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Mercury bioaccumulation by wild edible mushrooms

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ABSTRACT

Mercury (Hg) contents was determined in ten species of edible mushrooms in Zrin mountain, Croatia. The analyses of mercury were carried out by AAS mercury analyzer (Atomic Adsorption Spectrophotometer). The greatest mercury mean concentration of 2.28 mg kg^{-1} was determined in ectomycorrhizal species *Boletus edulis*. In terms of the anatomical parts of the fruiting body (cap-stipe), a considerably greater concentration of mercury was found in the cap then in other parts for all mushroom species. According to calculated bio-concentration factors, all the examined species were found to be bio-accumulators of mercury. The possibility of toxicological effects on human health consumption of investigated species are negligible.

Key words: mercury, edible mushrooms, bioconcentration factor, ecology

Introduction

Emissions of heavy metals from anthropogenic sources have been constantly increasing in recent decades. Heavy metals are very persistent in the environment and, due to the ability of accumulation, may affect living organisms. Mercury (Hg) along with other elements such as arsenic (As), lead (Pb) and cadmium (Cd) are important to consider in terms of food-chain contamination (McLaughlin et al., 1999.). The past 2-3 decades have witnessed increasing publications evaluating the Hg levels in both food and the environment at a national or a regional level. However, the recent publication of the United Nations Environment Programme indicated that this concern is a global issue as these contaminations result mostly from anthropogenic emissions of Hg (UNEP, 2013). Mercury is a trace element, natural and ubiquitous in the lithosphere and hydrosphere, with predilection to combine with sulphur (S) and selenium (Se) in the environment. Consequently it occurs in food and feedstuff. Mercury is readily biomethylated into methylmercury, which is then bioaccumulated (usually together with Se for which MeHg is an antagonist in selenocysteine) in the aquatic food chain (Falandysz and Borovička, 2013.). Differences in Hg distribution in soils is due to airborne Hg pollution (accumulation in litter and organic layer of soils) or from geogenic Hg (which occurs under the organic horizon layer). Important variables that determine the amounts of Hg observed in mushrooms depends on its availability to the mycelia, genetic factor, and adaptation to the geochemical composition and anomalies of soil background (Falandysz and Bielawski, 2007., Árvay et al., 2014., Wiejak et al., 2014., Falandysz and Drewnowska, 2015., Krasińska and Falandysz, 2016.). Both the mycorrhizal and non-mycorrhizal mushrooms are efficient in mobilizing and subsequently sequestering Hg and other elements from soil/litter substratum into their fruiting bodies. The mushroom mycelia can very efficiently mobilize Hg from mushroom substratum (soil, litter or wood) and translocate the same to the mushroom fruiting bodies thereby resulting in the observation of elevated amounts of Hg in the morphological parts of the mushroom (the cap and stipe) compared to the Hg levels in the substrate in some cases. However, mercury is one of the most toxic and dangerous environmental contaminants. Mercury can be efficiently bioaccumulated by many mushroom species, even if scarcely present in forest soils. For example, values of bioconcentration factors (BCF) for the genus *Boletus* can vary from 126 to 421 (Melgar et al., 2009.).

Species of the genus *Boletus* are capable of accumulating several times greater amounts of mercury than any other mushrooms species. Therefore, the objectives of this study were to (a) determine the mercury concentrations in wild edible mushroom species and the substrate on which they grow, (b) determine the accumulation capacity (bioconcentration or exclusion) of mercury in fruiting bodies of mushrooms, (c) determine the distribution of mercury in anatomical parts of fruiting bodies *(cap and stipe).*

Materials i methodes

The study was carried out in the area of Zrin mountain, Croatia. Mercury levels have been analysed in 80 samples of 10 edible mushroom species (8 samples per species). Among the sampled species, there were four terrestrial saprobes (*Agaricus campestris* L., *Clitocybe inversa* (Scop. ex Fr.) Pat.; *Clitocybe nebularis* Batsch. ex Fr. and *Macrolepiota procera* (Scop.) Singer, one lignicolous saprobe of the *Armillaria mellea* (Vahl) P. Kumm. group, and five ectomycorrhizal species (*Boletus aestivalis* Paulet ex Fries, *Boletus edulis* Bull., *Lactarius deterrimus* Gröger, *Tricholoma portentosum* (Fr.) Quelet, and *Tricholoma terreum* (Schaeff.) P. Kumm. At the same time, forest topsoil samples (0-10 cm layer) with organic and mineral parts were collected at the mushroom sampling sites. Collected specimens and topsoil samples were documented, oven dried (48 h; 60°C), ground with a laboratory Retch SM 2000 and placed into clean glass vessels until analysis. Laboratory glassware used for the preparation of samples for the determination of mercury was cleaned by soaking for 24 hours in a solution of ethylene-diamine-tetra-acetic acid (EDTA; Kemika, Croatia; 5% v/v) and subsequently for 24 hours in HNO_3 (10% v/v; TTT Ltd., Holy Sunday, Croatia). Soil samples, of 0.5 g weight were digested with 5 ml of HNO_3 (65%, Suprapur, Merck, Germany) in sealed PTFE vessels in a microwave oven for decomposition (Milestone microwave laboratory system, MLS 1200 mega, USA). After matrices decomposition in a microwave oven, the samples were cooled in a water bath, transferred quantitatively to plastic flasks and diluted to 25 mL using deionized water. The samples were transferred from volumetric flasks to plastic test tubes. Mercury content in samples of mushroom was measured without acid digestion using an AAS mercury analyzer (AMA 254 Advanced Mercury Analyser, Leco, Poland) that uses direct combustion of the sample in an oxygen rich atmosphere. The quality of analytical procedures was controlled by using blank samples, freshly prepared calibration curves and standards, and certified reference materials. Statistical analysis and all chartings were performed with the R Statistical Software by using two integral and three external statistical packages. The values of bioconcentration factors were calculated as a ratio between the mercury content in the mushroom and in the soil in which the mushroom grew

Results and discussion

Soil properties (pH value and organic matter content) and concentration of Hg in the area of Petrova gora are summarized in Table 1. The mean pH value of the soil substrate at Zrin mountain was 6.42, ranging between 4.21 and 7.52. Organic matter content of the soil underneath various mushroom species and areas in this study ranged from 2.38 % to 13.31 %, with a mean value of 6.21 %. The results of analysis of mercury concentration in the soil substrate show mean value of 0.054 mg kg^{-1}.

Table 1. pH, organic matter and mercury concentration (mg kg-1 dry matter) in soil from the study area.

	Zrin mountain			
	Mean ± sd	Min.	Max.	C.V. %
pH H_2O	6.42 ± 1.01	4.21	7.52	15.73
O.M. %	6.21 ± 3.34	2.38	13.31	53.78
Hg	0.054 ± 0.02	0.05	0.09	37.03

Mean – mean value; Sd – Standard deviation; Min. – Minimum value;
Max. – Maximum value; CV – Coeficient of variability.

Descriptive statistics of mercury concentrations and factors of bioconcentrations (BCF) are given in Table 2. It shows that mushroom species have various abilities to accumulate mercury. The highest concentration

of mercury was determined in *B. edulis* (2.28 mg kg^{-1}). In contrast, the parasitic fungus *A. mellea* had the lowest mercury content of 0.22 mg kg^{-1}. All species of mushrooms in this study accumulated mercury and BCF values were higher than one (Table 2). The highest BCF value of 45.19 was determined for *B. edulis*, while the lowest bioconcentration factor (BCF) of 3.89 was calculated for *T. terreum.* The determined BCF value in *B. edulis* was 12 times higher than the lowest value in *T. terreum*. The distribution of Hg between anatomical parts of the fruiting bodies *(cap and stipe)* of the investigated mushrooms are given in Table 2. A considerably higher accumulation of heavy metals in caps than in stipes was determined in all mushroom species.

Table 2. Mercury concentrations in the analysed species of mushrooms (mg kg^{-1} dry matter); mean ± SD (n=10), quotient (Qc/s) of cap to stipe, bioconcentration factor (BCF).

Species	Mecury		Qc/s	BCF	
	Cap	Stipe		Cap	Stipe
Agaricus campestris	1.43 ± 0.24	1.05 ± 0.12	1.36	26.48	19.44
Armillaria mellea	0.31 ± 0.14	0.13 ± 0.05	2.38	*	*
Clitocibe inversa	1.52 ± 0.10	0.86 ± 0.08	1.77	28.15	15.93
Clitocibe nebularis	1.13 ± 0.07	0.74 ± 0.06	1.53	20.93	13.70
Macrolepiota procera	1.51 ± 0.08	0.95 ± 0.07	1.59	27.96	17.59
Boletus aestivalis	2.33 ± 0.11	1.75 ± 0.21	1.33	43.15	32.41
Boletus edulis	2.44 ± 0.17	2.11 ± 0.11	1.16	45.19	39.07
Lactarius deterrimus	0.81 ± 0.09	0.66 ± 0.07	1.23	15.00	12.22
Tricholoma portentosum	0.91 ± 0.16	0.70 ± 0.07	1.30	16.85	12.96
Tricholoma terreum	0.40 ± 0.16	0.21 ± 0.11	1.91	7.41	3.89

*wood-decaying

The highest concentration levels of mercury were found in species of the genus *Boletus,* which is in accordance with those of Melgar et al. (2009.) and Širić et al. (2014.). However, there is still no scientifically based explanation for this phenomenon, although Falandysz et al. (2007.) consider that the tubes, which are part of the carpophore, are extremely rich with mercury. The species *A. mellea* and *T. terreum* had lowest average mercury contents (significant, $p<0.05$) (Figure 1), which is in agreement with the results of Falandysz et al., (2013) for *Armillaria solidipes*. Higher deposition of mercury in caps versus stipes of fruiting bodies determined in our study is in agreement with the results of Širić et al. (2016.). The caps (hymenophore) may generally accumulate higher amounts of mercury because it contains more mercury binding proteins and enzymes than the rest of fruiting body (Melgar et al., 2009.). The BCF for mercury of all mushroom species was greater than one. This result implies that all the investigated mushroom species are active bio-accumulators for Hg in soil substratum. In general, metal (mercury) levels in fruiting bodies of wild mushrooms are influenced considerably by the age of mycelium, interval between fructifications, and the species of mushrooms. These factors cause wide variability in mercury concentrations.

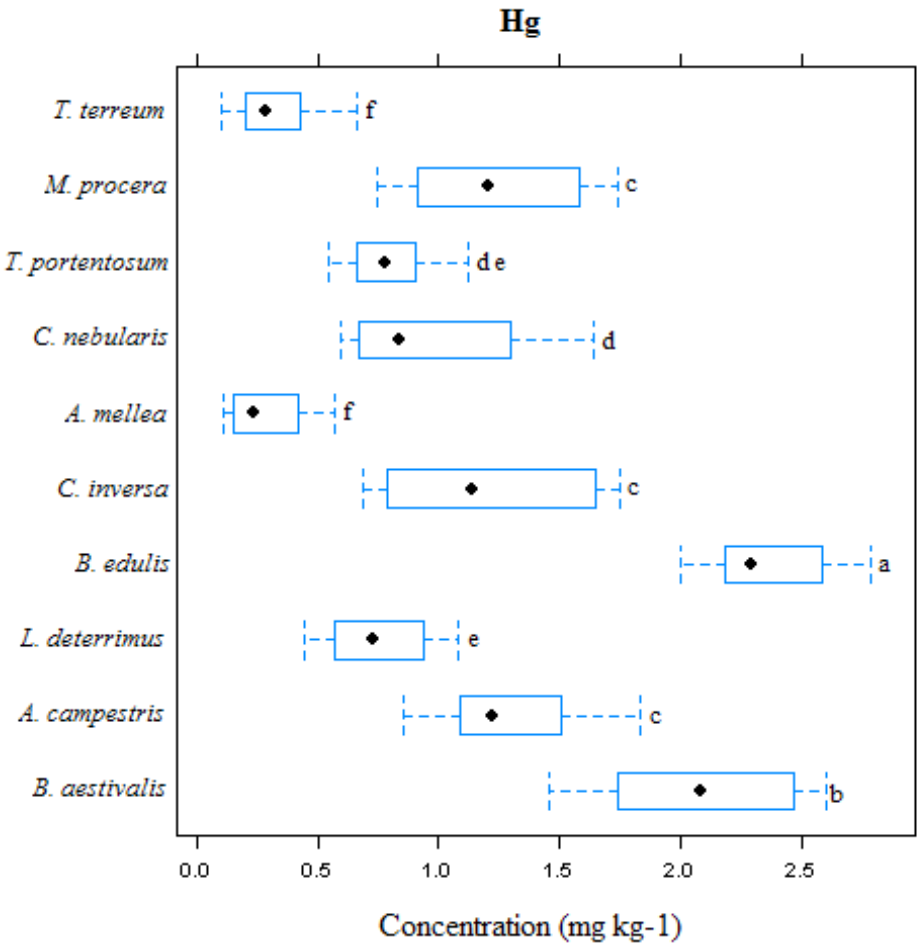

Figure 1. Box and whisker plots representing the distribution of Hg concentration in mushroom species: *Agaricus campestris; Armillaria mellea, Clitocybe inversa, Clitocybe nebularis, Macrolepiota procera, Boletus aestivalis, Boletus edulis, Lactarius deterrimus, Tricholoma portentosum* and *Tricholoma terreum*. Letters represent the results of Tukey's post-hoc comparisons of mean values among the species ($p<0.05$).

Conclusion

The mercury contents in the analysed mushrooms are mainly affected by the fungal species. All mushrooms species were bio-accumulation for mercury. The average concentrations of mercury metals were considerably different between the anatomical parts of the fruiting body (cap and stipe). Based on the determined concentrations of mercury and according to the regulations on maximum levels of certain contaminants in foodstuffs (NN 146/2012), it can be concluded that the consumption of the examined species of mushrooms do not pose a toxicological risk to humans. Nevertheless, the mercury concentration of wild edible mushrooms should be analysed more frequently in order to identify, evaluate and control the possible danger of exposure at the local or regional scale.

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