Scanning Electron Microscopy examination of the surface of softwood attacked by fungus

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Abstract. The morphological structure of the surface of four *Cedrus atlantica* softwood samples was monitored by scanning electron microscope. The obtained results have shown formation of cracks on the surface of samples with deterioration of the majority of cellulosic fibers. The alteration of wood structure and decomposition of carbohydrates exploited by scanning electron microscopy images could act as a confidential sign of an advanced stage of wood decay by biological attacks, leading upon time to the extinction of this natural wealth. It appeared that the studied samples were attacked by brown-rot fungus selectively decaying the cell wall of softwood materials. The scanning electron microscope morphological observation revealed that microfibril distribution was heterogeneous and dynamic contact angles increase significantly during exposure to the non-controlled environmental conditions.

1 Introduction

Since antiquity, softwoods are known to be one of the best natural materials that can be used for building purposes, because of their exceptional properties (high mechanical strength, fast growing, hygroscopic feature, etc.).

The decomposition of these materials depends directly on the frequent changes in temperature, UV light, humidity, etc. [1-3]. As a result, chemical processes and biological activities take place causing a serious deterioration of the chemical, physical and morphological structure of these materials [4]. The changes in relative humidity level can favor the development of different types of fungus that break down lignin and cellulose, causing deep defibrillation of the wooden materials [5, 6]. On the other hand, the pH of the wood is also an important factor that could have an effect on the type of fungus that may become established [7].

It was reported that three types of fungal wood decay can occur: soft-rot, white rot, and brown-rot [5, 8-10]. Soft-rot is a slow deterioration caused by metabolism of polysaccharides and lignin [11-12]. White-rot fungus deteriorates all structural cell walls of carbohydrates (cellulose and hemicelluloses) and lignin, whereas brown-rot fungus selectively deteriorate the carbohydrates structure, with limited lignin degradation [9].

The morphological changes of wood resulting from these biological decay have been investigated using different spectroscopic methods [9-10,13]. The Scanning Electron Microscopy (SEM) technique is the common technique used by different authors to characterize the morphology of archaeological objects such as wood. It was generally used as a powerful non-destructive method to verify the extent of structural decay and to identify the specie of wood, as well as to determine the nature of microbiological attacks [12,14-15]. By combining this analytical technique and energy dispersive X-ray spectrometry (EDX), we can obtain information about composing elements inside the wood [16].

The object of this study is to assess the changes that occur in the morphological structure, as well as to determine elemental composition, of eight samples of *Cedrus atlantica* softwood, as attacked superficially by fungus. The state of degradation of these samples is analyzed and the effect resulting from the natural degradation process is characterized experimentally. The evaluation is carried out by determining the microbial growth on the wood surface by means of the SEM.

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2 Materials and methods

2.1 Studied samples

Eight wood material samples were extracted from four cedar softwood (*Cedrus atlantica*) specimens originated from the Middle Atlas of Morocco and which are dated back to 16th, 17th, 19th and 21st centuries. They are considered as dead wooden materials, except for the latest sample dating from the 21st century which was taken from a living tree. The samples of degraded cedar wood were extracted from the outside surface (superficial face) which is exposed to the effects of environmental conditions. The non-degraded samples that are considered for comparison were taken from a position laying at 5cm of depth below the surface of the wood pieces. The characteristics of the analyzed samples are presented in Table 1.

Table 1. Description of cedar wood analysed samples; (D) degraded, (ND) non-degraded

Sample	State of analyzed sample	Age (Century)
A	ND	21st
A'	D	
B	ND	19th
B'	D	
C	ND	17th
C'	D	
D	ND	16th
D'	D	

2.2 SEM analysis

This analysis was performed by using a FEI Quanta 200 MK2. It is worth to note that the photographs were collected at the high resolution (x2000 magnification). For non-degraded samples, the observations were carried out directly on the wood sample and the morphological structure has been clearly characterized. However, a blurred and poor image takes place during analyzing degraded samples because of the accumulation of negative parasitic loads that produced the intense emission. Hence, a metallization process was intended to make the sample conductive of electrons by avoiding this accumulation, and consequently the obtaining of clear and explicating images. The samples were metalized by cathodic sputtering using an EDWARDS Scancoat Six SEM coater to cover them with a thin carbon layer (between 20 and 40nm), while respecting and maintaining their topography.

3 Results and discussions

The SEM analysis allowed achieving spatial and complementary information on the examined materials that are essential for studying the morphological changes occurring on wood material exposed to the factors of natural degradation. The SEM results showed that there are serious modifications of microstructure that have taken place because of natural degradation process; the surfaces of all wood samples were broken, and serious cracks and distortions appeared (Fig. 1).

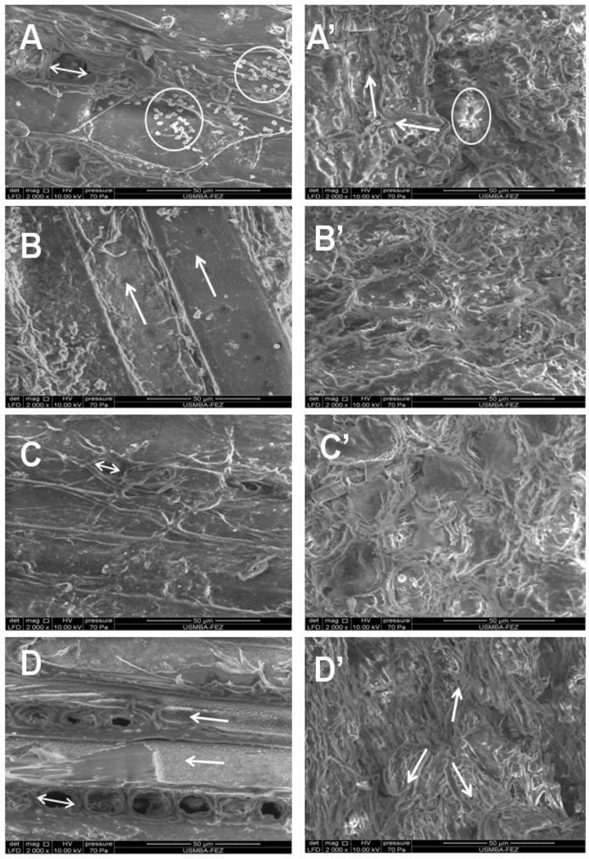

Fig. 1. Scanning electron micrographs characterizing the cedar softwood structure and comparing the morphological changes of non-degraded cedar wood samples and degraded ones (A and A': sample dating from the 21st, B and B' sample dating from the 19th, C and C' sample dating from the 17th, D and D' sample dating from the 16th century).

In the absence of an advanced degree of degradation process as shown in Fig. 1, for samples B, C and D which are non-degraded and dating, respectively, from the 16th, 17th and 19th centuries, a typical and organized structure can be seen regarding the random distribution of the fibers. This is formed by tracheids (thin cells) oriented in the same direction and arranged in longitudinal rows. It can be seen also that the tracheids diameter of the recent sample (Fig. 1A) is 16µm, and decreases to 12µm for the sample dating from the 17th century (Fig. 1C). For old samples dating from 16th century (Fig. 1D), the diameter increases and reaches 14 to 18µm. The heterogeneous surface in various regions and the distribution of fibers in different directions (marked with arrows in Fig. 1) can be observed for samples A' and D'. It appears more lucidly in the degraded sample than in the non-degraded one indicating the physical attacks of anatomical structure of all materials; whereas dynamic contact increases significantly as the age of sample progresses (Fig. 1D'). It is worth to note that the increase in heterogeneity was undoubtedly due to the strong physical, chemical and biological effects of degradation on the lignin-carbohydrate matrix.

Based on SEM pictures reported in Fig.1 for samples A and D, it can be observed that each tracheid contains numerous cavities which often follow a helical orientation. Under harsh conditions, the diameters of these pits extended and show irregular form providing direct evidence for remarkable deformation of the fiber borders (Fig. 1A and 1D). According to the literature [17], the fungal hyphae penetrated wood samples via ray and then spread through pits into the cell lumens, resulting in an alteration of wood structure and yielding changes of carbohydrate decomposition products. Subsequently, they degrade cell wall components with cellulolytic enzymes and/or low-molecular agents by growing inside the cell lumens [13].

In archaeological field, this state has prompted scientist researchers to focus on the study of the shape of these pits, which can vary from one group of conifers to another, as one of the most microscopic features used for characterization of softwood materials.

As reported by Tamburini and al. [18] , the opened structure observed in Fig.1 (samples A' and D') can be described as associated to the weakening of a fraction of the hydrogen bonds which can be likely attributed to water penetration into the cellulose fibers. Furthermore, the microscopic evaluation allowed investigating the biological attacks of wood. The colonization of fungal spore was observed on non-degraded part of recent sample (Fig. 1A) and the sample dating from the 19th century (Fig. 1B). Particularly for sample A, the distribution of the fungus spores covered an important space of wood surfaces (these are marked with circles) and the colonies are flat. These changes enhance the enzymatic saccharification and consequently generate abundant reactive sites on the fiber surface [19]. At the initial stages of the brown-rot fungus decay, the cell wall rapidly degraded and the softwood strength rapidly decreased [13, 20].

As found in previous studies about microbiology of the archaeological wood, the cellulolytic fungus attacking the wood produce *exo-* and *endo-* β-1,4-glucanase, which decompose cellulose fraction. The first enzyme provokes the initial stage of attack of crystalline form of cellulose. The latter enzyme achieves the degradation of the fibers resulting in short-chains called cellobiose [21]. On the other hand, wood-destroying fungus can metabolize the carbon-rich constituents of wood [14]. This observation clearly shows that our samples underwent a heavy biological degradation. Overall, the high concentration of extractives makes wood resistant to fungal attack; however, the exposure to natural degradation process for many years caused an important reduction in the amount of these extractives making, thus, wood less resistant to the deterioration.

Fig.1 for samples D and D' shows an advanced deterioration on microfibrils structure of sample dating from the 16th century which is in a state of extreme vulnerability may be due to its advanced age or to the microorganisms which have probably been active for many years within it. In advanced stages of simultaneous degradation, the SEM sections show that wood intensively attacked by fungus and fibers become hollow pores after degradation in the same patterns. It is of great importance to notice that biological attacks don't affect only the structure of wood but also the elemental composition of wood [14].

Generally, during the state of carbohydrate depolymerization, the decomposition of cellulose and hemicellulose could act as a startling sign for an advanced stage of softwood decay caused by brown-rot [13, 22, 23].

4 Conclusions

Morphological analysis by SEM showed that cedar wood, as softwood material, has a simple structure that is characterized by the presence of tracheids and which is responsible for sap transport with uniseriate or biseriate ligneous rays. The morphological changes in the structure of various cedar wood samples and their state of degradation were studied using SEM microscopy. The results show that the effect of the natural degradation process appears more pronounced at the surfaces of the old samples than the recent ones and leads to a deterioration of the majority of the cellulosic fibers, in particular for the sample dating from the 16th century.

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