Trace Element Contents in the Edible Mushroom *Boletus edulis* Bull. ex Fries

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Summary

Study was carried out on the trace element concentrations of edible mushroom *Boletus edulis* from Gorski kotar, Croatia. Samples were collected between March and December 2013. The analyses were carried out by Inductively Coupled Plasma – Optical Emission Spectrometry (ICP-OES). The results showed that the values of the studied elements decreased in the order: Cu (13.01) > Se (12.25) > Mn (9.19) > Al (4.62) > Ag (2.34) > Hg (2.18) > Cd (1.22) > Ni (0.99) > Pb (0.79) > Sb (0.60) > As (0.41) > Ba (0.38) > Cr (0.22) > Co (0.07). The essential elements in fruiting bodies *Boletus edulis* were a considerably higher than those of toxic elements. The consumption of wild edible mushroom *Boletus edulis* from Gorski kotar is safe for human health.

Key words

Boletus edulis, edible mushroom, consumption, human health

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Introduction

Mushrooms are a separate group of live organisms of considerable nutritive, pharmaceutical and ecological value. Mushrooms play vital roles in the majority ecosystem in the biosphere because they are able to biodegrade the substrate, and therefore use the wastes of agricultural production. Wild growing mushrooms are consumed as a delicacy in many countries. Numerous wild edible mushrooms are appreciated, not only for texture and flavor but also for their chemical and nutritional characteristics (Manzi et al., 2001). In addition, mushrooms have also been reported as therapeutic foods, useful in preventing diseases such as hypertension (Talpur et al., 2002), hypercholesterolemia (Jeong et al., 2010) and cancer (Sesli et al., 2008). Accordingly, nutritional quality of mushrooms, including its trace elements status is of interest for both agronomists and nutritionists. The consumption and collecting of wild edible mushrooms is increasing, due to good content of proteins as well as higher content of minerals (Kalač, 2010). Some species can accumulate high levels of toxic trace elements and pose a risk to human health. Compared with cultivated the trace element contents in the wild growing species are considerably higher and species dependent (Alonso et al., 2003; Garcia et al., 2009). Species of the genus *Boletus* are capable of accumulating several fold greater amounts of heavy metals (Alonso et al., 2000; Cocchi et al., 2006; Borovička and Randa, 2007; Falandysz et al., 2008; Melgar et al., 2009; Kalač, 2010; Širić et al., 2014; Širić et al., 2016), than in fruit and vegetables (Turgdogan et al., 2003). Therefore, investigating the composition of trace elements in wild edible mushrooms is important.

As in other countries, mushrooms picking in Croatia is very popular (Širić et al. 2014). Gorski kotar is located in the central part of Croatia. It is place that is covered with a well-preserved deciduous and mixed forest of *Quercus sp*., *Carpinus betulus* L., *Fagus sylvatica* L., and especially with *Picea abies* L., and *Abies alba* Mill. In the area of Gorski kotar there are a lot of species of edible mushrooms, especially from the genus *Boletus*, but there was no study on their trace element contents. *Boletus edulis* Bull. ex Fries is a ectomycorrhizal wild edible mushroom, which is one of the most popular in Croatia. It usually grows in summer and autumn, mostly in symbiosis with *Abies alba*. The fruiting body of *B. edulis* consists of cap and stipe. The brown in color cap is 5 to 30 cm in diameter. The stipe with light brown color is from 5 to 15 cm tall and from 2 to 7 cm wide (Božac, 2003). This species has been traditionally eaten, because of its delicious and delicate texture. However, little is known about trace element contents in *B. edulis* in Croatia. The aim of this study was to determine concentrations of seventeen elements in the fruiting bodies of edible mushroom *B. edulis*, and possible negative effects of toxic elements on human health.

Materials and methods

Fruiting bodies of *B. edulis* mushroom were collected in the central part of Gorski kotar area, Croatia. (Fig 1.). The study areas included forest distant from the sources of industrial pollution. Thirty-seven samples of investigated mushroom were collected and analyzed. Approximately 10–20 g of samples was taken. These samples were thoroughly cleaned (not washed) and cut into smaller pieces and placed into clean glass vessels. The samples were frozen and lyophilized before determination of the content trace elements at the Centre for Micro and Nano Science and Technology at the University of Rijeka. Freezing was carried out on the freezer (DW 1.0 to 110, Heto-Holten AIS Gydevang 17-19, Allerød, Denmark) at -120°C for 2–3 hours. The lyophilisation was performed in lyophilizer (UNICRYO MC2L, UNIEQUIP, Munich, Germany) at -60°C under pressure of 133 × 10^{-3} mBar, for 24 hours. The samples were stored in an exicator after lyophilization until analysis. Laboratory glass used for the preparation of samples for the determination of trace elements was cleaned by soaking for 24 hours in a solution of ethylene-diamine-tetra-acetic acid (EDTA; Kemika, Croatia; 5% v/v) and after 24 hours in HNO3 (10% v/v; TTT Ltd., Holy Sunday, Croatia). Samples, weight of 0.5 g, were digested with 5 ml of HNO_3 (65%, Suprapur, Merck, Germany) in sealed PTFE vessels in a microwave oven for destruction (Milestone microwave laboratory system, MLS 1200 mega, USA). After destruction in microwave oven, the samples were cooled in a water bath and over the funnel transferred to plastic flasks and diluted to 25 ml using deionized water. The samples were transferred from volumetric flasks to plastic test tubes and the content of metals were measured by inductively coupled plasma – optical emission spectrometry (ICP-OES; Optima 8000, Perkin Elmer, USA) equipped with autosampler (an automatic device for sampling). Mercury in lyophilized samples of mushroom was measured without destruction in acid using AAS mercury analyzer (AMA 254 Advanced Mercury Analyser, Leco, Poland) that uses direct combustion of the sample in a rich oxygen atmosphere. Four replications of the resulting sample were analyzed. The data obtained were analyzed by statistical program SAS V9.1.

Figure 1. Area of the sampling of *Boletus edulis* in Gorski kotar, Croatia

Results

Descriptive statistics on trace metal concentrations in *B. edulis* are given in Table 1. All examined elements content were determined on a dry weight of fruiting body. The results showed that, Cu (2.2–31.06 mg kg^{-1}), Se (7.06–20.57 mg kg^{-1}) and Mn (3.08–27.06 mg kg^{-1}) had the highest concentrations, followed by Al (0.09–17.30 mg kg^{-1}), Ag (0.64–4.80 mg kg^{-1}), Hg (0.73–5.71 mg kg^{-1}), Cd (0.27–5.81 mg kg^{-1}), Ni (0.22–1.41 mg kg^{-1}), Pb (0.3–1.39 mg kg^{-1}), Sb (0.23–1.02 mg kg^{-1}), As (0.20–0.58 mg kg^{-1}), Ba (0.07–0.92 mg kg^{-1}), and Cr (0.12–0.34 mg kg^{-1}), while Co (0.06–0.08 mg kg^{-1}) was found to have the lowest contents (Table 1). The concentration of V, Be and Tl were below the detection limit.

Table 1. Trace element concentrations (mg kg $^{-1}$, dry weight basis) in *Boletus edulis*

Element	Mean ± S.D.	Min.	Max.	CV%
Arsenic – As	0.41 ± 0.14	0.20	0.58	34.14
Lead – Pb	0.79 ± 0.35	0.31	1.39	44.30
Cadmium – Cd	1.22 ± 1.75	0.27	5.85	148.41
Chromium – Cr	0.22 ± 0.08	0.12	0.34	36.24
Vanadium – V	ND	ND	ND	ND
Manganese – Mn	9.19 ± 7.69	3.08	27.06	83.67
Cobalt – Co	0.07 ± 0.01	0.06	0.08	14.28
Nickel – Ni	0.99 ± 0.35	0.22	1.41	35.35
Cooper – Cu	13.01 ± 9.68	2.25	31.06	74.44
Silver – Ag	2.34 ± 1.48	0.64	4.80	63.24
Beryllium – Be	ND	ND	ND	ND
Barium – Ba	0.38 ± 0.42	0.07	0.92	110.52
Thallium – Tl	ND	ND	ND	ND
Antimony - Sb	0.60 ± 0.28	0.23	1.02	46.66
Selenium – Se	12.25 ± 4.70	7.06	20.57	38.36
Aluminum – Al	4.62 ± 5.22	0.09	17.30	112.55
Mercury - Hg	2.18 ± 1.53	0.73	5.71	70.18

Mean ± S.D. – Mean ± Standard deviation; Min. – Minimum value; Max. – Maximum value; CV – Coefficient of variation; ND – not determined

Discussion

Arsenic is a highly toxic element and its presence in the mushrooms is not well known, including the edible fungi. Arsenic values in samples of the fruiting bodies of *B. edulis* exceeded 0.50 mg kg^{-1} (Falandysz et al., 2006). The arsenic content in the presented study was found to be lower than that reported by the specified authors.

Lead is a heavy metal toxic to plants, animals and humans, and there is no evidence of its biological role in the human body. The lead level in our study ranged from 0.31 to 1.39 mg kg^{-1}, with the mean value of 0.79 mg kg^{-1}. Similar results were determined by Giannaccini et al. (2012) for *B. edulis* and Garcia et al. (2009) for *B. reticulatus*. In contrast of that, Širić et al. (2014) found considerably higher concentrations of lead in *B. reticulatus* and samples were collected from unpolluted areas Zrin Mountain, Croatia.

Cadmium is a highly toxic element and it has been probably the most determined trace element of mushrooms (Kalač et al., 2004). As results from data published until 2009 (Kalač 2010) cadmium contents varied between 0.5 and 5 mg kg^{-1} depending on the species of mushroom. Cocchi et al. (2006) reported contents of cadmium in genus *Boletus* between 0.54 (*B. erythropus)* and 4.39 mg kg^{-1} (*B. pinophilus*). The cadmium contents in this study are in agreement with the values reported by Cocchi et al. (2006).

Minimum and maximum mean values of chromium found were 0.86 mg kg^{-1} in *B. aureus* (Ouzuni et al., 2009) and 2.52 mg kg^{-1} in *B. reticulatus* (Širić et al., 2014), respectively. However, the chromium content determined in our study was considerably lower than reported levels.

Manganese values in wild growing mushrooms samples have been reported in the range of: 12.9–93.33 mg kg^{-1} (Kalač and Svoboda, 2000), 5–60 mg kg^{-1} (Tuzen, 2003), and 10–77 mg kg^{-1} (Sarikurkcu et al. 2011). According to the results of Turkekul et al. (2004), Tuzen et al. (2007) and Sarikurkcu et al. (2011), manganese values in some species from genus *Boletus* were a considerably higher than those observed in the presented study.

Kalač (2010) reported that the cooper levels in the most species from unpolluted areas are between 20 and 100 mg kg^{-1}. However, there are several accumulating species such as *A. arvensis, A. macrosporus, A. silvicola, Calvatia utriformis and Macrolepiota procera*, which accumulate a concentration of cooper above 100 mg kg^{-1} (Alonso et al., 2003 and Sarikurkcu et al., 2011). Observed cooper values in our study are in agreement with those reported by Širić et al. (2014) in species from genus *Boletus*.

The lower nickel value (1.61 mg kg^{-1}) was found in *B. aureus* Ouzuni et al. (2009), whereas the higher nickel value (5.96 mg kg^{-1}) was found in *B. chrysenteron* (Yamac et al., 2007). Our nickel value in *B. edulis* was a considerably lower than reported levels.

Cobalt content of macro fungi is relatively low, mostly below 0.6 mg kg^{-1} (Sesli and Tuzen, 1999; Demirbas, 2001; Nikkarinen and Mertanen, 2004; Vetter, 2005; Borovička and Randa, 2007). Our results are in essential agreement with results by Borovička and Randa (2007) for *B. edulis* species.

Silver is toxic to animal cells and Ag^+ ion is highly toxic to bacteria. This is because Ag ion has a high affinity for sulfhydryl, amino, and phosphate groups, and it readily complexes with many endogenous ligands of the mammalian body (Falandysz and Borovička, 2013). Silver contents of mushroom *B. edulis* in the literature were reported to be in the ranges 0.8 to 1.2 mg kg^{-1} (Cocchi et al., 2006; Giannaccini et al., 2012). Our silver levels are higher than those reported.

Mean barium content below 1 mg kg^{-1} in *B. edulis* was reported by Falandysz et al. (2008). The value of barium in this study was in the presented range.

In general, content of metalloid antimony in mushrooms were usual below 0.1 mg kg^{-1} (Borovička et al., 2006). The antimony level in the presented study was a considerably higher than reported concentration. However, chemical forms of antimony in mushrooms are not known.

Content of selenium in *B. edulis* ranged from 10 to 30 mg kg^{-1} (Borovička and Randa 2007; Szynkowska et al., 2008). Our selenium values are in agreement with those reported by specified authors, whereas a considerably higher selenium value was determined in *Albatrellus pescaprae* (200 mg kg^{-1}) (Kalač, 2010).

Mostly, Al concentrations were in the range from 35 to 365 mg kg^{-1} (Rudawska and Leski, 2005; Vetter, 2005; Falandysz et al., 2008). Wide variations within species were recorded in all the articles (Kalač 2010). In this study, the mean value of Al was a considerably lower than those reported in the literature by Kalač (2010).

Concentrations of Hg were in the range from 1 to 5 mg kg^{-1} depending on the species. Mercury values in genus *Boletus* have been reported in the ranges of: 1 to 2 mg kg^{-1} (Žarski et al., 1999), 2 to 5 mg kg^{-1} (Szynkowska et al., 2008; Melgar et al., 2009), and 5 to 10 mg kg^{-1} (Alonso et al., 2000). The content of mercury in the presented study is in agreement with the results reported by Szynkowska et al. (2008), and Melgar et al. (2009).

Conclusion

Seventeen trace elements (As, Pb, Cd, Cr, V, Mn, Co, Ni, Cu, Ag, Be, Tl, Sb, Se, Al and Hg) in *B. edulis* collected from Gorski kotar, Croatia, were determined. In the present study, the detected concentrations of Pb, Cd, Cu, Co, Ba, Se and Hg were generally in agreement with previously reported concentrations. The contents of As, Mn, Cr, Ni and Al were considerably lower than those reported in the literature, while the contents of Ag and Sb were a considerably higher than those reported in the literature. The contents of V, Be and Tl were below the detection limit. It can be concluded that the consumption of mushroom *B. edulis* cannot be considered as a toxicological risk from trace elements for human. The trace metal levels of wild edible mushrooms should be analysed more often in order to evaluate the possible danger to human health from them.

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