BIODETERIORATION OF WOODEN CHURCHES AND DEVELOPING A STRATEGY FOR RESTORATION. CASE STUDY: THE CHURCH OF URȘI

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Keywords: biodeterioration, biodeteriogens, brown rot fungi, white rot fungi, boring insects, mural painting, wooden church, Urşi

Abstract: Biodeterioration and biodeteriogens from the wooden church located in the village of Urşi, Co. Vâlcea are correlated with environmental conditions and are the consequence of the poor state of preservation of the mural paintings and the wooden structure of the monument. A high degree of biodeterioration was recorded both inside and outside the church. The main biodeteriogens are: basidiomycetes (*Antrodia sinuosa*, *Phellinus sp*), filamentous fungi (*Trichoderma viride*, *Aspergillus niger*, *Cladosporium sp*, *Penicillium sp*) and boring insects (*Hylotrupes bajulus*, *Annobium punctatum*). Investigations on one of the beams (*beam no. 2*) were performed both *in situ* and in the restoration studio, allowing us to develop a specific strategy for restoration and conservation.

Rezumat: Biodeteriorarea și biodeteriogenii din biserica de lemn din satul Urși sunt caracteristice condiţiilor de microclimat și sunt rezultatul stării precare de conservare a picturii murale și a structurii lemnoase. Atât în interior cât și la exterior s-a pus în evidenţă un stadiu foarte avansat de biodeteriorare. Principalii biodeteriogeni sunt specii de basidiomicete (*Antrodia sinuosa* și *Phellinus sp*), fungi filamentoși (*Trichoderma viride*, *Aspergillus niger*, *Cladosporium sp* și *Penicillium sp*) și insecte xilofage (*Hylotrupes bajulus* și *Annobium punctatum*). Cercetările efectuate *in situ* precum și în atelier pe una dintre bârne (notată cu nr. 2) au permis elaborarea strategiei de conservare restaurare.

Wooden churches are spread all over the world, but biodeterioration produced mostly by basidymycetes is always in connection with the climate and distribution of species, even enzymatic mechanism is involved in wood decomposition. In those areas of Chile where the climate is of Mediterranean type, Ortiz *et alii*[1] identified in wooden historical monuments built between 1700 and 1800 a total of 29 species of basidiomycetes and 18 species of ascomycetes.

In Romania, where the climate is Temperate Continental, there are over 1200 historical wooden churches of which about 650 are located in Transylvania and Banat; more than 490 are located in Oltenia, Walachia and Dobruja and about 300 are in Moldavia.[2] Bucşa *et alii*[3] found in Romanian wooden churches a total of 15 species of basidiomycetes and numerous microfungi; Cojocariu *et alii*[4] indicated 8 species of basidiomycetes in St. Nicholas church in Vrâncioaia (Vrancea County); Dăneasă[5] found different fungal species in the church of Boz (Hunedoara County). Other scientists found many species of basidiomycetes on wooden objects placed on or along the walls.[6]

Humar *et alii*[7] isolated *Antrodia vaillantii* and found that this species is able to hydrolyse cellulose and hemicelluloses very quickly, browning the wood; on the contrary, *Gloeophyllum trabeum*, being able to hydrolyse lignin, discolours the wood in the first stage and then it browns it.

In Latvia, where the climate is temperate (Humid Continental), *Serpula lacrymans* and *Antrodia*[8] are considered the most dangerous biodeteriogens of wood. Resistance of basidiomycetes to EU accepted and recommended biocides excludes chemical treatments for decontamination. Treatment with ionizing radiation (gamma rays 24kGy) was efficient for decontamination of wooden objects from churches in the areas of Cluj and Sibiu;[9] the same effect was obtained by exposing wooden objects to microwaves.[10]

Antrodia and *Serpula* are brown rot fungi and produce rapid depolymerisation of the polysaccharide component of wood.[11] In the early stages of degradation, non-enzymatic oxidative reactions are involved. This explains how to go beyond the barrier imposed by lignin as a result of the failure to produce ligninase and how basidiomycetes develop the strategy to protect endoglucanases, which have large molecules and cannot penetrate the way to the cellulose. Identification of hydrogen peroxides in the mycelium led to the hypothesis that degradation is based on the Fenton reaction. Brown rot fungi contain endoglucanases which act on cellulose molecules generating the reducing bonds attacked by exoglucanases. Cellobiose molecules are hydrolysed by β-glucosidases producing glucose molecules which are assimilated by hyphae.

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1 Ortiz *et alii* 2014, pp. 569-572.

2 See *Biserici de Lemn din România* (Wooden Churches of Romania), database maintained by the National Institute of Heritage. http://www.cimec.ro/Monumente/LacaseCult/lemnDefault_ro.htm (accessed 9 April 2014).

3 Bucşa, Bucşa 2010, p. 75.

4 Cojocariu *et alii* 2007, pp. 125-127.

5 Dăneasă 2013, pp. 53-64.

6 Gomoiu, Mohanu 2001, pp. 2-4; Gomoiu *et alii* 2010 pp. 88-90.

7 Humar *et alii* 2010, pp. 88-90.

8 Irbe, Andersone 2010, pp. 94-97.

9 Cutrubinis *et alii* 2010. pp. 82-87.

10 Merle Strätling *et alii* 2010, pp. 148-150.

11 Valášková, Baldrian 2006, pp. 3613-3622.

Fig. 1. Beam no. 2 shows the characteristic morphology of the biodeterioration process.

Fig. 2. Securing the pictorial layer before removing beam no. 2.

Fig. 3. Dismantling of beam no. 2.

The wooden church in the village of Urşi[12] (commune Popeşti, county Vâlcea) was built by the local community in 1795 or 1794 (7303 *Anno Mundi*, according to the date carved above the entrance to the church), and was repaired and painted soon after 1840 by a certain Nicholas Milcoveanu, a local historical figure of the 19th century. The painting is dated to 1843 by an inscription on the apse walls, signed by painters Gheorghe, Nicolae and Ioan. The three painted both the interior and exterior of the church in a mixed technique al fresco – al secco. As seen in archive images, the mural paintings and wooden substrate were in a continuous degradation process, as the structure suffered from an unstable foundation ground. The whole church went leaning to the north-west. In 1943 the shingle roofing had to be replaced, which did not address and solve the structural problems. Much later, in 2010, the first collapse episode occurred, when part of the apse vault fell in. Following the disaster, a rescue plan was initiated, within the *60 Wooden Churches* programme run by the Pro Patrimonio Foundation and the Romanian Chamber of Architects, a programme which relies on volunteering and creates partnerships between professional institutions and local communities.[13]

Preliminary measures were taken during the successive campaigns between 2010 and 2014, aiming to investigate and diagnose the degradation processes, and further to secure the painting in view of the interventions on the built structure – the temporary dismantling of the vault and iconostasis, lifting up and straightening of the church and its resettling on a new foundation.

Regarding the interior and exterior mural painting of the wooden church of Urşi, research started with the examination of the state of conservation. It was found that the biodeterioration process is expanded both inside and outside, being identified in the wooden structure and the mural painting, in the pronaos, naos, apse, vault and floor. The main biodeteriogens are basidiomycetes, filamentous fungi and boring insects. After *in situ* emergency interventions on the mural paintings, the need to lift and resettle the church on a new foundation required the dismantling of the vault and iconostasis, followed by the storage of the painted beams and planks in a space built for this purpose. The dismantled iconostasis was conserved inside the main church of the village. Later, the elements of the vault and iconostasis were gradually transported in the studios of the Conservation and Restoration Department of the National University of Arts, in Bucharest (NUAB). Each of these component parts was analysed and treated individually. A detailed photo survey of the vault was carried out, with the identification and tagging of each plank which carried mural painting.

[12] For a historical account on this church, a detailed analysis of its mural paintings and an inquiry into its conservation problems, see Mohanu *et alii* 2013, p. 249.

[13] The restoration project (by architects Ştefan Bâlici and Virgil Apostol of OPUS Architecture Studio, Bucharest) was prepared in cooperation with the Department of Conservation and Restoration of the National University of Arts in Bucharest (prof. dr. Dan Mohanu and prof. dr. Ioana Gomoiu). Chemical and physical analyses have been performed by the National Museum of Romanian History; see Mohanu *et alii* 2013, pp. 249-252.

Fig. 4. Wood sample covered by brown thick mycelium.

A special situation is presented by the beams with mural painting which make the dividing gable wall between pronaos and naos. Here, in order to restore the structural integrity of the church, one of the beams – tagged no. 2 – had to be removed for treatment, together with its paintings covering both faces. On the surface facing the pronaos, at both ends, around the joining points with the beams in the sidewalls, in the areas where the mural painting had disappeared, a typical morphology for the mycelium and fructification bodies could be identified (Fig. 1). On the surface facing the naos, as well, at both ends, a similar lack of painting (on a smaller area) and the same microbiologically characteristic morphology were revealed. Penetrating damp was a factor of degradation which contributed significantly to the growth and multiplication of the biodeteriogens in these areas. The spread of *Antrodia* species *in situ* was favoured by air currents that carried both spores generated by the fructification bodies, and mycelium fragments.

In order to establish the conservation-restoration protocol, *beam no. 2* was examined and then treated sequentially as follows: biodeterioration identification and location *in situ*, securing pictorial layer (Fig. 2), dismantling (Fig. 3), biocide treatment, packaging and transportation to NUAB. At the studios of the Conservation and Restoration Department of NUAB, *beam no. 2* was examined on both faces and all edges in order to reveal biodeteriogens, size of biodeteriorated areas and changes in the structure of wood.

Two distinct mycelium morphologies were identified: one with a crusty, thick, brown (in various shades) look and one with a felty, thin, white look. In addition, holes and

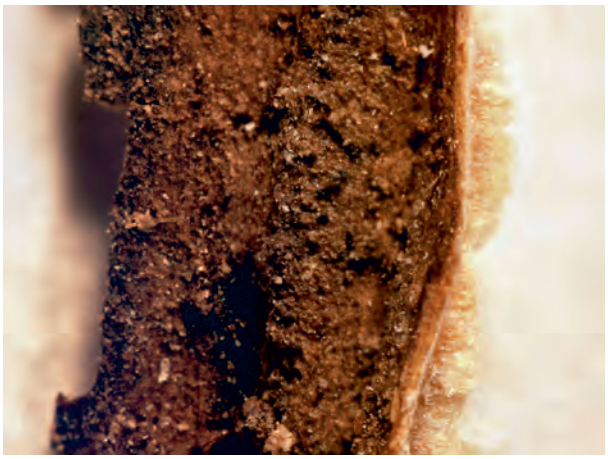

Fig. 5. The top surface of the sample confirms, by optical microscopy, the existence of thick, brown mycelium and deposits (*Phellinus sp.;* magnification x = 80).

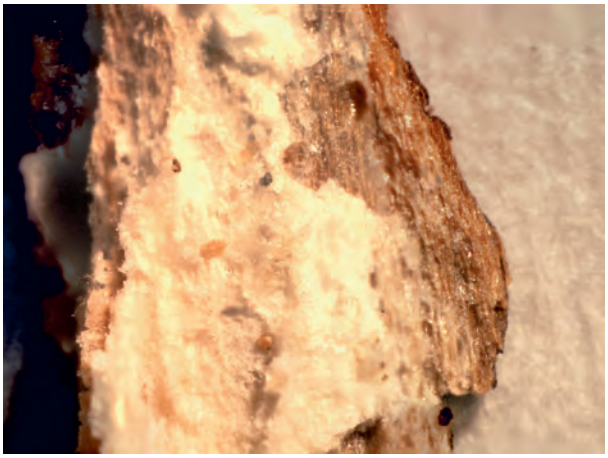

Fig. 6. The bottom surface of the sample reveals compact, white mycelium (*Phellinus sp.*; x = 80).

tunnels of boring insects were revealed. The wood samples collected from the entire surface of the beam, including from the areas close to the secured mural painting, were analysed by light microscopy as well as microbiologically (cultivation method).

The wood samples which were apparently hard but covered by thick, crusty and brown mycelium (Fig. 4 and Fig. 5) became friable during harvest activity. The seemingly normal appearance is given by the crusty mycelium which keeps the cellulose fibres in a common package, even though they are fragmented. After removing the crusts of mycelium, it was found that the wood was discoloured as a result of hydrolytic activity of ligninase and cellulases synthesized by the mycelium. On the underside of the sample, the mycelium was white with felty aspect (Fig. 6); it was protected by the crusty part, thus maintaining its viability. Microbiological analyses revealed that the area had been contaminated with *Phellinus sp.*

Fig. 7. On sample harvesting for microbiological analysis, it was found that the wood is friable.

Fig. 8. Deep into the wood consecutive layers of mycelium developed by *Antrodia sinuosa* (x = 80) were revealed.

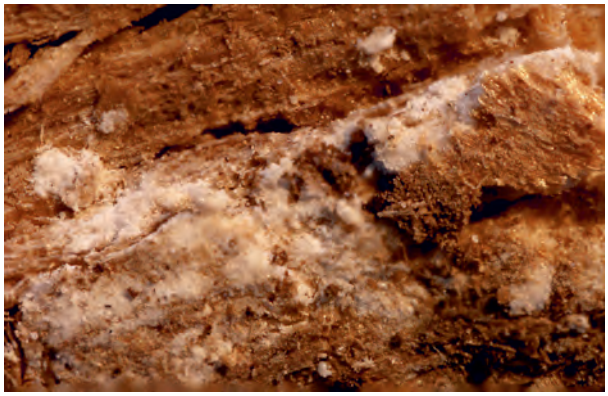

Fig. 9. Isolated and confluent colonies of *Phellinus sp.* (x = 80).

Those wood samples apparently hard but fragmented in cuboid forms covered by white mycelium with felty aspect (Fig. 7) became friable after collection, with visible discoloration. Within the wooden beam, layers of mycelium were revealed (Fig. 8), which developed in time depending on alternating environmental conditions, favourable to the species' biology or unfavourable. Contamination with *Antrodia sinuosa* was found in this area through microbiological analyses.

The samples of friable bleached wood are covered both on top and bottom surfaces by mycelium grown in isolated or confluent colonies (Fig. 9).The spread of mycelium deep into the wood occurs through larvae of xylophagous insects developing horizontal tunnels. Microbiological analyses established that this area was contaminated with *Phellinus sp.*

The samples of brown wood covered by white mycelia are bleached on the bottom part and are covered by compact masses of felty mycelium (Fig. 11). Microbiological analyses revealed that the upper part was contaminated with *Antrodia sinuosa* which is responsible of typical cuboid cracking and the lower part was contaminated with *Phellinus sp.* which produces discoloration of the wood.

The wood samples showing brown and discoloured areas covered by masses of white mycelium show successive layers of compact mycelium (Fig. 12) grown during periods when the water content was high. By microbiological analyses it was found that the upper part of the sample was contaminated with *Antrodia sinuosa* and the bottom with *Phellinus sp.*

The samples of wood covered with compact and brittle mycelium are contaminated in the depth with *Antrodia sinuosa*; both aerial and substrate mycelium was revealed (Fig. 13), because of air entering through the many cracks.

The samples of brown wood with cuboid cracking aspect become friable after harvest and are fully colonized with white and felty mycelium. This morphology of wood is specific for brown rot fungi and it is correlated with laboratory tests that identified *Antrodia sinuosa*.

The wood samples from areas close to the mural paintings are completely colonized with white, felty mycelium or with compact mycelium, both at surface as well as in depth (Fig. 14); microbiological analysis showed that the wood samples and the areas around them were contaminated with *Antrodia sinuosa*.

The wood samples with typical cuboid cracking covered by successive layers of mycelium (Fig. 15) reveal a similar in-depth stratification (Fig. 16), which proves that the colonization process initiated by *Antrodia sinuosa* took place in different stages related to the presence of water in the substrate; microbiological analyses shown that the samples were contaminated with *Antrodia sinuosa*.

The wood samples with almost normal appearance but covered with strands on the surface (Fig. 17) are completely

Fig. 10. Brown coloured wood sample colonized on the surface by white and viable mycelium of *Antrodia sinuosa* (x = 40).

Fig. 11. On the bottom surface the sample is colonized by white and viable mycelium of *Phellinus sp.* (x = 80).

Fig. 12. Wood sample colonized in-depth by consecutive masses of mycelium (*Phellinus sp.*; x = 80).

Fig. 13. Wood sample colonized by aerial and submerged mycelium (*Antrodia sinuosa;* x = 80).

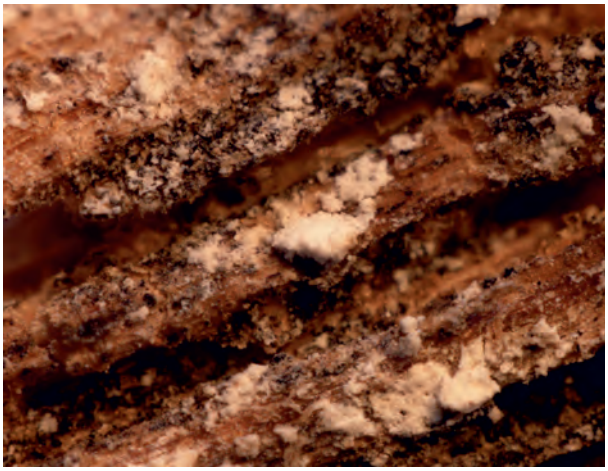

Fig. 14. *Antrodia sinuosa* mycelium growing both on the surface and in depth, having two distinct aspects: white and felty, respectively white and compact (x = 60).

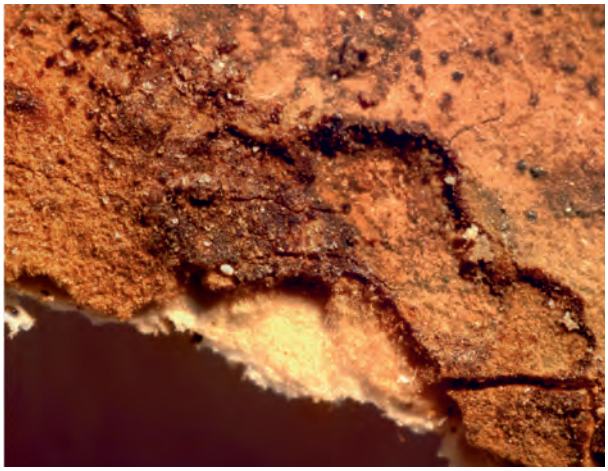

Fig. 15. The wood sample is covered at the surface successive layers of *Antrodia sinuosa* mycelium (x = 80).

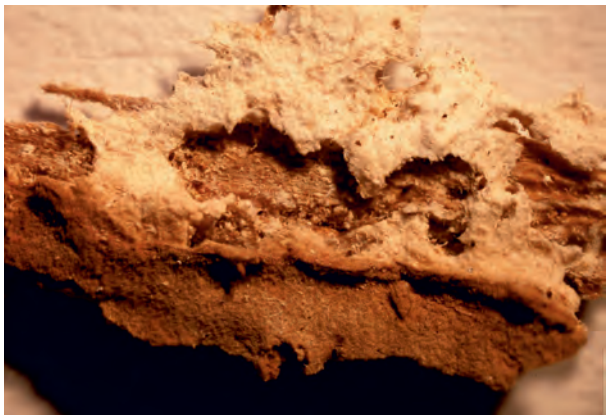

Fig. 16. The same sample revealed also in its depth successive layers of *Antrodia sinuosa* mycelium (x = 80).

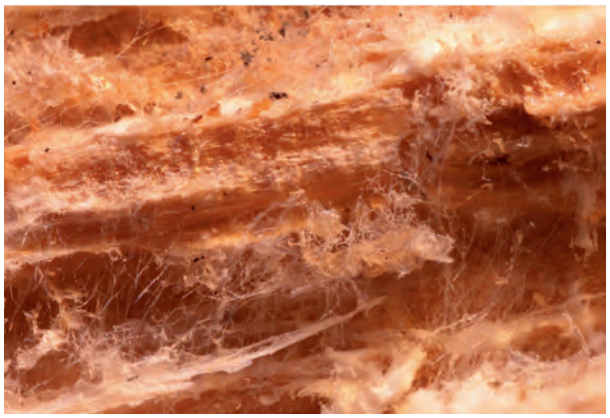

Fig. 18. In depth the wood is colonized by viable mycelium of *Phellinus sp.* (x = 80).

colonized by mycelium within, have a friable aspect and are discoloured (Fig. 18).

The biodeteriogens identified on *beam no. 2* belong to macroscopic fungi (basidiomycetes: brown rot fungi – *Antrodia sinuosa*; white rot fungi – *Phellinus sp.*), microscopic fungi (*Trichoderma viride*, *Aspergillus niger*, *Cladosporium sp*, *Penicillium sp.*) and insects (*Hylotrupes bajulus* and *Annobium punctatum*).

The biodeterioration caused by the fungus *Antrodia sinuosa* is a result of lack of maintenance of the monument, but also of improper interventions carried out over time. Fungi were identified in places where wood was soaked through infiltration and capillarity, or where condensation occurred.

At the first examination of the monument an increased amount of moisture was found in the floor, within the lower part of the walls, in the vault and the upper part of the walls (the latter being a result of roof deterioration and partial collapse). *Antrodia sinuosa* can colonize wood both with high water content (80-90%) and with low content (even 30%) but the growth rate is dependent on the water

Fig. 17. Strands of *Antrodia sinuosa* identified at the surface of the wood sample (x = 80).

content of the wooden substrate. A biological feature of the species that explains the massive biodeteriorationit causes, is the resistance to dryness,[14] which is higher than that of *Merulius lacrymans* species. Thus, *Antrodia sinuosa* withstands 6-7 years at temperature values between 7.5 to 27 ^{0}C, while *Merulius lacymans* withstands 8 years at 7.5 ^{0}C and only one year at 20 ^{0}C.

Antrodia species cause discoloration to wood. Thus the wood becomes brown with cuboid cracking easily revealed at a simple examination. The fungus produces white mycelium and strands usually leading to a misidentification as dry rot fungi. In fact *Antrodia* strands are flexible when dry and are pure white. The fruiting body is somewhat fleshy up to 12 mm in thickness, of white colour; the surface has a "honeycombed" shape, with angular and small pores (around 4 mm).

Wood colonization occurs along the medullar rays, and the spreading in tissues with longitudinal disposition occurs through the hyphae. These penetrate the tertiary wall (rich in lignin) and reach the inner layers of the secondary wall, where they completely degrade the carbohydrates, causing the wood to break in rectangular blocks.

The initial colonization began with spore germination of *Antrodia sinuosa*, most probably brought by air currents coming from outside the church. On the wet wood spores germinated and formed hyphae which, by multiple branches, developed aerial and submerged mycelium. The presence of spores and the process of germination cannot be identified with the naked eye and therefore the danger notification occurs only after the identification of mycelium, which is always correlated with olfactory perception.

Aerial mycelium continued the life cycle under favourable conditions (moisture and temperature) developing fructification bodies or remaining in the

[14] Schmidt 2006, p. 14.

same stage, maintaining viability for a certain period of time. Felty and yellow-orange mycelium identified after examination of the wood in the NUAB restoration studios was viable but the colour was the result of the toxic effect of biocides applied *in situ* or of intermediate products resulting from its decomposition. It was demonstrated that some strains of basidiomycetes (*Pleurotus ostreatus* and *Trametes versicolor*) have the ability to decompose biocides (sodium pentachlorophenol and lindane) applied as chemical treatment.[15]

Substrate mycelium developed in the surface layer of the wood where, by the enzymes it produced, it was able to decompose cellulose and lignin. The wood lost its consistency and has become soft; its colour changed to brown, and it became fragmented in geometric shapes (specific morphology, characteristic of wood deteriorated by basidiomycetes). It is obvious that the biodeterioration started on the surface of the beam and went in depth. In time, successive layers of mycelium were formed according to the microclimate conditions. The mycelium of *Antrodia sinuosa*, at the end of the biological cycle, after senescence stage, lost viability and was decomposed by filamentous fungi. This process was faster or slower depending on the microclimate. The aspect of dead mycelium may be similar to that of the viable one (felty and white), but it is friable (criterion suggesting suspicion of non-viable mycelium) or can be different (felty aspect with brown shades). The felty mycelium is formed when the favourable environmental conditions are maintained for a long period of time. In general, brittle and crusty mycelium is not viable; it is developed when the environmental conditions are favourable for a short period of time. In winter, when the temperature reaches freezing point, the aerial mycelium may lose viability but it acts as a protective layer for the submerged mycelium. The growth of submerged mycelium may be considered as a strategy to identify new nutrient sources, and in unfavourable environmental conditions as a defence strategy. The massive development of mycelium at the contact of wood and mural painting produced its slight detachment, becoming in time an irreversible loss.

The fungus *Antrodia sinuosa* spread inside the church by spores and mycelia fragments. The predominance of wood in the structure of the church and in religious objects, and thus its wide availability, guided the direction of the mycelium growth towards uncontaminated and healthy wood. This evidence explains a massive contamination of wood in general and of *beam no. 2* in particular. Transport vehicles were the air currents, rain and dew drops, larvae of boring insects as well as adult insects that accidentally came inside the church. Contamination occurred also through direct contact between human body and clothing, and areas containing active mycelium and fruiting bodies.

Research carried out on *beam no. 2* revealed that this was also contaminated with another basidiomycete belonging to the white rot fungi group: *Phellinus*. The wood deteriorated by *Phellinus* sp. is white and broken in thick and coarse fibres. Reddish-brown bundles were also found, especially near the cracks. The mycelium is white-brown or tawny, and is commonly structured in webs attached firmly to the surface of the wood.

International standards recommend eliminating the sources of moisture, removal or replacement of contaminated surfaces and applying a biocide with preventive role. In case of historical monuments and painted wood or furniture, these recommendations are not valid because it is of interest that the restoration and preservation to maintain the original shape. Presently, the methods recommended for the decontamination of natural wood colonized by basidiomycetes are based on its exposure to elevated temperature. Heating of the wood at 60 ^{0}C using a source of heat or microwaves is possible under surveillance but there is the risk of cracking, liquefaction and movement of resins, thermal damage to pigments and varnish. Irbe and Anderson[16] suggest the exposure of timber to 35-37 ^{0}C for 17 hours and then at 40-60 ^{0}C for 15 minutes. In Denmark electromagnetic therapy is applied,[17] while in Germany it is avoided because there is not enough scientific information to support it.

Viability tests showed that the mycelium identified on *beam no. 2* was viable and therefore we performed a chemical treatment with KOH (8%) only on the areas containing wood without mural painting but contaminated with mycelium.

Identification of *Antrodia sinuosa* mycelium and of tunnels of boring insects both on the surface and in the depth of the beam, correlated with the extension of biodeteriorated areas, led to the decision of extracting the mural painting and conserving the original beam in a museum. The place of the old beam has already been taken by a new oak beam, dressed and assembled *in situ*. To remove the mural painting layer from both sides of *beam no. 2* a facing was applied, consisting of a layer of Japanese paper and two layers of gauze (adhesive used for extraction: carboxymethylcellulose, 5%), all reinforced by transverse rods made of extruded polystyrene. After the application of the backing and removal of the facing, their relocation on the new beam follows *in situ*.

Microscopic fungi identified on *beam no. 2* as well as on dead mycelium of *Antrodia sinuosa* are:

[15] Pohleven *et alii* 2010, pp. 129-132.

[16] Irbe, Andersone 2010.

[17] Merle Strätling *et alii* 2010.

Trichoderma viride, *Aspergillus niger*, *Cladosporium* sp and *Penicillium* sp. They are characterized by the ability to synthesize cellulases and therefore are involved in the degradation of wood. This process is slower than in the case of *Antrodia sinuosa*, but cellulases complex is stronger because it contains exoglucanase.

The boring insects lay the eggs in the natural cracks or in those made by brown rot fungi, and their larvae are feeding with cellulose, starch and sugar from the wood. They make tunnels and grown up leaving the wood packed with fine powder. The winged adults leave the tunnels through the surface, and holes are putting in evidence. Two major types of insects have been found: *Hylotrupes bajulus* and *Annobium punctatum*, both preferring high moisture (15-30%).

The conservation and restoration strategy for *beam no. 2* belonging to the church in Urşi is based on well-defined activities, some performed *in situ* and others in the NUAB conservation studios. The main stages of the restoration strategy involved the following activities: biodeterioration identification by recognizing its specific morphology (*in situ*); emergency interventions (*in situ*); facing application (*in situ*); removal and storage in specially designed space (*in situ*); packaging and transport of the beam at the National University of Arts (*in situ*); analysis of state of conservation from biodeterioration point of view (NUAB); local and general chemical treatments for decontamination (NUAB); extraction of mural painting (NUAB); chemical treatment for decontamination (NUAB); mechanical cleaning of extracted mural painting; application of backing; removing of facing and minimum conservation activities; transportation *in situ* and replantation of extracted mural painting and performing conservation and restoration interventions.

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