



## Evaluation of trace metal contents of some wild edible mushrooms from Black sea region, Turkey

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### ABSTRACT

Fructification organs of *Calvatia excipuliformis*, *Lycoperdon perlatum*, *Laccaria amethystea*, *Armillaria mellea*, *Marasmius oreades*, *Xerula radicata*, *Cantharellus cibarius*, *Craterellus cornucopioides*, *Cantharellus tubaeformis*, *Hypholoma fasciculare*, *Clitocybe gibba*, *Collybia dryophila*, *Lepista nuda* and *Mycena aetites* were collected from different localities in Black sea region of Turkey. Their trace metals concentrations were determined by atomic absorption spectrometry after wet and microwave digestion. The results were (as mg/kg) 150–1741 for iron, 15.5–73.8 for copper, 28.6–145 for manganese, 43.5–205 for zinc, 4.8–42.7 for aluminium and 0.9–2.6 for lead. The levels of lead analyzed in some edible mushroom samples were found to be higher than legal limits. The relative standard deviations (R.S.D.) were found below 10%. The accuracy of procedure was confirmed by certified reference material.

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### 1. Introduction

Trace elements like iron, copper, zinc and manganese are essential metals since they play an important role in biological systems, whereas aluminium and lead are non-essential metals as they are toxic even in traces [1,2]. The essential metals can also produce toxic effects when the metal intake is excessively elevated [3,4].

The consumption of wild edible mushrooms is increasing, even in the developed world, due to a good content of proteins as well as a higher content of trace minerals [5]. Mushrooms have a long history of use in traditional Chinese medicine. Mushrooms have also been reported as therapeutic foods, useful in preventing diseases such as hypertension, hypercholesterolemia and cancer. These functional characteristics are mainly due to their chemical composition [6].

Mushrooms are the agents responsible for the breaking down of much of the organic matter and play an important part in the continual changes taking place in nature. They have a very effective mechanism to accumulate heavy metals from environment. Therefore, mushrooms can be used to evaluate the level of environmental

pollution [7]. On the other hand, many studies carried out to evaluate the possible danger to human health from the ingestion of mushrooms containing heavy metals [8–10].

Turkey can be separated into seven geographic regions. One of them is the Black sea region. In this region, the climate is mild and rainy. The seasons are normally wet with mild temperatures. The climate during the year, especially, in spring and autumn, is ideal for mushroom growth [7]. There are 1778 species of wild fungi (177 *myxomycetes* and 1601 *macromycetes*) growing in Turkey and some of them are a popular food source among the local people living in Black sea region of Turkey [11]. In spite of the popularity of this perfect food in the region, there is no enough data available regarding the levels of heavy metals. Some recent studies were carried out on the metal contents of mushrooms of Turkey [12–15].

The microwave digestion procedure was chosen for the samples because of more accuracy with respect to both time and recovery than wet digestion. The analytical parameters obtained make this method suitable for the determination of Cu, Mn, Fe, Zn, Pb and Al in mushroom samples.

In the present study, the contents of aluminium, lead, iron, copper, manganese and zinc in mushroom samples collected from Black sea region of Turkey were determined by flame and/or graphite furnace atomic absorption spectrometry (AAS) after microwave digestion.

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## 2. Experimental

### 2.1. Reagents

All reagents were of analytical reagent grade unless otherwise stated. Double deionized water (Milli-Q Millipore 18.2 MΩ/cm resistivity) was used for all dilutions. HNO<sub>3</sub> and H<sub>2</sub>O<sub>2</sub> were of suprapure quality (E. Merck, Darmstadt). All the plastic and glassware were cleaned by soaking in dilute HNO<sub>3</sub> (10%) and were rinsed with distilled water prior to use. The element standard solutions used for calibration were prepared by diluting stock solutions of 1000 mg/L of each element supplied from Sigma. Standard reference material (NIST-SRM 1515 Apple leaves) was used.

### 2.2. Apparatus

A PerkinElmer AAnalyst 700 atomic absorption spectrometer equipped with HGA graphite furnace and with deuterium background corrector was used. For flame measurements, a 10-cm long slot-burner head, a lamp and an air-acetylene flame were used. For graphite furnace measurements, argon was used as inert gas. The operating parameters for working elements were set as recommended by the manufacturer given in Table 1. Pyrolytic-coated graphite tubes (PerkinElmer part no. B3 001264) with a platform were used. Samples were injected into the graphite furnace using PerkinElmer AS-800 autosampler. The atomic absorption signal was measured as a peak height mode against an analytical curve.

Milestone Ethos D closed vessel microwave digestion system (maximum pressure 1450 psi, maximum temperature 300 °C) was used. Teflon reaction vessels used all the digestion procedures. The reaction vessels were cleaned using 5 mL of concentrated nitric acid before each digestion.

### 2.3. Samples

The samples of fructification organs were collected from Black sea region of Turkey during 2006. Ecological properties of species were noted at the field, then examined in the laboratory. The species (Table 2) were identified according to Breitenbach and Kränzlin [16,17].

**Table 1**  
Instrumental analytical conditions of investigated elements

Conditions for flame AAS				
Element	Acetylene (L/min)	Air (L/min)	Wavelength (nm)	Slit width (nm)
Fe	2.0	17.0	248.3	0.2
Cu	2.0	17.0	324.8	0.7
Zn	2.0	17.0	213.9	0.7
Mn	2.0	17.0	279.5	0.2
Conditions for graphite furnace AAS				
Instrumental conditions	Pb		Al	
Argon flow (mL/min)	250		250	
Sample volume (μL)	20		20	
Modifier (μL)	5		5	
Heating program temperature (°C) (ramp time (s), hold time (s))				
Drying 1	100 (5, 20)		100 (5, 20)	
Drying 2	140 (15, 15)		140 (15, 15)	
Ashing	700 (10, 20)		1700 (10, 20)	
Atomization	1800 (0, 5)		2500 (0, 5)	
Cleaning	2600 (1, 3)		2600 (1, 3)	

**Table 2**

Sample number, name, habitat and edibility of the mushroom

Sample number	Name of mushrooms	Habitat	Edibility
01	<i>Calvatia excipuliformis</i> (Scop.) Perdeck	On soil in forests	Edible
02	<i>Lycoperdon perlatum</i> Pers.	On soil in forests	Edible
03	<i>Laccaria amethystea</i> Cooke	In forests	Edible
04	<i>Armillaria mellea</i> (Vahl) P. Kumm.	On trees	Edible
05	<i>Marasmius oreades</i> (Bolton) Fr.	In meadows	Edible
06	<i>Xerula radicata</i> (Relhan) Fr.	On stumps in forests	Inedible
07	<i>Cantharellus cibarius</i> (Fr.) Quéf.	In forests	Excellent
08	<i>Craterellus cornucopioides</i> (L.) Pers.	On soil in forests	Good
09	<i>Cantharellus tubaeformis</i> (Bull.) Fr.	In forests	Edible
10	<i>Hypholoma fasciculare</i> (Huds.) Quéf.	On dead wood	Inedible
11	<i>Clitocybe gibba</i> (Pers.) P. Kumm.	In forests	Edible
12	<i>Collybia dryophila</i> (Bull.) P. Kumm.	In forests	Edible
13	<i>Lepista nuda</i> (Bull.) Cooke	On soil in forests	Excellent
14	<i>Mycena aetites</i> (Fr.) Quéf.	Among grasses	Edible

### 2.4. Digestion procedures

Two different types of digestion procedures were applied to the mushroom samples: wet and microwave digestions.

#### 2.4.1. Wet ashing

Wet digestion of mushroom samples was performed using an oxo-acid mixture of HNO<sub>3</sub>:H<sub>2</sub>O<sub>2</sub> (2:1) (16 mL for a 1.0 g sample). This mixture was heated until dryness for 4 h and brought to a volume of 10 mL with deionized water. Blank digestions were also carried out in the same way.

#### 2.4.2. Microwave digestion

One gram of sample was digested with 6 mL of HNO<sub>3</sub> (65%) and 2 mL of H<sub>2</sub>O<sub>2</sub> (30%) in microwave digestion system and diluted to 10 mL with deionized water. A blank digest was carried out in the same way. All sample solutions were clear. Digestion conditions for microwave system were applied as 2 min for 250 W, 2 min for 0 W, 6 min for 250 W, 5 min for 400 W, 8 min for 550 W, ventilation 8 min, respectively.

### 2.5. Analytical procedure

Detection limit is defined as the concentration corresponding to three times the standard deviation of 10 blanks [18]. Detection limit values of elements as (mg/L) in flame AAS were found to be 0.015 for Cu, 0.008 for Zn, 0.020 for Fe and 0.011 for Mn. Pb and Al were below detection limit of flame AAS. These elements were determined using graphite furnace AAS by autosampler. During analyses, internal argon flow rate through the graphite tube was 250 mL/min; gas flow was interrupted during atomization. Sample volume, ramp and hold times for the drying, ashing, atomization and cleaning temperatures were optimized before analysis to obtain maximum absorbance and minimum background. Matrix modifiers were added 200 μg NH<sub>4</sub>H<sub>2</sub>PO<sub>4</sub> for Pb and 50 μg Mg(NO<sub>3</sub>)<sub>2</sub> for Al. Most of the matrix was removed before the atomization step and less interference occurred during atomization. Each graphite furnace atomic absorption spectroscopic analysis calls for 20 μL of solution and 5 μL of the matrix modifier was added if necessary. The signals were measured as peak height. The absolute sensitivity is defined by the mass of an element, which gives a peak absorbance of 0.0044; it was found 12 pg for Pb and 16 pg for Al.

**Table 3**  
Trace metal contents with wet ashing and microwave methods in *Calvatia excipuliformis* sample (mean  $\pm$  S.D.,  $N=4$ )

Method	Concentrations ( $\mu\text{g/g}$ )					
	Fe	Cu	Mn	Zn	Al	Pb
Microwave	350 $\pm$ 30	25.2 $\pm$ 2.2	75.1 $\pm$ 6.5	52.7 $\pm$ 4.7	10.5 $\pm$ 0.9	1.7 $\pm$ 0.1
Wet ashing	342 $\pm$ 33	21.5 $\pm$ 2.1	70.2 $\pm$ 6.9	50.3 $\pm$ 4.9	9.9 $\pm$ 0.8	1.5 $\pm$ 0.1

**Table 4**  
Observed and certified values of trace metals in NIST-SRM 1515 Apple leaves,  $N=4$

Element	Certified value ( $\mu\text{g/g}$ )	Microwave digestion ( $\mu\text{g/g}$ )	Recovery (%)	Wet ashing ( $\mu\text{g/g}$ )	Recovery (%)
Cu	5.64	5.53 $\pm$ 0.30	98	5.46 $\pm$ 0.50	96
Zn	12.5	12.6 $\pm$ 1.0	101	11.9 $\pm$ 1.1	95
Mn	54	52.4 $\pm$ 2.3	97	51.4 $\pm$ 4.7	95
Fe	(83) <sup>a</sup>	82.2 $\pm$ 4.5	99	80.5 $\pm$ 6.3	97
Pb	0.47	0.45 $\pm$ 0.03	96	0.44 $\pm$ 0.04	94
Al	286	277.4 $\pm$ 8.6	97	270.5 $\pm$ 15.2	95

<sup>a</sup> The value in the parenthesis is not certified.

## 2.6. Statistical analysis

The whole data were subjected to a statistical analysis and correlation matrices were produced to examine the inter-relationships between the investigated trace element concentrations of the samples. Student's *t*-test was employed to estimate the significance of values.

## 3. Results and discussion

In the beginning of the work, the procedures were checked by recovery studies. The recovery values were nearly quantitative for all digestion methods. The relative standard deviations were less than 10% for all investigated elements. The performances of the two digestion procedures were compared by *t*-test ( $p < 0.05$ ). For this purpose, a sample (01) was digested by two methods. The results are given in Table 3.

In order to compare the results found by wet and microwave digestion procedures, NIST-SRM 1515 Apple leaves standard reference material was digested with two procedures. The results for this study are given in Table 4. The recovery values for the investigated metal ions were quantitative (>95%).

The comparison of wet and microwave digestion methods showed no statistically significant differences in results (Table 3). Therefore, the microwave digestion procedure was chosen for the samples because of more accuracy with respect to both time and recovery than wet digestion. The standard deviations of the wet digestion method are considerably higher than those of the microwave digestion method.

**Table 5**  
Trace metal levels (mg/kg) in mushroom samples from Black sea region, Turkey

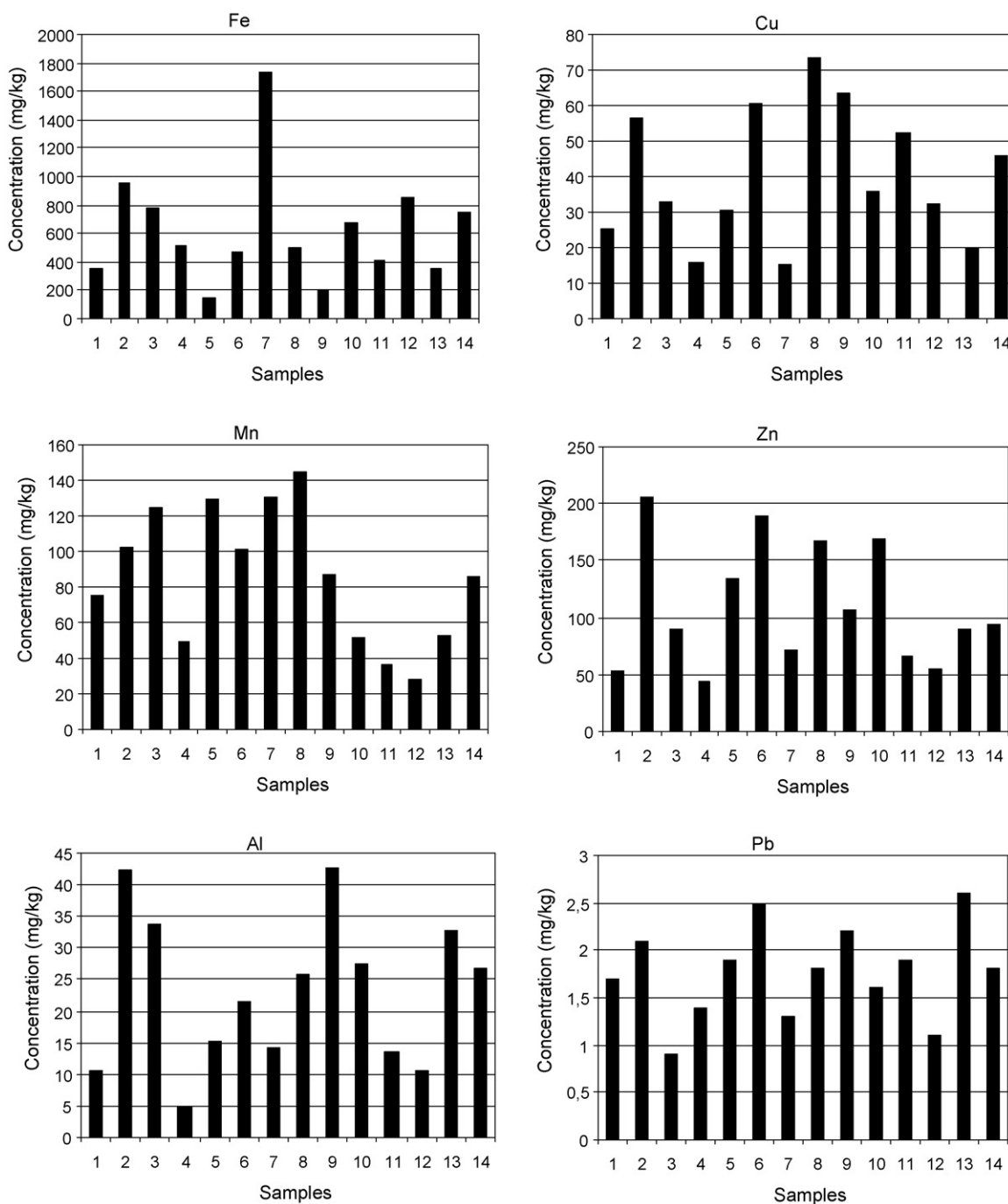
Sample number	Fe	Cu	Mn	Zn	Al	Pb
01	350 $\pm$ 30	25.2 $\pm$ 2.2	75.1 $\pm$ 6.5	52.7 $\pm$ 4.7	10.5 $\pm$ 0.9	1.7 $\pm$ 0.1
02	950 $\pm$ 80	56.2 $\pm$ 5.1	102 $\pm$ 10	205 $\pm$ 19	42.2 $\pm$ 3.2	2.1 $\pm$ 0.2
03	780 $\pm$ 75	32.8 $\pm$ 2.9	125 $\pm$ 16	89.2 $\pm$ 6.6	33.7 $\pm$ 2.8	0.9 $\pm$ 0.1
04	510 $\pm$ 42	15.6 $\pm$ 1.3	49.1 $\pm$ 3.6	43.5 $\pm$ 2.5	4.8 $\pm$ 0.5	1.4 $\pm$ 0.1
05	150 $\pm$ 13	30.5 $\pm$ 2.6	130 $\pm$ 10	135 $\pm$ 12	15.2 $\pm$ 1.3	1.9 $\pm$ 0.2
06	468 $\pm$ 41	60.3 $\pm$ 5.5	101 $\pm$ 9	190 $\pm$ 14	21.4 $\pm$ 1.9	2.5 $\pm$ 0.3
07	1741 $\pm$ 132	15.5 $\pm$ 1.3	131 $\pm$ 12	72.5 $\pm$ 6.5	14.2 $\pm$ 1.1	1.3 $\pm$ 0.1
08	502 $\pm$ 43	73.8 $\pm$ 5.6	145 $\pm$ 15	167 $\pm$ 14	25.9 $\pm$ 2.3	1.8 $\pm$ 0.2
09	205 $\pm$ 21	63.4 $\pm$ 5.3	87.4 $\pm$ 7.2	106 $\pm$ 10	42.7 $\pm$ 3.8	2.2 $\pm$ 0.2
10	674 $\pm$ 50	25.8 $\pm$ 2.3	51.5 $\pm$ 4.3	169 $\pm$ 15	27.3 $\pm$ 2.5	1.6 $\pm$ 0.1
11	406 $\pm$ 32	52.4 $\pm$ 4.3	36.9 $\pm$ 3.3	65.7 $\pm$ 5.1	13.6 $\pm$ 1.2	1.9 $\pm$ 0.2
12	852 $\pm$ 73	32.6 $\pm$ 2.9	28.6 $\pm$ 2.6	55.9 $\pm$ 4.6	10.5 $\pm$ 1.3	1.1 $\pm$ 0.1
13	258 $\pm$ 21	20.1 $\pm$ 1.9	52.4 $\pm$ 3.2	89.3 $\pm$ 7.7	32.8 $\pm$ 2.1	2.6 $\pm$ 0.3
14	743 $\pm$ 66	45.7 $\pm$ 4.3	85.6 $\pm$ 7.4	93.7 $\pm$ 7.1	26.9 $\pm$ 2.4	1.8 $\pm$ 0.1

Some species analyzed in this study were edible (*Calvatia excipuliformis*, *Lycoperdon perlatum*, *Laccaria amethystea*, *Armillaria mellea*, *Marasmius oreades*, *Cantharellus cibarius*, *Craterellus cornucopioides*, *Cantharellus tubaeformis*, *Clitocybe gibba*, *Collybia dryophila*, *Lepista nuda* and *Mycena aetites*), while others were inedible (*Xerula radicata* and *Hypholoma fasciculare*).

Lead, aluminium, iron, copper, manganese and zinc were chosen as representative trace metals whose levels in the environment represent a reliable index of environmental pollution [19–21]. The main sources of the toxic and essential metals in the environment are metallurgy industries, combustion of coal and high traffic density.

Table 5 and Fig. 1 present the results of the analysis of heavy metal levels (mg/kg). The order of the levels of heavy metals in the mushrooms samples was found to be as Fe > Zn > Mn > Cu > Al > Pb. The iron content of the samples ranged from 150 mg/kg in *M. oreades* to 1741 mg/kg in *C. cibarius*. The average iron content of the samples was 613.5 mg/kg. Iron values in mushrooms samples have been reported in the range of 31.3–1190 mg/kg [7], 568–3904 mg/kg [22] and 102–1580 mg/kg [23]. Our iron values are in agreement with literature values. The FAO/WHO has set a limit for heavy metal intake based on body weight. For an average adult (60 kg body weight), the provisional tolerable daily intake (PTDI) for lead, iron, copper and zinc are 214  $\mu\text{g}$ , 48 mg, 3 mg and 60 mg, respectively [24].

The copper levels ranged from 15.5 to 73.8 mg/kg for *C. cibarius* and *C. cornucopioides*, respectively. The average copper content of the samples was 39.3 mg/kg. Copper contents of mushrooms samples in the literature have been reported in the range of



**Fig. 1.** Levels of analyte ions in analyzed samples: (1) *Calvatia excipuliformis* (Scop.) Perdeck, (2) *Lycoperdon perlatum* Pers., (3) *Laccaria amethystea* Cooke, (4) *Armillaria mellea* (Vahl) P. Kumm., (5) *Marasmius oreades* (Bolton) Fr., (6) *Xerula radicata* (Relhan) Fr., (7) *Cantharellus cibarius* (Fr.) Quél., (8) *Craterellus cornucopioides* (L.) Pers., (9) *Cantharellus tubaeformis* (Bull.) Fr., (10) *Hypholoma fasciculare* (Huds.) Quél., (11) *Clitocybe gibba* (Pers.) P. Kumm., (12) *Collybia dryophila* (Bull.) P. Kumm., (13) *Lepista nuda* (Bull.) Cooke and (14) *Mycena aetites* (Fr.) Quél.

12–181 mg/kg [25], 10.3–145 mg/kg [7] and 13.4–50.6 mg/kg [23]. Our copper values are in agreement with literature values. Copper concentrations in the accumulating mushroom species are usually 100–300 mg/kg dry matter, which is not considered a health risk [26]. Copper contents in mushrooms higher than those in vegetables should be considered as a nutritional source of the element. Nevertheless, for people, bioavailability from mushrooms was reported to be low, due to limited absorption from the small intestine [27].

The manganese content of the samples ranged from 28.6 mg/kg in *C. dryophila* to 145 mg/kg in *C. cornucopioides*. The aver-

age manganese content for the samples was 85.8 mg/kg. The reported manganese values in the literature for mushrooms were 14.2–69.7 mg/kg [23] and 21.7–74.3 mg/kg [25]. Manganese, one of the least toxic metals, if inhaled as  $MnO_2$  dust is more hazardous than ingested manganese. Toxicity limits of manganese for plants are high (400–1000  $\mu g/g$ ). Our values are under toxicity limits.

The zinc content in our samples was the least (43.5 mg/kg) in *A. mellea*, whereas in *L. perlatum*, it was the highest (205 mg/kg). The average zinc content of the samples was 109.6 mg/kg. Zinc concentrations of mushrooms samples in the literature have been reported in the range of 33.5–89.5 mg/kg [23] and 45–188 mg/kg [25]. Zinc

**Table 6**  
Correlations between metal concentrations of mushroom samples

	Fe	Cu	Mn	Zn	Al	Pb
Fe	1.000					
Cu	-0.359	1.000				
Mn	0.227	0.297	1.000			
Zn	-0.051	0.568	0.437	1.000		
Al	-0.075	0.475	0.279	0.557	1.000	
Pb	-0.515	0.413	-0.026	0.463	0.387	1.000

is one of the most important trace metals for normal growth and development of humans. The deficiency of zinc can result from inadequate dietary intake, impaired absorption, excessive excretion or inherited defects in zinc metabolism. Mushrooms are known as zinc accumulator and sporophore:substrate ratio for zinc ranges from 1 to 10 mg/kg [28,29].

Aluminium content ranged from 4.8 mg/kg in *A. mellea* to 42.7 mg/kg in *C. tubaeformis*. The average aluminium content of the samples was 23 mg/kg. Aluminium contents of mushrooms samples have been reported in the range of 8.5–365 mg/kg [30]. Aluminium is not considered to be an essential element in humans. Exposure of aluminium has been implicated in a number of human pathologies including encephalopathy/dialysis dementia, Parkinson disease and Alzheimer's disease [31]. The permissible aluminium dose for an adult is quite high (60 mg per day) [32].

The lead content ranged from 2.6 mg/kg in *L. nuda* to 0.9 mg/kg in *L. amethystea*. The average lead content of the samples was 1.8 mg/kg. Lead contents of mushrooms samples in the literature have been reported in the range of 0.40–2.80 mg/kg [33] and 0.75–1.99 mg/kg [23]. The fact that toxic metals are present in high concentrations in mushrooms is of particular importance in relation to the standards [34–36] for Pb and Cd as toxic metals. The maximum permissible doses for an adult are 3 mg Pb and 0.5 mg Cd per week, but the recommended doses are only one-fifth of those quantities. Lead is known to induce reduced cognitive development and intellectual performance in children and increased blood pressure and cardiovascular disease in adults [37].

The trace metal contents in the mushrooms are mainly affected by acidic and organic matter content of their ecosystem and soil [8]. The uptake of metal ions in mushrooms is in many respects different from plants. For this reason the concentration variations of metals depend on mushroom species and their ecosystems [38–42].

A linear regression correlation test was performed to investigate correlations between metal concentrations. The values of correlation coefficients between metal concentrations are given in Table 6. There is a good correlation between zinc and copper ( $r=0.568$ ). There were positive correlations of aluminium–zinc, aluminium–copper and lead–zinc with corresponding  $r$  values of 0.557, 0.475 and 0.463, respectively. The negative correlations between lead–iron and copper–iron were found as  $-0.515$  and  $-0.359$ , respectively.

#### 4. Conclusion

The microwave digestion procedure was chosen for the samples because of more accuracy with respect to both time and recovery than wet digestion. The analytical parameters obtained make this method suitable for the determination of Cu, Mn, Fe, Zn, Pb and Al in mushroom samples. Relative standard deviations (R.S.D.) were found below 10%. Maximum lead level permitted for wild edible mushrooms is 1 mg/kg according to Turkish Food Codex [43]. The levels of lead analyzed in some edible mushroom samples were found to be higher than legal limits. Wild edible mushroom samples should be analyzed more often in Black sea region of Turkey with respect to toxic metals.

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