

EVALUATION OF *ARMILLARIA MELEA* SPECIES FEATURES CONCERNING SOME HEAVY METALS UP TAKE IN NATURAL CONDITIONS OF DIFFERENT FORESTRY ECOSYSTEMS

Gabriela BUSUIOC¹, Claudia STIHI¹, Carmen ELEKES¹, Andreea GEORGESCU¹

E-mail: g1busuioc@yahoo.com

Abstract

It was necessary to identify the metals (heavy, noble and rare metals) bioabsorption characteristics to the edible mushroom species *Armillaria melea*, in the view to relieve its quality as indicator. The samples were collected from some forestry ecosystems: Adanca, Gorgota, Manesti, Bucegi-Paduchiosu and Dambovita River vally from Dambovita County.

All over the world today are developing large studies concerning biochemical and physiological features of mushrooms in the view to promote them as biological tools in different types of environmental biotechnologies grace of their bioabsorption capacity for heavy, rare or noble metals.

This paper is about the elemental content of *Armillaria melea* in cap and stipe, bioabsorption factor, correlated to the mineral content of substrate and its natural pH value. The elemental content of biological and environmental samples was determined by spectrometry of fluorescence (EDXRF) using ELVA_X apparatus. Biological samples and their substrate samples have been dried at 60°C some hours first. After drying the solid samples have been grinded until to fine powder and weighed.

For the evaluation of EDXRF results was used a certified reference sample NIST SRM 1571- Orchard leaves. So, it was determinate the presence of some heavy metals as copper, manganese, lead.

Key words: *Armillaria melea*, bioabsorption factor, translocation factor, pH.

All over the world today are developing more and more the studies concerning biochemical and physiological features (2) of mushrooms (macromycetes) in the view to promote them as biological tools in different types of biotechnologies grace of their hyper accumulation capacity for biominerals, heavy, rare or noble metals. Piotr Krupa and Zofia Piotrowska-Seget in 2004 (9) put in evidence that mycorrhizal fungi existing in highly polluted soils developed a high tolerance against heavy metal (6) and they may play a big role in the bioremediation of polluted soils. The role of mycorrhizal fungi in reduction of metals toxicity is well known today (7- Jentschke G., Godbold D. in 2000). Also was studied the process of selected translocation of heavy metals by symbiotic fungi by Krupa P. In 1997 (8). So, it is important to identify mushrooms species which have a high bioabsorption factor in the view to use them in bioremediation process of cleaning up polluted soil with heavy metals (3, 10, 11, 12, 14). So, this paper is about bioabsorption factor for Co, Zn, and Pb at *Armillariella mellea*.

MATERIAL AND METHOD

Biological samples consisted in *Armillariella mellea* species samples and their substrate fresh harvested from some forestry ecosystems of Dambovita County: Adanca, Gorgota, Manesti, Bucegi - Paduchiosu and Dambovita River vally. Mushrooms species where identifying using some book guides very known in Europe published after 2000 (4, 5, 14). The samples were weighted first and then dried at 60°C for some hours. After drying operation the samples were weighted again. The method for analysing was chosen carefully and not at random. The analyses were made by EDXRF because this method is not destructive and on can use the same samples to be analyzed by others methods (1, 5, 13). The elemental content of biological and environmental samples was determined using Elva-X spectrometer having a X-ray tube with Rh anode. 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 mm Be window and a energy resolution of 200eV at 5.9 KeV 91,20 (2,19). Elva-X software was used to

¹ Universitatea VALAHIA din Targoviste

interpret the EDXRF spectra. The accuracy and precision of results were evaluated by measuring a certified reference sample (NIST SRM 1571-Orchard biological samples). By this method were obtained the concentration of studied metals. By these methods were registered all the quantities which were in a concentration higher than 1ppm. Every result represents the average of minimum 5 and maximum 10 determinations.

Bioabsorption factor was calculated after the following equation:

$$Fb\% = \frac{C_m}{C_s} \times 100$$

where: $Fb\%$ = bioabsorption factor

C_m = metal concentration in mushroom;

C_s = metal content of substrate.

$$Ft = \frac{C_c}{C_s}$$

where: Ft = translocation factor;

C_c = metal concentration in cap;

C_s = metal concentration in stipe/soil.

RESULTS AND DISCUSSIONS

On can see from figure 1 that the pH of soil had a very important effect on the up take of copper all over the area studied, that is releaved by the value of regression factor (0,868). pH value of soil was 6,03, easy acid in Gorgota forest and the lowest was 4,29, powerfull acide in Adanca. The greatest content of copper was finde in samples of *Armillariella mellea* harvested from Gorgota forest (35,11ppm in cap and 21,5ppm in stipe). The lowest concentrations of copper were determinated at exemplars growing on Adanca forest very closed as values from cap to stipe (13,88ppm repectively 13,4ppm). At *Armillariella mellea* fruitbodies from Manesti forest copper content in cap was 16,89ppm and in stipe was 14,47ppm. On can observe a importantd difference between the mushrooms growing in Gorgota and Adanca.

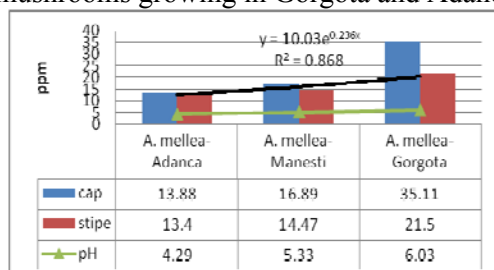


Figure 1 Correlation between pH value of soil and copper content at *A. mellea*

The value of bioabsorption factor both cap and stipe was highest at mushrooms growing in Adanca forest (figure 2). The lowest one was calculated for the exemplars growing in the others

two forest, Manesti and Gorgota, both cap and stipe with little differences from one to others. But translocation factor was also the highest one in case of mushrooms harvested from Adanca (2,29). The others were under 0,1. Also on can say that it is a significant difference between fruiting bodies of *Armillariella mellea* from Adanca forestry comparatively to the others.

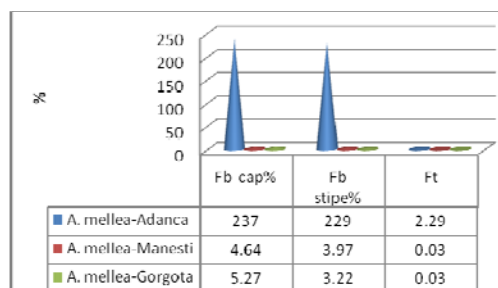


Figure 2 Bioabsorption and translocation factors for copper at *A. mellea*

Regression indicator (0,866) shows a great impact of pH of soil on translocation factor in all studied cases (figure 3).

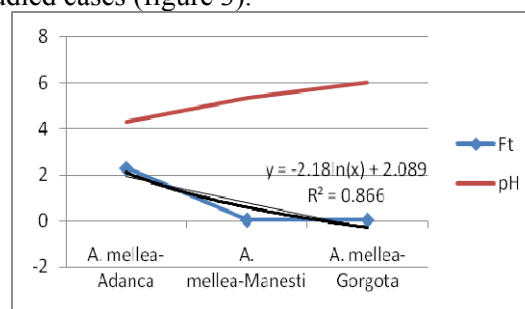


Figure 3 Correlation between pH of soil and translocation factor for Co at *A. mellea*

Concerning manganese content of *Armillariella mellea* on can see an important accumulation in caps and stipes in all cases (figure 4). The maximum concentrations was obtained in caps of mushrooms growing in Adanca forest (292ppm) and the lowest one in exemplars from Gorgota (158ppm). Speaking about stipe content of manganese, maximum of 410ppm was in mushrooms stipes from Gorgota and minimum of 385ppm in those from Adanca. In case of manganese storage pH values were stricktly involve in, this thing beeing releaved by regression factor very closed to 1 (meaning 0,977).

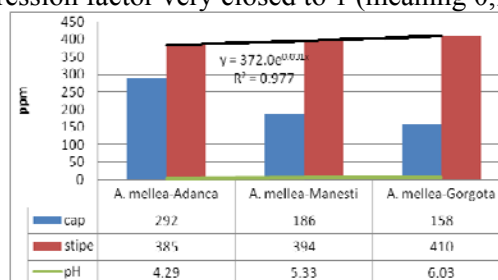


Figure 4 Correlation between pH value of soil and manganese content at *A. mellea*

Bioabsorption factor calculated for manganese was in all the others cases between 36 and 133% (figure 5). By exception, in case of mushrooms harvested from Adanca where the values were very higher, 1460% in cap and 1925% in stipe. A impressive value of translocation factor was 19 in case of mushrooms harvested from Adanca. The lowest one (0,77) was obtained at the exemplars from Manesti forest. A good translocation of 1,33 presented the mushrooms growing in Gorgota forest. So, absorption of manganese content was very different from a forest to other in all studied cases. Both pH of soil and manganese content influenced the accumulation of this metal species in high quantities inside fruiting body of these mushrooms.

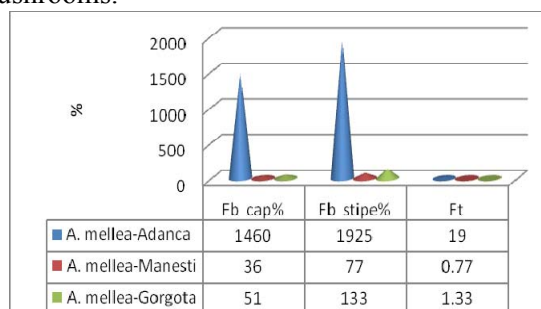


Figure 5 Bioabsorption and translocation factors for manganese at *A. mellea*

Speaking about the influence of soil pH on translocation factor for manganese, on can see that it is powerfull in all studied cases (figure 6). This is sow by the values of regression factor which is over 0,800. Blue line inside graphics reprezent translocation factor for manganese.

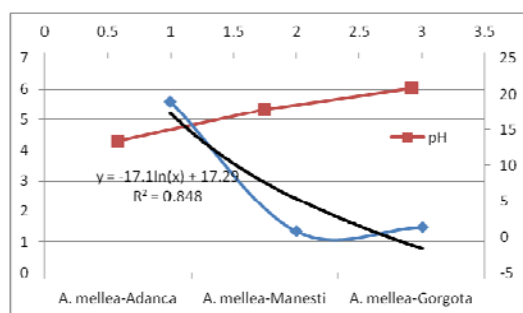


Figure 6 Correlation between pH of soil and translocation factor for Mn at *A. mellea*

The concentration of lead in soil was highest in Gorgota forest (2450ppm) and the minimum was determinated in mountains forest (Paduchiosu-440ppm) (figure 7). Maximum of lead content was determinated in fruiting body of mushrooms growing in mountains forest (640ppm) followed by those from Dambovita valey (630ppm) and minimum in *A. mellea* harvested from Gorgota forest (460ppm). Regression factor of 0,791 indicates a great influence of pH of soil on lead accumulation in mushrooms.

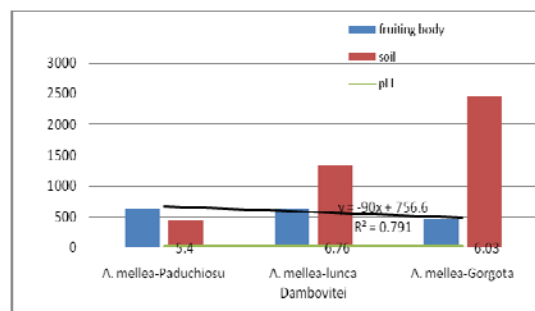


Figure 7 Correlations between pH of soil and Pb content of *A. mellea*

Bioabsorption factor for lead was under 50% at mushrooms harvested from Gorgota forest and Dambovita valey and over 100% in samples prelevated from mountains forest (figure 8).

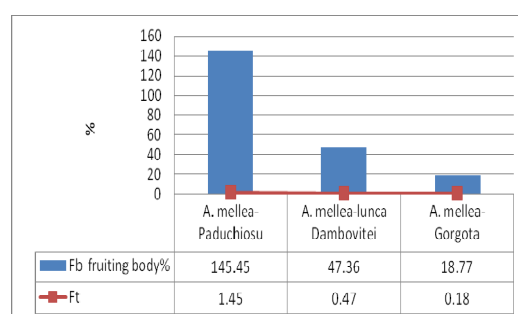


Figure 8 Bioabsorption and translocation factors for lead at *A. mellea*

The values of regression factor less than 0,5 shows that pH had no influence on translocation of lead into fruiting body of *A. mellea*, situation available in all studied cases (figure 9).

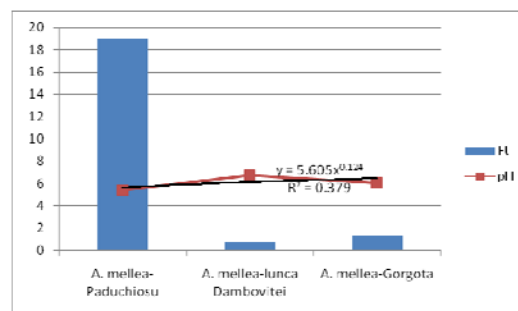


Figure 9 Correlation between pH of soil and translocation factor for Mn at *A. mellea*

It is at least strange this uncorrelation of pH with translocation factor, because its influence on accumulation of lead is certain if we think at figure 7 which put in evidence a clear influence of pH of soil on accumulation of lead in fruiting body also in this case.

CONCLUSIONS

Copper accumulation in *A. mellea* was moderate, a little higher in cap than in stipe.

Manganese was storage in higher quantities, but much more in stipe than in cap.

Lead was determined in higher and very higher concentration in fruiting body.

Bioabsorption factor for copper was over 100% only in mushrooms growing in Adanca forest.

Bioabsorption factor was over 100% at mushrooms from Gorgota and over 1000% at the exemplars harvested from Adanca forest.

Translocation factor for copper was over 1 in case of mushrooms from Adanca forest.

Translocation factor was over 1 at the mushrooms from Gorgota and even 19 at those from Adanca forest.

pH had a clear influence on accumulation of copper, manganese and lead, but with differences.

In case of lead accumulation inspite of pH influence on accumulation in fruiting body, concerning translocation factor it was find any correlations.

ACKNOWLEDGEMENTS

The financial support of these researches is UEFISCSU-CNCSIS by the project PN-II-ID-PCE-2008-2 ID-624 contract no 978/2009 entitled "The study of ubiquitarian macromycetes capacity for absorption heavy and rare metals in the view to be used them as bioindicators and bioaccumulators in environmental biotechnologies".

BIBLIOGRAPHY

- Arai, T., 2004** - *Analytical precision and accuracy in X ray fluorescence analysis*. Rigaku J., 21:26-38.
- Barros, L., Enturini, A.V., Baptista, P., Stevinho, L., Erreira, I., 2008** - *Chemical Composition and Biological Properties of Portuguese Wild Mushrooms: A Comprehensive Study*, J. Agric. Food Chem., 56 (10): 3856-3862.

- Demirba, Ayhan, 2002** - *Metal ion uptake by mushrooms from natural and artificially enriched soils*, Food Chemistry, Volume 78, Issue 1, Pages 89-93.
- Ettore, Bielli, 2009** - *Funghi*, ISBN: 8841859652, ISBN 13: 978884159650, Publisher: De Agostini.
- Gast, C.H., Jansen, E., Bierling, J., Haanstra, L., 1988** - *Heavy metals in mushrooms and their relationship with soil characteristics*, Chemosphere ISSN 0045-6535 CODEN CSMHAF, 17(4): 789-799 (27 ref.), Elsevier.
- Jentschke, G., Godbold, D., 2000** - *Metal toxicity and ectomycorrhizas*, Physiol.Plant, 109 (2): 107-116.
- Krupa, P., 1997** - *Inhibition of selected heavy metals translocation through mycorrhizal fungi and process dependence on the fungal symbiont*, Pol. J. Environ. Stud. 6: 35-38.
- Krupa, P., Zofia, Piotrowska-Seget, 2004** - *Identification of ectomycorrhizal fungi isolated from roots of birch growing on metallurgic heap*, Polish Journal of Ecology 52 /3 353-357.
- Ita B.N., Ebong, G.A., Essien, J.P., Eduok, S.I., 2008** - *Bioaccumulation Potential of Heavy Metals in Edible Fungal Sporocarps from the Niger Delta Region of Nigeria*, Pakistan Journal of Nutrition 7 (1): 93-97, ISSN 1680-5194.
- .Mustafa, Yamaça, Dilek, Yıldız, Cengiz, Sarıkürkcü, Mustafa, Çelikkollu, M. Halil, Solak, 2006** - *Heavy metals in some edible mushrooms from the Central Anatolia, Turkey*. 103(2): 263-267, ED. Elsevier.
- Sesli, Ertugrul, Tuzen, Mustafa, Soylak, Mustafa, 2008** - *Evaluation of trace metal contents of some wild edible mushrooms from Black sea region, Turkey Journal of Hazardous Materials, Volume 160, Issues 2-3, Pages 462-467.*
- Winefordner, J.D., 1999** - *Chemical analysis. X-ray Fluorescence Spectrometry*. JOHN Wiley and Sons, INC. USA.
- ***, **2001** - www.ecnc.nl/doc/projects/elisa.html, European Centre for Nature Conservation, ELISA: Environmental indicators for Sustainable Agriculture Final Report 2.<http://www.fungi.com/info/index.html>.
- ***, **2003** - **EURACHEM/CITAC Guide - Traceability in Chemical Measurement, A guide to achieving comparable results in chemical measurement**, Eds. S L R Ellison, B King, M Rösslein, M Salit, A Williams.