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BIODEGRADATION AND BIODETERIORATION OF WOODEN HERITAGE: ROLE OF FUNGAL SUCCESSION

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Abstract

Wood from heritage is usually attacked by wood-decay fungi generating mainly loss of dimensional and structural stability. The study of wood biodegradation process and its mechanism allow the obtaining of tools for wood conservation. In this paper, wood biodegradation and biodeterioration processes were studied in order to acquire a direct and visual indicator of the beginning of wood degradation. This indicator will allow the consolidation and protection of wood before it will be structurally compromised. Wood degradation conditions found in turntable were reproduced in laboratory by accelerated processes: environment degradation was developed by fluctuation cycles of humidity and temperature. Biological degradation was performed using wood decay fungus isolated from wooden heritage samples. The wood samples were inoculated with an equal amount of mycelia until abortive basidiomata emerged. The result analysis indicated that even though each species occupies particular niches, first settlers (environmental fungi) would generate a material more bioreceptive for wood decay fungi being replaced each other as dynamic communities. Consequently, environmental fungi allow the wood decay fungi to colonise and exploit better their ecological niche (succession). It was concluded that the appearance of first settlers is therefore a reliable visual indicator of the need of wood consolidation in order to preventing irreversibly wooden heritage loss.

Keywords: *Wooden artefact; Conservation state; Destruction and alteration; Wood decay fungi; Wood decay indicator; Wood preservation*

Introduction

Wood is a traditional building material and its deterioration and biodegradation are important critical factors for its durability. Wood structures are usually degraded by wood decay fungi and they generate mainly loss of dimensional and structural stability [1-3].

A wood biodegradation and biodeterioration effect depends on abiotic factors such as moisture and temperature, but also on the fungal decomposers which alter the chemical and physical properties of wood [4]. Some fungi species may be facilitated, inhibited or neutralised by the actions of other species [5].

In decay fungal communities there are not non-random co-occurrence patterns in the field as well as in laboratory studies, suggesting that the effects of primary species are important and may influence community structure in decomposer fungal communities [5-8], and indirectly also affect the nutrient and carbon dynamics in the ecosystem. This has been described in different substrate types, susceptible to fungal degradation, as cellulose film, complex substrates

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and many plants. Nevertheless, each succession event is unique because it dependents on the host material and its environment [9-11].

The main aim of the present study is to assess the species associations during the assembly of fungal communities on diverse type of decaying wood to obtain an indicator of wood biodegradation process beginning.

Experimental

Materials and methodology

This paper was based on the current status of the locomotive turntable found in the Provincial Railway Station La Plata. It was inaugurated on April 27, 1910 and it was declared as architectural heritage in 2006 (Fig. 1). The locomotive turntable is 200 meters from this station (34º 56'16'' S and 57º 56'13'' W) and it is a structure that consists in wooden slats of Schinopsis sp. into metal framework [12, 13]. Currently, this structure is in an advanced status of degradation caused by environmental and biological factors, as show in Figure 2. This was used as a model of biodegradation process.

Fig. 1. Actual photographs of La Plata Provincial Railway Station:
A - Front view of central station building (142,000 m^2); B - Back view of central station building;
C - Aerial view for location of locomotives turning table (34º 56'16'' S and 57º 56'13'' W)
D - locomotive turntable status.

Fig. 2. Detail of locomotive turntable status:
A - Structure consists in wooden slats of *Schinopsis sp*. within metal framework;
B - slats in advanced status of deterioration caused by environmental and biological factors;
C - Samples acquired for simulating the same degradation status than wooden turntable in laboratory;
D - Detail of wood decay fungi found and its biodegradation (inside view).

The deterioration status of the turntable was evaluated by studying samples and records acquired from nearby places (pieces of material degraded and detached from the structure). To characterise the degradation caused by wood decay fungi in the found wood, direct observation and stereoscopic magnifier were used. To establish the cellular degradation of wood and its fungal colonisation, scanning electron microscopy (SEM) images were analysed. The fungal attack was measured by area occupied by mycelium and basidiomata, (digital photography software analysis, ImageJ).

The same (*Schinopsis balansae*) and others wood species (*Pinus ponderosa, Araucaria angustifolia and Aspidosperma quebracho-blanco*) were used for biodegradation process study (n = 20 for each wood specie). The conditions were reproduced in laboratory by accelerated degradation process: environment degradation was performed by fluctuation cycles of humidity (from 15 to 80%) and temperature (from 18 to 35ºC). Biological degradation process was performed using the wood decay fungus isolated from the wooden heritage samples evaluated. It was determined that the fungus belongs to Hymenochaetaceae family [14], which produces white rot and has a very strong oxidative capacity according to Nobles method [15].

For fungus inoculation, the wood samples were divided in two groups to simulate the different colonisation possibilities: wood samples were sterilised and inoculated with wood decay fungus, Group I. Other samples were not sterilised and inoculated with wood decay fungus, Group II.

Other samples were exposed to fungi colonisation, present in the environment; wood samples were exposed to weather, in the same place where the turntable is, for 30 days. Then, environmental fungi species were isolated by incubation in culture medium with rose bengal agar supplemented with chloramphenicol for 7 days at 20±5°C, in order to identify them. This medium was selected in order to suppress the growth of the bacteria and to limit the size of the fast growing fungal colonies. This restriction helps isolating fungi with slower growth, facilitating the morphological identification. Finally, those wood samples colonised by environmental fungi were inoculated with wood decay fungus, Group III.

To obtain the inoculated biomass, mycelia were isolated from degraded wood areas of the turntable and it was incubated in liquid medium for 15 days. Subsequently, each wood sample was inoculated with same fungal biomass amount (1±0.5g). All samples were incubated until *abortive basidiomata* emerged (120 days). For biodegradation analysis, percentage area occupied by fungal growth was measured by ImageJ software and this was compared between different conditions. Isolated wood affected by fungus decay was also incubated in agar-malt medium as fungal viability control.

Results and discussion

Under laboratory conditions, the deterioration status of the turntable was reproduced: In the case of *Aspidosperma quebracho-blanco*, environmental fungi colonisation occurred later than in *Pinus ponderosa* and *Araucaria angustifolia* due to the different amount of extractives present in each one (3 months vs. 1-1.5 month). In *Schinopsis balansae*, the absence of environmental fungi colonisation during the employed period was observed, due to the presence of extractives. The accelerated removal of these compounds is not possible without altering the wood properties. Thus, the results obtained by this process do not describe the natural degradation of this wood species, but give a notion of elapsed time (years) in order for the turntable to reach its current conservation status.

To confirm that the deterioration status of the wood samples subject to accelerated degradation process is similar to that of the found heritage wood, SEM images were analysed (Fig. 3). In panels A and B, advanced deterioration status of structure heritage was observed, with high fungal colonisation (hyphae) and the loss of wood microstructure (the cell wall was degraded). In panels C and D, wood samples used in laboratory for simulating the same degradation showed similar degree of fungal colonisation and wood microstructure loss. These observations allow us to affirm that the deterioration status was accurately reproduced. In addition, images analysis allowed us to conclude that the development of fungi communities is responsible for physical-chemical changes in the deterioration and degradation of wood.

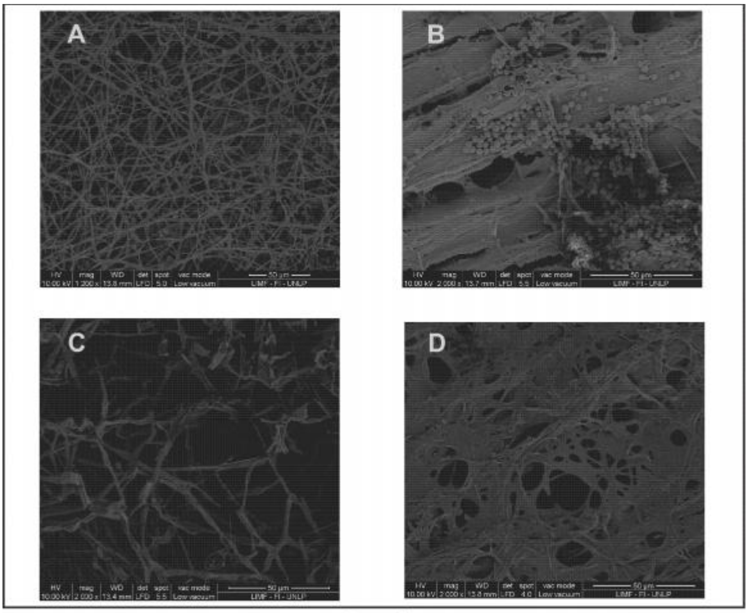

Fig. 3. Deterioration status of wooden turntable was reproduced, it is shown by scanning electron microscopy (SEM) of biodegraded wood samples: A and B - Wood found in locomotive turntable; C and D - Wood degraded in laboratory. Both showed similar degree of fungi colonisation and similar the wood microstructure loss.

Concerning the biological developing, it was observed that Group I (18±5% for P. ponderosa and *A. angustifolia*; 1±2% for *A. quebracho-blanco*) showed a lower development of wood decay fungi than Group II (41±5% for *P. ponderosa* and *A. angustifolia*; 10±4% for *A. quebracho-blanco*). According to these results, it was hypothesised that environmental fungi are necessary for wood decay fungi degradation since they will facilitate the colonisation and degradation process. This hypothesis was demonstrated by Group III conditions, where wood was previously colonised by environmental fungi and then inoculated with the wood decay fungus. In this group was obtained a complete wood decay in all samples (colonisation by wood decay fungus 100 ± 3% for all wood species).

The differences observed between groups indicated that environmental fungi would prepare the substrate for the subsequent colonisation of wood decay fungi. The first settlers increase and/or retain moisture of substrate, transforming it to be more bio-receptive (facilitation) (Fig. 4).

On the other hand, Group II showed a higher wood decay fungus development than Group I and lower than Group III. This co-existence between environmental and wood decay fungus is proof for the formation of biocoenosis with cooperative effect between species, as shown in Figure 4.

Finally, the complete colonisation of the substrate by wood decay fungus was observed in Group III, Figure 4: the first step of biocoenosis formation, the species coexist with cooperative effect between them. Subsequently, wood decay fungus could exploit resources better than environmental fungi due to their specific enzymatic battery, increasing their development and replacing environmental fungi in time (exclusion phenomenon). The differences observed between wood species are due to the different wood microstructure, cell morphology and amount of extractives present in each one.

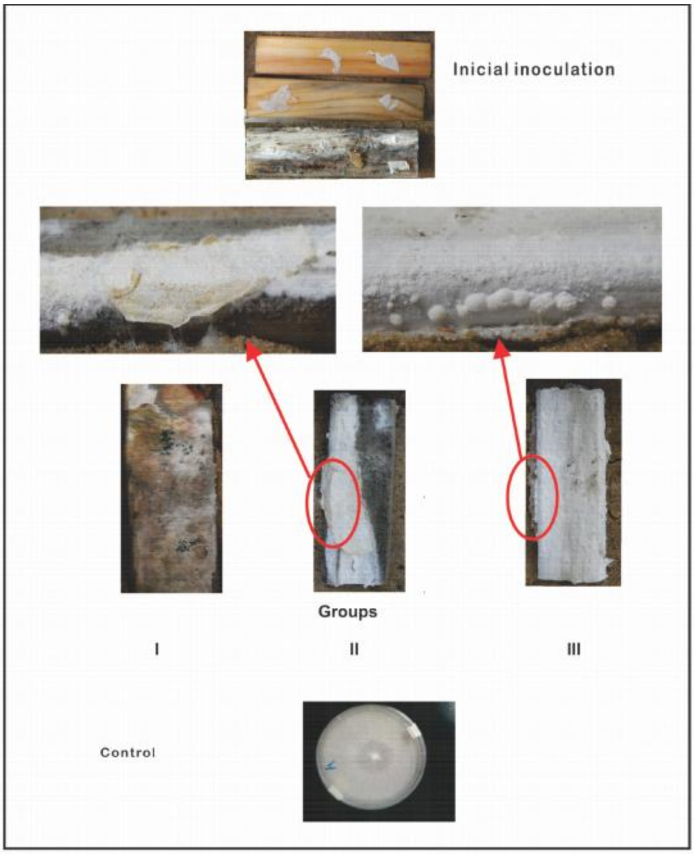

Fig. 4. Succession study: diverse groups represent fungi development in different inoculation condition: samples were sterilised and inoculated with wood-decay fungus (Group I); others were inoculated with wood decay fungus without sterilisation (Group II); and finally, another group was exposed to environmental fungi colonisation and once they were settled, they were inoculating with wood decay fungus (Group III).

Wood decay fungus culture in agar-malt medium was used as fungal viability control. Fungal succession was observed between environmental and wood decay fungus resulting in a necessary condition for wood biodegradation and biodeterioration process (note the fruiting bodies primordia).

Regarding diverse possibilities of colonisation to achieve the biodegradation status of wooden heritage, it was observed that the most representative condition was in Group III since the colonisation degree obtained matches with the conditions of turntable.

In regards to the environmental fungi, it was revealed that they belong to *mucoral* and *imperfecti* groups. The species determined were: *Alternaria sp.*, *Aureobasidium sp.*, *Cladosporium sp.*, *Mucor sp.*, *Rhizopus sp.* and *Epicoccum sp.*

The whole analysis of results establish that succession is a necessary phenomenon for wood biodegradation process: in early stages each species occupies particular niches, the first settlers (environmental fungi) would generate a better environment, which allows to wood decay fungi to colonise and exploit better their ecological niche. Biologically, succession has been defined as sequential occupation of the same site by different fungi or of different associations of fungi [16, 17]. Thus in wood biodegradation process, fungi are replaced by dynamic communities, alternating in space and time. Each species is adapted for occupying its particular niches and for competing for the resources used in common.

For the above mentioned, these results provide a visual indicator of the beginning of biodegradation process: the apparition of environmental fungi (Fig. 5). These fungi are easily detectable by human eyes, only affect the aesthetic appearance and they are easily removed by traditional clean protocols; while wood decay fungi often emerge (visually) when structural damage is irreversible. They are the principal consequence of the loss of wooden heritage.

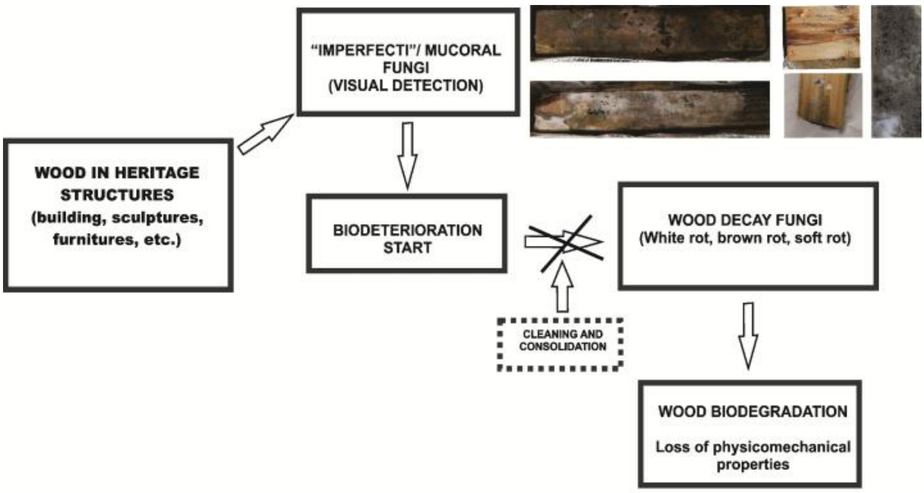

Fig. 5. Mechanism proposed for biodeterioration and biodegradation process. The appearance of first settler (environmental fungi) is therefore a reliable visual indicator of the needed of consolidate and protect wood. It is useful for preventing colonisation of degradation agent and to avoid structural irreversibly compromise of material.

In summary, the appearance of environmental fungi ("imperfecti"/mucoral fungi) is an indicator of the need to consolidate and protect heritage assets, to prevent the colonisation of specific degradation agent, who will be responsible for irreversible heritage loss. In recent years the study of methods and techniques of restoration and consolidation for heritage conservation was emphasised due to its high value for society. However, these studies suggest an intervention addressed usually without delving in the specific actions of each deterioration agent. Therefore, this study demonstrates that understanding mechanisms of damaging agents provides tools for the appropriate long-term prevention.

Conclusions

The importance of understand the degradation processes lies in obtaining tools for conservation of wood structures: in this paper it was evidenced by the obtaining of a new tool for recognised the biodegradation initial stage.

Biodegradation process is the result of the interaction between material, biota and climatic factors. The biodegradation initial stage is subject to emergence of "imperfecti"/mucoral fungi. Consequently, it is a visual indicator (detected by human eye) of the need to consolidate and protect wood. Therefore, front of this indicator, it required a rapid intervention to prevent wood degradation by decay fungi, which are the responsible of irreversibly damage of material.

Fungal succession occurs in wood biodeterioration process and it is necessary for wood decay fungi development. The degradation mechanism could be interpreted as a complex ecological pattern: few changes in the micro-environment can activate the succession by secondary settlers (wood decay fungi) since the first settlers change the bioreceptivity of material. It is the beginning of wood biodeterioration and biodegradation processes.

On the other hand, the different wood species is another factor to consider: the extractive substances that act as fungistatic biocides prevent the fungi development. Thus, there will be no colonisation of the first settlers until extractive substances are depleted, retarding colonisation of second settlers (wood decay fungi). This is one of the causes of the difference in decay resistance between wood species.

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