STATISTICAL ANALYSIS OF Cu AND Zn CONCENTRATIONS IN BLACK TRUMPETS (*CRATERELLUS CORNUCOPIOIDE*). ECOLOGICAL RISK ASSESSMENT

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Abstract: The aim of this study was to determine copper (Cu) and zinc (Zn) concentrations in wild edible black trumpet mushrooms (*Craterellus cornucopioides*) from the Batak mountain, Bulgaria. The preparation of samples was made by means of dry ashing and dissolution in 3 M HCl. An atomic absorption spectrometer "AAnalyst 800 with graphite furnace HGA" Company "Perkin Elmer was used for determination of Cu and Zn content in the mushroom samples. Wavelengths: Cu - 324.8 nm and Zn - 213.9 nm was used. The total Cu content in mushroom samples varied from 3.436 mg kg⁻¹ to 5.962 mg kg⁻¹, and the total Zn content of samples ranged between 9.636 mg kg⁻¹ and 13.981 mg kg⁻¹. According to WHO criteria, all measured Cu and Zn concentrations were under the permissible limits. SPSS (Statistical Package for Social Science) program for Windows was used for statistical data processing. The differences between the values of parameters are characterised by the range, dispersion, standard deviation and coefficient of variation

The ecological risk was calculated confirming that the Batak mountain is an ecologically pure region.

Keywords: Atomic absorption spectrometer, mushroom *Craterellus cornucopioides*, Batak mountain, Cu, Zn, Ecological risk.

1. INTRODUCTION

Variability in the chemical composition of mushrooms within a species is greater than that of plants, much more so than within cultivars of a crop. Each individual fruit body can result from the cross-breeding of different hyphae and so presents a distinct genotype [1-7]. Contents of a trace element in a mushroom species therefore vary widely, even by as much as 1 order of magnitude. Mushroom species, but most probably not genus, level of substrate composition, and local pollution with trace elements are the principal factors affecting trace element level in fruit bodies. The lifetime of most fruit bodies is usually only 10 – 14 days. Thus the role of fruit body age and size is of less importance owing to the limited proportion of contaminants originating from atmospheric depositions. However, this role may increase in some wood-growing species that have a longer lifespan than is usual in aboveground species [16-18,23-25,28,29]. Mushrooms not only accumulate elements from underlying substrate but also from host trees depending on the specificity of species. Elements detrimental to humans originate from both natural sources and anthropogenic activities. Motor transport delivers significant amounts of both gaseous pollutants and selected elements, which generally negatively influence human health and contribute to changes in climate (EPA) [23-25,32]. In Bulgaria, apart from the sale of cultivated mushrooms in shops, wild growing mushroom species are frequently sold along roadsides and in street markets.

The aim of this study was to determine copper (Cu) and zinc (Zn) concentrations in wild edible black trumpet mushrooms (*Craterellus cornucopioides*) from the Batak mountain, Bulgaria and calculated the ecological risk.

2. METHODS

2.1. Atomic absorption spectrophotometer apparatus and analysis conditions

For the elemental analysis, a Perkin-Elmer Analyst 800 atomic absorption spectrometer with deuterium background corrector was used in this study.

The operating parameters for working elements by graphite furnace were set as recommended by the application note. Atomic absorption spectrophotometer (AAS) graphite furnace operating parameters and temperature programme are given in Table 1, respectively.

Table 1. Instrumental analytical conditions of element analyses

AAS					
Element	Acetylene (l min ⁻¹)	Air (l min ⁻¹)	Wavelength (nm)	Slit width (nm)	Lamp current (mA)
Cu	2.0	17.0	324.8	0.7	15
Zn	2.0	17.0	213.9	0.7	15

2.2. Reagents

Reagents are qualified "AR" (pa Merck & Fluka). The starting standard solutions for AAS determination of Zn and Cu 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.

2.3. 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.

2.4. Sample preparation

Dry ashing

Procedure for dry ashing at 525 °C in a muffle furnace, following Bulgarian standard 11708 – 93, was used. Samples (0.5 g) were weighed in 50 ml glass beakers, charged on a hot plate with stepwise increasing temperature up to 350 °C for 4 hours and finally ashed in a muffle furnace at 525 °C for 1 hour. After cooling ashes were dissolved in 20 ml of in 3 M HCl.

2.5. Assessment of potential ecological risk

The assessment of potential ecological risk is performed by the following formulas:

$$R_i = \sum E_r^i \quad (1)$$

$$E_r^i = T_r^i C_f^i \quad (2)$$

$$C_f^i = C_o^i / C_n^i \quad (3)$$

The potential ecological risk index (R_i) was introduced to assess the degree of heavy metal pollution in mushrooms, according to the toxicity of heavy metals and the response of the environment. R_i is calculated as the sum of all risk factors for heavy metals in mushrooms, E_r^i is the monomial potential ecological risk factor, T_r^i is the toxic-response factor for a given substance, which accounts for the toxic requirement and the sensitivity requirement, as shown in Table 2. C_f^i is the contamination factor, C_o^i is the concentration of metals in the mushrooms, and C_n^i is a reference value for metals (Table 2).

Table 2. Reference values (C_n^i) and toxicity coefficients (T_r^i) of heavy metals in mushrooms

Heavy metals	Cu	Zn
C_n^i (mg kg ⁻¹)	10	40
T_r^i	5	1

Based on the reference values for these elements, the adjusted evaluation criteria for the ecological risk index R_i are listed in Table 3.

Table 3. Terminology used to describe the risk factor E_r^i and R_i

E_r^i	Potential ecological risk for single regulator	R_i	Ecological risk for all factors
$E_r^i < 20$	Low	$R_i < 47$	Low
$20 \leq E_r^i < 40$	Moderate	$47 \leq R_i < 94$	Moderate
$40 \leq E_r^i < 80$	Considerable	$94 \leq R_i < 188$	Considerable
$80 \leq E_r^i < 160$	High	$R_i \geq 188$	Very high
$E_r^i \geq 160$	Very high		

2.6. Statistical

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

We calculated the average value and the median being central features of the empirical distribution function. In order to measure the asymmetric distribution and excess, we used coefficients of asymmetry and excess. The differences between the individual indicators were characterized by the statistical dispersion, the variance, the standard deviation and the coefficient of variation, representing a measure of statistical dispersion. The greater the statistical dispersion, the higher the coefficient of variation.

The differences between the values of parameters are characterised by the range, dispersion, standard deviation and coefficient of variation.

3. EXPERIMENTAL

Table 4. Descriptive analysis of the results for Cu and Zn concentrations in the mushrooms *Craterellus cornucopioides*

	Cu	Zn
Mean	4.579	11.429
Standard Error	0.259	0.417
Median	4.431	11.427
Standard Deviation	1.002	1.616
Sample Variance	1.004	2.610
Kurtosis	-1.692	-1.443
Skewness	0.232	0.257
Range	2.526	4.343
Minimum	3.436	9.638
Maximum	5.962	13.981
Sum	68.688	171.437
Count	15.000	15.000
Confidence Level (95,0%)	0.555	0.895

Table 5. The values potential ecological risk index (R_i) of heavy metals in mushrooms *Craterellus cornucopioides*

	C_0 , mg kg ⁻¹	C_f	E_r	R_i
Cu	4.579	0.458	2.290	2.575
Zn	11.4.29	0.286	0.286	

4. RESULTS

The total Cu content in mushroom samples varied from 3.436 mg kg⁻¹ to 5.962 mg kg⁻¹ (Table 4). According to WHO criteria, all measured Cu concentrations were under the permissible limits.

The arithmetic mean is 4.5792 mg kg⁻¹, which value is greater than the median, ie there is a positive right asymmetry. So speaks the asymmetry coefficient (0.232). Coefficient of excess is negative (-1.692). Normal scattering values of copper to average leads to normal values of standard deviation and coefficient of variation, which are respectively 1.002 and 1.004.

Copper is the third-most abundant trace element in human body and small amount of Cu is found in the human body (50 – 120 mg), but it plays a critical role like vitamin in a variety of biochemical processes [20-22,26-28,30-33]. Copper forms part of at least 13 different enzymes, and its presence is needed for each if they are to function properly. It is known that Cu may be toxic to both humans and animals when its concentration exceeds the safe limits. Copper concentrations, accumulated in mushroom species, are usually 100 – 300 µg g⁻¹, which is not considered a health risk [30-33]. These levels are above the WHO permissible limits in foods, which is 40 µg g⁻¹ [19,37,38]. Copper contents of mushroom samples in the literature have been reported to be in the ranges: 4.71 – 51.0 µg g⁻¹, 10.3 – 145 µg g⁻¹, 12.0 – 181.0 µg g⁻¹, 13.4 – 50.6 µg g⁻¹, 24.1 – 86.2 µg g⁻¹, 10.6 – 144.2 µg g⁻¹, and 15.0 – 73.0 µg g⁻¹ (Sesli et al., 2008), respectively [1-7]. The Cu results of our study were in lower with those found in the literature [20-22, 26-28, 30-35].

The total Zn content in mushroom samples varied from 9.638 mg kg⁻¹ to 13.981 mg kg⁻¹ (Table 4). According to WHO criteria, all measured Zn concentrations were under the permissible limits [19].

The arithmetic mean is 11.429 mg kg⁻¹, which value is greater than the median, ie there is a positive right asymmetry. So talk and positive asymmetry coefficient (0.257). Coefficient of excess is negative (-1.443). Normal scattering values of zinc to average leads to normal values of standard deviation and coefficient of variation, which are respectively 1.616 and 2.610 (Table 4). The WHO permissible limit of Zn in foods is 60.0 µg g⁻¹. The values for Zn in some investigated mushroom samples were above the WHO's values. Zinc concentrations of mushroom samples in the literature have been reported to be in the ranges: 29.3 – 158.0 µg g⁻¹, 33.5 – 89.5 µg g⁻¹, 40.3 – 64.4 µg g⁻¹, 45.2 – 173.8 µg g⁻¹, and 43.5 – 205.0 µg g⁻¹, 42.9 – 94.3 µg g⁻¹, respectively. The Zn results of our study were in lower with those found in the literature [8-15].

In Table 5 are presented calculated values of C_f^i , E_r^i and R_i . It is seen that the values of E_r^i and the two elements are much smaller than 40, it means that Potential ecological risk for single regulator is Low. Also, it is seen that $R_i = 2.575$, which means that Ecological risk for all factors is also Low.

5. CONCLUSIONS

From the resulting value of R_i can say that locality Batak mauntain is ecologically clean area and very suitable for collecting wild edible mushrooms that we can use in our daily menu.

6. REFERENCES

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