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MICROSCOPIC INVESTIGATION OF DEGRADED WOOD FROM CULTURAL HERITAGE

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Abstract:

Microscopic examination is one of the most ready available and still reliable methods for wood investigation. Due to the friable nature of the degraded wood from artefacts, preparation of thin transparent micro-slides for microscopic examination may be very difficult. The present paper explores a technique of embedding of degraded wood with paraffin prior sectioning into transparent slides.

The wooden objects employed for the present research are traditional objects from Romanian households belonging to the open-air Astra Museum from Sibiu. A number of 6 objects were selected with the support of Museum's staff. They were: a shovel for bread baking, a shovel for embers, a churn, a stool, a crib, a salt fulling mill. Small samples were carefully extracted from all objects and further embedded in histological paraffin wax by vacuum impregnation procedure. Afterwards they were prepared for microscopic examination and investigated under an optical microscope fitted with a camera for image capture. The samples were observed in transmitted light at different magnifications to reveal the presence of biological degradation and relate it, if possible, the agents of decay.

The results indicated a fungal colonisation of wood from cultural heritage by detection of hiphae, cell wall thinning or disruption, destruction of parenchyma rays, cell separation. However, there were difficulties in clearly identifying the forms of decay.

Key words: *microscopy; degraded wood; decay; cultural heritage; paraffin embedding.*

INTRODUCTION

Cultural heritage represents a treasure of knowledge and significance of great importance for humanity. Since ancient history human being life has been strongly linked to the utilisation and processing of wood. Wooden artefacts and constructions are, therefore, a representative part of world cultural heritage that needs to be acknowledged and scientifically conserved. In a broad context of wood cultural heritage conservation, the ethnographic museums have a special role, mainly because of their broad approach to the perspective of culture (Svensson 2003, Krstovik 2011).

For Romania wood certainly represents an important traditional material, extensively used throughout history for various constructions from houses to the famous wooden churches from Maramures included in UNESCO acknowledged cultural heritage (http://www.cimec.ro/monumente/unesco/unescoro/indexMaram.htm), as well as for manufacturing very different artisan technical installations for the processing of crops and natural resources, for household objects and furniture. Unfortunately, many wooden historical artefacts have been lost or are at risk of being lost due to the fragile nature of the wooden material. Wood is susceptible to degradation by biotic and non-biotic factors, depending on the exposure situation. Many wooden objects of artistic or cultural value are seriously damaged by a variety of biological agents and the degradation is often irreversible as it affects not only the aspect but also the structural integrity and mechanical properties of wood. The resulting cultural and economic impacts are obvious. Therefore, conservation of cultural heritage is necessary and compulsory as a connection between generations and cultural survival of communities.

During the last decades the scientists have focussed on several problems related to preservation and conservation of wood from cultural heritage (Bisceglia *et al.* 2010). In Romania there is a permanent concern of scientists and specialists from museums about this issue (Olaru 2008, Sandu 2008), but microscopic investigations on wood from historic artefacts are limited. A few studies envisaged the occurrence and biological identification of fungi and insects on wooden monuments (http://www.transsylvanianostra.eu/download/05_livia_bucsa_degr_biologice_str_lemn.pdf), while other focussed only on the microscopic identification of wood species originated from different objects or furniture (Timar *et al.* 2012, Gurau *et al*. 2011).

Microscopic examination is one of the most ready available and still reliable methods for wood investigation. Transmitted light microscopy can provide information on the pattern of degraded wood cells, which is helpful in developing appropriate methods for conserving of wooden artefacts (www.ivalsa.cnr.it/en/research/diagnosis-and-conservation-of-wooden-cultural-heritage.html). Furthermore, it is possibly for the trained eye to identify the type of decay and determine the main types of degraders (Bjordal 2009).

Fungi and bacteria are considered the main degraders of wood. Three types of decay: brown rot, white rot and soft rot and two types of bacteria: tunnelling bacteria and erosion bacteria are commonly recognised. Fungi and bacteria leave unique fingerprints after decay of a cell wall (Nilsson *et al*. 1989, Obst *et al*. 1994, Srebotnik and Messner 1994, Anagnost 1998, Schwarze and Baum 2002, Biordal 2009).

Cellulose and hemicelluloses are broken down by brown rot fungi (*Basidiomycetes)*, so that the decayed wood acquires a brown colour and brittle consistency, cracks into cubes and finally crumbles into powder. Softwoods are more often affected than hardwoods. In cross section, it can be observed that the integrity and original form of fibres are slightly changed. In wet conditions S2 layer is dark, swollen and clearly affected and shows no birefringence in polarised light (is dark), indicating loss of crystalline cellulose (Anagnost 1998). In longitudinal section large bore holes between the fibres occur. Studies carried out on some commercial hardwood species showed that brown rot hyphae occur very sparsely in the wood, often restricted to the lumen of woody cells, the penetration of hyphae being detectable in correlation with vessel dimensions (Olfat 2011). The same studies indicated that even when the vessels are blocked by tyloses or other deposits penetration of hyphae may occur.

White rot is caused by certain *Basidiomycetes* and by certain *Ascomycete*s. The common feature of these fungi is that they can degrade lignin as well as cellulose and hemicellulose (Schwarze 2007). Hardwoods are more susceptible to white rot attack that cause “bleaching” of wood. Bleaching of wood is also caused by many simultaneous degraders. Formation of erosion troughs into cell walls is a characteristic morphological feature of this type of attack. The light microscopy combined with special colouring techniques can indicate *white-rot decay* by delignification (Srebotnik and Messner 1994). Same work suggests that rays contain more syringyl-type lignin than do other cells, and this lignin is more susceptible to white rot. In cross section a simultaneous decay of cell wall is present, observed as thinned cell walls, carries-like or broken cell walls. In longitudinal sections boreholes between fibres occur. *White rot* produces rounded erosion channels, U-shaped notches and rounded pit erosion (Anagnost 1998).

Soft rot fungi are active under wet conditions (wood in soil and water) and degrade mainly cellulose and hemicelluloses. They are acting often together with wood-degrading bacteria. Decayed wood is dark in colour, soft and develops cracks and shrinks on the surface. Cell wall erosion is the most common form of wood decay by soft rot fungi (Kim and Singh 2000, Nilsson *et al.* 1989). Microscopic examination of decayed

wood indicated two distinct types of degradations: formation of typical soft-rot cavities in the secondary cell-walls and a form of erosion starting from the lumen. The erosion varied from fairly even removal of cell wall material, to more localised attack resulting in more irregular troughs. S*oft-rot* fungi produce angular erosion channels, V-notches and diamond-shaped pit erosion (Anagnost 1998). Rays cell walls were degraded, but vessel walls appear to be little affected by erosion.

Due to the friable nature of the degraded wood from artefacts, preparation of thin transparent micro-slides for microscopic examination may be very difficult. The present paper explores a technique of embedding of degraded wood with paraffin prior sectioning into transparent slides. Paraffin embedding techniques are used not only for degraded wood (Wilcox 1964,1993, Cufar *et al*. 2008), but also for other soft organic tissues, being the most widely employed of all histological methods, to obtain serial sections from the investigated material by cutting with a microtome (Wilcox 1964).

A variety of references including manuals or guides (Sheenan 1987, www.bbka.org.uk/local/iceni/bm˜doc/microtomy_paper.pdf), protocols (Kulhmann 2008, http://bejerano.stanford.edu, http://www.daneprairie.com, http://www.publicbookshelf.com/public_html/Methods_in_Plant_Histology/planthist_d.html) have been consulted to develop the method employed in this paper.

Considering these aspects, the main objectives of the presented research can be summarized as follows:

- application, for the first time in our laboratory, of the wax embedding technique as a first step in the preparation of frail wood specimens for microscopic examination in transmitted light;
- microscopic investigation of the samples to reveal the type and severity of the degradation phenomena.

The degraded wooden samples investigated within this research were extracted from traditional artefacts belonging to the *Astra* Museum of Traditional Folk Civilisation, Sibiu (http://www.muzeulastra.ro), to be relevant for wooden cultural heritage investigation and conservation.

MATERIALS AND METHOD

The wooden objects employed for the present research are traditional objects from households from three Romanian geographic regions: Moldova, Transilvania and Oltenia belonging to the open-air *Astra* Museum of Traditional Folk Civilisation in Sibiu. These are objects exhibited in the respective houses in the museum (at least for the period April – November), in exposure situations characteristic to their former use. Thus, the most frequent exposure situation was wood in contact with soil because of the contact with clay flooring of old houses. Another usual exposure situation was wood outdoor, under cover on the veranda of the houses, only partially protected from the direct action of the climatic factors. It is easy to realise that this kind of museum and realistic exhibition make practically impossible the preventive conservation based on a controlled climate. Active conservation treatments are usually applied only during the winter period, when the objects are removed from the historic houses, disinfected, inspected and deposited in controlled conditions.

A number of 6 objects (coded a to f in Fig. 1) were selected with the support of Astra Museum's staff for this study. They were: a shovel for bread baking, a shovel for embers, a churn, a stool, a crib, a salt fulling mill. All these objects presented evidence of wood degradation due to the above mentioned considerations, being useful for the purpose of this research. Small samples of approximately (10-20 x 5-10 x 5) mm, with irregular shape, were carefully extracted from all the six objects, and coded to 1 to 6, as presented in Fig. 1. The areas where from the test samples were extracted were chosen to be representative for the most advanced visible degradation and at the same time minimum invasive and visible for the artefact. For example, the samples were extracted from the handle of the shovels, the bottom sides of the churn, stool legs, crib and fulling mill.

The very fragile wood samples were embedded in histological paraffin wax (from Chemical Company www.chemical.ro), with melting point of 54-58°C, by vacuum impregnation. Before impregnation the samples were dehydrated in a series of ethyl alcohol baths, with increasing concentrations (50%, 70%, 80% and finally absolute alcohol). After dehydration a clearing procedure by immersion in a xylene bath was applied to remove the alcohol from the samples. After clearing the wood samples were ready for paraffin impregnation.

Fig. 1

Wooden objects from ASTRA Museum and samples extraction:
a - shovel for bread baking (sample no 1); b - shovel for embers (sample no 2); c - churn (sample no 3); d - stool (sample no 4); e - crib (sample no 5); f - salt fulling mill (sample no 6).

Impregnation with melted paraffin was achieved under vacuum at 60-65°C in a vacuum oven with temperature control (Fig. 2). The samples were placed in porcelain crucibles and completely covered with melted paraffin. They were fixed with a sieve to be kept totally submerged. The treating procedure included an initial heating phase of 15 minutes, followed by a vacuum phase (p = 0.1 bar) of 20 minutes and a final phase at atmospheric pressure for 2 hours.

Fig. 2

The embedding procedure of wooden samples with paraffin:
a - crucibles with samples covered with paraffin; b - vacuum oven for samples impregnation; c - paraffin block including the impregnated sample; d - sample after removing the excess of paraffin.

Following this procedure and cooling at room temperature, paraffin blocks including the impregnated samples were obtained.

Once the blocks were hard, they appeared glassy clear and were kept for minimum 24 hours in laboratory conditions. Afterwards they were trimmed and heated to remove the excess of paraffin. Despite this procedure the impregnated samples could not be satisfactorily sliced into thin transparent micro-sections with a sliding microtome. Therefore transparent micro-sections for microscopy were cut by hand with a razor blade. The cut micro-sections were transferred directly to preheated microscopic slides to ensure their adherence to the slide.

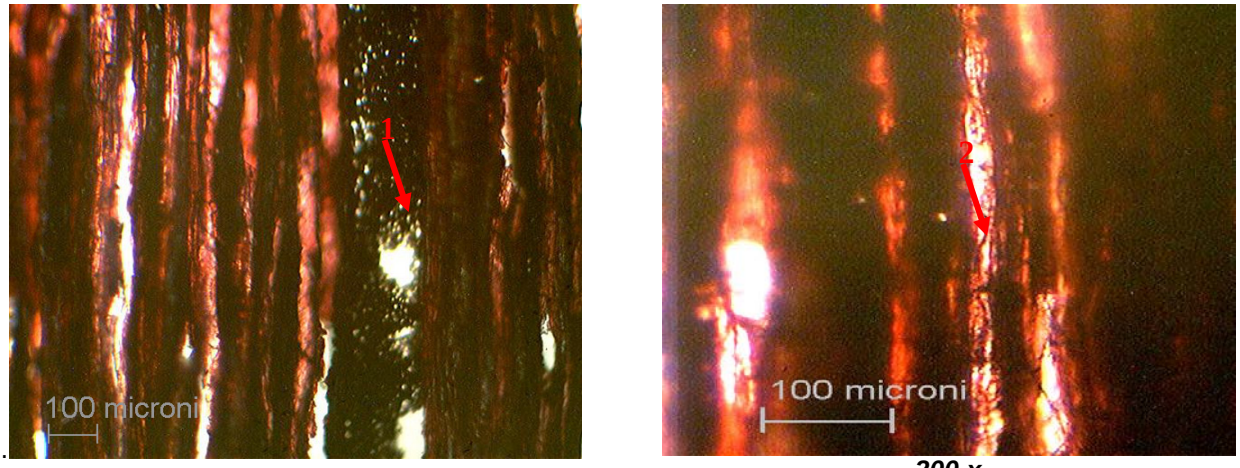
Safranin and Astra Blue were used in successive steps for samples staining in order to improve contrast and better visualise and differentiate sound and decayed areas (Schwarze 2007). Safranin stains in red lignin regardless whether cellulose is present or not and Astra-blue shows affinity for cellulose and is incorporated into cellulose fibres only in the absence of lignin (Srebotnik and Messner 1994). Following this procedure, de-lignified areas will be highlighted as blue areas on a red background.

Before staining the embedding wax had to be removed from the thin sections by careful washing in xylene, followed by immersions in ethyl alcohol and acetone. The washed micro-sections were transferred to microscopic slides for staining with aqueous solutions of 1% concentration of Safranin and Astra Blue, the colouring time being 15min for each. The micro-sections were washed with water after each staining step till the liquid was clear. The stained and washed micro-sections were temporarily mounted in water and glycerol and investigated under an optical microscope Biostar Optech B5 fitted with a camera for image capture. The samples were observed in transmitted light at different magnifications (40-200x).

RESULTS AND DISCUSSION

The samples collected from wooden objects had different macroscopic aspect and integrity. Time, domestic use and degradation phenomena put their marks to the wood. Though the objects employed for this study were generally in a good state of conservation, evident signs of biological degradation and mechanical deterioration were observed, as expected, especially in the bottom parts in contact with soil or the handle-ends. Discolouration from grey to black was present on all objects (Fig. 1), alongside insects attack, friable wood due to a possible decay process or crushed wooden tissues due to the intense use in service. Thus the investigated wooden objects presented sampling limitation. Wood species were not evident directly on objects or small samples. All these macroscopic observations represented an important set of information and a good reason for the microscopic investigation of samples to reveal the presence of biological degradation and relate it, if possible, to the agents of decay. The main features considered were detection of hyphae, their frequency and location, cell wall thinning or disruption, destruction of parenchyma rays, cell separation. Some recorded images are presented bellow.

The images for sample no 1 are given in Fig. 3. Only longitudinal tangential section could be obtained. The microscopic pictures reveal a hardwood species with vessels and large rays.



100 x ***200 x***

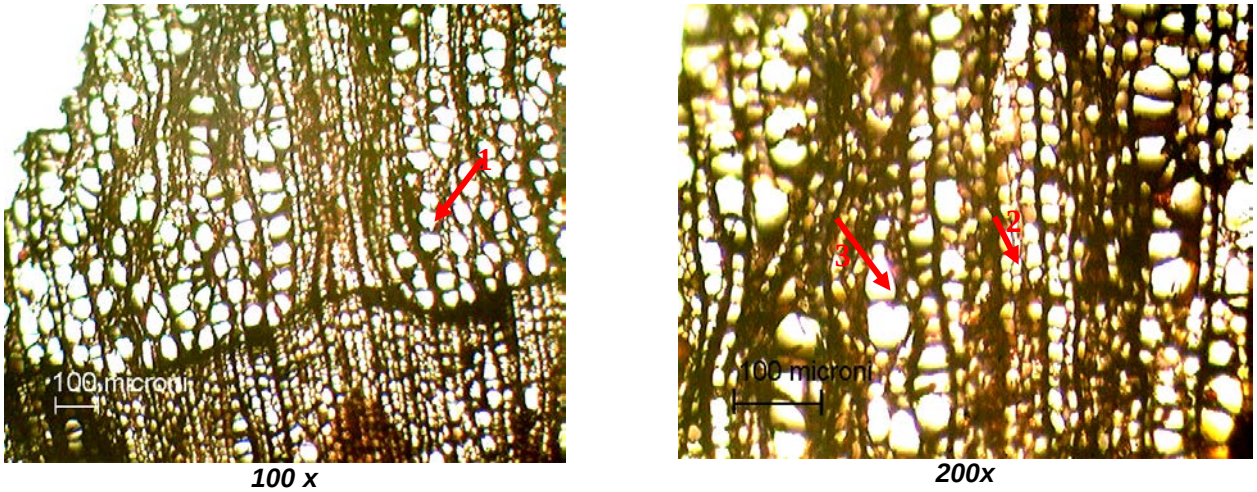
Fig. 3

Microscopic aspect of sample no 1 originated from the handle of shovel for bread baking- tangential section (original magnification 100x, 200x), showing: degradation of a parenchyma ray (left picture - arrow no 1), fungal colonization in vessels and possible pin holes (right picture - arrow no 2).

A big ray, very dark (blue) on a more reddish background, with evident broken tissue can be observed. Some darker filaments occur in vessel elements, alongside some possible pin holes suggesting fungal colonisation and degradation. The dark blue colour may indicate delignification, characteristic to white rot

fungi, degradation process which can be more advanced in the parenchyma rays tissue of hardwoods than other type of cells, according to literature data (Srebotnik and Messner 1994).

The sample no 2 (presented in Fig. 4) is a diffuse-porous hardwood species with growth rings more or less undulating. When examining the cross section it can be observed an advanced degradation of the wood structure. The walls of vessels and fibres are very thin, disrupted or broken. The blue areas show a delignification of cell wall. Some fibre areas and connection between fibres and vessels are broken. It certainly reflects the fact that considerable damage occurred. These features are characteristic to white-rot degradation.

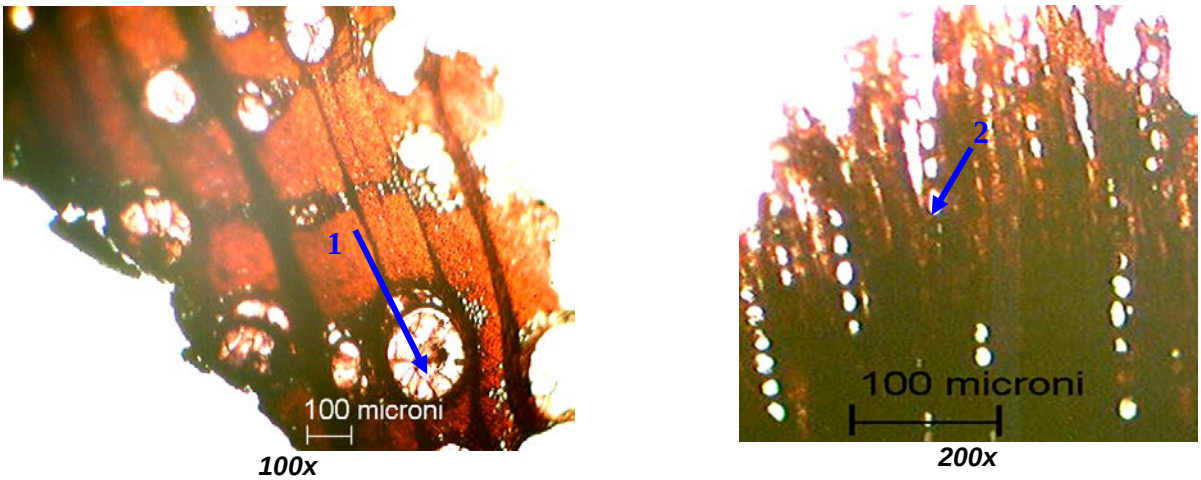


100 x *200x*

Fig. 4

Microscopic aspect of sample no 2 originated from the handle of shovel for embers - cross section (original magnification 100x, 200x) showing: thin wall vessel and fibres (arrow no 1 and 2 respectively), blue delignified areas (arrow no 3).

In the case of sample no 3, which seems to be hardwood species with large, round vessels, blocked by tyloses or other deposits, the penetration of hyphae is evident (Fig. 5). These appear as darker filaments inside of vessel. On the other hand, the general structure in the fibres area seems to be little affected. Slight degradation of cell walls and possibly partially delignified areas appear in the parenchyma cells. Therefore, it could be an incipient attack of white rot fungi. The reddish colour of the fibres areas could indicate a relatively sound wood (Olfat 2011).



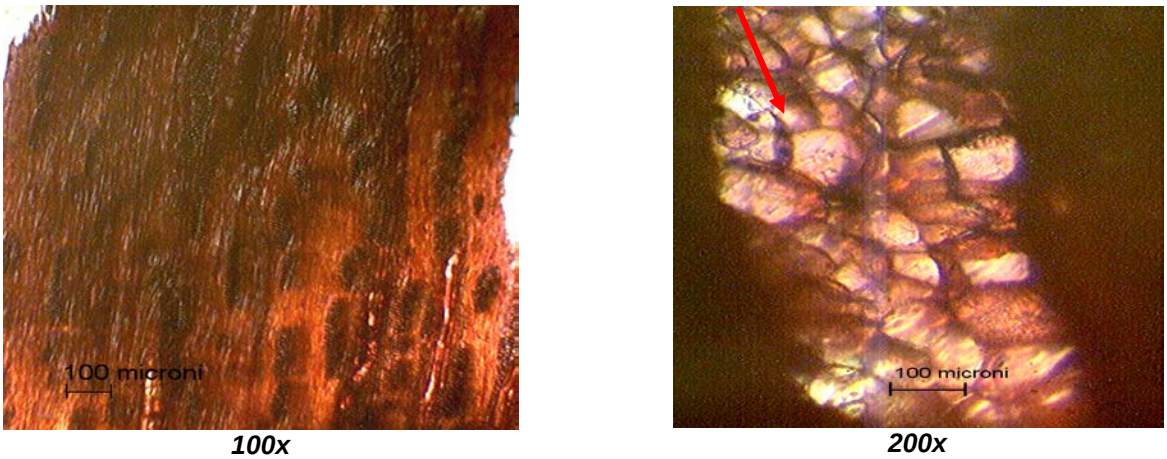
100x *200x*

Fig. 5

Microscopic aspect of sample no 3 originated from churn (original magnification 100x, 200x) - cross section (left picture) showing darker filaments of hiphae inside of vessel (arrow no 1) and tangential section (right picture) showing a delignification (arrow no 2).

For samples no 4 and 5 the microscopic investigation was difficult to be done because the sections were thick and very dark coloured. The identification of possible type of decay was very difficult. Therefore, no images are presented.

The Fig. 6 shows a tangential section of sample no 6, with numerous and homogenous rays and some darker filaments in vessel elements that could be hyphae. The blue colour indicates a possible degradation starting from the lumen of vessel, characteristic for an incipient fungi attack.



100x ***200x***

Fig. 6

Microscopic aspect of sample no 6 originated from salt fulling mill - tangential section (original magnification 100x, 200x): general image (left) and vessel (right) with hiphae that indicate an incipient attack of fungi (red arrow).

The results indicated a fungal colonisation of wood from cultural heritage. The absence of any change in wood structure does not negate the possibility of decay because it may not occur in the small samples of wood chosen for study. Degree of decay can vary considerably over short distances and microscopic diagnosis of no presence of decay may be unreliable (Carll *et al*. 1999).

CONCLUSSIONS

The present study was a challenge to test a new method in our laboratory, to learn and employ a technique that is commonly used in microscopic examination of degraded wood.

Embedding of wood in paraffin provided more or less quality of sections for microscopic investigation. Because the size of samples was too small, the current study will help to better understand its limitation in sampling by small areas observed in a sample, in conjunction with non-uniform spreading of fungal attack. This microscopic investigation must be correlated with other types of investigations such as: non destructive evaluation, radiography, tomography, density evaluation, mechanical testing. The observed degradation features in the samples from degraded wood could be possibly attributed to fungi. However, there were difficulties in clearly identifying the forms of decay.

Anyway, the experimental method employed in the present research will be improved on further wooden objects. A more detailed macroscopic examination of wood before sampling for microscopic investigation is required.

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