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MICROMORPHOLOGICAL AND CHEMICAL ASPECTS OF SOME LICHENIZED FUNGI SPECIES

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Abstract: At present, lichenized fungi are used in biomonitoring studies of air quality, being good receptors in the climate change. This paper aims to investigate surface micromorphology of *Xanthoria parietina* and *Phaeophyscia orbicularis* species (Lecanoromycetes, Ascomycota). The study also includes the investigation of selected chemical parameters as *p*H and conductivity of the lichenized fungi samples collected from various locations in the Iaşi County (Romania). Measurements of the *p*H provide information on the degree of pollution in the location of interest. Bark trees *p*H was also investigated in order to see if our matrix substrate influences the *p*H of the interest lichenized fungi samples.

Key words: lichenized fungi, Ascomycota, micromorphology, *p*H, conductivity

Introduction

Lichenized fungi, as unique forms in the plant world, are the result of the symbiosis between a fungus (mycobiont) and a cyanobacteria or green algae (phycobiont). The result of this symbiosis is a stable vegetative body having a specific structure and is known as the thallus [BRODO & al. 2001].

Lichenized fungi are the most complex forms of life, commonly found on about 8% Earth surface [AHMADJIAN, 1995]. They can survive under extreme conditions such as Polar Regions and in high mountains. These locations are characterized by severe abiotic conditions such as dehydration, extreme temperatures and high light intensities. Some authors claim that what really makes them special and what separates them from most other eukaryotic organisms is their ability to tolerate extreme conditions. For this reason, lichenized fungi were called "extremophilic organisms", which can develop in such conditions that would kill other organisms, less specialized [YOUNG, 2005].

Many authors have repeatedly drawn attention to the problem of using lichenized fungi morphology as a visual indication of air pollution effects. Changes in thallus morphology were observed in lichenized fungi transplanted to polluted areas, exposed to simulated acid rain and fumigated by gaseous pollutants. Reported morphological changes include a reduction in size, detaching from the substratum with margins upturned, changes in coloration, and development of chlorotic and necrotic patches [BENNETT & al. 1996; OTNYUKOVA, 2007].

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Given the importance of the above presented aspects, the purpose of this study is to observe in more details the structure of the interest lichenized fungi thallus (*Xanthoria parietina* and *Phaeophyscia orbicularis* species collected from Iaşi – Romania) to a better understand of those changes that occur in unfavorable environmental conditions for their development. Another aim is to investigate some chemical parameters (e.g., *p*H and conductivity), which might give us preliminary information about the chemical composition of the investigated matrix.

Material and methods

For an appropriate structural characterization of living organisms, microscopic investigations are requested. Thereby, specific information obtained from classical optical microscopy should be supplemented with information provided by Scanning Electron Microscopy (SEM) and Transmission Electronic Microscopy (TEM) techniques which are used depending upon the type and nature of the sample.

Microscopic investigations were performed on fresh lichenized fungi samples of *Xanthoria parietina* and *Phaeophyscia orbicularis* species (Lecanoromycetes, Ascomycota) collected from Iaşi (Romania). Surface micromorphology of the lichenized fungi species was investigated using a scanning electron microscope *FEI Quanta 250 FEG*.

Fresh thallus samples were cleaned, dried at room temperature and then segmented with a blade. Resulted segments were fixed on a copper stage using conductive carbon adhesive discs on both sides. Images were obtained using LFD (Large Field Detector) and GSED (Gaseous Secondary Electron Detector) detectors. Investigations on the chemical composition of the two lichenized fungi species was determined by EDX (Energy Dispersive X-ray Detector) detector.

Electrochemical parameters *p*H and conductivity of extracts can give preliminary indications on the chemical composition of the species of interest. Measurements of *p*H were made for both collected lichenized fungi and for the bark tree where sampling was conducted. *p*H was measured with a *p*H meter ION Meter Lab 450 and conductivity with a conductivity Lab Meter C 490. Each collected was analyzed in 10 replicates.

*Determination of lichenized fungi thallus p*H. Preparative steps were carried out in accordance with the suggestions from the literature [GAUSLAA, 1985; GILBERT, 1986; RIGA-KARANDINOS, 1998; CONTI & CECCHETTI, 2001; FRATI & al. 2006]. About 50 mg of lichenized fungi thallus was weighed with an *ADAM PW 254* balance. Weighed samples were homogenized, brought into contact with 10 ml of ultrapure water, ultrasonified 15 min and then centrifuged. After centrifugation at 1000 rpm for 10 min the supernatant was used for measurements the *p*H and conductivity.

*Determination of bark tree p*H. Bark tree samples were collected and used to determine the *p*H. 0.2 g of bark tree surface was placed in vials with 10 ml of ultrapure water and stirred for 1 h. Samples were then centrifuged for 10 min at 4000 rpm and clear liquid fraction was filtered (pore = 0.45 μm), and used for analysis.

Results and discussions

The thallus of *Xanthoria parietina* is usually 3–10 cm in diameter and closely adheres to the substrate. Apothecial discs (i.e. the hymenial layer) and the ostioles of pycnidia always revealed an intense yellow coloration due to the deposition of crystals of the anthraquinone parietin [SØCHTING, 1997]. It almost invariably produces apothecia (sexual reproduction organs) but no specialized organs for vegetative propagation. Fig. 1

clearly shows a lecanorin apothecia cup contoured on the lichenized fungi thallus surface of the *Xanthoria parietina* species.

Fig. 1. Thallus apothecia on the surface of *Xanthoria parietina*

The images presented in Fig. 2a & 2b were obtained with LFD detector at a voltage of 3.00 kV. Fig. 2a shows cortex morphology, with details at peripheral part revealing the presence of filamentous hyphal growth at the periphery and conglutinates zones in the microaerobic central part. Fig. 2b presents hyphae dimensions between 1.750 µm and 1.922 µm.

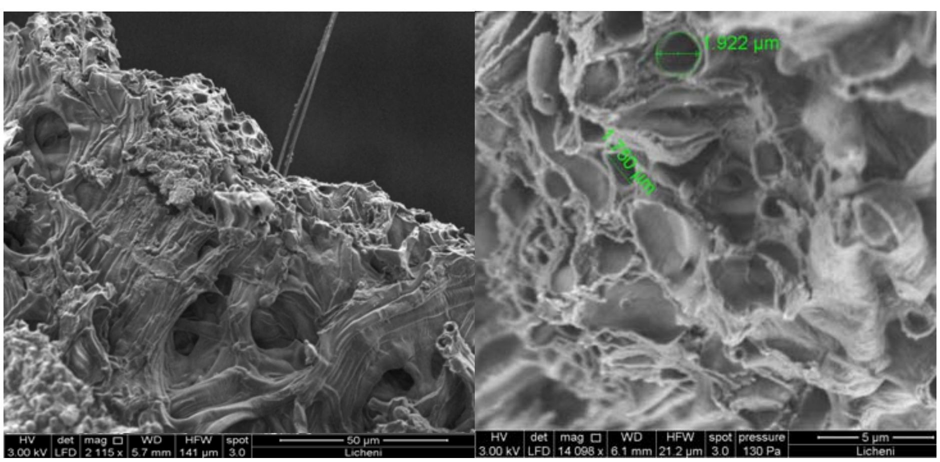

Fig. 2. (a, b) Surface micromorphology (upper cortex) of thallus of *Xanthoria parietina*

In Fig. 3, at a magnification of 470 x, the surface micromorphology highlighting the existence of a well-defined paraphyses, from *Xanthoria parietina* species with sizes of about 200 μm can be observed. It can be well observed that paraphyses present atmospheric particulate on the surface.

The thallus of the *Phaeophyscia orbicularis* is of foliose type, with a diameter up to 5 cm, irregular to more often orbicular lobes, in linear and discrete forms. Upper surface is gray to brown, sometimes with a whitish epinecral layer centrally formed. Apothecia are occasionally up to 2 mm in diameter, sessile, with circulars or lobules ascospores. Surface micromorphology of a *Phaeophyscia orbicularis* thallus is shown in Fig. 4. Existing lobes can be seen on the upper surface of the lichenized fungi.

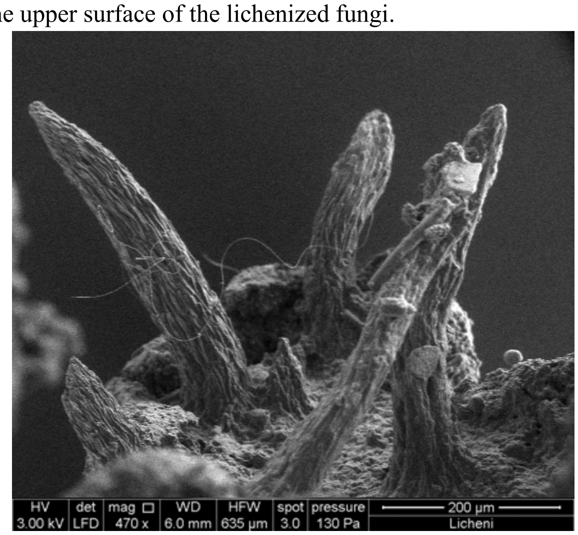

Fig. 3. Surface micromorphology of thallus: paraphyses

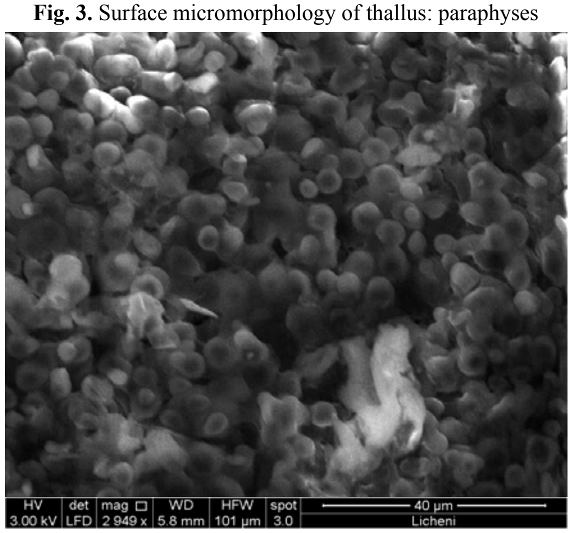

Fig. 4. Surface micromorphology of thallus of *Phaeophyscia orbicularis*

Investigations performed with an EDX detector for *Xanthoria parietina* (Fig. 5) and *Phaeophyscia orbicularis* (Fig. 6) species clearly reveal the presence of elements that probably can come directly from the thallus chemical composition (K, Mg, Ca, P, Fe) while the presence of elements such as Si and Al may be due to influences induced by the impact of atmospheric constituents (Si and Al are tracers of dust particles in the atmosphere).

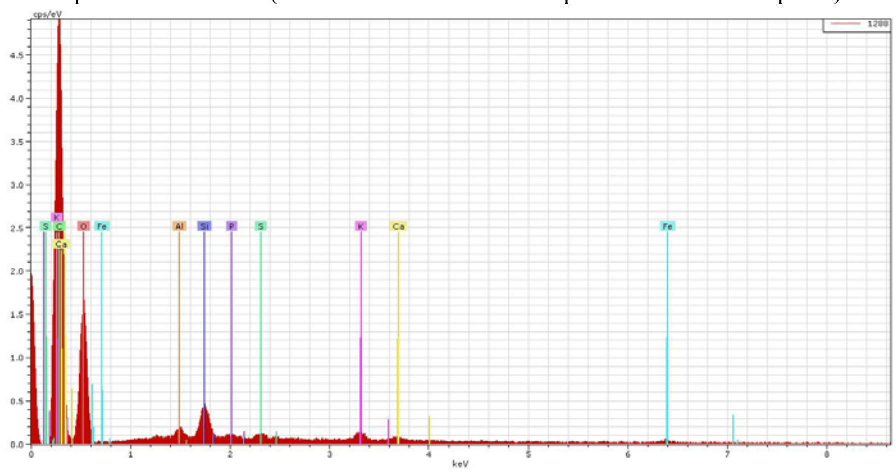

Fig. 5. The chemical composition of *Xanthoria parietina* thallus obtained using EDX detector

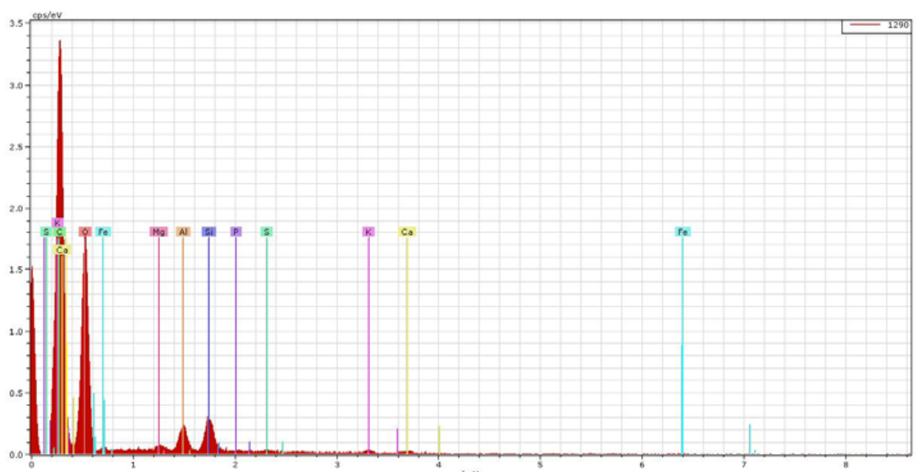

Fig. 6. The chemical composition of *Phaeophyscia orbicularis* thallus obtained using EDX detector

At Copou-Botanical Garden, Copou-Pârtie 1, Copou-Pârtie 2, Copou-Pârtie 3, Copou-Petru Poni, Copou-Penitenciar, Tudor Vladimirescu, Baza 3, Bucium, Dancu, Podul Roş, Galata sampling locations, sampling was performed for at least three month consecutively. At these sampling locations the results are presented as averages of the date obtained from the analysis of the total investigated replicates (i.e., 10 replicates). Especially the *p*H showed good stability within a three month period. For *Xanthoria parietina* the distribution of the *p*H (Fig. 7) and conductivity (Fig. 8) measurements of the samples collected are presented as averages of the total replicates (± SD-Standard Deviation) at the investigated sites.

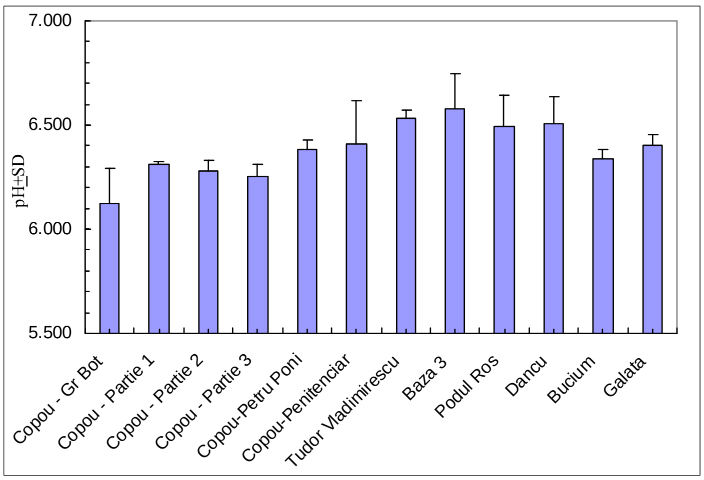

Fig. 7. Distribution of *p*H (mean ± SD) in *Xanthoria parietina* by sampling locations

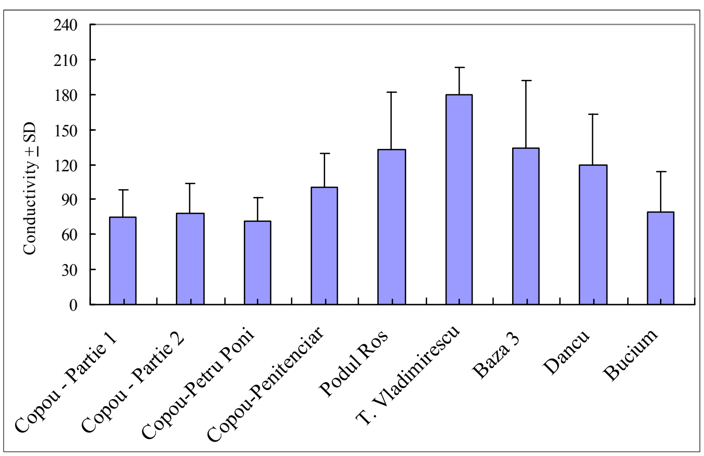

Fig. 8. Distribution of conductivity (mean ± SD) in *Xanthoria parietina* by sampling locations

Fig. 7 shows that statistically significant differences can be observed especially for samples collected from locations eventually heavily exposed to pollution (*e.g.* Copou-Penitenciar, Tudor Vladimirescu, Baza 3, Podul Roş which are locations susceptible to heavy car traffic induced pollution). The variability of the presented conductivity in Fig. 8 confirms the eventually intense exposure car traffic through a higher ionic components loading in the investigated species.

For *Phaeophyscia orbicularis* species simultan statistical calculations were performed, distribution of the interest species shown in Fig. 9 for *p*H and Fig. 10 for conductivity.

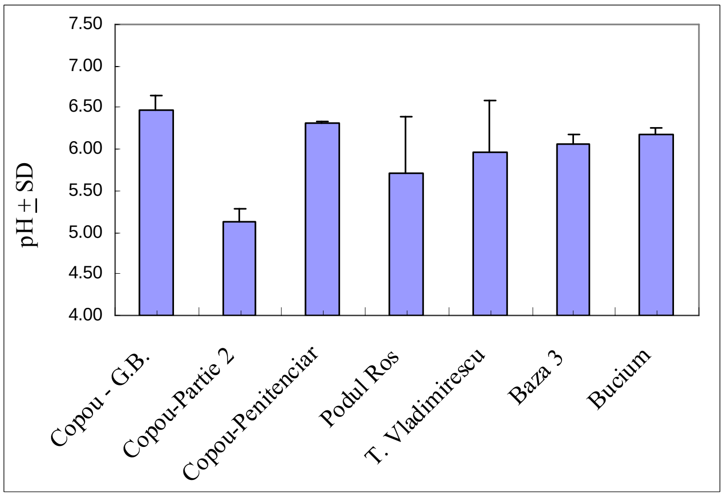

Fig. 9. Distribution of *p*H (mean ± SD) in *Phaeophyscia orbicularis* by sampling locations

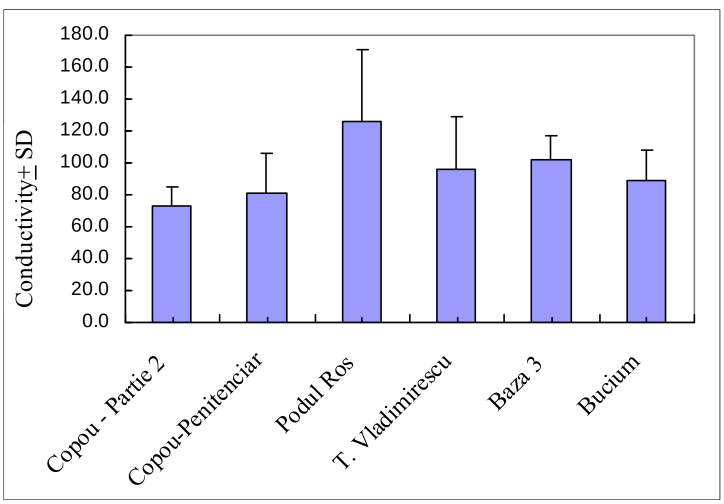

Fig. 10. Distribution of conductivity (mean ± SD) in *Phaeophyscia orbicularis* by sampling locations

For *Phaeophyscia orbicularis* species was observed that the *p*H variability ranged within the same limits with an exception of Copou-Pârtie 2 location. Distribution of the conductivity values shown in Fig. 10 suggests that, perhaps, also the pollution caused by an intensive road traffic Podul Roş, Tudor Vladimirescu, Baza 3 areas, could influence the values parameter in the collected samples from those areas.

Experimental investigations were also performed of the investigations in order to test the influence of the extraction matrix on the *p*H and conductivity. Fig. 11 presents the results obtained for *p*H variability in *Xanthoria parietina* species while Fig. 12 presents the results for the same investigations for the *Phaeophyscia orbicularis* species. Samples of interest species were extracted in ultrapure water and KCl 0.025 M solution.

The data presented in Fig. 11 and Fig. 12 allows us to suggest that the *p*H chemistry of the investigated biological samples can be experimentally performed by using as extraction agent either ultrapure water or potassium chloride solution.

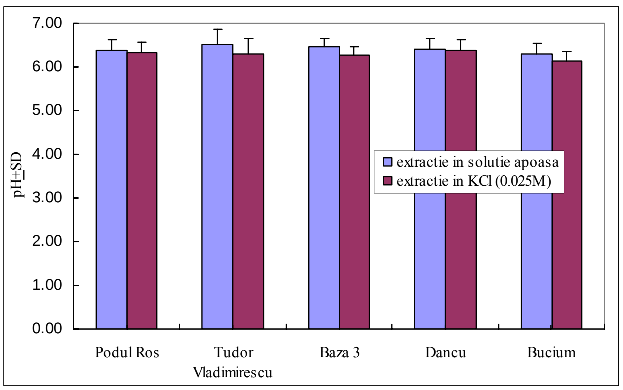

Fig. 11. *p*H variation (+ SD) in *Xanthoria parietina* species by sampling locations

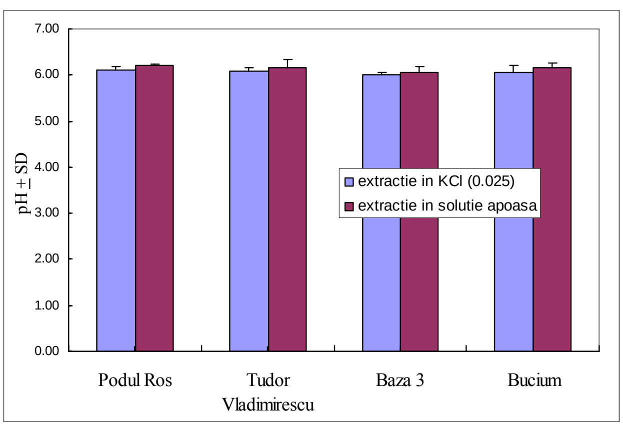

Fig. 12. *p*H variation (+ SD) in *Phaeophyscia orbicularis* species by sampling locations

Many studies [RIGA KARANDINO, 1998; VAN HERK, 2001; FRATI & al. 2006] report information on the possible influence of the substrate *p*H on the behaviour of the lichenized fungi thallus growing on that substrate. In the present work were undertaken also measurements of the bark tree *p*H from where lichenized fungi samples were collected. Data in Tab. 1 presents the *p*H values in the bark trees substrate and the corresponding collected lichenized fungi thallus. Tab. 1 shows the values of the interest statistical parameters, the average values for poplar bark substrate varying for example in the 5.6 to 6.3 range values. However, higher values were measured in bark substrates collected from locations with heavy traffic activities (*p*H = 6.035 in Podul Roş area and *p*H = 6.340 in Tudor Vladimirescu location). The variability shown by the average values specific for the investigated substrates and lichenized fungi samples highlights the existence of a statistically significant difference between the two types of matrices. This behaviour allow us to suggest that the chemical compositions of the investigated lichenized fungi samples were most probably affected mainly by the atmospheric deposition rather than the nature of the substrate on which it stands.

Tab. 1. Bark tree *p*H and thallus *p*H for investigated sampling locations.

Sampling location	Species	Type substrat	*p*H bark tree		*p*H thallus	
			mean	SD	mean	SD
Botanical G.	*Xanthoria parietina*	Oak	**5,591**	0,138	**6,125**	0,191
Botanical G.	*Phaeophyscia orbicularis*	Oak	**5,913**	0,204	**6,406**	0,146
Bucium	*Xanthoria parietina*	Poplar	**5,629**	0,048	**6,304**	0,231
T. Vladimirescu	*Xanthoria parietina*	Poplar	**6,340**	0,073	**6,513**	0,341
Baza 3	*Phaeophyscia orbicularis*	Poplar	**5,739**	0,167	**6,128**	0,190
Dancu	*Xanthoria parietina*	Lime	**6,035**	0,061	**6,385**	0,245
Podul Roş	*Phaeophyscia orbicularis*	Poplar	**5,817**	0,217	**6,412**	0,246

SD – Standard Deviation

Conclusions

Microscopic investigations performed in the present work highlighted interesting cortex morphology after sectioning the lichenized fungi thallus. The details observed at the peripheral part of a cross section of the thallus like fungal colony reveal filamentous hyphal growth at the periphery and conglutinate areas in the microaerobic central part. It was also possible to distinguish the existence of atmospheric particles on the thallus surface. The final assumption is that the morphology of lichenized fungi might have a complex structure that requires careful analysis.

Following the analysis of *p*H, it was observed that the bark tree nature does not influence the chemical behaviour of the interest lichenized fungi samples investigated in the present study.

Elevated values of the *p*H and conductivity chemical parameters were especially measured in *Xanthoria parietina* lichenized fungi samples collected from locations with particularly potential to be exposed to heavily pollution (Tudor Vladimirescu, Baza 3, Podul Roş locations susceptible to pollution induced by heavy car traffic).

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