# Element contents and health risk assessment in wild edible mushrooms of Bosnia and Herzegovina

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**Abstract:** The content of macro- and microelements in dry samples of mushrooms of the species *Macrolepiota procera*, *Boletus edulis* and *Cantharellus cibarius*, collected at different areas in Bosnia and Herzegovina, was determined using the ICP-OES method (inductively coupled plasma optical emission spectrometry). Of the macroelements, K is the most represented, followed by S, P, Mg, and the least represented Ca and Na. Zn is the most represented of the essential microelements, followed by Fe, Se, Cu, Mn and Co. Al is the most abundant of the other trace elements followed by Ni and Cr. Of the toxic metals, the most represented is Cd, followed by Pb and As. There are differences in the concentration of micro- and macroelements in the mushrooms analysed, depending on the area from which they were collected because natural geology and geochemistry influence the content of macro- and microelements in wild edible mushrooms. The results show that the analysed mushrooms can be considered a good source of essential elements. The study also assessed potential health risks of heavy metals and the target hazard quotient (THQ) for As, Cd, Pb, Cu, Zn, Ni and Cr in the analysed mushrooms was lower than the safe level. The carcinogenic risk index revealed that Cd and Ni are the most prevalent pollutants in the mushrooms studied.

Keywords: fungi; micro- and macronutrients; health hazard analysis

Bosnia and Herzegovina (B&H) have great resources of edible wild mushrooms. They are widely distributed due to the soil, climate and large forest area. Wild edible mushrooms are common food in many countries because they are rich in nutrients. They have balanced nutrient composition, because they are rich in minerals, polysaccharides, amino acids, proteins and fiber that gives them the status of low-calorie nutrients (Fu et al. 2020, Salihović et al. 2021). Mushrooms contain a high proportion of macro- and microelements in addition to the nutrients stated above. Microelements, micronutrients or trace elements are essential ingredients of food, necessary for the normal functioning of the organism and are needed in very small quantities (Mleczek et al. 2021). The quantity of elements in mushrooms usually depends on the geochemical properties of the soil or other food substrates on which the mushrooms grow, the content of organic matter, the presence of other types of mushrooms or plants, and the genetic characteristics resulting from the physiology of each species (Karaman and Matavulj 2005). Pollution caused by urban growth, industrial development, fuel combustion, the use of agrochemicals, mining of metal ores, processing and melting, and other anthropogenic activities contribute significantly to the increasing contamination of all parts of the environment with heavy metals (Árvay

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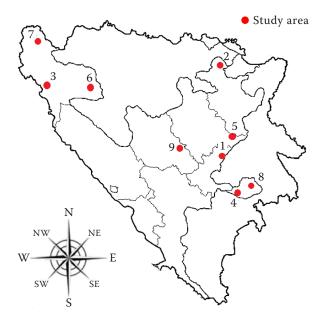


Figure 1. Area of the sampling of wild mushrooms in Bosnia and Herzegovina. 1 – Crepoljsko (Sarajevo); 2 – Gračanica; 3 – Bihać; 4 – Foča; 5 – Olovo; 6 – Ključ; 7 – Bužim; 8 – Goražde; 9 – Visoko

et al. 2015, Dowlati et al. 2021). Heavy metals such as Pb, Co, Mn, Cu, Cr, Cd, Zn and Fe are considered as the most harmful pollutants. Many researchers documented that mushrooms are susceptible to the bioaccumulation of heavy metals (García et al. 2013). These heavy metals, present even in very low concentrations, are thought to cause severe toxicological damage to health.

Macrolepiota procera, Boletus edulis and Cantharellus cibarius are predominant wild edible mushrooms consumed in B&H. Due to a lack of data, the goal of this study is to assess the content of micro- and macroelements in edible wild mushrooms from various areas. Furthermore, innovation in the study is using the obtained data for the calculation of a non-carcinogenic and carcinogenic health risk associated with the presence of heavy metals in wild edible mushrooms for adult inhabitants of the region.

## MATERIAL AND METHODS

**Mushroom samples collection.** Samples of wild edible mushrooms were collected randomly from different areas in B&H (Figure 1, Table 1). Data from a Federal Institute of Agropedology, Sarajevo B&H show the physicochemical characteristics of the soil from the sampling areas (Table 2). The pH values range from 3.72 to 7.41, soil organic carbon from 0.37% to 7.71%,  $CaCO_3$  from 0% to 38.11%,  $N_{org}$  from 0% to 0.54%, P from 0 to 20.53 mg/kg and K from 12.45 to 267.31 mg/kg. More detailed information on the chemical and physical characteristics of the soil from the sampling area is provided in Table 2.

After collection, the mushroom samples were cleaned of mechanical impurities and damaged parts and then subjected to a lyophilisation process. Identification of mushrooms was performed by the taxonomic keys of Đug (2013) and online keys (http://www.mycokey.com/).

**Sample preparation.** The process of drying mushroom samples: fresh and cleaned mushrooms (each species of 100 g) were frozen and immediately dried by lyophilisation (Lyophilizer Christ, Alpha 1–2 LD plus, Osterode am Harz, Germany). After the lyophilisation process, mushroom samples were vacuumed.

Digestion procedures: the dry mushrooms were soaked in 1 mL  $H_2O_2$  and 10 mL  $HNO_3$  (70%) and heated for 30 min in the microwave. Microwave digestion was performed in three steps: (*i*) the first 10 min the temperature was gradually raised to 200 °C; (*ii*) the temperature of 200 °C was maintained for the next 20 min; (*iii*) it was rapidly reduced to room temperature. After cooling, the clear solution was quantitatively transferred into 25 mL volumetric flasks and made up to the mark with ultrapure water.

Determination of the content of micro- and macroelements: it was performed using the inductively coupled plasma optical emission spectrometry (ICP-OES), by a Thermo Scientific iCAP 6500 Duo ICP (Thermo Fisher Scientific, Cambridge, UK). Two

Table 1. Geographical origin of the wild mushrooms studied

| Sample code | Mushroom species      | Study area |
|-------------|-----------------------|------------|
| S1          |                       | 1          |
| S2          | Macrolepiota procera  | 2          |
| S3          |                       | 3          |
| S4          |                       | 4          |
| S5          |                       | 5          |
| S6          | Boletus edulis        | 5          |
| S7          |                       | 7          |
| S8          |                       | 4          |
| S9          |                       | 8          |
| S10         | Cantharellus cibarius | 9          |
| S11         |                       | 5          |
| S12         |                       | 6          |

| 1 7  |                                   |                     |                   |                  |                   |                  |             |              |  |  |
|------|-----------------------------------|---------------------|-------------------|------------------|-------------------|------------------|-------------|--------------|--|--|
| Codo | Coordinates                       | Soil type           | ъЦ                | C <sub>org</sub> | CaCO <sub>3</sub> | N <sub>org</sub> | Р           | K            |  |  |
| Coue | Coordinates                       | Soil type           | pH <sub>KCl</sub> | (%)              |                   |                  | (mg/kg)     |              |  |  |
| 1    | 43°55'21''N,<br>18°28'4584''E     | Dystric<br>cambisol | 3.78-3.95         | 0.63-2.09        | 0                 | 0-0.12           | 0-1.75      | 12.45-62.26  |  |  |
| 2    | 44°42'29.1"N,<br>18°18'37"E       | Eutric<br>cambisol  | 3.90-5.10         | 3.53-4.27        | 0                 | 0.18-0.36        | 8.74-9.7    | 71.39-107.92 |  |  |
| 3    | 44°48'497''N,<br>15°52'19.7''E    | Calcomelanosol      | 7.41              | 3.62             | 38.11             | 0.30             | 10.0        | 97.13        |  |  |
| 4    | 43°30'22.98''N,<br>18°46'29.04''E | Ranker              | 3.81-4.08         | 1.55-7.71        | 0                 | 0.13-0.54        | 0.87-5.68   | 14.11-96.30  |  |  |
| 5    | 44°7'44.9''N,<br>18° 34'48.1''E   | Calcomelanosol      | 6.00-6.66         | 3.34-4.70        | 0-0.46            | 0.32-0.42        | 10.92-11.79 | 35.70-41.51  |  |  |
| 6    | 44°32'2.5''N,<br>16° 46'29.4''E   | Fluvisol            | 6.02-6.50         | 0.37-2.88        | 0                 | 0-0.30           | 8.30-12.67  | 20.75-267.31 |  |  |
| 7    | 45°3'33.2''N,<br>16° 1'54.9''E    | Podzol              | 3.86              | 5.10             | 0                 | 0.47             | 20.53       | 91.32        |  |  |
| 8    | 43°40'N,<br>18°59'E               | Calcomelanosol      | 4.52              | 2.71             | 0                 | 0.30             | 10.05       | 55.62        |  |  |
| 9    | 43°59'0''N,<br>18°10'0''E         | Dystric cambisol    | 3.72-4.29         | 0.63-2.09        | 0                 | 0-0.12           | 4.37-9.61   | 20.75-130.33 |  |  |

Table 2. Chemical and physical characteristics of soils from the sampling area

Source: Federal Institute of Agropedology, Sarajevo, Bosnia and Herzegovina

multi-element plasma standard solutions, Multi-Element Plasma Standard Solution 4, Specpure<sup>®</sup>, 1 000 µg/mL (Alfa Aesar GmbH & Co KG, Kandel, Germany), and ILM 05.2 ICS Stock 1 (VHG Labs, Inc- Part of LGC Standards, Manchester, USA), were used to prepare the solutions for calibration. For each mushroom sample, a measurement was performed with ICP-OES in triplicate. The reliability of measurement was confirmed by a relative standard deviation of less than 0.5%. Analytical quality control of the process, performed using the EPA Method 200.7 LPC solution certified reference material (CRM) for 30 analyses in different concentrations (ULTRA Scientific, Kingstown, USA), showed that the resulting concentrations were within 97–103%.

Potential effects of heavy metals in wild edible mushrooms on human health. Assessments of the impact of heavy metals on human health by consuming wild edible mushrooms were performed using the USEPA model which is used worldwide (Nowakowski et al. 2021). The potential non-carcinogenic and carcinogenic effects posed by the consumption of wild edible mushrooms contaminated with heavy metals were assessed by calculation of estimated daily intake (EDI) according to the equation (Fu et al. 2020):

$$EDI = \frac{c_{M} \times IR \times EF \times ED}{ET \times BW}$$
(1)

where:  $c_M$  – heavy metals concentrations in mushroom samples (mg/kg); IR – food ingestion rate, 6.60E-03 (kg/person/day); EF – exposure frequency, 365 (days/year); ED – exposure duration, 70 (years); ET – averaged exposure time, 25 550 (days/year) (Fu et al. 2020); BW – body weight, 70 (kg) (Nikkarinen and Mertanen 2004).

Non-carcinogenic health risk assessment of heavy metals in wild edible mushroom consumption was performed using the target hazard quotient value (THQ). THQ was calculated based on the equation from Fu et al. (2020):

$$THQ = \frac{EDI}{R_f D}$$
(2)

where:  $\rm R_fD$  – oral reference dose of the heavy metals (mg/ kg/day) (USEPA 2012).

The hazard index (HI) was calculated as the sum of THQs of all studied heavy metals that were found in the mushroom and the index was calculated based on the equation from Nowakowski et al. (2021):

$$HI = \sum_{i=k}^{n} THQ_{s}$$
(3)

The carcinogenic risk index (CRI) of potentially carcinogenic metals was calculated by multiplying the estimated daily intake by the corresponding oral cancer slope factor (CSF), and was calculated as (Fu et al. 2020):

$$CRI = EDI \times CS \tag{4}$$

The CRI was calculated for As, Pb, Cr, Ni and Cd. The values of CSF for As, Pb, Cr, Ni and Cd were defined by USEPA (2012) and USEPA (2017).

The total cancer risk index (TCRI) of potential carcinogens was calculated as the sum of the individual CRI values using the following equation (Fu et al. 2020):

$$TCRI = \sum CRI$$
(5)

## **RESULTS AND DISCUSSIONS**

Water content. The average water content for *M. procera* was 75.56% (range 68.7–83.5%), *B. edulis* 81.73% (range 77.9–86.4%), and *C. cibarius* 88.82% (range 87.9–90.5%). These values are different from sample to sample, which agrees with the fact that water contents and amount of dry matter, besides species and age of mushrooms, also depend on meteorological conditions (Stefanović et al. 2016).

**Macroelements (K, Na, Ca, Mg, S, P).** The results of the macroelements content in the analysed samples of mushrooms collected at different areas, are shown in Table 3. All examined elements' contents were determined of the mushrooms dry weight (DW).

The results showed that the obtained levels of macroelements in mushroom samples of *M. procera* (S1–S3) are in the following ranges (mg/kg, DW): K 11 764.4–20 212.2, Na 5.7–55.2, Ca 24.3–54.7, Mg 274.6–1 027.2, S 3 596.5–8 494.1, P 1 916.8–10 871.5; for the samples of *B. edulis* (S4–S7): K 16 021.6–24 940.4, Na 5.8–148.4, Ca 34.7–99.0, Mg 419.5–711.4, S 7 126.7–9 292.0, P 3 542.2–5 401.1; for the samples of *C. cibarius* (S8–S12): K 15 886.4–41 127.0, Na 17.8–94.7, Ca 42.1–582.1, Mg 548.5–897.1, S 559.4–3 136.9, P 3 627.5–4 844.2.

Of all the macroelements in the analysed mushroom samples, K has the highest content. The K content in sample S12 (*C. cibarius*) is the highest. The content of K in the mushroom species *B. edulis* and *C. cibarius* are consistent with the results of Bernaś et al. (2006) as well as *M. procera* with the results of Stefanovic et al. (2016).

Further, the results are consistent with the acceptable range for Na defined by the Scientific Committee for Food (SCF). Low concentrations of Na and the presence of large amounts of K in the tested samples of mushrooms allow their use in an antihypertensive diet (Lau et al. 2013). It is interesting that the Ca content in the species *C. cibarius* (S12) is higher than in other mushroom species studied. It is even about five times higher than in the species *M. procera* (S1–S3), but agrees with the results of Bernaś et al. (2006) and Stefanovic et al. (2016). Ca in the sample S6 of the species *B. edulis* is three times higher than in the sample S4 of the same species but agrees with the results of Bernaś et al. (2006).

Mg is the fourth in a row after K, P and S in the analysed dry mushroom samples. The content of Mg in sample S1 of the species *B. edulis* is four times higher than in sample S2 of the same species. Determined Mg content in all analysed mushroom species is consistent with the results of Gucia et al. (2012) and Reczyński et al. (2013).

In the dry mushroom samples analysed, S is the second most abundant macroelement. The results show that all mushrooms analysed are very rich in S; it is known that S has the ability to neutralise inflammation and also plays an important role in the prevention of heart disease (Panigrahi 2019). The analysed samples of dry mushrooms are also rich in P, which agrees with the results of Bernaś et al. (2006).

Table 3 shows that the content of the macroelements (K, Na, Ca, Mg, S, and P) for the same mushroom species varies by location, which may be related to the fact that samples of a particular mushroom species taken at different sites may contain the same elements in significantly different concentrations (Nikkarinen and Mertanen 2004). Similarly, large differences in the content of different elements in samples of the same mushroom species show that the properties of the elements themselves like the strength and type of binding in the soil or the mechanism of absorption of different elements in mushrooms, can affect their bioavailability (Haro et al. 2020).

**Essential microelements (Co, Cu, Fe, Mn, Se, Zn).** The results of the content of essential microelements in the examined mushroom samples are shown in Table 4.

The obtained levels of essential microelements in mushrooms are in the range (mg/kg, DW): for the samples of *M. procera* (S1–S3): Co 0.03–0.12, Cu 8.26–135.54, Fe 12.72–72.65, Mn 7.86–11.21, Se 1.75–12.13 and Zn 31.11–89.29; for the sample of *B. edulis* (S4–S7): Co 0.01–0.18, Cu 12.85–18.79, Fe 19.35–42.41, Mn 5.80–10.68, Se 11.9–26.06 and Zn 53.83–100.81; for the samples of *C. cibarius* (S8–S12): Co 0.11–0.37, Cu 20.23–60.74, Fe 25.96–138.99, Mn 11.79–47.17, Se 0.29–0.76 and Zn 43.02–74.57. In the samples of the same mushroom species, there are

| ard deviation | )                      |                 |                  |                    |                       |                       |
|---------------|------------------------|-----------------|------------------|--------------------|-----------------------|-----------------------|
| Sample code   | К                      | Na              | Ca               | Mg                 | S                     | Р                     |
| S1            | 19 994.1 ± 4.16        | $23.6\pm0.01$   | $46.74\pm0.01$   | $1\ 027.2\pm 0.18$ | 3 596.5 ± 0.07        | 10 871.5 ± 0.24       |
| S2            | $11\ 764.4\pm 0.76$    | $5.7\pm0.01$    | $54.7\pm0.01$    | $274.6\pm0.02$     | $8\ 494.1\pm 0.77$    | $1\ 916.8\ \pm\ 0.14$ |
| S3            | $20\ 212.2\ \pm\ 1.56$ | $55.2\pm0.02$   | $24.3\pm0.01$    | $906.7 \pm 0.10$   | $3\ 885.9\pm 0.17$    | $9\ 293.7\ \pm\ 0.32$ |
| S4            | $16\ 021.6\ \pm\ 2.03$ | $5.81 \pm 0.01$ | $34.7\pm0.01$    | $419.5 \pm 0.04$   | $7\ 126.7\ \pm\ 0.28$ | $3\ 542.2\ \pm\ 0.17$ |
| S5            | $24\;188.7\pm 4.11$    | $148.4\pm0.02$  | $69.67\pm0.01$   | $518.2\pm0.23$     | $7\ 320.8\pm 0.17$    | $4\ 848.4 \pm 0.17$   |
| S6            | $24\ 940.4\pm 4.59$    | $80.9\pm0.01$   | $99.0\pm0.02$    | $711.4\pm0.05$     | 8 737.1 ± 0.16        | $5401.1 \pm 0.02$     |
| S7            | $17\ 210.9\ \pm\ 1.2$  | $15.3\pm0.01$   | $53.7\pm0.01$    | $540.7 \pm 0.03$   | $9\ 292.0 \pm 1.02$   | $4\ 949.1\ \pm\ 0.49$ |
| S8            | $28\ 375.4\pm 4.35$    | $53.1\pm0.01$   | $266.3 \pm 0.05$ | $586.6 \pm 0.20$   | $1\ 627.1\pm 0.05$    | $3\ 794.0 \pm 0.05$   |
| S9            | $15\ 886.4 \pm 1.77$   | $25.9\pm0.01$   | $42.1\pm0.01$    | $548.5 \pm 0.05$   | $3\ 136.9\pm 0.14$    | $4\ 339.2\pm 0.16$    |
| S10           | $27\ 721.7\ \pm\ 1.18$ | $17.8\pm0.01$   | $351.5\pm0.14$   | $666.9 \pm 0.36$   | $795.4\pm0.02$        | $3\ 876.5\ \pm\ 0.08$ |
| S11           | $39\ 911.0\pm 9.49$    | $18.7\pm0.01$   | $352.5 \pm 0.06$ | $897.1 \pm 0.22$   | $559.4 \pm 0.02$      | $4\ 844.2\ \pm\ 0.14$ |
| S12           | $41\;127.0\pm 4.09$    | $94.7\pm0.01$   | $582.1\pm0.08$   | $746.9\pm0.14$     | $829.6 \pm 0.06$      | $3\ 627.5\ \pm\ 0.23$ |

Table 3. Macroelements contents (mg/kg, dry weight) in the wild mushroom samples analysed (mean ± standard deviation)

differences in the content of various essential microelements. It suggests that the area of the mushroom collection influence the macro- and microelement contents in mushrooms.

The obtained average concentrations of Co in *M. procera* are consistent with the literature data (Gucia et al. 2012, Stefanović et al. 2016). The content of Co in *B. edulis* is consistent with the results of Nikkarinen and Mertanen (2004), while the results of *C. cibarius* are consistent with the results of Ouzouni et al. (2009).

The content of Cu in *M. procera* sample S1 is higher than in other investigated mushrooms species. It is even about sixteen times higher than in sample S2 of the same mushroom species. The results for the Cu content in the species *C. cibarius* and *B. edulis* are however lower than the literature data (Nikkarinen and Mertanen 2004, Türkmen and Budur 2018, Sarikurkcu et al. 2020).

The results showed that the obtained average concentrations of Fe in all samples of *M. procera* and *C. cibarius* are lower than those reported by Gucia et al. (2012) and Türkmen and Budur (2018), while the samples of *B. edulis* are consistent with the results of Nikkarinen and Mertanen (2004). It is surprising that the Fe content in sample S10 (*C. cibarius*) is higher than in the others. It is even about seven times higher than sample S11 of the same species. Likewise, the Fe content in mushroom S3 of *M. procera* is about seven times higher than sample S2 of the same species.

Table 4. Essential microelements contents (mg/kg, dry weight) in the wild mushroom samples analysed (mean  $\pm$  standard deviation)

| Sample code | Co              | Cu                | Fe                | Mn               | Se               | Zn                |
|-------------|-----------------|-------------------|-------------------|------------------|------------------|-------------------|
| S1          | $0.12 \pm 0.01$ | $135.54 \pm 0.03$ | $64.11 \pm 0.02$  | $11.21\pm0.01$   | $1.75 \pm 0.01$  | $86.90\pm0.01$    |
| S2          | $0.11\pm0.01$   | $8.26\pm0.01$     | $12.72 \pm 0.07$  | $7.86\pm0.01$    | $12.13\pm0.01$   | $31.11 \pm 0.01$  |
| S3          | $0.03\pm0.01$   | $83.63 \pm 0.01$  | $72.65 \pm 0.01$  | $8.70\pm0.01$    | $2.32\pm0.01$    | $89.29 \pm 0.01$  |
| S4          | $0.12\pm0.01$   | $14.62\pm0.01$    | $35.09 \pm 0.01$  | $5.80\pm0.01$    | $17.53 \pm 0.01$ | $53.83 \pm 0.01$  |
| S5          | $0.02\pm0.01$   | $18.35\pm0.01$    | $19.35 \pm 0.01$  | $10.43\pm0.01$   | $21.19\pm0.01$   | $81.13\pm0.01$    |
| S6          | $0.18\pm0.01$   | $18.79\pm0.01$    | $42.41 \pm 0.01$  | $6.33\pm0.01$    | $26.06\pm0.01$   | $67.46 \pm 0.01$  |
| S7          | $0.01\pm0.01$   | $12.85\pm0.01$    | $25.44\pm0.01$    | $10.68\pm0.01$   | $11.90\pm0.01$   | $100.81 \pm 0.01$ |
| S8          | $0.30\pm0.01$   | $26.74\pm0.01$    | $138.99 \pm 0.02$ | $11.79\pm0.01$   | $0.45\pm0.01$    | $57.02\pm0.01$    |
| S9          | $0.37\pm0.01$   | $60.74\pm0.01$    | $25.96 \pm 0.01$  | $15.70\pm0.01$   | $0.76\pm0.01$    | $74.57 \pm 0.01$  |
| S10         | $0.35\pm0.01$   | $41.49\pm0.01$    | $213.07 \pm 0.07$ | $47.17 \pm 0.01$ | $0.56\pm0.01$    | $43.02\pm0.01$    |
| S11         | $0.11\pm0.01$   | $20.23\pm0.01$    | $39.14\pm0.01$    | $22.30\pm0.01$   | $0.29\pm0.01$    | $43.50\pm0.01$    |
| S12         | $0.14 \pm 0.01$ | $28.21 \pm 0.01$  | $116.46 \pm 0.01$ | $32.92 \pm 0.01$ | $0.54 \pm 0.01$  | $44.38 \pm 0.01$  |

The obtained average concentrations for Mn in *M. procera* are lower than those reported by Gucia et al. (2012). For *B. edulis*, the obtained average concentrations of Mn are consistent with the results reported by Nikkarinen and Mertanen (2004), while for species *C. cibarius*, they are consistent with those reported by Gas et al. (2000) and Türkmen and Budur (2018).

Most edible species of wild mushrooms are low in Se (< 1 mg/kg fresh weight), but there are species that are naturally rich in this element (Oteiza and Mackenzie 2005). The results show that all analysed mushrooms are rich in Se, which means that the need for Se can be met by consuming these mushrooms. The obtained average Se concentrations in *M. procera* are higher than in Stefanovic et al. (2016), while in *B. edulis* and *C. cibarius* they are consistent with the results of Gas et al. (2000) and Nikkarinen and Mertanen (2004).

The results of the investigation of the essential microelements content in analysed mushrooms show that Zn is the most abundant. The obtained average concentrations for Zn in *M. procera* and *C. cibarius* are however lower than those reported by Gucia et al. (2012) and Türkmen and Budur (2018), while the results for *B. edulis* are consistent with those of Nikkarinen and Mertanen (2004).

Some other trace elements (Al, Cr, Ni) and toxic metal (As, Cd, Pb). The results of other trace elements and toxic metals contained in the examined mushroom samples are shown in Table 5.

The obtained levels of some trace elements in the analysed mushroom species were as follows:

*M. procera* (S1–S3) samples are in the range (mg/kg, DW): Al 0.74–8.47, Cr 0.34–1.38 and Ni 0.16–1.04; *B. edulis* (S4–S7) samples: Al 5.33–30.86, Cr 0.21–0.69 and Ni 0.91–1.28; *C. cibarius* (S8–S12) samples: Al 11.68–175.41, Cr 0.22–3.63 and Ni 0.43–4.42. It is noted that there are also considerable differences in the content of listed trace elements in the samples of the same mushroom species, depending on the collection site.

The obtained concentrations of toxic metals in the analysed mushroom species ranged as follows: *M. procera* (S1–S3) samples (mg/kg, DW): As 0.29– 1.10, Cd 0.10–1.79 and Pb < 0.02–10.97; *B. edulis* (S4–S7) samples: As 0.17–0.42, Cd 0.77–3.00 and Pb 0.04–0.42: *C. cibarius* (S8–S12) samples: As < 0.02-0.17, Cd 0.10–3.20 and Pb 0.13–1.12. Toxic metals in samples of the same mushroom species also show a significant difference in their content in relation to the collection site.

The examined mushroom samples exceeded the Al acceptable limit (according to the EFSA in 2008). High Al content could be caused, for example, by soil that has penetrated the pores of the mushroom and has not been cleaned. The highest Al content was reported in *C. cibarius*, sample S10, but still lower than the results published by Gas et al. (2000). Then follows *B. edulis*, where the Al content is higher than in the results of Širić et al. (2016). *M. procera* has the average Al content lower than the results of Gucia et al. (2012).

The Cr content in the sample of *C. cibarius* (S10) was the highest compared to other analysed mush-rooms; it was even sixteen times higher than in the

| Sample code | Al                | Cr              | Ni            | As                | Cd            | Pb                |
|-------------|-------------------|-----------------|---------------|-------------------|---------------|-------------------|
| S1          | $8.47 \pm 0.01$   | $1.38\pm0.01$   | $0.16\pm0.01$ | $0.53 \pm 0.01$   | $1.79\pm0.01$ | $10.97 \pm 0.01$  |
| S2          | $0.74\pm0.05$     | $0.63 \pm 0.01$ | $1.04\pm0.01$ | $0.29\pm0.01$     | $0.10\pm0.01$ | $< 0.02 \pm 0.01$ |
| S3          | $4.13\pm0.01$     | $0.34\pm0.01$   | $0.31\pm0.01$ | $1.10\pm0.01$     | $0.32\pm0.01$ | $0.34\pm0.01$     |
| S4          | $10.73 \pm 0.01$  | $0.46\pm0.01$   | $0.91\pm0.01$ | $0.42\pm0.01$     | $1.12\pm0.01$ | $0.12\pm0.01$     |
| S5          | $5.33 \pm 0.01$   | $0.26\pm0.01$   | $1.28\pm0.01$ | $0.28\pm0.01$     | $3.00\pm0.01$ | $0.42\pm0.01$     |
| S6          | $30.86 \pm 0.01$  | $0.69\pm0.01$   | $1.04\pm0.01$ | $0.21\pm0.01$     | $0.81\pm0.01$ | $0.04\pm0.01$     |
| S7          | $15.84\pm0.01$    | $0.21\pm0.01$   | $1.06\pm0.01$ | $0.17\pm0.01$     | $0.77\pm0.01$ | $0.17\pm0.01$     |
| S8          | $18.65 \pm 0.01$  | $0.45\pm0.01$   | $0.92\pm0.01$ | $0.03\pm0.01$     | $0.65\pm0.01$ | $0.27\pm0.01$     |
| S9          | $11.68 \pm 0.01$  | $0.22\pm0.01$   | $0.43\pm0.01$ | $< 0.02 \pm 0.01$ | $3.20\pm0.01$ | $1.12\pm0.01$     |
| S10         | $175.41 \pm 0.05$ | $3.63\pm0.01$   | $4.42\pm0.01$ | $0.17\pm0.01$     | $0.32\pm0.01$ | $0.51\pm0.01$     |
| S11         | $42.18\pm0.01$    | $3.05\pm0.01$   | $1.87\pm0.01$ | $< 0.02 \pm 0.01$ | $0.10\pm0.01$ | $0.13\pm0.01$     |
| S12         | $135.74\pm0.01$   | $0.58\pm0.01$   | $1.75\pm0.01$ | $0.10\pm0.01$     | $0.38\pm0.01$ | $0.17\pm0.01$     |

Table 5. Some other trace elements and toxic metal contents (mg/kg, dry weight) in the wild mushroom samples analysed (mean ± standard deviation)

sample of the same species (S9). This difference can be interpreted by the origin of the samples, i.e. sample S10 was collected in an urban environment and sample S9 in an area where the anthropogenic factor (rural environment) is not present. The average Cr concentrations obtained in M. procera species are consistent with those in Gucia et al. (2012) while B. edulis species are larger than those of Širić et al. (2016), and Nikkarinen and Mertanen (2004).

The results show that Ni is present in all the analysed mushrooms samples. The Ni content was highest in sample S10 (C. cibarius) and least in sample S1 (M. procera). The average concentrations of Ni obtained in *M. procera* species correspond to those of Gucia et al. (2012). In samples of B. edulis, the concentrations of Ni are higher than in Nikkarinen and Mertanen (2004), while those of C. cibarius are lower compared to Türkmen and Budur (2018).

In this study, the average concentrations of toxic metals in all analysed mushroom samples are in accordance with the previously recorded values (Giannaccini et al. 2012, Falandysz and Rizal 2016). In the case of As, only the S3 sample of the *M. pro*cera contains increased concentrations of this metal.

Further, the results show that the highest Pb content is present in sample S1 (M. procera). The average concentrations of Pb obtained in all analysed mushroom samples are consistent with the results of Gas et al. (2000), with the exception of sample S1. The concentration of Pb in sample S1 of the mushroom species *M. procera* represents a toxicological risk due to the content of this metal.

The highest Cd concentration is found in the S9 sample (C. cibarius), even ten times higher than in the S10 sample of the same species. In samples of B. edulis and M. procera the concentrations of Cd are consistent with Sirić et al. (2016) and Gucia et al. (2012), while they are higher in *C. cibarius* than at Gas et al. (2000).

Mushroom chemical composition is mostly affected by the elements present in the substrate on which they grow, and it evolves precisely in accordance with their absorption capability, which is attained through their diet. They accumulate specific macroor microelements through various mechanisms they have created during evolution as a defense mechanism (Karaman and Matavulj 2005). The above is confirmed by the obtained results.

Wild edible mushrooms can contain large amounts of macro- and micronutrients, but also toxic metals. At the same time, the essential elements can be

Table

1.98E-02 l.07E-02 1.43E-02 8.30E-03 2.18E-02 6.73E-03 1.41E-02 6.82E-03 9.59E-02 1.84E-02 4.35E-02 1.14E-01 Ľ 4.88E-03 l.45E-03 4.28E-03 6.05E-03 4.88E-03 4.98E-03 4.33E-03 2.01E-03 2.09E-02 8.81E-03 03 7.78E-04 8.26EïŻ consumption of wild edible mushrooms 2.81E-02 1.69E-02 1.35E-02 1.37E-02 2.73E-02 9.78E-03 2.55E-02 2.12E-02 3.17E-02 1.79E-02 2.34E-02 L.39E-02 Zn THQ 3.45E-02 3.03E-02 4.77E-02 1.95E-02 4.32E-02 4.43E-02 6.30E-02 9.78E-02 1.97E-01 6.65E-02 3.19E-01 I.43E-01 Ca 1.08E-04 9.27E-03 3.23E-03 L.08E-03 4.63E-03 3.45E-03 1.12E-02 7.25E-03 1.38E-02 2.96E-01 3.02E-02 4.66E-03 Pb 7.61E-02 9.62E-03 9.62E-03 2.97E-02 2.83E-01 7.21E-02 6.17E-02 3.00E-02 1.06E-01 59E-02 3.02E-01 1.69E-01 Cd metals *via* 8.89E-02 -02 9.21E-02 1.32E-01 6.66E-02 5.37E-02 1.01E-02 1.26E-03 5.37E-02 1.26E-03 1.65E-01 3.45E-01 3.11E-As values of heavy 2.49E-05 -05 1.30E-04 5.95E-05 3.21E-05 4.30E-05 6.54E-05 2.02E-05 4.22E-05 2.05E-05 3.43E-04 2.88E-04 51Eü LC. and Target hazard quotient (THQ) 1.56E-05 9.77E-05 2.90E-05 8.55E-05 1.21E-04 9.77E-05 9.97E-05 4.17E-04 1.76E-04 -048.66E-05 4.02E-05 1.65EïŻ 2.93E-03 8.42E-03 5.08E-03 7.65E-03 6.36E-03 4.10E-03 8.19E-03 9.50E-03 5.38E-03 7.03E-03 4.06E-03 4.18E-03 Zn (mg/kg) 7.79E-04 7.89E-03 1.38E-03 1.73E-03 L.77E-03 I.21E-03 3.91E-03 1.91E-03 1.28E-02 2.52E-03 5.73E-03 66E-03 EDI G 3.24E-05 1.13E-05 3.93E-05 3.77E-06 1.62E-05 1.06E-04 1.21E-05 1.03E-03 3.77E-07 2.54E-05 4.84E-05 1.63E-05 daily intake (EDI) Pb 9.62E-06 2.97E-05 2.83E-04 7.61E-05 3.02E-04 9.62E-06 -05 1.69E-04 1.06E-04 7.21E-05 6.17E-05 3.00E-05 59E-Cd Estimated 4.96E-05 2.76E-05 3.95E-05 2.00E-05 l.04E-04 2.67E-05 1.61E-05 3.02E-06 l.61E-05 -06 3.77E-07 3.77E-07 9.33E-As <u>.</u> Sampl code 510 S11 S1S2 S3 **S4** S5 S6 S7 S8 S9

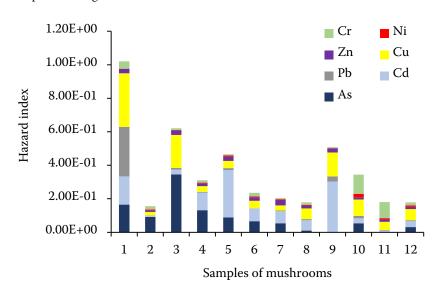


Figure 2. Hazard index for noncarcinogenic risk to humans due to heavy metals intake *via* consumption of wild edible mushrooms collected in Bosnia and Herzegovina

potentially toxic if found in high concentrations. Concentrations of certain heavy metals in the human body are relatively high because they are involved in metabolic processes and regulate the immune system, while long-term intake of heavy metals is harmful to human health (Gall et al. 2015). Thus, we assessed the potential health risks of heavy metals in wild edible mushrooms from B&H.

Human health risk assessment. To assess the impact of heavy metals in wild edible mushrooms on the human health, hazard index for non-carcinogenic compounds and carcinogenic risk index for carcinogenic compounds was calculated. For the analysis of the non-carcinogenic and carcinogenic health risks posed by heavy metals in the wild edible mushrooms, the EDI of heavy metals was calculated which describes the intake of individual metals by humans *via* mushrooms consumption (Demková et

al. 2021). The EDI of the heavy metals for mushroom samples are presented in Table 6 and they are within the limits of the maximum tolerable daily intake (TDI) (FAO/WHO 2011).

**Non-carcinogenic health risk analysis.** The obtained results for THQ of the seven heavy metals are presented in Table 6. The value of THQ higher than 1 indicates a probability that the consumption of a wild edible mushroom could produce negative health effects (Chen et al. 2020). Since the THQ value for all tested metals from individual mushrooms was less than 1, it shows that the consumption of these mushrooms does not pose non-carcinogenic health risks.

The results for HI of the analysed samples are shown in Figure 2. The HI values for wild edible mushrooms are ranged from 1.56E-01 to 1.02E+00. In the present study, the highest and the lowest

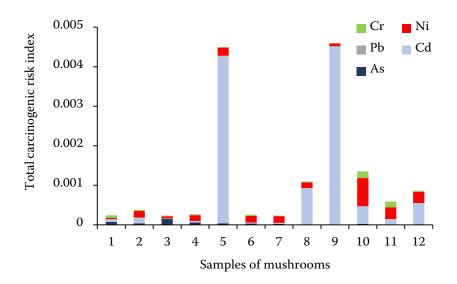


Figure 3. Total carcinogenic risk index for carcinogenic risk to humans due to heavy metals intake *via* consumption of wild edible mushrooms collected in Bosnia and Herzegovina

HI values were found for the mushroom species M. procera, sample S1 and S3, respectively. The results obtained for HI can be related to the fact that some metal content in mushrooms depends on the spices of mushrooms and other factors such as geographical area, soil composition, i.e. substrate, or the content of acidic and organic compounds in the soil ecosystem, the age of mushrooms, and sources and distances of pollution. The HI is considered to be a significant risk and potential hazard to health when it reaches a level above 1 (Ahmed et al. 2019). Hazard indexes concerning the level of heavy metals in the analysed wild edible mushrooms were determined to be < 1, except for *M. procera*, sample S1 where HI is >1. Árvay et al. (2015) from Slovakia have also found that consumption of M. procera may have a negative impact on the human health due to the high concentration of heavy metals. Similar HI values were reported from other countries in the region Croatia (0.506), Serbia (2.30) and Slovakia (1.655) (Dowlati et al. 2021).

**Carcinogenic health risk analysis.** The obtained results of the cancer risks for individual elements (As, Cd, Pb, Ni and Cr) are presented in Figure 3. The cancer risk varied from minimum value 3.21E-09 for Pb in *M. procera*, sample S3, to the maximum value 4.52E-03 for Cd in *C. cibarius*, sample S9. Compared to the other three metals (As, Pb and Cr) Cd and Ni seemed to be the leading contaminants that created a relatively higher risk, followed by Cr and As.

TCRI values calculated to assess heavy metals' total carcinogenic health risk due to consumption of wild edible mushrooms from different locations were presented in Figure 3. A wide range of TCRI was calculated, from 2.31E-04 to 4.60E-03. The highest values were found for C. cibarius, sample S9, while the lowest values were obtained for *B. edulis*, sample S7. The results showed higher carcinogenic risks than a tolerable range of 1E-06 to 1E-04 (USEPA 2012). Such high values of TCRI indicated that the human population consuming wild edible mushrooms from the study area was at a significantly high carcinogenic risk. High values of TCRI in the analysed samples suggest that further control needs to be carried out to determine the scale of heavy metals pollution of food in B&H.

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