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RESEARCH ARTICLE

Element concentration, daily intake of elements, and health risk indices of wild mushrooms collected from Belgrad Forest and Ilgaz Mountain National Park (Turkey)

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Abstract

The aim of this study was to determine the element content of wild edible and inedible mushroom species (*Agaricus campestris*, *Armillaria ostoyae*, *Boletus reticulatus*, *Bondarzewia mesenterica*, *Bovistella utriformis*, *Cantharellus cibarius*, *Marasmius oreades*, *Megacollybia platyphylla*, *Meripilus giganteus*, *Neoboletus erythropus*, *Panellus stipticus*, *Phaeotremella foliacea*, *Pleurotus ostreatus*, *Podoscypha multizonata*, *Russula aurea*, *R. chloroides*, *R. virescens*, *T. versicolor*, *Trametes gibbose*, and *Trichaptum biforme*) collected from the Belgrad Forests and the Ilgaz Mountain National Park. Based on the results of elemental analyses, daily metal intake (DMI) and health risk index (HRI) values of edible mushrooms collected from both localities were also calculated. As, Cd, Cr, Se, P, Hg, Cu, Mn, Fe, Zn, Al, Ca, Mg, and K contents of mushrooms were in the ranges of 0.16–3.45, 0.09–2.4, 0.15–2.34, 0.3–8.13, 0.28–11.44, 14.03–37.81, 3.87–108.57, 6.18–149.77, 11.9–776.1, 5.4–317.4, 7.4–355.2, 15.4–3517.3, 266.0–2500.0, and 628.0–24083.0 mg/kg dry weight, respectively. As a result of the DMI and HRI analyses, Cu concentration of *B. utriformis* (DMI: 46.53 μg/kg body weight/serving, HRI: 1.16) and Cd concentrations of *A. campestris* (DMI: 0.49 μg/kg body weight/serving, HRI: 1.36), *A. ostoyae* (DMI: 1.03 μg/kg body weight/serving, HRI: 2.86), *B. utriformis* (DMI: 0.52 μg/kg body weight/serving, HRI: 1.44), and *P. ostreatus* (DMI: 0.45 μg/kg body weight/serving, HRI: 1.24) were found to exceed the legal limits determined by authorities. It was concluded that the species collected from the regions in question should be consumed in a controlled manner.

Keywords Edible mushrooms · Metal concentration · Daily intakes of element · Health risk index · Belgrad Forest · Ilgaz Mountain

Introduction

One of the most important problems faced by people living in the world is malnutrition and hunger that occurs due to this phenomenon. Especially, people living in developing countries face these problems more due to income inequality. Due to the problems experienced in livestock and agricultural activities, people cannot adequately meet their animal and vegetable protein needs. This has increased the demand for alternative protein sources such as mushrooms. The fact that mushrooms are also more nutritious than many other foods in these countries has made mushrooms more and more popular in terms of nutrition (Wani et al. 2010).

In developing countries, in addition to the problems experienced in the agriculture and farming depending on the geographical conditions, the population growth rate is very high due to the low education level. In these countries, the demand for natural resources and industrialization is increasing gradually to meet the need for goods and services. With the increasing industrialization, wastes rich in toxic metals are released to the environment. Ecosystem pollution negatively

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affects the life of all living things in that ecosystem. This situation poses a great threat to all living individuals of the ecosystem (Ndimele et al. 2017a).

The distribution of toxic elements in the ecosystem is determined by some physical and chemical processes. Among these processes, diffusion, absorption, precipitation, and dilution are of special interest. In addition, the uptake of these toxic elements by organisms living in the ecosystem is one of the factors affecting the distribution of toxic elements in the ecosystem (Ndimele et al. 2017b). Some elements such as manganese (Mn), zinc (Zn), copper (Cu), cobalt (Co), and iron (Fe) are required for catalytic activity of some enzymes at low concentrations. However, the presence of these elements in high concentrations is toxic to the organism (Bryan 1976). On the other hand, some elements such as lead (Pb), mercury (Hg), and cadmium (Cd) do not have a known role in metabolism and show toxic effects even when taken in low concentrations. According to the researchers, the toxic elements in question are toxic to organisms living in both aquatic and terrestrial ecosystems. These elements cause serious changes on metabolic activities in organisms and have a negative effect on biochemical parameters (Nemcsok and Hughes 1988).

There are numerous studies showing that wild mushrooms accumulate higher amounts of some elements both from the soil and from other agricultural products (Huang et al. 2010; Ouzouni et al. 2009; Svoboda and Kalac 2003; Zhu et al. 2011). As mentioned above, toxic elements are ecosystem elements whose concentrations must be kept under control due to the risk of environmental pollution. Some studies showed that the concentration of toxic elements exceeds legal limits in some geographic areas (Chen et al. 2009a; Zhang et al. 2012; Zhang and Chen 2012). Scientists agree that wild mushrooms that grow near mining fields, urbanized areas, or industrial establishments have higher concentrations of toxic elements (Chen et al. 2009b; Zhang et al. 2010; Zhu et al. 2011). Therefore, wild mushroom species consumed with great pleasure by people should be constantly monitored for their element concentrations.

The purpose of this study was to determine the element contents of wild mushroom species collected from Belgrad Forests (*Neoboletus erythropus*, *Marasmius oreades*, *Megacollybia platyphylla*, *Meripilus giganteus*, *Panellus stipticus*, *Pleurotus ostreatus*, *Podoscypha multizonata*, *Trametes gibbosa*, *T. versicolor*, *Trichaptum biforme*, *Russula aurea*, *R. chloroides*, *R. virescens*, *Phaeotremella foliacea*) and the Ilgaz Mountain National Park (*Boletus reticulatus*, *Armillaria ostoyae*, *Cantharellus cibarius*, *Agaricus campestris*, *Bondarzewia mesenterica*, *Bovistella utriformis*). Additionally, the daily metal intake (DMI) and health risk index (HRI) values of the edible mushrooms from the analyzed mushrooms were also determined and some inferences were made for the consumption of the mentioned mushrooms according to the results.

Materials and methods

Collection, identification, digestion, and elemental analysis of mushroom species

Fully matured fruiting bodies of mushrooms were collected between October 20 and 30, 2020, from Belgrad Forest, Istanbul—Turkey—and Ilgaz Mountain National Park, Kastamonu—Turkey. Information on the habitats of the samples and the substance on which they are grown (substrate) are given in Table 1. Table 1 also shows the families, edibility, and herbarium numbers of mushrooms. Identification, digestion, and elemental analysis of mushrooms are detailed in the supplementary file. Additionally, information on the reference material used (recovery rates and analytical outputs) is also provided in the Table S1 of the supplementary file.

Determination of DIM and HRI values

DIM and HRI analyses of the samples were performed by using the methods recommended by Cui et al. (2004) and Liu et al. (2015). The calculation of the DIM value was based on the RfD^{o} values set by the competent authorities (USEPA 2002). Details regarding the calculation of DIM and HRI values are given in the supplementary file.

Statistical analyses

One-way ANOVA by Tukey's tests (at 5% significance level) test was performed for variance analysis of the data obtained from the mushroom samples. The principal component analysis (PCA) and the hierarchical cluster analysis (HCA) were carried out to comprehend the similarity among the mushroom species in terms of the mineral contents. PCA was done after pareto standardization of data and HCA was based on the Ward rule and Euclidean distance. The statistical analyses were done using SIMCA v. 14.1 and R software v. 3.6.2 respectively.

Results and discussion

Element concentrations of the mushrooms

In this section, element concentrations of twenty different mushroom species collected from the Belgrad Forest (Istanbul, Turkey) and the Ilgaz Mountain National Park (Kastamonu, Turkey) were presented. The data on the mushrooms (edibility, habitat, substrate, and locality) are given in Table 1. Half of the fourteen mushrooms collected from the Belgrad Forest and five of the six mushrooms collected from the Ilgaz Mountain National Park were edible. Element concentrations of

Table 1 Families, edibility, habitats, substrates, and harvesting area of wild mushroom species

Family	Species	Edibility	Habitat	Substrate	Locality
Agaricaceae	*Agaricus campestris* Scop.	Edible	Meadow	On soil	Ilgaz Mountain National Park
Boletaceae	*Neoboletus erythropus* (Pers.) C. Hahn	Poisonous	Beech forest	On soil	Belgrad Forest
	Boletus reticulatus Schaeff.	Edible	Fir forest	On soil	Ilgaz Mountain National Park
Bondarzewiaceae	*Bondarzewia mesenterica* (Schaeff.) Kreisel	Inedible	Fir forest	On fir root	Ilgaz Mountain National Park
Hydnaceae	*Cantharellus cibarius* Fr.	Edible	Oak forest	On soil	Ilgaz Mountain National Park
Lycoperdaceae	*Bovistella utriformis* (Bull.) Demoulin & Rebriev	Edible	Meadow	On soil	Ilgaz Mountain National Park
Marasmiaceae	*Marasmius oreades* (Bolton) Fr.	Edible	Meadow	On soil	Belgrad Forest
	Megacollybia platyphylla (Pers.) Kotl. & Pouzar	Edible	Oak forest	On soil	Belgrad Forest
Meripilaceae	*Meripilus giganteus* (Pers.) P. Karst.	Edible	Beech forest	On beech wood	Belgrad Forest
Mycenaceae	*Panellus stipticus* (Bull.) P. Karst.	Inedible	Fir forest	On fir wood	Belgrad Forest
Physalacriaceae	*Armillaria ostoyae* (Romagn.) Herink	Edible	Fir forest	On fir stump	Ilgaz Mountain National Park
Pleurotaceae	*Pleurotus ostreatus* (Jacq.) P. Kumm.	Edible	Oak forest	On oak wood	Belgrad Forest
Podoscyphaceae	*Podoscypha multizonata* (Berk. & Broome) Pat.	Inedible	Beech forest	On beech root	Belgrad Forest
Polyporaceae	*Trametes gibbosa* (Pers.) Fr.	Inedible	Beech forest	On beech wood	Belgrad Forest
	Trametes versicolor (L.) Lloyd	Inedible	Beech forest	On beech wood	Belgrad Forest
	Trichaptum biforme (Fr.) Ryvarden	Inedible	Oak forest	On oak wood	Belgrad Forest
Russulaceae	*Russula aurea* Pers.	Edible	Beech forest	On soil	Belgrad Forest
	Russula chloroides (Krombh.) Bres.	Edible	Beech forest	On soil	Belgrad Forest
	Russula virescens (Schaeff.) Fr.	Edible	Beech forest	On soil	Belgrad Forest
Tremellaceae	*Phaeotremella foliacea* (Pers.) Wedin	Inedible	Beech forest	On beech wood	Belgrad Forest

mushrooms are given in Table 2. The data in the table show that the As, Cd, Cr, Se, P, Hg, Cu, Fe, Zn, Mg, and K contents of the mushrooms collected from the Ilgaz Mountain National Park were higher than those collected from the Belgrad Forest. The elemental concentrations of mushrooms were discussed one by one at the element level in the following subsections.

Concentration of macro elements

P concentration of mushrooms

P is an important element that is among the basic components of many organic compounds in living organisms. It serves as the functional unit of many compounds, from genetic material to ATP or GTP, which are the leading actors of bioenergetics. Approximately 80% of the P in the body is found in bones as hydroxyapatites and the remaining 20% in cells, cellular fluids, and cell membranes. P also acts as an excellent buffering agent in the body (Breves and Schröder 1991). According to the data obtained from the present study, the P contents of edible and inedible mushrooms were between 2.05–11.44 and 0.28–6.43 mg/kg dry weight, respectively. The data in Table 2 show that, in general, the P contents of edible mushrooms were higher than inedible ones. *B. utriformis* was the richest mushroom species with a P concentration of 11.44 mg/kg dry weight. *M. giganteus* and *T. gibbosa* were found not contain P.

Ca concentration of mushrooms

Ca is involved in many important metabolic processes in the body. Muscle contraction is a physiological process that requires Ca. The contraction of the heart muscle also depends on the presence of Ca. It has also an important role in the transmission of the impulse in the nervous system. The main factor that makes bones and teeth strong is Ca. This element is also involved in oocyte activation and blood coagulation. Children, pregnant, and breastfeeding women need Ca. In case of not getting enough Ca, the bone structure deteriorates, the fragility of the bones increases, and osteoporosis and rachitis (as a child disease) occur (Pravina et al. 2013). The data in Table 2 show that some inedible mushrooms had higher Ca concentrations than edible ones. Ca concentrations of edible and inedible mushrooms were in the ranges of 48.4–913.7 and 15.4–3517.3 mg/kg dry weight, respectively. Among the edible mushrooms, the richest in Ca was *M. oreades* with 913.7 mg/kg of dry weight, while among the inedible ones, *P. stipticus* stood out with a Ca content of 3517.3 mg/kg of dry weight.

Mg concentration of mushrooms

Mg is an important element needed to maintain electrolyte balance in the body. It acts as a cofactor in many enzymatic reactions. Many clinical symptoms are seen in individuals

Table 2 Element concentrations of wild mushroom species (mg/kg dry weight)[1]

Mushrooms	As	Cd	Cr	Se	P	Hg	Cu
A. campestris	0.91 ± 0.21^{ab}	1.15 ± 0.08^{f}	1.30 ± 0.11^{f}	1.17 ± 0.48^{abcd}	7.98 ± 0.05^{m}	14.95 ± 0.42^{a}	27.29 ± 0.27^{i}
A. ostoyae	0.47 ± 0.06^{a}	2.40 ± 0.12^{g}	nd	nd	5.13 ± 0.05^{i}	15.14 ± 0.66^{a}	17.35 ± 0.12^{f}
B. reticulatus	0.23 ± 0.09^{a}	0.48 ± 0.05^{cd}	0.73 ± 0.06^{de}	8.13 ± 0.71^{e}	6.95 ± 0.04^{l}	37.81 ± 0.90^{e}	18.58 ± 0.12^{g}
B. mesenterica	2.18 ± 0.55^{bc}	0.33 ± 0.05^{bc}	nd	0.75 ± 0.19^{abcd}	6.43 ± 0.10^{k}	14.59 ± 0.20^{a}	18.43 ± 0.14^{g}
B. utriformis	3.45 ± 0.48^{c}	1.21 ± 0.06^{f}	0.59 ± 0.06^{cd}	1.54 ± 0.24^{cd}	11.44 ± 0.13^{o}	21.45 ± 0.26^{d}	108.57 ± 0.60^{n}
C. cibarius	0.74 ± 0.18^{a}	nd	0.26 ± 0.05^{ab}	nd	2.18 ± 0.02^{e}	15.42 ± 0.65^{abc}	20.00 ± 0.17^{h}
M. oreades	0.85 ± 0.41^{a}	0.09 ± 0.08^{a}	0.40 ± 0.06^{bc}	0.30 ± 0.20^{a}	8.63 ± 0.03^{n}	15.66 ± 0.62^{abc}	32.81 ± 0.27^{l}
M. platyphylla	0.70 ± 0.18^{a}	0.18 ± 0.06^{ab}	nd	0.98 ± 0.20^{abcd}	2.05 ± 0.02^{de}	14.94 ± 1.12^{a}	8.21 ± 0.07^{d}
M. giganteus	1.38 ± 0.61^{ab}	nd	2.34 ± 0.07^{g}	0.44 ± 0.11^{ab}	nd	17.46 ± 0.82^{bc}	3.87 ± 0.03^{a}
N. erythropus	nd	nd	0.15 ± 0.08^{a}	1.38 ± 0.14^{bcd}	5.23 ± 0.02^{i}	15.27 ± 0.58^{ab}	37.56 ± 0.12^{m}
P. stipticus	0.85 ± 0.09^{a}	0.30 ± 0.06^{b}	0.17 ± 0.05^{a}	0.52 ± 0.17^{abc}	1.19 ± 0.02^{c}	14.94 ± 1.26^{a}	9.96 ± 0.09^{e}
P. foliacea	0.16 ± 0.02^{a}	nd	nd	0.35 ± 0.18^{ab}	2.02 ± 0.01^{d}	14.03 ± 0.86^{a}	5.02 ± 0.07^{b}
P. ostreatus	nd	1.04 ± 0.07^{ef}	nd	0.61 ± 0.26^{abc}	2.65 ± 0.02^{f}	14.61 ± 0.43^{a}	5.55 ± 0.01^{b}
P. multizonata	0.87 ± 0.42^{ab}	0.92 ± 0.02^{e}	0.84 ± 0.08^{e}	1.15 ± 0.29^{abcd}	3.01 ± 0.02^{g}	15.28 ± 0.30^{ab}	6.62 ± 0.07^{c}
R. aurea	1.22 ± 0.70^{ab}	0.25 ± 0.03^{ab}	nd	1.79 ± 0.13^{d}	3.41 ± 0.02^{h}	15.01 ± 0.58^{a}	37.18 ± 0.31^{m}
R. chloroides	0.42 ± 0.10^{a}	nd	0.32 ± 0.05^{ab}	0.52 ± 0.26^{abc}	2.67 ± 0.01^{f}	14.34 ± 0.58^{a}	37.47 ± 0.16^{m}
R. virescens	nd	0.63 ± 0.03^{d}	nd	0.54 ± 0.18^{abc}	2.97 ± 0.03^{g}	17.63 ± 1.10^{c}	28.95 ± 0.16^{k}
T. gibbosa	nd	nd	nd	0.91 ± 0.35^{abcd}	nd	14.98 ± 0.65^{a}	6.88 ± 0.09^{c}
T. versicolor	0.68 ± 0.48^{a}	0.19 ± 0.03^{ab}	nd	0.70 ± 0.32^{abc}	0.28 ± 0.01^{a}	14.58 ± 0.73^{a}	6.80 ± 0.06^{c}
T. biforme	0.64 ± 0.07^{a}	1.22 ± 0.03^{f}	nd	0.58 ± 0.42^{abc}	0.79 ± 0.01^{b}	15.39 ± 0.54^{ab}	6.85 ± 0.03^{c}
Mushrooms	Mn	Fe	Zn	Al	Ca	Mg	K
A. campestris	7.81 ± 0.07^{b}	87.2 ± 0.4^{f}	67.9 ± 0.7^{l}	62.3 ± 0.5^{f}	696.8 ± 0.9^{m}	1159 ± 3^{p}	18160 ± 17^{p}
A. ostoyae	14.19 ± 0.10^{d}	85.7 ± 0.3^{f}	51.5 ± 0.7^{i}	52.3 ± 0.4^{e}	48.4 ± 0.5^{b}	1022 ± 1^{m}	20232 ± 23^{t}
B. reticulatus	23.94 ± 0.13^{fgh}	236.3 ± 0.6^{o}	105.6 ± 1.4^{o}	199.8 ± 1.0^{p}	179.0 ± 1.0^{g}	737 ± 2^{g}	13684 ± 33^{n}
B. mesenterica	25.77 ± 0.14^{i}	92.6 ± 0.2^{g}	60.2 ± 0.4^{k}	26.2 ± 0.3^{c}	97.8 ± 1.2^{d}	2500 ± 1^{t}	18660 ± 10^{r}
B. utriformis	23.24 ± 0.14^{f}	114.5 ± 0.4^{h}	317.4 ± 2.2^{s}	8.2 ± 0.2^{a}	85.2 ± 0.4^{c}	1535 ± 3^{s}	15595 ± 49^{o}
C. cibarius	18.37 ± 0.16^{e}	141.8 ± 0.8^{k}	37.8 ± 0.5^{f}	120.1 ± 0.9^{l}	526.6 ± 0.9^{k}	876 ± 3^{ik}	18822 ± 48^{s}
M. oreades	32.17 ± 0.23^{l}	123.1 ± 1.1^{i}	85.9 ± 0.5^{m}	113.7 ± 1.2^{k}	913.7 ± 4.1^{o}	1046 ± 5^{n}	12219 ± 50^{l}
M. platyphylla	24.21 ± 0.11^{gh}	52.1 ± 0.3^{c}	29.5 ± 0.1^{d}	20.0 ± 0.1^{b}	187.2 ± 1.1^{g}	783 ± 2^{h}	12600 ± 9^{m}
M. giganteus	23.51 ± 0.14^{fg}	155.6 ± 1.1^{l}	21.7 ± 0.2^{c}	76.5 ± 0.6^{i}	348.5 ± 1.9^{i}	988 ± 5^{l}	628 ± 3^{a}
N. erythropus	8.31 ± 0.04^{b}	61.2 ± 0.7^{d}	88.7 ± 0.6^{n}	49.2 ± 0.3^{d}	15.4 ± 0.3^{a}	618 ± 3^{f}	12665 ± 45^{m}
P. stipticus	149.77 ± 0.57^{p}	246.9 ± 1.0^{p}	32.4 ± 0.3^{e}	180.1 ± 0.8^{n}	3517.3 ± 8.0^{r}	866 ± 2^{i}	2995 ± 8^{e}
P. foliacea	44.72 ± 0.26^{m}	75.2 ± 0.2^{e}	40.5 ± 0.1^{g}	72.0 ± 0.7^{h}	1155.3 ± 0.9^{p}	520 ± 2^{d}	6647 ± 12^{f}
P. ostreatus	12.33 ± 0.10^{c}	203.4 ± 0.9^{n}	37.3 ± 0.3^{f}	154.7 ± 1.3^{m}	316.5 ± 2.3^{h}	1178 ± 6^{r}	10976 ± 55^{h}
P. multizonata	44.05 ± 0.23^{m}	776.1 ± 4.6^{r}	132.4 ± 0.8^{r}	355.2 ± 1.9^{r}	107.0 ± 2.0^{e}	1140 ± 7^{o}	8955 ± 59^{g}
R. aurea	12.59 ± 0.08^{c}	33.8 ± 0.1^{b}	109.4 ± 1.1^{p}	7.4 ± 0.3^{a}	84.7 ± 1.1^{c}	883 ± 3^{k}	24083 ± 90^{u}
R. chloroides	28.72 ± 0.13^{k}	173.0 ± 0.7^{m}	47.4 ± 0.2^{h}	193.0 ± 0.6^{o}	95.6 ± 1.2^{d}	590 ± 3^{e}	12019 ± 43^{k}
R. virescens	6.18 ± 0.02^{a}	11.9 ± 0.1^{a}	69.0 ± 0.5^{l}	9.6 ± 0.1^{a}	55.0 ± 1.1^{b}	464 ± 3^{c}	11696 ± 51^{i}
T. gibbosa	48.97 ± 0.39^{n}	88.8 ± 0.3^{f}	5.4 ± 0.2^{a}	66.4 ± 0.7^{g}	131.0 ± 1.0^{f}	266 ± 1^{a}	956 ± 4^{b}
T. versicolor	58.62 ± 0.50^{o}	73.9 ± 0.4^{e}	11.4 ± 0.2^{b}	54.5 ± 0.5^{e}	622.7 ± 4.1^{l}	381 ± 1^{b}	1461 ± 6^{c}
T. biforme	24.25 ± 0.10^{h}	73.2 ± 0.6^{e}	20.1 ± 0.1^{c}	47.4 ± 0.5^{d}	905.1 ± 4.2^{n}	515 ± 2^{d}	1595 ± 2^{d}

[1] The values indicated by different superscripts within the same columns show significant difference at $p < 0.05$. *nd*, not determined

with low Mg levels. It also plays a critical role in preventing various disorders such as cardiovascular diseases, osteoporosis, migraine, bronchial asthma, and diabetes (Al Alawi et al. 2018). As a result of elemental analysis, it was determined that the Mg contents of edible and inedible mushroom species examined in the present study were in the ranges of 464.0–1535.0 and 266.0–2500.0 mg/kg, respectively. In general, edible mushrooms had higher Mg content than inedible ones.

Among the edible and inedible mushrooms, *B. utriformis* (1535.0 mg/kg dry weight) and *B. mesenterica* (2500.0 mg/kg dry weight) were the richest in Mg, respectively.

K concentration of mushrooms

K is an important element that plays a role in the regulation of blood pressure in the body. It also plays an important role in generating the cell membrane potential. Therefore, K is one of the leading actors in muscle contraction and impulse transmission. In case of excessive intake, it may cause cardiac arrhythmia and subsequent death. Na and Cl are needed in the excretory system to expel excess K from the body (Nomura et al. 2019). According to the data in Table 2, the edible mushrooms richest in K were *A. ostoyae* and *R. aurea* (20232.0 and 24083.0 mg/kg dry weight, respectively). The majority of inedible mushrooms had lower K concentrations than the edible ones. Among the inedible mushrooms, *N. erythropus* and *B. mesenterica* had the highest K concentration (12665.0 and 18660.0 mg/kg dry weight, respectively).

Concentration of micro elements

As concentration of mushrooms

As is an element with high environmental abundance. Therefore, people can be easily exposed to this element. As is one of the toxic elements that cause chronic health problems. In addition, this element has genotoxic effect due to its oxidant properties. It acts on the DNA methylation profile and changes the proliferation characteristics of the cell. Therefore, As is considered one of the major causes of cancer cases (Hughes 2002). According to the data in Table 2, As contents of edible and inedible mushroom species were in the range of 0.23–3.45 and 0.16–2.18 mg/kg dry weight, respectively. While the mushroom with the lowest As content was *P. foliacea*, the As content of *B. utriformis* was higher than the other mushroom species. In addition, the As content of *B. mesenterica* (synonym: *B. montana*), an inedible mushroom species, was found to be higher than other mushroom samples (2.18 mg/kg dry weight). It was also determined that *N. erythropus*, *P. ostreatus*, *R. virescens*, and *T. gibbosa* did not contain As.

Se concentration of mushrooms

Se is an essential element in the human body that helps some important metabolic enzymes, primarily glutathione peroxidase (GPx). It is found in the organic Se forms as selenomethionine and selenocysteine in grains, nuts, and seafood. The amount of Se in foods varies depending on the Se content of the soil (Barclay et al. 1995; Rayman et al. 2008). Keshan disease, a type of cardiomyopathy, is among the most typical clinical signs of Se deficiency. This disease mostly affects young children and women of childbearing age (Yang and Xia 1995). Se also protects the cells against lipid peroxidation, and is involved in the modulation of the inflammatory response, T cell immunity, and thyroid hormone metabolism (Brown and Arthur 2001; Curran et al. 2005; Deagen et al. 1993; Kryukov et al. 2003; Shrimali et al. 2008). According to the results of the elemental analysis carried out in the present study, it was determined that the Se contents of edible mushrooms were between 0.3 and 8.13 mg/kg dry weight, while those of inedible mushrooms were in the range of 0.35–1.38 mg/kg dry weight. *B. reticulatus* was the mushroom species with the highest Se content with a Se concentration of 8.13 mg/kg dry weight. *A. ostoyae* and *C. cibarius* did not contain Se.

Cu concentration of mushrooms

Cu is one of the essential elements for human metabolism. Since this element is the basic structural component of metalloenzymes, it plays a critical role in nutrition. However, both its deficiency and excess cause some negative effects on human health. While humans may be exposed to Cu toxicity by accidental ingestion, toxicity may also result from consumption of food and drinking water containing excess Cu (Stern et al. 2007). There are data that acute Cu toxicity may be associated with some prion-borne diseases such as bovine spongiform encephalopathy (BSE) and neuronal disorders such as Alzheimer's disease (Llanos and Mercer 2002). According to the data presented in Table 2, Cu contents of edible mushroom species were in the range of 3.87–108.57 mg/kg dry weight. Cu content of inedible mushroom species was also determined to be between 5.02 and 37.56 mg/kg dry weight. Among the mushroom samples, *B. utriformis* had the highest Cu content with 108.57 mg/kg of dry weight.

Mn concentration of mushrooms

Mn is one of the most important elements involved in glucose and lipid metabolism in humans. It also acts as the cofactor for some enzymes. The most typical example of this is Mn-superoxide dismutase (SOD). It is responsible for eliminating reactive oxygen species (ROS). Therefore, the deficiency of this element leads to the emergence of some clinical symptoms. On the other hand, it has been reported that overexposure to Mn may lead to neurological disorders or ROS formation (Li and Yang 2018). The data in Table 2 show that Mn contents of inedible mushroom species were higher than the edible ones. Edible mushrooms were found to have Mn concentrations in the range of 6.18–32.17 mg/kg dry weight, while those inedible were between 8.31 and 149.77 mg/kg dry weight. *P. stipticus* was the richest mushroom species in terms of this element, with a Mn content of 149.77 mg/kg dry weight.

Fe concentration of mushrooms

Fe provides the fulfillment of many vital functions in the human body. It participates in the structure of hemoglobin and takes part in DNA synthesis and electron transport processes. However, since it has the potential to generate free radicals in the body, its concentration in tissues must be within certain limits. While Fe deficiency causes various metabolic dysfunctions, especially anemia, in its excess, some neurodegenerative diseases may occur (Abbaspour et al. 2014). Fe concentrations of edible mushroom species analyzed in the present study were between 11.9 and 236.3 mg/kg dry weight. Fe contents of inedible mushrooms were between 61.2 and 776.1 mg/kg dry weight. *P. multizonata* had Fe content of 776.1 mg/kg dry weight, by far higher than other mushroom species.

Zn concentration of mushrooms

Zn is one of the indispensable components of human metabolism. Zn deficiency usually occurs due to insufficient and unbalanced nutrition. The Zn requirement is higher especially in adolescents, babies, pregnant, and breastfeeding women. The most typical clinical sign of Zn deficiency is growth retardation. Many systems in the body are affected by zinc deficiency, especially the nervous, skeletal-muscular, reproductive, and gastrointestinal systems (Roohani et al. 2013). Elemental analysis showed that the Zn contents of the mushrooms ranged from 5.4 to 317.4 mg/kg dry weight. Edible mushrooms had higher Zn content than inedible ones. Among edible mushrooms, especially *B. utriformis* was the richest one in Zn content with 317.4 mg/kg of dry weight.

Concentration of trace/toxic elements

Cd concentration of mushrooms

Cd is one of the environmental pollutants that cause serious health problems. It causes acute poisoning in the human body. In case of short-term exposure to Cd, the lung, liver, kidneys, and testicles are adversely affected. When exposed for a long time, it has toxic effect on the renal, immune systems, and bones, and triggers tumor formation. Cd also contributes to the generation of reactive oxygen species, increasing oxidative stress (Patra et al. 2011). The data in Table 2 showed that the Cd contents of edible mushrooms were between 0.09 and 2.4 mg/kg dry weight. Cd content of inedible mushroom species was determined to be between 0.19 and 1.22 mg/kg dry weight. While the mushroom with the lowest Cd content was *M. oreades*, the Cd content of *A. ostoyae* was the highest. *C. cibarius*, *M. giganteus*, *N. erythropus*, *P. foliacea*, *R. chloroides*, and *T. gibbose* did not contain Cd.

Cr concentration of mushrooms

Scientists think that Cr is an important micronutrient in terms of health. However, there are various concerns that exposure to excessive amounts of Cr^{6+} may lead to cancer and may be associated with some pathologies in the body. Although the mechanism of action of this element in humans has not been fully elucidated, it is suggested that it causes cancer formation, genomic instability, and epigenetic modifications (Pavesi and Moreira 2020). Elemental analysis showed that half of the mushroom species analyzed in the current study did not contain Cr. The Cr contents in the rest of the edible and inedible mushrooms were between 0.26–2.34 and 0.15–0.84 mg/kg dry weight, respectively. However, the Cr contents of *A. campestris* and *M. giganteus* were significantly higher than the other samples (1.3 and 2.34 mg/kg dry weight, respectively).

Hg concentration of mushrooms

Hg is one of the most toxic of toxic elements and is considered a silent threat to human life. It has a negative effect on almost all organs and tissues in the body. Hg is usually taken into the body by consuming seafood. Prolonged exposure to excessive amounts of Hg causes toxic effects on the gastrointestinal, cardiovascular, urinary, and nervous systems and the skin. Exposure to Hg is more common in occupational groups dealing with metals (dentists, jewelers, etc.) (Kim et al. 2016). Hg concentrations of most of the mushroom species analyzed in the present study were found to be close to each other. Hg concentrations of edible and inedible mushrooms were between 14.34–37.81 and 14.03–15.39 mg/kg dry weight, respectively. Especially the Hg contents of edible mushrooms were higher than inedible ones. While *R. chloroides* had the lowest Hg content among edible mushrooms, it was determined that the Hg contents of *B. utriformis* and *B. reticulatus* were significantly higher than the others (21.45 and 37.81 mg/kg dry weight, respectively).

Al concentration of mushrooms

Al is one of the elements that are not essential for life but are the most abundant in the earth. Al prevents the fulfillment of many important biological functions. It is stated that there is a correlation between excessive exposure to this element and the emergence of some neurodegenerative disorders. These disorders include Alzheimer's, dementia, encephalopathy, and amyotrophic lateral sclerosis. Therefore, it is recommended to avoid unnecessary Al exposure (Kawahara et al. 2007). According to the data in Table 2, the Al contents of edible and inedible mushrooms were between 7.4–199.8 and 26.2–355.2 mg/kg dry weight, respectively. It has been determined that *R. aurea*, *B. utriformis*, and *R. virescens* contained low amounts of Al. Al contents of *R. chloroides* and *B. reticulatis* were found to be quite high (193.0 and 199.8 mg/kg dry weight, respectively). *P. multizonata*, an inedible mushroom, was the richest one in terms of this element with a concentration of 355.2 mg kg dry weight.

Literature discussion on the metal content of some mushroom species analyzed in the present study

When the data presented in Table 2 are evaluated as a whole, it is seen that the metal content of some mushroom species is higher than others. Among them, *B. utriformis* (syn: *Calvatia utriformis*) stood out. *B. utriformis* is known as the ectotrophic mycorrhizal symbiont of *Pinus sylvestris* and *C. excipuliformis* (Trappe 1962). Some researchers have reported that Lycoperdaceae members, including *B. utriformis*, accumulate higher rates of Hg than other mushroom species (Aichberger 1977; Seeger 1976). Falandysz et al. (2003) reported that this species was the highest Hg accumulator among fourteen mushroom species tested. According to these researchers, the Hg concentration of this mushroom is 900 times higher than the substrate. Findings reported by Pokorny et al. (2004) also support these data. Similar results were obtained in studies conducted to determine the As, Pb, and Cd content of the same mushroom species (Pokorny et al. 2004). It was concluded that the data obtained from the present study were compatible with those in the literature.

According to the data in Table 2, other mushroom species with high element content were *B. reticulatus*, *M. oreades*, *N. erythropus*, *R. aurea*, *R. chloroides*, and *R. virescens*. According to Širić et al. (2014), Cd is the most determined trace element of *Boletus* species. Literature data indicate that the Cd content of this species is in the range 0.54–4.39 mg/kg dry weight (Cocchi et al. 2006; Kalač 2001; Kalač 2010). The data obtained from the present study are compatible with the literature data. A similar situation is valid for Cu, Fe, Hg, and Zn concentrations of this species in the literature (Blanuša et al. 2001; García et al. 2009; Kalač 2010; Nikkarinen and Mertanen 2004; Szynkowska et al. 2008).

There is no specific data in the literature that *M. oreades* and *N. erythropus* accumulate certain elements in high amounts. However, it is a well-known fact that *Russula* species accumulate certain elements in large quantities. Researchers agree that these species accumulate particularly high amounts of Cu, Hg, Zn, and Cd (Busuioc et al. 2011; Kalač and Svoboda 2000). Although the amounts of these elements vary at the species level, the concentrations of these elements in the current study were higher than those reported by Kalač and Svoboda (2000).

DMI and HRI values of the edible mushrooms

DMI and HRI values were calculated in terms of Zn, Mn, Fe, Cu, Cr, and Cd contents of the edible mushroom species analyzed in the present study and the results are given in Table 3. According to the data in the table, the Zn, Mn, and Fe concentrations of the mushrooms were found within the legal limits determined by JECFA (1993) and USEPA (2002). However, both Cu and Cd content of *B. utriformis* were determined above the dose limits determined by the USEPA (2002) and EFSA (2009) (46.53 and 0.52 μg/kg body weight/serving, respectively). HRI values of *B. utriformis* in terms of Cu and Cd were also above 1.0 (1.16 and 1.44, respectively). In addition, the Cd contents of *A. campestris*, *A. ostoyae*, and *P. ostreatus* were above the dose limits determined by the EFSA (2009) (DMI: 0.49, 1.03, and 0.45 μg/kg body weight/serving, HRI: 1.36, 2.86, and 1.24, respectively).

There is no evidence in the literature that DMI and HRI values exceed legal limits due to the high Cu and Cd content of *B. utriformis*. However, there are some reports that the Zn content of this mushroom species may be high (Alonso et al. 2003; Semreen and Aboul-Enein 2011). According to Alonso et al. (2003), since Zn interacts antagonistically with some other toxic elements (Cd, Pb, Ni), it helps to reduce the risk associated with the presence of these elements in high amounts. *B. utriformis* is the edible mushroom species with the highest Zn content in the present study.

Unsupervised multivariate analysis

The exploratory multivariate method (principal component analysis) was employed to explain differentiation between mushrooms and to obtain more information on the toxic elements that mainly influence the mushrooms similarities and differences. PCA permits us to realize a reduction in dimensionality, and an extraction of a limited number of dimensions that are necessary to explain the greater part of variability with a little loss of information (Rodríguez-Delgado et al. 2002). The PCA was done on the whole set of average values with 74.15% in the first four dimensions (Table 4). These dimensions gave Eigenvalues greater than 1.0. Figure 1 shows interrelation among analyzed toxic elements and each dimensions. Eight (Cd, Cr, Se, P, Cu, Zn, Mg, K), eleven (Cd,

Table 3 Daily metal intake and health risk indexes in wild edible mushroom species

Edible mushrooms	Daily metal intake (DMI, µg/kg body weight/serving)						Health risk indexes (HRI)					
	Zn	Mn	Fe	Cu	Cr	Cd	Zn	Mn	Fe	Cu	Cr	Cd
A. campestris	29.08	3.35	37.39	11.69	0.56	0.49	0.10	0.02	0.12	0.29	0.19	*1.36*
A. ostoyae	22.07	6.08	36.74	7.43	nd[1]	1.03	0.07	0.04	0.12	0.19	nd	*2.86*
B. reticulatus	45.25	10.26	101.26	7.96	0.31	0.21	0.15	0.07	0.34	0.20	0.10	0.57
B. utriformis	136.01	9.96	49.05	46.53	0.25	0.52	0.45	0.07	0.16	*1.16*	0.08	*1.44*
C. cibarius	16.20	7.87	60.76	8.57	0.11	nd	0.05	0.06	0.20	0.21	0.04	nd
M. oreades	36.84	13.79	52.76	14.06	0.17	0.04	0.12	0.10	0.18	0.35	0.06	0.11
M. platyphylla	12.63	10.38	22.31	3.52	nd	0.08	0.04	0.07	0.07	0.09	nd	0.22
M. giganteus	9.31	10.08	66.68	1.66	1.00	nd	0.03	0.07	0.22	0.04	0.33	nd
P. ostreatus	15.97	5.29	87.18	2.38	nd	0.45	0.05	0.04	0.29	0.06	nd	*1.24*
R. aurea	46.87	5.40	14.50	15.93	nd	0.11	0.16	0.04	0.05	0.40	nd	0.30
R. chloroides	20.31	12.31	74.16	16.06	0.14	nd	0.07	0.09	0.25	0.40	0.05	nd
R. virescens	29.55	2.65	5.12	12.41	nd	0.27	0.10	0.02	0.02	0.31	nd	0.75
R_fD^{o}[2] (µg/kg body weight/day)	300[3]	140[3]	300[4]	40[3]	3[3]	0.36[5]						

[1] *nd*, not determined

[2] R_fD^{o}, reference dose

[3] USEPA (2002)

[4] JECFA (1993)

[5] EFSA (2009)

Cr, Se, P, Cu, Mn, Fe, Zn, Al, Ca, Mg), seven (Cd, P, Cu, Mn, Zn, Ca, Mg), and five (Cd, Fe, Al, Mg, K) metals were grouped on right side of the dimensions 1, 2, 3, and 4 respectively. In contrast, four (Mn, Fe, Al, Ca), one (K), five (Cr, Se, Fe, Al, K), and seven (Cr, Se, P, Cu, Zn, Ca, Mn) metals were aggregated on left side of the dimensions 1, 2, 3, and 4 respectively. However, each dimension was dominated by a few metals which can be summarized as follows: Dimension 1 had high loading of P, Cu, Zn, and K; dimension 2 was mostly characterized by Fe and Al; dimension 3 was predominantly determined by Mn and Ca; and dimension 4 was especially characterized by Cd and Se. This finding indicates that the four dimensions differentiate the mushrooms in terms of these toxic elements that are strongly linked to them. The score plots representing the positioning of analyzed mushrooms in comparison to each other are displayed in Fig. 2. Overall, there is variability between the samples since some species are segregated from other species along the different retained dimensions. This implies that there are a few homogeneous groups encompassing some species, which have certain similarities in term of metal contents. Furthermore, a few observation can be stress, indeed, *Bovistella utriformis*, *Panelus stipticus*, and *Podoscypha multizonata* was determined as an outlier suggesting these three species strongly differs from the others mushrooms. Following PCA, hierarchical clustered analyze (HCA) was carried out for a better visualization of a different groups of mushrooms. The dendogram generated by HCA revealed five clusters when considering the truncation point of 1 (Fig. 3). The graph presented two large clusters (A and B) formed by eight species, respectively. Cluster C, including one species, formed with clusters A and B; a large group entitled cluster I. Three species constituting cluster II was clearly separated from the members of cluster I; this reinforces the earlier observation when analyzing PCA score plots. Two sub-clusters were distinguished; cluster C contains two species and cluster D was determined by one species.

The exploratory multivariate analysis revealed a strong variability between the species studied in term of the toxic element concentration. According to Gebrelibanos et al. (2016), the factors related to environment (i.e., PH, concentration of metal in oil, organic matter amount) as well as the factors related to fungi involving types of

Table 4 Percentage of variability and eigenvalue explained by each dimension of principal component analysis

	Dim 1	Dim 2	Dim 3	Dim 4	Dim 5
Eigenvalue	3.92	2.33	1.49	1.16	0.95
Percentage of variance	32.69	19.39	12.43	9.64	7.93

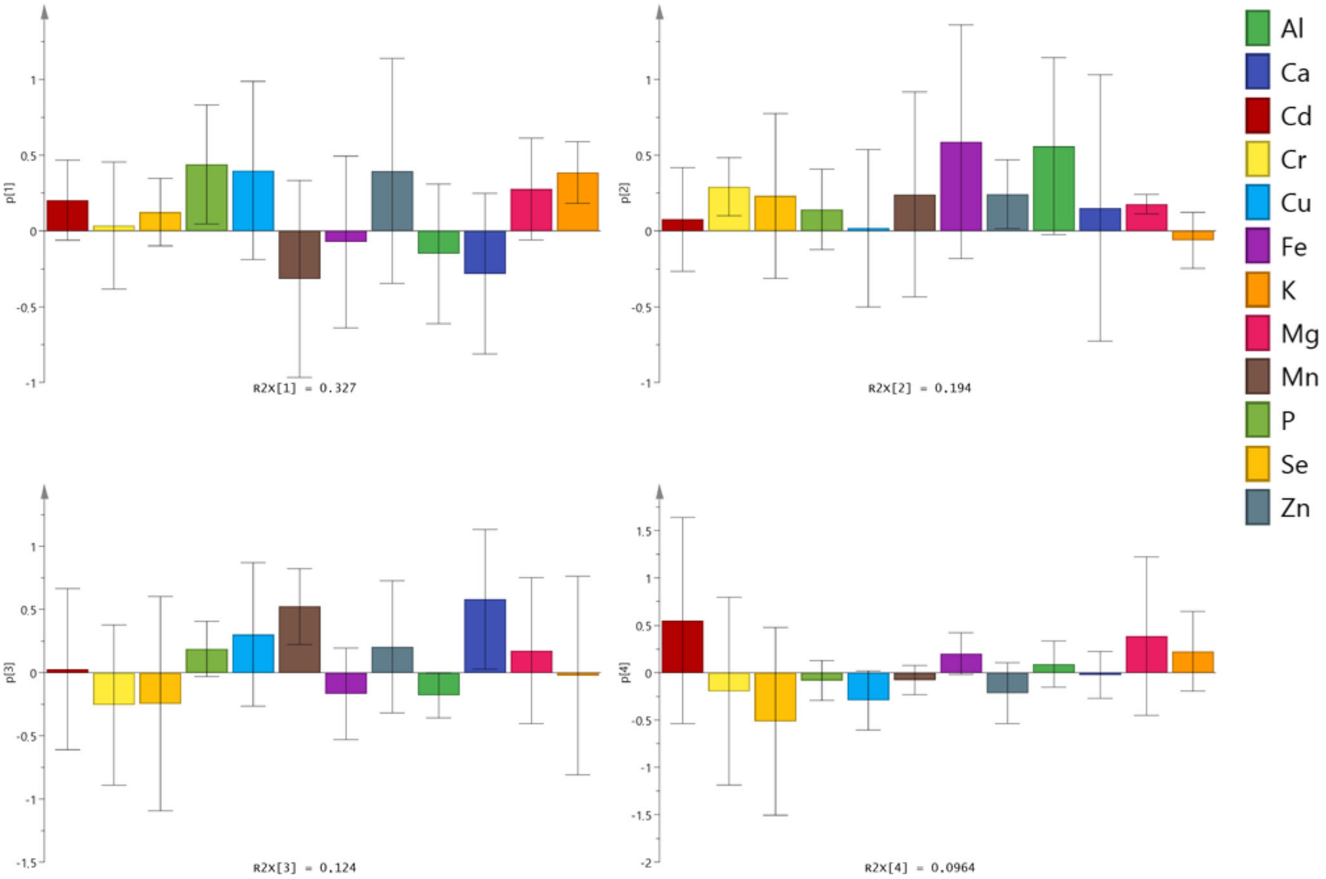


Fig. 1 Relation between the toxic elements and the first five significant dimensions

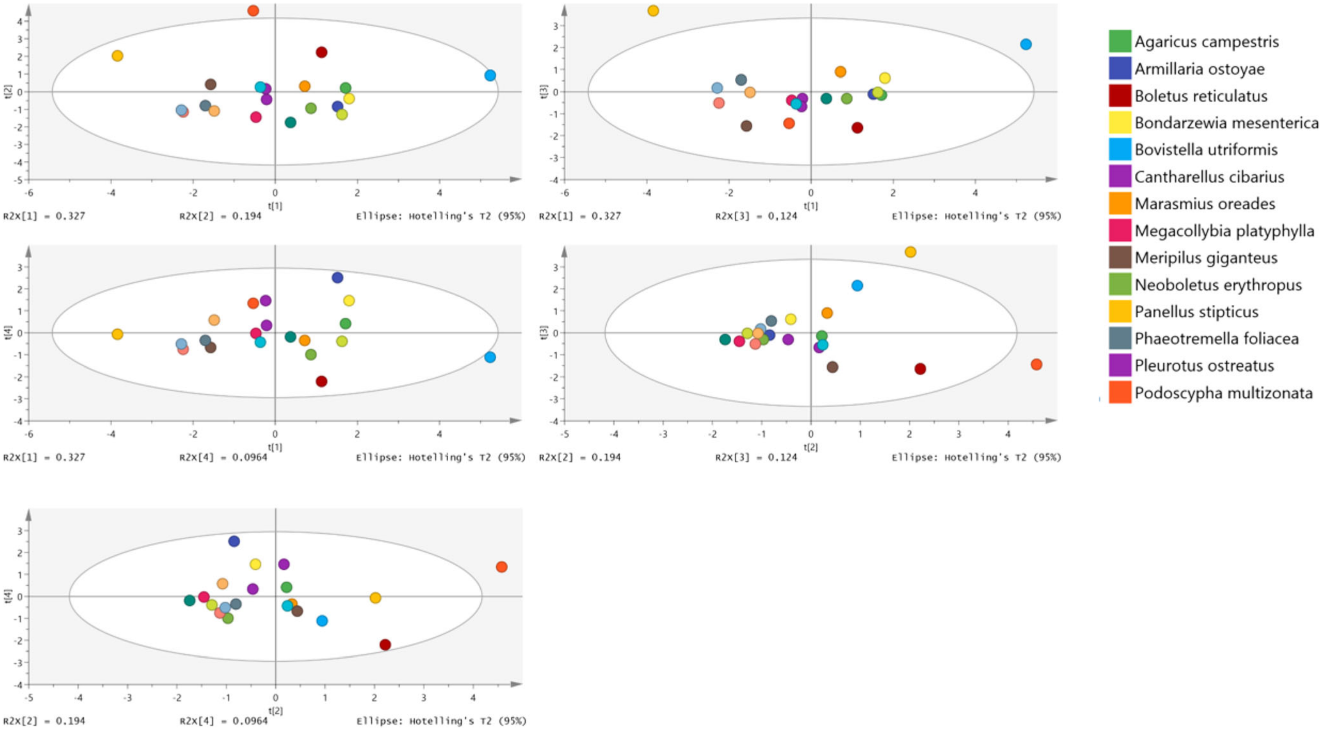


Fig. 2 Score plots of principal component analysis

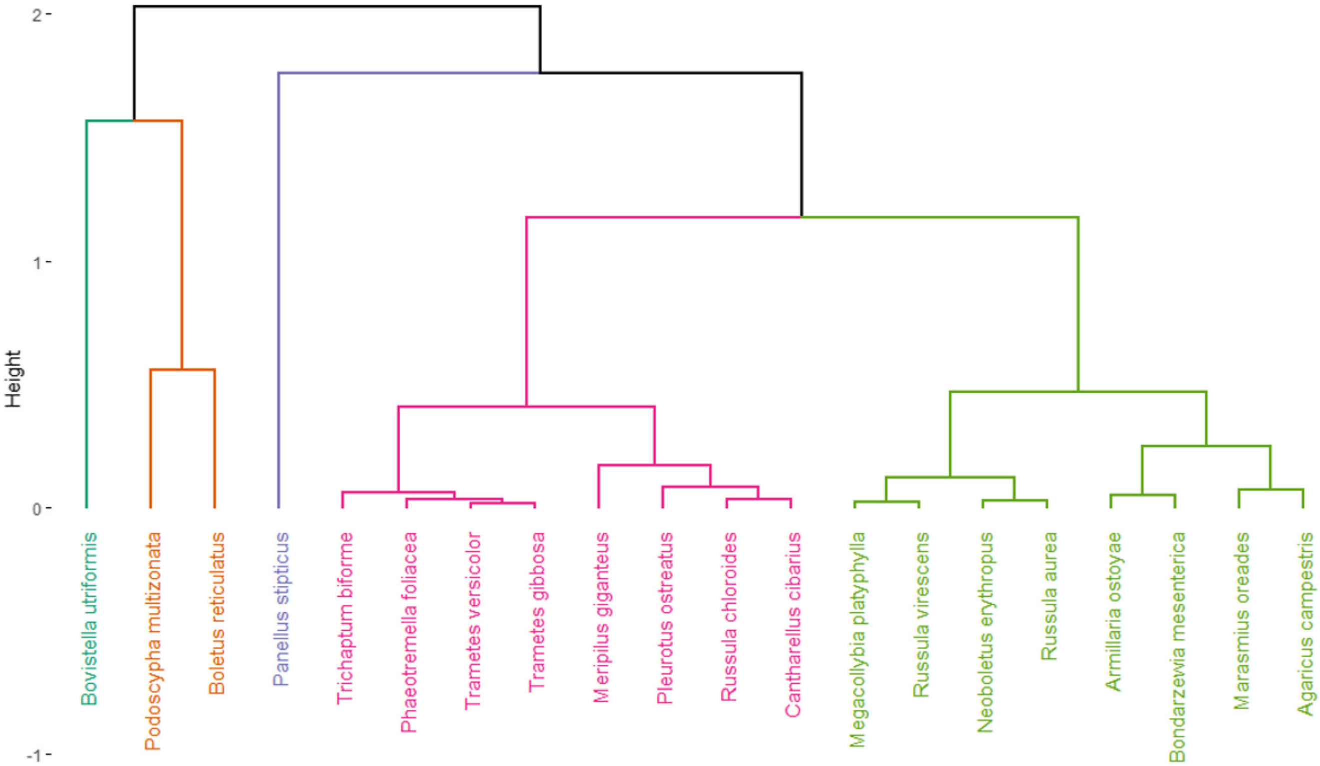


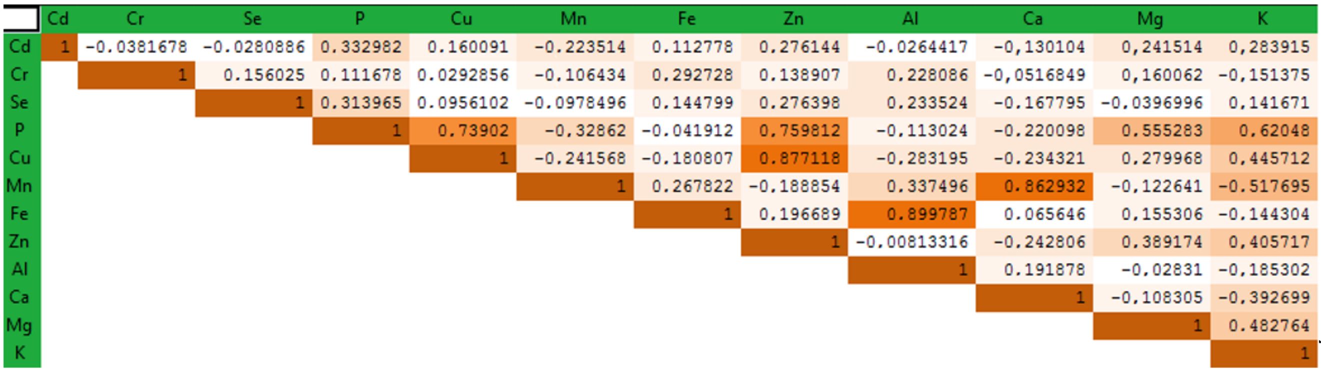
Fig. 3 Dendogram of hierarchical clustering analysis on toxic element composition of mushroom species

mushrooms (i.e., growth phases, age and density of mycelium, biochemical structure, morphological part of fruiting body) are the two main factors impacting the toxic element accumulation in mushrooms. Moreover, Semreen and Aboul-Enein (2011) suggest that toxic element contents in mushrooms vary with the distance from the source of contamination and the uptake of metal in mushrooms. The pattern of uptake of toxic element ions in mushrooms relies on the kind of plant (Dowlati et al. 2021). Accordingly, depending on the surrounding ecosystems and the mushroom species, the amount level of metals is variable. In the context of their harm to human health, it is strongly advised that appropriate steps should be taken to control, monitor, and decrease the amount of

Afterwards, Pearson coefficient was calculated to check the relationship between the metals (Fig. 4). The results showed that ZN had significantly positive correlation with P and Cu, respectively. Fe was significantly bound with Al, Ca had significant positive correlation with Mn, while Cu was significantly linked with P.

Conclusions

In this study, the element concentrations of edible and inedible mushroom species collected from the Belgrad Forest and Ilgaz Mountain National Park were documented. Additionally, DMI and HRI values of the edible ones were calculated based these analyses, Cu concentration of *B. utriformis* and Cd



	Cd	Cr	Se	P	Cu	Mn	Fe	Zn	Al	Ca	Mg	K
Cd	1	-0.0381678	-0.0280886	0.332982	0.160091	-0.223514	0.112778	0.276144	-0.0264417	-0,130104	0,241514	0,283915
Cr		1	0.156025	0.111678	0.0292856	-0,106434	0,292728	0,138907	0,228086	-0,0516849	0,160062	-0,151375
Se			1	0.313965	0.0956102	-0.0978496	0,144799	0,276398	0,233524	-0.167795	-0,0396996	0,141671
P				1	0.73902	-0,32862	-0.041912	0,759812	-0,113024	-0.220098	0.555283	0,62048
Cu					1	-0.241568	-0,180807	0.877118	-0,283195	-0.234321	0,279968	0,445712
Mn						1	0.267822	-0,188854	0.337496	0.862932	-0,122641	-0.517695
Fe							1	0,196689	0.899787	0.065646	0,155306	-0.144304
Zn								1	-0,00813316	-0.242806	0.389174	0,405717
Al									1	0.191878	-0,02831	-0,185302
Ca										1	-0,108305	-0,392699
Mg											1	0.482764
K												1

Fig. 4 Pearson coefficient depicting the correlation between the toxic elements

concentrations of *A. campestris*, *A. ostoyae*, *B. utriformis*, and *P. ostreatus* were found to exceed the legal limits determined by authorities. Therefore, it was concluded that the consumption of these mushrooms collected from the localities in question should be consumed in a controlled manner.

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Availability of data and materials All data generated or analyzed during this study are included in this published article and its supplementary information file.

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Declarations

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Competing interests The authors declare no competing interests.

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