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HEAVY METALS AND MACROELEMENTS IN SOME MACRO-FUNGI OF NATIONAL PARK FRUSHKA GORA (SERBIA)

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ABSTRACT

Considering the importance of Basidiomycotina fungi as bioindicators and the increasing tendency of air and soil contamination nowadays, content of macroelements: N, P, K, Ca, Mg and Na, and some of heavy metals (microelements): Fe, Pb, Cu, Cr, Zn in sporocarps of 22 species of macrofungi from Serbian National park Frushka Gora (Autonomous province of Voyvodina) was analyzed in this work. Majority of them are lignicolous species from the class: Homobasidiomycetes order: Aphyllophorales s. lato and two of them belong to subdivision Ascomycotina. Special attention was given to the medically important fungal species broadly distributed in this area: Ganoderma lucidum, Ganoderma applanatum, Coriolus versicolor, Flammulina velutipes, Meripilus giganteus and Omphalotus olearius. Species that accumulate microelements were found to be: Meripilus giganteus (exept for Zn), Schizophyllum commune (exept for Pb), and Ganoderma applanatum (exept for Fe and Zn). Superaccumulators of Fe were lignicolous, medically imortant species: M. giganteus, G. lucidum, Sch. commune and tericolous ones: C. atramentarius, F. velutipes and P. vernalis. Good Cu accumalotors were tericolous species: Psathyrella vernalis, Morchella vulgaris and Coprinus atramentarius, then the species possesing the rhizomorphs: Armillaria polymyces and Omphalotus olearius, and finally lignicolous species: Ganoderma applanatum and Pseudotrametes gibbosa. The highest accumulation of Zn was recorded for the species Schizophyllum commune, while the fungal species expressed the smalest tendecy of accumulation of Pb, exept one tericolous, saprophytic species C. atramentarius. The greatest accumulation of Cr was found in tericolous species, especially C. atramentarius.

KEY WORDS:
Basidiomycotina, sporocarps, bioindication, fungi, heavy metals, macroelements, Frushka Gora

1. INTRODUCTION

Fungi are ubiquitous in natural environments and important in industrial processes, which together with other microorganisms can accumulate metals and radionuclides from their environment. The principal factors influencing the accumulation of heavy metals in macrofungi are environmental factors (metal concentration in substrate, pH, organic matter, and contamination by atmospheric deposition), and *fungal factors* (fungal structure, biochemical composition, decomposition activity, development of mycelium and sporocarps, etc.), although

physiological mechanisms of metal ions uptake are still not determined. Fungi are very important in natural cycle of metal ions since they are very good in accumulation of metals and could be used as bioindicators species of heavy metals in soils [1,7,15,16]. Due to mycelium, which has the great surface of hyphae that could adsorb and accumulate metals, the majority of them (Cu, Zn, Cd) are captured in fungal biomass in the layer of humus. Fungi *Basidiomycotina* degrade the upper layer of humus e.g. polyphenolic compounds (lignin, humin acid, fulvic acid, humin) by enzymes (phenoloxidases) which are dedicated of effective binding of ions by means of ion exchange and forming of chelates. Hence, the high tolerance against heavy metals can be realized among the fungi that have ability of polyphenol degradation [8].

Measurements of macroelements and heavy metals in biological and soil samples are inevitable in order to investigate the effect of migration of chemical elements, especially the artificial radionuclides. High concentrations and bioaccumulation of different major and trace elements were reported in European forests [4,6,9,15,]. and also in Japanese [16,17]. Many of them demonstrated that mushrooms tended to accumulate Cu, Zn, Rb, Cd and Cs. Kalac *et al.* [9]. observed that Hg, Pb, and Cu were accumulated by tericolous *Lepista nuda* and *Lepiota rhacodes*. Higher concentrations of Pb, Cd, Zn and Hg are found in macrofungi (prevalent ectomycorhizal that are in tense contact with roots of wood) from urban or industrial areas [5,12,13], but also in wood ecosystems which are influenced by contaminants. In the present study the mineral concentrations of certain lignicolous and tericolous species were investigated. The aim of this study was to compare a fungi on the basis of their mineral concentrations and to determine whether these species have availability for metal ion bioaccumulation.

2. MATERIAL AND METHODS

2.1. Fungi and soil sampling

The sporocarps of investigated fungal species were collected from four sampling sites, locations: (*L1 - Elektrovoyvodina* (Irishki Venac), *L2 - Mali spomenik* (Irishki Venac), *L3 - Zmayevac, L4 - Paragovo*) in the National park Frushka Gora and from one urban location *L5 - Ribarsko Ostrvo*. Sampling sites on the Frushka Gora mountain are situated within area under the first degree of protection, far from the potential pollution sources (industrial facilities or heavy traffic). The sites were visited in the course of autumn 1999 and in spring 2000. Predominately sporocarps of species growing on stumps, fallen logs, tree-stump roots etc. were collected, identified and listed in Table 1. with data about date, location and substrate from which they were sampled. The samples were dry cleaned in order to remove attached soil and humus. After cleaning, samples were air dried, pulverized and then dried in a dry-kiln (105^{o}C) to constant mass. Since fungi take macro- and microelements from the

substrate, the tree and soil samples were also sampled and prepared using the same procedure. Soil samples were taken from a 25cm depth randomly, covering the surface of approximately 300m in diameter and from the centers within the location. From every sampling site twenty soil samples were taken. These samples were mixed in one composite sample for each location, air dried and ground to Ø1mm.

2.2. Mineralisation of mushrooms and substrates

Approximately 3g aliquots of homogenized dry biological material or substrates were weighed to 0.1mg accuracy, placed in a porcelain crucible and ashed, first on a stove to total carbonization and then in an oven at 425-440 ^{o}C for 2h. After cooling, 1ml H_2O_2 was added to each crucible and ashed in oven for 30 min. In each vessel 10ml 25% HCl was added and slowly heated on a stove to evaporate 1/2 to 1/3 of acid volume. The residues were transferred into a 50ml volumetric flasks making up the level with boiling deionized water [14] · All samples were performed in triplicate. Heavy metals content in soil samples was analyzed by use of atomic absorption spectrometry (AAS, Varian spectra 600 type). Soil mineralisation was done by method of Alloway [1] using HNO_3.

2.3. Analyses

Concentrations of K were analyzed by flame photometry and concentrations of P were determined spectrophotometrically. Trace elements (Pb, Cu, Cr, Zn) and macroelements (Fe, Ca, Mg) were measured by atomic emission spectrometry at the Lab of Physical Chemistry, Institute of Nuclear Sciences, Vincha, Belgrade. Direct-current U-shaped arc plasma was used as the excitation source. A PGS-2 plane grating spectrograph (Carl Zeiss, Jena) with an attachment for photoelectric detection having a single (laboratory-made) exit slit was used as the monochromator. The solution was aspirated by an argon stream through a concentric glass Meinhard type nebulizer and the aerosol obtained was introduced into the plasma column [11]. The original sample solutions were diluted 2.5 times for mushroom samples and 1.5 times for substrate samples. These solutions also included a fixed concentration of 0.5% KCl as buffer. The reference and blank solutions also included the same concentration of buffer.

3. RESULTS AND DISCUSSION

3.1. Contents of ash

The solubile fraction of ash was found to range between 0.70% and 22.77%, while the unsolubile fraction was between 0.32 and 42.14%. It could be asumed that recorded variability in the ash content was determined by the specific features of different fungal species, because the same species from different sites (*S. hirsutum*, *C. versicolor*, *L. sulphureus*) had very similar values of ash content. Generally, lignicolous species recover less amount of ash then tericolous species what direct to the conclusion that they have less concentrations of mineral elements. Species with rizomorphs (*A. polymyces)* had higher ash content then

strictly lignicolous ones, probably due to mycelial cords along which the accumulation influx of mineral elements is significantly, aproximately 10 times stronger from the environmental substrates.

3.2. Concentrations of macroelements

The fungi expressed the affinity for accumulation of K (0.05% in *Daedalea quercina* to 6.38% in *Psathyrella vernalis,* in average 1.83%d.m.), and P (0.02% in *D. quercina* to 0.53% in *M. giganteus,* in average 0.3%d.m.) followed by N (1.43% in *C versicolor* to 5.19% in *Morchella vulgaris,* in average 3.08%d.m.), with lower variability recorded than for Ca (156.44 mg/kg d.m. in *Pholiota squarrosa* to11946.07 mg/kg d.m. in *Coprinus atramentarius*, in average 2226.85 mg/kg d.m.), Mg (377.73 mg/kg d.m. in *D. quercina* to 4531.94 mg/kg d.m. in *C. atramentarius*, in average 1384.24 mg/kg d.m.), and Na (1.42 mg % in *Panellus stypticus* to 120.42 mg% in *Omphalotus olearius* , in average 15.09 mg%). This indicates the essential importance of these elements in fungal metabolic processess, independently from ecological fungal group and habitats, so the fungal sporocarps contained them in higher concentrations then the substrate they grow on.

3.3. Protein content

As a result of analyses of N content in studied fungal species, the protein content varied from 6.26 to 22.73%, according to Breene [3]. The highest protein content was recorded for edible species: *M. vulgaris* (22.73%), *A. polymyces* (22.56%), *P. ostreatus* (18.57%) and *O. olearius* (18.09%). High content of N in analyzed sporocarps could be the consequence of N translocation from mycelium to metabolicaly active hyphae of sporocarps, while the young part of vegetative body have intensive cell divisions and contain the higher N concentrations due to intensive synthesis of proteins. Generally, analyzed tericolous fungi had the highest N concentrations than lignicolous ones.

According to literature data [2,3,15], analyzed sporocarps contained smaller concentrations of N and P, simmilar values for content of K and Na and higher concentrations of Ca (10 times) and Mg., what could be explained by the two facts: lignicolous species were dominant in total number of analyzed species and as a substrate they they use wood characterized by increasing content of Ca with the age. The concentration of Ca in tericolous fungi was found to be was very high (10.51 % $CaCO_3$).

Species *M. giganteus* could be pointed as a good accumulator of almost all of macroelements, (exept Ca), while *P. ostreatus* is a good accumulator only for N and P. *P. squarrosa* and *P. vernalis* are distinguishing themselves as good accumulators of K, while the extreme accumulators of Ca turned to be species: *G. applanatum* and *P. stypticus*, and especially *S. hirsutum* (superaccumulator). The species *C. atramentarius* was found to be the greatest accumulator of Ca and Mg, and a species *P. vernalis* the greatest accumulator of K and Na. Abundant in Na content were found to be species: *A. polymyces* and *O. olearius.* Lignicolous species, accumulating majority of macroelements in small

ammounts, turned to have good uptake of Ca, such as: *D. quercina*, *C. versicolor*, *P. gibbosa* (indicator species). Only the species which are in contact with soil, along the rizomorphs (Lig/Ter) or by the mycelium could be considered as agood macroelement accumulators such as: *A. polymyces* and *O. olearius*.

3.4. Concentrations of microelements

Considering the concentration of microelements, content of Fe was dominating: *Fe* (from 102.67µg/g in *Ph. squarrosa* to 4276.61µg/g in *C. atramentarius*), following by content of *Zn* (from 6.57µg/g in *D. quercina* to160.96µg/g in *M.conica* var. *cristata*), *Cu* (from 1.40µg/g in *D. quercina* to 95.86µg/g in *P. vernalis*), *Cr* (0.96µg/g in *S. hirsutum* to13.36µg/g in *C. atramentarius*) and *Pb* (from 1.93µg/g in *S. hirsutum* to 9.7µg/g 2 in *C. atramentarius*).

Tericolous species from the sampling site 5 showed the highest content, both for the majority of microelements and macroelements concentration as well as for the total ash content. According to our results, the chemical composition in different fungal species (ecotypes) dominantely depends on awailability of these elements in supstrates. These are accumulator (ecotypes) of specific element (superaccumulator, accumulator, bioindicator), which, in the course of evolution create the defense mechanisms or stress adaptation by which they could exclude or amortise unfavorable conditions in the environment.

In comparison with previous studies [2,3,16,17], recorded results were high for Fe and Pb, but also for Cu, Cr and Zn in some fungal species. Species wich contained 1000µg/g Fe (what would be toxic for plants) could be considered as superakumulators**,** such as some lignicolous medically important species: *M. giganteus*, *G. lucidum*, and *Sch. commune* and tericolous ones*: C. atramentarius*, *F. velutipes* and *P. vernalis*. The highest accumulation that was recorded for the species *C. atramentarius* from the location 5, was not just the consecuence of the property of this species characterized as superaccumulator, but also the result of specific chemical composition of soil (although conc. of Fe was not high, pH<7, and lower concentration of Mn and Zn influenced the higher accumulation of Fe. However, the fact that saprophytic species could change physico-chemical composition of environment, revealed their influence on availability of other elements in substrate and their accumulation in metabolically active hyphae.

The content of Pb in tericolous species was twice higher in studied fungi but none of concentration was not critical nor toxic exept for *C. atramentarius* (Tf - 7.22). Uptake was species specific, what was predictable due to the fact that Pb belong to the group of elements, which in low concentrations are not dangerous but could be harmful wen present in higher concentrations.

Lignicolous fungi mainly were not accumulating microelements (exept *G. applanatum*, for Cu, *Sch. commune* for Zn and *A. polymyces*, *O.*

olearius for both of them). The recorded content of Cr for the species *C. atramentarius* was a toxic concentration. There are data about the synergistic efect of accumulation of metals on the cell surface what was confirmed by our results. The highest significant correlation was noticed for synergistic uptake of Fe and Cr (0.83) and for Fe together with Cu and Zn.

As a species accumulators of microelements the following species could be listed: *M. giganteus* (exept for Zn), *Sch. commune* (exept for Pb), and *G. applanatum* (exept for Fe and Zn). Fungi that accumulate all of microelements were the species which had the higher surface of mycelium due to ryzomorphs: *A. polymyces*, and *O. olerius*, and one strictly lignicolous fungi, *S. hirsutum*, that could be asigned as a superacumulator of microelements, especially Pb and Cr. The best tericolous accumulator species were: *C. atramentarius* (especcialy for Fe, Pb and Cr) and *P. vernalis* (especially for Pb and Cu). Since the concentration of Pb on the urban location was high, these species could be bioindicators of its presence in the substrate.

The content of all of microelements varied significantly among the species, but differs in the same species from the different sampling site, especially in regard of the Fe and Pb content (i.e. *C. versicolor*, *S. hirsutum*). We assume that different ecotypes of one species are distinguishing in ability of metal uptake from substrata.

3.5. Mineral contents of fungi against the contents of substrata

Concentrations of macro and microelements were lower in substrata than in fungi (especcialy for P, K and N). The content of Ca and Pb was significantlly higher. Lignicolous fungi were not accumulating Fe from wood exept the species of *G. lucidum* (Tf = 19, 3-5 time higher than in other species).

3.6. Mineral content of fungi agianst the content of soil

As a result of an Tf values in relation to soil analyzed fungi tended to accumulate K, P, N, Cu i Zn, and partialy Cr. As a very good accumulators of Cu in relation to tericolous species: *P. vernalis*, *M. vulgaris* and *C. atramentarius*, than the species with rysomorphs: *A. polymyces* and *O. olearius*, and finally the strictly lignicolous fungi: *G. applanatum* and *P. gibbosa*. While the concentration of Cu in soil on all of locations was simmilar we could assume that the Cu accumulation by fungi was detrmined by ecologigal features of fungi, first of all by the surface of mycelium. These species could found the application in removal of elements from polluted areas.

The highest accumulation of Zn was recorded for saprophytic lignicolous fungi *Sch. commune* (Tf = 10.48). In relation to Pb, fungi showed lower tendency of accumulation, so the higher concentration of this element could be expected only in a havilly (motorway) polluted

areas. The highest recorded Tf values wasfound in *S. hirsutum* (0.46) and *C. atramentarius* (0.40).

Cluster analysis classified fungi mostly by location (sampling sites). Some species from different locations, (*Stereum hirsutum*, *Coriolus versicolor)* showed different mineral contents and were classified on the separated branches of the dendrogram. These data indicate that the accumulation ability is not only genetically coded, but also influenced by environmental factors and therefore that the same species of fungi and also the fungi with the same means of life could be ecologically different.

TABLE 1. SPECIES, ECOLOGY, UTILITY, LOCALITY, DATE AND SUBSTRATE OF MYCELIUM OF INVESTIGATED FUNGI

SPECIES	Locality, date	eco. group	habitat of mycelium	utility
Meripilus giganteus(Pers.exFr.)Karst	L 1 $_1$07.10.99	Ter/Lig/sap	soil nearby Acer stump	*/L
Pseudotrametes gibbosa (Pers.exPers.)Bond.&Sing.	L 1 $_2$07.09.99	Lig/sap (p)	Fagus stump	( )
Coriolus versicolor(L.exFr.)Quel.	L 1 $_3$ 07.09.99	Lig/sap	dead Fagus trunk	( )/L
Ganoderma applanatum (Pers.exWallr.)Pat	L 1 $_3$07.09.99	Lig/sap	dead Acer trunk	( )/L
Stereum hirsutum (Wild.exFr.)S.F.Gray	L 1 $_3$07.09.99	Lig/sap (p)	dead Fagus brunch	( )
Pholiota squarrosa (Muller.exFr)Kummer	L 1 $_4$ 10.10.99	Lig/p (sap)	alive Fagus trunk	*
Ganoderma lucidum (Curt.exFr.)Karst	L 1 $_2$ 07.09 99	Lig/sap	dead Acer trunk	( )/L
Daedaleopsis confragosavar. tricolor (Bull.)Bond.	L 1 $_3$07.09.99	Lig/sap	dead Tilia brunch	( )
Armillaria polymyces (Pers.exS.F.Gray)Sing.	L 2 $_1$10.10.99	Ter/Lig/sap	base of Querqus trunk	*/L
Daedalea quercina L.exFr.	L 2 $_2$10.10.99	Lig/sap (p)	dead Querqus trunk	( )
Pseudotrametes gibbosa (Pers.exPers.)Bond.&Sing	L 2 $_3$10.10.99	Lig/sap (p)	fallen Querqus trunk	( )
Coriolus versicolor (L.exFr.)Quel.	L 2 $_3$10.10.99	Lig/sap	dead Prunus trunk	( )/L
Stereum hirsutum (Wild.exFr.)S.F.Gray	L 2 $_3$10.10.99	Lig/sap (p)	dead Fagus brunch	( )
Omphalotus olearius(D.C.exFr.)Sing.	L 2 $_4$10.10.99.	Lig/Ter/sap (p)	the base of Fagus trunk	++/L
Morchela conicavar. costata Pers. (Focht)	L 5 $_1$8/4/00	Ter /sap	soil, sand	**
Coprinus atramentarius(Bull.)Fr.	L 5 $_1$9/4/00	Sap	soil, sand	*
Psathyrella vernalis(Lge.)Mos	L 5 $_1$9/4/00	Ter /sap	soil	( )
Morchella vulgaris Pers.	L 5 $_1$16/4/00	Ter/sap	soil, sand	**
Flammulina velutipes(Curtis) Karst	L 5 $_3$ 9/1/00	Lig /sap	dead Salix trunk	**/L
Laetiporus sulphureus (Bull.exFr.)Murr.	L 3 $_4$ 27/4/00	Lig/ p	alive Prunus trunk	**
Schizophyllum commune Fr.	L 1 $_3$27/4/00	Lig /sap	dead brunch	( )/L
Pleurotus ostreatus (Jacq.exFr.)Kummer	L 1 $_3$27/4/00	Lig/sap	dead trunk Fagus	**
Panus tigrinus (Bull.exFr.)Singer	L 1 $_3$ 27/4/00	Lig/sap	dead trunk Fagus	( )
Panellus stypticus (Bull.exFr.)Karst	L 1 $_3$ 27/4/00	Lig/sap	dead brunch	( )
Laetiporus sulphureus (Bull.exFr.)Murr.	L 4 $_4$ 2/5/00	Lig/P	alive Prunus trunk	**
Polyporus tuberaster (Pers.)Fr.	L 4 $_3$ 2/5/00	Lig/sap	dead Fagus brunch	( )

Legend: sap = saprophytic species, p = parasitic species, Ter = tericolous species, Lig = lignicolous species, * = edible species, M = medically important, ( ) = not edible, ++ = poisonous, L1 = species collected on locality 1, L2 = species collected on locality 2, L3 = species collected on locality 3, L4 = species collected on locality 4, L5 = species collected on locality 5, microhabitat of mycelium L $_1$ = soil, L $_2$ = stump, L $_3$ = dead trunk, L $_4$ = alive trunk or soil nearby the lived trunk. Fungi that are marked Lig/Ter forming rihzomorphs. Fam. Polyporaceae s. stricto, Fam. Polyporaceae s. lato, Fam. Ganodermataceae, Fam. Tricholomataceae, Fam. Stereaceae, Fam. Pleurotaceae, Fam. Coprinaceae, Fam. Strophariaceae, Fam. Schizophyllaceae, Fam. Morchellaceae, Fam. Omphalotaceae.

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