HEAVY METALS BIOACCUMULATION IN SPECIES OF WILD GROWING MUSHROOMS

Carmen Cristina ELEKES [1], Gabriela BUSUIOC [1]

[1] Faculty of Environmental Engineering and Biotechnologies, University Valahia of Târgovişte

Abstract

The study of some wild growing species of mushrooms from the Bucegi Mountain show important concentrations of heavy metals in the fruiting bodies. The zinc concentration range between 6.92 and 74.25 mg/kg (the highest concentration was for *Calvatia excipuliformis*); copper has concentration between 16.24 - 226.30 mg/kg (the highest values was also for species *Calvatia excipuliformis*); and tin concentration range between 16.23 - 14048.1 mg/kg (the highest values was for *Hygrophorus virgineus* species). The bioaccumulation factor of these metals in the fruiting body of analyzed species of mushrooms range according the metal concentrations in macrofungus and the metal content in soil. For the analyzed mushrooms, the bioaccumulation factor of zinc has values between 0.04 and 0.46, no results are important for bioremediation. The highest value was for *Calvatia excipuliformis* species. The copper bioaccumulation factor range between 0.83 and 3.19, the majority of analyzed species shows values of this factor higher than 1, and the most important results was for species *Collybia butyracea*. The bioaccumulation factor of tin has values between 0.06 and 49.61, only few species have this factor higher than 1. *Hygrophorus virgineus* species shows a very important value of tin bioaccumulation factor, up to 50, which make this species very efficient in bioremediation technologies.

Key words: Heavy metals, bioaccumulation, mushrooms

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 growing mushrooms (Diez, V.A. and Alvarez, A., 2001). Because of these, it is necessary to investigate the level of metal concentrations in the wild growing mushrooms. They are known to accumulate high levels of several heavy metals like copper, mercury, lead, zinc and cadmium (Kalač, P. and Svoboda, L.A., 2000).

Numerous data on metals concentrations in the fungal fruiting bodies were published (Garcia, A.M. et al., 1998; Alonso, J. et al., 2003; Isildak Ö. et al., 2004; Soylak, M. et al., 2005; Cocchi, L. et al. 2006; Svoboda, L. 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, they affecting the elements absorption and translocation (Malinowska, E. et al., 2004). Mushrooms represent responsible agents for the breaking down of the organic matter and play an important role in the continual changes of the nature. Heavy metals concentrations in mushrooms are, frequently higher than those in the agricultural crop plants, vegetable and fruits.

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 and the correlation that exist between the concentration of these elements in the analyzed macro fungus species and the soil parameters.

MATERIAL AND METHOD

Species and ecology

Five species of edible mushrooms were harvested 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 (*Marasmius oreades* 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 sporophore, and were collected the whole fruiting bodies, caps and stipes.

Analytical methods

For each mushroom, we sample 6-9 exemplars from different places to form 3 samples

for each species, 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 60ºC and then grinding to fine powder. The soil samples were dried at 40ºC 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 express in mg of metal related with kg of dry soil or plants. The minimal detection limits of device range according the analyzed element and is 0.4 mg/kg for Cu and Zn; 0.6 mg/kg for Sn.

RESULTS AND DISCUSSIONS

The reported metal concentrations vary over a wide range within the mushrooms species, because of many factors affecting the accumulation rate. Density and depth of the mycelium living in the soil for several months or even years influence the metals contents in the fruiting bodies. In addition, 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 M. et al., 2009).

Some of the soil characteristics from Bucegi Massif on the sites where the fruiting bodies were harvest are present in *table 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, A. and Pendias, H., 1993).

Table 1

Humidity (%), pH and heavy metals contents (mg/kg) in the soil from the Bucegi Massif, near Sinaia, Romania

Soil parameters	Mean	SD	Range	Minimum	Maximum
Humidity	47.11	9.38	26.48	31.48	57.96
pH	6.69	0.14	0.44	6.48	6.90
Zn	130.80	26.59	67.71	94.63	162.34
Cu	46.02	38.24	100.56	9.68	110.24
Sn	495.14	457.47	1234.69	58.68	1293.37

In the fruiting body of mushrooms, the metals are accumulated in different quantities comparing the cap and the stipe, according the species and the metal concentrations in the substratum that the mushroom grew on. As we can see from *table 2*, the level of copper concentration in the stipe is higher than in the cap of fruiting body, showing a low mobility of copper into the mushroom. The copper concentration in the cap of some species of analyzed species of mushrooms was under the detection limit of method (*Boletus griseus* and *Hygrophorus virgineus*). The highest copper concentrations were founded in *Calvatia excipuliformis* species, as in cap, as in stipe of fruiting body (207.73 mg/kg, 244.86 mg/kg respectively).

The zinc shows a higher mobility in the analyzed species of mushrooms than the copper, because of the highest concentration of this element in the cap of fruiting body for the analyzed wild growing species of mushrooms. The levels of zinc concentration in these species vary between 10.98 mg/kg in *Collybia butyracea* species and 92.19 mg/kg, the highest value showing the superior part of *Calvatia excipuliformis*.

The concentrations of tin in the cap are 2-5 times the concentration in stipe of the fruiting

body. For the majority of species, the concentration in the stipe shows values under the detection limit of method. The most significant values of tin concentration has *Hygrophorus virgineus* species, 18535.80 mg/kg of dry weight in the cap and 9560.30 mg/kg dry weight in the stipe of mushroom, values 100 –fold higher than for the other analyzed species.

Comparing the concentration in the fruiting body and the concentration in the substratum that the mushrooms grew on, we obtain the bioaccumulation factor, for each studied heavy metal. The bioaccumulation factor represents the pollutant concentration in mushrooms comparing with the environment concentration (in soil) (Scragg, A., 2005). For a plant or mushroom to be efficient tool in the polluted soil bioremediation, the bioaccumulation factor have to be higher than 1 (Scragg, A., 2005).

The bioaccumulation factor of the analyzed species is different according the metal that is concentrate in the fruiting body of mushrooms (table 2). For copper, the bioaccumulation factor has higher than 1 values for the majority of analyzed species of mushrooms (exception is *Hygrophorus virgineus* species), taking values between 1.55 and 3.19 – the highest value is for *Collybia butyracea* species. The bioaccumulation factor of zinc and tin in the fruiting body of analyzed species has sub unitary values, because the level of zinc and tin the fruiting body of these species is lower than the concentrations in substratum. An exception is *Hygrophorus virgineus* species which has a bioaccumulation factor of tin about 49.6, value that indicate the hyper capacity of this species to absorb and accumulate Sn in its fruiting body.

Table 2

Heavy metal concentrations in the fruiting body of some wild growing mushrooms (mg/kg of DW), the concentration of metals in substratum (mg/kg) and the bioaccumulation factor for the analyzed samples

Metal concentration		Boletus griseus	Collybia butyracea	Calvatia excipuliformis	Marasmius oreades	Hygrophorus virgineus
Cu	Cap	0	27.33 ± 1.88	207.73 ± 3.53	106.41 ± 1.88	0
	Stipe	32.48 ± 0.94	84.52 ± 0.97	244.86 ± 4.26	157.57 ± 1.30	45.40 ± 0.51
	Substratum	10.44 ± 0.84	17.49 ± 0.69	109.34 ± 0.89	65.59 ± 0.40	27.23 ± 0.57
	BF	*1.5596 ± 0.0800*	*3.1977 ± 0.0511*	*2.069 ± 0.0186*	*2.0121 ± 0.0118*	*0.8335 ± 0.0082*
Zn	Cap	13.84 ± 0.35	11.62 ± 0.34	92.19 ± 0.21	46.27 ± 0.25	25.91 ± 0.39
	Stipe	0	10.98 ± 0.23	56.31 ± 0.27	25.34 ± 0.35	15.40 ± 0.30
	Substratum	157.29 ± 2.70	131.52 ± 1.17	160.41 ± 1.83	95.39 ± 0.88	109.38 ± 0.97
	BF	*0.0440 ± 0.0003*	*0.0859 ± 0.0013*	*0.4629 ± 0.0038*	*0.3753 ± 0.0007*	*0.1888 ± 0.0019*
Sn	Cap	48.67 ± 0.50	109.58 ± 1.81	32.47 ± 1.15	147.27 ± 1.66	18535.80 ± 73.83
	Stipe	0	0	0	27.77 ± 0.39	9560.30 ± 32.59
	Substratum	188.35 ± 1.50	660.25 ± 4.74	60.59 ± 1.94	1283.40 ± 13.17	283.14 ± 2.57
	BF	*0.1291 ± 0.0003*	*0.0829 ± 0.0007*	*0.2679 ± 0.0016*	*0.0681 ± 0.0002*	*49.6168 ± 0.2781*

Near the species, the soil characteristics influence in different way and range the accumulation of heavy metals in the fruiting body of mushrooms. The concentration of copper in mushrooms positively is influenced by the three studied parameters: heavy metal concentrations in substratum, pH and soil moisture. The first two parameters have a strong influence ($R^2 = 0.953$, $R^2 = 0.815$ respectively) (*figure 1*), which mean that the level of concentration in mushrooms has increase with the increase of copper content in soil and with the increase of pH. In addition, an increase of the soil moisture has positively influence the increase of copper mobility and facilitates the absorption and accumulation of this heavy metal.

The influence of soil characteristics on zinc accumulation in the fruiting body of mushrooms is not significant, like for copper, and negative ($R^2 = 0.86$) (figure 2). For an increase of zinc content in the soil with 20-25 mg/kg, the zinc concentration in the fruiting body decreased with 10-15 mg/kg. The pH and soil moisture positively influenced the accumulation of zinc in the fruiting body, but the correlation is not significant ($R^2 < 0.7$).

For tin, the analyze of soil characteristics influences on the metal accumulation was done separately, for the all studied species, apart of *Hygrophorus virgineus*, because the tin concentration for this species has too high level comparing with the rest of analyzed species.

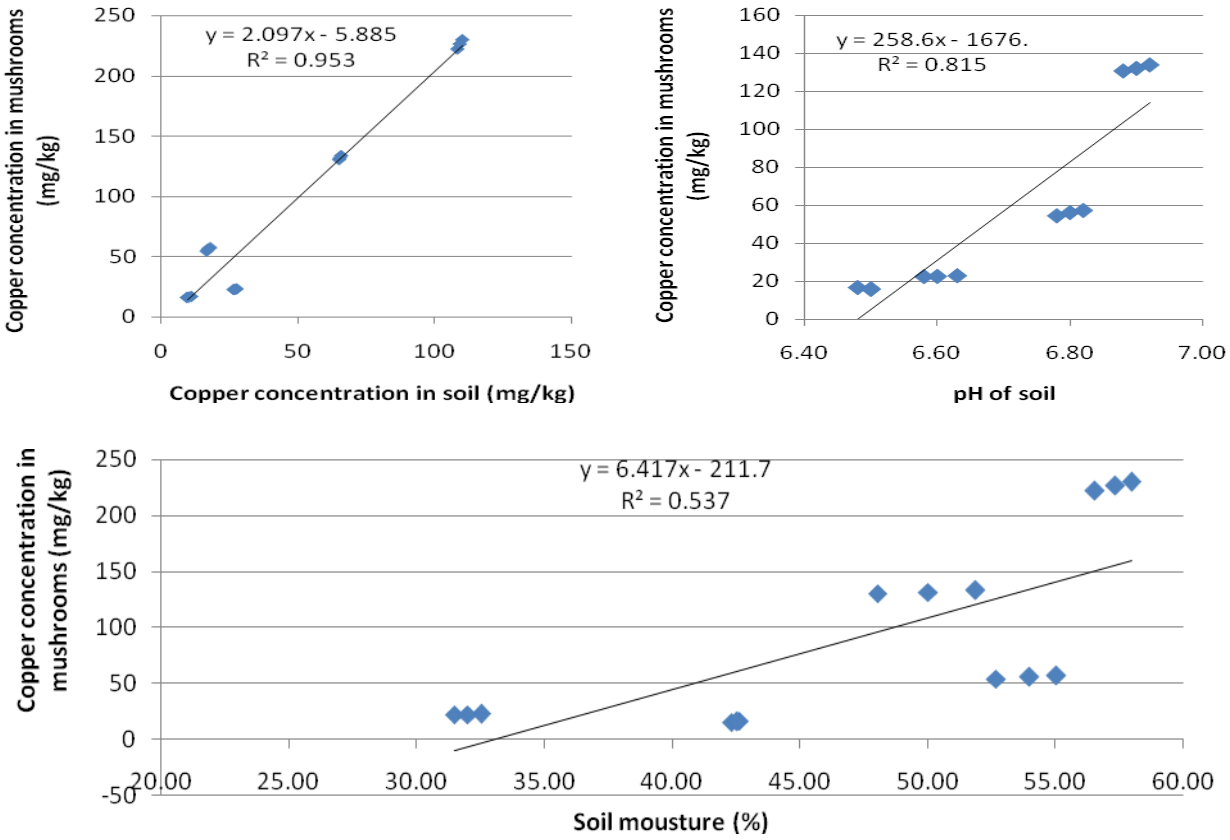

Figure 1 **The influence of soil parameters on the copper concentration in the fruiting body of wild growing mushrooms**

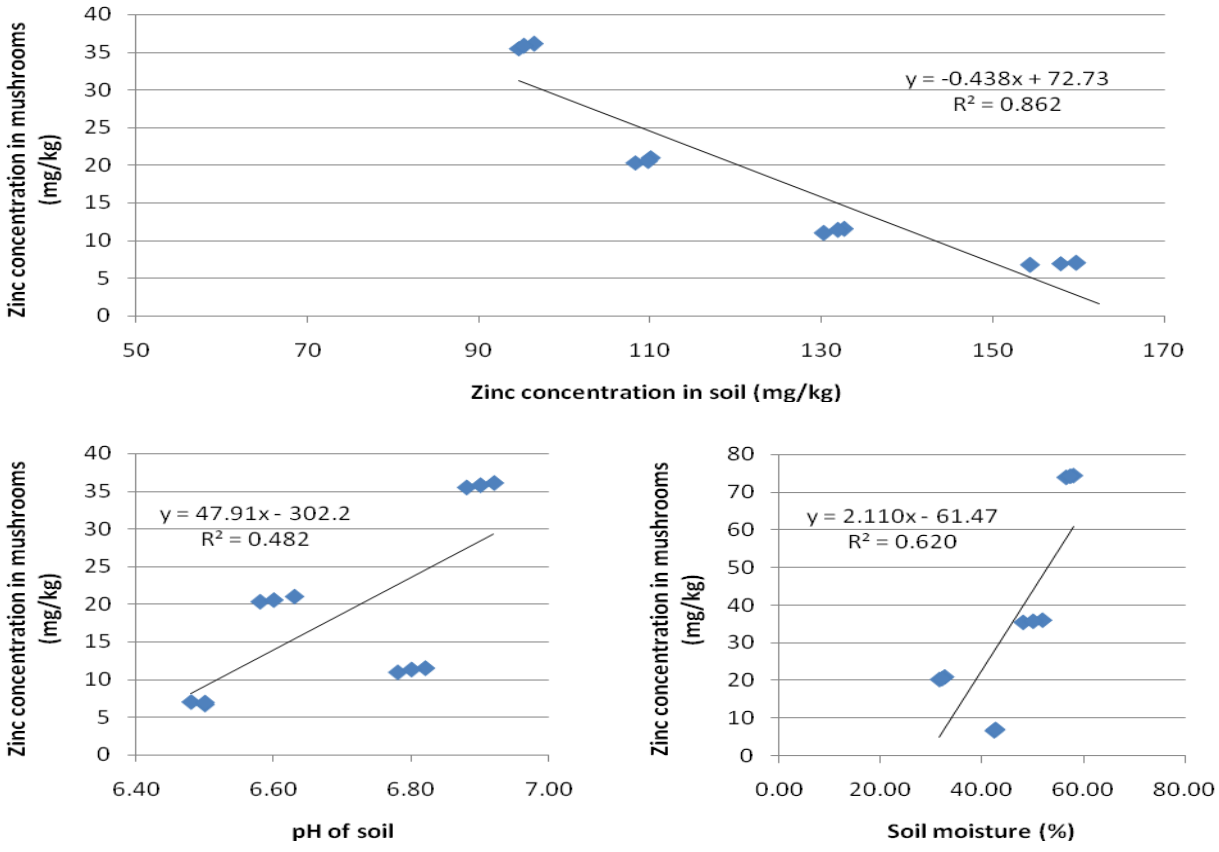

Figure 2 **The influence of soil parameters on the zinc concentration in the fruiting body of wild growing mushrooms**

Therefore, for *Hygrophorus virgineus* species, the regression values show an intraspecies comparison of the soil parameters influences on the tin concentration in mushroom. As we can see in figure 3, the metal concentration in soil had a positive and significant influence on the tin concentration in the fruiting body of analyzed species of mushrooms. The same correlation is also for *H. virgineus* species. The soil moisture and pH had a moderate significant influence on the tin concentration in the fruiting body (R^2 = 0.855, R^2 = 0.647 respectively). The tin concentration in mushroom increased with the increase of soil concentration and pH and when the moisture of soil has decreased.

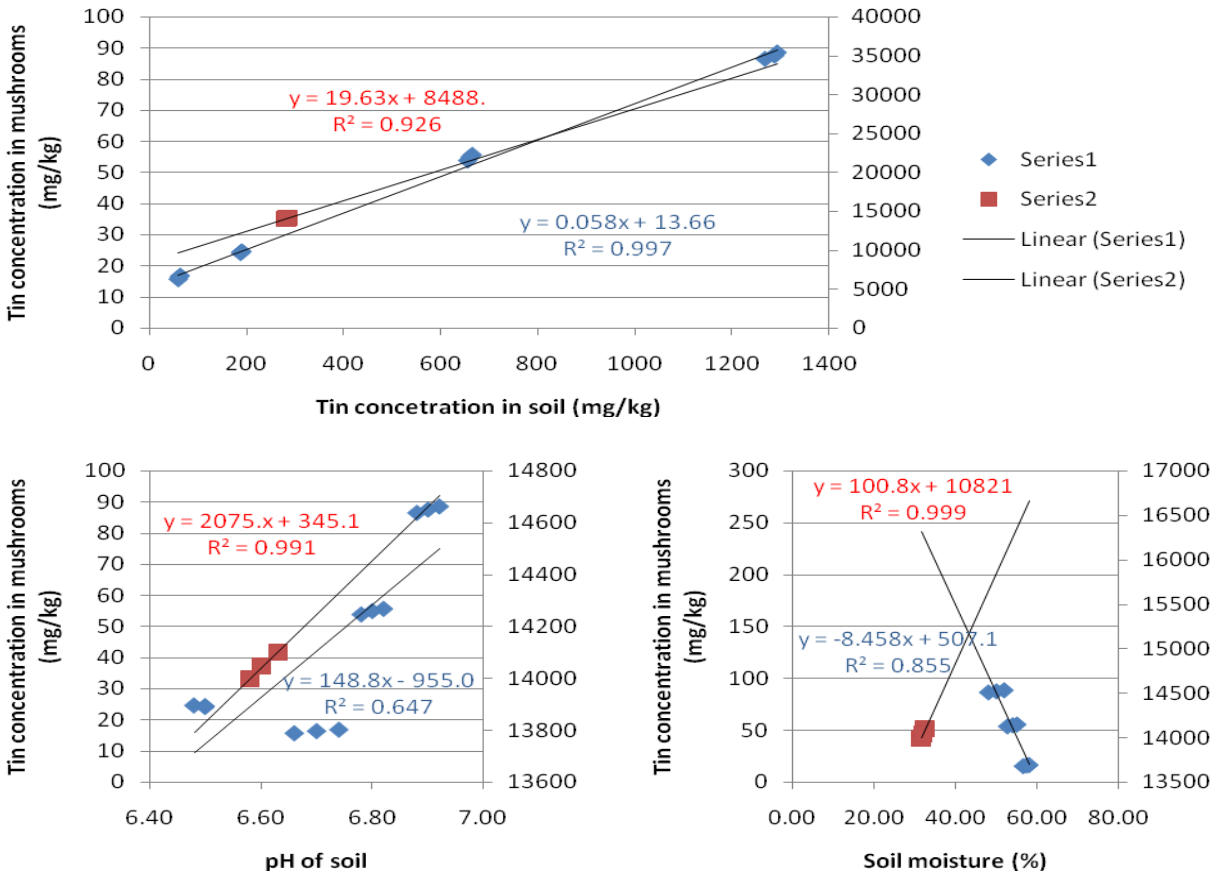

Figure 3 **The influence of soil parameters on the tin concentration in the fruiting body of wild growing mushrooms** (Series 1: *Boletus griseus, Collybia butyracea, Calvatia excipuliformis* and *Marasmius oreades;* Series 2: *Hygrophorus virgineus* on secondary axis)

The values of translocation factor of heavy metals in the fruiting body of analyzed mushrooms vary according with the species. For copper the values of translocation factor were zero for *Boletus griseus* and *Hygrophorus* species, or sub unitary, which show a lower concentration of copper in the superior part of the fruiting body. The translocation factor for Zn and Sn, had higher than 1 values, which means that the level of concentration for these metals was higher in the superior part of the fruiting body (in cap), comparing with the stipe of mushroom.

Table 3

The translocation factor of heavy metals in the fruiting body of edible mushrooms

Translocation factor	Boletus griseus	Collybia butyracea	Calvatia excipuliformis	Marasmius oreades	Hygrophorus virgineus
Cu	0	0.3231 ± 0.0186	0.8483 ± 0.0034	0.6753 ± 0.0064	0
Zn	*	1.0584 ± 0.0119	1.6373 ± 0.0044	1.8260 ± 0.0157	1.6822 ± 0.0077
Sn	*	*	*	5.3029 ± 0.0213	1.9388 ± 0.0028

** - The metalic concentration in stipe is under the detection limit of method*

CONCLUSIONS

The level of copper, zinc and tin concentration in the fruiting body of wild growing edible mushrooms is influenced by the species and the individual capacity of absorption for heavy metals and by the soil characteristic, such as metal content of the soil, pH and soil moisture.

The translocation factor is influenced by the nature of studied metal and its mobility in the fruiting body of mushroom, and is different from one species to another, varying in a wide range.

Acknowledgements

This work was supported by CNCSIS – UEFISCSU, project number PNII – IDEI 624/2008, coordinated by Ass. Prof. Gabriela Busuioc.

BIBLIOGRAPHY

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

Angeles, Garcia, M., Alonso, J., Melgar, J.M., 2009 - *Lead in edible mushrooms. Levels and bioaccumulation factors*, J. Hazard. Mater.

Cocchi, L., Vescovi, L., Petrini, L.E., Petrini, O., 2006 - *Heavy metals in edible mushrooms in Italy*, Food Chem., 98, 277-284.

Diez, V.A. and Alvarez, A., 2001 - *Compositional and nutritional studies on two wild edible mushrooms from northwest Spain*, Food Chem., 75, 417-422.

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

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

Kabata-Pendias, A., Pendias, H., 1993 - *Biogeochemistry of trace elements*, Warsaw: PWN.

Kalač, P., Svoboda L.A., 2000 - *Review of trace element concentrations in edible mushrooms*. Food Chem., 62, 273-281.

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

Scragg, A., 2005 - *Environmental Biotechnology*, Oxford University Press, New York.

Soylak, M., Saraçoğlu, S., Tüzen, M., Mendil, D., 2005 - *Determination of trace metals in mushroom sample from Kayseri,* Turkey*,* Food Chem. 92, 649-652.

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