The Mycoremediation of Metals Polluted Soils Using Wild Growing Species of Mushrooms

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Abstract: - Many of mushrooms species from the forest area of Bucegi Mountains are consumed by the native population without thinking about the heavy metals uptake in the human body. From all the edible species we choose eight mushrooms, part of them considered edible and part with uncertain edibility. Heavy metals concentration in the fruiting body of mushrooms are different from one species to another and shows mean values of 11.94 mg/kg for Ti, 1.07 mg/kg for Sr, 1163.86 mg/kg for Bi and 17.49 mg/kg for Mn. The bioconversion factor of heavy metals represent the level of metals concentration in the mushrooms body correlated with the metallic element in the soil on which the fungus growth, and has the highest values in *Marasmius oreades* species for bismuth and titanium.

Key-Words: - heavy metals, macrofungi, soil, ICP-AES method, bioconversion factor

1 Introduction

The mushrooms can be used to evaluate the level of environmental pollution [14] and to remediate the metal polluted soil. Also, many studies carried out to evaluate the possible danger to human health from the ingestion of mushrooms containing heavy metals [8, 13, 4]. Numerous data on metals contents in fungal fruiting bodies were published previously [1, 5, 7, 9, 11, 15, 16], and the reported metal concentrations vary over a wide range within the mushrooms species, because of many factors affecting the accumulation rate.

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 [18]. Also, high level of metals concentration was observed in the vicinity of metals polluted area and metals smelter [10, 17, 6].

Density and depth of the mycelium living in the soil for several months or even years influence the metals contents in the fruiting bodies. Also, the soil properties, such as pH, redox potential, organic matter content, clay mineralogy, cation exchange capacity of the soil phase, competition with other metal ions and composition of the soil solution concentrations influence metals absorption of the mushrooms [2]. Because macrofungi 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 [12].

2 Problem Formulation

2.1 Species and ecology

Some species of mushrooms were harvested from a wooded area, near Sinaia city, from Bucegi Massif of Carpathian Mountains. All these species of macro fungi were founded 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 deadwood and leaves from the ground. The analyzed species are edible (*Marasmius oreades, Hypholoma capnoides Cortinarius largus, Cortinarius armillatus* and *Boletus griseus*), edible with low nutritive value (*Collybia butyracea,* and *Hygrphorus virgineus*) or with conditioned edibility (*Calvatia excipuliformis* – can be used only when is very young).

The harvested mushrooms were mature, with developed spore forming part, and we collecte the whole fruiting bodies, cap and stipe. The species of mushrooms were identified using some guides book [3] and the Internet [19].

2.2 Analytical methods

For each mushroom we sample 6-9 exemplars from different points of the same area, and the substratum near the mycelium, down to the depth of 5 cm. Both the samples of mushrooms and soil, and them processing were done with plastic, glass and pottery instruments to avoid any metal contact which can influence the final results. After harvesting, the mushrooms were clean up by the soil particles, dried at 60 ºC and then grinding to fine powder. The myceliumsurrounding soil samples were dried at 40 ºC until the complete process, then grinding to a fine powder and sieved at 250 µm (conform SR ISO 11464).

The estimation of metallic content in the analyzed mushroom and soil was done by the Inductively Coupled Plasma - Atomic Emission Spectrometry method (ICP-AES). 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 established 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.

3 Problem Solution

In the first stage of our experiments we analyzed the metallic content of the soil and its pH. Due to the position of these soils in a mountain wooded area the soil reaction was acid to weak acid, with a pH values quite low, varying in a wide range from 4.79 to 6.83. Also the metal concentrations in the soil were founded in a wide range for the analyzed metals. Titanium had values of 15.13 – 111.84 mg/kg of dry soil, with a range of 96.71 mg/kg between minimum and maximum concentrations; and a mean of 60.70 ± 31.15 (standard deviation). Strontium was founded in concentrations between 31.85 and 186.13 mg/kg of dry soil, with a range of 154.28 mg/kg. The mean of this metal content in soil is 135.17 ± 51.63 (SD). The bismuth concentrations in soil had values from 930.48 to 1 891.43 mg/kg, with a range of 960.95 mg/kg and a mean value of 1374.62 ± 411.75 (SD). The manganese was in the highest amount in the analyzed soil compared with the other three metals, with values of concentrations between 1375.35 and 4305.42 mg/kg. The range of manganese content of the soil is about 2930.07 mg/kg, and the mean is 2374.18 ± 1056.66 (SD).

As we can see from table 1, the wide range of metals concentrations in the fruiting body of mushrooms is respected, as in the soil. The results from this table show a value of titanium concentration in mushrooms from 5.76 to 19.94 mg/kg of dry weight; the lowest value shows *Calvatia excipuliformis* species and the highest value was in *Hypholoma capnoides*, an edible mushroom species. The values of concentration for strontium in the fruiting body of analyzed mushrooms are between 0.15 mg/ kg in *Boletus griseus* and *Collybia mutyracea* and 3.70 mg/kg of dry weight in *Hypholoma capnoides*. The bismuth concentrations are in a wide range of values from 235.86 mg/kg in *Calvatia excipuliformis* to 8894.29 mg/kg in *Marasmius oreades* species. Manganese shows values of concentration in the fruiting body between under detection limit of device and 87.34 mg/kg (*Hypholoma capnoides*) with a mean value of 17.49 mg/kg of dry weight.

Table 1 The metallic content of the fruiting body in wild mushroom species from the Carpathian Mountain, Romania (milligram of metal per kilogram of dry weight of mushroom)

Species	Ti	Sr	Bi	Mn
Calvatia excipuliformis	5.76	0.28	235.86	25.39
Marasmius oreades	6.89	0.36	8894.29	12.16
Boletus griseus	8.68	0.15	298.13	0.00
Collybia butyracea	15.29	0.15	445.73	0.00
Hygrophorus virgineus	15.65	0.89	571.09	15.04
Hypholoma capnoides	19.94	3.70	990.72	87.34
Cortinarius largus	8.10	2.76	1251.66	0.00
Cortinarius armillatus	15.26	0.30	623.44	0.00
Mean ± SD	**11.94 ± 5.19**	**1.07 ± 1.37**	**1663.86 ± 2941.50**	**17.49 ± 29.75**
Range	**14.18**	**3.55**	**8658.43**	**87.34**
Minimum	**5.76**	**0.15**	**235.86**	**0**
Maximum	**19.94**	**3.70**	**8894.29**	**87.34**

The bioconversion factor of the analyzed mushrooms was calculated as a ratio between the metal concentration in the fruiting body and the content of metal in the under-stipe soil. For a biosystem to be efficient in the remediation of polluted soil, the bioconversion factor must have values higher than 1, as this indicator is higher, as the species is more efficient in the technology of "cleaning up". For titanium the bioconversion factor of analyzed mushrooms is lower than 0.5 (table 2), ranging from 0.0795 to 0.4554, the lowest value showing *Calvatia excipuliformis* and the highest value *Marasmius oreades*. For strontium and manganese, the bioconversion factor of these species of mushrooms shows very low values, in the most of the species lower than 0.01, values which are not significant for a remediation method. The bioconversion factor of bismuth has values ranging from 0.1576 in *Boletus griseus* to 4.9547 for *Marasmius oreades*, species which has an efficient result for the remediation process. Also an significant value of the bioconversion factor for bismuth has *Cortinarius largus*, which shows a ratio between the metal concentration in fruiting body and metallic content of the soil higher than 1 (1.2208).

In table 3 are represented the result of a *t* test on the matrix correlation between the metal concentrations in the soil and the bioconversion factor for the studied metals. For Sr, Bi and Mn the Pearson's coefficient shows low degree of correlation, the bioconversion factor of these metals in the fruiting body of mushrooms is independent on the metallic content of the soil. The highest degree of correlation is between the Ti concentration in the soil and its bioconversion factor in the fruiting body, and between the Sr concentration in the soil and its bioconversion factor. The studied correlations have significance at level of 0.1% and lower ($p < 0.0001$).

Table 2 The bioconversion factor for Ti, Sr, Bi and Mn of some species from Carpathian Mountain, Romania

Species	Ti	Sr	Bi	Mn
Calvatia excipuliformis	0.0795	0.0015	0.2312	0.0180
Marasmius oreades	0.4554	0.0027	4.9547	0.0049
Boletus griseus	0.3357	0.0013	0.1576	0.0000
Collybia butyracea	0.3555	0.0009	0.3690	0.0000
Hygrophorus virgineus	0.2253	0.0088	0.6138	0.0064
Hypholoma capnoides	0.1786	0.1162	0.7939	0.0635
Cortinarius largus	0.1128	0.0149	1.2208	0.0000
Cortinarius armillatus	0.2006	0.0019	0.3319	0.0000
Mean ± SD	**0.2428 ± 0.1289**	**0.0185 ± 0.0397**	**1.0840 ± 1.6021**	**0.0116 ± 0.0218**
Range	**0.3758**	**0.1152**	**4.7970**	**0.0635**
Minimum	**0.0795**	**0.0009**	**0.1576**	**0.0000**
Maximum	**0.4558**	**0.1161**	**4.9546**	**0.0635**

Table 3 The Pearson's coefficient of the correlation between the metal concentration in the soil and the bioconversion factor of mushrooms species

Bioconversion factor	**Metal concentration in soil**			
	Ti	**Sr**	**Bi**	**Mn**
Ti	-0.7955*	-0.1623**	0.5828**	0.3849*
Sr	0.6973*	-0.7950**	-0.1897**	-0.4311*
Bi	-0.4878*	-0.0405**	0.3008**	-0.0867*
Mn	0.6904*	-0.7430**	-0.2413**	-0.4979*

*** $p \leq 0.001$, ** $p \leq 0.0001$**

4 Conclusion

- *Hypholoma capnoides*, an edible species of mushrooms, has the highest concentrations of Ti, Sr and Mn, comparing with the other analyzed mushrooms species from Carpathian area, and the highest concentration of Bi was founded in *Marasmius oreades* species.
- *Marasmius oreades* species shows the most important values of the bioconversion factor of Bi and Ti in the fruiting body.
- Between the Ti, Sr, Bi, and Mn concentrations in the soil and theirs bioconversion factor in the fruiting

body of mushrooms is a medium and low degree of correlation, with statistically significance difference ($p < 0.001$, and $p < 0.0001$).

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References:

[1] Alonso J.,Garcia A.M., Pérez-López M., Melgar M.J., The concentrations and bioconcentration factors of copper and zinc in edible mushrooms, Arch. Environ. Contam. Toxicol. 44 (2003) 180-188;

[2]Angeles Garcia M., Alonso J., Melgar J.M., Lead in edible mushrooms. Levels and bioaccumulation factors, J. Hazard. Mater. (2009)

[3] Bielli E., translate by Gadei R., Funghi, All Educational Press, Bucharest, 1999, ISBN 973-684-137-5.

[4] Borovička J., Řanda Z., Distribution of iron, cobalt, zinc and selenium in macrofungi, Mycol.Prog. 6 (2007) 249-259;

[5] Cocchi L., Vescovi L., Petrini L.E., Petrini O., Heavy metals in edible mushrooms in Italy, Food Chem., 98 (2006) 277-284

[6] Collin- Hansen C., Andersen R. A., Steinnes E., Isolation and N-terminal sequencing of a novel cadmium- binding protein from Boletus edulis. J Phys. IV France 107, 2003, 311- 314.

[7] Garcia A.M., Alonso J., Fernández M.I., Melgar M.J., Lead content in edible wild mushrooms in northwest Spain as indicator of environmental contamination. Arch. Environ. Contam. Toxicol. 34 (1998) 330-335;

[8] Gast C.H., Jansen E., Bierling J., Haanstra L., Heavy metals in mushrooms and their relationship with soil characteristics, Chemosphere 17 (1998) 789-799;

[9] Isildak Ö., Turkekul I., Elmastas M., Tuzen M., Analysis of heavy metals in some wild-grown edible mushrooms from the Middle Black Sea region, Turkey, Food Chem. 86 (2004) 547-552;

[10] Kalač P., Niznanska M., Bevilaqua D., Staskova I., Concentrations of mercury, copper, cadmium and lead in fruiting bodies of edible mushrooms in the vicinity of a mercury smelter and a copper smelter. The Science of the Total Environment, 177, 1996, 251-258.

[11] Kalač P., Svoboda L., A review of trace element concentrations in edible mushrooms, Food Chem. 69 (2000) 273-281;

[12] Malinowska E., Szefer P., Falandysz J., Metals bioaccumulation by bay bolete, *Xerocomus badius*, from selected sites in Poland, Food Chem. 84 (2004) 405-416;

[13] Ouzouni P.K., Veltsistas P.G., Paleologos E.K. Riganakos K.A., Determination of metal content in wild edible mushrooms species from region of Greece, J. Food Compos. Anal. 20 (2007) 480-486;

[14] Sesli E., Tuzen M., Levels of trace elements in the fruiting bodies of macrofungi growing in the East Black Sea region of Turkey, Food Chem. 65 (1999) 453-460;

[15] Soylak M., Saraçoğlu S., Tüzen M., Mendil D., Determination of trace metals in mushroom sample from Kayseri, Turkey, Food Chem. 92 (2005) 649-652;

[16] Svoboda L., Havličková B., Kalač P., Contents of cadmium, mercury and lead in edible mushrooms growing in a historical silver-mining area, Food Chem., 96 (2006) 580-585;

[17] Svoboda L., Zimmermanniva K., Kalač P., Concentrations of mercury, cadmium, lead and copper in fruiting bodies of edible mushrooms in an emission area of a copper smelter and a mercury smelter, The Science of the Total Environment, 246, 2000, 61-67.

[18] Thomet U., Vogel E., Krähenbühl U., The uptake of cadmium and zinc by mycelia and their accumulation in mycelia and fruiting bodies of edible mushrooms. Eur. Food Res. Technol. 209, 1999, 317 – 324.

[19] The Free Encyclopedia Wikipedia, http://en.wikipedia.org