Annals. Food Science and Technology
2011

COPPER BIOABSORPTION IN SOME MACROMYCETES SPECIES GREW IN LABORATORY CONDITIONS

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Abstract
The metal content of fungi's fruiting body is dependent on species, mode of nutrition and soil properties – pH and metal content. The pH influences the metal bioavailability in a direct way. The studied species are wild growing fungi from Dâmbovița County forestry, in the view of using them in environmental remediation biotechnologies. The sample with young mushroom exemplars from wild were grew in laboratory controlled condition for few days, until the egg mushrooms become a mature fruiting body. During this period the egg mushrooms were sprinkled with $CuSO_4$ solution, 6% concentration and Pb acetate solution, 1% concentrate. By the treatment of mushrooms with $CuSO_4$ and Pb acetate solutions, the concentration of Cu and other elements increase or decrease according with the synergic or antagonistic effect that can have Cu and Pb on the bioaccumulation of other elements. The sample of Boletus edulis treated with $CuSO_4$ solution showed a concentration of Cu in fruiting body over 100 times higher comparing with the blank. For the sample of Russula foetens which was treated with Pb acetate, the Zn concentration increase 2 times, which highlight the synergic effect of lead in soil on the Zn bioaccumulation. The bioaccumulation of Fe, Cu, Zn, Mn and Ni in Cantharellus cibarius, Russula foetens and Lactarius piperatus species is influenced by the Cu and Pb concentration in soil and for Boletus edulis the accumulation is depending on the species metabolism.

Keywords: macromycetes, bioaccumulation, heavy metals

Submitted: 04.10.2011 Reviewed: 07.11.2011 Accepted: 07.12.2011

1. INTRODUCTION

Last researches in this field demonstrate that many fungus species accumulate metallic elements in higher amounts comparing with other biosystems. The metal content of fungi's fruiting body is dependent on species, mode of nutrition and soil properties – pH and metal content (Gast et al., 1998). Nearby the metallurgical units of Targoviste city, the concentration of heavy metals in soil exceeds the normal limits (Elekes et al., 2010). In order to reduce the high concentrations of these elements in soil some biotechnologies of remediation can be apply, using microorganisms, plants or fungus (USDI, 2009).

The previous studies showed that bioabsorption rate can be increased by nutrients apply (Doble and Kumar, 2005). The organic matter from the soil have an important role in the metal accumulation because they can delay metal absorption and transfer in soil solution and also influence the chemical form of metals in soil. The metal toxicity can be reduced or increased by the organic matter. The pH influences the metal bioavailability in a direct way: in acid soils the elements are more soluble so more easily they go in the soil solution (Müller, 1965). The Zn solubility in soil was studied by Herms and Brummer in 1984, showing the extent to which this element is dissolved by high acidity of soil and made available for plants to absorb it.

The aim of this paper is to highlight the capacity of some macromycetes species to absorb and accumulate Cu from soil, when the concentration of this element in the soil solution is critical. The studied species are wild growing fungi from Dâmbovița County forestry, in the view of using them in environmental remediation biotechnologies.

2. MATERIAL AND METHOD

Four species of wild growing mushrooms were taken from forestry ecosystem, 20 km from Targoviste City, Romania, between 44°57´45´´ - 44°57´50´´ N, and 25°19´00´´ - 25°19´10´´ E. The studied area is at 12 km NW from the metal smelter of Targoviste, 3.5 km SW from an oil extraction platform and 2 km NE from a high traffic road. Because of the relatively high distance from the metal smelter, there is a wick influence of the emissions deposition. Also a wick influence has the oil extraction activities and the traffic because the studied area is in the middle of forest, at least 2 km of forest until the edge of sampling points.

The sampling of mushrooms and soil and them processing were done with plastic, glass and pottery instruments to avoid any metal contacts which could influence the results.

We harvested few mature exemplars as blank sample, and very young exemplars (egg stage) were taken with 6 dm^3 of substratum (20X30 cm and 10 cm deep).

The sample with young mushroom exemplars were grew in laboratory controlled condition: 25°C and 75% humidity during the day; 22°C and 80% humidity during the night, for few days, until the egg mushrooms become a mature fruiting body.

During 2-4 days, the egg mushrooms were sprinkled with $CuSO_4$ solution, 6% concentration and Pb acetate solution, 1% concentrate.

After harvesting the blank sample and the treated ones, the fresh mushrooms were clean with deionised water to remove the soil particles, dried at 60ºC and then ground to fine powder. The soil samples under the stipes were completely dried at 40ºC and ground to a fine powder and sieved at 250 µm (conform SR ISO 11464).

2.2 Analytical procedure

The elemental content of samples was established by Energy Dispersive X-Ray Fluorescence method (EDXRF) (Ene et al., 2009), using ElvaX Spectrometer having a X-ray tube with Rh anode operated at 50 kV and 100 µA. Two grams of each sample were pressed manually, without any chemical treatment, in a plastic vial with Mylar on the bottom. The samples were excited for 300s and the characteristic X-rays were detected by a multichannel spectrometer based on a solid state Si-pin-diode X-ray detector with a 140 µm Be window and an energy resolution of 200eV at 5.9 keV. The accuracy and precision of the results were evaluated by measuring a certified reference sample NIST SRM 1571 – Orchard leaves (table 1).

In this way were registered all the elements which were in a concentration higher than 1 mg/kg. Every result is the average of three determinations. The final results were reported to dry substances and calculated in mg of metal per kg of dry weight (mushrooms or soil) – mg/kg.

Table 1 Observed and Certified values of trace metals in NIST-SRM 1571 Orchard leaves (n=3)

Element	Certified value (mg/kg)	EDXRF value (mg/kg)	Recovery (%)
Cu	12.00 ± 1.0	11.75 ± 1.6	98
Fe	300.00 ± 20.0	307.22 ± 18.3	102
Zn	25.00 ± 3.0	23.96 ± 2.2	96

2.3 Data analysis

The bioaccumulation factor (BF) of the studied mushrooms, for each metal is expressed by the equation (1):

$$BF = \frac{C_M}{C_S} \quad (1)$$

where: BF represents the bioaccumulation factor of mushrooms; C_M is the metal concentration in mushroom and C_S is the metal content of substrate (soil).

The translocation factor (TF) represents the ratio of metal concentration in cap and stipe and is calculated according to the equation (2):

$$TF = \frac{C_{cap}}{C_{stipe}} \quad (2)$$

where: TF represents the translocation factor of mushrooms; C_{cap} is the metal concentration in the cap and C_{stipe} is the metal concentration of the stipe of fruiting body.

3. RESULTS AND DISCUSSIONS

The iron concentration in analyzed mushrooms is different from one species to another, and varies in the fruiting body (table 2). The Fe concentration in the stipe of mushrooms is higher than the concentration in the cap, for the studied species, excepting *Boletus edulis*. The lower concentration of Fe was in *Lactarius piperatus* (71.352 mg/kg) specie and the highest in *Russula foetens* (397.440 mg/kg).

The mean concentration of Cu in mushrooms is comparative, even lower comparing with previous published results 15.5-73.8 mg/kg (Sesli et al., 2008) or 13.4-50.6 mg/kg (Soylak et al., 2005). The highest Cu concentration was founded in *Cantharellus cibarius* species, 21.817 and 15.361 mg/kg in cap and stipe respectively.

The mean concentration of Zn in analyzed mushrooms is comparative with the range of published values 28.6-179.0 mg/kg (Rudawska and Leski, 2005), 43.5-205.0 mg/kg (Sesli et al., 2008) and 45-188 mg/kg (Tuzen, 2003), and excels these values in the cap of *Cantharellus cibarius* species, 235.884 mg/kg.

Mn concentration in studied mushrooms excels the references data (10-60 mg/kg - Kalač, 2010; Sesli et al., 2008). The maxim value of Mn concentration was founded in stipe of *Russula foetens* species, 154.834 mg/kg, value which is similar with the previously results for *Boletus edulis* and *Macrolepiota procera*, which exceed 100 mg/kg (Kalač, 2010; Sesli et al., 2008).

Ni concentrations are in the range of normal values, < 15 mg/kg (Kalač, 2010), for all of studied species. The lower values were founded in *Russula foetens* and *Lactarius piperatus* species, < 1 mg/kg, and the highest values were for *Cantharellus cibarius* species, 1.993 and 2.025 mg/kg for cap and stipe respectively.

Table 2 Metal concentration (mg/kg) in four wild growing species of mushrooms

Species	Fe		Cu		Zn		Mn		Ni	
	cap	stipe	cap	stipe	cap	stipe	cap	stipe	cap	stipe
Cantharellus cibarius	173.118	272.149	21.817	15.361	235.884	87.195	50.942	86.767	1.993	2.025
Russula foetens	204.831	397.440	5.039	3.640	45.635	43.785	82.004	154.834	0.770	0.905
Lactarius piperatus	71.352	149.263	8.531	5.422	38.846	17.780	33.728	70.121	0.919	0.759
Boletus edulis	233.911	197.354	5.467	8.658	17.043	-	93.757	56.796	0.904	1.556

By the treatment of mushrooms with $CuSO_4$ and Pb acetate solutions, the concentration of Cu and other elements increase or decrease according with the synergic or antagonistic effect that can have Cu and Pb on the bioaccumulation of other elements.

For *Cantharellus cibarius*, the sample was treated with $CuSO_4$ solution, and after few days, the concentration of Cu in fruiting body increase almost 100 times, to 1306.64 and 1020.06 mg/kg for cap and stipe respectively (fig. 1). Also, the concentration of Fe, Mn and Ni increase in the fruiting body of this species, especially in the stipe, because of the synergic effect of Cu in soil on the bioaccumulation of these elements. Because of the antagonistic effect between Cu and Zn, the Zn concentration in fruiting body decrease to 65.19 mg/kg and under the detection limit of method for cap and stipe respectively. The same pattern of metal bioaccumulation was observed by analyzing the bioaccumulation factor, which increases to 3.47 and 2.71 for Cu and decrease 30 times for Zn (table 3). The translocation factor shows comparable values between blank and sample exemplars.

For *Russula foetens*, was one sample treated with $CuSO_4$ solution and one treated with Pb acetate (fig. 2). For the sample which was treated with $CuSO_4$ solution, the concentrations of Cu, Mn and Ni show an increase and the concentration of Zn show a slight decrease according with the pattern of synergic and antagonistic effect of Cu in soil on the bioaccumulation of these elements. For the sample which was treated with Pb acetate, the Zn concentration increase 2 times, which highlight the synergic effect of lead in soil on the Zn bioaccumulation. The bioaccumulation factor increase for Cu in the sample treated with $CuSO_4$ solution and for Zn in sample treated with Pb acetate (table 4).

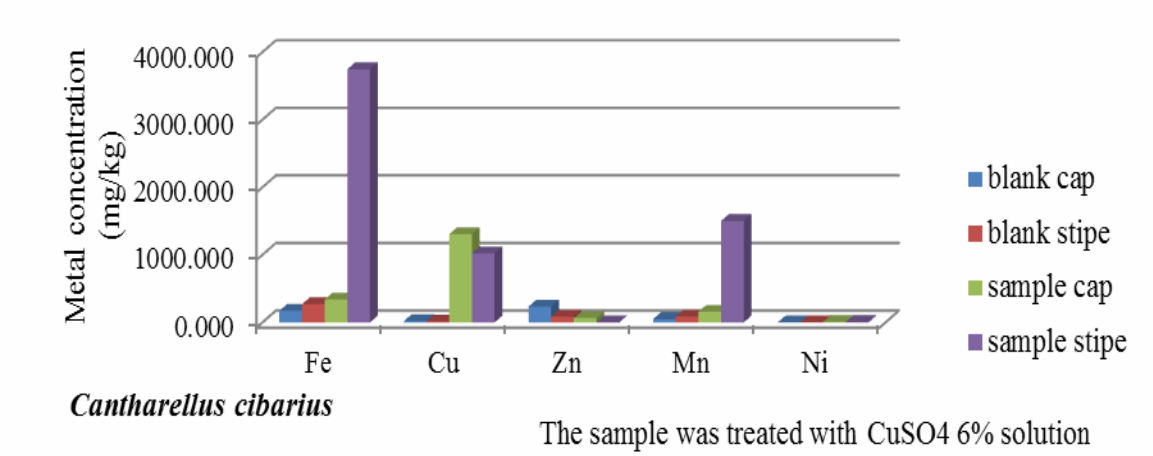

Figure 1 Metal concentrations in blank and treated sample of *Cantharellus cibarius* species

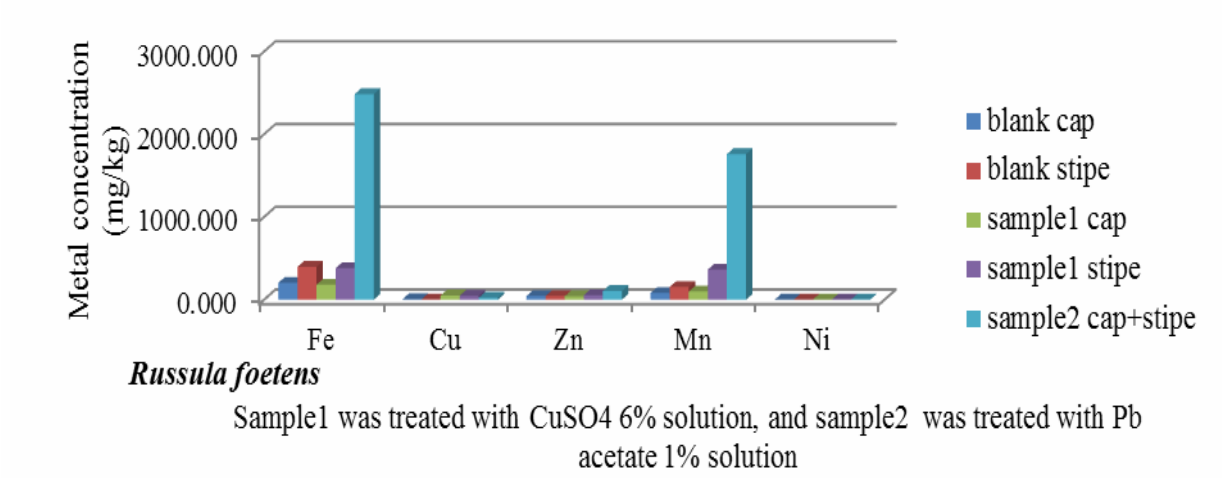

Figure 2 Metal concentrations in blank and treated samples of *Russula foetens* species

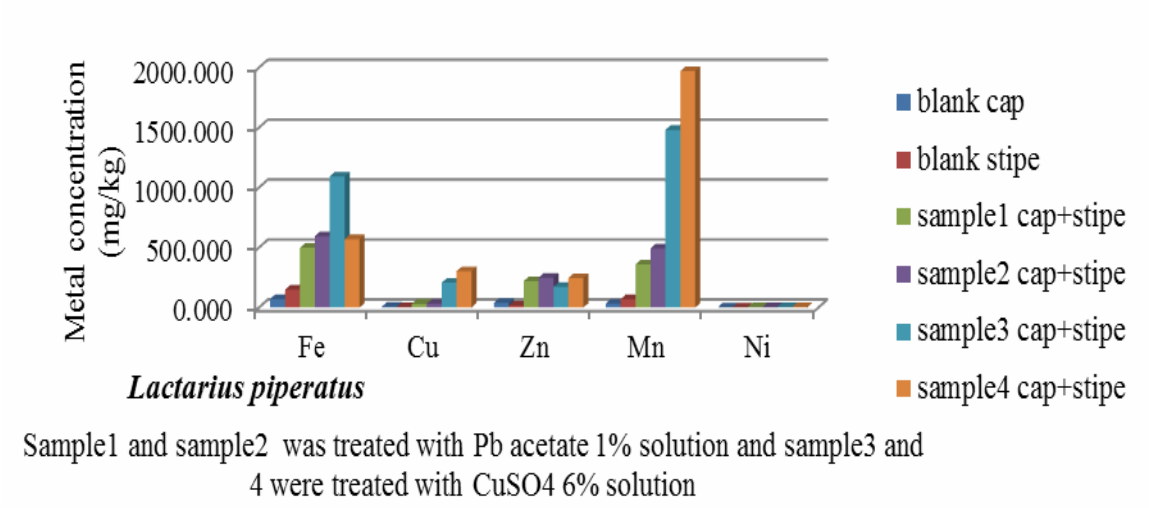

Figure 3 Metal concentrations in blank and treated samples of *Lactarius piperatus* species

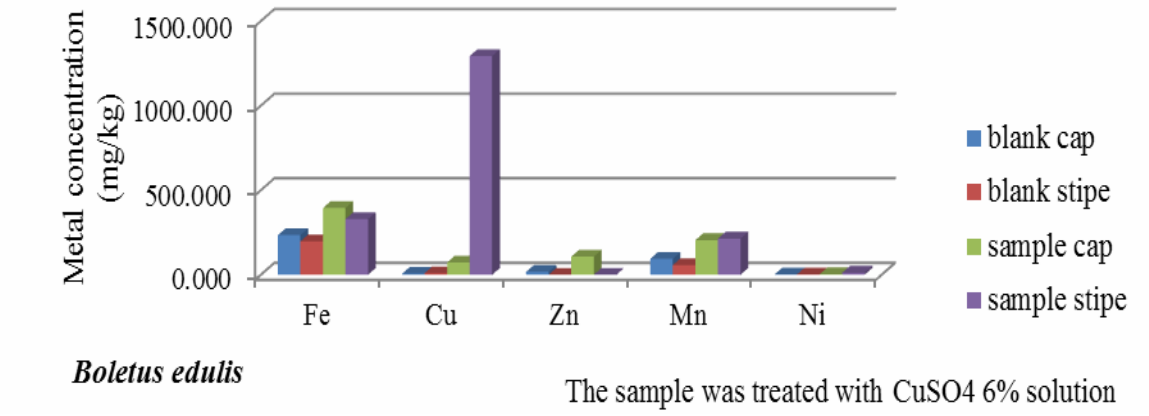

Figure 4 Metal concentrations in blank and treated sample of *Boletus edulis* species

For *Lactarius piperatus* species, two samples were treated with $CuSO_4$ and two with Pb acetate. In the two samples treated with $CuSO_4$, the Cu, Fe and Mn concentration were the highest, increasing by 5 to 30 times comparing with the blank exemplars (fig. 3).

Table 3 Bioaccumulation (BF) and translocation (TF) factors of *Cantharellus cibarius* species

Metal	Blank			Sample		
	BF cap	BF stipe	TF	BF cap	BF stipe	TF
Fe	0.002	0.003	0.636	0.003	0.037	0.091
Cu	0.059	0.042	1.420	3.475	2.713	1.281
Zn	0.743	0.275	2.705	0.026	-	-
Mn	0.103	0.176	0.587	0.294	2.783	0.106
Ni	-	-	0.984	-	-	1.243

Table 4 Bioaccumulation (BF) and translocation (TF) factors of *Russula foetens* species

Met	Blank			Sample1			Sample2		
al	BF cap	BF stipe	TF	BF cap	BF stipe	TF	BF		
Fe	0.003	0.006	0.515	0.002	0.005	0.476	0.032		
Cu	0.016	0.012	1.384	0.111	0.103	1.069	0.051		
Zn	0.012	0.012	1.042	0.017	0.022	0.768	0.042		
Mn	0.156	0.295	0.530	0.148	0.546	0.270	2.638		
Ni	-	-	0.851	-	-	0.528	-		

In the samples treated with Pb acetate, all the metals concentration increase comparing with the blank, but the Zn concentration was higher comparing with the Zn concentration in the other two samples. When the Cu and Pb concentration in soil increase, the bioaccumulation factor increase also for other elements, but these two metals has an synergic effect on the bioaccumulation of Mn, the bioaccumulation factor increase from <0.2 to >0.9, even to 5.268 (table 5).

Table 5 Bioaccumulation (BF) and translocation (TF)

factors of *Lactarius piperatus* species

Metal	Blank			Samp 1	Samp 2	Samp 3	Samp 4
	BF cap	BF stipe	TF	BF	BF	BF	BF
Fe	0.001	0.002	0.478	0.007	0.008	0.016	0.008
Cu	0.023	0.014	1.573	0.041	0.043	0.271	0.397
Zn	0.011	0.005	2.185	0.116	0.131	0.091	0.129
Mn	0.077	0.159	0.481	0.963	1.320	3.959	5.268
Ni	0.001	0.001	1.211	-	-	-	-

Table 6 Bioaccumulation (BF) and translocation (TF) factors of *Boletus edulis* species

Metal	Blank			Sample		
	BF cap	BF stipe	TF	BF cap	BF stipe	TF
Fe	0.003	0.003	1.185	0.006	0.005	1.200
Cu	0.009	0.015	0.631	0.106	1.902	0.056
Zn	0.005	-	-	0.044	-	-
Mn	0.284	0.172	1.651	0.344	0.360	0.957
Ni	-	-	0.581	-	-	0.217

The sample of *Boletus edulis* was treated with $CuSO_4$ solution and the concentration of Cu in fruiting body increase over 100 times, to 1293.26 mg/kg (fig. 4). Also, the concentration of Fe, Zn, Mn and Ni increase in the fruiting body of this species, especially in the stipe, because of the synergic effect of Cu in soil on the bioaccumulation of these elements. Even the antagonistic effect of Cu in soil on the bioaccumulation of Zn, the Zn concentration increase in the sample treated with $CuSO_4$. The same pattern of metal bioaccumulation was observed by analyzing the bioaccumulation factor, which increases to 1.9 for Cu in the stipe of fruiting body (table 6). The translocation factor shows comparable values between blank and sample exemplars.

4. CONCLUSIONS

- For *Cantharellus cibarius* the Cu, Mn and Ni concentration in the fruiting body increase with the increasing of Cu concentration in substratum.
- *Russula foetens* species hyperaccumulate Mn if the Cu concentration increases in soil. The Cu concentration in fruiting body increase with the increasing of Cu concentration in soil, but this species is not accumulator for Cu.
- For both the Pb and Cu concentration increasing in soil, the bioaccumulation factor of Mn indicates the hyperaccumulation capacity of *Lactarius piperatus* species for Mn.
- The accumulation of Zn in *Boletus edulis* species was not influenced by the synergic effect of Cu in soil and also the synergic effect of Cu in soil on the bioaccumulation of Fe and Mn was weak.
- The bioaccumulation of Fe, Cu, Zn, Mn and Ni in the first 3 species is influenced by the Cu and Pb concentration in soil and for *Boletus edulis* the accumulation is depending on the species metabolism.

5. ACKNOWLEDGEMENTS

This work was supported by CNCSIS – UEFISCSU, project number PNII – IDEI 624/2008, coordinated by Associate Professor Gabriela BUSUIOC. It is a good occasion to thank to Mr. Mihai DUMITRU, PhD in Geobotanics field, for the identification of fungi species analyzed in this work.

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