The Physico-Chemical Composition and the Level of Metals From (Buletus Edulis) and (Cantharellus Cibarius) From the Vatra Dornei Area

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The Physico-Chemical Composition and the Level of Metals From (Boletus Edulis) and (Cantharellus Cibarius) From the Vatra Dornei Area

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Abstract

In general, wild mushrooms are considered a good source of valuable nutritional compounds but can exert toxic potential due to the accumulation of heavy metals. In the present study the basic physicochemical composition (fat, protein, and moisture) and heavy metal content profile (Pb, Cd, Cs) of two popular edible wild mushrooms of the species Boletus edulis and Cantharellus cibarius harvested from the forests of Vatra Dorna, Romania were determined. Both investigated mushrooms samples demonstrated a good protein source with the means ranged from 1.58-2.91. In addition, analyzed mushrooms showed a low-fat content presenting values of 0.41-0.45. Inductively coupled plasma mass spectrometry technique (ICP-MS) presents heavy metal content from B. edulis and C. cibarius samples ranged from 0.9 - 0.12 for Pb and 0.16 - 0.25 for Cd, respectively.

Keywords: Boletus edulis, Cantharellus cibarius, ICP-MS, physicochemical composition.

1. Introduction

The metal content derived from wild mushrooms represents a good source of micronutrients and macronutrients required for a balanced diet (Kalac, 2010; Chang și Miles, 2004). Zavastin et al. (2018) concluded after investigations done on fungi of the species Cantharellus cibarius and Boletus edulis, that the most important nutritive components for the human body are considered carbohydrates and proteins.

Due to the fact that most species of edible wild mushrooms contain biologically active compounds, their benefits to the human body are numerous. According to Vamanu et al. (2012), during chemical analysis of the content for Boletus edulis harvested from Romania, showed a new evidence of the appearance of rosmarinic acid that is considered a strong antioxidant.

First of all, the biologically active compounds present in these fungi contribute to maintaining the physical and mental health of the population (Bernas et al., 2006; Chang and Miles, 2004). Secondly, because of high calcium and low content of phosphorus, copper, and cobalt, those mushrooms consumed in a balanced diet can contribute for maintaining the health of the bones and
joints, additionally to maintain the circulatory system through the high iron content. In the study by Zavastin et al. (2018), it has been observed that a high concentration of magnesium, zinc, and selenium contributes to the production of antibodies by stimulating the immune system, helping to increase their number and cell metabolism. Authorities responsible for food safety are obligated to analyze the mushrooms for chemical content, regardless of where they came from, due to mushroom ability to accumulate toxic elements. Toxicity of elements such as lead and cadmium causes the appearance of diseases and deficiencies with serious repercussions for the human body. Alimentary toxicity of the human body with cadmium causes diseases such as breast cancer, cardiovascular and pulmonary diseases, can induce loss of renal function, and bone fractures. The ingestion of high lead concentrations in children can downgrade the development of the body and can affect the growth process, and in adults, the most well-known diseases resulting from lead-induced poisoning are paralysis, anorexia, premature birth and sleep disorders (ATSDR, 2007). Edible wild mushrooms contain significant amounts of compounds possessing antioxidant activities reason for which may be used for the purpose of extracting these compounds and their use as functional ingredients that can fight or prevent some diseases. At the same time, edible wild mushrooms can be included in the human diet due to the beneficial properties of bioactive molecules present in their composition (Ferreira et al., 2009). The discovery of bioactive compounds from *Cantharellus cibarius*, from the research done by Lemieszek et al. (2018), demonstrated new evidence for neuroprotective effects of these mushrooms that can be a novel therapeutic strategy in order to fight neurodegenerative diseases.

## 2. Material and Method

**Chemicals.** All the chemical substances from the present study were of analytical reagent grade. Nitric acid HNO3 65% (Merk, Germany), Hydrochloric acid fuming HCl 37% (Merk, Germany), Hydrogen peroxide H2O2 30% (Merk, Germany), ICP multi-element standard solution 1000 mg/l (Merk, Darmstadt, Germany) and ultra pure water, Milli-Q (Millipore, Bedford, MA, USA).

**Determination of heavy metals.** Determination of heavy metals from mushrooms was performed by inductively coupled plasma mass spectrometry technique or ICP-MS used for the identification and quantification of Pb, Cd, Cu and Zn elements at a concentration level of ng/l and μg/l or higher concentrations by the appropriate dilution of the sample. Evaluation of lead (Pb) and cadmium traces (Cd) was determined according to SR EN 14082: 2003; LOD - 0.05 mg/kg; LOQ -0.1 mg/kg.

**Mushroom samples.** A total of 10 samples of *Boletus edulis* (King bolete, Penny bun) and 10 samples of *Cantharellus cibarius* were collected from the Vatra Dornei area. The mushrooms were cleaned from the forest detritus using a plastic knife and transported to in a laboratory within 12 hours of collection. Mushrooms of the species *Cantharellus cibarius* and *Boletus edulis* were recognized by the Department of Animal Science and Biotechnology on the territory of the University of Agricultural Sciences and Veterinary Medicine, Cluj-Napoca. All the samples were dried before performing the analysis.

**Physicochemical composition.** The following physicochemical parameters were analyzed: (fat, protein, and humidity). Fat and protein content was analyzed by the Soxhlet and Gerber method. Determination of moisture content was evaluated by the air-oven method, according to SR ISO 24557. Evaluation of lead (Pb) and cadmium (Cd) was determined according to SR EN 14082: 2003; LOD - 0.05 mg/kg; LOQ -0.1 mg/kg.

**Laboratory equipment.** As equipment used in that study Berghoff MWS-3+ Microwave Digestor (Eningen, Germany) was used followed by ICP-MS ELAN DRC II Perkin-Elmer.

**Mineralization of samples.** The mushroom samples were crushed and milled and subjected to microwave digestion with 8 ml Nitric acid (65% HNO3) and 2 ml Hydrogen peroxide (30% H2O2). The digestion program is presented in Table 1. After cooling to ambient temperature, the sample was diluted with 25 ml of ultra-pure water, then filtered through a 0.45 μm cellulose membrane filter. At the same time, blank samples were prepared. Concentrations of the trace elements from the mineralized solutions were determined by ICP-MS.

### Table 1. Operating conditions of microwave digestion system

| Phase | 1  | 2  | 3  | 4  |
|-------|----|----|----|----|
| Pressure (bar) | 30 | 30 | 30 | 0  |
| Ramp time /min | 5  | 1  | 1  | 1  |
| Maintenance time/min | 15 | 10 | 15 | 10 |
For the quantitative determination of the desired elements, the external calibration method was performed.
With the help of interpolation, the concentration of the analyte in the unknown sample can be easily determined.
Therefore, calibrations were performed with multi-element standard solutions at different concentration levels and then the calibration curves were drawn.

3. Results and Discussions

Evaluation of physicochemical parameters of *Boletus Edulis* and *Cantharellus cibarius* obtained from Vatra Dorna area. Fig. 1 and 2 show the fat, protein and moisture values for *Boletus Edulis* and *Cantharellus cibarius* mushrooms in the Vatra Dornei area. The protein ranged from 1.58-2.91, 0.41-0.45 fat and 12.17-17.01 moisture. These results are similar to those reported in the literature.

**Figure 1.** The average values for fat, protein, and moisture in *Boletus Edulis* mushrooms

**Figure 2.** The average values for fat, protein, and moisture of *Cantharellus cibarius* mushrooms

Evaluation of the level of Cd, Pb, Cs 135 and 137 of *Boletus Edulis* and *Cantharellus cibarius* wild mushrooms obtained from Vatra Doina area. Figures 3-6 show the values for Pb, Cd, Cs137 and Cs134 from the *Boletus Edulis* and *Cantharellus cibarius* mushrooms in the Vatra Dornei area. In analyzed mushrooms, Pb ranged from 0.9-0.12 and Cd in the range of 0.16-0.25. Cs 137 was in the range of 8.02-10.34 and Cs134, 1.21-2.03 (Fig. 3-6).
Figure 3. The mean values for Pb and Cd in *Boletus edulis* mushrooms

Figure 4. The mean values for Pb and Cd in *Cantharellus cibarius* mushrooms

Figure 5. The mean values for Cs137 and Cs134 in *Boletus edulis* mushrooms
According to the investigation of Falandysz et al. (2017) on mushrooms of the species, *Cantharellus cibarius* harvested from Poland and China, detectable Pb levels were lower than Cd. The lead presented values of 0.17-0.66 mg Pb / kg/dw for the samples from Poland compared with China sample with the mean 1.1 mg Pb / kg/dw and for the concentrations of cadmium was, respectively 0.20-0.23 mg Cd / kg/dw in Polish mushrooms and 0.58 mg Cd/kg/dw for China specimen. A recent study carried out by Zavastin et al. (2018) on *Cantharellus cibarius* harvested from Suceava, Romania, showed significantly lower concentrations for Cd, 0.08 mg/kg/dw, respectively compared to those obtained by us. Another study from Yunnan Province of China indicated significantly higher concentrations of Cd compared to our results, numbers of Cd 7.7-85.4 mg kg−1 and 3.7-91.5 mg kg−1 for caps and stipes of porcini mushrooms using ICP-AES (Su et al 2018). The present study shows that the Cd levels obtained for the samples analyzed by us are high due to the intensive pollution of the soil and air in the areas where the mushrooms were collected. (L. Svboda, 2006; In the study of Li et al. (2017), the values for the content of Cadmium from the fruiting body of *Boletus edulis* were recorded between 5.93-46.07 mg/kg−1. The abundant content of Hg from the Boletus edulis caps collected in Western China areas was reported in the study of Falandysz et al, 2015 with the values of 22 mg/kg−1 of dry weight. Falandysz et al. (2017) obtained values for mercury derived from dry biomass of fresh *Cantharellus cibarius* (0.017 ± 0.003) mg/kg−1, respectively. Interestingly, for these results, the blanching procedure of mushrooms prior for gastronomical use can decrease the content of Hg. An elevated content of Hg was observed in the caps of *B. edulis* obtained from a polluted area of Idria (Slovenia) with the numbers of 98.9 ±5.1 (mg/ kg/dw) compared to caps harvested from an unpolluted area of Ljubljana 7.85 ±1.2 (mg/kg/dw), respectively. Another study demonstrated that pickling and blanching processes applied for *Cantharellus cibarius* can significantly diminish the content of cadmium, showing 72–91%, median values, respectively. The data obtained from this study indicated the cadmium concentrations of *C. cibarius* between (0,17 ± 0,01 and 0,27 ± 0,01) mg/kg−1 obtained from dry biomass. According to Záhorcová et al, 2016 hymenophores of *Cantharellus cibarius* harvested from mining areas of Slovakia presented the content of lead and cadmium with values of 4.07 mg/kg and 0.56 mg/kg, respectively. Fruit bodies of chanterelles obtained from the polluted region of Slovakia presented Cadmium content of 1.1 mg/kg/dm and lead content of 0.8 mg/kg/dm. Fruit bodies of Chantarelle mushrooms from unpolluted regions of Poland showed values of Cd and Pb ranged between 0.21- 0.41 mg/kg/dm and 0.28-0.50 mg/kg/dm.

Proximate composition of *Boletus edulis* (king bolete) from Taiwan in one study showed the percentage of crude protein, fat, carbohydrates and moisture having values of (18.54 ± 0.35), (5.76 ± 0.19), (56.16 ± 0.23) and (11.97 ± 0.18), respectively. Similar results were found in the study of Ouzoni et al. (2007) presenting the protein content of 3.15±0.10 for fresh weight and 26.49±0.16 for dry weight, fat content showed numbers of 0.33±0.04 f.w. and 2.77±0.02 d.w. g/100 g for *Boletus edulis* samples collected from Greece. In addition, the level of Cd and Pb was low in this study, presenting means 0.29 ± 0.01 µg/g−1 and 1. 29±0.02 µg/g−1, respectively. In
the study realized by Beluhan et al. (2010) which reported parameters of nutritive composition for Croatian wild edible mushrooms. Investigated B. edulis and C. cibarius demonstrated good protein levels 36.91 ± 0.02 and 30.91 ± 0.28 g/100 g, also these mushrooms showed very low-fat content 2.7 ± 0.56 and 1.9 ± 0.61 g/100 g.

Barros et al. (2008) reported nutritional values for C. cibarius mushrooms gathered from Portugal comprising the fat content of (0.22 ± 0.04), protein (4.09 ± 0.09), moisture (92.38 ± 0.31) and carbohydrates (2.44 ±0.33) g/100 g of fresh weight, respectively. Interestingly, another study has revealed significantly high content of crude protein in wild Chanterelle mushrooms having values of 69.14±3.26 g/100 g. Furthermore, the author suggested that wild mushrooms contain a potent source of nutraceuticals such as phenolics, tocopherols, carotenoids and could be used furtherly as phytonutrients versus microbial diseases. Another study reported data for the basic composition of Cantharellus cibarius acquired from Macedonia and Greece, presenting values for proteins 21.57 ± 0.21, fat 2.88 ± 0.02 and carbohydrates 66.07 ± 0.23 g/100 g in dry weight. No lead concentrations were detected in these mushrooms, and significantly lower concentrations of Cd was observed 0.38 ± 0.10 µg/g in a dry mass, respectively.

Melinda Nagy, in the Ph.D. thesis, studied the physicochemical parameters for three species of edible wild mushrooms, including the Chanterelle. In the analyzes of the protein content for Chanterelle was 21.3 g / 100 g dw. Based on this result, Melinda NAGY concluded that the Chanterelle is a protein-rich vegetable source that can successfully replace the meat protein. The metallic content of the Boletus edulis mushrooms does not depend only on the age of the mushrooms or the size of the fruit body but also depends on the periods between fructifications. (Kalac, 2010; Chang and Miles, 2004). The most abundant microelements in Boletus edulis mushrooms are copper, iron, manganese, and zinc. The concentration of copper found in King bolete cultivated in Romania was much higher than that reported in China - 12.0 mg/kg dw (Liu et al., 2015) or Turkey - 20.1 mg/kg dw (Tuzen et al., 2007). Wang XM et al, 2015 reported high concentrations of Cu in the caps and stips of Boletus edulis mushrooms with values ranged between 17-884 mg kg\(^{-1}\) and 8.6-703 mg kg\(^{-1}\) of dry mass. In addition, Su et al. (2018) recorded Copper values of 24-53 mg kg\(^{-1}\) and 16-84 mg kg\(^{-1}\) for caps and stips. Falandysz et al, 2015 reported values for Cu and Zn obtained from Chanterellus cibarius fruit bodies with variations from 35-49 mg/kg and 74-120 mg/kg for Zn. Wang XM et al, 2015 found that Manganese appeared to be more cumulative in the stalks compared for caps of Boletus mushrooms, and higher concentrations of minerals such as Cu, Zn, K, Mg, and P can be detected in the caps. For manganese content, the recorded values for Romanian Porcini was lower compared to the mushrooms harvested in Turkey - 96.3 mg/kg dw (Tuzen et al., 2007), but higher than reported in China - 7, 15 mg/kg dw (Liu et al., 2015).

The zinc content reported for fungi grown in Romania were significantly lower than those reported in fungi from Turkey - 158 mg/kg dw (Tuzen et al., 2007) and China - 138 mg/kg dw (Liu et al., 2015). Moreover, Wang XM et al, 2015 using inductively coupled plasma atomic emission spectroscopy observed an increased amount of Zn in stipes and caps of Boletus edulis with the values ranged between 35-332 mg kg\(^{-1}\) and 54-534 mg kg\(^{-1}\). Lately, Wang et al. 2015 performed the same experiment and the data obtained for Zn regarding stipes and caps was 41 mg/kg and 88 mg/kg. The author concluded that the level of Zn derived from caps is more concentrated than in stalks for Boletus mushrooms. The content of metals from B. edulis, reported by Širić et al. (2016) from northwest Croatia for copper, iron, and zinc was 22.56±7.26, 69.39±26.01 and 82.93±13.70 mg/kg\(^{-1}\), respectively. Regarding commercial species of Cantharellus cibarius analyzed by Barros et al. 2008, we can observe the an elevated fat content between 4.49 and 4.60 g / 100 g / dw. The reason for the increase in fat content is the cultivation of chanterelle mushrooms on an industrial scale due to the increasing demand of consumers for this species of mushrooms. Crude water and ethanolic extracts from porcini mushrooms demonstrated antiproliferative effects against MCF-7 tumor cells. Ethanolic extracts from the study of Zavastin et al. (2016) and the methanolic ones by Kozarski et al. (2015) have shown that the mushrooms mentioned above have an antimicrobial activity versus gram-positive microorganisms. Ethanolic extracts were the most effective against Escherichia coli ATCC 25922 and Pseudomonas aeruginosa ATCC 27853 with minimal inhibitory concentrations of 15 µg/mL and 13 µg/mL, respectively. Therefore, methanolic extracts from Chantarelle provided a potent antibacterial effect against Enterococcus faecalis ATCC 29212 indicating a significant inhibition zone of 21.2 ± 0.2 mm determined by the disc diffusion method. In addition, ethanolic and methanolic extracts obtained from Cantharellus cibarius contains compounds with bioactive potential that exerts antioxidant and anti-inflammatory activities. Interestingly, Choma et al. (2018) observed after metal biosorption assay from an alkali-soluble polysaccharide obtained from penny bun, the values for kinetic adsorption for Pb2+ was 35.49 and 33.2 µg h\(^{-1}\) mg\(^{-1}\) for Cd2+, respectively. Moreover, that author reports, the ability to bound particular
heavy metals in cellular structures of *Boletus edulis* is attributed due α-(1 → 3)-d-mannogluccans.

**Conclusions**

The assimilation of Pb and Cd in *Boletus edulis* and *Cantharellus cibarius* is also influenced by the physico-chemical composition, but for samples analyzed, there were no significant differences between the two types of mushrooms.

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