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Wild mushrooms from Eastern Black Sea Region (Türkiye): Element concentrations and their health risk assessment

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Doğu Karadeniz Bölgesi (Türkiye)'den yabani mantarlar: Element konsantrasyonları ve sağlık risk değerlendirmesi

Abstract: The aim of this study is to determine the mineral contents of wild edible mushrooms. The potassium (K), magnesium (Mg), calcium (Ca), manganese (Mn), iron (Fe), zinc (Zn), copper (Cu), nickel (Ni), cadmium (Cd) and lead (Pb) contents of twenty four edible mushrooms, collected from East Black Sea Region, Türkiye, were analyzed. The studied mushrooms varied widely in their content of both essential and toxic deleterious elements. The minimum and maximum mineral contents of mushrooms were determined as mg/kg dw for K (4573-15645), Mg (173-1421), Ca (24-711), Mn (5.34-90.64), Fe (44.78-236.95), Zn (24.81-119.03), Cu (11.02-174.01), Ni (0.95-2.86), Cd (0.05-22.57) and Pb (0.01-2.07). The potassium content was found to be higher than those of the other minerals in all the mushrooms. In addition to the metal contents, the daily intakes of metal (DIM) and Health Risk Index (HRI) values of edible mushrooms were also calculated. Lead and cadmium were present but at concentrations that are not hazardous to human health except for *Russula vinosa*. The K, Mg, Zn, and Ni concentrations were determined to be high in *Russula integra*. Mushrooms have become increasingly attractive as functional foods for their potential beneficial effects on human health. Due to the toxic minerals they carry, mushrooms should be taken into consideration during their consumption as human food. The differences and similarities between mineral contents were established by Principal Component Analysis. Also, mushrooms are important in the ecosystem because they are able to biodegrade the substrate and to collect heavy metal.

Key words: Edible mushrooms, minerals, toxic element

Özet: Bu çalışmanın amacı, yenilebilir yabani mantarların mineral içeriklerini belirlemektir. Doğu Karadeniz Bölgesi'nden toplanan yirmi dört yenilebilir mantarın mineral içerikleri Potasyum (K), magnezyum (Mg), kalsiyum (Ca), mangan (Mn), demir (Fe), çinko (Zn), bakır (Cu), nikel (Ni), kadmiyum (Cd) ve kurşun (Pb) analiz edilmiştir. İncelenen mantarlar, hem temel hem de toksik elementleri büyük farklılıklar göstererek içermektedirler. Mantarların minimum ve maksimum mineral içerikleri K (4573-15645), Mg (173-1421), Ca (24-711), Mn (5.34-90.64), Fe (44.78-236.95), Zn (24.81-119.03), Cu (11.02-174.01), Ni (0.95-2.86), Cd (0.05-22.57) ve Pb (0.01-2.07) için mg/kg km olarak belirlenmiştir. Tüm mantarlarda potasyum içeriği diğer minerallerden daha yüksek bulunmuştur. Metal içeriklerinin yanı sıra yenilebilir mantarların Günlük Metal Alımları ve Sağlık Risk İndeksi değerleri de hesaplanmıştır. Kurşun ve kadmiyum *Russula vinosa* dışında insan sağlığına zararlı olmayan konsantrasyonlarda belirlenmiştir. *Russula integra* da K, Mg, Zn ve Ni konsantrasyonlarının yüksek olduğu belirlenmiştir. Mantarlar, insan sağlığı üzerindeki potansiyel yararlı etkileri nedeniyle fonksiyonel gıdalar olarak giderek daha çekici hale gelmiştir. Mantarlar, taşıdıkları toksik mineraller nedeniyle insan gıdası olarak tüketilmeleri sırasında dikkate alınmalıdır. Mineral içerikleri arasındaki farklılıklar ve benzerlikler Temel Bileşen Analizi ile de belirlenmiştir. Ayrıca mantarlar, ağır metal toplamak için substratı biyolojik olarak parçalayabildikleri için ekosistemde önemlidirler.

Anahtar Kelimeler: Yenen mantarlar, mineraller, zehirli element

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1. Introduction

Wild grown mushrooms are considered as a popular delicacy in many countries mainly in Europe, Asia and Africa. Their therapeutic application includes the prevention and treating of diseases such as hypertension, hypercholesterolemia, and cancer. It has been documented that these curational properties are mainly due to their chemical composition. Mushrooms are organisms that can grow both in uncontaminated rural ecosystems and in urban areas with high industrial pollution (Karaman et al., 2012; Rakić et al., 2014). They are important in terms of pharmaceuticals as well as their ecological values (Sirić et al., 2016). Therefore, it is thought that mushrooms have a high potential to be used in the treatment of some diseases such as cancer, obesity, hypertension, and hyperglycemia, which threaten human beings and have a high prevalence (Guggenheim et al., 2014; Guillamón et al., 2010).

Mushrooms are considered not only as spice and food ingredients, but also as a nutritional supplement in the human diet which also plays the role of functional foods. Both wild and cultivated mushrooms have many beneficial compounds for human health. For thousands of years mushrooms have constituted a valuable source of nutrients since they contain carbohydrates, protein, dietary fibre, vitamins and minerals, but are low in calories, fat and contain negligible amounts of cholesterol (Kozarski et al., 2015; Nowakowski et al., 2021). Since mushrooms are low-calorie foods, they are particularly preferred in diets. In addition, they are rich in vitamins, elements (both macro and microelements), and proteins (Gargano et al., 2017).

Some mushroom species have therapeutic value due to their primary (e.g., polysaccharides and polysaccharide–protein complexes, etc.) and secondary metabolites (e.g., alkaloids, terpenoids, phenolic compounds, etc.) and are also known as medicinal mushrooms in the literature (De Silva et al., 2013; Duru and Çayan, 2015; Gargano et al., 2017). Studies have shown that mushrooms have many biological/pharmacological activities (such as neuroprotective, cardiovascular, antioxidant, antimicrobial, antitumor, etc.). These activities are thought to be due to compounds that they contain (Gargano et al., 2017; Muszynska et al., 2018; Nowakowski et al., 2021).

In addition to the benefits mentioned above, fungi are also responsible for the cycle of elements in nature, as they also fulfill the functions of breaking down organic materials (Sesli et al., 2008). With the increasing interest of humans in wild mushroom species due to their nutritional properties, researchers have started to focus on whether the concentrations of elements accumulated in these organisms pose a risk to human health (Işıldak et al., 2004; Kalač and Svoboda, 2000; Mleczek et al., 2016a; Severoğlu et al., 2013; Sirić et al., 2016). Some mushroom species can accumulate certain metals at higher concentrations than other organisms living in the same ecosystem (Falandysz et al., 2008; Kalać, 2010; Rakić et al., 2014; Sesli et al., 2008; Severoğlu et al., 2013; Sirić et al., 2016). The biosorption of elements by mushroom species is a well-known mechanism studied by many researchers (Mleczek et al., 2016a; Sesli and Dalman, 2006; Sesli et al., 2008). When toxic metals and metalloids such as Hg, Pb, Cd, As, etc. accumulate in fruiting bodies of mushrooms at high concentrations, people who feed on these mushrooms can also accumulate them in their bodies. In this way, these metals can cause various adverse effects on human metabolism (Falandysz and Borovicka, 2013; Mleczek et al., 2016b; Rzymski et al., 2016). This makes controlling the toxic element content of wild mushroom species a priority issue (Agrawal and Dhanasekaran, 2019; Rashid et al., 2018). High metal accumulation in mushrooms is also under the scrutiny of researchers as they are indicators of metal pollution in the ecosystem as well as their negative effects on human health (Li et al., 2017).

Despite many positive aspects of mushroom consumption, there are risks associated with their ingestion, such as poisoning with pesticides or harmful elements, e.g. mercury (Hg), lead (Pb), cadmium (Cd) and arsenic (As), which might accumulate in mushrooms (Sirić et al., 2017).

Wild mushroom species can be classified as edible, inedible, poisonous, soil-grown, wood-grown, parasitic or saprophytic, etc.. These differences in the way of life of mushrooms have a major impact on their metal accumulation capacity (Kalać, 2010; Mleczek et al., 2016a). In addition, areas where mushroom species grow provide important clues in environmental researches in terms of metal accumulation (Kalac, 2001; Rakič et al., 2014; Sirič et al., 2016).

The purpose of this study was to determine K, Mg, Ca, Mn, Fe, Zn, Cu, Ni, Cd and Pb in some edible collected from Bayburt, Gümüşhane, Rize and Artvin (East Black Sea, Turkey). In addition, the daily intakes of metal (DIM) and Health Risk Index (HRI) values of edible mushroom species were also calculated, and their potential effects on human health were discussed.

2. Materials and Method

2.1. Samples

Fully matured fruiting bodies of mushroom samples were collected from Rize, Artvin, Gümüşhane and Bayburt Eastern Black Sea, Turkey. Information on the habitats and taxonomic records of mushroom species are given in Table 1. Colour slides of the macrofungal specimens were taken in their natural habitats during fields studies. After relevant notes were taken of their morphological and ecological features, they were put in private prepared boxes and brought to the laboratory. Their spore prints were taken and spore dimensions were measured using an ocular micrometer. Then, dried specimens were placed in locked polyethylene bags and kept in a deep freezer at -20 ℃ to protect against parasites.

Identification of the specimens was performed according to Breitenbach and Kranzlin (1984–2000), Bresinsky and Besl (1990), Buczacki (1989) and Dahncke and Dahncke (1989). All specimens are kept in the Herbarium of Yuzuncu Yıl University, Department of Biology (VANF). Table 1 shows the taxa of wild edible macrofungi.

2.2. Method

Atomic absorption spectrophotometer (Varian Techtron Model AAS 1000, Varian Associates, Palo Alto, CA) was used for the determination of the minerals (Ca, Mg, K, Fe, Zn, Cu, Mn, Pb, Ni and Cd) in dried fruit bodies of macrofungi. Each dried mushroom sample was weighed as 4-5 g and placed in a porcelain crucible and ashed at 550 °C for 18-20 h. then the ash was dissolved in 1ml concentrated HNO_3, evaporated to dryness, heated again at 550 °C for 4 h, treated successively with 1 ml HNO_3 and 1 ml H_2O_2 and then diluted with double deionized water up to a volume of 25 ml. Three blank samples were treated in the same way. The species, which were digested in an acid solution of HNO_3 were determined by the AAS system using different lamps, and calibrated with related minerals in different concentrations for different micronutrients (AOAC, 1990). To check for possible contamination by reagents or glassware, blanks containing 4ml of ultrapure concentrated HNO_3 and 4 ml H_2O_2 were run together with analytical samples and every batch of analytical samples was run together with the standard matrix. The values of Ca, Mg, K, Fe, Zn, Cu, Mn, Pb, Ni and Cd were calculated as mg/kg dw. Detection limit is defined as the concentration corresponding to three times the Standard deviation of ten aborbance measurements of blank solution divided by the slope of calibration curve for each element ($3xs_b/m$). Detection limits of elements as mg/kg by AAS were found to be 0.012 for K, 0.003 for Mg, 0.015 for Ca, 0.029 for Mn, 0.060 for Fe, 0.013 for Zn, 0.041 for Cu, 0.063 for Ni, 0.032 for Cd and 0.10 for Pb. The results were within limits of quantification for above minerals (calculated as 10-fold of standard deviation from ten replicates of the instrumental blank solution: $10xs_b/m$) 0, 2, 4, 8 and 16 mg/g or mg/kg, respectively. Mushrooms were selected normally harvested for consumption (pileus+stipe). For all the mushroom species, at least three samples were analysed.

2.3. Determination of DIM and HRI values

DIM and HRI analyses of mushrooms were performed following the method given in the literature (Cui et al., 2004; Liu et al., 2015). While calculating DIM values of which details were also given in the supplementary file, RfD^o values set by USEPA (2002) were taken into consideration.

Table 1. Taxa of wild edible macrofungi collected from Bayburt, Gümüşhane, Rize and Artvin regions.

	Macrofungi taxa	Collection locality, habitat/substrate
1	*Agaricus bisporus* (J.E. Lange) Imbach	Gümüşhane, Torul, Köprübaşı district, apple trees garden
2	*Chlorophyllum rhacodes* (Vittad.) Vellinga	Rize, İkizdere, Çamlık district, in conifer forest
3	*Macrolepiota procera* (Scop.) Singer	Artvin, Şavşat, Sahara National Park, in conifer forest
4	*Amanita rubescens* Pers.	Rize, Çamlıhemşin, Ayder Plateau, in conifer forest
5	*Armillaria ostoyae* (Romagn.) Herink	Gümüşhane, Kürtün, Örümçek Forest, in conifer forest
6	*Pleurotus dryinus* (Pers.) P. Kumm.	Gümüşhane, Özkürtün, Karaçukar Forest, in conifer forest
7	*Pleurotus ostreatus* (Jacq.) P. Kumm.	Bayburt, Akşar, on willow
8	*Cerioporus squamosus* (Huds.) Quél.	Bayburt, Akşar, on stumps of poplar tree
9	*Boletus edulis* Bull.	Artvin, Şavşat, Karagöl National Park, in conifer forest
10	*Neoboletus praestigiator* (R. Schulz) Svetash., Gelardi, Simonini & Vizzini	Gümüşhane, Zigana Mountein, in conifer forest
11	*Leccinum scabrum* (Bull.) Gray	Gümüşhane, Torul, Günay Village, mixed forest
12	*Suillus luteus* (L.) Roussel	Gümüşhane, Torul, Günay Village, mixed forest
13	*Lepista nuda* (Bull.) Cooke	Rize, Çamlıhemşin, Ayder Plateau, in conifer forest
14	*Lepista personata* (Fr.) Cooke	Bayburt, Aydıntepe, Centrum, garden
15	*Hydnum repandum* L.	Artvin, Şavşat, Karagöl National Park, in conifer forest
16	*Lactarius deliciosus* (L.) Gray	Gümüşhane, Torul, Günay Village, mixed forest
17	*Lactifluus piperatus* (L.) Roussel	Rize, Çamlıhemşin, Ayder Plateau, in conifer forest
18	*Lactarius salmonicolor* R. Heim & Leclair	Gümüşhane, Zigana Mauntein, in conifer forest
19	*Lactifluus volemus* (Fr.) Kuntze	Rize, Çamlıhemşin, Ayder Plateau, in conifer forest
20	*Russula delica* Fr.	Rize, Çamlıhemşin, Ayder Plateau, in conifer forest
21	*Russula integra* (L.) Fr.	Rize, İkizdere, Çamlık village, in conifer forest
22	*Russula adusta* (Pers.) Fr.	Rize, Centrum, mixed forest
23	*Russula vinosa* Lindblad	Rize, Çamlıhemşin, Ayder Plateau, in conifer forest
24	*Neoboletus erythropus* (Pers.) C. Hahn	Rize, Çamlıhemşin, Ayder Plateau, in conifer forest

3. Results and discussion

Information about the taxonomic details, substrates, and edibility of the mushroom species analyzed in this study are given in Table 1. The concentrations of K, Mg, Ca, Mn, Fe, Zn, Cu, Ni, Cd and Pb in mushrooms are presented in Table 2 (in mg/kg dry weight). Additionally, DIM and HRI values of mushrooms were calculated and documented in Table 3.

Data on potassium and nine metals most frequently determined in mushrooms from Gümüşhane, Rize, Artvin, Bayburt in Turkey are given for 24 species in Table 2. All the metal concentrations were determined on a dry weight basis. The metal contents varied across the locations and the mushroom species sampled. The variations could be as a result of differences in substrate composition which is determined by the ecosystem as well as the differences in the absorption of individual metals by the mushroom species.

Heavy metals can pass through to mushrooms from the contaminated soil and environment. Soil's composition, contaminated water, air and soil, metal based pesticides and fertilizers, industrial emissions are the main factors that affect the heavy metal concentrations in mushrooms.

The acid–base balance, the blood pressure and the regulation of osmotic pressure of the organism are controlled by potassium, which is mainly present in intracellular fluid (Yellen, 2002). Potassium content was higher than other minerals in all mushrooms in this study, varying between 4573 (*Lepista personata*) and 15645 mg/kg dw (*Russula integra* (L.) Fr.

In general, most of the mushrooms studied contained considerably high amounts of minerals. The levels of essential elements in mushroom species were higher than those of toxic elements. Gençcelep et al. (2009) reported the potassium contents of wild edible mushrooms as being between 12600 and 29100 mg/kg dw. Wang et al. (2014) found that potassium content was between 16000 and 37000 mg/kg in dry matter. Sanmee et al. (2003) reported that potassium accumulation in mushrooms could rise up to 45200 mg/kg. Liu et al. (2012) reported that the lowest potassium value (1300 mg/kg dw) was measured in *Melanoleuca gigantea* and *Melanoleuca arcuata*, the highest potassium value (4600 mg/kg dw) was found in *Boletus griseus*. The greatest concentrations of K were obtained in *C. cibarius* (41823.3 mg/kg), whereas the lowest was in *Boletus edulis* (11266.9 mg/kg) (Cvetkovic at al., 2015). Usual potassium content in mushrooms varies between 20000 and 40000 mg/kg dw (Gençcelep et al., 2009; Kalač, 2009). Literature data showed that K concentration of mushroom samples was between 19000 and 54073 mg/kg (Alaimo et al., 2018; Mleczek et al., 2020). In this study, potassium levels were lower than reported values in the literature. Showing that mushrooms are an excellent source of potassium in the human diet.

Magnesium content 173 mg/kg dw in *Cerioporus squamosus* and 1421 mg/kg dw in *Lactarius volemus*. The level of magnesium reported in this study was relatively low compared to earlier published reports (Demirbaş, 2000) which was magnesium content ranged from 330 mg/kg dw in *Tricholoma anatolicum* to 6560 mg/kg dw in *Morchella*

Table 2. Amounts of total K, Mg, Ca, Mn, Fe, Zn, Cu, Ni, Cd and Pb of wild edible mushrooms from Bayburt, Gümüşhane and Rize Region of Turkey (mg/kg dw).

No	Mushrooms	K	Mg	Ca	Mn	Fe	Zn	Cu	Ni	Cd	Pb
1	*Agaricus bisporus*	7854	880	138	14.30	140.04	46.00	23.46	1.84	0.25	1.06
2	*Chlorophyllum rhacodes*	10900	536	309	13.83	147.92	66.15	18.01	2.19	0.12	1.13
3	*Macrolepiota procera*	6810	738	197	11.21	83.47	60.22	47.56	1.55	0.34	0.67
4	*Amanita rubescens*	15527	256	262	18.46	236.95	61.07	34.47	2.54	0.14	0.96
5	*Armillaria ostoyae*	8749	255	51	17.76	218.25	33.45	35.96	1.09	6.30	1.03
6	*Pleurotus dryinus*	8098	441	338	21.42	197.76	57.66	24.35	1.91	0.84	1.19
7	*Pleurotus ostreatus*	9042	704	452	18.75	160.01	53.97	36.55	1.74	0.16	1.66
8	*Polyporus squamosus*	7348	173	24	10.08	201.25	25.03	20.99	1.52	0.18	1.04
9	*Boletus edulis*	6140	394	270	20.14	142.18	39.45	22.58	1.91	0.05	1.30
10	*Neoboletus praestigiator*	7275	231	96	9.40	71.45	42.95	23.31	0.95	0.36	1.64
11	*Leccinum scabrum*	6005	373	163	16.20	64.04	33.58	35.94	1.39	0.46	1.07
12	*Suillus luteus*	11127	393	117	12.02	225.17	57.86	33.21	1.64	0.09	1.19
13	*Lepista nuda*	7322	420	146	33.25	119.18	58.64	23.38	2.08	0.83	2.07
14	*Lepista personata*	4573	667	306	90.64	49.94	24.81	14.64	1.59	0.96	0.48
15	*Hydnum repandum*	11455	1145	47	23.31	64.43	79.05	39.34	1.66	0.52	1.61
16	*Lactarius deliciosus*	4867	661	73	15.94	77.42	28.35	11.02	1.31	1.38	0.46
17	*Lactarius piperatus*	7364	773	289	14.10	165.05	55.23	17.34	2.54	0.59	1.05
18	*Lactarius salmonicolor*	11947	781	199	38.05	119.12	65.10	40.31	2.28	0.47	0.59
19	*Lactarius volemus*	8437	1421	711	6.38	59.08	63.49	145.92	2.12	0.72	0.12
20	*Russula delica*	7887	438	133	5.34	101.88	61.56	14.89	2.03	1.24	0.97
21	*Russula integra*	15645	1249	275	11.77	44.78	119.03	72.01	2.86	0.16	0.73
22	*Russula nigricans*	11133	731	177	25.41	102.54	39.38	12.05	2.22	2.73	1.05
23	*Russula vinosa*	8673	1190	574	32.22	89.09	77.77	174.01	2.30	22.57	1.37
24	*Neoboletus erythropus*	6815	417	309	9.32	91.69	46.58	22.69	1.45	0.33	0.01
	Mean±SD	8791 ±2885	636 ±343	235.6 ±166.1	20.38 ±17.47	123.86 ±59.11	54.01 ±20.67	39.33 ±39.72	1.86 ±0.47	1.74 ±4.62	1.01 ±0.48
	Minimum	4573	173	24	5.34	44.78	24.81	11.02	0.95	0.05	0.01
	Maximum	15645	1421	711	90.64	236.95	119.03	174.01	2.86	22.57	2.07

deliciosa. In our previous study, the concentration levels of Mg in *Morchella vulgaris* 1920 mg/kg, *Helvella lacunosa* 1190 mg/kg, *Lepista nuda* 3410 mg/kg were found (Gençcelep et al., 2009). Liu et al. (2012) reported that the magnesium contents of the mushrooms ranged from 84 mg/kg dw in *Aspropaxillus giganteus* (Sowerby) Kühner & Maire to 550 mg/kg dw in *Macrocybe gigantea*.

Previously reported magnesium contents in mushrooms varied between 800 and 1800 mg/kg dw (Kalač, 2009). The lowest magnesium value, 248 mg/kg dw *Boletus tomentipes* Earle, was found by Li et al. (2011). Sanmee et al. (2003) reported that mature *Astraeus hygrometricus* (Pers.) Morgan had 1600 mg/kg of Mg concentrations. In this study, magnesium concentrations of same mushrooms species were found low. As a result, environmental factors are very important to amount of metal concentrations in mushrooms. Magnesium levels in this study are in agreement with the value reported in the literature.

In the present study, the calcium contents of the mushrooms ranged from 24 mg/kg dw in *Cerioporus squamosus* to 711 mg/kg dw in *Lactarius volemus*. In our previous study, the concentration levels of Ca in *Morchella vulgaris* 870 mg/kg, *Helvella lacunosa* 470 mg/kg, *Lepista nuda* 8800 mg/kg were found (Gençcelep et al., 2009). Previously reported calcium contents of mushrooms varied from 100 to 500 mg/kg dw (Kalač, 2009). The calcium contents in our mushroom samples are lower than the values reported in the literature. The accumulation of metals in mushrooms has been found to be affected by environmental and fungal factors. But, it seems to be higher when compared to the concentrations obtained by Sanmee et al. (2003) (100-2400 mg/kg dw). The results of nutritionally valuable minerals show that twenty four mushroom species contained high amounts of potassium, calcium, magnesium and iron. Most of them contain little lead, nickel, cadmium or copper. Minerals in the diet are required for metabolic reactions, transmission of nerve impulses, rigid bone formation and regulation of water and salt balance (Kalač et al., 2004).

Manganese was also determined in all mushrooms. The manganese content of the mushrooms studied in the present work ranged from 5.64 mg/kg dw in *Russula delica* to 90.64 mg/kg for *Lepista personata*. The Mn toxic limits for plants is 400–1000 mg/kg (Zhu et al., 2011). Some literature reported Mn levels in mushrooms of as high as 21.7–74.3 mg/kg (Mendil et al., 2004), and 14.2–69.7 mg/kg (Soylak et al., 2005) and 7.1-81.3 mg/kg, 5.54-135 mg/kg dw (Falandysz et al., 2017). Literature data showed that the Mn concentrations of the mushrooms ranged from 0.081 to 188.8 mg/kg (Ayaz et al., 2011; Wang et al., 2017). Other studies have also reported varying metal concentrations in mushrooms (Falandysz et al., 2017; Pelkonen et al., 2006). The manganese values in this study are found almost the same in the literature.

Fe is found in the structure of hemoglobin, whose main function is to carry oxygen, and therefore is an important element. It is known that about 70% of the Fe in the human body is used for hemoglobin production. Fe is the main structural component of myoglobin, which is abundant in muscle cells, as well as hemoglobin. Fe deficiency causes anemia in organisms (Gupta, 2014). The Fe contents of the mushrooms analyzed in the present study were found to range between 44.78 (*Russula integra*) and 236.95 (*Amanita rubescens*) mg/kg dw. According to the literature data, Fe concentrations of the mushroom samples were between 0.04 and 10558 mg/kg (Niemiec et al., 2018; Rasalanavho et al., 2020). Iron values in mushroom samples (as reported) ranged from 31.3-1190 mg/kg (Sesli and Tüzen, 1999), 56.1-7162 mg/kg (Işıloğlu et al., 2001), 50.1-842.0 mg/kg (Gençcelep et al., 2009), respectively. The iron values in the present study are in lower than with reported values in the literature.

Mushrooms are known as zinc accumulators and the sporophore: substrate ratio for Zn ranges from 1 to 10 mg/kg (Işıloğlu et al., 2001). The zinc content was the lowest (24.81 mg/kg dw) in *Lepista personata*, whereas it was highest (119.03 mg/kg dw) in *Russula integra*. The reported literature zinc content ranged between 22.10 and 185 mg/kg dw (Gençcelep et al., 2009; Kalač et al., 2004; Kaya and Bağ, 2010). Sarıkürkçü et al. (2012) found the highest Zn values in *Helvella leucopus* and *Tricholoma auratum* (354 and 356 mg/kg, respectively). The lowest Zn content was found in *Lyophyllum decastes* and *Rhizopogon roseolus* (46 and 47 mg/kg, respectively). In this study, some mushroom species have higher zinc content more than 50 mg/kg, this counts total samples of %50. Therefore, metal accumulation may be more owing to soil pollution. Zn is an essential nutrient that has an important role in biological systems. Zinc is necessary for the functioning of various enzymes and plays an essential role in DNA, RNA, and protein synthesis. The major symptoms of zinc deficiency are delayed growth and slow maturation (WHO, 2004).

Copper with other metals could be transported from the anthropogenic sources of emission to remote areas with moving air masses, but they are largely deposited locally (Nygård et al., 2012). Minimum and maximum values of copper were 11.02 and 174.01 mg/kg dw in *Lactarius deliciosus* and *Russula vinosa*. Copper contents of mushroom samples in the literature have been reported to be in the range of 4.71-51.0 mg/kg (Tüzen et al., 1998) and 10.3-145 mg/kg (Sesli and Tüzen, 1999). Copper contents found in this study parallel those reported in the literature. In this study, *Leccinum versipelle* (102.40 mg/kg) and *Russula delica* (128.94 mg/kg) were collected near the downtown of Erzincan, therefore copper contents of these samples were found higher than the others. In our previous study, the concentration levels of Cu in *Pleurotus ostreatus* 47.1 mg/kg and *Lepista nuda* 26.6 mg/kg were found (Gençcelep et al., 2009). Cu is an essential element. Enzymes containing copper are important for the body to transport and use iron. In 1996, a joint FAO/International Atomic Energy Agency/ WHO official report set an upper limit for the safe range of population mean exposures for adults of 0.2 mg/kg body weight per day (WHO, 1996). According to Gast et al. (1988) Cu may be harmful to both humans and animals when its concentration exceeds the safe limits. Our results are lower than some values reported in literature: 13.4–50.6 mg/kg (Soylak et al., 2005) and 15–73 mg/kg (Sesli et al., 2008).

Nickel was determined all mushrooms. *Russula integra* var. *integra* contained high nickel content with an amount of 2.86 mg/kg dry matter. The reported Ni values for wild-growing mushrooms were 0.4-15.9, 0.4-2, 1.72-24.1 mg/kg (Işıloğlu et al., 2001; Kalač et al., 2004; Soylak et al., 2005), respectively. The Ni levels are generally in agreement with previous studies. The obtained Ni levels in almost all mushrooms are higher than (1 mg/kg dw) the allowed amount (0.05-5 mg/kg) of National Academy of Sciences (1975) for plants and foods (1975) (Table 2). Nickel has been linked to lung cancer and the tolerable upper intake level for this toxic element is reported as 1 mg/day.

Cd is mostly used in batteries, TV screens, lasers, cosmetics, paint and galvanized steel. The most common route of Cd exposure is inhalation and therefore, cigarette smoking is the primary source of contact with the element. Foods most commonly contaminated with Cd include leafy vegetables, crustaceans, offal, rice, water and dietary supplements (Abernethy et al., 2010). Cd exposure induces oxidative stress, deregulation of transport pathways and modification of DNA expression. It also inhibits the synthesis of heme component, deregulates mitochondrial potential by inducing apoptosis and binding with sulfhydryl groups. From a clinical viewpoint, Cd adversely affects kidneys, bones, hematopoiesis, cardio-vascular, immune and endocrine systems. The toxicity of Cd is enhanced by interaction with Pb and As (Bernhoft, 2013). It is a human carcinogen belonging to Group 1 according to the IARC classification (IARC, 2020). Cadmium is known as a principal toxic element, since it inhibits many life processes. Mushroom, in particular, can be very rich in cadmium. Cadmium was measured as small amount detected (0.05 mg/kg dw) in *Boletus edulis* and it was the highest in *Russula vinosa* (22.57 mg/kg dw) which is relatively high compared to reported literature data (Mendil et al., 2005; Kalač et al., 2004). Cd levels were found generally lower than 1.0 mg/kg for the other mushrooms species, in this study. Long-term exposure to high levels of Cd may lead to considerable accumulation in the liver and kidneys, particularly the renal cortex, resulting in kidney damage (WHO, 1989). Thus, cadmium seems to be the most deleterious one among heavy metals in mushrooms. It is acceptable daily or weekly intake may be easily reached by consumption of an accumulating mushroom species (Kalač et al., 2004).

The presence of Pb in the central nervous system may result in behavioural and developmental disorders. Long-term exposure to Pb can cause learning disabilities, attention deficit disorders, brain damage, muscle weakness, anaemia, and renal dysfunction. The symptoms of Pb poisoning include insomnia, depression, headache, fatigue, memory loss, hypertension and cardiovascular diseases. Lead is a cumulative toxin that can primarily affect the blood, nervous system, and kidneys. In the blood at high concentrations, lead inhibits red blood cell formation and eventually results in anemia.The main sources of Pb exposure are urban pollution, some medicines and supplements, electric cables, batteries, petrol, paint, water,

Table 3. DIM and HRI values of wild edible mushroom species

		DIM (µg/kg body weight/ serving)							HRI						
	Ebdible Mushroom	Mn	Fe	Zn	Cu	Ni	Cd	Pb	Mn	Fe	Zn	Cu	Ni	Cd	Pb
1	*Agaricus bisporus*	6.12	60.02	19.71	10.05	0.79	0.11	0.45	0.043	0.20	0.07	0.25	0.04	0.21	0.12
2	*Chlorophyllum rhacodes*	5.92	63.40	28.35	7.71	0.93	0.05	0.48	0.042	0.21	0.09	0.19	0.04	0.10	0.13
3	*Macrolepiota procera*	4.80	35.77	25.81	20.38	0.66	0.14	0.28	0.034	0.12	0.09	0.51	0.03	0.29	0.07
4	*Amanita rubescens*	7.91	101.5	26.17	14.77	1.08	0.06	0.28	0.056	0.34	0.08	0.37	0.05	0.12	0.07
5	*Armillaria ostoyae*	7.61	93.54	14.33	15.41	0.46	2.70	0.44	0.054	0.31	0.05	0.39	0.02	5.40	0.12
6	*Pleurotus dryinus*	9.18	84.76	24.71	10.43	0.82	0.36	0.51	0.065	0.28	0.08	0.26	0.04	0.72	0.14
7	*Pleurotus ostreatus*	8.03	68.58	23.13	15.66	0.75	0.07	0.71	0.057	0.23	0.07	0.39	0.03	0.14	0.20
8	*Polyporus squamosus*	4.32	86.25	10.72	9.00	0.65	0.07	0.45	0.031	0.28	0.04	0.23	0.03	0.14	0.12
9	*Boletus edulis*	8.63	60.94	16.90	9.68	0.82	0.02	0.55	0.061	0.20	0.06	0.24	0.04	0.04	0.15
10	*Neoboletus praestigiator*	4.02	30.62	18.40	10.00	0.40	0.15	0.70	0.028	0.10	0.06	0.25	0.02	0.31	0.19
11	*Leccinum scabrum*	6.94	27.44	14.39	15.40	0.06	0.20	0.45	0.049	0.10	0.05	0.39	0.03	0.40	0.12
12	*Suillus luteus*	5.15	96.51	24.79	14.25	0.70	0.04	0.51	0.036	0.32	0.08	0.36	0.04	0.07	0.14
13	*Lepista nuda*	14.25	51.08	25.13	10.02	0.89	0.35	0.88	0.101	0.17	0.08	0.25	0.05	0.71	0.24
14	*Lepista personata*	38.84	21.40	10.63	6.27	0.68	0.41	0.20	0.277	0.07	0.03	0.16	0.03	0.82	0.05
15	*Hydnum repandum*	10.00	27.18	33.88	16.86	0.71	0.22	0.70	0.071	0.09	0.11	0.42	0.03	0.44	0.19
16	*Lactarius deliciosus*	6.83	33.18	4.72	4.72	0.56	0.59	0.19	0.048	0.11	0.02	0.12	0.02	1.18	0.05
17	*Lactarius piperatus*	6.08	70.74	7.43	7.43	1.09	0.25	0.45	0.043	0.24	0.03	0.18	0.05	0.50	0.12
18	*Lactarius salmonicolor*	16.32	51.05	17.27	17.27	0.98	0.20	0.25	0.116	0.17	0.06	0.43	0.05	0.40	0.07
19	*Lactarius volemus*	2.73	25.32	27.21	62.54	0.90	0.31	0.05	0.019	0.09	0.09	1.56	0.05	0.61	0.08
20	*Russula delica*	2.28	43.66	26.38	6.38	0.87	0.53	0.41	0.016	0.15	0.09	0.16	0.04	1.06	0.11
21	*Russula integra*	5.04	19.20	51.01	30.86	1.22	0.07	0.31	0.036	0.06	0.17	0.77	0.06	0.13	0.08
22	*Russula adusta*	10.89	43.95	16.87	5.16	0.95	1.17	0.45	0.077	0.15	0.06	0.13	0.05	2.34	0.12
23	*Russula vinosa*	13.80	38.18	33.33	74.58	0.98	9.67	0.58	0.098	0.13	0.11	1.86	0.05	19.34	0.16
24	*Neoboletus erythropus*	4.00	39.30	19.96	9.72	0.62	0.14	0.01	0.028	0.13	0.07	0.24	0.03	0.28	0.01
	RfD^0 (µg/kg body weight/day)	140[3]	300[2]	300[3]	40[3]	20[2]	0.5[2]	3.6[2]							

dust, air, soil, cereals and vegetables grown in soil rich in Pb. A significant source of exposure to lead is via the diet (Markowitz, 2000; Miracle, 2017).

Pb concentrations of mushroom samples were generally low, except *Lepista nuda* with an amount of 2.07 mg/kg dw. The Pb levels of all other samples were not higher compared to the reported Pb values for mushrooms by Tüzen et al. (1998) (2.35 mg/kg), Kalaĉ and Svoboda (2000) (0.5-20 mg/kg) and Kaya and Bağ (2010) (2.166 mg/kg). Sarıkürkçü et al. (2012) found the lowest Pb value in *Lyophyllum decastes* (0.5 mg/kg). This is followed by *Morchella esculenta* (0.9 mg/kg). In *Rhizopogon roseolus*, *Volvoriella gloiocephala* and *Cyclocybe cylindracea*, Pb contents were found equal or above 4.0 mg level (6.2, 5.9 and 4.0 mg/kg), respectively. Pb is used for a number of industrial, domestic, and rural purposes for example, in lead batteries and in leaded petrol (Çayır et al., 2010). The Food and Agriculture Organization and World Health Organization (2004) have set the provisional tolerable weekly intake of Pb at 25 µg/kg bodyweight. For a person with a bodyweight of 60 kg, these mushrooms do not pose a health risk.

The present work, therefore, aims: (1) to evaluate the metal content in several species of wild edible mushrooms harvested from Türkiye, (2) to assess the contribution of mushrooms to the daily intake of several toxic elements, (3) to determine the possibility of evaluated mushrooms as bioindicators of environmental contamination.

3.1. DIM and HRI of the mushrooms

Some metal species are accumulated at high rates by wild edible mushrooms. This can threaten human health. Some metals such as Cu and Cd are found in high concentrations in the human body to perform some metabolic functions. However, the presence of some metals such as Cd and Cr in high concentrations in the body has a toxic effect (Fu et al., 2020). Therefore, DIM and HRI values for fungi species examined in this study were calculated and the results are given in Table 4. According to the data in the Table 4, the amounts of all metals except Cu and Cd in mushrooms were not at levels that pose a threat to human health. However, the Cd concentration in *Armillaria ostoyae* (DIM: 2.70 µg/kg body weight/ serving), *Russula adusta* (DIM: 1.17 µg/kg body weight/serving), *Lactarius deliciosus* (DIM: 0.59 µg/kg body weight/serving), *Russula delica* (DIM:

0.53 µg/kg body weight/serving), and *Russula vinosa* (DIM: 9.67 µg/kg body weight/serving); the Cu concentration in *Russula vinosa* (DIM: 74.58 µg/kg body weight/serving); and *Lactifluus volemus* (DIM: 62.54 µg/kg body weight/serving) were above the reference doses. The reference doses in question can be updated by the competent authorities by following up-to-date developments. For example, while DIM determined for Cd according to USEPA (2002) was 1.0 μg/kg body weight/serving, this value was updated as 0.36 μg/kg body weight/serving according to EFSA (2011). It was also determined that the HRI values of the Cu concentration in *Lactifluus volemus* (HRI: 1.56), *Russula vinosa* (HRI: 1.86), *Macrolepiota procera* (HRI: 0.51) and *Russula integra* (HRI: 0.77); the Cd concentration in *Armillaria ostoyae* (HRI: 5.40), *Lactarius deliciosus* (HRI: 1.18), *Russula delica (*HRI: 1.06), *Russula adusta* (HRI: 2.34), and *Russula vinosa* (HRI: 19.34) were above 1.0. Therefore, consumption of these mushroom species was considered to be risky in terms of these metals.

3.2. Principal component analysis

Principal component analysis (PCA) is a procedure which is used to reduce dimensions of multivariate data allowing to understand trends and variations in the data set by a reduced number of latent variables. In order to determine whether there exist trends and variations amongst the elements accumulated, PCA was carried out. PCA showed three components accounting for 68.977% of the variability (Fig. 1). A component loading of greater than 0.7 matrix indicates elements which are closely related to each other. The first principal component (36.285%) showed Zn (0.871), K (0.870) and Ni (0.850). The second principal component (19.754%) showed a closer relationship amongst Cu (0.910), Ca (0.694), Mg (0.497) and Cd (0.865). The third principal component (12.938%) showed Pb (0.796) and Fe (0.698) to have a common association in mushrooms.

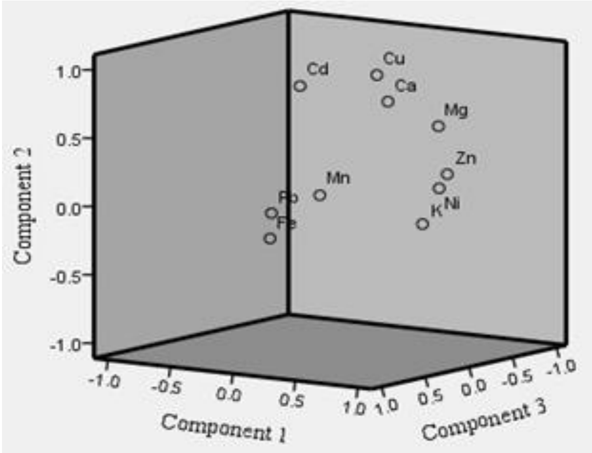

Figure 1. Principal component analysis threeplot of minerals contents of wild mushroom

4. Conclusions

In this study, metal contents of 24 different edible mushroom species collected from Bayburt, Gümüşhane, Rize and Artvin Region of Türkiye were analyzed, and using the data obtained, DIM and HRI values were calculated. According to JECFA (1993), Cd concentrations of *Armillaria ostoyae*, *Russula adusta*, *Lactarius deliciosus, Russula delica* and *Russula vinosa*; the Cu concentration in *Russula vinosa* and *Lactarius volemus* were above legal limits. Based on these values, HRI values of Cu in *Lactifluus volemus*, *Russula vinosa*, *Macrolepiota procera* and *Russula integra;* the Cd concentration in *Armillaria ostoyae*, *Lactarius deliciosus*, *Russula delica*, *Russula adusta* and *Russula vinosa* were also found to be above 1.0. Therefore, it was concluded that these species collected from Rize Ayder Plateau have been polluted by Cd and Cu, and their long-term consumption may have adverse effects on the biosynthesis reactions in the human body, trigger cancer formation, and cause gastrointestinal symptoms and liver toxicity. These findings reveal that urgent measures should be taken in terms of industrial pollution in the area where samples are collected. Since the sampling area has high traffic intensity, it has been concluded that the emissions of the vehicles should be controlled in terms of legal limits and that the consumption of mushrooms in this region should not be preferred until necessary measures are taken.

Conflict of Interest

Authors have declared no conflict of interest.

Authors' Contributions

The authors contributed equally.

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