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Micro- and Macroelements Content and Health Risk Assessment of *Morchella esculenta* and *Lactarius piperatus* from Bosnia and Herzegovina

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Abstract

The content of micro- and macroelements in dry wild and edible *Morchella esculenta* and *Lactarius piperatus* mushrooms collected in Bosnia and Herzegovina was determined using the ICP-OES (inductively coupled plasma optical emission spectrometry) technique. The contents of microelements in *M. esculenta* and *L. piperatus* expressed in mg kg^{-1} DW (dry weight) were as follows: Co 0.08 and 0.28, Cu 37.35 and 27.66, Fe 174.29 and 28.11, Mn 21.26 and 19.31, Se 0.46 and 0.52, Zn 122.84 and 45.06, Al 27.80 and 24.80, Cr 0.83 and 1.06, Ni 0.99 and 0.96, As 0.32 and 0.09, Cd 0.48 and 0.13, and Pb 0.61 and 0.12, respectively, while the contents of macroelements were: K 26989.48 and 36117.20, Na 70.85 and 28.60, Ca 643.48 and 271.93, Mg 684.16 and 840.64, S 2329.33 and 610.42, and P 10339.35 and 5107.63, respectively.

In this study, the potential health risks of heavy metals were assessed, and target hazard quotient (THQ) for As, Cd, Pb, Cu, Zn, Ni, and Cr in the tested mushrooms was lower than the safe level. Edible wild mushrooms *M. esculenta* and *L. piperatus*, according to this study, could be used in human nutrition due to their favourable characteristics. Based on the accumulations of heavy metals in the tested mushrooms, it was shown that the collection surfaces are environmentally acceptable. Mushrooms collected from this area are generally safe to eat and pose no health risks to humans.

Keywords

Wild mushrooms, macroelements, microelements, ICP-OES, health risk assessment

1 Introduction

Wild edible mushrooms are abundant in our country. Because of the soil, climate, and extensive forest area, they are widely spread.[1,2] Due to their biological activity, wild mushrooms are known to be pharmaceutically valuable, and are used to cure a variety of diseases.[3,4] Since wild edible mushrooms are high in nutrients, they are a popular food in many places. As they are abundant in minerals, polysaccharides, amino acids, proteins, and fibre, and contain a high percentage of macro- and microelements, they have a balanced nutritious content.[5,6] Microelements, macronutrients, and trace elements are important nutritional constituents that are required in extremely minute amounts for the regular functioning of the body.

The geochemical structures of the land, the level of organic substance, the attendance of other varieties of mushrooms or plants all impact the content of elements in mushrooms.[7,8]

Pollution generated by urbanization, industrialization, fuel combustion, the use of agrochemicals, metal ores mining, processing, and melting, and other human activities all contribute to the rising levels of heavy metal pollution.[2,9] Heavy metal contamination in edible mushrooms can harm the heart and kidneys, as well as cause skeletal, digestive, neurological, and immune system failure.[10] Studies have proven that mushrooms growing in soils highly contaminated with heavy metals, including Cd, Cr, Pb, and Hg, acquire these metals at a high rate. Essential elements (Zn, Fe, Cu) play a crucial role in plant and animal metabolism as long as they stay within regulatory limits. Heavy metals in excess of regulatory limits, on the other hand, are poisonous and have a negative impact on human health.[11,12]

Wild edible mushrooms *Morchella esculenta* L., *1753* and *Lactarius piperatus* (L.) Pers., *1797,* are popular in Bosnia and Herzegovina (B&H).[13] They were collected in B&H's cities Visoko and Rudo. The forests, glades, and meadows of B&H are rich in excellent examples of mushrooms, and the country's regular rains, humid air, and high temperatures give optimal meteorological conditions for mushroom yields. Mushroom picking is a major source of income for many locals, particularly in rural regions, such as those in Visoko and Rudo, who harvest mushrooms in addition to their normal agricultural work. Visoko is a town in B&H of the Zenica-Doboj Canton, part of the Sarajevo-Zenica industrial zone. Rudo is a municipality in the Republika Srpska's east of the country, near the borders of B&H, Serbia, and Montenegro.

The objective of this research was to determine the content of micro- and macroelements in *M. esculenta* and *L. piperatus*. The data was used to assess non-carcinogenic and carcinogenic health risks for adult residents of the region linked with the presence of heavy metals in *M. esculenta* and *L. piperatus*.

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2 Materials and methods

2.1 Mushroom samples collection

Samples of *Morchella esculenta* were collected in Visoko and samples of *Lactarius piperatus* in Rudo, B&H, in 2020 (Fig. 1). After being collected, the mushroom samples were cleaned to remove mechanical contaminants and damaged sections before being lyophilized. The taxonomic keys of Đug[14] and Moser[15] as well as internet keys (http://www.mycokey.com/) were used to identify the mushrooms.

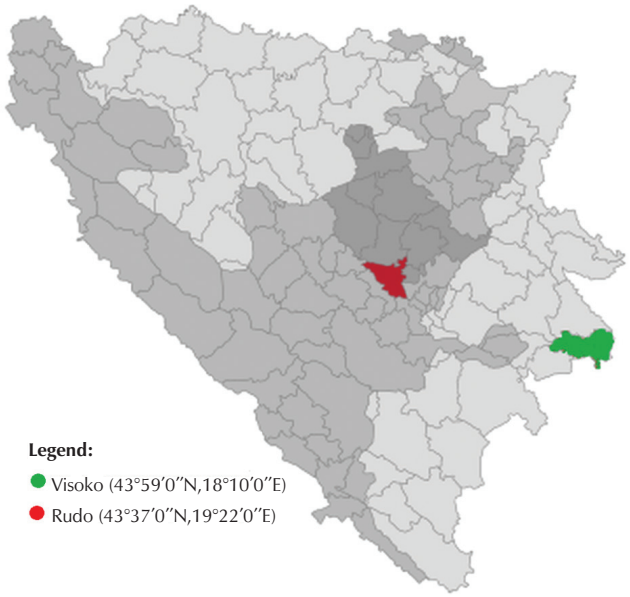

Fig. 1 – Area of sampling of mushrooms *M. esculenta i L. piperatus* in B&H
Slika 1 – Područje uzorkovanja gljiva *M. esculenta* i *L. piperatus* u BiH

2.2 Sample preparation

The drying of mushroom samples included the following steps: Fresh and cleaned mushrooms (each weighing 100 g) were frozen and lyophilized immediately (Lyophilizer CHRIST, Alpha 1-2 LD plus). Mushroom samples were vacuumed after the lyophilization procedure.

Digestion procedures: Microwave digestion was performed in three steps: In the first step, the temperature was gradually raised to 200 °C for the first 10 min, in the second step, the temperature was maintained at 200 °C for the next 20 min, and in the third step, the temperature was rapidly reduced to room temperature.

Determination of micro- and macroelement content: A Thermo Scientific iCAP 6500 Duo ICP was used to determine micro- and macroelement content using inductively coupled plasma optical emission spectrometry (ICP-OES) (Thermo Fisher Scientific, Cambridge, United Kingdom). The calibration solutions were prepared using two multi-element plasma standard solutions: Multi-Element Plasma Standard Solution 4, Specpure®, 1000 g ml^{-1} (Alfa Aesar GmbH & Co KG, Germany) and ILM 05.2 ICS Stock 1 (VHG Labs, Inc- Part of LGC Standards, Manchester, NH 03103 USA). ICP-OES measurements were conducted in triplicate ($n = 3$) on each mushroom sample. A relative standard deviation of less than 0.5 % proved measurement accuracy. The analytic quality control of the process was carried out using EPA Method 200.7 LPC Solution certified reference material (CRM) for 30 analytes at various concentrations (ULTRA Scientific, USA), which revealed that the obtained concentrations were between 97–103 %.

2.3 Potential effects of heavy metals in wild edible mushrooms on human health

The USEPA model[18] was used to assess the influence of heavy metals on human health when wild edible mushrooms were consumed. The possible non-carcinogenic and carcinogenic effects of the consumption of *M. esculenta* and *L. piperatus* polluted with heavy metals were analysed using Eq. (1) to calculate estimated daily intake (EDI).[16]

$$\mathrm{EDI}\left(\frac{\mathrm{mg}}{\mathrm{kg}}\right) = \frac{c_M \cdot IR \cdot EF \cdot ED}{ET \cdot BW} \quad (1)$$

The definitions and reference values for some health risk assessment parameters are shown in Table 1.

Table 1 – Reference values of variables used for health risk assessment of heavy metals from wild edible mushrooms
Tablica 1 – Referentne vrijednosti varijabli za procjenu zdravstvenog rizika od teških metala za samonikle jestive gljive

Variable (abbreviation)	Value used	Units	Ref.
c_M – heavy metals concentrations in mushroom samples	–	mg kg^{-1}	this study
IR – food ingestion rate	$6.60 \cdot 10^{-3}$	kg/person/day	7
EF – exposure frequency	$3.65 \cdot 10^{2}$	days	16
ED – exposure duration for adult	$7.00 \cdot 10^{1}$	years	16
BW – body weight	$7.00 \cdot 10^{1}$	kg	17
ET – averaged exposure time	$2.56 \cdot 10^{4}$	days	16

The target hazard quotient (THQ) value was used to estimate the non-carcinogenic health risks of heavy metals upon consumption of *M. esculenta* and *L. piperatus*. Eq. (2)[16] was used to compute THQ:

$$\mathrm{THQ} = \frac{\mathrm{EDI}}{R_fD} \quad (2)$$

where R_fD is the heavy metal oral reference dose expressed in mg *per* kg of body weight and *per* day. As,

Cd, Pb, Cu, Zn, Ni, and Cr had R_iD values of $3.00 \cdot 10^{-4}$, $1.00 \cdot 10^{-3}$, $3.50 \cdot 10^{-3}$, $4.00 \cdot 10^{-2}$, $3.00 \cdot 10^{-1}$, $2.00 \cdot 10^{-2}$, and $3.00 \cdot 10^{-3}$, respectively.[18,19]

The hazard index (HI) was determined as the sum of THQ values of all heavy metals that were identified in the tested mushrooms, using Eq. (3).[20]

$$HI = \sum_{i=k}^{n} THQ_i \quad (3)$$

The carcinogenic risk index (CRI) of potentially carcinogenic metals was determined by multiplying the estimated daily intake by the corresponding oral cancer slope factor (CSF), as given in Eq. (4).[16]

$$CRI = EDI \cdot CSF \quad (4)$$

For As, Pb, Cr, Ni, and Cd, the CRI was determined. According to USEPA 2017 and USEPA 2010, the CSF values for As, Pb, Cr, Ni, and Cd were 1.50, $8.50 \cdot 10^{-3}$, $5.00 \cdot 10^{-1}$, 1.70, and $3.80 \cdot 10^{-1}$ $(mg/kg/day)^{-1}$, respectively.[21,22]

The total cancer risk index (TCRI) of possible carcinogens was determined as the total of the individual CRI values according to Eq. (5).[23]

$$TCRI = \sum_i CRI_i \quad (5)$$

3 Results and discussion

3.1 Water content

The average water content of *M. esculenta* was 78 %, whereas that of *L. piperatus* was 82 %. These values vary from mushroom to mushroom, which is consistent with the notion that water content and dry matter content, in addition to mushroom species and age, are influenced by climatic circumstances.[24]

3.2 Micro- and macroelements in analysed mushroom samples

The contents of micro- and macroelements in *M. esculenta* and *L. piperatus* samples that were collected in Visoko and Rudo are shown in Tables 2 and 3.

The microelements of mushrooms can play an important role in the prevention of many diseases of the biological system. It has been found that the content of some of the microelements in mushrooms are high, such as Se, about 47 % of the recommended daily intake, and that they are an excellent source of Cu, about 55 % of the recommended daily intake. They contain essential Zn, about 11 % of the total daily intake. Micronutrient deficiency can lead to heart disease, malignant tumours, type-2 diabetes, and hypertension.[25,26]

Most edible species of wild mushrooms are low in Se (< 1 mg kg^{-1} fresh weight), but there are species that are naturally rich in this element.[27] Our results showed that the analyzed mushrooms were rich in Se, meaning that the need for Se can be met by consuming these mushrooms. Our results indicated that Se concentrations in *M. esculenta* and *L. piperatus* were higher than those reported by *Strapáč et al.*[28] and *Konuk et al.*[29], but Cu concentrations were lower. Zinc (Zn) is one of the most abundant microelements in the mushrooms analyzed, but the concentrations were lower than those reported by *Strapáč et al.*[28] and *Konuk et al.*[29]. Furthermore, the macroelements concentrations of Co, Ni, and Al in both mushrooms matched those reported by *Strapáč et al.*[28] and *Konuk et al.*[29], while the Fe and Mn concentrations were similar to those found by *Mohammad et al.*[30] and *Demirbaş*,[31] respectively. It's optimistic that the toxic microelement concentrations (As, Cd, Cr, and Pb) in mushrooms examined in this study were lower than those reported by *Mohammad et al.*[30], *Demir-*

Table 2 – Microelements content (mg kg^{-1}, dry weight) in analysed wild mushroom samples (mean ± standard deviation)

Tablica 2 – Sadržaj mikroelemenata (mg kg^{-1}, suhe težine) u analiziranim uzorcima divljih gljiva (srednja vrijednost ± standardna devijacija)

Element (Symbol)	*M. esculenta*	*L. piperatus*
Cobalt (Co)	0.08 ± 0.01	0.28 ± 0.01
Copper (Cu)	37.35 ± 0.01	27.66 ± 0.01
Iron (Fe)	174.29 ± 0.03	28.11 ± 0.01
Manganese (Mn)	21.26 ± 0.01	19.31 ± 0.01
Selenium (Se)	0.46 ± 0.01	0.52 ± 0.01
Zinc (Zn)	122.84 ± 0.01	45.06 ± 0.01
Aluminium (Al)	27.80 ± 0.01	24.80 ± 0.01
Chromium (Cr)	0.83 ± 0.01	1.06 ± 0.01
Nickel (Ni)	0.99 ± 0.01	0.96 ± 0.02
Arsenic (As)	0.32 ± 0.01	0.09 ± 0.01
Cadmium (Cd)	0.48 ± 0.01	0.13 ± 0.03
Lead (Pb)	0.61± 0.01	0.12± 0.01

Table 3 – Macroelements contents (mg kg^{-1}, dry weight) in analyzed wild mushroom samples (mean ± standard deviation)

Tablica 3 – Sadržaj makroelemenata (mg kg^{-1}, suhe težine) u analiziranim uzorcima divljih gljiva (srednja vrijednost ± standardna devijacija)

Element (Symbol)	*M. esculenta*	*L. piperatus*
Potassium (K)	26989.48 ± 4.91	36117.20 ± 1.43
Sodium (Na)	70.85 ± 0.02	28.60 ± 0.01
Calcium (Ca)	643.48 ± 0.14	271.93 ± 0.09
Magnesium (Mg)	684.16 ± 0.08	840.64 ± 0.18
Sulphur (S)	2329.33 ± 0.07	610.42 ± 0.04
Phosphorus (P)	10339.35 ±0.18	5107.63 ± 0.38

baş,[31] and *Konuk et al.*[29]. However, it should be mentioned, that the chemical composition of mushrooms, independent of species, is influenced by the elements present in the substrate in which they grow and develop. In addition, it has been found that mushrooms are capable of accumulating specific macro- or microelements through a variety of mechanisms that they have developed as a defensive mechanism during evolution.[8]

Macroelements are elements that are necessary, in relatively large quantities, for the normal functioning of physiological processes in the human body. The content of macroelements in mushrooms, such as Na, K, and P is approximately constant, while the content of Ca, Mg, and S varies depending on the composition of the substrate in which the mushroom grows.[32,33]

The content of macroelement K was the highest in both mushroom species, followed by P and S. Our results for K content are consistent with the results of *Strapáč et al.*[28] and *Demirbaş*.[31] Interestingly, our results indicated that Na content in both mushroom species was lower than those reported by *Mohammad et al.*[30] and *Demirbaş*[31]. The Na content in mushrooms is low, which is why this food is highly recommended,[34] and our results were consistent with the aforementioned authors. Low concentrations of Na and the presence of large amounts of K in the tested mushroom samples allow their use in an antihypertensive diet.[35] Furthermore, our results showed that the Ca content in both mushroom species was higher, but the Mg content was lower compared to that published by *Strapáč et al.*[28] and *Demirbaş*.[31]

It is well understood that high concentrations of some essential microelements may be toxic. Long-term exposure to high levels of some heavy metals can be hazardous to human health.[36] Therefore, we assessed the potential health risks of heavy metals in *M. esculenta* and *L. piperatus* from B&H.

3.3 Health risk assessment

Heavy metals can be increased and amplified by consuming wild edible mushrooms, posing a major health risk to humans.[2] The concentrations of some heavy metals in the human body are relatively high because they are involved in human metabolism, while long-term consumption of these elements through different sources is harmful to human health.[16] Thus, we assessed the potential health risks of As, Cd, Pb, Cu, Zn, Ni, and Cr in *M. esculenta* and *L. piperatus* from B&H. The HI for non-carcinogenic risk and CRI for the carcinogenic risk were calculated to assess the health risk of heavy metals *via* consumption of *M. esculenta* and *L. piperatus*.

The EDIs of heavy metals, which reflect the intake of individual metals *via* mushroom consumption, was calculated for the analysis of non-carcinogenic and carcinogenic health risks. Fig. 2. shows the EDI of heavy metals in mushroom samples, which were below the limits of the maximum tolerated daily intake (TDI).[37]

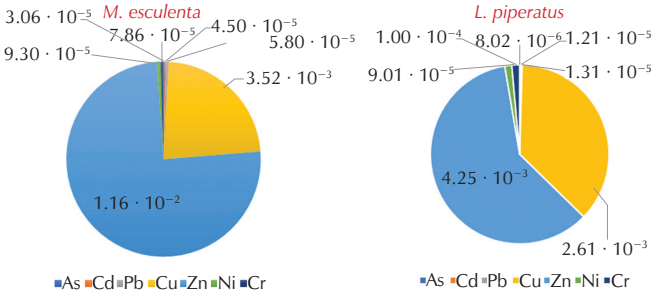

Fig. 2 – EDI values (mg kg^{-1}) of heavy metals by consumption of *M. esculenta* and *L. piperatus*

Slika 2 – Vrijednosti EDI (mg kg^{-1}) teških metala konzumacijom *M. esculenta* i *L. piperatus*

Fig. 3 shows the THQ results for the heavy metals that were tested in two wild mushroom species. The non-carcinogenic risk, THQ values for heavy metal in this study were found in the order of Ni ($9.16 \cdot 10^{-3}$) < Pb ($1.98 \cdot 10^{-2}$) < As ($2.73 \cdot 10^{-2}$) < Zn ($5.28 \cdot 10^{-2}$) < Cd ($5.71 \cdot 10^{-2}$) < Cr ($5.96 \cdot 10^{-2}$) < Cu ($1.53 \cdot 10^{-1}$). The THQ values for analyzed mushroom samples were highest for Cu and lowest for Ni. THQ values for all tested heavy metals were less than 1. Any THQ value greater than value 1 indicates a potential health risk.[38] Thus, heavy metals do not pose a potential health risk to long-term consumption of *M. esculenta* and *L. piperatus* from B&H.

Furthermore, the values for HI were $2.19 \cdot 10^{-1}$ of *M. esculenta* and $1.60 \cdot 10^{-1}$ of *L. piperatus*. When the HI reaches a level of 1, it is considered a potential risk and probable health hazard.[39] *M. esculenta* and *L. piperatus* had HI values less than 1, indicating that long-term consumption of these wild edible mushrooms has no non-carcinogenic health impacts on humans.

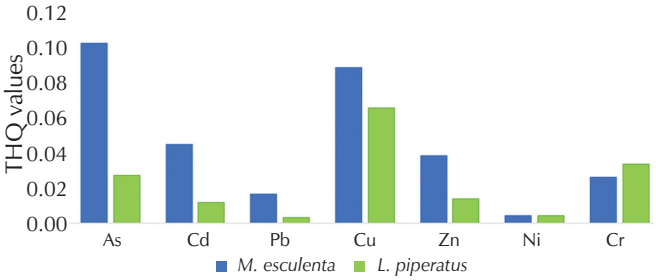

Fig. 3 – THQ values of heavy metals *by* consumption of *M. esculenta* and *L. piperatus*

Slika 3 – Vrijednosti THQ teških metala konzumacijom *M. esculenta* i *L. piperatus*

Fig. 4 displays the results of the cancer risk assessments for individual elements: As, Pb, Cr, Ni, and Cd. The cancer risk varied from a minimum value of $9.62 \cdot 10^{-8}$ for Pb in *L. piperatus*, to the maximum value of $6.75 \cdot 10^{-4}$ for Cd in

M. esculenta. According to our results, the primary pollutants that pose a higher risk were Cd and Ni, followed by Cr and As, and Pb.

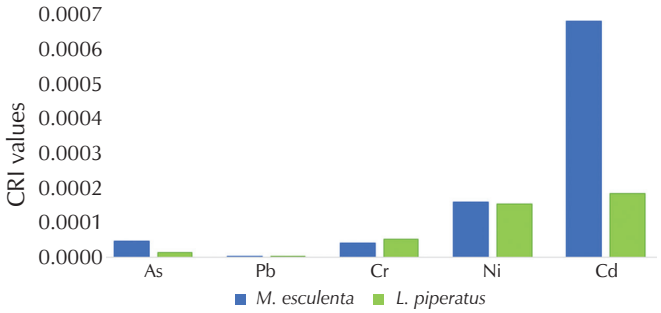

Fig.4 – CRI values of heavy metals by consumption of *M. esculenta* and *L. piperatus*
Slika 4 – CRI vrijednosti teških metala konzumacijom *M. esculenta* i *L. piperatus*

The TCRI values obtained to assess heavy metals overall carcinogenic health risk due to intake of *M. esculenta* and *L. piperatus* were $9.18 \cdot 10^{-4}$ and $3.97 \cdot 10^{-4}$, respectively. The TCRI was found to be higher than the acceptable range of $1.00 \cdot 10^{-6}$ to $1.00 \cdot 10^{-4}$.[40] A higher TCRI value for *M. esculenta* may be related to the fact that some metal content in mushrooms depends on the area where that mushroom was sampled.[2] It is well-known that *M. esculenta* is a mushroom that accumulates hazardous components from the environment in which it grows, and it may be used as a pollution indicator.[28] The increased values of TCRI found in the samples suggest that more testing is needed to identify the range of heavy metal pollution in mushroom species originating from Visoko and Rudo.

4 Conclusion

The results of this study revealed that wild edible mushrooms *M. esculenta* and *L. piperatus*, independent of where they were collected, are rich in micro- and macroelements. Natural geology and geochemistry, as well as the mushroom species influence the content of macro-and microelements in wild edible mushrooms. Analyzed mushrooms differ considerably in their content of macro- and microelements in different locations. Variations in the concentrations of most of the elements studied in *M. esculenta* and *L. piperatus*, collected in different areas, indicate the existence of complex mechanisms of the accumulation of elements by these mushrooms, which depend on the composition and characteristics of the soil. The most prominent macroelements found in the mushrooms examined were K, followed by S, and P, Mg, Ca, and Na. The important data in terms of microelements is that the mushrooms analyzed were rich in Zn and Se. The results indicate that these mushroom species can be a rich source of essential elements, such as Zn, Mn, Fe, K, Mg, and Se.

According to the Hazard Index, the examined mushrooms, (HI > 1), can be considered safe for human consumption in terms of heavy metals. However, in this study, the carcinogenic risk analysis (TCRI) represented by the carcinogenic elements revealed increased values. This leads to the conclusion that more testing is necessary to determine the extent of contamination with such elements in mushroom species originating from Visoko and Rudo.

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List of abbreviations
Popis kratica

ICP-OES – inductively coupled plasma optical emission spectrometry
– induktivno spregnuta plazma optičko emisijski spektrometar

CRM – certified reference material
– certificirani referentni materijal

EDI – estimated daily intake
– procijenjeni dnevni unos

IR – food ingestion rate
– stopa uzimanja hrane

EF – exposure frequency
– učestalost izloženosti

ED – exposure duration for adult
– trajanje izloženosti za odrasle

BW – body weight
– tjelesna težina

ET – averaged exposure time
– prosječno vrijeme izloženosti

THQ – target hazard quotient
– kvocijent ciljane opasnosti

HI – hazard index
– indeks opasnosti

CRI – carcinogenic risk index
– indeks karcinogenog rizika

CSF – cancer slope factor
– faktor nagiba raka

TCRI – total cancer risk index
– indeks ukupnog rizika od raka

EDI – estimated daily intake
– procijenjeni dnevni unos

TDI – tolerated daily intake
– prihvatljiv dnevni unos

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SAŽETAK

Sadržaj mikro- i makroelementa te procjena zdravstvenog rizika *Morchella esculenta* i *Lactarius piperatus* prikupljenih na području Bosne i Hercegovine

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Sadržaj mikro i makroelemenata u sušenim divljim jestivim gljivama *Morchella esculenta* i *Lactarius piperatus* prikupljenim u Bosni i Hercegovini određen je tehnikom ICP-OES (induktivno spregnuta plazma s optičkom emisijskom spektrometrijom). Sadržaj mikroelemenata kod *M. esculenta* i *L. piperatus* izražen u mg kg^{-1} suhe težine bio je sljedeći: Co 0,08 i 0,28, Cu 37,35 i 27,66, Fe 174,29 i 28,11, Mn 21,26 i 19,31, Se 0,46 i 0,52, Zn 122,84 i 45,06, Al 27,80 i 24,80, Cr 0,83 i 1,06, Ni 0,99 i 0,96, As 0,32 i 0,09, Cd 0,48 i 0,13, te Pb 0,61 i 0,12, dok je sadržaj makroelemenata bio: K 26989,48 i 36117,20, Na 70,85 i 28,60, Ca 643,48 i 271,93, Mg 684,16 i 840,64, S 2329,33 i 610,42, te P 10339,35 i 5107,63.
U ovom radu procijenjeni su potencijalni zdravstveni rizici teških metala, a kvocijent ciljane opasnosti (THQ) za As, Cd, Pb, Cu, Zn, Ni i Cr u ispitivanim gljivama bio je niži od sigurnosne razine. Prema rezultatima istraživanja, divlje jestive gljive *M. esculenta* i *L. piperatus*, zbog svojih povoljnih karakteristika, mogle bi se upotrebljavati u ljudskoj prehrani. Podatci o akumuliranosti teških metala u ispitivanim gljivama pokazali su da su sabirne površine ekološki prihvatljive, a gljive sigurne za jelo.

Ključne riječi
Divlje gljive, makroelementi, mikroelementi, ICP-OES, procjena zdravstvenog rizika

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