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Determination and evaluation of Cu, Mn, Zn, Cd, Pb and Ni contents in wild-grown edible mushroom species from Cappadocia, Turkey

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Abstract

The aim of this study was to determine the Cu, Mn, Zn, Cd, Pb and Ni concentrations in sixteen different wild-grown edible mushroom species grown in Niğde and Nevşehir. In the sample preparation step, the samples were dried, ground and sieved by 200 meshed sieve, consecutively. The extractions of metals were conducted by acid mineralization using concentrated nitric acid and hydrogen peroxide in microwave digestion unit. The determinations of metals were performed by flame atomic absorption spectrometry. Among the mushroom samples that were analyzed, Cu, Mn, Zn, Cd, Pb, and Ni concentrations were determined in the range of 6.7-1353, 4.7-109, 44.8-406, 0.14-6.4, 4.28-25.6 and 1.7-11.0 mg/kg, respectively. The accuracy and precision of the proposed method for metal determinations were validated by using the NIST SRM 1573a Tomato Leaves certified standard material. The obtained results were evaluated in terms of human health and compared with each other and previously reported values in the literature. In addition, the habitats of the mushroom samples were identified.

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1. Introduction

In the world, food production and consumption are increasing due to population growth. Increased population, growing industrialization, developments in technology, new industrial factories and their activities bring a lot of advantages and adversely affect to environment and also inhabitants. As a result of these activities considerable amount of trace metal can be released to environment. So, environment has been polluted mostly in terms of heavy metals. The majority of heavy metals (Cd, Ni, Pb, etc.) have toxic effects on living organisms [1]. On the other hand, some of these metals (Cu, Mn, Zn, etc.) are essential for human health [2]. Heavy metals are introduced to the human body via inhalation, the food chain, drinking water and skin contact and accumulate in vital organs (the brain, liver, bones and kidneys) [3]. Accumulated heavy metals can lead to diseases and cancers [4].

Trace metal pollution in the world is the biggest problem and also inevitable for environment and inhabitants. Heavy metals are found naturally in the earth and become concentrated with increasing anthropogenic and industrial activities [5]. Concentrated heavy metals can be accumulated by some plants [6,7] and animal species [8,9]. When the mushrooms are compared in the plant family, they can accumulate a considerable amount of heavy metal such as Zn, Cu and Pb [10,11]. Wild-grown edible mushrooms are a dietetic food and have high nutritional value, low calories and pharmacological characteristics. Therefore, mushrooms are highly preferred by people as a natural nutrient [12,13]. For these reasons, the determination and evaluation of heavy metal levels in wild-grown edible mushrooms are very important.

Great efforts have been made to determine the trace metal levels of wild-grown edible mushrooms by using flame atomic absorption spectrometry [14], graphite

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furnace atomic absorption spectrometry [15], inductively coupled plasma mass spectrometry [16,17] and inductively coupled plasma optic emission spectrometry [18,19] after application of different digestion methods such as wet digestion [20], dry ashing [21] and microwave digestion procedures [22]. The wet digestion and dry ashing methods are complicated and more time-consuming than the microwave digestion method without having any advantages. The obtained results in the literature from standard reference material analysis show that the microwave digestion method provided the best quantitative recovery among these digestion methods [23].

In this study, evaluation of the heavy metal pollution level in Cappadocia was performed. The trace metal levels of wild-grown edible mushroom species in Cappadocia, Turkey were determined by using flame atomic absorption spectrometry. Mushroom samples were digested by a microwave digestion unit. In addition, the metal contents of mushroom species were compared with previously reported results in the literature.

2. Materials and Methods

2.1. Apparatus

A Perkin Elmer AAnalyst 700 (Waltham, Massachusetts, ABD) flame atomic absorption spectrometer was used for the determination of the heavy metal levels in mushroom species. Milestone Ethos D (Milestone, Sorisole, Italy) microwave digestion unit was used for the digestion process. The Milli-Q Millipore ultrapure distilled water system (Darmstadt, Germany, resistivity of 18.2 MΩ-cm) was used throughout the experiments.

2.2. Reagents and solutions

The chemicals and acid solutions used in the experiments were of analytical grade and obtained from Merck (Darmstadt, Germany) and Sigma-Aldrich (St. Louis, MO). Ultrapure distilled water was used for all dilutions, preparation of solutions and washing processes. Glassware and plastic equipment were washed with a 10% HNO_3 (Merck) solution, rinsed with ultrapure water and dried in an oven before use throughout the experiments. In atomic absorption measurements, standard metal solutions for the calibration graph were prepared daily by dilution of 1000 µg/mL stock metal solutions from Merck and Sigma. NIST SRM 1573a Tomato Leaves were used as a certified reference material to check the accuracy of the method.

2.3. Collecting site and sampling

Nevşehir and Niğde are small cities which are located in the touristic Cappadocia region of Turkey. Domestic and foreign visitors come to the area throughout the year.

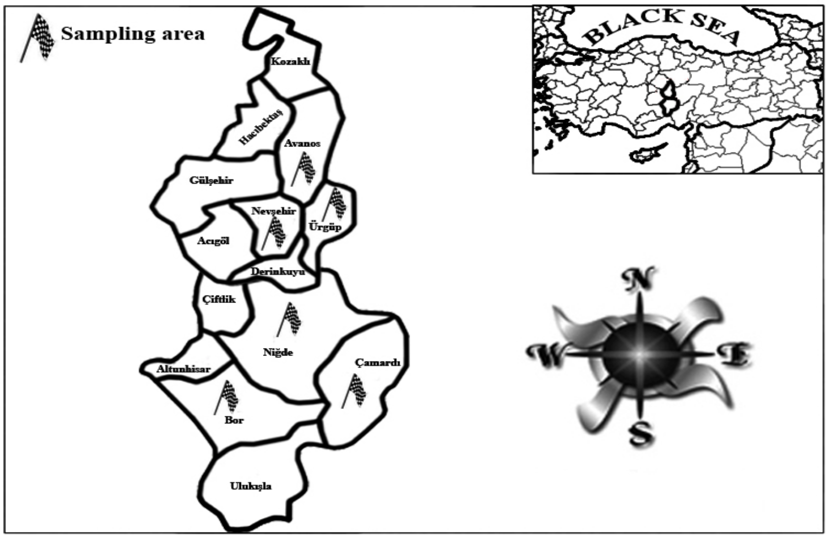

Figure 1. Map of the study area

A total of 187 living fresh samples from 16 different species of mushrooms (*Agaricus campestris*, *Agrocybe aegerita*, *Agrocybe dura*, *Armillaria mellea*, *Boletus edulis*, *Boletus luteus*, *Coprinus comatus*, *Lactarius piperatus*, *Lactarius salmonicolor*, *Lactarius volemus*, *Marasmius oreades*, *Panellus stipticus*, *Piptoporus betulinus*, *Pleurotus ostreatus*, *Rhizopogon luteolus*, *Russula delica*) were collected from different sites of Cappadocia (Nevşehir 38° 42’ North, 34° 50’ East and Niğde 37° 57’ North, 34° 40’ East). The locations of the collected mushroom samples are given in Figure 1. In addition, 10±2 g mushroom samples were collected from each species and sampling study was performed during March-April 2015. The collected mushroom samples were stored in polyethylene bags.

2.4. Preparation of mushroom samples

The collected fresh mushroom samples were kept for 48 hours in an oven at 80 °C for the drying process. Dry mushroom samples were homogenized in a porcelain agate homogenizer. Samples were stored in polyethylene bags after being sieved through a 200 mesh sieve. The samples were kept in these bags at room temperature until the digestion process.

2.5. Digestion process

The digestion procedure was performed by using a microwave digestion unit [24]. 1.0±0.1 g. of each dry mushroom sample, 6 mL of concentrated (65%) HNO_3 and 2 mL of (30%) H_2O_2 solution were added in the teflon reaction vessel of the microwave digestion system and diluted to 10 mL of final volume with ultrapure distilled water after applying the digestion process.

Table 1 Digestion conditions for Milestone Ethos D microwave digestion unit

Digestion Step	Time, (Min)	Power, (W)
1	2	250
2	2	0
3	6	250
4	5	400
5	8	550
Ventilation	8	0

Table 2 Obtained and certified metal levels of NIST SRM 1573a Tomato Leaves, N=4

Element	Certified value (µg/g)[a]	Obtained value (µg/g)[a]	Recovery (%)[a]
Cu	4.7	4.60±0.28	98±2
Zn	30.9	29.7±1.5	96±3
Mn	246	238±15	97±1
Cd	1.52	1.52±0.10	100±1
Ni	1.59	1.55±0.10	97±3

[a]Mean ± Standard deviation

The analyses of blank samples and standard reference material were performed at the same conditions. The digestion conditions of the microwave unit and analysis results of the certified reference material are given in Tables 1 and 2, respectively.

3. Results and Discussion

As shown in Table 2, the results obtained from certified reference material analyses were in good agreement with the certified levels. The recovery values of the investigated metal ions were quantitative (>95%). The relative standard deviations were less than 10% for the investigated metals. The families of wild-grown mushroom species were identified and given in Table 3 with their habitats. The results obtained from the analysis are given in Tables 4 and 5. They were compared with each other and also with previously reported results in the literature.

Table 3 Habitats of the edible mushroom species

Mushroom species	Habitat
Agaricus campestris	Among grass
Agrocybe aegerita	In forests
Agrocybe dura	Roadside or in meadows
Armillaria mellea	In dense clusters on or around tree trunks
Boletus edulis	In pine forests
Boletus luteus	In broad-leaved woods
Coprinus comatus	In meadows
Lactarius piperatus	On ground or native trees
Lactarius salmonicolor	Under trees
Lactarius volemus	In pine forests
Marasmius oreades	Often forming rings in the short grass of pasture or lawns
Panellus stipticus	On dead wood
Piptoporus betulinus	On wood or trees
Pleurotus ostreatus	On wood or trees
Rhizopogon luteolus	In soil
Russula delica	In coniferous and mixed woodland

The Cu concentrations in the mushroom samples were found in the range of 58-1353 and 6.7-250 mg/kg for Niğde and Nevşehir, respectively. The Cu contents of mushroom samples in Niğde were higher than the mushroom samples of Nevşehir. When the copper concentrations were compared with other studies, the copper levels of the mushroom samples were slightly higher than previously reported studies [25]. Only *Agaricus campestris* had a considerably high concentration of copper (1353 mg/kg). In a previous study, copper concentrations in wild-grown edible mushrooms were found to be between 100 and 300 mg/kg, which were not considered a health risk [26].

The Mn levels of the mushroom samples were between 7.9 and 109 mg/kg for Niğde, 4.7 and 55.6 mg/kg for Nevşehir. The Mn concentrations of the mushroom samples grown in Niğde city were relatively higher than the mushroom samples of Nevşehir. The manganese levels of the analyzed mushrooms, when compared with other studies, showed that the results were in agreement with previous studies [27,28]. The reported manganese concentrations for edible mushrooms were between 13.5 and 113 mg/kg on dry weight basis [29].

The Zn contents in mushroom samples were determined to be between 44.8 to 406 mg/kg and 49.2 to 147 mg/kg for Niğde and Nevşehir, respectively. The Zn levels of the mushroom samples were almost equal. Only one species of mushroom which was *Agaricus campestris* had a high concentration of zinc. The zinc contents of the analyzed mushroom samples, when compared to other studies, showed that the zinc levels were consistent with previously reported studies [30,31]. The determined Zn levels in edible mushroom samples ranged from 35.8 to 410 mg/kg in a previously reported study [32].

The Cd contents of mushroom samples were determined to be between 0.14 and 2.01 mg/kg on a dry weight basis for Niğde. The Cd levels of Nevşehir mushrooms were determined to be between 1.2 and 6.4 mg/kg. Levels in Nevşehir mushroom samples were higher than the mushroom samples of Niğde. The cadmium levels of mushrooms in Niğde were lower than those previously reported in the literature [33] while the Cd concentrations of the mushroom samples grown in Nevşehir were higher than previous studies [34]. According to Maximum Levels of Contaminants in Foods (GB2762-2005) and Hygienic Standard for Edible Fungi (GB7096-2003), the safe limit was 0.2 mg/kg for Cd. High levels of cadmium in analyzed mushroom species may be sourced from fertilizer.

The minimum and maximum Pb levels in mushroom samples were found as 4.3-25.6 mg/kg and 7.9-22.2 mg/kg for Niğde and Nevşehir, respectively. The Pb concentrations in edible mushrooms grown in both cities were the same and there was no significant difference in terms of Pb concentration. When the lead contents of mushrooms were compared with other studies, lead levels were higher than previously reported in the literature [35]. In another previous study Pb levels were determined between 0.1 to 40 mg/kg [36]. According to reported values, the Pb concentrations of the mushroom samples were found at lower levels. High levels of lead in analyzed mushroom samples can be sourced from traffic.

The Ni concentrations of mushroom samples were determined in the range of 2.8 and 11.0 mg/kg for Niğde and 1.7 and 10.5 mg/kg for Nevşehir, respectively. The Ni concentrations in mushroom samples from both cities were approximately the same. The nickel concentrations of the analyzed mushroom samples were lower than in a previous study [37]. In previously reported studies, nickel concentrations were determined to be between 8.2-21.6 mg/kg and 0.4-15.9 mg/kg [38].

Table 4 Trace metal contents (mg/kg) of mushroom species from Niğde, N=4

Sample Name	Cu[a]	Mn[a]	Zn[a]	Cd[a]	Pb[a]	Ni[a]
Agaricus campestris	1353±32	50.0±3.7	406±33	0.83±0.05	25.6±1.1	10.3±0.7
Agrocybe aegerita	137±10	10.7±0.8	76.5±5.9	0.74±0.03	6.9±0.3	*BDL
Armillaria mellea	154±9	81.3±6.0	50.3±5.5	0.53±0.03	20.8±1.3	4.2±0.3
Boletus edulis	425±29	109.0±8.0	211±18	0.44±0.02	22.1±1.7	11.0±0.8
Coprinus comatus	214±8	7.9±0.4	95.4±7.4	2.01±0.05	4.3±0.2	4.2±0.3
Lactarius piperatus	161±11	25.2±1.9	90.6±8.2	1.28±0.03	4.3±0.4	6.2±0.4
Lactarius volemus	258±15	67.9±5.3	93.1±7.9	0.85±0.06	11.9±0.8	6.2±0.5
Panellus stipticus	374±10	67.1±5.6	59.0±4.6	1.48±0.05	9.7±0.7	9.6±0.6
Pleurotus ostreatus	58±4	16.8±1.2	44.8±4.1	0.24±0.02	4.3±0.3	4.2±0.3
Rhizopogon luteolus	159±9	8.2±0.7	49.9±3.0	0.14±0.01	6.8±0.4	2.8±0.2
Russula delica	592±43	71.9±7.7	172±14	0.98±0.04	14.4±1.6	7.6±0.6
Detection limit	0.08	0.07	0.31	0.05	0.58	0.63

*BDL: Below detection limit
[a]Mean±standard deviation

Table 5 Trace metal contents (mg/kg) of mushroom species from Nevşehir, N=4

Sample Name	Cu[a]	Mn[a]	Zn[a]	Cd[a]	Pb[a]	Ni[a]
Agrocybe aegerita	250.0±23.0	55.6±6.4	147±15	4.6±1.0	22.2±2.5	7.8±2.4
Agaricus campestris	103.0±16.0	36.8±3.6	119±7	2.5±0.5	14.9±0.9	5.2±0.8
Agrocybe dura	21.2±1.5	11.3±1.3	79.1±2.5	1.5±0.5	10.6±0.2	3.7±1.4
Armillaria mellea	42.2±2.8	9.9±0.9	87.4±8.1	6.4±0.5	12.8±0.8	5.2±1.0
Boletus luteus	34.4±2.8	12.4±1.1	91.5±8.7	2.8±0.5	11.1±0.3	4.8±1.6
Coprinus comatus	90.3±7.1	21.2±3.7	89.4±7.8	1.8±0.3	13.1±1.1	5.5±0.9
Lactarius volemus	64.3±2.0	8.2±2.1	94.3±4.7	1.7±0.6	12.4±1.4	4.2±0.8
Lactarius salmonicolor	6.7±1.1	10.5±2.6	82.7±6.0	1.2±0.3	7.9±0.9	1.7±0.6
Marasmius oreades	49.5±1.7	28.1±2.4	56.4±5.7	3.0±0.5	13.0±1.1	2.1±0.8
Piptoporus betulinus	32.6±2.5	4.7±1.1	49.2±10.5	1.3±0.4	10.3±1.0	10.5±0.2
Rhizopogon luteolus	26.7±0.9	14.0±2.8	88.4±3.5	3.3±0.5	12.0±0.5	1.9±0.3
Russula delica	31.3±3.2	14.1±2.0	95.2±6.3	3.6±0.5	10.6±0.3	2.8±0.3
Detection limit	0.08	0.07	0.31	0.05	0.58	0.63

*BDL: Below detection limit
[a]Mean±standard deviation

4. Conclusions

Among the six metals, Cd, Pb and Ni are potentially hazardous. These heavy metals in wild-grown edible mushrooms may enter the human body via food chain and seriously damage human health. Therefore, it is necessary to periodically evaluate the metal contents and health risks of these mushrooms. Heavy metal pollution in wild-grown edible mushrooms from Cappadocia Region of Turkey has become a serious problem. The essential element concentrations (Mn, Cu, and Zn) in the mushrooms were determined to be at typical levels. However, the concentrations of toxic metals (Cd, Pb) in nearly all of the mushroom samples exceeded safe limits. The wild edible mushrooms in the study area have been contaminated with heavy metals so that they pose a threat to human health. Among the toxic heavy metals, Pb and Cd were accumulated by the mushrooms. Pb and Cd in wild edible mushrooms may pose a higher health risk than Ni. Intakes of Pb and Cd by consuming wild edible mushrooms from the study area may cause serious health problems.

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Conflict of interest

The authors declare that they have no conflict of interests.

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