Heavy Metals, Trace and Major Elements in 16 Wild Mushroom Species Determined by ICP-MS

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ABSTRACT

Eight minerals (Na, Mg, Ca, V, Mn, Fe, Zn, Se) and eight heavy metals (Al, Cr, Ni, As, Sr, Co, Cu, Pb) were studied in 16 wild mushroom species: *Agaricus bisporus, Agaricus bitorquis, Agaricus essettei, Armillaria tabescens, Craterellus cornucopioides, Hebeloma eburneum, Hebeloma fragilipes, Hypholoma fasciculare, Lepista nuda, Leucoagaricus leucothites, Melanoleuca graminicola, Omphalotus olearius, Phellinus torulosus, Ramaria flava, Russula foetens,* and *Trametes versicolor* collected from Usak, Turkey, using ICP-MS. The mineral content of the mushroom samples was determined by ICP-MS and ranged from 46–1717 mg/kg for Na, 122–1121 mg/kg for Mg, 15–316 mg/kg for Ca, 0.09–1.04 mg/kg for V, 3.60–76.40 mg/kg for Mn, 17.3–395 mg/kg for Fe, 3.4–70.7 mg/kg for Zn, 0.03–0.56 mg/kg for Se. The heavy metals contents in the mushroom species were between 108–575 mg/kg for Al, 0.08–0.61 mg/kg for Cr, 0.18–3.95 mg/kg for Ni, 0.19–4.43 mg/kg for As, 2.41–14.9 mg/kg for Sr, 0.02–3.44 mg/kg for Co, 0.17–9.89 mg/kg for Cu, 0.05–3.33 mg/kg for Pb. All values of the inedible mushrooms, except the Cr and Pb concentrations of *Leucoagaricus leucothites*, were within the permitted WHO limits. Thus, the mushrooms analyzed are safe for human consumption.

INTRODUCTION

Edible mushrooms are valued all over the world as a health food since they are low in calories, low in fat and fatty acids, and rich in proteins, vitamins, and minerals such as iron (Fe), potassium (K), phosphorus (P), magnesium (Mg), manganese (Mn), zinc (Zn) and calcium (Ca) (1, 2). However, inedible and poisonous mushrooms can be used for environmental cleaning by absorbing or chelating heavy metals. Metals, such as iron, copper, zinc, and manganese are essential metals since they play an important role in biological systems. However, taken in excess some of these essential metals, including the heavy metals, can also produce toxic effects and lead to health problems (3-5).

The 16 studied mushroom species are known to contribute to favorable biological activities (see Table I) such as antioxidant (1, 6–13), antitumor (14, 15), anticholinesterase (6, 13), anti-inflammatory (16), antimicrobial (6, 9, 10, 17-19), antihypertensive (20), immunomodulating (21), antihyperglycemic (22), pancreatic lipase inhibitor (23), tyripsin inhibitor (24), nematicidal (25), α-amylase inhibitor and α-glucosidase inhibitor (26), antiproliferative (27) and antidiabetic activities (28).

The aim of this study was to determine the concentrations of eight minerals (Na, Mg, Ca, V, Mn, Fe, Zn, and Se) and eight heavy metals (Al, Cr, Ni, As, Sr, Co, Cu, and Pb) in 16 mushrooms collected in the area of Usak, Turkey, using inductively coupled plasma mass spectrometry (ICP-MS). The mushroom species of *Hebeloma eburneum, Hebeloma fragilipes,* and *Melanoleuca graminicola* have, to our knowledge, never been studied. Various reports have been published on the other 13 mushroom species (3, 29, 30-50) but some of the metals have not yet been investigated such as V, Al, As, Sr for *A. bisporus*; Na, V, Cr, As, Sr, Co for *A. bitorquis*; Na, Mg, Ca, V, Mn, Fe, Zn, Al, Cr, Ni, As, Sr, Ag, Co, Cu for *A. essettei*; Na, Mg, Ca, V, Mn, Fe, Zn, Se, Cr, Ni, As, Sr, Cd, Cu, Pb for *A. tabescens*; Na, Mg, Ca, V, Se, Cr, Co for *C. cornucopioides*; Se, As, Sr for *H. fasciculare*; V, Se, Cr, As, Sr, for *L. nuda*; Na, V, As, Sr for *L. leucothites*, Na, Mg, Ca, V, Mn, Se, Al, Ni, As, Sr, Cd, Co for *O. olearius*; Na, Ca, V, Fe, Se, Al, Ni, As, Sr, Co, Pb for *P. torulosus*; V, Al, Cr, Ni, As, Sr, Cd, Pb for *R. flava*; Na, Mg, V, Se, Al, Cr, Ni, As, Sr, Co for *R. foetens*; Na, Mg, Ca, V, Se, Al, As, Sr, Co for *T. versicolor*) (Table II). The goal of this study is to make a complete scientific report on the metal concentrations of all the studied mushroom species grown in Turkey. Table II lists all of the metals previously studied and those studied in the present report.

EXPERIMENTAL

Instrumentation

The metals analyses were performed using an Agilent 7700x inductively coupled mass spectrometer (Agilent Technologies, Inc., U.S.). The instrumentation and the operating parameters are listed in Table III.

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TABLE I
Collection Localities and Dates, Family, and Edibility of the Studied Mushroom Species

No.	Mushroom	Collection Localities and Dates	Family	Edibilty	Biological Activity
1	*Agaricus bisporus*	Uşak, Banaz, December 2007	Agaricaceae	Edible	[1,6,7,8,14,16,20,21]
2	*Agaricus bitorquis*	Uşak, Banaz, December 2007	Agaricaceae	Edible	[6]
3	*Agaricus essettei*	Uşak, Banaz, December 2007	Agaricaceae	Edible	[6]
4	*Armillaria tabescens*	Uşak, Banaz, September 2009	Marasmiaceae	Poisonus	[11, 18]
5	*Craterellus cornucopioides*	Uşak, Banaz, November 2009	Cantharellaceae	Edible	[7, 16, 23, 24]
6	*Hebeloma eburneum*	Uşak, Banaz, November 2009	Bolbitiaceae	Not edible	-
7	*Hebeloma fragilipes*	Uşak-Banaz, November 2009	Strophariaceae	Not edible	-
8	*Hypholoma fasciculare*	Uşak, Banaz, November 2009	Strophariaceae	Poisonus	[8, 19]
9	*Lepista nuda*	Uşak, Banaz, December 2008	Tricholomataceae	Edible	[8, 17, 22, 24]
10	*Leucoagaricus leucothites*	Uşak, Banaz, September 2008	Agaricaceae	Edible	[9]
11	*Melanoleuca graminicola*	Uşak, Banaz, November 2008	Tricholomataceae	Not edible	-
12	*Omphalotus olearius*	Uşak-Banaz, November 2008	Omphalotaceae	Poisonus	[25]
13	*Phellinus torulosus*	Uşak-Banaz, December 2008	Hymenochaetaceae	Not edible	-
14	*Ramaria flava*	Uşak-Banaz, September 2008	Ramariaceae	Edible	[10, 26]
15	*Russula foetens*	Uşak, Banaz, September 2009	Russulaceae	Not edible	[15, 26]
16	*Trametes versicolor*	Uşak, Banaz, December 2008	Polyporaceae	Not edible	[12, 13, 27, 28]

TABLE II
The Studied Metals Versus the Studied Mushroom Species in the Literature [a]

	Mushroom species															
Metals	***A. bisporus***	***A. bitorquis***	***A. essettei***	***A. tabescens***	***C. cornucopioides***	***H. eburneum***	***H. fragilipes***	***H. fasciculare***	***L. nuda***	***L. leucothites***	***M. graminicola***	***O. olearius***	***P. torulosus***	***R. flava***	***R. foetens***	***T. versicolor***
The minerals																
Na	[31]	-	-	-	-	-	-	[40,41]	[43]	-	-	-	-	[48]	-	-
Mg	[30,31]	[30,35]	-	-	-	-	-	[40,41]	[30,43,44]	[30,45]	-	-	[47]	[48]	-	-
Ca	[30,31]	[30,35]	-	-	-	-	-	[40,41]	[30,43,44]	[3,30,45]	-	-	-	-	[3]	-
V	-	-	-	-	-	-	-	[41]	-	-	-	-	-	[48]	-	-
Mn	[30-34]	[30]	-	-	[38,39]	-	-	[34,39,40,42]	[30,39,43,44]	[3,30,45,46]	-	-	-	[48]	[3,34,42]	[49]
Fe	[30-33]	[30,35]	-	-	[38,39]	-	-	[39-41]	[30,39,43,44]	[3,30,45,46]	-	-	-	[48]	[3]	-
Zn	[30,32-34]	[30]	-	-	[38,39]	-	-	[34,39-42]	[30,39,43,44]	[3,30,45,46]	-	-	[47]	[48]	[3,34,42]	[50]
Se	[31]	[36]	[36]	-	-	-	-	-	-	[3]	-	-	-	[48]	[3]	-
The heavy metals and others																
Al	-	[35]	-	[37]	[39]	-	-	[40,41]	[37,39]	[30,45,46]	-	-	-	-	-	-
Cr	[33]	-	-	-	-	-	-	[41]	-	[45]	-	-	-	-	-	[49]
Ni	[30,32,33]	[30]	-	-	[38]	-	-	[41]	[30]	[30,45]	-	-	[47]	-	-	[49]
As	-	-	-	-	[38]	-	-	-	-	-	-	-	-	-	-	-
Sr	-	-	-	-	[38]	-	-	-	-	-	-	-	-	-	-	-
Co	[32,33]	-	-	-	-	-	-	[41]	[37,43]	[37,45]	-	-	-	[48]	-	-
Cu	[31-33]	[30]	-	-	[38,39]	-	-	[34,40-42]	[30,39,43,44]	[30,45]	-	[3]	[47]	[48]	[34,42]	[49,50]
Pb	[30-33]	[30,36]	[36]	-	[38,39]	-	-	[34,40,41]	[30,39]	[30,45,46]	-	-	-	-	[34,42]	[49,50]

[a] The literature of studied metals of mushrooms were given in the cells.
-: Not studied in previous studies, and studied in this study.

Reagents, Standards, and Samples

Nitric acid (HNO_3, 65%) and hydrogen peroxide (H_2O_2, 30%) were obtained from E. Merck (Darmstadt, Germany). All other chemicals and solvents were of analytical reagent grade. For calibration plots, the standard solutions were prepared by diluting the stock solutions with ultrapure water containing 0.2% HNO_3 and 0.3% HCl. In order to check the accuracy of the method, the certified reference materials (CRM) NIST-CRM-1203 Drinking Water, NIST-CRM-1570a Spinach Leaves, and NIST-CRM-1573a Tomato Leaves were used (National Institute of Standards and Technology, Gaithersburg, MD, USA). Agilent No. 5188-6525 was used as the internal standard for scandium, germanium, indium, and bismuth. The names of the species and their family classification, collection dates and localities, as well as the edibility of the 16 mushroom species, are listed in Table I. The voucher specimens of these species have been deposited in the Fungarium at the Department of Biology, Mugla Sıtkı Koçman University, Mugla, Turkey.

Sample Preparation

The collected samples were cleaned, sliced, and dried in an oven (Nüve, Istanbul, Turkey) at 105 ºC for 24 hours. The dried samples were homogenized using a homogenizer (IKA, Staufen, Germany), sifted using a 10-mesh sieve, and (particle size of 1600 µm, average) stored in pre-cleaned polyethylene bottles for analysis. All aqueous solutions were prepared by using deionized water (18.2 $M\Omega.cm^{-1}$) from a Milli-Q® system (Human Power I Plus, Korea). All plastic and glassware were cleaned by soaking in 10% nitric acid solution overnight and rinsing with deionized water. For digestion, a CEM Mars 5 microwave closed system (CEM, Matthews, NC, USA) was used. Samples of 0.25 g were digested with 9 mL of HNO_3 (65%) and 1 mL of H_2O_2 (30%) in the microwave for 23 minutes, then diluted to 25 mL with deionized water. A blank digest was carried out in the same way. The microwave digestion temperature was set to 180 ºC for 5 minutes and kept constant for 2 minutes. This digestion heating program was used for all samples.

TABLE III
ICP-MS Instrumental Operating Conditions

Instrument	Agilent™ 7700x ICP-MS
RF power	1600 W
RF match	2.10 V
Sampling depth	10.0 nm
Nebulizer gas	0.57 L/min
S/C temperature	2 ºC
Nebulizer type	MicroMist
Spray chamber	Scott-type double-pass
Ar flow rate	Plasma: 15 L/min
	Auxiliary: 0.9 L/min
	Nebulizer: 1.0–1.1 L/min
Solution uptake rate	1.8 mL/min
VacuumInterface	4 torr, quadrupole: 2 105 torr
Data acquisition	Peak hopping
	Replicate time 200 ms
	Dwell time 200 ms
	Sweeps/reading 3
	Readings/replicate 3
	Number of replicates 3
Analytical masses	^{23}Na, ^{24}Mg, ^{43}Ca, ^{51}V, ^{55}Mn, ^{56}Fe, ^{66}Zn, ^{69}Ga, ^{82}Se, ^{27}Al, ^{52}Cr, ^{60}Ni, ^{75}As, ^{88}Sr, ^{206}Pb, ^{59}Co, ^{63}Cu

ICP-MS Analysis Procedure

For the elemental analysis, an Agilent 7700x ICP-MS was used. All metal concentrations were determined on a dry weight basis. The procedures from sampling to analysis were also applied to blanks to evaluate any metal contamination during the analytical procedure. The limit of detection (LOD) is defined as the concentration corresponding to three times the standard deviation of 10 blanks. The ICP-MS detection limits (µg/L) of the elements were found to be 0.0098 for Pb, 0.1151 for Zn, 0.0069 for Fe, 0.0108 for Mn, 0.0029 for Cu, 0.0162 for Cr, 0.0311 for Ni, 0.0006 for Co, 0.7630 for Na, 0.1468 for Mg, 0.7821 for Al, 0.7393 for Ca, 0.0103 for V, 0.0245 for As, 0.0219 for Se, and 0.0213 for Sr. The ICP-MS operating conditions are listed in Table III. The standard reference material (SRM) NIST-CRM-1203 Drinking Water, and NIST-CRM-1570a Spinach Leaves, and NIST-CRM-1573a Tomato Leaves were used to check the accuracy of the analytical method. Agilent No. 5188-6525 was used as the internal standard. The results obtained are listed in Table IV. The relative standard deviation (RSD) was below 8% (51).

In ICP-MS, most elements have multiple isotopes which are corrected mathematically for both isobaric and polyatomic interferences. By considering the natural abundance of different isotopes and

TABLE IV
Certified and Experimental Values of Studied Metals in NIST-CRM 1203 Drinking Water, NIST-CRM 1570a Spinach Leaves, and NIST-CRM 1573a Tomato Leaves (mg/kg)[a]

Metals	NIST-CRM 1203 Drinking Water (mg/kg)			NIST-CRM 1570a Spinach Leaves (mg/kg)			NIST-CRM 1573a Tomato Leaves (mg/kg)		
	Certified Value (mg/kg)	Experimental Value + S.D. (mg/kg)[b]	Recovery Value (%)	Certified Value (mg/kg)	Experimental Value + S.D. (mg/kg)[b]	Recovery Value (%)	Certified Value (mg/kg)	Experimental Value + S.D. (mg/kg)[b]	Recovery Value (%)
Al	250.5±1.3	251.6±2.01	100.44	310±14	285±15	91.2	598±12	596±2	99.7
As	15.00±0.08	14.48±0.35	96.53	0.068±0.012	0.066±0.014	95.1	0.112±0.004	0.111±0.008	99.1
Ca	99.78±0.50	100.42±0.95	100.64	-	-			-	-
Co	5.00±0.03	5.22±0.18	104.40	0.39±0.05	0.38±0.07	94.8	0.57±0.02	0.55±0.08	96.5
Cr	50.09±0.25	49.92±0.56	99.66	-	-	-	1.99±0.06	2.02±0.12	101.5
Cu	2000±10	202.9±0.12	101.45	12.2±0.7	12.0±0.8	97.3	4.70±0.14	4.71±0.05	100.2
Fe	200.3±1.0	199.89±2.05	99.94	-	-	-	368±7	373±2	101.4
Mg	99.77±0.50	100.68±1.02	100.23	-	-	-	-	-	-
Mn	50.17±0.25	50.02±0.75	99.67	76±1.4	75±2.6	98.6	246±8	243±3	99.8
Na	49.68±0.25	50.01±0.78	100.66	-	-	-	136±4	138±2	101.5
Ni	50.16±0.25	4.93±0.61	97.05	2.14±0.07	2.22±0.15	103.6	1.59±0.07	1.56±0.03	98.2
Pb	24.99±0.12	2.44±0.68	97.99	-	-	-	0.075±0.025	0.079±0.012	101.4
Se	10.00±0.05	10.08±0.24	106.93	0.116±0.009	0.120±0.010	103.7	0.054±0.003	0.055±0.007	101.9
Sr	-	-	-	55.5±0.6	54.9±1.8	101.8	-	-	-
V	-	-	-	0.57±0.02	0.56±0.04	98.4	0.835±0.010	0.839±0.009	100.5
Zn	1000±5	1003.1±7.8	102.59	82±3.9	80±5	97.5	30.9±0.7	31.5±1.0	101.5

[a] Ten times dilution of Certified NIST-SRM 1203 Drinking Water, NIST-CRM 1570a Spinach Leaves, and NIST-CRM 1573a Tomato Leaves (mg/kg).
[b] Average of triplicate measurements of certified material ($p<0.05$).

TABLE V
Mineral Content (mg/kg Mushroom of Dry Weight) of Mushroom Species[a]

No	Na	Mg	Ca	V	Mn	Fe	Zn	Se
1 [e]	883 ± 7	323 ± 3	52.2 ± 2	0.36 ± 0.02	3.60 ± 0.12	17.3 ± 1	9.71 ± 0.4	0.03 ± 0.01
2 [e]	487 ± 5	297 ± 3	63.2 ± 2	0.72 ± 0.05	10.2 ± 0.51	114 ± 3	13.4 ± 0.5	0.21 ± 0.02
3 [e]	235 ± 3	190 ± 3	19.1 ± 1	0.58 ± 0.04	4.92 ± 0.14	66.1 ± 2	10.1 ± 0.6	0.16 ± 0.01
4 [p]	1717 ± 10	795 ± 5	69.2 ± 2	0.91 ± 0.03	18.3 ± 0.22	218 ± 6	15.9 ± 1.1	0.19 ± 0.01
5 [e]	619 ± 6	498 ± 4	41.4 ± 1	0.19 ± 0.01	76.4 ± 2.84	77.1 ± 3	16.7 ± 0.8	0.04 ± 0.01
6 [ne]	562 ± 5	666 ± 5	46.2 ± 2	0.25 ± 0.01	8.21 ± 0.40	231 ± 3	21.7 ± 0.9	0.08 ± 0.02
7 [ne]	228 ± 3	735 ± 5	75.1 ± 2	0.76 ± 0.03	18.7 ± 0.82	172 ± 2	43.3 ± 2.4	0.56 ± 0.01
8 [p]	121 ± 2	122 ± 2	15.1 ± 1	1.04 ± 0.07	38.3 ± 1.11	198 ± 3	3.91 ± 0.1	0.29 ± 0.01
9 [e]	401 ± 5	600 ± 5	113 ± 4	0.29 ± 0.02	13.6 ± 0.83	174 ± 4	21.2 ± 0.8	0.16 ± 0.01
10 [e]	722 ± 5	1050 ± 8	142 ± 5	0.56 ± 0.03	8.61 ± 0.31	115 ± 1	52.4 ± 2.5	0.14 ± 0.01
11 [ne]	426 ± 4	788 ± 6	105 ± 2	0.62 ± 0.02	44.4 ± 1.52	395 ± 3	35.9 ± 1.2	0.13 ± 0.01
12 [p]	274 ± 3	205 ± 3	18.1 ± 1	0.12 ± 0.01	5.05 ± 0.14	84.3 ± 2	3.4 ± 0.1	0.03 ± 0.01
13 [ne]	46 ± 1	301 ± 3	316 ± 3	0.18 ± 0.01	4.32 ± 0.13	41.2 ± 1	7.71 ± 0.2	0.04 ± 0.01
14 [e]	307 ± 3	804 ± 6	19.1 ± 1	0.21 ± 0.01	22.5 ± 1.43	90.1 ± 2	14.5 ± 1.0	0.19 ± 0.01
15 [ne]	731 ± 5	765 ± 5	57.1 ± 2	0.21 ± 0.01	8.92 ± 0.53	198 ± 3	13.7 ± 0.3	0.21 ± 0.01
16 [ne]	177 ± 2	1121 ± 7	109 ± 3	0.09 ± 0.01	9.81 ± 0.62	96.1 ± 1	70.7 ± 2.6	0.06 ± 0.01

[a] Values expressed are means ± S.E.M. of three parallel measurements ($p<0.05$).
[e] Edible mushroom species; [p] Poisonous mushroom species; [ne] not edible mushroom species; [NA] Not Available.

measuring the intensity of a non-interfered isotope, the extent of the interference and the contribution to yield is then subtracted and the concentration calculated. Moreover, the nebulizer gas of Ar may combine with other atoms and leads to some interferences. For example, ArO, ArC, and ArCl may occur in the plasma, and act on behalf of Fe, Cr, and As, respectively. The developed ORS (Octopole Reaction System) in ICP-MS separates these polyatomic interferences by using helium as a collision gas. Thus, any possible interferences are prevented by using the ORS.

RESULTS AND DISCUSSION

The concentration of 8 minerals (Na, Mg, Ca, V, Mn, Fe, Zn, and Se) and 8 heavy metals (Al, Cr, Ni, As, Sr, Pb, Co, and Cu) was studied in mushroom species. Their concentrations in the various mushroom species are listed in the Tables V and VI as mg/kg dry weight, respectively.

Mineral Element Concentrations

Sodium (Na) is an essential macro element for humans and animals and has an important physiological effect on different organs and the cellular mechanisms, such as the K/Na pump of cell membranes (52). The Na concentrations ranged between 46±1 to 1717±10 mg/kg in the mushroom species. The highest and lowest Na concentrations were obtained in *A. tabescens* and *P. torulosus*, respectively (Table V). The recommended minimum daily intake for Na is about 1500 mg and the maximum requirement is 2400 mg per day (53).

Magnesium (Mg) is a major mineral for the human body and the daily dietary intake is between 168-319 mg, with an estimated average requirement for males of 330-350 mg/day, for females of 255-265 mg/day (54). Mg is important for the formation of the skeletal structure with regard to bone quality. Dietary deficiency of Mg has been implicated as a risk factor for osteoporosis (55). The Mg concentration of the mushroom species ranged from 122±2 to 1121±7 mg/kg. The highest and lowest of Mg levels were found in *T. versicolor* and *H. fasciculare*, respectively.

Calcium (Ca) is an element whose behavior seems to be very interesting because there are quite substantial differences between both the phylogenetic position and lifestyle (41). The Ca content in the mushroom samples was between 15±1 to 316±3 mg/kg. The lowest and highest Ca values were observed in *H. fasciculare* and *P. torulosus,* respectively. The recommended daily dietary intake of Ca is between 466–880 mg, and the adequate intake for adults is 1–1.2 g (54).

TABLE VI
Heavy Metal Content (mg/kg Mushroom of Dry Weight) of the Mushroom Species[a]

No	Al	Cr	Ni	As	Sr	Co	Cu	Pb
1 [e]	440 ± 4	0.14 ± 0.01	0.19 ± 0.01	0.43 ± 0.02	5.66 ± 0.12	0.02 ± 0.00	9.89 ± 0.65	0.43 ± 0.03
2 [e]	491 ± 4	0.30 ± 0.02	0.68 ± 0.02	0.91 ± 0.05	9.71 ± 1.05	0.24 ± 0.01	5.03 ± 0.24	1.34 ± 0.05
3 [e]	129 ± 2	0.34 ± 0.02	0.36 ± 0.02	0.26 ± 0.01	6.87 ± 0.36	0.23 ± 0.01	7.37 ± 0.36	0.31 ± 0.02
4 [p]	144 ± 3	0.35 ± 0.01	0.57 ± 0.04	2.65 ± 0.12	13.4± 1.02	0.30 ± 0.02	2.32 ± 0.06	1.74 ± 0.06
5 [e]	388 ± 2	0.15 ± 0.01	0.18 ± 0.01	0.19 ± 0.01	7.43 ± 0.47	0.06 ± 0.00	0.58 ± 0.04	0.07 ± 0.00
6 [ne]	398 ± 3	0.56 ± 0.03	3.40 ± 0.09	0.87 ± 0.45	4.55 ± 0.24	0.37 ± 0.02	0.57 ± 0.02	0.11 ± 0.00
7 [ne]	122 ± 2	0.33 ± 0.02	0.58 ± 0.03	4.43 ± 0.25	10.0 ± 0.28	0.38 ± 0.03	1.87 ± 0.08	0.18 ± 0.01
8 [p]	360 ± 2	0.17 ± 0.01	0.39 ± 0.01	1.96 ± 0.08	2.41 ± 0.09	0.97 ± 0.05	1.01 ± 0.08	0.29 ± 0.02
9 [e]	432 ± 4	0.20 ± 0.01	0.55 ± 0.03	0.29 ± 0.01	14.9 ± 1.19	0.13 ± 0.01	0.21 ± 0.01	0.58 ± 0.03
10 [e]	575 ± 5	0.61 ± 0.04	0.69 ± 0.04	0.46 ± 0.03	4.53 ± 0.14	0.29 ± 0.02	1.45 ± 0.05	1.91 ± 0.05
11 [ne]	108 ± 2	0.51 ± 0.02	0.57 ± 0.04	1.20 ± 0.05	10.1 ± 0.79	0.43 ± 0.03	0.72 ± 0.03	3.33 ± 0.06
12 [p]	145 ± 3	0.19 ± 0.01	0.43 ± 0.01	0.38 ± 0.02	7.97 ± 0.62	0.05 ± 0.00	0.20 ± 0.01	0.20 ± 0.01
13 [ne]	143 ± 1	0.08 ± 0.00	0.69 ± 0.03	0.29 ± 0.02	9.07 ± 0.32	0.10 ± 0.01	1.27 ± 0.08	0.05 ± 0.00
14 [e]	352 ± 2	0.25 ± 0.01	1.61 ± 0.08	0.69 ± 0.05	3.45 ± 0.11	3.44 ± 0.18	8.03 ± 0.56	1.56 ± 0.10
15 [ne]	451 ± 4	0.49 ± 0.03	3.95 ± 0.09	0.29 ± 0.01	4.92 ± 0.35	0.53 ± 0.04	0.24 ± 0.01	0.26 ± 0.02
16 [ne]	169 ± 5	0.28 ± 0.01	0.96 ± 0.05	0.24 ± 0.01	6.78 ± 0.14	0.14 ± 0.02	0.17 ± 0.01	0.27 ± 0.01
WHO values (mg/kg)	< 5000 [67] [b]	< 0.5 [76] [b]	70.0 [69] [b,c]	< 4 [71] [b]	NA	NA	< 10 [64] b	1.75 [75] b, c

[a] Values expressed are means ± S.E.M. of three parallel measurements ($p<0.05$). [b] citied reference in the text; [c] mg/kg/body weight; [e] Edible mushroom species; [p] Poisonous mushroom species; [ne] not edible mushroom species; [NA] Not Available.

Vanadium (V) is present at very low concentrations in virtually all cells in plants and animals. Numerous studies have demonstrated that compounds of the trace element V exert antidiabetic effects *in vitro* and *in vivo* (56). However, the toxicity associated with V limits its role as a therapeutic agent for diabetic treatment (57). The V concentrations in the studied mushroom species ranged from 0.09±0.01 to 1.04±0.07 mg/kg. The recommended V concentration in foods is between 19 to 50 µg/day (58).

Manganese (Mn) is an essential component of many enzymes and also activates numerous enzymes (32). The minimum and maximum Mn levels found were 3.60±0.12 mg/kg and 76.40±2.84 mg/kg for *A. bisporus* and *C. cornucopioides*, respectively. The mean concentration range of Mn in vegetables is between 0.42 – 6.64 (mg/kg). Several investigations of adult diets exhibited that the average daily consumption of Mn is between 2.0 to 8.8 mg (59).

Iron (Fe) is essential for nearly all living organisms. It is known that adequate Fe in a diet is very important for decreasing the incidence of anemia. Iron deficiency occurs when the demand for iron is high, e.g., for growth, due to high menstrual loss and pregnancy, and the intake is quantitatively inadequate or contains elements that render the iron unavailable for absorption (60). The Fe concentrations in this study ranged from 173±1 and 395±3 mg/kg and was highest in *M. graminicola* and lowest in *A. bisporus*. According to the National Research Council (61), the recommended daily intake of iron in foods ranges from 10 to 14 mg.

Zinc (Zn) is an essential nutrient that plays an important role in the biological systems (62). It is a central metal of various enzymes which are synthase or degrade the carbohydrates, lipids, proteins, and nucleic acids in the biological fluids. Another role of Zn is maintenance of cell and organ integrity by stabilizing the molecular structure of cellular components. In addition, Zn plays a central role in the immune system (63). Some mushrooms are known as Zn accumulators, and the mushroom-to-underlying soil ratio for zinc ranges from 1 to 10 mg/kg. The highest zinc level was found in *L. leucothites* (52.4±2.5 mg/kg), and the lowest Zn content was found in *O. olearius* (3.4±0.1 mg/kg). The calculated daily dietary intake of Zn is 16 mg for males and 12 mg for females (64).

Selenium (Se) is an essential nutrient, and Se deficiency is associated with general impairment of the immune system and has a protective role in preventing carcinogenesis and other chronic diseases. There is evidence that Se also plays an antioxidant role (65). The minimum and maximum Se concentrations were 0.03±0.01 mg/kg in *A. bisporus* and 0.56±0.01 mg/kg in *H. fragilipes*, respectively. The daily recommendation of Se intake for adult females and males is 26 and 35 µg, respectively (66).

Heavy Metals Concentrations

The heavy metals concentrations of the mushroom species are listed in Table VI including the WHO recommended limits. Aluminum (Al) is not considered to be an essential element in humans. Exposure of Al has been implicated in a number of human pathologies including dementia, Parkinson's disease, and Alzheimer's disease. Aluminum is naturally present in large amounts in potatoes, spinach, and tea which are generally considered highly preferred foods (67). Al is not normally added to foods but can be a contaminamt in some processed foods during the production process (60). The Al content of the mushroom species ranges from 108±2 mg/kg in *M. graminicola* to 575±5 mg/kg in *L. leucothites*. Daily dietary intake of Al for adults varies from-country to country. Al intake in Australia is 1.9–2.4, while in Europe it is 3.1 – 13, and in the USA it is 7.1–8.2 mg/day. Consumption of 5 g/day is considered to be high (67).

Chromium (Cr) is an essential mineral for humans and has been related to the carbohydrate, lipid, and protein metabolism. The recommended daily intake is 50–200 µg (60). The minimum and maximum contents of Cr found were 0.14±0.01 mg/kg in *A. bisporus* and 0.61±0.04 mg/kg in *L. leucothites*, respectively.

Trace amounts of nickel (Ni) may be beneficial as an activator of some enzyme systems, such as urease (68). The tolerable daily intake level of Ni is given as 1 mg per kg of body weight per day (69). At upper limits, it is considered toxic. It accumulates in the lungs and may cause bronchial hemorrhage or collapse (60). The minimum and maximum contents of Ni in mushroom species were 0.18±0.01 and 3.95±0.09 mg/kg. The highest value was found in *C. cornucopioides*, whereas for *R. foetens,* it was the lowest value.

Arsenic (As) is generally found at low concentrations (0–20 mg/kg), with the exception of rice (150–25 mg/kg) and certain edible mushrooms (several milligrams per kilogram) in the terrestrial environment (70). The As content in this study ranged from 0.19±0.01 and 4.43±0.25 mg/kg and was highest in *H. fragilipes* and lowest in *C. cornucopioides*. The promulgated daily limits by WHO for As is less than 4 mg/kg in foods for adults (71).

The highest (14.9±1.19 mg/kg) and the lowest (2.41±0.09 mg/kg) concentrations of strontium (Sr) were found in *L. nuda* and *H. fasciculare*, respectively. Exposure to high levels of Sr during infancy and childhood can affect bone growth and cause dental changes, while there is no evidence to suggest that ingestion of foods that naturally contain traces of strontium is harmful. Effects during adulthood are less well understood, but there is some evidence that strontium increases bone density. According to the U.S. Environmental Protection Agency (EPA), water with Sr levels higher than 4 mg/L cannot be used in the preparation of beverages and foods. This means that for an adult having a 130 µg/kg body weight, the amount of 4 mg/L Sr is toxic.

Cobalt (Co) is an important trace element in nature and can be either essential or toxic for many biological systems, depending on its concentration range (68). For example, Co is essential for nitrogen fixation by symbiotic systems, blue-green algae, and free-living bacteria. In higher plants, Co is a necessary element for legumes (72). Cobalt exposes toxicity over oxidant-based and free radical-based processes as hypothesis. It also affects genes sensitive to oxidant status. This leads to apoptosis. Neuromuscular transmissions can be affected by cobalt (72). The average content of Co was found to be 1.09 mg/g and the highest concentration was in *R. flava* (3.44±0.18 mg/kg) and lowest in *A. bisporus* (0.02±0.00 mg/kg). The recommended daily intake for Co is between 0.005 to 0.008 mg (73).

Copper (Cu) is an essential element. Enzymes containing copper are important for the body to transport and use iron (47). Minimum and maximum copper levels were found as 0.17±0.01 mg/kg and 9.89±0.65 mg/kg for *T. versicolor* and *A. bisporus*. For adults, long-term dietary intake of Cu at concentrations of 1–10 mg/day has no apparent adverse effects (64).

Lead (Pb) is used for a number of industrial, domestic, and rural purposes. A significant source of exposure to Pb is through diet (62). Lead is a cumulative toxin that primarily affects the blood, nervous system, and kidneys. In the blood at high concentrations, Pb inhibits red blood cell formation and eventually results in anemia (74). The minimum and maximum lead concentration in the mushroom species of this study was in the range of 0.05±0.00-3.33±0.06 mg/kg. These values were determined in both *P. torulosus* and *M. graminicola*. According to the Joint Food and Agriculture Organization of the United Nations [FAO)/WHO Expert Committee on Food Additives (JECFA)], the provisional tolerable weekly intake of Pb is 25 µg/kg body weight (75).

CONCLUSION

Turkey has an appropriate climate for mushroom growth. The studied 16 mushroom species grow naturally in the western part of Turkey. The data obtained in this study show which heavy metals and what amounts are absorbed by edible, inedible, and poisonous mushrooms. The ICP-MS method, with the accuracy verified by comparing the results with the values of certified materials, was successfully applied for the analysis of mushroom species. In this study, the WHO limits were used to compare the safe levels of heavy metals in mushroom species. Accordingly, the heavy metals concentrations in the studied edible mushrooms grown naturally and cultivated in Turkey are at safe levels for public consumption, except *Leucoagaricus leucothites*. In this mushroom, the Cr and Pb levels were slightly over the WHO limits. Moreover, among the inedible mushrooms, the Cr levels in *Hebeloma eburneum* and *Melanoleuca graminicola*, and the As levels in *Hebeloma fragilipes,* exceeded the WHO limits. This method can also be recommended for use in the routine determination of trace elements in similar matrices. In addition, the study completed the scientific report on the metal concentrations of studied mushroom species.

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