PHYSIOLOGICAL AND BIOCHEMICAL ASPECTS IN THE MACROMYCETES SPECIES COLLECTED FROM CĂLIMANI NATIONAL PARK (THE ORIENTAL CARPATHIANS)

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Abstract: In this paper we present the results of certain physiological and biochemical parameters (water content, dry matter, total mineral elements, organic substance and soluble proteins, and the respiration intensity) in eight macromycetes species collected from Călimani National Park. The results emphasize, for all the investigated species, specific variations of the aimed parameters, the determined values presenting comparable amplitudes for each parameter.

INTRODUCTION

The present paper continues our previous researches regarding certain physiological and biochemical aspects in macromycetes species collected from Călimani National Park (Stratu et al., 2008). Our researches belong to an ample study which aimed the potential of mycoremediation of certain polluting substances from the landfills resulted from mining.

The species analyzed belong to six families from Basidiomycota class: Gloeophyllaceae (*Gloeophyllum sepiarium* (Wulfen) P**.** Karst.), Polyporaceae (*Hapalopilus nidulans* (Fr.) P. Karst.), Fomitopsidaceae (*Fomitopsis pinicola* (Sw.) P. Karst.), Suillaceae (*Suillus grevillei* (Klotzsch) Singer; *Suillus variegatus* (Sw.) Kuntze; *Suillus luteus* (L.) Roussel), Hydnangiaceae (*Laccaria laccata* (Scop.) Cooke.) and Strophariaceae (*Hebeloma subsaponaceum* P. Karst.)(http://www.indexfungorum.org).

From the analyzed species, five are mycorrhizal (*Suillus grevillei, Suillus variegatus, Suillus luteus*, *Laccaria laccata, Hebeloma subsaponaceum*) some of them with role in the bioconversion of certain pollutants from the environment and three are lignicolous (*Gloeophyllum sepiarium*, *Hapalopilus nidulans, Fomitopsis pinicola)* contributing to the wood decomposition (Sing and Ward, 2004).

MATERIALS AND METHODS

The physiological and biochemical researches were made on certain species of macromycetes that were collected from Călimani National Park. The samples collected from the Reservation with Pinus cembra represented control assays and those collected from areas with high soil pollution degree (Ilva Waste Pad, Dumitrelu Waste Pad, Pinului Waste Pad) represented the proper assays. The biological material was collected in August and October 2007.

In the harvesting stage at the end of August, we collected the control assays (*Fomitopsis pinicola* şi *Hapalopilus nidulans*) and assays from Ilva, Dumitrelu and Pinului waste pads ((*Suillus luteus, Suillus variegates, Suillus grevillei, Laccaria laccata*)*.* The control samples collected in October were represented by *Laccaria laccata, Hebeloma subsaponaceum, Fomitopsis pinicola, Gloeophyllum sepiarium* species and those collected from waste pads by *Laccaria laccata* and *Hebeloma subsaponaceum.*

The analysis was carried out on freshly collected biological material, represented by the macromycetes fructification corpuses. The following physiological and biochemical parameters were determined: the water and dry matter content, the respiration intensity, the total mineral elements content, the organic substance content, the soluble protein content. The water and dry matter contents was determined gravimetrically through the maintenance of the biological material to 105^{o}C up to the constant weight. For measuring the respiratory process intensity we used the manometric method. The method principle consists in determining the quantity of gases produced or consumed in a closed system, by measuring the pressure variation in constant volume and temperature conditions. (Boldor et al., 1981). The total mineral elements content was determined by assessing the calcinated residues at 550^{o}C. The method mainly consists in maintaining the sample to analyze at the calcination temperature which leads to the loss of organic substances and of a part of the volatile mineral substances, after which we proceed to the gravimetric evaluation of the residue (Boldor et.al., 1981; Mănescu et al., 1994). The organic substance content was calculated through the difference between the dry matter content and the ash content. The quantitative determination of soluble proteins was achieved through the

Bradford method (Bradford, 1976). The principle of the method is based on the colour reaction of the protein extract with solution of Coomassie Brilliant Blue G250.

RESULTS AND DISCUSSIONS

The presence of water of the vegetal organisms conditions the normal deployment of chemical reactions involved in the metabolism. The water quantity is correlated with a multitude of factors of which we mention the species particularities, the analyzed organ, the climate conditions, the ecosystem from which the biological material is collected etc.

The results regarding the **water content** of the biological material collected in August 2007 from the Ilva, Dumitrelu and Pinului waste pads emphasize amplitudes that vary, in the majority of cases, around the value of 50g% or are situated under this value (fig. 1).

In the *Suillus luteus* species collected from 3 different locations we discover the fact that, at the highest water content we registered the samples collected from Waste Pad Dumitrelu. If we compare the species of the *Suillus* genus collected from the same waste pad (Pinului Waste Pad) we ascertain that the *Suillus variegates* species has the highest degree of hydration, followed, in decreasing order, by *Suillus luteus* and *Suillus grevilei.*

There is only one experimental variant (*Hapalopilus nidulana*), collected as a control sample from the Reservation with *Pinus cembra*, in which case the water content exceeds 50g% (73,50g %).

We consider that the amplitudes of the values registered by us show that this indicator is correlated with the species particularities, the age of the fructification body, the sensitivity of its tissues, and with the mycelium sensitivity compared to the presence of water in the atmosphere or the substrate. In the species, the water content reflects the characteristics of coniferous wood which is more porous and absorbs the water from the environment more easily.

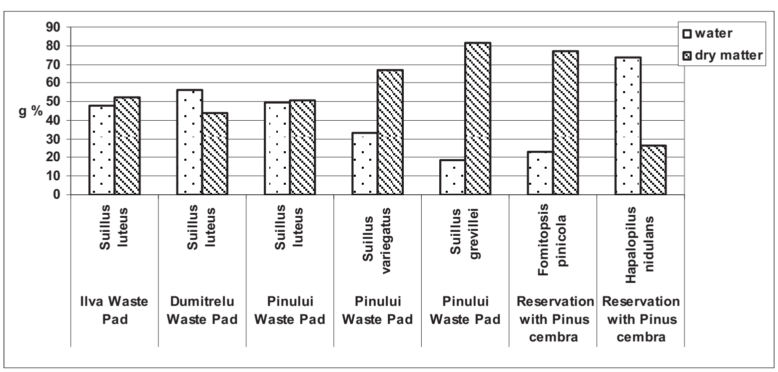

Fig.1. Water and dry matter content from macromycetes species collected in August

Regarding the macromycetes samples collected in October 2007, the analysis of the results registered do not indicate significant differences regarding the water content of the analyzed biological material between the control samples and those collected from the Waste pads (fig. 2).

We also ascertain that *Lacaria lacata* has water content around 80g%, the species *Hebeloma subsaponaceum* and *Fomitopsis pinicola* around 50g%, and the minimum value of the indicator is registered for *Fomitopsis pinicola* species.

From the interpretation of the results obtained for our experimental model, we deduce that the substratum does not influence the water content of the analyzed biological material.

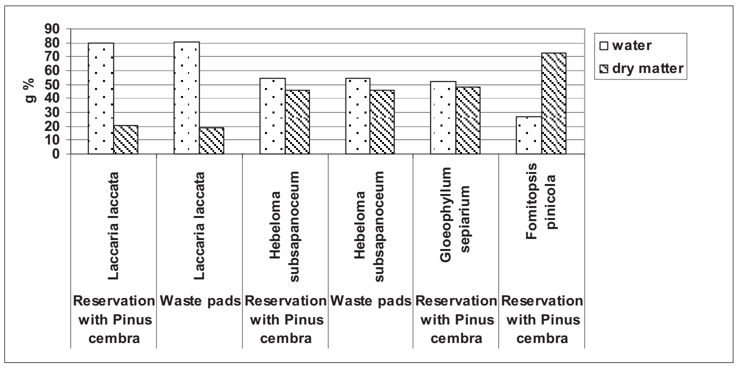

Fig.2. Water and dry matter content from macromycetes species collected in October

The dry substance content represents an indicator that characterizes the level of organic and mineral constituents. The species analyzed by us are characterized through a generally high content of dry substance, with slightly lower values in the case of species collected in October. Among the lignicolous species *Fomitopsis pinicola* (66,05g% - 77,09g%) are emphasized and among the mycorrhizal ones *Suillus grevillei* and *Suillus luteus* (Pinului Waste Pad), the latter species presenting the highest values of the organic substance content (fig. 1,2).

The **respiration intensity** is an indicator of the metabolic activity of live organisms and indirectly, it can represent an indicator of the stress state.

The respiration intensity has higher values in the mycorrhizal species, compared with the lignicolous ones (fig. 3). These differences could be determined by the species particularities, by the age of the fructification body, its consistence and chemical composition, its hydration degree, the abiotic factors etc.

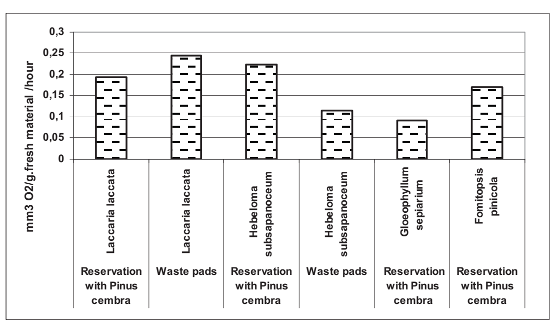

Fig. 3 Intensity of respiration from macromycetes species collected in October

The results obtained in the determinations made in October 2007 emphasize a higher respiratory intensity in the mycorrhizal species, behaviour determined probably by the oxidative stress caused by the presence in the soil of some pollutants or by the environment conditions from the period of the biological material collection (fig. 3)

The process is only triggered at a certain hydration degree of tissues, and its intensity increase with the water content growth. This fact is explained through the fact that the enzymatic complex involved in the oxidoreduction reduction processes characteristic to respiration can only function in the aqueous medium.

The data present in the specialty literature (Li Xiong, 2000) regarding the fresh comestible mushrooms (full or cut) indicate the fact that, in normal conditions, the respiration intensity is high, compared with that of some vegetable species (tomatoes or salad).

According to the literature data (Breene, 1990; Matilla et al., 2001 cited by Bernas et al., 2006), the fructification bodies of mushrooms present a high content of mineral elements (phosphorus, potassium, calcium, magnesium, sulphide, sodium, iron, zinc) which differ however according to the species, age of the mushroom, diameter of the cap, substrate etc. The distribution of these elements in the fructification bodies vary, their content being higher in the cap than in the leg.

The total mineral elements content determined in the vegetal material from our experimental model registers higher values in the mycorrhizal species (5,98g% - 16,53g%) compared with the lignicolous ones (1,54g% - 3,03g%), situation valid in the case of the two intervals of the biological material collection intervals (fig. 4, 5).

This behaviour denotes differences as regards the chemical nature of the substrate and the capacity of mineral elements exploitation. The mycorrhizal species present a higher potential of minerals substrate use, fact confirmed by the results of some literature studies (Prasad and Freitas, 1999). Some macromycetes species such as the mycorrhizal *Suillus bovinus* and *S. luteus* with *Pinus sylvestris* reduce the heavy metals toxicity (in the case presented by the authors – tolerance to Cd) (Colpert and Van Asssche 1992 cited by Prasad and Freitas, 1999).

They have an extremely important ecological role, contributing to the remedy polluted soils with heavy metals and to the increase of efficiency of mineral elements use (by the superior plants – through the solubilisation of difficulty soluble elements) from the low trophicity soils.

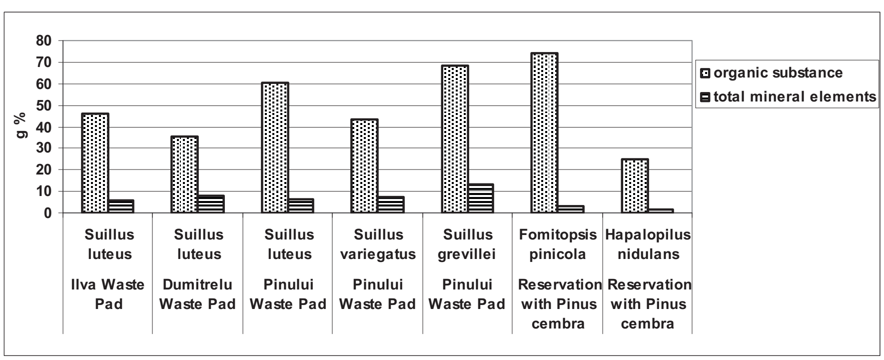

Fig. 4 Mineral and organic substances from macromycetes species collected in August

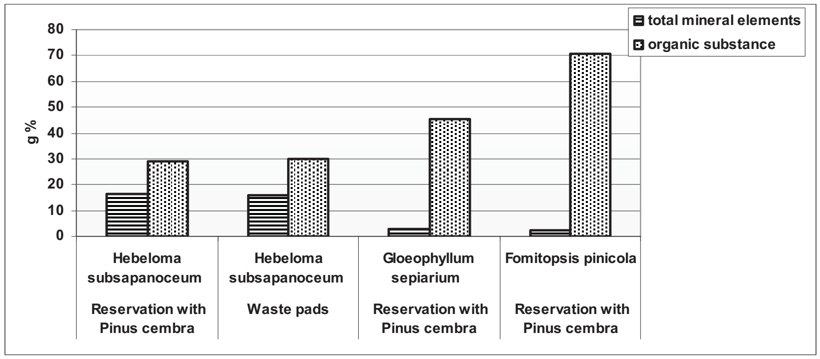

Fig. 5 Mineral and organic substances from macromycetes species collected in October

We mention that the determinations made in August, in parallel, on samples collected from the same species, from the same location, reveal the capacity of some species (*Suillus luteus* - Ilva Waste Pad; *Laccaria laccata* – Ilva Waste Pad) to accumulate large quantities of metals (Cu, Fe, Zn) (Mircea et al., 2007).

Among the mycorrhizal species from the sterile waste pads we emphasize *Suillus grevillei* collected in August from Pinului Waste Pad (13,12g%) and *Hebeloma subsaponaceum* collected in October (15,99g%). Among the Suillus genre species, *Suillus grevillei* collected from Pinului Waste Pad is characterized through the highest degree of total mineral elements.

Regarding the *Suillus luteus* species, collected from three different locations, we ascertain slightly higher values in the case of samples collected from Dumitrelu Waste Pad (8,02g%). In the *Hebeloma subsaponaceum* species the mineral elements content registers insignificant differences between the control and the sample.

The organic substances are represented by proteins, poly-carbohydrates, lipids, aminoacids, vitamins, phenolic compounds (flavonoids, lignins, phenolic acids), substances that give the mushrooms flavour etc (Bernas et al., 2006).

The organic substances content present high values in the two categories of analyzed species. We appreciate that this behaviour emphasizes an intense metabolic activity and presents a special ecological importance, since the organic matter contributes to the soil formation, to the improvement of its physical-chemical characteristics, favouring the installation of a specific vegetation and to the remedy in time of the degraded habitat as a result of the mining activity.

Among the mycorrhizal species collected from the waste pads *Suillus grevillei* (68,35g%) and *Suillus luteus* (60,61g%) are distinguished (Pinului Waste Pad) for the August assay.

Among the lignicolous species *Fomitopsis pinicola* distinguishes itself, assayed from the control area – the resort with *Pinus cembra,* with valid situation in the case of the two time intervals studied (fig. 4; 5).

The quantity of the **soluble protein** determined in the macromycetes samples collected in August led to obtaining some values with different amplitudes.

The species *Suillus luteus* collected from the Pinului Waste Pad presents a total content of soluble proteins of approximately six times bigger compared with the same species collected from Ilva Waste Pad, and 4.7 times bigger compared to those collected from Dumitrelu Waste

Pad. The presence of these values could be explained by the fact that the soil presents a high pollution degree, which denotes a more intense protein metabolism. We also collected from Pinului Waste Pad other two species belonging to the same genre, *S. variegates* and *S. grevillei*, which presented extremely different protein content, 148.11 mg% and, respectively, 748.82 mg% (fig. 6).

The protein content variation, in the case of determinations from August, is correlated with the results regarding the concentrations of nitrates, nitrites and sulphites obtained through determinations made in parallel, on samples collected from the same macromycetes species (Mircea et. al., 2007).

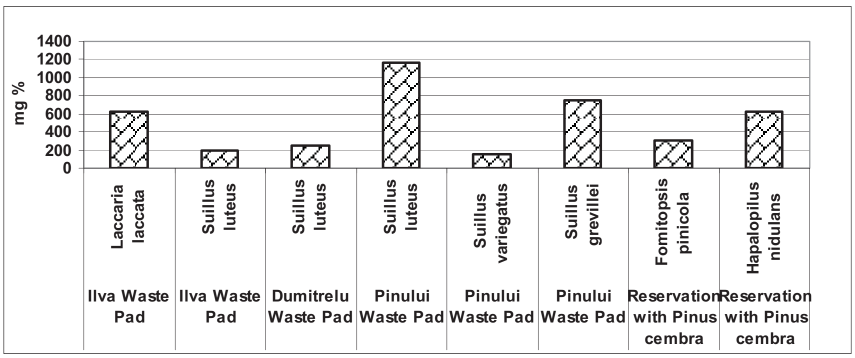

Fig. 6 Soluble protein content from macromycetes species collected in August

The high proteins content in the species collected from the Pinului and Dumitrelu Waste Pads could be due to the higher content of nutrients in the soil, as a result of the activity of microorganisms involved in the nitrogen, carbon and sulphur cycle (Ulea and Lipşa 2009).

In the Pinus cembra reservation area, considered as control area, we did not find species belonging to the *Suillus* genre but only *Fomitopsis pinicola* and *Hapalopilus nidulans*, which had soluble proteins content of 299,1mg% and respectively 619.54mg%. This value difference can be explained through the species specificity or the age of macromycetes, knowing that together with age, the protein content is decreased.

In the case of assays from October, in the same species, the soluble protein content is maintained at a high level both in the control samples and in those collected from the waste pads and depends on the species. Nevertheless, we notice that the *Laccaria laccata* species presents a higher quantity of soluble protein in the samples collected from the waste pads, compared with the samples collected from the area without pollution of Călimani National Park (853,56mg% compared with 611,62mg%) (fig. 7).

In the *Hebeloma subsaponaceum* species the situation is the other way round, the protein content being bigger in the samples collected from Călimani National Park, in comparison with the samples collected from the waste pads (271,79mg% compared to 1028,26mg%). We can consider that, due to the presence in small quantity of soil pollutants, the protein metabolism is intensified and thus the protein content grows (fig. 7).

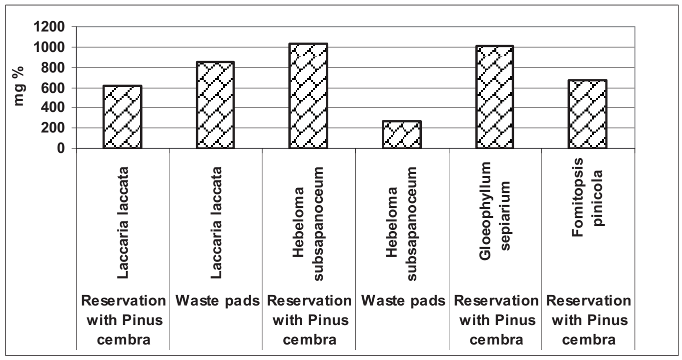

Fig. 7 Soluble protein content from macromycetes species collected in October

CONCLUSIONS

Our results emphasize for all the investigated species, specific variations of the aimed parameters, the determined values presenting comparable amplitudes for each parameter.

In *Lacaria lacata* and *Hebeloma subsaponaceum* species (samples and control) we ascertain positive correlation between the respiration intensity, the water content and soluble proteins.

The more intense physiological and metabolic activity, emphasized in the species collected from Pinului and Dumitrelu waste pads, correlated with the presence of microorganisms involved in the nutrient cycle creates the premises of the ecological reconstruction of the degraded soils in which the macromycetes species have an important role.

We consider that the characteristic manifestations of the investigated parameters are determined, to a great extent, by the species particularities and by the abiotic conditions of the areas from which the biological material was harvested.

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Names of fungi and author’s abbreviations follow the CABI database (http://www.indexfungorum.org).

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