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THE HEAVY METALS CONTENT IN WILD GROWING MUSHROOMS FROM BURDENED SPIŠ AREA

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ABSTRACT

In this work, we evaluated the rate of entry of heavy metals into the edible parts of wild mushrooms, from central Spiš area. The area is characterized by extremely high content of heavy metals particularly mercury in abiotic and biotic components of ecosystems. The toxicity of heavy metals is well known and described. Known is also the ability of fungi to accumulate contaminants from substrates in which mushrooms grow. We have collected commonly consumed species of mushrooms (*Russula vesca., Macrolepiota procera, Lycoperdon pyriforme, Lecinum piceinum, Boletus reticulatus*). Sampling was conducted for two years 2012 and 2013. The samples taken mushrooms and substrates on which to grow, we determined heavy metal content (Cd, Pb, Cu), including total mercury content modified by atomic absorption spectrometry (AMA - 254). In the substrate, we determined the humus content and pH value. The heavy metal content in soils were evaluated according to Law no. 220/2004 Z.z The exceedance limit values of Cd, Pb, Cu and Hg was recorded. Most significantly the respective limit was recorded in soil samples in the case of mercury. The determined concentration Hg was 39.01 mg.kg^{-1}. From the results, we evaluated the degree of ability to bioaccumulate heavy metals different kinds of fungi. We also evaluated the health safety of the consumption of these fungi on the comparison with the limit values provided in the food code of SR. We recorded a high rate of accumulation of mercury in the species Boletus reticulatus and Macrolepiota procera. For these types we recorded the most significant than allowed concentrations of mercury in mushrooms. The highest recorded concentration reached 17.64 mg.kg^{-1} Hg in fresh matter. The limit value was exceeded also in the case of copper. We do not recommend to increased consumption of wild mushrooms in the reference area.

Keywords: Mushrooms; heavy metals; soil; food chain; mercury; midle Spiš

INTRODUCTION

The area of middle Spiš is significantly burden by heavy metal in content of soils **Hronec et al., (2008)**. In addition to air pollution, there are other sources of pollution. They came from the extraction of minerals, their modification and processing. From the iron manufactory in Rudňany were emitted into air 120 tons of mercury. These data prepared by measuring groups of Research Institute of Mineral (now Research Institute of Geotechnics SAS) in Košice. These amounts have been challenged by State Inspection of Slovak Republik Based on the balance of atmospheric emission of mercury they calculated, that in the environment received 40 tons Hg per year. It means this is the highest source of mercury pollution in Europe **Závodský, (1991)**. In this area, the specific sources of pollution endogenous geochemical anomalies, especially in the area of Rudňany, Poráč, Gelnice, Slovinky and Krompachy **(Čurlík and Šefčík, 1999)**. In these days, the highest producer of air pollution is town Krompachy, where Kovohuty Krompachy and iron foundry Slovak energy manufactory (SEZ) produce 90% of total emissions (Hronec et al. 2008). Toxic metals can enter the human body by consumption of contaminated food crops, water or inhalation of dust and cause damage to the organism, its toxic efects (**Mahmoode et al. 2013; Timoracká et al., 2011)**. Most dangerous elements in terms of their content in the soil to a possible accumulation of plants in this area appear Hg, Cd, Pb, and Cu. Toxic effect of Hg and its compounds is largely the reaction of Hg ion with SH-groups of biomolecules with subsequent changes in the permeability of cell membranes and damage cellular enzymes. Mercury has the ability to accumulate in the human body and leads to toxic manifestations of brain damage and peripheral nerves **Zahir et al., (2005)**. All compounds of cadmium are toxic. Cd has a high acute toxicity tests and certified short current (short-term) inhalation of high levels may result in the human body resulting in lung damage. It is highly toxic, causing inhibition of sulfhydryl enzymes in particular. Binds in the liver, but also affects the metabolism of carbohydrates and inhibits insulin secretion **Godt et al., (2006)**. Depending on the amount of exposure, lead can adversely affect the nervous system, kidneys, the immune system, reproductive and developmental systems and the cardiovascular system. Its toxic effects vary from subtle changes in neurocognitive function in low-level exposures to a potentially fatal encephalopathy in acute lead poisoning **Gillis et al., (2012)**. Copper is one of the essential elements for humans, but many copper compounds are potentially toxic. Excessive intake of copper is manifested neurological disorders **(Shaligram and Campbel, 2013)**. As risky part of the food chain can be considered by **Rieder (2011), Kalač (2010)** edible mushrooms due to their properties accumulation characteristics to heavy metals. The ability of fungi to accumulate in their fruiting body of heavy metals relates to the content of heavy metal in the soil, its link to the soil structure (fulvo and humic acids, clay particles etc.) soil pH, as well as the way of nutrition of the species of fungi. It was found significantly higher accumulation of heavy metals in certain species of fungi **(Falandysz and Gucia, 2008; Melgar et al., 2009)**. Heavy metals concentrations in mushrooms are also considerably higher than those in agricultural crop plants, vegetables and fruits. This suggests that mushrooms possess a very effective mechanism that enables them readily to concentrate certain heavy metals from the ecosystem, compared to green plants growing in similar conditions. According to the mechanism by which some heavy metals are accumulated is somewhat obscure although it seems to be associated with a chelation reaction with sulfhydryl groups of protein and especially with methionine. Thus considerable effort has been focused to evaluate the possible risk to human health from the consumption of mushrooms with regard to their heavy metal content **Paraskevi et al., (2007)**. Mushrooms are a popular part of the menu of the population and in particular mushroom pickers are therefore described as a vulnerable population in terms of income food of heavy metals **Ostos et al., (2015)**.

MATERIAL AND METHODOLOGY

Collection and processing of samples

Samples of fungi (n = 20) were collected, then cleaned of soil, and allowed to dry at 45 °C in a tray drier.

Samples of the substrates (n = 20) were taken from the same sites as samples of fungi, we left the air-dry and the final drying (50 °C) the samples were sifted through a sieve (particle diameter = 2 μm). The samples were collected during 2012 and 2013. Mushrooms species were collected: *Russula vesca*, *Macrolepiota procera*, *Lycoperdon pyriforme*, *Lecinum piceinum*, *Boletus reticulatus*.

The water content of the samples was determined by the moisture analyzer DLB 160-3A (Kern, Germany). Homogenized mushroom samples (1.000 g) were mineralized in a closed system of microwave digestion using Mars X-Press 5 (CEM Corp., USA) in a mixture of 5 cm^3 HNO_3 (Suprapur, Merck, Germany) and 5 cm^3 deionized water (0.054 $mS.cm^{-1}$) from Simplicity 185 (Millipore, UK). Digestive conditions for the applied microwave system comprised heating to 160 °C for 15 minutes and keeping it constant for 10 minutes. A blank sample was treated in the same way. The digested substances were subsequently filtered through a quantitative filter paper Filtrak 390 (Munktell, Germany) and filled up with deionized water to a volume of 50 cm^3.

Analytical procedure

Metal determinations were performed in a Varian AA240Z (Varian, Australia) atomic absorption spectrometer with Zeeman background correction. The graphite furnace technique was used for the determination of Cd and Pb, whereas the flame AAS Varian AA240FS (Varian, Australia) was used for the determination of Cu (detection limits for FAAS: 2.0 $mg.kg^{-1}$), and GF-AAS: 10.0 and 10.0 $ng.kg^{-1}$ for Cd and Pb, respectively). The total content of Hg was determined in the homogenized dried samples of mushrooms (0.005 – 0.01 g) using a cold-vapor AAS analyzer AMA 254 (Altec, Czech Republic).

We conducting determination of organic carbon and humus in the soil according to Turin and the modifications by Nikitin.

Soil organic carbon is oxidized with oxygen in chromo-sulfuric mixture. The amount of oxygen consumed in the oxidation is determined by the difference consumed and unconsumed chromo-sulfuric mixture. Humus content was found by calculation according to the following of relations:

$$c_{ox} = \frac{(a-b) \times 0{,}03 \times 1{,}17 \times f_{mohr}}{n}$$

(a - b) = difference between sample and blank test
f_{mohr} = accurate substance concentration of Mohr salt
n = sample weight in g
humus content = $c_{ox} \times 1{,}724$ [%]

c_{ox} = percentage of oxidizable carbon
1,724 = conversion factor to humus content in the soil sample.

Determination of active reactions in soil (pH/H_2O)

Twenty grams of soil samples were taken. Subsequently 50 ml of H_2O was added. The suspension was allowed 10 minutes to shake by shaker Heidolphpromax 1020 at a frequency of 180 oscilation per minute. After shaking and settled solution we filtered suspension through filter paper *FILTRAK* 390. After filtering the suspension, the pH of the filtrate was measured on the pH meter Metrohm 691, we calibration aparat to two buffers at pH 4 and 7. The resulting values were subtracted from pH meter display with two decimal places.

Bioavailability calculation (Baf): the heavy metal content in mushroom (fresh veight) / heavy metal content in substrat.

RESULTS AND DISCUSSION

The contents of heavy metals (Table 1) in substrates were evaluated according to the Annex. 2 of the Slovak decree no. 220/2004 col. Type of soil samples from the point of delivery 1 was sandy-loam, loam. Soils are predominantly slightly acidic. The humus content is very high. The minimum content of Cd was 0.03 $mg.kg^{-1}$ and maximum content of Cd was was 6.07 $mg.kg^{-1}$. The limit value from Cd in soil exceeded 47% of the samples. Pb minimum in soil we recorded 0,10 $mg.kg^{-1}$ and maximum in value172.50 $mg.kg^{-1}$. Only one sample exceeded the limit value from Pb in soil. Copper minimum in soil was 0.04 $mg.kg^{-1}$ and maximum 145.20 this sample exceeded the limit value from copper in soil 2.42 times. Minimum of mercury content was 0.24 $mg.kg^{-1}$ and maximum was 39.01 $mg.kg^{-1}$. In our study 19 from 20 samples exceeded the limit value for Hg in soil. The soils in middle Spiš area are extremely contaminated with mercury.

Extremely contamined soil by mercury, for which they were collected Wild mushrooms were also recorded in the

Spanish province of Asturias, where the values of Hg concentrations in the soil reach values 1 – 895 mg.kg^{-1}, is also contamination of anthropogenic origin **(Ordóñez et al., 2013)**.

Contents of the monitored heavy metals in mushroom samples varied at different intervals depending on the mushroom species (Table 2). Mushrooms are generally considered extensive accumulators of heavy metals. The level of the transfer of heavy metals into fruiting bodies is affected by a large number of factors, such as mushroom species, chemical parameters of the substrate (substrate composition, heavy metals content, pH, humus content), the age of the mycelium and, probably, the interval between fructification events. **Kalač, 2010**. Content of heavy metals in samples of mushrooms were evaluated according to the Food codex of the Slovak republic (decree of the Ministry of Agriculture and the Ministry of Health no. 608/3/2004-100 of 15 March 2004).

Cadmium

Minimum concentration of cadmium we recorded in sample *Boletus reticulatus* 0.027 mg.kg^{-1} and maximum in (0.48 mg.kg^{-1}) sample *Russula vesca.* No sample exceeded the limit value. The highest mean content we determined in species *Russula vesca.* Biovailability declined from species: *Boletus reticulatus* > *Russula vesca* > *Lycoperdon pyriforme* > *Lecinum piceinum* > *Macrolepiota procera*.

High content of Cadmium (4.27 mg.kg^{-1}) in *Russula* species determined in work from Turkia **(Tüzen, 2003)**.

Lead

The minimum concentration of lead we recorded in sample Boletus reticulatus 0.01 mg.kg^{-1} and maximum in sample Macrolepiota procera 0.51 mg.kg^{-1}.

The highest mean content of lead we determined in species Macrolepiota procera. Analysed samples do not exceed the limit value. Biovailability declined from species: Boletus reticulatus > Lycoperdon pyriforme > Macrolepiota procera > Russula vesca > Lecinum piceinum. High level of lead in species Macrolepiota procera determined also García et al., 2008.

Copper

Minimum content of copper we determined in sample *Lecinum piceinum* 1.61 mg.kg^{-1}. Maximum content (35.86 mg.kg^{-1}) we determined in sample *Lycoperdon pyriforme*, this sample exceede the limit value 3,58 times. The highest mean content we determined in species *Lycoperdon pyriforme.* Three samples exceeded the limit value (*Macrolepiota procera* (2) and *Lycoperdon pyriforme*). Biovailability declined from species: *Boletus reticulatus* > *Macrolepiota procera* > *Russula vesca* > *Lycoperdon pyriforme* > *Lecinum piceinum.* Comparable Cu concentration in mushrooms also mentioned **Kalač 2010.**

Mercury

Minimum content of mercury we determined in sample *Lecinum piceinum* (0.009 mg.kg^{-1}) and maximum mercury content (17.64 mg.kg^{-1}) we determined in sample *Macrolepiota procera* this sample exceeded the limit value 70.56 times.

High mean content we determined in species *Macrolepiota procera* (10.91 mg.kg^{-1}). 90% of samples exceeded the limit value from mercury in wild growing mushrooms. Bioavailability declined from species: *Macrolepiota procera* > *Lycoperdon pyriforme* > *Boletus reticulatus* > *Lecinum piceinum* > *Russula vesca.*

Table 1 Substrate parameters from mushrooms sampling.

Mushroom species	Substrate parameter					
	pH (H_2O)	Humus content %	Cd (mg.kg^{-1}) *LV (0.7)	Pb (mg.kg^{-1}) *LV (70)	Cu (mg.kg^{-1}) *LV (60)	T-Hg (mg.kg^{-1}) *LV (0.5)
Russula vesca						
range	4.44 – 7.29	2.48 –12.28	0.09 – 4.29	0.20 – 61.00	0.71 – 17.80	6.07 – 39.01
mean	5.93	7.70	1.72	25.64	10.33	20.06
SD	1.40	3.74	1.99	30.34	10.55	13.88
Macrolepiota procera						
range	3.73 – 6.18	1.75 – 7.32	0.04 – 4.10	0.10 – 64.50	0.08 – 32.30	0,50 – 18.83
mean	4.72	4.53	2.57	41.32	16.52	10.20
SD	1.15	2.36	1.83	30.05	13.17	7.65
Lycoperdon pyriforme						
range	4.63 – 6.17	2.42 – 6.60	0.03 – 6.07	0.13 – 39.70	0.14 – 145.20	1.26 – 10.66
mean	5.55	4.28	2.05	13.36	48.48	6.39
SD	0.71	1.72	3.48	20.80	83.76	4.75
Lecinum piceinum						
range	4.30 – 6.38	5.33 –14.70	0.09 – 3.44	0.48 – 172.50	0.19 – 37.50	0.24 – 19.30
mean	4.92	7.88	1.39	49.52	11.98	6.98
SD	0.89	3.94	1.59	8.20	17.55	8.66
Boletus reticulatus						
range	4.16 – 5.10	6.29 –10.05	0.14 – 0.14	0.31 – 2.14	0.04 – 0.23	2.33 – 36.35
mean	4.63	8.17	0.14	1.23	0.13	19.34
SD	0.66	2.65	0.00	1.29	0.13	24.05

Note: SD – standard deviation, * LV – limit value (Annex. 2 of the Slovak decree no. 220/2004 Col., Cd, Pb and Cu after aqua regia extraction).

Table 2 The heavy metals content in mushrooms samples (fresh matter).

Mushrooms species	Heavy metals content in fresh matter (mg.kg^{-1})			
	Cd (mg.kg^{-1}) *LV(1.0)	Pb (mg.kg^{-1}) *LV(1.0)	Cu (mg.kg^{-1}) *LV(10.0)	T – Hg (mg.kg^{-1}) *LV(0.25)
Russula vesca				
range	0.05 – 0.48	0.02 – 0.11	3.66 – 6.12	1.65 – 3.54
mean	0.18	0.06	4.65	2.33
SD	0.20	0.03	1.04	0.83
Macrolepiota procera				
range	0.08 – 0.16	0.16 – 0.51	3.09 – 20.93	1.24 – 17.64
mean	0.10	0.37	11.46	10.91
SD	0.03	0.16	9.03	7.14
Lycoperdon pyriforme				
range	0.08 – 0.21	0.12 – 0.26	7.71 – 35.86	0.56 – 6.96
mean	0.13	0.20	17.10	3.61
SD	0.06	0.07	16.24	3.20
Lecinum piceinum				
range	0.03 – 0.11	0.03 – 0.11	1.61 – 7.14	0.009 – 2.86
mean	0.06	0.06	3.28	1.32
SD	0.03	0.03	2.58	1.49
Boletus reticulatus				
range	0.027 – 0.10	0.01 – 0.07	1.78 – 2.10	1.62 – 14.35
mean	0.06	0.04	1.94	7.99
SD	0.05	0.04	0.22	9.00

Note: SD – standard deviation, * LV – limit value (Decree of the Ministry of Agriculture and the Ministry of Health of the Slovak republic no. 608/3/2004-100 of 15 March 2004).

Table 3 Pearson corelation coeficients between heavy metals conntent in mushrooms and substrates

corelation	Cd (mushrooms)	Pb (mushrooms)	Cu (mushrooms)	Hg (mushrooms)	Cd (substrates)	Pb (substrates)	Cu (substrates)	Hg (substrates)
Cd (mushroom)								
Pb (mushrooms)	0.03							
Cu (mushroom)	0.16	0.27						
Hg (mushroom)	0.015	0.67*	0.09					
Cd (substrates)	0.27	0.44	0.47	0.18				
Pb (substrates)	0.17	0.21	0.08	-0.04	0.65*			
Cu (substrates)	0.29	0.22	0.82*	0.04	0.76*	0.35		
Hg (substrates)	-0.10	-0.08	-0.14	0.41	-0.22	-0.34	-0.16	1

Note: * p <0.05

Contamination of wild mushroom species (*Boletus* and *Agaricus*) with mercury dealt **Melgar et al. (2009)**, with the highest concentration of mercury found in species of the genus *Boletus* and *Agaricus* in the range of 2.0 to 6.9 mg.kg $^{-1}$. The lowest concentrations reported in species of the genus Fistulina 0.22 mg.kg^{-1}. Bioavailability was also recorded the highest in the species of the genus *Boletus* and *Macrolepiota*. The observed area was northwest Spain. Their findings are comparable to our resultes.

We demonstrated high correlation (Table 3) between content of lead and mercury in mushrooms. We found high correlation between copper content in mushrooms and substrates on which they grow. We found high correlation between the cadmium content and lead, copper content in substrates.

CONCLUSION

Mushrooms as a popular culinary raw material is in our country a source of heavy metals, particularly mercury, in the food chain. This is due to their ability to accumulate well heavy metals from substrates on which they grow. This is confirmed by their biovailability. Mushrooms are therefore a real risk to human health following exposure to heavy metals in the target area. We don´t have known data about year-round consumption of mushrooms for better risk assessment. It would be appropriate to continue to complement those data and thus continue in this direction of research.

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