.87 ± 0.16	1.83 ± 0.14	93.6
Cu	18.2 ± 0.8	18.1 ± 0.7	99.4
Co	0.429 ± 1.4	0.420 ± 0.02	98.0
Zn	43.3 ± 2.1	44.1 ± 1.6	101.8
Mn	79.7 ± 2.6	77.5 ± 2.1	97.2
Pb	22.1 ± 1.2	23.0 ± 0.8	104
Cd	1.52 ± 0.17	1.50 ± 0.05	98.7

Table 4. Concentration of heavy metals in mushroom samples (*Morchella esculenta*) collected from Batak Mountain, Bulgaria (n = 15)

Element	$\overline{X}$, mg kg^{-1}	SD, mg kg^{-1}	- 95% Confid.	+ 95% Confid.
Fe	17.01	± 3.65	14.99	19.03
Ni	0.46	± 0.11	0.40	0.52
Cr	0.28	± 0.18	0.18	0.38
Cu	0.18	± 0.02	0.17	0.19
Co	0.28	± 0.10	0.22	0.34
Zn	0.82	± 0.22	0.70	0.95
Mn	0.96	± 0.39	0.74	1.17
Pb	0.28	± 0.11	0.22	0.34
Cd	0.10	± 0.02	0.09	0.11

Fe was found to be the dominant ion as compared with other heavy metals in mushrooms, followed by manganese and zinc ions. Iron is vital for almost all living organisms, participating in a wide variety of metabolic processes, including oxygen transport, DNA synthesis, and electron transport. It is known that adequate iron in a diet is very important for decreasing the incidence of anemia. Iron deficiency occurs when the demand for iron is high, e.g., in growth, high menstrual loss, and pregnancy, and the intake is quantitatively inadequate or contains elements that render the iron unavailable for absorption. High concentrations of iron may lead to tissue damage, as a result of the formation of free radicals. The amount of Fe in the mushroom species varied from 14.99 to 19.03 mg kg^{-1}. Our data on the values of iron are lower than the values given in the cited reports. The reported iron values for mushroom samples were 1.190-628 mg kg^{-1} [7, 26–30].

Cr is a trace metal necessary for the normal metabolism of cholesterol, fat, and glucose. Chromium deficiencies in the diet produce elevated circulating insulin concentrations, hyperglycemia, elevated body fat, decreased sperm counts, reduced fertility, and shortened life span. The concentration of chromium in the mushroom species varied between 0.18 and 0.38 mg kg^{-1}. In other studies, chromium concentrations between 0.1 and 4 mg kg^{-1} were recorded [30, 33, 34].

Ni has no specific function in humans; however, it is a co-factor for some microbial intestine enzymes. Ni content in the adult human body should remain below 0.1 mg per day, and excess may cause damages to DNA and cell structures. The daily intake of Ni was estimated as 0.046 mg, which represents approximately 3.3 % of R_fD established in 0.02 mg kg^{-1} per day, equivalent to 1.4 mg per day for a 70 kg adult [35]. Maximum and minimum values of Ni in the mushrooms were 0.40 and 0.52 mg kg^{-1}. Ni values found by the authors are in accordance with those of previous studies [30, 33, 34, 36].

Cu is an essential metal, which is a constituent of some metalloenzymes, and is required in hemoglobin synthesis and catalysis of metabolic growth. The average copper content of the analyzed mushroom samples was 0.18 mg kg^{-1}, which is below the safe limit of 40 mg kg^{-1} set by the WHO [35] in foods. According to the review reported by Kalac [36], copper concentrations accumulated in mushroom species usually varied from 20.0 to 100.0 mg kg^{-1}. Other workers have presented copper contents of mushroom samples to be in the ranges: 16.1-144.9 mg kg^{-1} [37], 2.6-72.4 mg kg^{-1} [5], 1.8-22.3 mg kg^{-1} [38], 5.0-83.0 mg kg^{-1} [30] and 13.7-182.4 mg kg^{-1} [39]. The results obtained in the current study indicated that copper content of the investigated mushroom samples was comparable to those reported in the literature.

The average concentration of Co in the analysed mushroom samples was 0.28 mg kg^{-1}, which is below the safe limit of 1 mg kg^{-1} set by the WHO [35] in foods. Based on the results from papers published until 2013, the reported Co content was found to vary between 0.1 and 7.0 mg kg^{-1} [36]. Cobalt is a constituent of vitamin B12.

Zn is one of the most important minerals needed by our body systems due to the fact that it is highly associated with protein- and carbohydrate-rich foods. Zinc is also used in medicines that treat rashes, acne, dandruff and athlete's foot [38]. It has biological significances for living organisms and mushrooms are known as good zinc accumulators. The average Zn content recorded in the studied samples was 0.82 mg kg^{-1}, respectively, which is below the safety limit of 60 mg kg^{-1}, determined by the WHO [35]. Zn levels reported in the literature were 29.3-158.0 mg kg^{-1} [7, 30, 34, 36, 38].

Mn is an essential metal needed for biological systems such as metalloproteins [34]. The average Mn content recorded in the studied samples was 0.96 mg kg^{-1}, which is below the toxicity limit of 400.0-1000.0 mg kg^{-1}. The reported levels of Mn in the literature are 12.9-93.3 mg kg^{-1} [7, 35-38].

Cd is accumulated mainly in kidneys, spleen and liver and its blood serum level increases considerably following mushroom consumption [25, 36-40]. Thus, cadmium seems to be the most deleterious among heavy metals in the mushrooms. It accumulates in bones and can take the role of calcium. The average concentration of Cd present in the studied wild mushrooms was 0.1 mg kg^{-1}, which is far below the 0.2 mg kg^{-1} limit set by the WHO [35].

The average concentration of Pb present in the studied wild mushrooms was 0.28 mg kg^{-1}, which is far below the 10.0 mg kg^{-1} limit set by the WHO [35]. Pb levels reported in the literature are 0.4-7.77 mg kg^{-1} [35-40]. Pb creates health disorders such as sleeplessness, tiredness, hearing and weight loss [26].

Hazard index

In order to assess the contribution of some heavy metals to the health risk of mushroom consumption, we calculated the hazard quotients. We evaluated the health risk of mushroom consumption concerning different age-groups relying on the hazard index (Table 5). 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 = 0.0143 kg day^{-1} fresh weight) for adults and adolescents beyond 14, and of 0.5 kg (DI = 0.0071 kg day^{-1} fresh weight) for children and teenagers.

CONCLUSIONS

Results of the studied area showed that the selected metals concentrations were below the safe limits of WHO/FAO set for edible mushrooms and for foodstuffs. The lower concentrations of essential and toxic metals in morels resulted in low HI values. This could be attributed to the lack of anthropogenic input like mining and industry and low-scale agricultural activities. From the obtained concentrations of heavy metals one can conclude that the locality Batak mountain is an ecologically clean area very suitable for collecting wild edible mushrooms that we can use in our daily menu.

Table 5. The hazard index values for mushrooms grown in the Batak mountain.

	Hazard index (HI)				
Age groups	1-3	3-10	10-14	14-18	adult
Average body weight (kg)#	12	23	43	61	70
Morchella esculenta	0.665	0.347	0.186	0.131	0.230

#Source: European Food Safety Authority Journal

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РАСТЕЖ НА ОБИКНОВЕНАТА СМРЪЧКУЛА В БЪЛГАРИЯ: ХИМИЧЕСКИ ПРОФИЛ И ЗДРАВЕН РИСК

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(Резюме)

Обикновената смръчкула е една от най-високо оценените диви ядливи гъби. Гъбата е известна със своите лечебни свойства, антиоксидантна активност, хепатопротективна активност, антимикробни свойства и антитуморен ефект. Обикновената смръчкула е богата на въглехидрати (79.06 g 100 g^{-1} сухо тегло), последвани от протеини (11.19 g 100 g^{-1} сухо тегло) и пепел (7.32 g 100 g^{-1} сухо тегло). Съдържанието на мазнини е 2.43 g 100 g^{-1} сухо тегло. Също така беше определена влагата (89.41%) и изчислена общата енергия (1624.14 kJ 100^{-1} g^{-1} сухо тегло). Беше определена концентрацията на тежките метали в обикновената смръчкула: Pb (0.28 mg kg^{-1}), Cd (0.10 mg kg^{-1}), Cu (0.18 mg kg^{-1}), Co (0.28 mg kg^{-1}) , Ni (0.46 mg kg^{-1}), Cr (0.28 mg kg^{-1}), Fe (17.01 mg kg^{-1}) и Mn (0.96 mg kg^{-1}). Стойностите на здравния риск са изчислени за деца на възраст от 1 до 3 години (0.665), 3-10 години (0.347), 10-14 години (0.186), 14-18 години (0.131) и възрастни над 18 години (0.230). Според това изследване, обикновената смръчкула е годна за консумация и може да се използва в храненето на хората поради добрите си параметри. Съдържанието на тежки метали в пробите показва, че Баташката планина е екологично чист район в България и поради това гъбите, събрани от това място, могат да се консумират без риск за човешкото здраве.