BIO- AND TOXIC ELEMENTS IN EDIBLE WILD MUSHROOMS (*SUILLUS LUTEUS*)

Penko Papazov

Department of Organic Chemistry and Inorganic Chemistry, Technological Faculty,
University of Food Technology
26 Maritsa Blvd., 4000 Plovdiv, Bulgaria
e-mail: penko_papazov@abv.bg

Abstract: The aim of this study was to perform analysis by flame and graphite furnace AAS the content of Pb, Cd, Ni, Cr, Mn, Co, Cu, Zn and Fe in wild edible mushrooms *Suillus luteus*. The samples were collected from the Batak Mountain. All metals concentrations were determined on a dry weight basis. The average content of studied elements: Pb, Cd, Ni, Cr, Mn, Co, Cu, Zn and Fe in *Suillus luteus* samples were: 0.66 mg kg^{-1} , 0.52 mg kg^{-1} , 0.55 mg kg^{-1} , 0.51 mg kg^{-1} , 11.33 mg kg^{-1} , 0.98 mg kg^{-1} , 19.28 mg kg^{-1} , 51.67 mg kg^{-1} , and $114.41 \text{ mg kg}^{-1}$.

Keywords: Atomic absorption spectrometry, *Suillus luteus*, heavy metals, dry ashing.

1. INTRODUCTION

Wild-growing saprobic and symbiotic mushrooms may accumulate in their fruiting bodies' considerable amounts of metallic elements and metalloids due to specificities in their physiology [1-4]. Mushrooms are relatively rich in mineral constituents, both edible and inedible or poisonous species which all are eaten by game animals (wild boars, stags, and others) and may end up in humans via the food chain [4-7]. This is spectacularly evidenced for typical toxic metals such as cadmium (Cd), lead (Pb), mercury (Hg), or silver (Ag), which are well bioconcentrated from soil by many mushrooms [8-12].

Decomposition of solid samples is an important step in combined analytical methods. In most cases, when using highly sensitive measuring methods, such as flame atomic absorption spectrometry (FAAS), graphite furnace AAS, ICP-OES, ICP-MS, the sample is measured in an aqueous solution [13-15]. Combined analytical methods are favoured for multi element analysis of environmental and biological samples at very high speed. Sequential and simultaneous determinations of the elements can be made using the above analytical techniques [16-20].

The aim of this study was to perform analysis by flame and graphite furnace AAS the content of Pb, Cd, Ni, Cr, Mn, Co, Cu, Zn and Fe in wild edible mushrooms *Suillus luteus*. The samples were collected from the Batak Mountain.

2. EXPERIMENTAL

2.1. Sampling

Mushroom samples were collected in 2017 and 2018 from the Batak Mountain by the authors themselves.

Mushroom samples were washed with distilled water and dried at 105°C for 24 h. The dried samples were ground, then homogenized using an agate pestle and stored in polyethylene bottles until analysis.

2.2. 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. HNO₃, H₂SO₄, H₂O₂, HF, HClO₄ and HCl were of supra pure quality (E. Merck). All the plastic and glassware was cleaned by soaking in dilute HNO₃ (1+9) and rinsed with distilled water prior to use. The element standard solutions used for calibration were prepared by diluting a stock solution of 1000 mg l⁻¹ (Pb, Cd, Co, Cr, Mn, and Ni) supplied by Sigma and (Cu, Zn, Fe) by Aldrich.

2.3. Apparatus

A Perkin Elmer AAnalyst 800 atomic absorption spectrometer with deuterium background corrector was used in this study. Pb, Cd, Co, Cr, Mn and Ni in plant samples were determined by HGA graphite furnace using argon as inert gas. Other measurements were carried out in an air/acetylene flame. The instrumental parameters and operating conditions are given in Table 1.

Table 1. Instrumental analytical conditions of element analyses

FAAS						
Element	Wavelength (nm)	Lamp current (mA)	Slit width (nm)	Air (l min ⁻¹)	Acetylene (l min ⁻¹)	
Pb	283.3	30	0.7	17.0	2.0	
Cd	228.8	4	0.7	17.0	2.0	
Ni	232.0	25	0.2	17.0	2.0	
Cr	357.9	25	0.7	17.0	2.5	
Mn	279.5	20	0.2	17.0	2.0	
Co	240.7	30	0.2	17.0	2.0	
Cu	324.8	15	0.7	17.0	2.0	
Zn	213.9	15	0.7	17.0	2.0	
Fe	248.3	30	0.2	17.0	2.0	
GFAAS						
Instrumental conditions	Pb	Cd	Ni	Cr	Mn	Co
Argon flow (ml min ⁻¹)	250	250	250	250	250	250
Sample volume (μl)	20	20	20	20	20	20
Modifier (μl)	5	10	5	5	5	5
Heating program temperature, °C (ramp time (s), hold time (s))						
Drying 1	110 (1, 20)	110 (1, 20)	110 (1, 20)	110 (1, 20)	110 (1, 20)	110 (1, 20)
Drying 2	130 (5, 30)	130 (5, 30)	130 (5, 30)	130 (5, 30)	130 (5, 30)	130 (5, 30)
Pretreatment	850 (10, 20)	700 (10, 20)	1100 (10, 20)	1500 (10, 20)	1300 (10, 20)	1400 (10, 20)
Atomization	1500 (0, 5)	1400 (0, 5)	2300 (0, 5)	2500 (0, 5)	1900 (0, 5)	2400 (0, 5)
Cleaning	2400 (1, 2)	2400 (1, 2)	2400 (1, 2)	2400 (1, 2)	2400 (1, 2)	2400 (1, 2)

2.4. Digestion procedures

One gram of mushroom sample was placed into a high form porcelain crucible. The furnace temperature was slowly increased from room temperature to 450 °C in 1 h. The samples were ashed for approximately 4 h until a white or grey ash residue was obtained. The residue was dissolved in 5 ml of HNO₃ (25% v/v) and the mixture, when necessary, was heated slowly the residue. The solution was transferred a 25-ml volumetric flask and made up to volume. A blank digest was carried out in the same way.

2.5. Analytical procedure

Detection limit is defined as the concentration corresponding to three times the standard deviation of ten blanks. Detection limit values of elements as microgram per litre in flame AAS were found to be 0.025 for Cd; 0.127 for Co; 0.083 for Cr; 0.072 for Cu; 0.111 for Fe; 0.058 for Mn; 0.145 for Ni; 0.450 for Pb; and 0.021 for Zn. The concentrations of Cu, Zn and Fe were determined in the plant samples using FAAS. The other elements (Cd, Pb, Co, Cr, Mn and Ni) were below the corresponding detection limits of FAAS. These elements in plant samples were determined using graphite furnace AAS by autosampler.

During analyses, internal argon flow rate through the graphite tube was 250 ml min⁻¹; 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 0.050 - mg NH₄H₂PO₄ + 0.003 mg Mg(NO₃)₂ for Pb; 0.050 - mg NH₄H₂PO₄ + 0.003 mg Mg(NO₃)₂ for Cd; 0.015 mg Mg(NO₃)₂ for Co; 0.005 mg Pd + 0.003 mg Mg(NO₃)₂ for Mn; 0.015 mg Mg(NO₃)₂ for Ni; and 0.015 mg Mg(NO₃)₂ for Cr. Most of the matrix was removed before the atomization step and less interference occurred during atomization. Each graphite furnace AAS analysis calls for 20 µl of solution and 5–10 µl of the matrix modifier. As Table 1 shows, matrix modifier was used for all 6 elements determined by GFAAS.

Characteristic mass for 0.0044 absorbance was found to be 1.3 pg for Cd; 17.0 pg for Co; 7.0 pg for Cr; 20.0 pg for Ni; 6.3 pg for Mn; and 30 pg for Pb.

2.6. Statistical processing

SPSS (Statistical Package for Social Science) program for Windows was used for statistical data processing.

3. RESULTS AND DISCUSSION

It is desirable to use a higher ashing temperature in graphite furnace in order to remove the matrix efficiently for many analytes in food, biological and environmental samples. The ashing and atomization temperatures of heavy metals were increased using different chemical modifiers.

Table 2. Concentration of heavy metals in mushroom samples (*Suillus luteus*) collected from Batak mountain, Bulgaria (n = 135)

	Pb	Cd	Ni	Cr	Mn	Co	Cu	Zn	Fe
$\bar{X}$ mg kg ⁻¹	0.66	0.52	0.55	0.51	11.33	0.98	19.28	51.67	114.41
SD mg kg ⁻¹	0.38	0.37	0.22	0.46	8.97	0.72	16.93	32.91	53.89
Min	0.63	0.21	0.08	0.06	0.18	0.08	4.11	51.72	74.56
Max	0.94	0.42	0.22	0.11	2.76	0.12	8.93	69.26	105.13
95% Confid. Level	57.62	70.88	40.72	90.12	79.19	73.81	87.83	63.69	118.11

Living organisms require varying amounts of “heavy metals”. Iron, cobalt, copper, manganese, molybdenum and zinc are required by humans, but excessive levels can be damaging to the organism. Other heavy metals such as mercury, plutonium and lead are toxic metals and their accumulation over time in the bodies of animals can cause serious illnesses. Copper (Cu) as stated is an essential metal, which serve as a constituent of some metal-loenzymes, and is required in hemoglobin synthesis and catalysis of metabolic growth [21-25]. The average concentration of Cu in the studied samples was 19.28 mg kg⁻¹, which was far below the safe limit of 40 mg kg⁻¹ set by WHO [26]. Copper levels in mushrooms reported in literature are 4.71–181.0 mg kg⁻¹ [20-25].

Iron (Fe) is an essential metal involved in biochemical processes. The average Fe content recorded in the studied samples was 114.41 mg kg⁻¹, respectively, which are above the safety limit of 15.0 mg kg⁻¹, determined by the WHO. Levels of Fe reported in literature were 31.3–1190.0 mg kg⁻¹ [27-29].

Manganese (Mn) is an essential metal needed for biological systems such as metalloproteinases [25-29]. The average Mn content recorded in the studied samples was 11.33 mg kg⁻¹, respectively, which are also below the toxicity limit of 400.0–1000.0 mg kg⁻¹. The literature has reported levels of Mn as 12.9–93.3 mg kg⁻¹ [20-29].

According to Vonugopal and Lucky [30], lead (Pb) is toxic even at trace levels and the impairment related to lead toxicity in humans include abnormal size and hemoglobin content of the erythrocytes, hyper stimulation of erythropoiesis and inhibition of haem synthesis. The maximum level of Pb present in the studied wild mushrooms was 0.66 mg kg⁻¹, which is far below the 10.0 mg kg⁻¹ limit set by the WHO [26]. Lead levels reported in literature are 0.4–7.77 mg kg⁻¹ [20-29].

Zinc is an essential metal and a component of a wide variety of different enzymes in which it is involved in catalytic, structural and regulatory roles. The average Zn content recorded in the studied samples was 51.67 mg kg⁻¹, respectively, which are above the safety limit of 60 mg kg⁻¹, determined by the WHO [26]. Zn levels reported in literature were 29.3–158.0 mg kg⁻¹ [20-29].

The contents of cadmium (Cd), chromium (Cr), nickel (Ni) and cobalt (Co) in mushroom *Suillus luteus* were found to be 0.52; 0.51; 0.55 and 0.98 mg kg⁻¹, respectively. The literature has reported levels of as 0.01–7.77 mg kg⁻¹ [31-33].

The trend in decreasing order of elements present in wild samples was Fe > Zn > Cu > Mn > Co > Pb > Ni > Cd > Cr. Mushrooms are known to possess a very effective mechanism that enables them to readily take up some heavy metals from the ecosystem [21-30]. The accumulation of heavy metals in mushrooms seems to be affected by environmental and fungal related factors. Environmental factors such as organic matter content, pH and metal

concentration in soil and fungal factors such as the species, morphological part of the fruit body, developmental stages, age of mycelium, intervals between fructifications and biochemical composition [27-35].

4. CONCLUSIONS

The heavy metals in mushrooms were at concentrations similar to those determined for the same or similar species elsewhere in Europe. According to this study, the edible wild mushroom *Suillus luteus* could be used in human nutrition due to its good parameters. Heavy metal content of samples indicated that the Batak Mountain was an ecologically pure region of Bulgaria, and therefore the mushrooms collected from this location could be consumed without any risk for human health.

5. REFERENCES

- [1] Frankowska A., Ziółkowska J., Bielawski L. & Falandysz J. (2010). Profile and bioconcentration of minerals by King Bolete (*Boletes edulis*) from the Płocka Dale in Poland. *Food Addit Contam B*, Vol. 3, 2010, pp. 1-6.
- [2] Aloupi M., Koutrotsios G., Koulousaris M. & Kalogeropoulos N. (2011). Trace metal contents in wild edible mushrooms growing on serpentine and volcanic soils on the island of Lesbos, Greece. *Ecotox Environ Safe*, Vol. 78, 2011, pp. 184-194.
- [3] Kalač P. (2016). *Edible mushrooms, chemical composition and nutritional value*. Academic Press, London, 2016.
- [4] Dospatliev L. & Ivanova M. (2017). *Boletus pinophilus* growing in Bulgaria: Chemical profile and hazard index. *Comptes rendus de l'Académie bulgare des Sciences*, Vol. 70, No. 6, 2017, pp. 795–802.
- [5] Brzostowski A., Jarzyńska G., Kojta A.K. & Wydmańska D. (2011). Variations in metal levels accumulated in Poison Pax (*Paxillus involutus*) mushroom collected at one site over four years. *J Environ Sci Health Part A*, Vol. 46, 2011, pp. 581–588.
- [6] Falandysz J., Zhang J., Wang Y., Krasińska G., Kojta A., Saba M., Shen T., Li T. & Liu H. (2015). Evaluation of the mercury contamination in mushrooms of genus *Leccinum* from two different regions of the world: accumulation, distribution and probable dietary intake. *Sci Total Environ*, Vol. 537, 2015, pp. 470-478.
- [7] Dospatliev L. & Ivanova M., Petkova Z., Angelova-Romov M. & Antova G. (2018). Fatty acid composition in medicinal mushrooms (*Craterellus tubaeformis*). *Applied Researches in Technics, Technologies and Education*, Vol. 6, No. 1, 2018, pp. 35-39.
- [8] Solomko E.F., Grodzinskaya A.A., Paschenko L.A. & Pchelintseva R.K. (1986). Mineralnij szosztav nekotarih kultivirujemih i dikorasztusih gribov klassa Basidiomycetes. (Mineral composition of some cultivated and wild fungi of the class Basidiomycetes). *Mikologia i fitopatologii*, Vol. 20, 1986, pp. 474-478.
- [9] Malinowska E., Szefer P. & Bojanowski R. (2006). Radionuclides content in *Xerocomus badius* and the other commercial mushrooms from several regions of Poland. *Food Chem*, Vol. 97, 2006, pp. 19-24.
- [10] Borovička J., Kotrba P., Gryndler M., Mihaljevič M., Řanda Z., Rohovec J., Cajthaml T., Stijve T. & Dunn C.E. (2010). Bioaccumulation of silver in ectomycorrhizal and saprobic macrofungi from pristine and polluted areas. *Sci Total Environ*, Vol. 408, 2010, pp. 2733-2744.
- [11] Vinichuk M., Taylor A.F.S., Rosén K. & Johanson K.J. (2010). Accumulation of potassium, rubidium and caesium (¹³³Cs and ¹³⁷Cs) in various fractions of soils and fungi in a Swedish forest. *Sci Total Environ*, Vol. 408, 2010, pp. 2543-2548.

- [12] Dospatliev L. & Ivanova M. (2017). Concentrations and risk assessment of lead and cadmium in wild edible mushrooms from the Batak mountain, Bulgaria. *Oxidation Communications*, Vol. 40, No. 2, 2017, pp. 993-1001.
- [13] Tuzen M., Sesli E. & Soylak M. (2007). Trace element levels of mushroom species from East Black Sea region of Turkey. *Food Control*, Vol. 18, 2007, pp. 806-810.
- [14] Kaya A. & Bağ H. (2010). Trace element contents of edible macrofungi growing in Adiyaman, Turkey. *Asian Journal of Chemistry*, Vol. 22, 2010, pp. 1515-1521.
- [15] Dospatliev L. & Ivanova M. (2017). Determination of heavy metals in mushroom samples by Atomic Absorption Spectrometry. *Bulgarian Chemical Communications*, Vol. 49, No. G, 2017, pp. 5-9.
- [16] Knapp G. (1991). Mechanized techniques for sample decomposition and element preconcentration. *Microchim. Acta*, Vol. 2, 1991, pp. 445-455.
- [17] Tüzen M., Özdemir M. & Demrbaş A. (1998). Study of heavy metals in some cultivated and uncultivated mushrooms of Turkish origin. *Food Chemistry*, Vol. 63, 1998, pp. 247-251.
- [18] Gucia M., Jarzyńska G., Rafał E., Roszak M., Kojta A.K., Osiej I. & Falandysz J. (2012). Multivariate analysis of mineral constituents of edible Parasol Mushroom (*Macrolepiota procera*) and soils beneath fruiting bodies collected from Northern Poland. *Environ. Sci. Pollut. Res.*, Vol. 19, 2012, pp. 416-431.
- [19] Dospatliev L. & Ivanova M. (2017). Statistical analysis of Cu and Zn concentrations in black trumpets (*Craterellus Cornucopioides*). *Ecological risk assessment. Applied Researches in Technics, Technologies and Education*, Vol. 5, No. 1, 2017, pp. 72-78.
- [20] Gąsecka M., Rzymiski P., Mleczek M., Siwulski M., Budzyńska S., Magdziak Z., Niedzielski P. & Sobieralski K. (2017). The relationship between metal composition, phenolic acid and flavonoid content in *Imleria badia* from non-polluted and polluted areas. *J. Environ. Sci. Heal B*, Vol. 52, 2017, pp. 171-177.
- [21] Silvestre M.D., Lagarda M.J., Farra R., Martinez-Costa C. & Brines J. (2000). Copper, iron and zinc determination in human milk using FAAS with microwave digestion. *Food Chemistry*, Vol. 68, No. 1, 2000, pp. 95-99.
- [22] García-Lafuente A., Moro C., Villares A., Guillamón E., Rostagno M.A., D'Arrigo M. & Martínez J.A. (2011). *American Journal of Community Psychology*, Vol. 48, 2011, pp. 125-141.
- [23] Sarikurkcü C., Tepe B., Kocak M.S. & Uren M.C. (2015). Metal concentration and antioxidant activity of edible mushrooms from Turkey. *Food Chem.*, Vol. 175, 2015, pp. 549-555.
- [24] Dospatliev L. & Ivanova M. (2016). ICP determination of Pb, Mn, Cu, Zn, Fe, K, Na, P, Mg and Ca in edible wild mushroom (*Boletus pinophilus*). *International Journal of Scientific and Research Publications*, Vol. 6, No. 12, 2016, pp. 266-269.
- [25] Stefanova-Bahchevanska T., Ahmedov R., Zaruba S., Andruch V., Delchev V., Dospatliev L. & Gavazov K. (2018). A cloud-point extraction-chromogenic system for copper(II) based on 1-(2-thiazolylazo)-2-naphthol. *Bulgarian Chemical Communications*, Vol. 50, No. B, pp. 38-43.
- [26] WHO. (1982). World Health Organization Evaluation of Certain Foods Additives and Contaminants (Twenty-Six Report of the Joint FAO/WHO Expert Committee on Food Additives); WHO Technical Report Series, No. 683; WHO Library Cataloguing-in-Publication Data; WHO: Geneva, Switzerland, 1982.
- [27] Yamaç M., Yıldız D., Sarıkürkcü C., Çelikkollu M. & Solak M.H. (2007). Heavy metals in some edible mushrooms from the Central Anatolia, Turkey. *Food Chemistry*, Vol. 103, 2007, pp. 263-267.

- [28] Zhu F., Qu L., Fan W., Qiao M., Hao H. & Wang X. (2011). Assessment of heavy metals in some wild edible mushrooms collected from Yunnan Province, China. *Environmental Monitoring and Assessment*, Vol. 179, No. 1-4, 2011, pp. 191-199.
- [29] Dospatliev L. & Ivanova M. (2017). ICP determination of Pb, Mn, Cu, Zn, Fe, K, Na, P, Mg and Ca in edible wild mushroom *Cantharellus Cibarius*. *Applied Researches in Technics, Technologies and Education*, Vol. 5, No. 1, 2017, pp. 66-71.
- [30] Vonugopal B. & Luckey T. (1975). Toxicity of Non-Radioactive Heavy Metals and Their Salts in Heavy Metals Toxicity, Safety and Hormology; Coulston F., Ed., Georg Thieme: Stuttgart, Germany. Academic Press: New York, NY, USA, 1975.
- [31] Falandysz J. & Drewnowska M. (2015). Macro and trace elements in Common Chanterelle (*Cantharellus cibarius*) mushroom from the European background areas in Poland: Composition, accumulation, dietary exposure and data review for species. *Journal of Environmental Science and Health, Part B*, Vol. 50, No. 5, 2015, pp. 374-387.
- [32] Dospatliev L. & Ivanova M. (2017). Spectrophotometric investigations on liquid-liquid extraction systems containing cobalt and tetrazolium salts. Application of the developed method for analysis of the cobalt content of biological samples (mushrooms and tobaccos). *Bulgarian Chemical Communications*, Vol. 49, No. 4, 2017, pp. 787-791.
- [33] Massadeh A.M. & Al-Massaedh A.T. (2018). Determination of Heavy Metals in Canned Fruits and Vegetables Sold in Jordan Market. *Environ. Sci. Pollut. Res.*, Vol. 25, No. 2, 2018, pp. 1914-1920.
- [34] Türkmen M. & Budur D. (2018). Heavy Metal Contaminations in Edible Wild Mushroom Species from Turkey's Black Sea Region. *Food Chem.*, Vol. 254, 2018, pp. 256-259.
- [35] Dospatliev L., Gavazov K & Ivanova M. (2018). Correlation between Cambisols soil characteristics and lead content in wild edible mushrooms (*Cantharellus cibarius*, *Tricholoma equestre*, *Craterellus cornucopioides*). *Bulgarian Chemical Communications*, Vol. 50, No. B, pp. 32-37.