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Heavy metals in edible mushroom *Boletus reticulatus* Schaeff. collected from Zrin mountain, Croatia

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Abstract

Background and Purpose: *Trace element contents in genus Boletus from Croatia are very limited. The aim of this study is to give detailed trace element concentrations in ectomychorrhizal edible mushroom* Boletus reticulatus *from Zrin mountain.*

Material and Method: *Fruiting bodies of the* Boletus reticulatus *were collected in a study area of Zrin mountain (Croatia). The contents of Cd, Cr, Cu, Fe, Hg, Ni, Pb and Zn in fruiting bodies of ectomychorrhizal species were carried out by atomic adsorption spectrometry.*

Results and Conclusions: *The results showed that concentrations of the studied elements decreased in order: Zn (91,31 mg kg^{-1}) > Fe (57,27 mg kg^{-1}) > Cu (13,80 mg kg^{-1}) > Pb (4,20 mg kg^{-1}) > Ni (2,67 mg kg^{-1}) > Cr (2,52 mg kg^{-1}) > Hg (2,38 mg kg^{-1}) > Cd (1,66 mg kg^{-1}). The essential elements in fruiting bodies of* Boletus reticulatus *were much higher than those of toxic elements. The possibility of toxicological effects on human health consumption of investigated species (*Boletus reticulatus*) is negligible.*

INTRODUCTION

Wild growing mushrooms are a popular and favourite delicacy in many countries in the world. The consumption and collecting of wild edible mushrooms is increasing, due to a good content of proteins as well as a higher content of minerals *(1).* As in other countries, picking mushrooms has become a national hobby in Croatia. Zrin mountain is one of the richest edible mushrooms resources in Croatia, especially with genus *Boletus.* It is a place that was hard affected by the war, and there may be increased concentrations of same heavy metals in mushrooms. Investigated species from genus *Boletus* is used as a food in Croatia. After that, mushrooms have been also reported as therapeutic foods, useful in preventing diseases such as hypertension, hypercholesterolemia, and cancer in China *(2, 3).* These functional characteristics are mainly due to their chemical composition *(4).* However, data on trace element content in genus *Boletus* from Croatia are very limited and fragmentary. Compared with cultivated mushrooms, the trace element contents in the wild growing species are high and species dependent *(5, 6, 7, 8, 9, 10).* Extensive research has been carried out on trace elements for searching edible wild growing species accumulating high levels of some minerals, and for investigating the level of risk elements *(11, 12, 13).*

Cadmium, chromium, copper, iron, mercury, nickel, lead zinc were chosen as representative trace metals whose levels in the environment represent a reliable index of environmental pollution. Metals such as iron, copper and zinc are essential metals since they play an important role in biological systems, whereas cadmium, mercury and lead are non-essential metals as they are toxic even in traces *(14)*. The essential metals can also produce toxic effects when the metal intake is excessively elevated *(15, 16)*.

Boletus reticulatus is an ectomychorrhizal edible mushroom, which is very popular in Croatia. It usually grows up under the mixed deciduous coniferous forests, especially in symbiosis with oak (*Quercus sp.*), beech (*Fagus sylvatica*), hornbeam (*Carpinus betulus*) and chestnut (*Castanea sativa*). The fruiting body of *Boletus reticulatus* consists of cap and stipe. The cap with brown in colour is 8 to 30 cm in diameter. The stipe with light brown colour is from 5 up to 20 cm tall *(17)*. This species has been traditionally eaten by some mushroom fanciers because of its delicious and delicate textures. However, little is known about trace element in *Boletus reticulatus* in Croatia. In this study, the concentrations of eight trace elements in the fruiting bodies of *B. reticulatus* from Zrin mountain (Croatia) were determined.

MATERIAL AND METHODS

The study included forty-seven samples (n = 47) of edible mushrooms *Boletus reticulatus* Schaeff. Fruiting bodies were collected during the extraordinarily humid autumn of 2012 (October and November) in a study area of Zrin mountain (Croatia) (Fig. 1). The study areas included forest distant from the sources of industrial pollution. These samples were thoroughly cleaned (not washed), cut and dried at 60 °C for 48h until the samples reached a constant weight. After that samples were powered at the laboratory mill (Retsch SM 200) through mesh in diameter 0,5 mm. Four sample replications of the resulting were stored in hermetic plastic bags.

Heavy metals were determined in each dried fruiting bodies samples by AAS – Atomic Absorption Spectrometry (SOLAR-THERMO SCIENTIFIC, Type M5 AA System). 0,5 g powered dry samples were weight into glass beakers. 6 ml HNO_3 and 1 ml $HClO_4$ were added to the samples. The prepared mixture was burned in a microwave oven and then cooled in a digester. After cooling, digested samples were diluted to 50 ml using deionized water, and stored in 50 ml flasks until analysis. The contents of heavy metals in the solution were directly read on AAS – Atomic Adsorption Spectrometer. The AAS analysis analyses were conducted in an accredited (ISO 17025:2009) laboratory.

Heavy metals levels in the samples were calculated by the following formula:

$$\text{mg metal/kg dry weight} = (AV)/W;$$

where A = µg/l of metal, V = dilution volume of sample, l, and W = dry weight of sample, g.

The data obtained were analyzed by statistical program SAS V9.1

RESULTS

All examined elements contents were determined on a dry weight of fruiting body. The results showed that, among these elements, Zn (14,30 – 147,20 mg kg^{-1}) and Fe (23,54 – 93,20 mg kg^{-1}) had the highest concentra-

TABLE 1

Trace element content (mg kg^{-1}, dry weight basis) in *B. reticulates.*

Element	Mean ± S.D.	Min.	Max.	CV%
Cadmium – Cd	1.66 ± 0.28	1.22	2.47	16.64
Chromium – Cr	2.52 ± 0.18	2.15	2.78	7.11
Cooper – Cu	13.80 ± 7.33	4.76	23.11	53.14
Iron – Fe	57.27 ± 22.95	23.54	93.20	40.08
Mercury – Hg	2.38 ± 0.37	1.58	3.67	15.84
Nickel – Ni	2.67 ± 0.51	2.10	3.74	19.14
Lead – Pb	4.20 ± 0.91	2.90	6.31	21.56
Zinc – Zn	91.31 ± 36.68	14.30	147.20	40.17

Mean ± S.D. – Mean value ± Standard deviation; Min. – Minimum value; Max. – Maximum value; CV – Coefficient of variation.

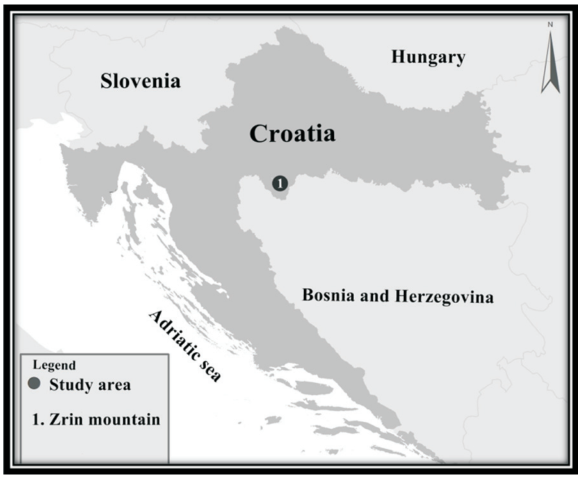

Figure 1. *Area of the sampling of Boletus reticulatus in Zrin mountain, Croatia.*

tions followed by Cu (4,76 – 23,11 mg kg^{-1}), Pb (2,90 – 6,31 mg kg^{-1}), Ni (2,10 – 3,74 mg kg^{-1}), Cr (2,15 – 2,78 mg kg^{-1}), Hg (1,58 – 3,67 mg kg^{-1}), while Cd (1,22 – 2,47 mg kg^{-1}) was found to have the lowest contents (Table 1)

DISCUSSION

Cadmium is a highly toxic element and its presence in the mushrooms is well known, including the edible fungi. It has been probably the most determined trace element of mushrooms. As reported *(18)*, content of cadmium in mushrooms of the genus *Boletus* were between 0,54 *(B. erythropus)* and 4,39 mg kg^{-1} *(B. pinophilus).* Kalac and Svoboda, *(19)*, reported that the Cd concentration of the mushrooms of the genus *Boletus* in the range from 1 to 2 mg kg^{-1}. Cadmium levels were found to be in agreement with contents reported in the literature *(13).*

Minimum and maximum values of chromium found were 0,89 mg kg^{-1} in *Boletus aureus (20)* and 4,2 mg kg^{-1} in *Boletus pinophilus (9)* in the literature, respectively. The chromium contents in our samples were found to be lower than concentrations reported by *(9)* in *Boletus aestivalis* species.

Copper values in some species of genus *Boletus* samples from unpolluted areas are between 20 and 75 mg kg^{-1} *(13)*. The copper contents obtained in this study were lower than those reported in the literature.

Mostly, Fe concentrations are in the range from 50 to 150 mg kg^{-1} depending on the species *(13, 21, 22, 23*). However, extraordinarily high Fe content (1304 – 2075 mg kg^{-1}) was found in *Suillus variegatus (24)*. Our iron values are in agreement with contents between 50 and 150 mg kg^{-1} reported in the literature *(13).*

Mercury values in genus *Boletus* samples have been reported in the ranges of 1 to 2 mg kg^{-1} *(25)*, 2 to 5 mg kg^{-1} *(26, 27)*, and from 5 to 10 mg kg^{-1} *(28)*. The analysis of Szynkowska *et al*., *(26)* and Melgar *et al*. *(27)* were similar with the data in this study.

The lower nickel value (1.61 mg kg^{-1}) was found in *Boletus aureus (20)* whereas the higher nickel value (5.96 mg kg^{-1}) was found in *Boletus chrysenteron (29)*. The nickel levels in our works are in agreement with literature values.

Lead is especially toxic to the growing brain and can affect the behavioral development of the children, even at the low concentration. Garcia *et al*. *(8)* found low concentrations Pb < 1 mg kg^{-1} for ectomycorrhizal fungi *Boletus reticulatus*. In our work we detected means values Pb of 4,20 mg kg^{-1} for *B. reticulatus*, which fluctuated between 2,90 and 6,31 mg kg^{-1}.

Zinc is an element which plays an important role in human nutrition a metabolism. Zinc values in samples of genus *Boletus* have been reported to be in the ranges between 50 and 350 mg kg^{-1} *(6, 20, 24, 30, 31, 32)*. The values of Zn in this study were in the presented range.

CONCLUSIONS

Wild growing mushrooms have been a widely consumed delicacy in many countries of central, eastern and south-eastern Europe. The present results are based on analyses of samples originated from unpolluted areas Zrin mountain, Croatia. The essential elements (Zn, Fe and Cu) in samples of this mushrooms *Boletus reticulatus* were much higher than those of toxic elements. The toxic elements (Cd, Hg, Pb) concentrations in analyzed samples can be considered sufficiently low. It can be concluded that the consumption of mushroom *Boletus reticulatus* can not be considered as a toxicological risk form Cd, Hg and Pb content for human.

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