OXYDOREDUCTASE ACTIVITY OF THE SOME FUNGI HARVESTING FROM DIFFERENT CĂLIMANI NATIONAL PARK AREAS

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Abstract: The paper presents the results of the determinations regarding the activity of some oxydoreductases (superoxyd dismutase, and catalase peroxydase- indicators of the oxydative stress) involved in the metabolism of some fungi harvested from different areas of the National Park Călimani.

INTRODUCTION

Micro-organisms (bacteria, fungi, actinomicete, protozoa, etc.) have the capacity to recycle organic matter creating conditions for other plants to grow. The fungi are considered ideal for soil bioremediation polluted due to rapid colonization and because their metabolism. Studies with ligninolytic fungi have shown that these organisms produce extracellular enzymes with a very low specificity of substrate (Fan and Krishnamurthy, 1995). Most fungi are robust body and generally more tolerant to high concentrations of polluting chemicals than are bacteria which explain why fungi have been extensively investigated for their bioremediation capacities (Gadd, 2001, Čeněk Novotný et. al., 2004, Singh A., Ward O.P., 2004, Baldrian P, et. al., 2000, Nemergut D. R., et. al., 2000).

Pollutants are stress factors and their action disturbed the protective mechanisms of living organisms, establishing the high levels of reactive oxygen species. But living organisms have specific biochemical mechanisms by which these toxic agents are neutralizing. Under the action of superoxide dismutase, catalase, peroxidase and other enzymes or enzymatic system, such molecular species are decomposed leading to the formation of compounds simpler and non-toxic that can be removed later in the environment or into different sequences of metabolic biosynthesis and/or degradation.

Catalytic activity of enzymes may have value situated outside of the normal physiological ranges where the bodies shall act in various physical, chemical or biological environmental factors. This is why for the determination of enzyme activity or dosage of plastic molecules with a role and/or functional, is a way of assessing the degree of pollution of habitat species.

The present paper study presents the study of oxido-reductase enzymes (superoxide dismutase, catalase) activity in the biological material represented by fungi harvested from Călimani National Park.

MATERIALS AND METHODS

Biochemical researches have been effect with some species of fungi that have been harvested from the National Park Călimani. Samples collected from the *Pinus cembra* reservation were control and those harvested from areas with high pollution of the soil were evidence itself (Ilva landfill, Dumitrelu landfill, Pinului landfill). The collections of biological material were made from June to September of 2007, in four stages.

At the end of June (first harvesting) was collected from the same substrate (coniferous wood), a single fungal species, *Gleophyllum odoratum*. Fungus has been harvested from three different areas of the National Park Călimani. In the last decade of July (second harvesting) samples analyzed was represented by fungi *Fomitopsis pinicola*. We mention that the small number of samples harvest in the first two stages was due to drought conditions which have allowed the development of mushrooms. In the next stage of harvest, which was held at the end of August (third harvesting), were taken as evidence control *Fomitopsis pinicola* and *Hapalopilus nidulans*, and the evidence itself of landfills Ilva, Dumitrelu and Pinului: *Suillus luteus, Suillus variegates, Suillus grevillei and Laccaria laccata*. In September, was conducted last sampling of mushrooms (fourth harvesting) and the biochemical analysis was executed on the evidence of control represented by *Laccaria laccata, Habeloma subsaponaceum, Fomitopsis pinicola, Gloeophyllum sepiarium* and the species *Hebeloma subsaponaceum* and *Laccaria laccata,* too.

The enzymes studied for the degree of pollution in landfill National Park Călimani are superoxide dismutase and catalase. For each sample analyzed the biological material was homogenised achieving the average of samples that were made three determinations. The results were reported in the quantity of fresh material.

The superoxide dismutase activity (EC 1.15.1.1) was determined measuring the percent of the superoxide dismutase induced inhibition of nitro blue tetrazolium reduction by the superoxide radicals resulted in the reaction of riboflavin photoreduction (Winterbourne et al., 1975, and Minami Yoshikawa, 1979). Determination of catalase activity

(EC 1.11.1.6) was performed by the iodometric method and the results obtained were reported as catalase unities reported to 1g of fresh material (Artenie et al., 2008).

RESULTS AND DISCUSSIONS

Interpreting of the results of superoxide dismutase and catalase activity is seen as an expression of oxidative stress induced mainly by the drought conditions. Superoxide radicals (O_2^-) are the molecular species highly toxic to the cell alive. In normal physiological conditions they are formed in relatively small quantities being distorted just under catalytic action of superoxide dismutase. If the different kind of oxidizing substances entering into a living organism, the superoxide anionic concentration in the body increases suddenly and, implicitly there is a corresponding of superoxide dismutase activity development.

For *Gleophyllum odoratum* species (control) harvested at the end of June, 2007 in three areas of the National Park Călimani but on the same substrate the wood of conifers, superoxide dismutase activity was values significantly different (Table 1). Amplitude of these values are comparable to zones 1 and 3, while the evidence recovered from zone 2 enzyme activity decreases slightly.

Table 1. The superoxid dismutase activity at the fungi harvested at the end of June 2007

Species of fungi	Zone of collection	Superoxid dismutase activity (U/g)
Gloeophyllum odoratum	*Pinus cembra* rezervation (control) – zone 1	137,23
Gloeophyllum odoratum	*Pinus cembra* rezervation (control) – zone 2	112,32
Gloeophyllum odoratum	*Pinus cembra* rezervation (control) – zone 3	149,41

The catalase is presence in the samples collected from zones 1 and 3. In zone 2 wasn't detected catalase activity, the detoxifiant role of toxic products resulting from the cell respiration processes being taken probably, by the peroxidase, the next enzyme entering the activity (Table 2).

Table 2. The catalase activity at the fungi harvested at the end of June 2007

Species of fungi	Zone of collection	catalase activity (U/g)
Gloeophyllum odoratum	*Pinus cembra* rezervation (control) – zone 1	15.62
Gloeophyllum odoratum	*Pinus cembra* rezervation (control) – zone 2	-
Gloeophyllum odoratum	*Pinus cembra* rezervation (control) – zone 3	7,54

Towards the end of July it was make another sampling (second harvesting). Drought has affected for this time, too, the development of fungi, which is why biochemical tests were performed on a single species. Thus, *Fomitopsis pinicola* was harvested from the control area of the National Park Călimani where the environmental conditions are natural, without human intervention with different chemicals pollute that can induce oxidative stress. For these sample

have shown superoxid dismutase activity who oscillate around 56 U/g, values which demonstrates the lack of strong oxidants pollute on the environment (Table 3).

Table 3. The superoxid dismutase activity at the fungi harvested at the end of July, 2007

Species of fungi	Zone of collection	Superoxid dismutase activity (U/g)
Fomitopsis pinicola	*Pinus cembra* rezervation (control)	56,53

Catalase activity is low behavior that indicates a level of normal aerobic metabolism of species *Fomitopsis pinicola* in climatic conditions of the July month (Table 4).

Table 4. The catalase activity at the fungi harvested at the end of July 2007

Species of fungi	Zone of collection	catalase activity (U/g)
Fomitopsis pinicola	*Pinus cembra* rezervation (control)	490,61

By the end of August when the weather conditions are changing were harvested samples for enzyme analysis (third harvesting) and the number of samples collected has increased because the development of fungi. Thus, were collected *Suillus luteus* samples from Ilva, Dumitrelu and Pinului landfills, *Laccaria laccata* from Ilva landfill, *Suillus variegatus* from Pinului landfill and *Suillus greviellei* from Pinului landfill. Also, were collected two controls *Fomitopsis pinicola* and *Halopilus nidulans* from *Pinus cembra* reservation - Călimani National Park.

As regards the superoxide dismutase activity (Table 5) it was high or very high (3 to 40 times higher) in samples collected from Ilva, Pinului and Dumitrelu landfills compared with control collected from the Călimani National Park. It is very possible that landfill contain a series of chemical pollute with strong oxidizing, after absorption by the mushrooms, to induce activation of superoxide dismutase that response to the emergence of the fungal body superoxide radicals. All these values of enzymatic activity strengthen our hypothesis that, species of landfill absorb chemical pollutants from the soil with oxidizer character, which implicit induce oxidative stress.

Table 5. The superoxid dismutase activity at the fungi harvested at the end of August, 2007

Species of fungi	Zone of collection	Superoxid dismutase activity (U/g)
Suillus luteus	Ilva landfill	939,35
Suillus luteus	Ilva landfill	305,58
Laccaria laccata	Ilva landfill	1881,64
Suillus luteus	Dumitrelu landfill	984,78
Suillus variegates	Pinului landfill	153,69
Suillus luteus	Pinului landfill	993,46
Suillus grevillei	Pinului landfill	1218,81
Fomitopsis pinicola	*Pinus cembra* (control) rezervation	47,92
Hapalopilus nidulans	*Pinus cembra* (control) rezervation	87,77

If SOD activity offer direct information regarding the absorbed chemical pollute with oxidizer character capacity of this fungi, the catalase activity offer signs on fungus capacity to metabolized further on this pollutes who lead at appearance of toxic molecular species.

For samples recovered from Ilva, Dumitrelu and Pinului landfills, catalase activity have shown that the values between 3539 U/g and 23395 U/g, being generally higher comparatively with control catalase activity represent by other species (Table 6). Great value to catalase activity recorded *Hapalopilus nidulans* may due to a stress factor.

Table 6. The catalase activity at the fungi harvested at the end of August, 2007

Species of fungi	Zone of collection	catalase activity (U/g)
Suillus luteus	Ilva landfill	5428
Suillus luteus	Ilva landfill	6195
Laccaria laccata	Ilva landfill	23395
Suillus luteus	Dumitrelu landfill	3539
Suillus variegates	Pinului landfill	13455
Suillus luteus	Pinului landfill	6421
Suillus grevillei	Pinului landfill	10849
Fomitopsis pinicola	*Pinus cembra* (control) rezervation	3269
Hapalopilus nidulans	*Pinus cembra* (control) rezervation	11707

At the end of September (fourth harvesting) was collected the species *Laccaria laccata*, *Hebeloma subsaponaceum*, *Fomitopsis pinicola* and *Gleophyllum sepiarium* from the *Pinus cembra* reservation (trial witness) and from the landfills.

If in regard to the superoxide dismutase activity is not notable evidence differences between control and the fungi collected from landfill (Table 7), the catalase activity presents a totally different picture. Thus, in the case of control samples *Laccaria laccata* the catalase activity is zero but at the same species collected from the landfill, this enzyme has a very high activity behavior that demonstrates the existence of an aerobic metabolism particularly intense.

Table 7. The superoxid dismutase activity at the fungi harvested at the end of September, 2007

Species of fungi	Zone of collection	Superoxid dismutase activity (U/g)
Laccaria laccata	*Pinus cembra* (control) rezervation	1010,16
Laccaria laccata	Pinului landfill	1035,60
Hebeloma subsaponaceum	*Pinus cembra* (control) rezervation	520,91
Hebeloma subsaponaceum	Pinului landfill	365,38
Fomitopsis pinicola	*Pinus cembra* (control) rezervation	102,16
Gloeophyllum sepiarium	*Pinus cembra* (control) rezervation	341,69

The same major differences are recorded in the case of other species taken in the study: *Hebeloma subsaponaceum* (21,770 U/g), *Fomitopsis pinicola* (11,231 U/g), respectively, *Gloeophyllum sepiarum* (25,575 U/g) comparatively with the value 138 U/g recorded in the analysis of control sample (Table 8).

Table 8. The catalase activity at the fungi harvested at the end of September, 2007

Species of fungi	Zone of collection	catalase activity (U/g)
Laccaria laccata	*Pinus cembra* (control) rezervation	lipsă

Laccaria laccata	Pinului landfill	19,187
Hebeloma subsaponaceum	*Pinus cembra* (control) rezervation	138
Hebeloma subsaponaceum	Pinului landfill	21,770
Fomitopsis pinicola	*Pinus cembra* (control) rezervation	11,231
Gloeophyllum sepiarium	*Pinus cembra* (control) rezervation	25,575

CONCLUSIONS

Biological techniques that use clean capacity with the growing mushrooms on the contaminated soil presents a particular interest, which lies essentially in that it requires no cutting, no transport and the implementation is less expensive.

The biochemical studies effect lead us to conclude that enzymes activity is different depending on the nature of mushrooms analyzed, the degree of development of their abiotic conditions of the ecosystems of which they were harvested and weather conditions, too.

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