

Aspects regarding micromycetes involvement in wood biodeterioration

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Abstract. The micromycetes, as a result of mycelia growth and development on wooden surfaces, produce their physical and chemical degradation. We studied various wood samples, on different deciduous types: linden (*Tilia platyphyllos*) used as prop for icons in our country, hornbeam (*Carpinus sp.*) and oak (*Quercus sp.*). The samples have different durability, density, strength and degrees of humidity. After 30 days of incubation at a temperature of $30\pm 2^{\circ}\text{C}$ and a relative humidity of the air of over 90%, which are favorable conditions for the development of fungi, the samples of lime and hornbeam being used on a Czapek-Dox medium of culture (no extra carbon source), there were isolated lignolitic fungi – *Chaetomium globosum* and *Paecilomyces variotii*, as well as species that degrade pulp: *Trichoderma viride*, *Myrothecium verrucaria*, and species of *Aspergillus* and *Penicillium*. The quantification of the weight variation in wood pieces, as well as in sawdust, and the optical microscopy showed the involvement of fungi in the structural changes of wood. By the methods that we used, we tried to point out, on one hand, the load level of the mycoflora depending on the wood type (healthy, not attacked) and on its humidity and, on the other hand, the beginning of some bio-deterioration processes (changes in the structure of pulp). The purpose of this work is the application of a proper antifungic treatment to lime wood that is used as a prop for icons.

Key Words: Micromycetes, wood, linden, hornbeam, oak, biodeterioration.

Rezumat. Micromicetele, prin creșterea și dezvoltarea miceliilor pe suport lemnos, produc degradări fizico-chimice ale acestuia. Au fost studiate probe de lemn de diferite esențe de foioase: tei (*Tilia platyphyllos*) utilizat ca suport pentru icoane la noi în țară, carpen (*Carpinus sp.*) și stejar (*Quercus sp.*). Probele au o durabilitate, densitate, duritate și grade de umiditate diferite. După 30 de zile de incubare la o temperatură de $30\pm 2^{\circ}\text{C}$ și U.R. a aerului de peste 90 %, condiții favorabile creșterii și dezvoltării ciupercilor, de pe probele de lemn de tei și carpen aplicate pe mediu Czapek-Dox (fără sursă suplimentară de carbon) au fost izolate atât ciuperci lignolitice – *Chaetomium globosum* și *Paecilomyces variotii*, cât și specii implicate în degradarea celulozei: *Trichoderma viride*, *Myrothecium verrucaria*, specii de *Aspergillus* și *Penicillium*. Determinarea variației de masă pe bucăți de lemn ca și pe rumeguș, cât și microscopia optică au evidențiat implicarea ciupercilor în modificările de structură ale lemnului. Prin metodele utilizate am încercat să evidențiem pe de-o parte gradul de încărcare cu micofloră în funcție de esența de lemn – sănătos, neatacat și umiditatea acesteia, iar pe de altă parte, inițierea unor procese de biodeteriorare (modificarea structurii celulozei). Scopul lucrării este aplicarea unui tratament antifungic corespunzător în cazul lemnului de tei utilizat ca suport pentru icoane.

Cuvinte cheie: Micromicete, lemn, tei, corn, stejar, biodeteriorare.

Introduction. The development of mushrooms micelles on wood surface which they feed with involves two way of attack, according to the enzymes which occur in degradation mechanisms of cellulose and lignin.

From this point of view we deal with cellulose fungi which also attack other cellulose substrata, species of *Trichoderma*, *Aspergillus*, *Alternaria*, *Chaetomium*, *Aureobasidium*, *Fusarium*, *Myrothecium*, *Penicillium* and so on and lignin fungi – *Chaetomium globosum*, *Paecilomyces variotii* and *Stachybotrys atra* (www.indexfungorum.org). They degrade the lignin, causing weight losses and implicitly changes in the physical-chemical properties of the wood support.

The wood decay is characterized through texture loss, density decrease, loss of some mechanical features, wood becomes breakable, brittle, changing its color.

It is known the fact that a biodeterioration process is characterized through physical, chemical changes and also functional changes. The most important aspect to be

underlined in case of wood, as a substratum attacked by both microorganisms, mushrooms and by caries, is the brittle feature of the wood (Vornicu & Bibire 2002).

It's about a succession of populations, an interaction among the organisms that populate this substratum.

The presence and activity of micromycetes can be primary or secondary, according to the mechanical action performed by caries in the sense that the adults of the insects from Anobiidae family bring in their digestive tube spores of micromycetes also, infecting in this way the substratum attacked. The presence in the sawdust of these spores is the proof of this action (Bucşa 1981).

Concerning the three wood essences of deciduous trees in this study – linden (used as support for icons in our country), oak and hornbeam for comparison, the durability (natural resistance) of live wood is known to be of 15 years for oak whereas for linden and hornbeam is between 0 and 5 years. The oak and the hornbeam are considered the "heavy wood" as hardness, while the linden is called "semi light" of "soft" (Bucşa 1980).

Through the methods used we tried to outline on one hand the loading degree with micro-flora depending on the wood essence – healthy, unaffected – and on its humidity, and on the other hand the initiation of some biodeterioration processes.

Material and Method. The witness samples in this study belong to three essences of deciduous trees, linden (*Tilia platyphyllos*), oak (*Quercus sp.*) and hornbeam (*Carpinus sp.*) with a different durability (natural resistance), hardness and density. These samples have different degrees of humidity, being drawn in certain stages of their processing. Thus, with a certain degree of humidity were the samples: linden from sawmill, oak and hornbeam. Since in our country linden wood is used as support for icons, it was studied under three different forms: dried linden in workshop of different ages (1-4), dried linden with preparation layer, dried linden in workshop ready for painting.

Depending on the purpose there were used the following classic methods, according to www.indexfungorum.org (SR EN ISO 846/2000):

- exposure in Czapek-Dox environment, without supplementary carbon supply, in order to establish the biological loading (samples not inoculated);
- exposure in complete Czapek-Dox environment, with saccharose as supplementary carbon supply present also in the inoculum obtained from a spores suspension of rock salt – in order to establish the resistance to mouldiness in conditions of stimulated contamination;
- exposure in conditions of maximum humidity on different supports, especially arranged (for large samples an exsiccator was also used) and inoculation with spores + rock salt + saccharose for physical-chemical determinations.

The Micromycetes whose spores were used for inoculation belong to the following species: *Aspergillus niger* van Tieghem, *Aspergillus amstelodami* (Mangin) T. and C., *Aspergillus flavus* Link ex Fr, *Aureobasidium pullulans* Viala et Boyer, *Botrytis cinerea* Persoon ex Fries, *Chaetomium globosum* Kunze; Fries, *Cladosporium herbarum* Link ex Fries, *Memnoniella echinata* Rivolta, *Myrothecium verrucaria* Tode, *Paecilomyces variotii* Mainier, *Penicillium cyclopium* Westling, *Stachybotrys atra* Corda, *Trichoderma viride* Persoon ex Fries.

Incubation conditions were as follows: equipment – hydraulic thermostat type ITM Temperature, $30 \pm 2^{\circ}\text{C}$; relative humidity of air – over 90 %; exposure duration – 30 days.

The samples were periodically positioned (7, 14, 21 and 30 days) at a stereomicroscope with a magnifying power of X50 and they were photographed.

Results and Discussion. First we established the biological loading. The results obtained are presented in table no. 1 and figures no. 1-7.

Table 1

Species of micromycetes with biodeteriogen potential for wood

No.	Sample	Species of micromycetes from spontaneous flora
1	Linden from sawmill	<i>Trichoderma sp.</i> , <i>Aspergillus niger</i> , <i>Penicillium sp.</i> , unidentified species 1 (long conidioforo and small black conidia - possibly <i>Stachybotrys sp.</i>)
1a	Linden from sawmill (whole wood)	Loose micelle with beige fructifications <i>Paecilomyces variotii</i>
2	Oak	Unidentified species no. 2 - colonies which cover 100 % the sample with small greenish-beige conidi
3	Hornbeam	<i>Alternaria sp.</i> (thin branchless chains of inhomogeneous black conidia 100 %
3a	Hornbeam (whole wood)	<i>Chaetomium sp.</i> – perithecia, <i>Alternaria sp.</i> , covering 100 %
4	Dried linden in workshop of different ages	
4a	1	Perithecia of <i>Chaetomium sp.</i> covering 100 %
4b	2	<i>Chaetomium sp.</i> , <i>Mucoraceae-Rhizopus sp.</i> , <i>Aspergillus sp.</i> , <i>Trichoderma sp.</i>
4c	3	<i>Aspergillus niger</i> , <i>Chaetomium sp.</i> , <i>Penicillium sp.</i> , <i>Paecilomyces variotii</i>
4d	4	<i>Aspergillus niger</i>
5	Dried linden in workshop with preparation layer	<i>Aspergillus niger</i>
6	Dried linden in workshop ready for painting	<i>Aspergillus niger</i> , <i>Paecilomyces variotii</i>

What is interesting to observe is the presence of colonies of *Paecilomyces variotii* on the samples: linden from sawmill, dried linden in workshop ready for painting, as well as the perithecia of *Chaetomium sp.* seen on three of the four types of dried linden in workshop of different ages and on the hornbeam sample (Bucşa & Bucşa 2005). These species are the only ones from micromycetes which together with *Stachybotrys atra* determine the "soft rot" at wood. *Chaetomium*, belonging to the category Ascomycetes colonizes the substratum stretching the hyphae to the interior of the wood realizing an oblique penetration of cell walls, using as source of food the reserve matters that the wood has. This type of attack is frequently met and it produces mechanical changes at the surface, a softening of the wooden tissue, followed by massive loss of weight, but they are not very active lignolytically (Vornicu & Bibire 2002).

These surface changes after a short delay of exposure – 30 days – were highlighted by optical microscopy, in the case of hornbeam and the linden dried in workshop 3 (figures 7 and 8). On the other hand, fungi like *Trichoderma viride* (on wood there is also the species *Trichoderma lignorum*), *Aspergillus niger*, *Penicillium* species which colonized the samples analyzed as well as *Aureobasidium sp.*, *Fusarium sp.* and so on, from Deuteromycetes category are responsible also for a secondary attack regarding the usage degree of the cellulose in wood after a partial depolymerization caused by other microorganisms, completing the destruction. From those which come back periodically we mention *Trichoderma viride* si *Gliocladium roseum* (Vornicu & Bibire 2002).

In the pictures taken after 7 days there can be distinguished the species with rapid growth *Aspergillus niger* and *Trichoderma viride* (figures 1-7) whereas in the pictures made at stereomicroscope - after 3 months - we notice the presence of perithecia of *Chaetomium* (figure 8).



Figure 1. Linden from sawmill.

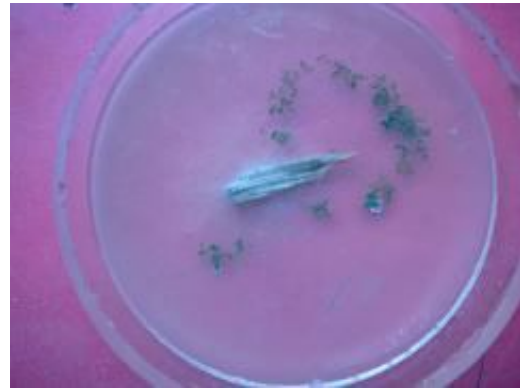


Figure 2. Hornbeam.



Figure 3. Dried linden 1.



Figure 4. Dried linden 2.



Figure 5. Dried linden 3.



Figure 6. Dried linden 4.



Figure 7. Dried linden ready for painting.

From the numeric point of view, the most aggressed by the colonies of micromycetes was the linden, wood with soft essence and the least colonized the oak sample, which though in conditions of U.R. of over 90 % colour the culture or water environment, in brown, eliminating quercitine, probably tannin (Şesan & Tănase 2006; Tănase & Şesan 2006).

Further on, we determined the resistance to mouldiness in conditions of stimulated contamination. The results obtained are presented in table no. 2.

Table 2

Resistance to mouldiness in conditions of stimulated contamination

Sample	Covering degree (%)	Predominant micromycetes
Linden from sawmill	100	<i>Aspergillus niger</i> , <i>Trichoderma viride</i> , <i>Penicillium cyclopium</i> , <i>Myrothecium verrucaria</i>
Dried linden 1	50-75	<i>Aspergillus niger</i> , <i>Trichoderma viride</i> , <i>Paecilomyces variotii</i>
Dried linden 2	50	<i>Aspergillus niger</i> , <i>Trichoderma viride</i> , <i>Penicillium cyclopium</i>
Dried linden 3	100	<i>Aspergillus niger</i> *
Dried linden 4	100	<i>Aspergillus niger</i> , <i>Trichoderma viride</i> **
Hornbeam	100	<i>Alternaria sp.</i> ***, <i>Aspergillus niger</i> , <i>Trichoderma viride</i> , <i>Penicillium sp.</i> , <i>Myrothecium sp.</i> , <i>Cladosporium sp.</i>
Oak	50-75	<i>Aspergillus niger</i> , <i>Paecilomyces variotii</i>
Dried linden in workshop ready for painting	75-100	<i>Aspergillus niger</i> , <i>Trichoderma viride</i>
Dried linden in workshop ready for painting, with preparation layer	75-100	<i>Paecilomyces variotii</i> , <i>Trichoderma viride</i> (prevails on the prepararion layer), <i>Penicillium cyclopium</i>

* *Aspergillus niger* – ubiquitous mushroom with a very rich enzyme equipment,

** *Trichoderma viride* – ubiquitous mushroom, aggressive,

*** *Alternaria sp.* – comes from the infection of the spontaneous flora sample, not from the inoculum.

The results presented in table no. 2 point out the presence of colonies of *Myrothecium verrucaria*, species known as cellulosolitic, on the samples with a certain humidity (linden from sawmill and hornbeam). Another aspect is represented by the different degrees of coverage with colonies of micromycetes.

Some aspects of sample behavior after 30 days of exposure in the conditions method 3 used by us are presented in figures 8-12.



Figure 8. *A. niger* on oak.



Figure 9. Dried linden in workshop ready for painting.



Figure 10. Hornbeam inoculated in exsiccator.



Figure 11. *A. flavus* on linden from sawmill.



Figure 12. *Trichoderma viride* on dried linden 3.

Then, we determined the weight loss. We used the method SR EN ISO 846/2000 which was also used in the case of biodegradable composites having as filling wood flour. There were prepared lots of 4 samples (2 witnesses and 2 inoculated samples) for each type of wood analyzed. They were analyzed from this point of view: hornbeam, dried linden ready for painting and sawdust obtained from the linden piece from sawmill. These samples were weighed with an electronic balance with a 4 decimal accuracy, model A & D – series HR before and after the exposure at the action of some species of micromycetes, for a 30 day period.

There were performed weighing at regular lapses of time (24 h) till we got a constant mass, with an accuracy of 0,1 mg. Between weighing the samples were kept in an exsiccator with CaCl_2 for humidity absorption.

The mass variation was calculated according to the formula: $\frac{\Delta \bar{m}_i - \Delta \bar{m}_s}{\bar{m}_e}$ in which:

$\Delta \bar{m}_i$ - mass variation of inoculated samples; $\Delta \bar{m}_s$ - mass variation of sterile samples;
 \bar{m}_e - average of initial masses of test-piece.

For all determinations made after the accelerated ageing (temperature, air U.R. and the presence of mould spores) the samples were washed with tap water, then immersed for an hour in mercuric chloride (for spores destruction), washed again and left to dry on a filter paper at room temperature. In table no. 3 there are presented the average values of weight loss.

Table 3

Weight loss determination

Wood sample	Weight loss (mg)
Hornbeam	0.4
Dried linden ready for painting	1.5
Sawdust (linden form sawmill)	12.9

The results obtained in a very short period of time, confirm the fact that the linden sample – essence used as wood support for icons, as soft wood, losses 4 times more of its weight than the hornbeam sample. In the case of sawdust, the large contact surface with extra-cellular enzymes produced by mushrooms as well as the crystalline structure destroyed by the mechanical activity, determines a significant weight loss.

The study of structural changes of different nature occurred at wood samples as a result of mould activity

Performed determinations:

- micro-hardness – measurements made on hornbeam and linden form sawmill samples before and after the mould activity, to see if there are differences in the change of hardness value for samples of different nature, as a result of mould activity;
- density – measurements made on linden form sawmill and dried linden samples before and after the mould activity, to see the influence of drying degree of the sample on the hydrostatic density;
- phase quality analysis – measurements of X-rays diffraction on all samples, before and after the mould activity, to see the possible changes in the crystalline structure of samples;
- optical microscopy – optical microscopy images on all sample, before and after the mould activity, to see possible changes in the microscopic structure of samples as a result of mould activity.

Analyzed samples were as follows: linden from sawmill, dried linden in workshop ready for painting, dried linden in workshop ready for painting with preparation layer, dried linden 1, dried linden 2, dried linden 3, dried linden 4, hornbeam, oak.

Equipment was represented by Microdurometer type FM 700, environment conditions: $t = 20.8^{\circ}\text{C}$, humidity 37 %, trial charge: 25 gf, indenter: Vickers, impression length: 20 s. Results are noted in table 4.

Table 4

HV0.025/20						
Sample	Measured values					Average value
Hornbeam	6.8	7.9	7.2	8.0	9.0	7.8
Linden form sawmill	5.6	5.0	5.0	4.8	4.6	5.0

Environment conditions: $t = 21.4^{\circ}\text{C}$, humidity 42%

Trial charge: 25 gf

Indenter: Vickers

Impression length: 20 s

Results after mould activity

HV0.025/20						
Sample	Measured values					Average value
Hornbeam	8.0	9.3	8.3	8.8	8.4	8.6
Linden form sawmill	5.9	5.7	4.4	5.0	5.5	5.3

Opinions and interpretations

As a result of analyses made on hornbeam and linden we notice a growth of micro-hardness both for hornbeam and for linden, with a value of 10% for hornbeam and 6% for linden.

Hydrostatic density

Equipment: Analytical balance type XS 204 with density kit

Environment conditions: $t = 20.3^{\circ}\text{C}$, humidity 39%

Immersion liquid: water

Water temperature: 20.0°C

Results

Table 4 – Continued

Sample	Hydrostatic density [g/cm^3]		
	Measured value		Average value
Linden from sawmill	0.543	0.541	0.542
Dried linden	0.555	0.551	0.553

Environment conditions: $t = 21.1^{\circ}\text{C}$, humidity 46%

Immersion liquid: water

Water temperature: 20.0°C

Results after mould activity

Sample	Hydrostatic density [g/cm^3]		
	Measured values		Average value
Linden from sawmill	0.569	0.573	0.571
Dried linden	0.368	0.562	0.465

Opinions and interpretations

Following the analyses performed on linden from sawmill and dried linden ready for painting we notice that as a result of mould activity, the density of linden from sawmill grows. At samples of dried linden the 2nd sample it gave an increased value of density whereas for the 1st sample the value was lower. Since this method of density determination implies sample immersion into a liquid, in our case distilled water, such a variation in density can be caused only by the porosity growth of the 1st sample.

Phase qualitative analyses through X rays diffraction

Equipment:

Diffractometer Bruker AXS D8 ADVANCE

- X rays tube with anode of Cu $\lambda = 1.5406 \text{ \AA}$

- 40kV / 40 mA

- filter K_{β} Ni

- speed: 2 sec/pas

- pas: 0.04°

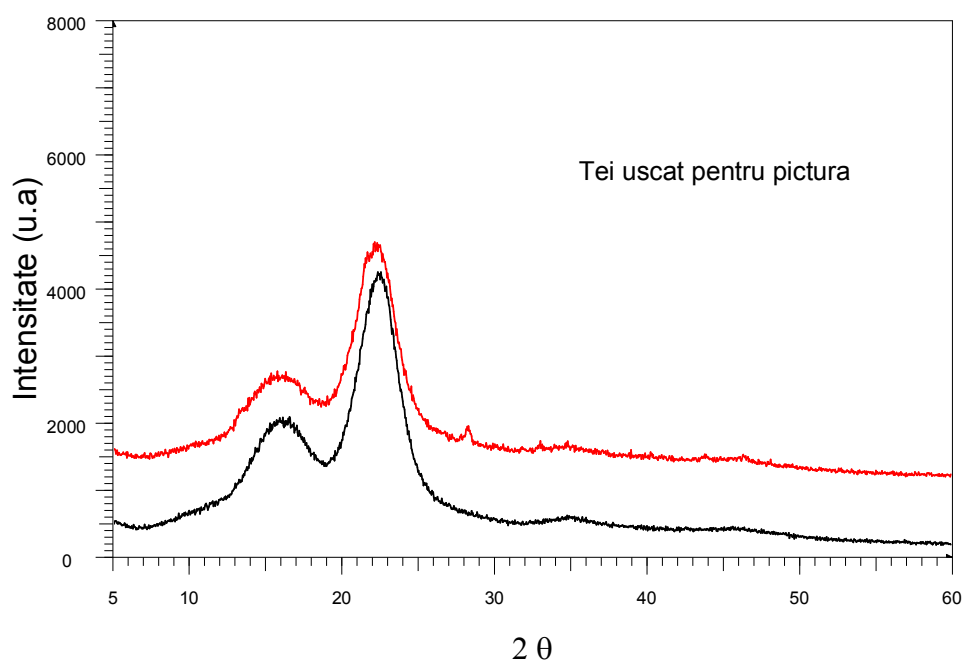


Figure 13. Comparison between spectrums of X rays diffraction obtained on the sample of dried linden in workshop ready for painting, before (black) and after (red) mould activity

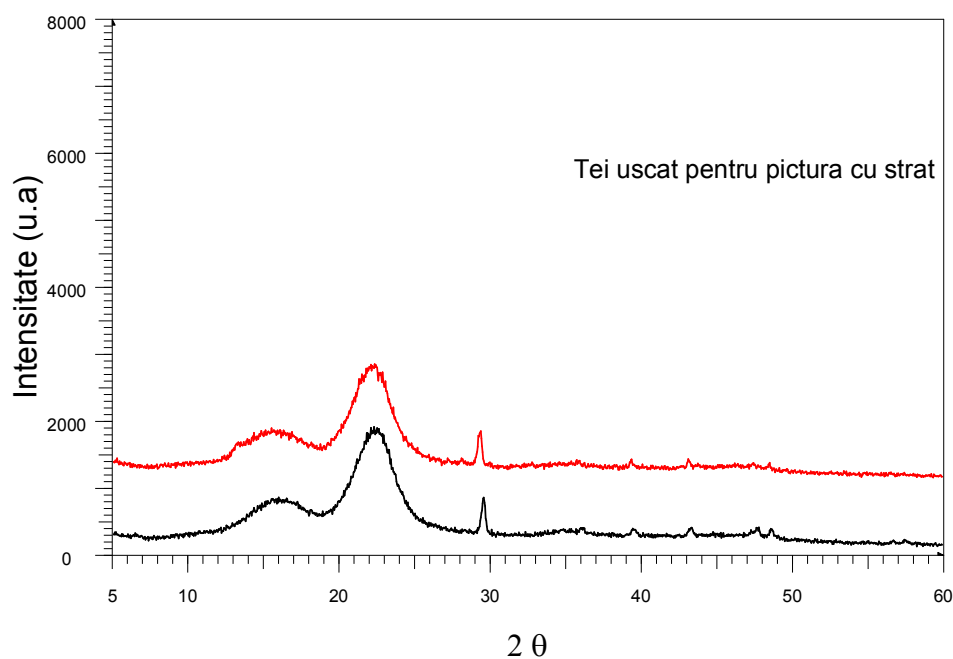


Figure 14. Comparison between spectrums of X rays diffraction obtained on the sample of dried linden in workshop ready for painting with preparation layer, before (black) and after (red) mould activity

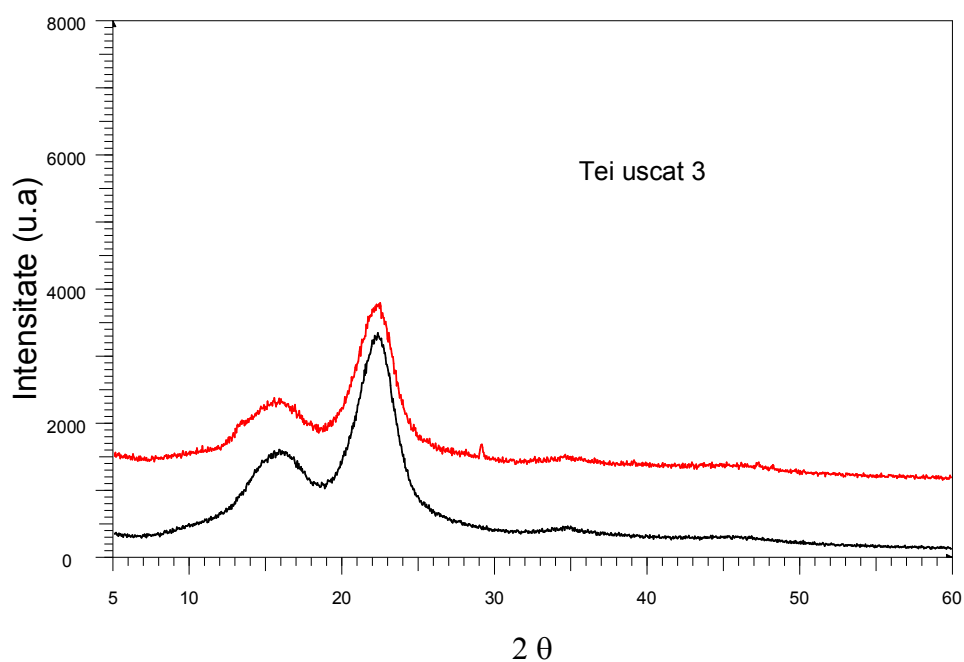


Figure 15. Comparison between spectrums of X rays diffraction obtained on the sample of dried linden 3, before (black) and after (red) mould activity

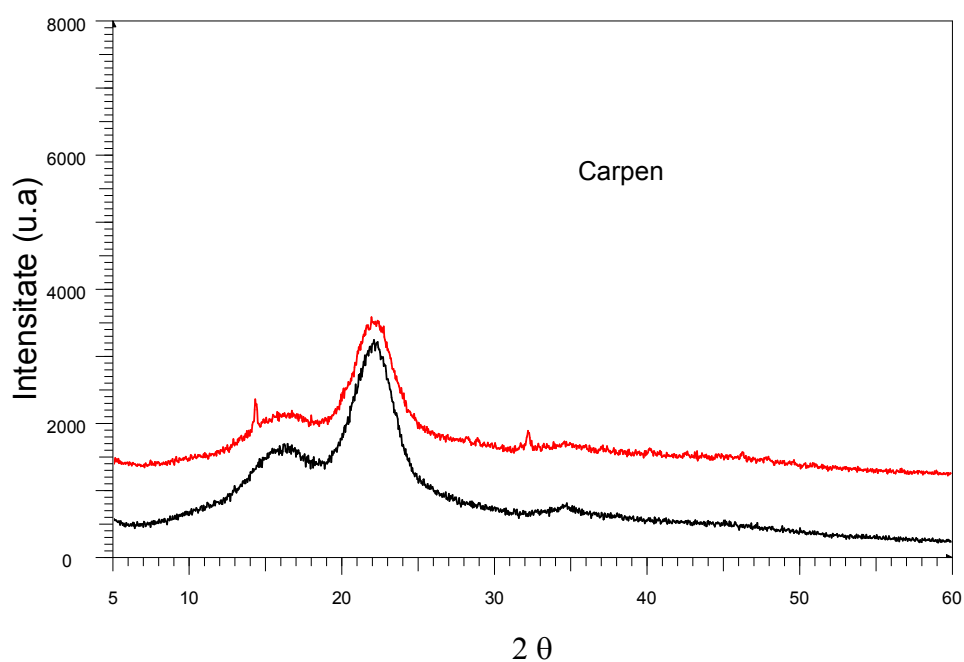


Figure 16. Comparison between spectrums of X rays diffraction obtained on the hornbeam sample, before (black) and after (red) mould activity

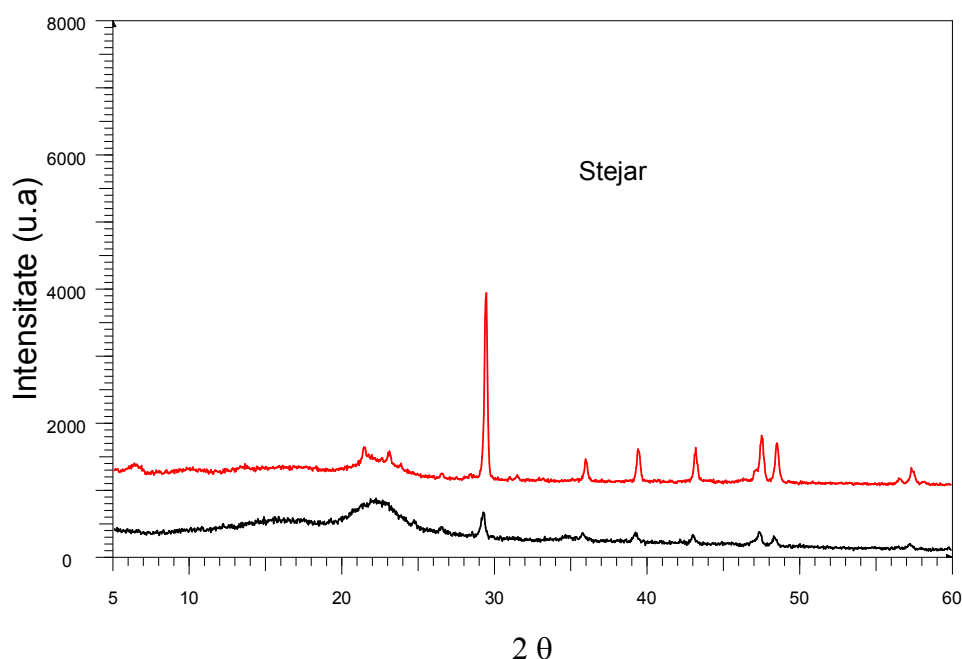


Figure 17. Comparison between spectrums of X rays diffraction obtained on the oak sample, before (black) and after (red) mould activity

Opinions and interpretations

The wood, whatever its nature (in this case linden, oak or hornbeam) presents at X rays diffraction only one structure. As a result of mould activity no change of this structure can be noticed, possibly because of the short period of exposure. The sample of dried linden in workshop ready for painting with preparation layer presents a supplementary phase resulting from the preparation layer.

Only at some samples the occurrence of a secondary phase can also be noticed as a result of mould activity. At samples of dried linden ready for painting and dried linden 3 some diffraction peaks can be noticed which were identified as a rhombohedral structure of CaCO_3 (figures 13, 14 and 15). At the hornbeam sample a tetragonal structure can be noticed which was identified with $\text{C}_2\text{CaO}_4 \cdot 2\text{H}_2\text{O}$ (fig.16).

The analysis of oak wood sample is considered unconvincing because of the small sample quantity (figure 17). After the mould activity a prevailing phase of CaCO_3 can be noticed. The reason for which such a big quantity of CaCO_3 can be noticed is just the fact that the sample analyzed is too small. That's why the results obtained on this sample can't be compared with the results obtained on the other samples.

Optical microscopy

The images of optical microscopy were obtained with the same device with which the micro-hardness was determined, because the microdurometer FM 700 is also equipped with CCD camera and software for image processing.

The images were obtained at different optical magnifying levels: 50x, 100x, 200x, 500x (ocular eye glass with 10x magnifying glass, and lens with optical magnifier of 5x, 10x, 20x and respectively 50x).

We present further on only the images obtained at 200x enlargement, which was considered optimum for observing samples' microstructure (figures 18, 19 and 20).

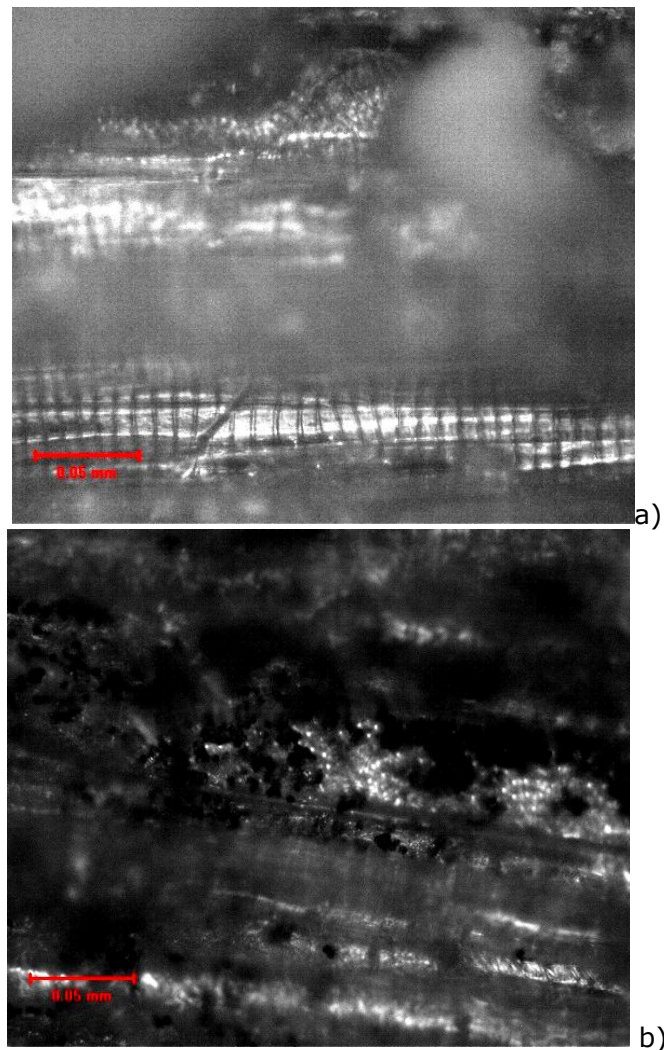


Figure 18. Dried linden in workshop ready for painting with preparation layer before (a) and after (b) mould activity

Opinions and interpretations

As a result of optical microscopy analysis, the only samples which present obvious changes following the mould activity are the samples of dried linden in workshop ready for painting with preparation layer, dried linden 3 and hornbeam.

Conclusions. Through combined biological methods with optical microscopy techniques and determinations of weight loss, micro-hardness, hydrostatic density as well as the qualitative analysis of stage through X diffraction there were studied a series of witness samples belonging to 3 deciduous essences of wood – linden, hornbeam and oak.

We noticed the presence of micromycetes which produce the wood “soft rot” namely lignin species: *Chaetomium globosum* and *Paecilomyces variotii* on linden and oak samples.

Obvious changes in wood microstructure owing to mould activity were found on the samples of dried linden in workshop ready for painting with preparation layer, dried linden of various ages, sample 3 and hornbeam.

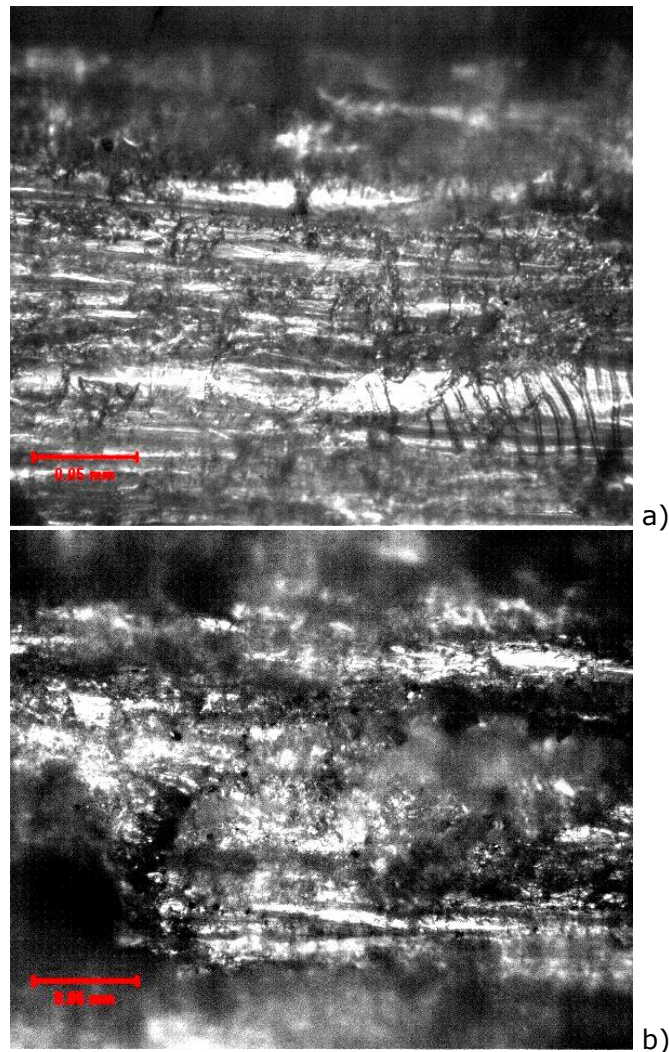


Figure 19. Dried linden 3 before (a) and after (b) mould activity

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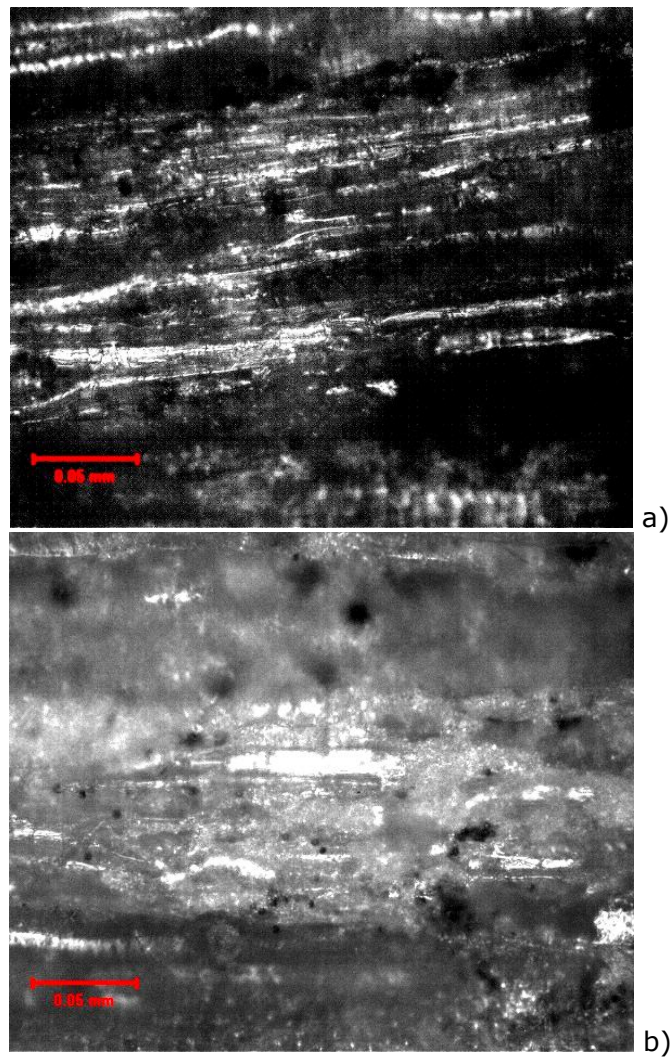


Figure 20. Hornbeam before (a) and after (b) mould activity

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