ICP DETERMINATION OF Pb, Mn, Cu, Zn, Fe, K, Na, P, Mg AND Ca IN EDIBLE WILD MUSHROOM *CANTHARELLUS CIBARIUS*

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Abstract: The aim of this study was to perform ICP analysis of the content of Pb, Mn, Cu, Zn, Fe, K, Na, P, Mg and Ca in wild edible mushrooms *Cantharellus cibarius*. The samples were collected from the Batak mountain, Bulgaria. The preparation of samples was done by microwave mineralisation. All mineral concentrations were determined on a dry weight basis (d.w.). The average content of studied elements: Pb, Mn, Cu, Zn, Fe, K, Na, P, Mg and Ca in *Cantharellus cibarius* samples was: 0.272 mg kg⁻¹, 18.319 mg kg⁻¹, 27.568 mg kg⁻¹, 78.829 mg kg⁻¹, 82.295 mg kg⁻¹, 31308.130 mg kg⁻¹, 139.431 mg kg⁻¹, 2713.615 mg kg⁻¹, 626.292 mg kg⁻¹ and 459.594 mg kg⁻¹.

Keywords: ICP, mushroom *Cantharellus cibarius*, Batak mountain, mineral concentrations.

1. INTRODUCTION

Minerals occur naturally in the environment and are present in rocks, soil, plants and animals. Minerals occur in different forms: as ions dissolved in water, as vapours or as salts or minerals in rock, sand and soil. They can also be bound in organic or inorganic molecules, or attached to particles in the air. Both natural and anthropogenic processes emit metals into air and water. It is already generally known that some living organisms possess the ability to take up and accumulate, in their structures, certain elements especially metals, at high concentrations [4]. Fungi are present almost everywhere and they are important in ecosystems because they are able to biodegrade the substrate [1,2]. Mushrooms have been a food supplement in various cultures for a long time. Edible mushrooms are cultivated and consumed by many people around the world. All essential amino acids for human health are present as well as watersoluble vitamins and all the essential minerals [1]. In addition, some mushrooms are reputed to possess due to properties of antioxidant, antimicrobial, antiallergenic and anticancer [7,10,16]. On the other hand, mineral concentrations in mushrooms are considerably higher than those in agricultural crops. Macrofungi possess a very effective mechanism that enables them readily totake up some minerals from the ecosystem compared to green plants growing in similar conditions [19]. Recently, some studies have drawn attention to the occurrence of the mineral contents of macrofungi [5,26, 25]. The concentrations of minerals in macrofungi were found to depend on the physiology of the species and particularly on its ecosystem pattern [14]. High accumulating ability observed in several species promoted their screening as bioindicators. Several works were reviewed with the conclusion that no macrofungi species can be considered as an exact indicator of environmental metal pollution but their fruiting bodies can be useful for distinguishing between polluted and unpolluted areas [8].

Mushrooms have been reported as therapeutic foods useful in preventing diseases and they exhibit varied biological properties such as antibacterial, antimutagenic, antitumoral and antiviral activities [9,12]. These functional characteristics are mainly due to their chemical composition [4].

The aim of this study was to perform ICP analysis of the content of Pb, Mn, Cu, Zn, Fe, K, Na, P, Mg and Ca in wild edible mushrooms *Cantharellus cibarius*. The samples were collected from the Batak mountain.

2. METHODS

In determining the amount of elements in samples of wild edible mushroom *Cantharellus cibarius* e used ICP Optima model 7000, at wavelengths: Pb – 220.353 nm, Fe – 238.204 nm, Zn – 213.857 nm, Mn – 257.610 nm, Cu – 327.395 nm, K – 766.491 nm, Na – 589.592 nm, Ca – 317.933 nm, P - 213.618 nm and Mg – 279.553 nm.

2.1. Reagents

Reagents are qualified "AR" (pa Merck & Fluka). The starting standard solutions for ICP determination of Pb, Fe, Zn, Mn, Cu, K, Na, Ca, P and Mg at concentrations of 1000 mg l⁻¹ were supplied by Merck, Darmstadt Germany.

All chemicals were at least of analytical-reagent grade. Water was de-ionized in a Milli-Q system (Millipore, Bedford, MA, USA) to a resistivity of 18.2 MV cm.

All plastic and glassware were cleaned by soaking in diluted HNO₃ (1/9, v/v) and were rinsed with distilled water prior to use.

2.2. Samples

One hundred and fifty mushroom samples were collected in 2014 and 2015 from the Batak mountain by the authors themselves.

The samples were prepared for the experiment in a standard way; they were dried at 65°C in a fan oven and stored in dark polyethylene bottles.

2.3. Sample preparation

Microwave acid digestion method was used for sample preparation. An amount of 0.2 g of samples was taken into digestion tubes and 5 ml of HNO₃ (65%), 1 ml of HCl and 3 ml of H₂O₂ (30%) were added. The samples were digested in a microwave closed system Multiwave 3000 (Anton Paar, Germany) according to the programme given in Table 1. After digestion, the samples were diluted up to 25 ml with 2 ml l⁻¹ HNO₃. Duplicated analysis was performed on the samples. Blank digestion was also carried out in the same way.

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

3. EXPERIMENTAL

The results for the efficiency of microwave mineralization for Pb, Fe, Zn, Mn, Cu, K, Na, Ca, P and Mg determination in the certified referent material - Virginia tobacco CTA- VTA-2 are displayed in Table 2.

The results from the descriptive analysis of the concentration of Pb, Fe, Zn, Mn, Cu, K, Na, Ca, P and Mg in *Cantharellus cibarius* samples are presented in Table 3.

Table 2. Effectiveness of microwave mineralization in the determination of Pb, Fe, Zn, Mn, Cu, K, Na, Ca, P and Mg in Virginia Tobacco-CTA-VTA-2 certified reference material

Element	Certified value	Observed value	Recovery (%)
Pb	22.1 ± 1.2 mg kg ⁻¹	23.1 ± 1.2	104.5
Fe	1083 ± 33 mg kg ⁻¹	1160 ± 44 mg kg ⁻¹	103
Zn	43.3 ± 2.1 mg kg ⁻¹	44.1 ± 1.6 mg kg ⁻¹	101.8
Mn	79.7 ± 2.6 mg kg ⁻¹	77.5 ± 2.1 mg kg ⁻¹	97.2
Cu	18.2 ± 0.8 mg kg ⁻¹	18.1 ± 0.7 mg kg ⁻¹	99.4
K	1.03 ± 0.04 %	1.0 ± 0.03 %	97.1
Na	312 mg kg ⁻¹	302.64 ± 12.64 mg kg ⁻¹	97
Ca	3.60 ± 0.15 %	3.23 ± 0.1 %	89.6
P	2204 ± 78 mg kg ⁻¹	23550 ± 110 mg kg ⁻¹	106.8
Mg	0.510 ± 0.023 %	0.531 ± 0.013 %	104.1

Table 3. Descriptive analysis of the results for Pb, Fe, Zn, Mn, Cu, K, Na, Ca, P and Mg concentrations in *Cantharellus cibarius* samples

Елемент	$\bar{X}$ mg kg ⁻¹	SD mg kg ⁻¹	-95% Confid.	+95% Confid.
Pb	0.272	0.113	0.210	0.335
Fe	82.295	6.753	78.556	86.035
Zn	78.829	3.857	76.694	80.965
Mn	18.319	4.239	15.971	20.666
Cu	27.568	4.956	24.824	30.313
K	31308.13	1185.239	30651.77	31964.5
Na	139.431	5.021	136.651	142.212
Ca	459.594	17.346	449.988	469.199
P	2713.615	168.836	2620.117	2807.113
Mg	626.055	65.292	589.898	662.212

4. RESULTS

The variations and mean concentrations of ten macro- (sodium, potassium, calcium, phosphorus and magnesium) (Table 3) and micro- elements (iron, zinc, copper and manganese), and the heavy metal lead (Table 3) were examined in the wild species of the mushrooms under study. The samples were shown to be rich in potassium. The trend in decreasing order of macro elements content for the studied mushrooms was K > P > Mg > Ca > Na. Living organisms require varying amounts of "heavy metals". Iron, cobalt, copper, manganese, molybdenum, and zinc are required by humans, but excessive levels can be damaging to the organism. Other heavy metals such as mercury, plutonium, and lead are toxic metals and their accumulation over time in the bodies of animals can cause serious illnesses. Copper as stated is an essential metal, which serve as a constituent of some metalloenzymes, and is required in haemoglobin synthesis and catalysis of metabolic growth

[15]. The average concentration of Cu in the studied samples was $27.568 \text{ mg kg}^{-1}$, which was far below the safe limit of 40 mg kg^{-1} set by WHO [24]. Copper levels in mushrooms reported in literature are $4.71\text{--}51.0 \text{ mg kg}^{-1}$ [21], $13.4\text{--}50.6 \text{ mg kg}^{-1}$ [28], and $12.0\text{--}181.0 \text{ mg kg}^{-1}$ [29]. These values recorded for wild mushrooms correspond to samples collected near industrial sites. Iron (Fe) is an essential metal involved in biochemical processes. The average Fe content recorded in the studied samples was $82.295 \text{ mg kg}^{-1}$, respectively, which are above the safety limit of 15.0 mg kg^{-1} , determined by the WHO. Levels of Fe reported in literature were $146.0\text{--}835.0 \text{ mg kg}^{-1}$ [20], $31.3\text{--}1190.0 \text{ mg kg}^{-1}$ [13], and $180.0\text{--}407.0 \text{ mg kg}^{-1}$ [6]. Manganese (Mn) is an essential metal needed for biological systems such as metalloproteins [22]. The average Mn content recorded in the studied samples was $18.319 \text{ mg kg}^{-1}$, respectively, which are also below the toxicity limit of $400.0\text{--}1000.0 \text{ mg kg}^{-1}$.

The literature has reported levels of Mn as $14.2\text{--}69.7 \text{ mg kg}^{-1}$ [17], $12.9\text{--}93.3 \text{ mg kg}^{-1}$ [20], and $14.5\text{--}63.6 \text{ mg kg}^{-1}$ [6]. According to Vonugopal and Lucky [23], lead (Pb) is toxic even at trace levels and the impairment related to lead toxicity in humans include abnormal size and haemoglobin content of the erythrocytes, hyperstimulation of erythropoiesis and inhibition of haem synthesis. The maximum level of Pb present in the studied wild mushrooms was 0.442 mg kg^{-1} , which is far below the 10.0 mg kg^{-1} limit set by the WHO [26]. Lead levels reported in literature are $0.75\text{--}7.77 \text{ mg kg}^{-1}$ [21], $1.43\text{--}4.17 \text{ mg kg}^{-1}$ [20], and $0.40\text{--}2.80 \text{ mg kg}^{-1}$ [18]. Zinc is an essential metal and a component of a wide variety of different enzymes in which it is involved in catalytic, structural and regulatory roles. The average Zn content recorded in the studied samples was $78.829 \text{ mg kg}^{-1}$, respectively, which are above the safety limit of 60 mg kg^{-1} , determined by the WHO [26]. Zn levels reported in literature were $45.2\text{--}173.8 \text{ mg kg}^{-1}$ [17], $33.5\text{--}89.5 \text{ mg kg}^{-1}$ [20], and $29.3\text{--}158.0 \text{ mg kg}^{-1}$ [6].

The trend in decreasing order of elements present in wild samples was $\text{Fe} > \text{Zn} > \text{Cu} > \text{Mn} > \text{Pb}$. Mushrooms are known to possess a very effective mechanism that enables them to readily take up some heavy metals from the ecosystem [27]. The accumulation of heavy metals in mushrooms seems to be affected by environmental and fungal related factors. Environmental factors such as organic matter content, pH and metal concentration in soil and fungal factors such as the species, morphological part of the fruit body, developmental stages, age of mycelium, intervals between fructifications and biochemical composition [11].

5. CONCLUSIONS

According to this study, the edible wild mushroom *Cantharellus cibarius* could be used in human nutrition due to its good parameters. Heavy metal content of samples indicated that the Batak mountain was an ecologically pure region of Bulgaria, and therefore the mushrooms collected from this location could be consumed without any risk for human health.

6. REFERENCES

- [1] Buigut S. K. (2002). Mushroom production in sustainable small-scale farming system opportunities and constraints: a survey of Uasin Gishu district. Proceedings of the Horticulture seminar on sustainable horticultural production in the tropics at Jomo Kenyatta University of Agriculture and Technology, Juja, Kenya, 3-6 October, (2001), pp. 1-5.
- [2] Demirbaş A. (2001). Concentrations of 21 metals in 18 species of mushrooms growing in the East Black Sea region. Food Chemistry, Vol. 75, (2001), pp. 453 – 457.
- [3] Falandysz J., Szymczyk K., Ichihashi H., Bielawski L., Gucia M. & Frankowska A. (2001). ICP/MS and ICP/AES elemental analysis (38 elements) of edible wild

- mushrooms growing in Poland. *Food Additives & Contaminants*, Vol. 6, (2001), pp. 503 – 513.
- [4] García-Lafuente A., Moro C., Villares A., Guillamón E., Rostagno M. A., D'Arrigo M. & Martínez J. A. (2011). Mushrooms as a source of anti-inflammatory agents. *American Journal of Community Psychology*, Vol. 48, (2011), pp. 125–141.
 - [5] Isildak Ö., Turkekul I., Elmastas M. & Tuzen M. (2004). Analysis of heavy metals in some wild-grown edible mushrooms from the middle black sea region, Turkey. *Food Chemistry*, Vol. 86, (2004), pp. 547 – 552.
 - [6] Isiloğlu M., Yilmaz F. & Merdivan M. (2001). Concentrations of trace elements in wild edible mushrooms. *Food Chemistry*, Vol. 73, (2001), pp. 169 – 175.
 - [7] Jiskani M. M. (2001). Energy potential of mushrooms. *The DAWN Economic and Business Review*, October 15-21, (2001).
 - [8] Kalac P. & Svoboda L. (2000). A review of trace element concentrations in edible mushrooms. *Food Chemistry*, Vol. 69, (2000), pp. 273 – 381.
 - [9] Manzi P., Aguzzi A. & Pizzoferrato L. (2001). Nutritional value of mushrooms widely consumed in Italy. *Food Chemistry*, Vol. 73, (2001), pp. 321 – 325.
 - [10] Mau J. L., Lin H. C. & Song S. F. (2002). Antioxidant properties of several specialty mushrooms. *Food Research International*, Vol. 35, (2002), pp. 519-526.
 - [11] Radulescu C., Stihl C., Popescu I.V., Busuioc G., Gheboianu A. I., Cimpoca V. G., Dulama I. D. & Diaconescu M. (2010). Determination of heavy metals content in wild mushrooms and soil by EDXRF and FAAS techniques. *Ovidius University Annals of Chemistry*, Vol. 21, (2010), pp. 9 – 14.
 - [12] Schillaci D., Arizza V., Gargano M. L. & Venturella G. (2013). Antibacterial activity of Mediterranean Oyster Mushrooms, Species of genus *Pleurotus* (Higher Basidiomycetes). *International Journal of Medicinal Mushrooms*, Vol. 15, (2013), pp. 591–594.
 - [13] Sesli E. & Tüzen M. (1999). Levels of trace elements in the fruiting bodies of macrofungi growing in the east Black sea region of Turkey. *Food Chemistry*, Vol. 65, (1999), pp. 453 – 460.
 - [14] Sesli E. (2006). Trace element contents of some selected fungi in the ecosystem of Turkey. *Fresenius Environmental Bulletin*, Vol. 15, No 6, (2006), pp. 518 – 523.
 - [15] Silvestre M. D., Lagarda M. J., Farra R., Martinezze-Costa C. & Brines J. (2000). Copper, iron and zinc determination in human milk using FAAS with microwave digestion. *Food Chemistry*, Vol. 68, (2000), pp. 95–99.
 - [16] Solak M. H., Kalmış E., Sağlam H. & F. Kalyoncu (2006). Antimicrobial activity of two wild mushrooms *Clitocybe alexandri* (Gill.) Konr. and *Rhizopogon roseolus* (Corda) T.M. Fries collected from Turkey. *Phytotherapy Research*, Vol. 20, (2006), pp. 1085 – 1087.
 - [17] Soylak M., Saracoglu S., Tüzen M. & Mendli D. (2005). Determination of trace metals in mushroom samples from Kayseri, Turkey. *Food Chemistry*, Vol. 92, (2005), pp. 649 – 652.
 - [18] Svoboda L. & Kalac P. (2003). Contamination of two edible *Agaricus* spp. mushrooms growing in a town with cadmium, lead and mercury. *Bulletin of Environmental Contamination and Toxicology*, Vol. 71, (2003), pp. 123 – 130.
 - [19] Svoboda L., Zimmermannova K. & Kalac P. (2000). Concentrations of mercury, cadmium, lead and copper in fruiting bodies of edible mushrooms in an emission area of a copper smelter and a mercury smelter. *Science of the Total Environment*, Vol. 246, (2000), pp. 61 – 67.
 - [20] Tüzen M. (2003). Determination of heavy metals in soil, mushroom and plant samples by atomic absorption spectrometry. *Microchemical Journal*, Vol. 74, (2003), pp. 289 – 297.

- [21] Tüzen M., Özdemir M. & Demirbas A. (1998). Study of heavy metals in some cultivated and uncultivated mushrooms of Turkish origin. *Food Chemistry*, Vol. 63, (1998), pp. 247 – 251.
- [22] Unak P., Lambrecht F. Y., Biber F. Z. & Darcan S. (2007). Iodine measurements by isotope dilution analysis in drinking water in Western Turkey. *Journal of Radioanalytical and Nuclear Chemistry*, Vol. 273, (2007), pp. 649–651.
- [23] Vonugopal B. & Luckey T. (1975). *Toxicity of Non-Radioactive Heavy Metals and Their Salts in Heavy Metals Toxicity, Safety and Hormology*; Coulston, F., Ed., Georg Thieme: Stuttgart, Germany. Academic Press: New York, NY, USA (1975).
- [24] WHO. World Health Organization Evaluation of Certain Foods Additives and Contaminants (Twenty-Six Report of the Joint FAO/WHO Expert Committee on Food Additives); WHO Technical Report Series, No. 683; WHO Library Cataloguing-in-Publication Data; WHO: Geneva, Switzerland, (1982).
- [25] Yamaç M., Yıldız D., Sarıkürkcü C., Çelikkollu M. & Solak M. H. (2007). Heavy metals in some edible mushrooms from the Central Anatolia, Turkey. *Food Chemistry*, Vol. 103, (2007), pp. 263 – 267.
- [26] Yılmaz F., Işıloğlu M. & Merdivan M. (2003). Heavy metal levels in some macrofungi. *Turkish Journal Of Botany*, Vol. 27, (2003), pp. 45 – 56.
- [27] Zhu F., Qu L., Fan W., Qiao M., Hao H. & X. Wang (2011). Assessment of heavy metals in some wild edible mushrooms collected from Yunnan Province, China. *Environmental Monitoring and Assessment*, Vol. 179, (2011), pp. 191 – 199.