

DETERMINATION OF HEAVY METALS IN SEVERAL SPECIES OF WILD MUSHROOMS AND THEIR INFLUENCE ON PEROXIDASE ACTIVITY

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Abstract

The heavy metals (Zn, Fe) content of some wild mushrooms (*Amanita caesarea*, *Rusulla virescens*, *Rusulla cyanoxantha*, *Rusulla vesca*, *Rusulla alutacea*, *Rusulla foetens*, *Lepiota procera*, *Lepiota excoriata*, *Armillaria mellea*, *Cantharellus cibarius*, *Boletus edulis*, *Pleurotus ostreatus*, *Hydnum repandum*, *Lactarius piperatus*, *Lactarius vellereus*) of four sites from Dambovită county Romania, were analyzed. Elements concentrations were determined by Energy Dispersive X-ray Fluorescence spectrometry (EDXRF). In fruiting body of these mushrooms, the highest mean concentration of macroelements was found for Zn and Fe. *Hydnum repandum* species accumulated zinc at a level three times higher than average concentration of species of fungi studied, 248 mg/kg and *Cantharellus cibarius* species concentrate the metal values of two times higher than average concentration. *Rusulla cyanoxantha* species concentration values of iron is four times higher, respectively *Cantharellus cibarius*, *Lactarius piperatus* species have values three times higher than average concentration of this metal. We can say that these three species of this metal are good bioaccumulation, developing normally, even at high concentrations of iron.

Peroxidase activity of fungi was determined in order to find correlations between the size of oxidoreductase activity and concentration of heavy metals. Enzymatic activity was enhanced by higher concentrations of metals accumulated in most species studied macromycetes.

Key words: wild mushroom, EDXRF, heavy metal, peroxidase

Recent years have put great emphasis on the use of biotechnology for remediation of soils. Biotechnologies that are based biosystems that are live microbial cells, plants or animals. An organically polluted soil remediation and recovery of heavy metals is based on the use of fungi. They are accumulated mineral elements in general and in particular, are several species of fungi that accumulate heavy metals (Kalač P. et al., 1991; Kalač P. et al., 2005).

Fungi are natural indicators of pollution. Mushrooms easily accumulate heavy metals, pesticides, radioactive substances. A chemical analysis of fungi can show the state of pollution of a habitat. Thus, research on the remediation environment deals with the development of biotechnology based on reducing the heavy metal content in soil with mushroom crop. Composition and content of heavy metals in enzymes of fungi different by species, ontogenetic stage of development. They are correlated with different parts (cap, stipe) of the fruiting body, microclimate conditions, and nutritional substrate on which it grows.

Biological material was subjected to study the species of fungi of the families: *Russulaceae*, *Lepiotaceae*, *Tricholomataceae*, *Pleurotaceae*,

Amanitaceae, *Cantharellaceae*, *Boletaceae*, *Hydnaceae*, all belonging to the order *Basidiomycota*. Harvested mushrooms were edible and inedible species but also some semicomestibile, such as *Lactarius piperatus*, *Lactarius vellereus*, *Hydnum repandum* and *Russula foetens*. Fungal species analyzed were collected from Adanca, Ungureni, Mănești, Picior de Munte of Dambovită county, species that have been indicated by the literature as frequently consumed in Romania's rural population. Samples will be collected so as to contain all the fruiting body: cap, stipe, blades, cuticle. The amount of sample taken in the analysis will be an average body elements of fructification.

MATERIAL AND METHOD

Materials

Was collected a total of 14 species of mushrooms from each species looking at several individuals from the same location, but also from different locations (table 1). Samples were collected in order to contain all the fruiting body: cap, stipe, blades, cuticle. The amount of sample taken in the analysis was an average of fructification body elements.

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Table 1

Species of mushrooms used for analysis

Family	Genus	Mushrooms species
<i>Familia Russulaceae</i>	<i>Genul Russula</i>	<i>Russula virescens</i> (C) <i>Russula cyanoxantha</i> (C) <i>Russula vesca</i> (C) <i>Russula alutacea</i> (C) <i>Russula foetens</i> (N)
<i>Familia Lepiotaceae</i>	<i>Genul Macrolepiota</i>	<i>Lepiota procera</i> (C) <i>Lepiota excoriata</i> (C)
<i>Familia Tricholomataceae</i>	<i>Genul Armillaria</i>	<i>Armillaria mellea</i> (C)
<i>Familia Pleurotaceae</i>	<i>Genul Pleurotus</i>	<i>Pleurotus ostreatus</i> (C)
<i>Familia Amanitaceae</i>	<i>Genul Amanita</i>	<i>Amanita caesarea</i> (C)
<i>Familia Cantharellaceae</i>	<i>Genul Cantharellus</i>	<i>Cantharellus cibarius</i> (C)
<i>Familia Boletaceae</i>	<i>Genul Boletus</i>	<i>Boletus edulis</i> (C)
<i>Familia Hydnnaceae</i>	<i>Genul Hydnum</i>	<i>Hydnum repandum</i> (SC)
<i>Familia Russulaceae</i>	<i>Genul Lactarius</i>	<i>Lactarius piperatus</i> (SC) <i>Lactarius vellereus</i> (N)

Heavy metal content of samples was determined by using energy dispersion X-ray - EDXRF - Energy Dispersive X-ray Fluorescence. Macromycetes freshly harvested specimens were made within one hour at laboratory research "Biological Evaluation of Environmental and Food Safety" of the Faculty of Environmental Engineering and Biotechnology where to performed the cleaning and sorting. Each specimen was carefully cleaned of vegetal wastes and soil using deionized water and plastic tools. Then all specimens were approximately the same size (1-2 mm difference). For each particular analysis samples were formed average number of 5-20 samples, depending on the density of the species growing on an area of 1 m². They weighed 100 g sample of each species and were introduced to the oven at 60 ° C (Binder drying system) for 24-48 hours until the total elimination of water from tissues. After drying, the samples were mortars until a fine powder that was stored in plastic bottles with a stopper. Powders thus obtained were weighed and then analyzed by EDXRF spectrometry.

To determine the peroxidase activity using fresh biological material and peroxidase activity was determined by reading absorbance at the wavelength of 420 nm, using SPEKOL spectrometer.

Reagents

Reagents used were deionized water with a resistivity better than 17.5 MΩcm, distilled water, alcoholic solution of guaiacol (1%), hydrogen peroxide (0,5 M), sodium chloride (2%).

Methods**Energy Dispersive X-ray Fluorescence**

Were weighed 1-2g powder of each species harvested, depending on grain. For a correct reading, the powder must be distributed evenly across the surface capsule, as do a reading spectrometer sample surface.

The elemental content of samples was determined by Energy Dispersive X-Ray Fluorescence (EDXRF) technique, using the ElvaX spectrometer

having a X-ray tube with Rh anode, operated at 50 kV and 100μA. Samples were excited for 300 s and the characteristic X-rays were detected by a multichannel spectrometer based on a solid state Sipin-diode X-ray detector with a 140 μm Be window and a energy resolution of 200eV at 5.9 KeV. ElvaX software was used to interpret the EDXRF spectra. The accuracy and precision of the results were evaluated by measuring a certified reference sample (NIST SRM 1571-Orchard leaves).

Determination of peroxidase activity

The method chosen is a colorimetric method and is based on property peroxidase to oxidize in the presence of hydrogen peroxide or other peroxide compounds such as aromatic. Quinones which are formed by polymerization of compounds give brown (melanin). Take 5g sample, 25mL mix fine with sodium chloride (2%). Pass the mortar contents quantitatively to a 100mL volumetric flask and dilute to the mark with distilled water. Mix and leave 15 minutes for extraction. 10mL of the filtrate are taken in a 100mL Erlenmeyer glass, add 1 mL hydrogen peroxide(0,5 M), 3 mL guaiacol solution (1%), mix and leave the sample so obtained, covered, at room temperature for 30 minutes. Will see a brown, more or less intense, depending on how active enzyme.

Absorbance of the solution was determined with the spectrometer Spekol, at the wavelength of 420 nm. Reference sample to the absorbance reading is made is distilled water.

RESULTS AND DISCUSSIONS

Minerals enter into the composition of chemical compounds with structural role, but have great physiological importance, as activators or inhibitors of the enzymatic systems or components of enzymes, coenzymes, pigments, etc.. Mineral content is characteristic for a species or a plant organ and varies quite large, depending on climatic factors, soil, crop technology.

Determined by on selected species of fungi shows that they are rich in Fe, Zn, metals that have a role in activation of enzymes and enzyme systems. Metals are important for living organisms, but, if they exceed a certain level after bioaccumulation, are toxic (Ita B.N., et al., 2006; Kalač P., et al., 1991; 2005).

Zinc has an important role in the metabolism of fungi, is an active component of enzymes, including dehydrogenase, dipeptidaze, fosfohidrolaze, peptidases. Thus, the basic functions of zinc are related to the metabolism of

carbohydrates, proteins and phosphates. This metal influences and stabilize cellular membrane permeability. Also, zinc stimulates resistance to diseases and pests, high temperatures and drought.

The mean concentration of Zn in fungi is 82.7 to 85 mg / kg. Zinc deficiency is seen in fungi generally they contain less than 20mg/kg, and toxic effects are observed at concentrations exceeding 300-400 mg / kg. Because zinc deficiency, remain small and underdeveloped fungi (Cocchi L. et al., 2006; Ita B.N. et al., 2006).

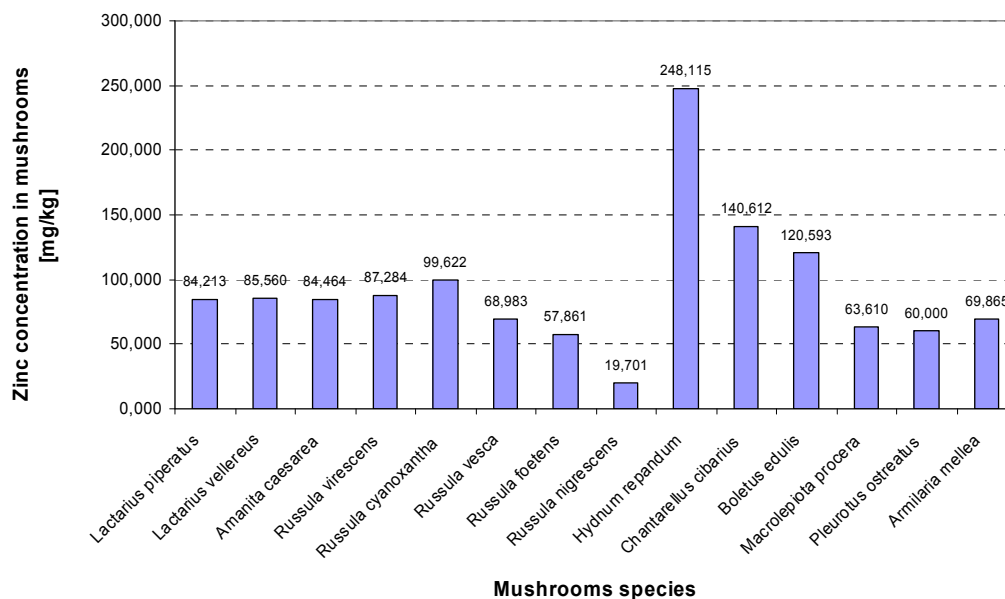


Figure 1 Zinc concentration in mushrooms

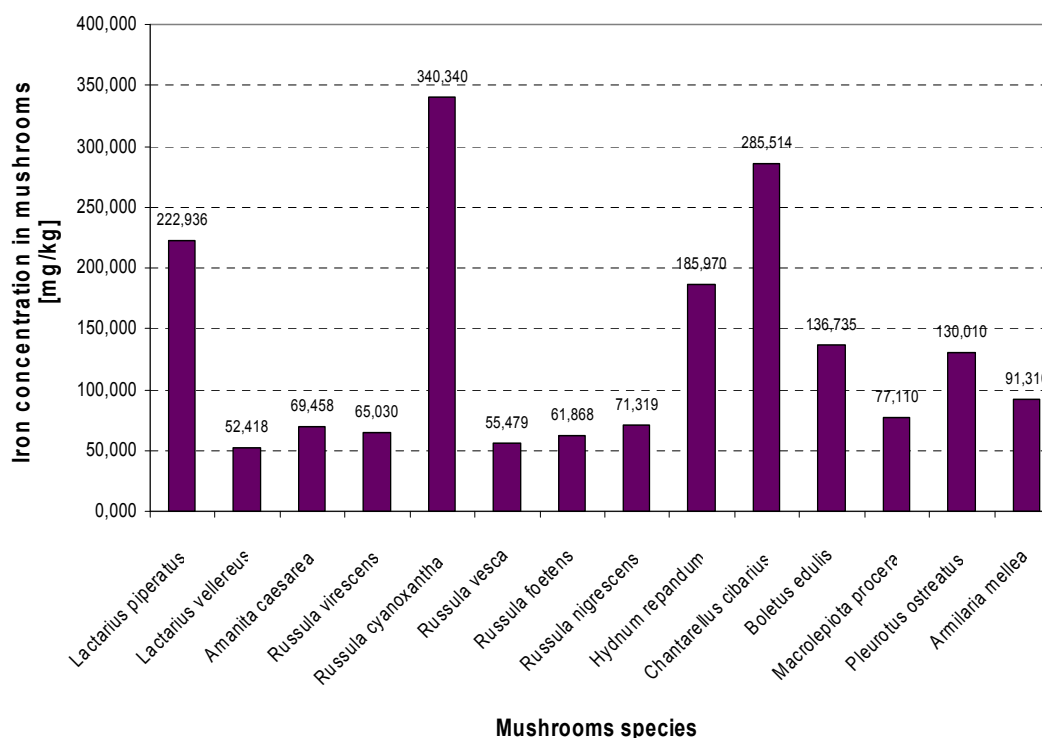


Figure 2 Iron concentration in mushrooms

It is noted that for most species, the concentration of zinc accumulated by mushrooms is close and is close to the average concentration (fig. 1). *Hydnum repandum* species accumulated zinc at a level three times higher than average concentration, 248 mg/kg and *Cantharellus cibarius* species is the metal concentration values twice higher than average concentration.

Iron redox processes involved in plant tissues, by passing from form to form ferric ferrous and vice versa. Enter into the composition of enzymes (catalase and peroxidase) and of hemoprotein.

From the literature studied, mushrooms contain 90-95 mg / kg Fe (Borovička J., 2007).

The concentration of iron species analyzed is almost four times higher in *Russula cyanoxantha* species and three times higher in species *Chantarellus cibarius*, *Lactarius piperatus* (fig. 2). These macromycetes have values close to maximum, respectively over a maximum, being

able to say that there are species of this metal accumulators, developing normally, even at high concentrations.

Values less than the average encounter *Lactarius vellereus* species, *Amanita caesarea* and the species of the genus *Russula*, 52-71 mg / kg Fe.

Russula cyanoxantha species peroxidase activity has increased 1.14 U / g. It is a species that has accumulated high concentrations of metals Fe and Zn. For other species of the genus *Russula* do not does respect these rules. It is noted that the species *Pleurotus ostreatus* has the lowest peroxidase activity (fig. 3).

Lactarius piperatus is a fungus that edible also large concentrations of Fe and Zn metals involves increased peroxidase activity.

From fig.3., *Hydnum repandum* is observed that the species has high peroxidase activity it with metals Fe and Zn concentration in very large, in fact the highest concentration for zinc of 248,115 mg / kg.

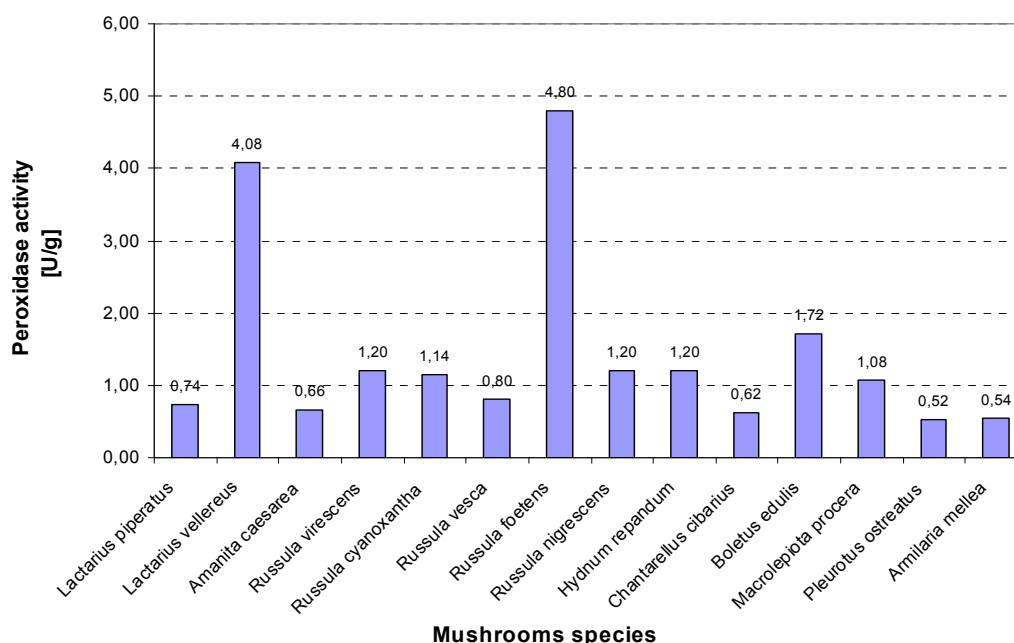


Figure 3 Results to determine the peroxidase activity the species studied macromycetes

CONCLUSIONS

An ecological approach to soil remediation and recovery of heavy metals was the use of fungi. Because remediation using fungi to have a satisfactory result, both in terms of clean soil, and from economically, have used species of fungi that are bioaccumulation of metals.

Metals can be accumulated in mushrooms and this accumulation is dependent, in general, the metabolism is strongly affected species and the

chemical composition of the substrate on which fungi obtain nutrients. Mushrooms are considered healthy, especially when young, with a high content of protein and carbohydrates. They are also rich in minerals and dietary fiber.

The results of this study have shown that some species of wild mushrooms are bioaccumulation of metals: *Hydnum repandum* species accumulated zinc at a level three times higher than average concentration of species of fungi studied, 248 mg / kg and *Cantharellus*

cibarius concentrate this metal species at levels two times higher than average concentration; - we studied 14 species of fungi, of which 11 species have values close to the average Fe concentrations (90-95 mg / kg), and *Russula cyanoxantha* species is four times higher values, respectively *Chantarellus cibarius*, *Lactarius piperatus* species have values three times the average concentration, one can say that these three species of this metal bioaccumulation, developing normally even high concentrations of iron; - also been observed that enzymatic activity was enhanced by higher concentrations of metals accumulated in most species studied macromycetes.

In conclusion, the species of fungi studied species bioaccumulation of metals are: *Hydnum repandum*, *Cantharellus cibarius*, which accumulate large amounts Zn and Fe, respectively *Russula cyanoxantha* and *Lactarius piperatus* are bioaccumulation of Fe. These species have high peroxidase activity.

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