THE EFFECT OF METALLIC CONTENT OF SOIL ON THE ABSORPTION AND ACCUMULATION FOR SOME SPECIES OF FUNGI USED IN SOIL'S BIOREMEDIATION

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Abstract

The remediation of heavy metals polluted soil using biosystems involves the use of plants and fungi species for the extraction of metals from soil. To streamline the process of bioremediation of soil polluted with heavy metals using higher fungi, those species should be chosen with a high capacity to absorb metals and environmental factors can be controlled so that the accumulation of these elements to be favored. Heavy metal absorption by higher fungi is influenced primarily by the species, but also by soil pH and concentration of other metals in the soil. The interaction of chemical elements can be synergistic and / or antagonistic, and the reactions that lead to the creation of a chemical imbalance are a chemical stress for the fungi. The main correlations were observed between the Cu content in soil and concentration of Cu and Zn in fungi, elements which have a moderate positive correlation, but also between Sn content in the soil and the content of Co and Ni in the fungus. Moderate synergistic effects were observed between Co and Cr content in the soil on the absorption of Mn in higher fungi. Iron content in the soil has a synergistic effect on the absorption of Cr, Mn, Sn and antagonistic effect on the absorption of Cu, Co, Ni and Zn. Macronutrients affect the bioavailability of heavy metals in soil by changing the soil reaction. Fe and Mg have a synergistic effect on the absorption of most metals, while P, K and Ca have an antagonistic effect. Based on the synergistic and antagonistic effects between the soil components on metal bioavailability to higher fungi may be a modeling process of absorption and accumulation of heavy metals in tissues of biosystems.

Key words: bioremediation, fungus, heavy metals, absorption

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). 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).

Also, the 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 influence the metal bioavailability in a direct way: in acid soils the elements are more soluble so more easy the 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 correlations between the metal content of the soil and absorption/accumulation of heavy metals by some wild growing fungi in Dâmbovita County, in the view of using them in environmental remediation biotechnologies.

MATERIAL AND METHOD

Sampling and preparation

The studied fungus species were harvested from forestry area, nearby the metallurgical units of Targoviste city (fig. 1). When the mushrooms were harvested, also the substratum they grew on was sampled. After harvesting, the fresh fungi sample were cleaned with deionized water to remove the soil particles and leaves, dried at 60°C for few hours, ground to fine powder and analyzed to establish the metal concentrations. The fungus substratum samples were dried at 40°C for 24 hours, ground to fine powder, sieved at 250 μm

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(conform SR ISO 11464), then analyzed to establish the metal concentrations and pH.

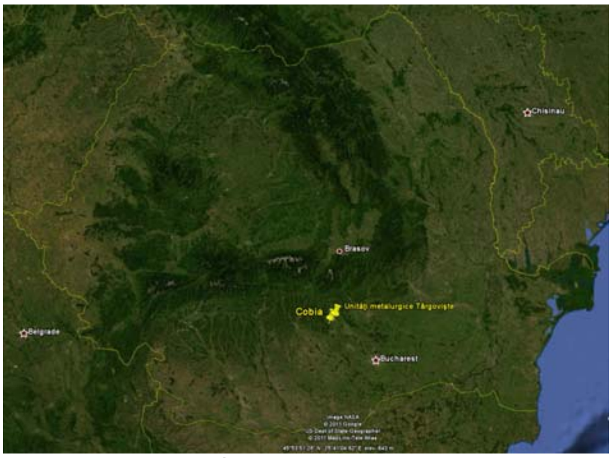

Figure 1 **Sampling point location (Cobia and Priseaca forest located at 6-9 km distance from metallurgical units, Targoviste)**

Analytical procedure

Determination of heavy metals, in both fungi and substratum they grew on, was done by Inductively Coupled Plasma - Atomic Emission Spectrometry method (ICP-AES). For analyze, the samples were mineralized in Berghof microwave digester, fungi by mixture with 10 ml of nitric acid concentrated 65% and 2 ml of hydrogen peroxide, and substratum in mixture 1:1 with nitric acid (according with Berghof method). The advantage of this method is the multielementale detection, which give the possibility, in one shot, to read a wide range of elements.

For this research, analyze was done with Liberty 110 spectrometer of Varian brand. The minimal detection limits of device range according to the analyzed element and is 0.4 mg/kg for Zn, Mn and Cu; 0.5 mg/kg for Cr and Co; 0.6 mg/kg for Sn, Pb, Mo and Ni. The concentrations values for analyzed metals are expressed in milligrams of metal per kilogram of dry soil or fungi (mg/kg).

The soil pH determination was done with portable pH-meter WTW 3110 SET 2, with a precision of 0.01 units. For pH analyze, 5 g of each substratum sample were mixed with 50 ml KCl 0.1N, F 1000, Tt 0.0056 g/ml and homogenized for 15 minutes with a magnetic stirrer.

Data analysis

Statistical interpretation of results was done with Microsoft Office Excel (2007) and CAMO UnscramblerX, 10.1 version software. The first software was used to calculate the Pearson's coefficient of correlation between the soil content and metal concentration in fungi, and the second one for descriptive statistics of metal concentrations and Principal Component Analysis (PCA) of the correlation between the metallic content of the soil and metal concentrations in studied fungi.

RESULTS AND DISCUSSIONS

Substratum characteristics

Heavy metal concentrations in fungi vary in a wide range according with species and the characteristics of the substratum they grow on. The presence in soil of some metals influence the absorption of other metals because of the ion competition in soil solution and the pH has an important role for the chemical state of elements in soil solution.

Heavy metals and macronutrients concentrations in the substratum that fungi grew on are shown in table 1. The substratum represents forest soil sampled from 6-9 km distance from metallurgical units of Targoviste city, a significant heavy metals pollution source. The effects of pollution are highlight by the mean heavy metals concentration in soil which exceed the normal values of 13-24 mg/kg for Cu (Kabata-Pendias, 2001), respective 650 mg/kg for Mn (FOREGS, 2005). For Sn, values of concentration in analyzed substratum exceed the maxim values, 106 mg/kg, founded in Europe soils by FOREGS project (2005). For Zn, Co, Ni and Cr, the concentration in some sampling points exceed the tolerance limits: 13-149 mg/kg for Zn, 3-44 mg/kg for Co, 10-92 mg/kg for Ni, 41-131 mg/kg for Cr respective (Fadigas et al., 2006).

Table 1

Metal concentration in substratum and the pH

Metal	Substratum concentration				
	min	max	mean	SD	unit
Fe	1.54	51.45	18.17	12.49	g/kg
Ca	7.92	59.33	36.11	14.09	g/kg
Mg	2.35	8.05	3.98	1.24	g/kg
P	1.08	7.32	2.54	3.17	g/kg
K	1.54	12.25	3.34	2.74	g/kg
Cu	10.27	109.33	37.67	22.79	mg/kg
Zn	75.37	211.67	129.44	32.92	mg/kg
Sn	40.08	1427.34	467.88	388.00	mg/kg
Co	0.52	24.51	7.61	6.13	mg/kg
Ni	0.62	126.02	24.45	30.09	mg/kg
Cr	0.58	54.32	14.31	14.74	mg/kg
Mn	1122.93	4305.41	2160.71	779.91	mg/kg
pH	6.50	6.97	6.74	0.31	

Heavy metal concentrations in fungi

Fungi samples were harvested from forestry area, with heavy metals polluted soils. Studied species, the harvesting location and the edibility of each species are showed in table 2.

Table 2

Analyzed fungi species

Species	Harvesting location	Edibility
Hypholoma capnoides	Erdemir Plant	Edible*
Hypholoma fasciculare	Priseaca Forest, Cobia Forest	Toxic
Cortinarius callisteus	Cobia Forest	Toxic
Cortinarius largus	Cobia Forest	Non-edible
Cortinarius armillatus	Priseaca Forest	Non-edible
Cortinarius subfulgens	Priseaca Forest	Poisoning
Calvatia excipuliformis	Priseaca Forest	Edible*
Tricholoma flavovirens	Cobia Forest	Toxic
Collybia butyracea	Cobia Forest	Edible*
Marasmius oreades	Cobia Forest, Priseaca Forest	Edible
Boletus griseus	Priseaca Forest, Cobia Forest	Edible
Hygrophorus virgineus	Priseaca Forest	Edible*
Tapinella atrotomentosus	Cobia Forest	Non-edible
Paxillus involutus	Cobia Forest	Non-edible

* Conditioned edibility or low nutritive value

Statistical results of heavy metals concentrations in studied fungi are presented in table 3 and figure 2. Because of the high Cu content in substratum, mean concentration of this element in fungi, 80.13 mg/kg, is higher 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 *Calvatia excipuliformis* species, 226.52 mg/kg, value that far exceeds the results of Tuzen (2003), 181 mg/kg.

The mean concentration of Zn in studied fungi, 32.59 mg/kg and the maxim value of concentration, 124.30 mg/kg founded in *Calvatia excipuliformis* species also, are in the lower half of 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).

Mean value of concentration for Sn was 283.63 mg/kg with a maximum level at *Hygrophorus virgineus* species, 2809.34 mg/kg.

The mean value of concentration for Co, 3.85 mg/kg exceed the normal values of 0.5 mg/kg (Svoboda and Chrastný, 2008), but the maxim value, 5.42 mg/kg, founded in *Marasmius oreades* species is similar with the high values of 7.2, 5.8, 3.3 and 2 mg/kg previously founded in *Ramaria largentii* (Ouzuni et al. 2009), *Agaricus arvensis* and *Cantharellus cibarius* (Borovička and Řanda, 2007).

Ni concentrations are in the range of normal values, < 15 mg/kg (Kalač, 2010), for the majority of studied species. For *Marasmius oreades* species, the concentration of this element far exceed the highest values previously founded in *Coprinus comatus*, 58,6 mg/kg (Yamaç et al., 2007).

Mn concentration in studied fungi has normal values comparing with the references data (10-60 mg/kg - Kalač, 2010; Sesli et al., 2008), with a mean value of 32.47 mg/kg. The maxim value of Mn concentration was founded in *Hypholoma capnoides* species, 93.85 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)

The mean value of Cr concentration was 9.10 mg/kg, which is similar with previously results of 5-10 mg/kg, for part of the studied species (Isildak et al., 2007; Konuk et al., 2007; Ouzuni et al., 2009).

Table 3

Statistical results of heavy metals concentrations in wild growing fungi from Dambovita County (data are in mg/kg)

Statistics	Cu	Zn	Sn	Co	Ni	Mn	Cr
Mean	80.13	32.59	283.63	3.85	1300.53	32.47	9.10
Max	226.52	124.30	2809.34	5.42	2958.82	93.85	32.64
Min	15.24	9.14	31.66	2.71	1.58	12.16	0.64
Range	211.28	115.16	2777.68	2.71	2957.24	81.69	32.00
SD	58.78	30.59	540.91	1.40	1282.82	26.63	12.20
Skewness	1.06	1.88	4.38	1.21	0.0806	1.82	1.26
Kurtosis	0.49	2.84	20.81	0.00	-1.8264	2.28	-0.1824
Median	59.29	19.31	116.34	3.44	1678.26	25.29	3.1850
Q1	30.33	14.27	74.12	3.07	1.67	16.32	0.80
Q3	118.61	33.68	276.27	4.43	1862.35	28.42	11.31

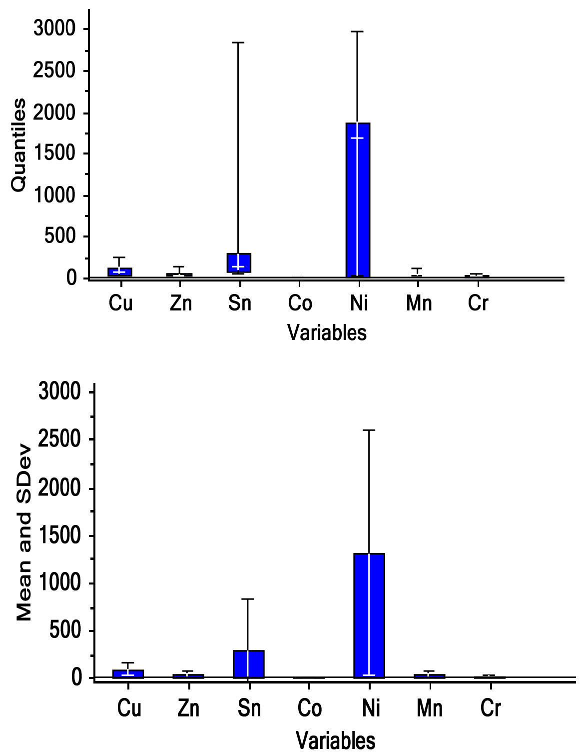

Figure 2 **Statistical results of heavy metals concentration in fungi (data are in mg/kg)**

The influence of metals in soil on the absorption and accumulation of heavy metals in fungi

In general, the heavy metals absorption fungi are influenced by species because in analyzed species a wide range of metal concentration was found. Also, a very important role is played by the metal concentration in the substratum they grew on. Near these two factors, the concentration of other heavy metals and macronutrients is important, because of the antagonistic/synergic effect that they have on the metal bioavailability (table 4).

Table 4
The effect of heavy metals in soil on the absorption of fungi (Pearson's coefficient)

		Heavy metals in fungi						
		Cu	Zn	Sn	Co	Ni	Mn	Cr
Heavy metals in substratum	Cu	0.598	0.645	-0.080	0.146	0.243	0.199	0.266
	Zn	-0.141	0.407	-0.160	0.000	0.044	0.294	0.220
	Sn	0.272	0.232	-0.160	0.767	0.749	-0.226	0.067
	Co	-0.345	-0.087	0.277	-0.401	-0.405	0.588	0.020
	Ni	0.054	-0.078	0.014	-0.075	-0.070	-0.277	-0.082
	Mn	-0.280	-0.301	-0.035	-0.160	-0.052	-0.329	-0.322
	Cr	-0.280	0.032	0.195	-0.268	-0.264	0.495	0.024

The most evident correlations that were observed between the heavy metals in soil and them presence in studied fungi were a medium positive one, between Cu in soil and Cu and Zn in fungi (synergism), where the value of Pearson's coefficient was higher than 0.50. Between Sn concentration in soil and Co, Ni concentration in fungi, were strong positive correlations, with Pearson's value > 0.75. A medium positive correlation was between Co content of the soil and Mn concentration in fungi (> 0.5), and also a weak-medium positive correlation was between the Cr content of the soil and Mn concentration in fungi (< 0.5). The other positive correlations were very weak, with a low level of Pearson's coefficient, which indicate a low rate of synergic effect of metals in soil on the metals uptake.

Negative weak-medium correlation were found between the Co content of the soil and fungal concentration of Cu (-0.345), Co (-0.401) and Ni (-0.405), and also between the Mn content of soil and the concentration of Zn (-0.301), Mn (-0.329) and Cr (-0.322) in studied fungi.

The negative values of Pearson's coefficient of correlation between the metallic content of soil and metals concentration in fungi show a possible antagonistic effect of some metals in soil on the fungal uptake. Very weak antagonistic effect were between Co – Cu, Mn – Cu, Cr – Cu, Mn – Zn, Cr – Co, Cr – Ni, Co – Ni, Sn – Mn, Ni – Mn and Mn – Cr.

In the principal component analysis of the correlation between the metallic content of soil and metals concentration in fungi (fig. 3) can be observed a strong positive correlation Sn – Ni and Sn – Co, correlation highlighted also by the Pearson's coefficient. Another positive correlation were observed in the relation Cu – Co and Cu – Ni, but, because the short distance between the intersection of two axes and the point of Cu concentration scores, we can say that is a weak correlation.

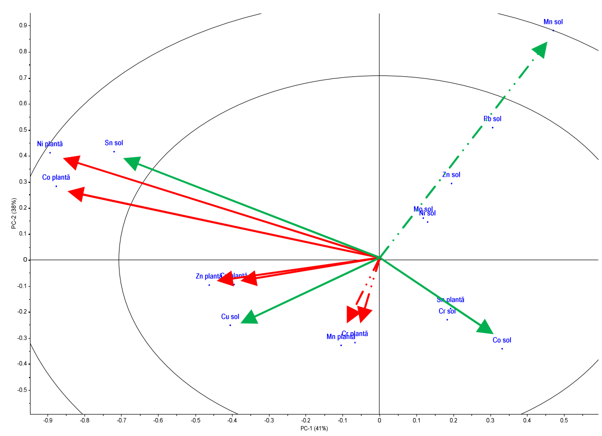

Figure 3 **The effect of heavy metals from soil on the metal absorption of fungi**

Between the Mn content of the soil and Mn/Cr concentration in fungi were a weak negative correlation showed by the position, respective to the intersection of axes, of two points representing the intersection of metal concentration scores. Also a negative correlation can be observed between Co content in the soil and Co/Ni concentrations in fungi.

Regarding the influence of soil pH and macronutrients in soil on heavy metals uptake, were observed very low values of Pearson's coefficient (tab. 5). The most significant influence on the metals uptake was from Fe content in the soil on Co and Ni absorption, from Ca content of the soil on the Mn and Cr absorption and from K content of the soil on the Zn absorption. All these correlation are very weak, with low negative values of Pearson's coefficient < 0.5, which indicate a weak antagonistic effect of macronutrients in soil on the heavy metals uptake.

Table 5

The effect of pH and macronutrients in soil on the heavy metals bioaccumulation factor for fungi (Pearson's coefficient)

		Cu	Zn	Sn	Co	Ni	Mn	Cr
	pH	0.001	0.065	0.019	-0.112	-0.107	0.257	0.022
Heavy metals in substratum	Fe	0.004	0.008	0.034	-0.216	-0.230	0.041	-0.008
	Ca	-0.010	-0.027	-0.015	-0.008	-0.007	-0.324	-0.170
	Mg	0.013	0.012	0.008	-0.067	-0.071	0.007	-0.054
	P	-0.001	-0.005	-0.023	-0.006	-0.006	-0.015	-0.007
	K	-0.001	-0.135	-0.007	0.001	0.006	0.012	0.000
		Bioaccumulation factor						

The pH influence on the heavy metals uptake is very weak for Cu, Zn, Sn and Cr and weak for Co, Ni and Mn. For Co and Ni, the values of Pearson's coefficient are negative (-0.112, -0.107 respectively), and for Mn the correlation was positive (0.257).

CONCLUSIONS

The heavy metals concentration in soil is influenced by the metallurgical activities, increasing its values, and because of that, the heavy metals concentrations in fungi increase also, exceeding the normal values in this biosystems, comparing with values from previously study on wild growing mushrooms.

The heavy metals content of the soil has a moderate influence on the heavy metals uptake of studied fungi, most of the correlation showing synergic effects, and some antagonistic effects.

The influence of macronutrients and pH of the soil on the heavy metals accumulation is lower comparing with the influence of heavy metals from the soil. The correlations are in most of the cases negative which means that a macronutrient applying method will induce the decreasing of heavy metals accumulation in studied fungi.

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