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The Bioaccumulation of Some Heavy Metals in the Fruiting Body of Wild Growing Mushrooms

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Abstract

Due to their effective mechanism of accumulation of heavy metals from soil, the macrofungi show high concentrations of metals in their fruiting body. According with this ability, the mushrooms can be used to evaluate and control the level of environmental pollution, but also represent danger for human ingestion. We analyzed some macrofungi species from a wooded area to establish the heavy metal concentrations and ability of bioaccumulation and translocation for Zn, Cu and Sn in fruiting body. The metallic content was established by the Inductively Coupled Plasma-Atomic Emission Spectrometry method (ICP-AES). The minimal detection limits of method is 0.4 mg/kg for Zn and Cu and 0.6 mg/kg for Sn. Heavy metals concentrations in the fruiting body ranged between 6.98-20.10 mg/kg for Zn (the higher value was for *Tapinella atrotomentosa*); 16.13-144.94 mg/kg for Cu (the higher value was for *Hypholoma fasciculare*); and 24.36-150.85 mg/kg for Sn (the higher value was for *Paxillus involutus*). The bioaccumulation factor has important values (higher than 1) only for copper in all the analyzed species (between 1.30 and 8.86) and for tin in *Paxillus involutus* species (1.19). The translocation factor shows that zinc and tin were accumulated in higher concentrations in cap of mushrooms and the copper had higher concentrations in stipe.

Keywords: macromycetes, bioaccumulation, translocation factor, zinc, copper, tin

Introduction

The mushrooms are consumed because of their chemical and nutritional properties, as for their therapeutic and preventing disease characteristics due to the chemical composition (Agrahar-Murugkar and Subbuakshmi, 2005; Manzi *et al.,* 2001). There is a well-established consumer acceptance of cultivated mushroom, such as *Agaricus bisporus*, *Pleurotus* spp., *Lentinus edodes* and other, but some specific groups of people, seasonally, are traditionally eating wild mushrooms (Diez and Alvarez, 2001).

Accurate food composition data are estimating the adequacy of essential nutrients intakes and assessing exposure risk from intake of toxic non-essential metals (Onianwa *et al.,* 2001; Soylak *et al.,* 2005). Trace elements above threshold concentration level, can cause morphological abnormalities and reduce growth and increase mortality and mutagenic effects in human bodies (Olumuyiwa *et al.,* 2007). The bioavailability of iron in mushrooms is therefore high and human body can absorb up to 90% of the present iron (Kalač and Svoboda, 2000).

Because of these, it is necessary to investigate the level of metals concentration in the wild growing mushrooms. They are known to accumulate high levels of several heavy metals like copper, mercury, lead, zinc and cadmium (Kalač and Svoboda, 2000).

Numerous data on metals concentrations in the fungal fruiting bodies were published (Alonso *et al.,* 2003; Cocchi *et al.,* 2006; Garcia *et al.,* 1998; Isildak *et al.,* 2004; Soylak *et al.,* 2005; Svoboda *et al.,* 2006). Because the macro fungi are integral part of the forest ecosystems, sometimes the soil-to-mycelium transfer of metals depends on relationship between mycelium and symbiotic plants species affecting element absorption and translocation (Malinowska *et al.,* 2004). In addition, the metals are distributed unevenly within the fruiting body, the highest concentrations have been observed in the spore-forming part, but not in the spore, a lower content in the rest of the cap and the lowest level in the stipe (Thomet *et al.,* 1999). High level of metals concentration was observed near metals polluted area and metals smelter (Collin- Hansen and Andersen, 2003; Kalač *et al.,* 1996; Svoboda *et al.,* 2000).

The purpose of this paper is to identify the level of toxic elements like copper, zinc and tin which are concentrated in the fruiting body of some mushrooms collected from a forest area of Carpathian Mountain, Bucegi Massif. A comparison between the level of heavy metals in the edible mushrooms and the toxic mushrooms will be done to underline the possible danger of the wild growing mushrooms consumption.

Materials and methods

Biological material

Six species of wild growing mushrooms were harvest from a wooded area, near Sinaia city, from Bucegi Massif of Carpathian Mountains. All these macro fungus were found in deciduous forest, at 800 m altitude, relatively close to the road Targovisite-Sinaia. They growth in a cold period, in November, on the soil, but the mycelium was founded also in the mixture of litter wood and leaves. The analyzed species are edible (*Collybia butyracea* and *Boletus griseus*), non-edible (*Tapinella atrotomentosus* and *Paxillus involutus*) or toxic (*Hypholoma fasciculare* and *Tricholoma flavovirens*). The harvested mushrooms were mature, with sporophore, and were collected the whole fruiting bodies, caps and stipes.

Analytical methods

For each mushroom, we sample 6-9 exemplars from different places and the substratum near the mycelium, down to the depth of 5 cm. Both the samples of mushrooms and soil, and them processing were did with plastic, glass and pottery instruments to avoid any metal contacts that can influence the results.

After harvesting, the mushrooms were clean up by the soil particles, dried at 60°C and then grinding to fine powder. The soil root surrounding samples were dried at 40°C until the complete process, then grinding to a fine powder and sieved at 250 μm (conform SR ISO 11464).

The Inductively Coupled Plasma - Atomic Emission Spectrometry method (ICP-AES), did the estimation of metallic content in the analyzed mushroom and them soil. For the analyzes with ICP-AES method, the biological samples (mushrooms) were mineralized, in Berghof microwave digestor, by mixture with 10 ml of nitric acid concentrated 65% and 2 ml of hydrogen peroxide, and for the soil samples were done hot extractions with nitric acid 1:1.

In present paper, the metals contents of mushrooms were establish with a 110 Liberty Spectrometer type of Varian brand. To disintegrate the sample in constituents atoms or ions is used a plasma source, which will stir up them on superior energetic layer. They will revert to the initial form by the emission of characteristic energy photon, emission recorded by an optical spectrometer. The radiation intensity is proportional with each element concentration in the sample and is intern calculated by a couple of calibration curves to obtain directly the measured concentration.

The concentrations represent the mean of many exemplars and are expressed in mg of metal related with kg of dry soil or plants. The minimal detection limits of the device ranged according the analyzed element and was 0.4 mg/kg for Cu and Zn; 0.6 mg/kg for Sn.

Results and discussion

Soil characteristics

The metal concentrations in the fruiting body of mushrooms vary over a wide range within the species, because of many factors affecting the absorption and accumulation rate. The soil properties, such as pH, redox potential, organic matter content, clay mineralogy, caution exchange capacity of the soil phase, competition with other metal ions and composition of the soil solution influence the absorption of metals in mushrooms (Angeles Garcia *et al.,* 2009).

Some of the soil characteristics from the sites where the mushrooms were harvested are present in Tab. 1. The humidity of the analyzed sites has the mean value of 47.53% because of the high ratio of leaf litter in the analyzed substratum, and the soil pH reaction is 6.70 due to the high content of the biological material. The mean amount of trace metals in the soil was for Zn higher than the normal value for an organic soil (57-100 mg/kg), and did not reached this limit for Cu (1-115 mg/kg) (Kabata-Pendias and Pendias, 1993).

Tab. 1. Humidity (%), pH and heavy metals contents (mg/kg) in the studied sampling points of soil from the Bucegi Massif, Romania

Soil parameters	Mean	Range	Minimum	Maximum
Zn	130.80	67.71	94.63	162.34
Cu	46.02	100.56	9.68	110.24
Sn	495.14	1234.69	58.68	1293.37
pH	6.69	0.44	6.48	6.90
Humidity	47.11	26.48	31.48	57.96

Metal concentrations in mushrooms

In the culinary domain, the mushrooms are very appreciated because of their concentration in minerals. Besides water (75-95% fresh weight), they has an important content of carbohydrates (39% dry weight), proteins (17.5% dry weight) and a low content of lipids (2.9% dry weight) (Latiff *et al.,* 1996). The amount of dry matter of mushrooms is species dependent, but also depends on the age and meteorological condition. A mean percentage of dry weights for each species of analyzed mushrooms are: *Boletus griseus* - 26.25%, *Collybia butyracea* - 33.59%, *Tapinella atrotomentosus* - 9.07%, *Paxillus involutus* - 18.01%, *Hypholoma fasciculare* - 18.23% and *Tricholoma flavovirens* - 16.17%.

Zinc is one of the important trace metals for a normal growth and development of humans and mushrooms are known as well accumulators for this element. Zinc content in the analyzed mushrooms from Bucegi Massif varies in the fruiting body of each species. The results obtained for the zinc concentration (Fig. 1) are in accordance with the concentrations from literature, which have been reported in the range of 28.6-179.0 mg/kg (Rudawska and Leski, 2005), 43.5-205.0 mg/kg (Sesli *et al.,* 2008) or 45-188 mg/kg (Tuzen, 2003). The zinc concentration is higher in the cap of the fruiting body than in stipe for all the analyzed species of mushrooms. For *B. griseus* and *T. flavovirens*, the zinc concentration in the stipe was under the detection limit of method.

The highest concentration of zinc was founded in non-edible and toxic species of mushrooms, *T. atrotomentosa* (30.05 mg/kg) and *T. flavovirens* (31.85 mg/kg), and the lowest concentrations of this element were founded in the edible species, *B. griseus* and *C. butyracea.*

Copper concentrations in the accumulating mushrooms species are usually 100-300 mg/kg of dry matter, which is not considered a risk for human health (Kalač and Svoboda, 2000) and a concentration higher than those in vegetable should be considered as a nutritional source of this element (Sesli *et al.,* 2008). For wild growing mushrooms, the copper content range between 'not detectable' and 169.80 mg/kg, in agreement with the literature values 15.5-73.8 mg/kg (Sesli *et al.,* 2008), 12-181 mg/kg (Tuzen, 2003) or 13.4-50.6 mg/kg (Soylak *et al.,* 2005). In fig. 1 we can see the differences of copper accumulation in the fruiting body of mushrooms, according with them edibility. The lower copper concentrations were founded in the edible mushrooms, and the highest concentrations in the toxic species of analyzed mushrooms. In addition, the copper is accumulated in higher quantities in the stipe of the fruiting body, for all the analyzed species of mushrooms.

The concentration of tin in the wild growing species of mushrooms ranged between 48.73 mg/kg for *B. griseus* and 301.70 mg/kg for *P. involutus*, the lowest concentrations are also in the edible species of mushrooms. This element is accumulated in the cap of the fruiting body; and the concentrations in the stipe of analyzed mushrooms were under the detection limit of method.

The bioaccumulation factor

The bioaccumulation factor represents the pollutant concentration in mushrooms comparing with the environment concentration (in soil) (Scragg, 2005). The bioac-

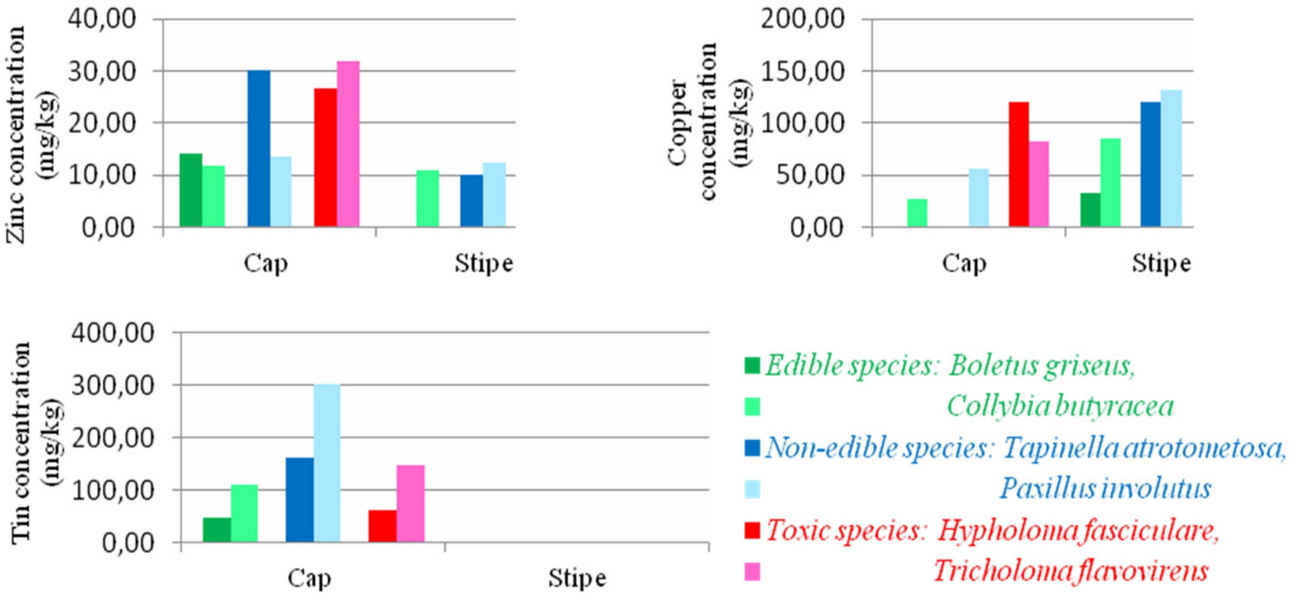

Fig. 1. The metal concentrations in the fruiting body of some wild growing mushrooms

Tab. 2. The bioaccumulation factor of edible and non-edible wild growing species of mushrooms

Bioaccumulation factor		Edible species		Non-edible species		Toxic species	
		Boletus griseus	Collybia butyracea	Tapinella atrotomentosa	Paxillus involutus	Hypholoma fasciculare	Tricholoma flavovirens
Zn	Cap	0.0885	0.0892	0.3258	0.0988	0.2199	0.2655
	Stipe	0	0.0834	0.1101	0.0906	0.0862	0
Cu	Cap	0	1.5823	0	5.2789	2.2705	2.6297
	Stipe	3.1314	4.8738	2.6001	12.4508	3.2104	4.0097
Sn	Cap	0.259	0.1663	0.1967	2.3877	0.23	0.0867
	Stipe	0	0	0	0	0	0

Tab. 3. The correlation between the metal contents in soil and pH with the metal concentration in the fruiting body (Pearson's coefficient)

Mushroom concentrations		Soil concentration			pH
		Zn	Cu	Sn	
Zn	Cap	-0.7631[a]	0.8205	0.6594[d]	0.1610[d]
	Stipe	-0.3526[a]	0.2011[d]	-0.4471[d]	0.3283
Cu	Cap	-0.0976[d]	0.4445	0.1304	0.5120
	Stipe	-0.6113	0.6939[b]	0.1457	0.5596[b]
Sn	Cap	-0.1614	-0.2825[d]	-0.0136	0.4558[d]
	Stipe	*	*	*	*

[a] $p < 0.001$; [b] $p < 0.005$; [c] $p < 0.01$; [d] $p < 0.05$; *the concentration in mushrooms is under the detection limit of method

cumulation factor of the analyzed species is different according the metal that is concentrate in mushrooms (Tab. 2). The bioaccumulation factor of analyzed mushrooms for zinc and tin has low values, under 0.5, comparing with vegetables and perennial plants. The values of copper bioaccumulation factor are more significant, they are higher than 1, which means that mushrooms can be considered accumulators and hyperaccumulators of this element. The highest values of the copper bioaccumulation factor were for P. invollutus species, as for cap (5.2789), as for the stipe of the fruiting body (12.4508 respectively).

Tab. 4. The correlation between the metal contents in soil and pH with the bioaccumulation factor in the fruiting body (Pearson's coefficient)

Bioaccumulation factor	Soil concentration			pH
	Zn	Cu	Sn	
Zn	-0.9771[a]	0.8602[d]	0.3681[d]	0.1904[a]
Cu	0.257[a]	-0.4581[d]	-0.2703[d]	0.4705[d]
Sn	0.2631[a]	-0.4685[d]	-0.4726[d]	0.1724[a]

[a] $p < 0.001$; [b] $p < 0.005$; [c] $p < 0.01$; [d] $p < 0.05$

The Pearson's coefficient of correlations (Tab. 3) between the metal contents in the soil and the metal concentrations in the fruiting body show that the metals concentrations is influenced by the species, morphological part of the fruiting body and by the soil characteristics, as metal content and pH. The correlation has significance at level 0.1% for zinc, and 5% for copper and tin.

The correlation between the metal contents of the soil and the bioaccumulation factor has high degree of correlation only between copper and zinc in soil and the bioaccumulation factor of zinc in the fruiting body of analyzed species of mushrooms, with statistically significant differences, $p < 0.05$.

Conclusions

The zinc and copper contents of the soil from the wooded area of Bucegi Massif are comparable, even higher than the maximum values of metals concentration in this category of soil. The toxic analyzed species grew on the soil with higher content of heavy metals than the edible species.

The lowest content of heavy metals was founded in edible species of mushrooms, and the highest in the toxic species. The concentrations of these elements increased with the increasing of the toxicity of analyzed species of mushrooms.

The bioaccumulation factor is comparable for the analyzed species of mushrooms concerning the three heavy metals, which means that the concentrations in mushrooms edible, non-edible or toxic, increase with the increase of metal content in the soil.

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