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ICP determination of Pb,Mn, Cu, Zn,Fe, K, Na, P, Mgand Cain edible wild mushroom

(Boletuspinophilus)

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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, MgandCa in wildediblemushrooms *Boletus pinophilus*. The samples were collected from the Batak mountain, Bulgaria. The preparation of samples was done by microwave mineralisation. Allmineral concentrations were determined on a dry weight basis (d.w.). The average content of studied elements: Pb, Mn, Cu, Zn, Fe, K, Na, P, MgandCain Boletus pinophilus samples was: 0.579 mg kg^{-1}, 10.415 mg kg^{-1}, 23.669 mg kg^{-1}, 69.003 mg kg^{-1}, 98.418 mg kg^{-1}, 30555.6 mg kg^{-1}, 94.749 mg kg^{-1}, 7347.933 mg kg^{-1}, 647.256 mg kg^{-1} and 256.960 mg kg^{-1}.

Index Terms- ICP, mushroom Boletus pinophilus, Batakmountain, mineralconcentrations

I. Introduction

Wild edible mushrooms are prized for their desirable taste, withtheir annual consumption exceeding 10 kg per individual in somecountries[1]. In addition to their flavor andtexture, the nutritional value of the fruiting bodies is muchappreciated[2].Mushrooms have also been proven to have therapeutic properties,counteracting diseases such as hypertension, hypercholesterolemiaand cancer[3]. It shouldbe noted that some studies have suggested that heavy metal accumulationmay be species-specific[4,5],and that while metal levels in mushrooms may be an importantindicator of environmental pollution, some wild mushroomspeciescan exceed legal limits for heavy metals even in non-polluted areaswhere heavy metal concentrations in soils are low [6-13].

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 *Boletus pinophilus*. The sampleswerecollectedfromtheBatakmountain.

II. MATERIALS AND METHODS

In determining the amount of elements in samples of wild edible mushroom *Boletus pinophilus*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.

Reagents

Reagents are qualified "AR" (pa Merck &Fluka). The starting standard solutions for ICP determination ofPb, Fe, Zn, Mn, Cu, K, Na, Ca, P and Mg atconcentrations of 1000 mg l^{-1} were suppliedby 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 indiluted HNO_3 (1/9, v/v) and were rinsed with distilledwater prior to use.

Samples

One hundred and fifty mushroom samples were collected in 2014 and 2015 from the Batak mountain by the authors themselves.The mushroom samples were cleaned from forest debris (without washing) with a plastic knife, transported to the laboratory within 4 h of collectionand placed temporarily in glass vessels at 18 C.

The samples were prepared for the experiment in a standard way; they were dried at 65^0C in a fan oven and stored in dark polyethylene bottles.

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 HNO_3 (65%), 1 ml of HCl and 3 ml of H_2O_2 (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} HNO_3. Duplicated analysis was performed on the samples. Blank digestion was also carried out in the same way.

Table I: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

Accuracyandprecision

Comparison of the experimental data to the certified reference material data In order to validate the accuracy, reliability and sensitivity of the above analytical methods the certified reference material (CRM) CTA-VTA-2 (Virginia Tobacco) was used. The CRM was stored under specified controlled conditions to ensure its stability. Ten measurements on the CRM were performed and the results were compared with the certified values.

III. RESULTS AND DISCUSSION

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*Boletus pinophilus*samplesarepresentedinTable 3.

TableII:Effectiveness of microwavemineralizationinthedetermination of Pb, Fe, Zn, Mn, Cu, K, Na, Ca,P andMginVirginia Tobacco-CTA-VTA-2 certifiedreferencematerial(n=10)

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	312mg 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 III:Concentration of heavy metals in mushroom samples (*Boletus pinophilus)* collectedfromBatak mountain, Bulgaria (n=15)

Елемент	$\bar{X}$ mg kg^{-1}	SD mg kg^{-1}	-95% Confid.	+95% Confid.
Pb	0.579	0.287	0.421	0.739
Fe	98.418	32.564	80.384	116.451
Zn	69.003	6.292	65.519	72.488
Mn	10.415	1.57	9.545	11.285
Cu	23.669	6.151	20.263	27.075
K	30555.600	2735.731	29040.600	32070.600
Na	94.749	23.569	81.696	107.801
Ca	256.960	50.856	228.797	285.124
P	7347.933	807.505	6900.752	7795.115
Mg	647.256	98.788	592.549	701.963

Table 3 presents the mineral composition (mg $kg^{-1}$of dry weight)of the investigated mushrooms. Mushroomswere reported to be a good source of minerals, and thelevels of these studied mineral element meet well the recommendeddietary allowances of NRC/NAS [14]. 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 edible wild mushroom (*Boletus pinophilus*) understudy. 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.This agrees with previous reports [15-17], which found the highest mineral to be K invarious species of edible mushrooms analysed. Based on previousreports, the Na/K ratio was often varied from 0.01 to 0.2 [15-17]. While in the present study,the Na/K ratio is very low (0.003) and Chenet al. [17]indicated it is considered to be an advantage from thenutritional point of view, since the intake of sodium chloride anddiets with a high Na/K ratio have been related to the incidence ofhypertension.

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 [18,19]. The average concentration of Cu in the studied samples was 23.568 mg kg^{-1}, which was far below the safe limit of 40 mg kg^{-1}set by WHO [6]. Copper levels in mushrooms reported in literature are 4.71–181.0mg kg^{-1} [20-24]. Iron (Fe) is an essential metal involved in biochemical processes. The average Fe content recorded in the studied samples was 98.418 mg kg^{-1}, respectively, which are above the safety limit of 15.0mg kg^{-1}, determined by the WHO. Levels of Fe reported in literature were 31.3–1190.0mg kg^{-1} [25-27]. Manganese (Mn) is an essential metal needed for biological systems such as metalloproteins [28,29]. The average Mn content recorded in the studied samples was 10.415 mg kg^{-1}, respectively, which are also below the toxicity limit of 400.0–1000.0mg kg^{-1}.The literature has reported levels of Mn as 12.9–93.3 mg kg^{-1}[20-29]. 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 erythropoisis and inhibition of haem synthesis. The maximum level of Pb present in the studied wild mushrooms was 0.579mg kg^{-1}, which is far below the 10.0 mg kg^{-1}limit set by the WHO [6]. Lead levels reported in literature are 0.4–7.77 mg kg^{-1}[20-29]. 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 69.003mg kg^{-1}, respectively, which are above the safety limit of 60 mg kg^{-1}, determined by the WHO [6]. Zn levels reported in literature were 29.3–158.0mg kg^{-1}[20-29].

The trend in decreasing order of elements present in wild samples was Fe > Zn > Cu > Mn > Pb. Mushrooms are known to possess a very effective mechanism that enables them to readily take up some heavy metals from the ecosystem [18-29]. 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 [7-15].

IV. CONCLUSIONS

According to this study, the edible wild mushroom *Boletus pinophilus*couldbeusedinhumannutritiondue to itsgoodparameters. 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.

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