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DETERIORATION OF WOOD BY MICROORGANISMS IN A HISTORICAL BUILDING ON THE EXAMPLE OF A HISTORICAL HEALTH RESORT VILLA

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Abstract

This paper discusses the deterioration of historical wooden buildings by microorganisms. The subject was presented on the example of a historical wooden villa in the Polish health resort of Rabka-Zdrój. The 'Wawel' Villa is a historical building which, due to its material value, is under strict conservation. During its restoration process, due to its unsatisfactory technical condition, microbiological testing of its wooden substance was performed. It was aimed at assessing the degree of the wood's microbiological contamination and its suitability in the process of the building's restoration. The findings were confronted with current potential for wood disinfection so as to preserve it as one of the key values of the building. They can be used in the process of restoring historical wooden architectural heritage.

Keywords*: Wooden architecture conservation; Wood decay; Biodeterioration of wood; Management of monument protection*

Introduction

This paper discusses the biodeterioration of wooden historical structures via the destruction of their material by microorganisms. The problem in question shall be presented on the example of the historical 'Wawel' villa located in Rabka-Zdrój, Poland, a popular health resort. At present, a process of the gradual destruction of historical health resort development is being observed here. The wooden villa buildings of Rabka-Zdrój, associated with its initial function, is deteriorating and its owners and users do not always want or are able to allocate the necessary funds to disinfect the historical materials so as to preserve and conserve the original historical substance.

It should be noted that the biodeterioration of wood as the substance of historical buildings is commonplace and is a considerable challenge to designers and conservators in Poland [1-4]. Together, they strive to preserve the greatest possible portion of the original structure of historical buildings, including their material, which is often one of its fundamental values. One of the elements of this process is the analysis of wood mould contamination, which shall be presented in this paper, in addition to the best method of its disinfection, which is to allow protecting and preserving historical materials.

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Thus far, the problem associated with the biodeterioration of wood, with a particular focus on historical substance, was discussed by: *A. Blanchette* [5], *A. Koziróg et al* [6], *T. Rosado et al* [7], *I. Irbe and I. Andersone* [8]; G. Alfredsen et al [9] and *K. Sterflinger and G. Piñar* [10]. They highlighted the considerable threat that microorganisms pose to wood. They also pointed to the need to conduct multi-directional analyses of substances that should lead to choosing the best method of protecting or disinfecting it.

'Wawel' Villa

The problem signalled above also appeared during the restoration of the historical 'Wawel' villa (Figs. 1 and 2), located along one of the main streets of the Rabka-Zdrój health resort.

Fig. 1. 'Wawel' villa at the start of the twentieth century.

a b

Fig. 2. 'Wawel' villa in the present day, prior to its restoration

Rabka-Zdrój is a village whose history dates back to the Middle Ages, and is located in the south of the Lesser Poland Voivodeship. In 1861, on the initiative of Julian Zubrzycki, it became an important health resort due to springs of iodine and bromide-infused brine that had been discovered here [11, 12].

The villa is surrounded by development typical of health resorts in the form of guest houses and former sanatoriums with interesting architecture typical for this form of use from the turn of the twentieth century.

The building in question was constructed at the start of the twentieth century as a guesthouse. It featured rooms for the health resort's guests, a common area (a large hall with a dining room) and technical rooms. After the Second World War, after years of prosperity, the guesthouse's situation began to worsen. Its technical condition significantly deteriorated during a period when it was used by a primary school. After its liquidation in the first years of the twenty-first century, the building was not in use for several years until a private investor appeared in 2010 and purchased it from the municipal government. The villa thus has a chance to survive and, most importantly, return to the condition it was in during its best years [13].

The villa is a two-storey building with a habitable attic. It also has a partial basement. The building has a composite structural system. The villa has a wooden purlin-and-rafter roof truss that supports a steep gable roof that is currently covered with trapezoidal metal sheets that have replaced traditional shingles. The building was erected using traditional timber technology with log walls. It has wooden beam decks. In the basement section the walls are made of crushed stone, similarly to the foundations [13].

Due to the fact that the villa is a timber building, it was necessary to perform an analysis of the technical condition of its structural system and substance. These studies were preceded by a survey and inspections at the site. The analyses indicated that the building is in an unsatisfactory technical condition, which led to the necessity of performing microbiological testing of its wooden substance.

Microorganisms that cause the biodeterioration of wood

Wood is a material that has been used as a structural material to build houses, ships, machines and tools for centuries. It is also a base for painterly and sculptural works of art. As an organic material, it is easily colonised by living organisms: microorganisms, insects, algae and lichens, for which it forms an important niche [14].

The colonisation of wood and wood products by microorganisms and their development in favourable conditions leads to the biodeterioration of wood, which is primarily based on the degradation of the wood's main chemical components: cellulose, hemicelluloses and lignin, which are treated as nutrients. The cellulose content in wood as the substance of cell walls is similar and amounts to 40–50%, but the content of the remaining components depends on the class and species of a given plant. Softwoods contain less hemicelluloses and more lignin than hardwoods. The differences in the chemical composition of these components in the cellular structure, e.g. the orientation of microfibrils and the presence of specific wood extractives affect the degree of susceptibility to and the character of the degradation process [5].

The deterioration of wood is primarily caused by extracellular enzymes produced by microorganisms that decompose the cell walls of wood cells. This leads to aesthetic changes that are visible on wood surfaces, such as peeling, delamination and discoloration, which are significant in the deterioration of wood products [15].

Structural and mechanical changes lead to the loss of structural strength and hardness, and thus to the suitability of wood as a structural material [5, 6, 16].

Due to the character of changes and the course of the biodegradation process, microorganisms that cause wood decay can be divided into three categories. The broadest scope of damage is caused by taxonomic fungi classified as *Basidiomycota* and described as white- and brown-rot fungi. White-rot fungi degrade all the components of cell walls, including lignin, which is not a common ability in other microorganisms. They cause the bleaching of wood's otherwise normal colour and the progressive erosion of the cell wall layer, along with significant loss of wood structural strength appears during the advanced stages of the degradation process. In the natural environment, white-rot fungi are common wood parasites and the decomposers of woody debris. Brown-rot fungi cause the depolymerisation of cellulose, but do so quickly, already during the wood colonisation stage. The loss of structural strength is thus very rapid, and often occurs before any signs of contamination become visible. Lignin is not decomposed, degraded wood takes on a brown colour, becomes softer and cracks and crumbles into multi-sided pieces when it dries. Brown-rot fungi are a frequent cause of damage to historical buildings [17].

This group includes the indicator fungus *Serpula lacrymans*, which commonly attacks structural wood and can spread to other natural surfaces that are less susceptible to microbiological degradation, such as masonry walls, concrete or carpets, transporting nutrients and water across considerable distances of even as much as a few metres through its mycelium. The use of wood with this type of contamination is particularly dangerous as it can lead to its spread and the deterioration of many of the building's structural elements. Although fruiting bodies and mycelium are easy to remove, mycelium cords remain in the material and, in favourable conditions, can redevelop. This fungus is particularly dangerous as it has low humidity demand, producing the water it requires to grow during the decomposition of cellulose. Colonising the environment of dwellings, it causes not only significant wood deterioration but also, having spread throughout the building, it can cause disorders of the respiratory system and discomfort in residents, as the decomposition of wood is accompanied by the emission of considerable amounts of carbon dioxide, organic acids and, most importantly, odours. Another important member of this group of fungi is *Coniophora puteana*, which develops on the dead wood of coniferous trees, quickly infects timber structures and as a domestic fungus is often encountered in basements, cellars or when there is accumulation of humidity, for instance due to an improperly applied damp-proof course. It can grow in low temperatures which is why it is common across the northern hemisphere.

The third group of fungi that causes the biodeterioration of wood are soft-rot fungi, which taxonomically belong to *Ascomycota* and *Deuteromycota*, which contaminate wood during highly damp conditions. One of the types of changes these fungi cause are longitudinal cavities that form on the interior of the secondary wall of wood cells, and the other is the erosion of the entire secondary wall, without the degradation of the middle lamella, which distinguishes these changes from those caused by white-rot fungi. The deterioration is accompanied by a pronounced loss of structural strength with a visible growth of the mycelium, but wood can also be damaged by moulds when mycelium growth is not observed. The fungi that cause these changes belong to the genera *Acremonium*, *Cladosporium*, *Alternaria*, *Humicola*, *Penicillium* and *Chaetomium* [18]. In quantitative testing performed by Piotrowska and Żakowska, which assessed the degree of fungus contamination of the wooden surfaces of historical floors of barracks buildings, no active growth of bacteria and mould was observed although signs of degradation were visible. The degradation process occurred when the microorganism count determined on the basis of cultivation tests was between 1.1 and 3.5×10^3 cfu $100cm^{-2}$ of surface [19]. Soft-rot fungi can reveal their destructive potential even in a polar climate. This was confirmed by studies of Antarctic huts from period of historical polar expeditions. The conservation of these buildings required proofing not only from wind and salt degradation, but also biodegradation caused by fungi from the genera *Cladosporium* and *Phialophora* [20].

Fungi are considered to be the main cause behind the degradation of wood in human surroundings. In aquatic environments and soils, wood is more often attacked by bacteria, particularly those that do not require oxygen. Bacteria can cause erosion, cavitations or tunnelling within wood cell walls [5, 21]. In environments with access to sunlight, algae, moss and lichens also play a large role [14].

Protection of historical wood from biodegradation

The effective protection of wood is not easy to attain. Prevention requires maintaining proper temperature and humidity that limits the development of microorganisms. This solution is a fundamental preventive measure in storage conditions and is possible to maintain in exhibit conditions in museum spaces, but under external exposure or use in a historical building it is almost unachievable. In most cases, under changing weather conditions in seasons during which humidity and air temperature enable the development of microorganisms and the biodeterioration of wood, all that remains is chemical intervention.

Proofing wood using chemical agents has been known for centuries. Many of the chemicals used in the past included heavy metals such as arsenic or chrome or often in the form of chemical compounds that are toxic to humans [22, 23]. To limit the negative impact on the environment, EU regulations have considerably limited the groups of materials that can be used to produce agents [24]. New, more eco-friendly solutions are pursued, and natural substances such as essential oils or agents that increase the hydrophobic properties of wood that are also biostatic are re-entering use. Essential oils of the tea tree, lavender and thyme, when introduced into cellulose materials, are capable of preventing the development of cellulolytic fungi at very low concentrations. For instance, tea tree essential oils were effectively used to conserve a paper manuscript and were proposed as an anti-microbial agent that can be applied to cellulose masses in infills in the conservation of paper-based monuments [25]. Innovative formulations of proofing agents based on silanes (methyltriethoxysilane, MTES and n-octyltryethoxysilane, OTES) developed for waterproofing impregnation and the consolidation of historical wood and that does not require the application of toxic solvents were also proven to protect against the activity of white- and brown-rot fungi [26]. Attempts are made to use natural extracts from decay-resistant woods to re-proof historical wood against fungi, particularly in cases where these extracts have been released over time. Studies in this field were performed where acacia wood was treated with a combination of Paraloid B-72, an acrylic resin commonly used in conservation, and methanol extracts from different parts of *Morus alba*, *Maclura pomifera* and *Cupressus sempervirens* trees, which produced satisfactory results of constraining the development of the cellulolytic fungus *Trichoderma harzianum* [27]. There are also studies on new chemical formulations intended to proof and protect wood such as ionic fluids, fatty acids or modifications of quaternary ammonium salts.

In the aspect of proofing historical wood, derivatives of quaternary ammonium salts are studied to the greatest extent. In studies by Koziróg et al. 2016 concerning the effects of anti-microbial agents dedicated to this use, it was demonstrated that polyamine N-(3-aminopropyl)-N-dodecylpropane-1,3-diamine (APDA) and didecyldimethylammonium chloride (DDAC), a QAC are characterised by highest biocidal properties in comparison to other chemical substances used in wood protection: sodium hypochlorite, hydrogen peroxide, glutaraldehyde, boric acid or lactic acid. Further studies tested anti-microbial activity of eight commercially available preparations that included DDAC and APDA, identified during the first stage, as their agents against bacteria: *Staphylococcus equorum*, *Pseudomonas aeruginosa*, *Bacillus cereus*, and fungi: *Alternaria alternate*, *Cladosporium cladosporioides*, *Engyodontium album*, *Chaetomium globosum* and *Penicillium citreonigrum*. After application to wood colonised by microorganisms, preparations such as ABM-1 and ABM-2 solution 2–6 % (MDA, Poland), 8% Rocima 101 (H.S.H., Poland), 12% Preventol R80 (Bayer AG, Germany), 15% Acticide 706 LV (THOR, Germany) and 30% Boramon (Altax, Poland) were demonstrated to be effective disinfectants. The effectiveness of DDAC-based disinfectants with additions of EDTA (ethylenediaminetetraacetic acid) as a chelating agent in the anti-microbial proofing of historic wood was also confirmed by Rajkowska et al. 2016. Despite the wide spectrum of activity indicated, limited biocidal activity of these preparations against *Aspergillus fumigatus* was observed. It is one of the most dangerous and toxin-producing fungi responsible for the deterioration of historic objects in museum collections. Among the biocides from the QAC group, DDAC is a biocide with proven effectiveness against phototrophic organisms such as cyanobacteria and algae responsible for the biofouling of historical wood and brick. At a concentration of >14% (vv^{-1}) it shows activity as an antifouling agent that protects wooden buildings from the formation of 'green' coatings [28].

The selection of a proper preparation to secure materials that are a part of cultural heritage is a process that requires multi-stage studies. Upon application to a material, a biocide must not only demonstrate an appropriate anti-microbial effectiveness and maintain this effect over time, but also cannot adversely affect the materials. In such studies, typically after application to a model material (often subjected to artificial aging) the preparation is tested at different concentrations and changes in coloration and brightness of the material or structure are tested, including using microscopic techniques [26, 29].

The criterion of the assessment of these changes is different and depends on the state of preservation of historical substance. According to the Procedure for the protection of historical buildings from biodeterioration formulated by Koziróg et al [6], when discoloration of model wood ΔE* measured using the CIE L*a*b* system never exceeds 2, and ΔL* brightness is lower than 1 while the reduction of microorganisms in culture-dependent testing methods exceeds 3 log, the preparation is considered effective and can be used to disinfect an object. n the colorimetric evaluation of old wood there are a series of published works on chromatic deviations under the influence of various factors [30-32].

The final application of the chosen biocide is not always performed. Disinfection is a costly procedure, which can be beyond the financial ability of a historical object's owner, particularly when it is a private property. Studies on the effectiveness and safety of conservation-related application of individual disinfectants dedicated to specific types of materials, whose examples have been discussed above, are highly valuable. They facilitate decision-making and lower cost by the expenses incurred on the testing of individual applications of proofing agents and shorten conservation procedures, which in the case of the necessity of individually confirming the durability of a proposed biocidal measure can even be as long as several months.

Experimental - substance testing

Sampling

Due to the previously mentioned concerns about the state of historical substance, a decision was made to perform microbiological testing.

The test was applied to structural wood from the nearly century-old building the 'Wawel' villa. Its objective was to assess the degree of the wood's microbiological pollution and its suitability in the process of the building's restoration. The testing was performed for four samples that were aseptically collected into sterilised containers from places shown on photographs (Fig. 3). The material was collected from elements with visible signs of damage or covered in fungal hyphae.

Biological contamination assessment

Under laboratory conditions, the collected wood samples were placed on microbiological fungus cultivation media (MEA - Malt Extract Agar and PDA - Potate Dextrose Agar). In order to facilitate the development of microorganisms with cellulolytic properties and confirm their deterioration potential, the wood samples were placed in a Greathouse liquid mineral nutritional medium without a source of carbon. All of the samples were subjected to incubation in laboratory incubators at a temperature of 28±2°C, awaiting the development of microorganisms. Qualitative and quantitative analysis was performed on the basis of macroscopic observation of the colony morphological structures and microscopic samples of distinct structures used for propagation. An optical microscope and a Discovery VMS-004 (veho) mobile stereoscopic microscope were used to perform the observations.

Results and discussion

Changes in wood structure and colour visible on photographs (Fig. 3) such as brittleness and browning of the wood suggested deterioration of wood caused by brown-rot fungi. The macroscopically observable mycelium growth on the surface of the material and grey fruitage indicated colonisation of the material by moulds and biodegradation distinct for soft-rot fungi. The intensive mycelium growth in places of the collection of samples caused concern about the state of preservation of the wood, as the material was collected during cold winter months when negative temperatures were recorded, which are conditions that are not conducive to fungal growth.

Fig. 3. Microbiological testing sample collection sites with visible alterations showing signs of biodeterioration.

Under laboratory conditions, numerous microorganisms were cultivated from the collected samples. During the first week of incubation, the samples and media displayed intensive multicultural fungal growth that confirmed significant contamination of the wood (Fig. 4). The fungal growth was so intensive that the wood samples became completely covered and obscured by it, hence they are not visible the photographs (Fig. 4b-c).

The cultures based on the mineral medium also displayed rapid development of cellulolytic microorganisms with a predominance of fungi from the genera *Trichoderma* and *Penicillium*. The development of these fungi on wood placed in the medium, whose only source of carbon were the wood elements present in the sample, confirm their potential for inducing biodeterioration (Fig. 5). One of the tested samples was very highly contaminated with a fungus from the genus *Penicillium*. Its growth covered a considerable surface of wooden beams, which was macroscopically observable on photograph (Fig. 3). This type of infection was confirmed in laboratory cultures (Fig. 4c) and in microscopic observations (Fig. 5b) wherein similar, brush-like conidiophores of *Penicillium* covered the wood surface. Fungi from the genus *Penicillium* are common in historical wooden structures [33]. They were frequently isolated on the surfaces of historical wooden buildings of the Auschwitz-Birkenau concentration camp. Over a several-months-long study of this complex, researchers demonstrated a significant share of fungi of the genus *Penicillium* among the factors responsible for the biodeterioration of historical wood, followed by fungi from the genera *Cladosporium* and *Alternaria* [14].

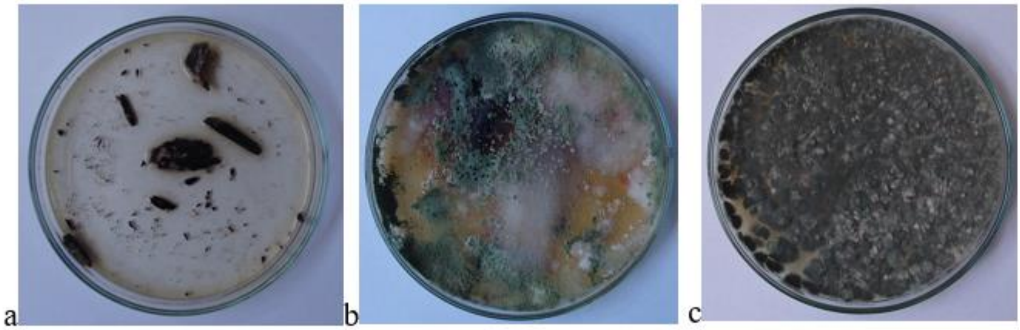

Fig. 4. Fungal growth on wood samples after placement in cultivation media for 8 days of incubation, wood sample prior to incubation (a), after incubation on a MEA medium (b) and after cultivation on a PDA medium (c)

Penicillium strains, alongside *Cladosporium* and *Mucor*, were also dominant fungi in the process of the biodeterioration of the altar from the Espirito Santo church in Ewor, Portugal. Enzymatic activity studies of these fungi confirmed their high cellulolytic activity and ability to cause structural wood damage [7]. The occurrence of numerous *Penicillium* sp. was noted on the surface of the exhibition hall of the Museum of Natural Science of La Plata, Argentina, at showcases with Egyptian mummies, which facilitate the accumulation of the spores of these fungi, carried in from the external environment, although the presence of other actively cellulolytic moulds such as *Chaetomium globosum* or *Stachybotrys* sp. was being actively limited in the space [34].

Fig. 5. Fungal growth on wood samples in a mineral medium

The predominance of moulds from the genus *Penicillium* in the biodeterioration of the material under study does not preclude the participation of other fungi in this process. In spite of not detecting the presence of domestic fungi *Serpula lacrymans* or *Coniophora puteana* that typically infect wood, this does not mean that the wood under study was free of them. White mycelium covering wooden logs at the sample collection site (Fig. 3) or the compact mycelium seen in laboratory cultures (Fig. 5d) that resembled the mycelium of *Serpula lacrymans* in initial development stages could have suggested the presence of fungi that did not develop distinct structures such as fruiting bodies with spores that could allow their identification. In the search for the direct symptom of wood deterioration that would indicate a need for wood proofing and consolidation prior to the appearance of observable material damage, Alfieri et al. 2016 noted that environmental fungi, as first wood colonisers, prepare the medium for secondary colonisers, which are fungi directly responsible for biodeterioration and it is their presence that can be seen as a cause for initiating protective actions [35].

According to the procedure proposed by *A. Koziróg at al.* [6], when assessing the degree of wood deterioration from a microbiological perspective and when making the decision as to indicating disinfection, one should analyse two major criteria: quantitative criteria that pertain to the number of microorganisms present on the material, and qualitative criteria that pertain to the presence of strains responsible for wood biodeterioration.

In the presented studies, the author's isolated fungi responsible for the biodeterioration of wood in the wood samples collected from the 'Wawel' villa, and the intensity with which they grew on media in the cultures and the historical substance in situ showed a high degree of wood contamination in quantitative terms. As stipulated in the procedure, the presented findings justify the necessity of performing disinfection with the intent of preventing biodeterioration in the historical building under study.

Conclusions

In summary, it should be noted that knowledge of the specific character and scope of alterations caused in historical wooden substance by microbiological contamination allows the assessment of the possibility of protecting or conserving it using a properly selected method.

In the case of the 'Wawel' villa, whose historical substance was subjected to microbiological testing, the authors observed potential for the use of preparations that can save the original substance of the building. Such preparations include Boramon (Altax, Poland) or Rocima 101 (HSH, Poland), which are proven to protect wood for up to several months post application without altering the colour or brightness of the wood [6, 30-32].

It should also be noted that their potential use should be included in managing the process of conserving and restoring historical structures. Such procedures should be enacted at the start of this process and account for the need for unforeseen conservation work and additional testing, including microbiological testing, and their consequences. These include disinfection, which is very costly and is to be performed by the developer. However, in the case of buildings as valuable as the 'Wawel' villa, which has retained its functio-spatial character and material to this day, this possibility should be taken under consideration to ensure the continued existence of this historical building [33-37].

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