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CHARACTERIZATION OF ARCHAEOLOGICAL WOOD STAINED WITH BAT EXCRETIONS USING VARIOUS ANALYTICAL TECHNIQUES

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Abstract

This study was conducted to diagnose and evaluate the deterioration of archaeological wood caused by bat excretions. The samples were collected from the ceiling of Mohamed Ali palace (1812), which is located in Suez, and Baron Empain palace (1911), which is located in Cairo, Egypt. The wooden samples were covered with a preparation layer (gesso) on one side. Parts from both sides of the wooden samples that suffered from the accumulation of bat excretions were examined. The effects of bat excretions on the anatomical structure and chemical composition of the wooden samples were examined and evaluated using various analytical methods, such as scanning electron microscopy (SEM), Fourier transform infrared spectroscopy (FTIR), and X-ray diffraction (XRD). In addition, a microbiological study was undertaken to identify microbial activity on the wood surface. The results showed that the wood suffered changes in its anatomical structure in addition to scratches on the wood surface that were caused by the bats' claws. Changes in the molecular bonds of both lignin and cellulose were noted in the wood. In addition, the polymerization degree of cellulose was reduced.

Keywords: *Archaeological wood; Bat excretion; SEM; FTIR; XRD; Microbial activity*

Introduction

Archaeological wood is subjected to different types of decay, resulting in distinct changes within the wood structure [1]. The degradation patterns that occur in wood structures vary according to the wood type, the usage of the wood material (i.e., whether the wood was used in buildings as an architecture element, in panel paintings, in sculptures or in any other objects), and deterioration factors that affect the wood (i.e., weathering when the wood is exposed to natural elements, e.g., air, water and light), physical, chemical, and mechanical factors, and biological agents).

Weathering has a destructive effect on the wood which can undergo severe changes of its physical and structural properties due to the combined effect of sunlight, oxygen, moisture, atmospheric pollutants and micro-organisms during outdoor exposure [2]. Iron or other metal corrosion products can weaken wood and cause significant cell wall alterations. Metal ions are active catalysts promoting chemical reactions that initiate a non-biological type of cell wall degradation. Moisture and soluble chlorides accelerate the corrosion process and the deterioration of wood [3].

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A few studies indicated that chemical hydrolysis was most likely responsible for some degradation patterns observed in the wood structure, when the wood is in contact with limestone, gesso, or various minerals over long periods of time [3-5], but the results of a recent study indicated that preparation layers cause serious damage to the adjacent wood surface. The patterns and the degradation rate of the wood covered with chalk- and gypsum- based layers differ according to the chemical composition of the layer which can be maximized if the layer contains both minerals [6].

Wooden elements in historic building are susceptible to a complex series of deterioration and degradation factors according to their structure, their location and their function [7- 9]. In addition, in many bat-inhabited historical buildings in Egypt, bat colonies are responsible for depositing large amounts of droppings (guano), urine and blood, causing aesthetic and biological damage [10].

Various methods can be used to characterize the surface properties of wood; these methods may be divided into three broad categories: microscopic, spectroscopic, and thermodynamic. Microscopic methods provide information about the surface morphology, spectroscopic methods provide information about the surface chemistry, and thermodynamic methods provide information about the surface energy [11]. Monitoring the anatomical features associated with decayed wood and estimating the changes of the chemical components in wood that are caused by different deterioration factors and treatments are helpful strategies for determining the degradation level within the wood structure, which enhances our understanding of the mechanisms that underlie the processes of degradation and deterioration.

Based on various analytical methods, this study aims to assess the deteriorations and degradation of wood stained with bat excretions, which is essential for understanding the characteristics of this wood and for planning appropriate conservation procedures for these valuable items.

Materials and Methods

Sampling

The samples were collected from the ceiling of Mohamed Ali palace (1812), which is located in Suez, and Baron Empain palace (1911), which is located in Heliopolis, Cairo, Egypt. The wooden samples were covered with a preparation layer (gesso) on one side. Parts from both sides of the wooden samples that suffered from the accumulation of bat excretions were examined and compared with unaffected wood (control sample) that was obtained from the wood sample's core away from the substrate. The wood samples that were chosen for this study were desiccated and brittle; therefore, it was not possible to carry out standard sampling techniques or to make thin sections in order to identify the wood. Four wooden samples were examined. The first two samples were taken from the side that was covered with the gesso layer, which was stained with bat guano (excrement), whereas the other two samples were collected from the back side of the wood sample, which was directly covered with bat blood and excretions (Table 1).

Table 1. Samples used for the SEM study

Samples	Location	Sampling region
Sample no. 1	Mohamed Ali palace	From the wood directly beneath the gesso layer.
Sample no. 2	Mohamed Ali palace	From the wood directly beneath the bat excretions (blood and guano).
Sample no. 3	Baron Empain palace	From the wood directly beneath the gesso layer.
Sample no. 4	Baron Empain palace	From the wood directly beneath the bat excretions (blood and guano).

Investigation and Analytical Methods

Environmental Scanning Electron Microscope (ESEM) and EDX

All the samples were prepared for ESEM by fixing small pieces of an appropriate size on stubs with double-sided cellophane tape. The samples were examined using a Quanta 250FEG ESEM. The ESEM study was performed to monitor significant changes in these samples. In addition, the elemental composition of some areas of the bat excretion layer that accumulated on the wood surface of samples no. 2 and 4 was analyzed using an EDX Ametek Octane Pro.

X-ray Diffraction (XRD)

The mineral composition and percentages of the preparation layers were also identified, and measurements of the cellulose crystallinity in the wood samples were performed via powder XRD using a Philips Analytical X-Ray B.V., the PC-APD diffraction software, and a type PW 1840 diffractometer with a Cu tube anode, a generator tension of 40kV and a generator current of 25mA. The CuKα radiation consists of Kα1 (0.154056nm) and Kα2 (0.154439nm) components. Scans were obtained from 5 to 60 degrees 2θ in 0.03-degree steps for 0.3 seconds per step. The crystalline index (crystalline to amorphous ratio) was calculated as described by Segal et al. 1959 (i.e., CI% = ((I002 -I18)/I002)X100, with the diffraction intensities I002 at 002 peak position (2Θ = 22.5º) and I18 at 2Θ = 18º (angle of amorphous cellulose)) [12].

Fourier Transform Infrared Spectroscopy (FTIR)

FTIR was used to monitor the chemical characterization and changes that occurred in the wood due to the bat excretions. The samples were analyzed with an FTIR spectrometer (Model 6100, Jasco, Japan). The spectra were obtained in the transmission mode with a TGS detector using the KBr method and represent (2mm/Sec) co-added scans in the spectral region from 4,000 to 400cm^{-1}, with a resolution of 4cm^{-1}.

Microbiology study

This study was conducted to investigate the presence of microbial infection in the wood due to bat excretions. For microbiology studies, the samples were cultivated on Potato Dextrose Agar (PDA) medium. After 10 days of incubation at 25-30ºC, the microorganisms developed. The medium showed the growth of different kinds of bacteria. The developed bacteria were isolated, purified and identified.

Results

Environmental Scanning Electron Microscope (ESEM) and EDX

ESEM was used to identify and examine the wooden samples. The results showed clearly that the samples from both palaces were softwood *Pinus halepensis L.*

The wood samples that were collected from the ceiling of Mohamed Ali's palace (samples no. 1 and 2) were nearly completely covered with bat excretions. SEM was used to examine the wood surface. The results clearly demonstrate that the surface of the wood (sample no.1) that adheres directly to the preparation layers undergoes distinct anatomical changes, as the sample exhibited progressive erosion and loss in the middle lamella region, which cause separations between cells. In addition, loss and degradation were observed in some parts of the cell walls. The cell wall layers became disrupted and dispersed due to the extensive degradation. In addition, the partial collapse of the wood structure was observed (Fig. 1).

The SEM images of wood sample number 2 show the bat guano layer (excrement) that covers the wood surface. The remains of an insect, especially the antenna and legs, appeared clearly within the bat guano layer (Fig. 2a). Some areas of the bat excretion layer were analyzed using SEM-EDX (Fig. 2b, Table 2). The loss of numerous parts of the wood structure and the presence of cracks, fractures, and separations along the wood cells were noticed. In addition, microscopic scratches were found on the wood cells due to the bats' claws (Fig. 2c).

The precipitation of bat guano on the wood cells was apparent in the SEM images. A parallel series of separations occurred along and within the cell walls, which appeared due to the loss of the middle lamella layers that caused adjacent cells to separate from each other. The pits appeared to be nearly intact, although the openings and unevenness were increased. Additionally, aggressive erosion and cell loss occurred due to the partial breakdown of the wood cells in some regions that became easily fragmented. Cracks and fractures developed within the cell walls in the sample, especially beneath the bat guano (Fig. 2d, e and f). Bacterial attack that affected the wood structure and resulted in erosion and the loss of parts of cell walls was evident in the SEM images (Fig. 2g and h).

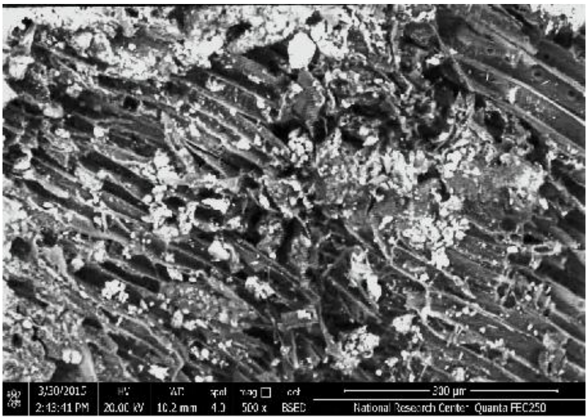

Fig. 1. SEM image of longitudinal section of sample no. 1 shows loss in the middle lamella region which causes separations between cells. Loss and erosion in parts of the cell walls are evident.

Table 2. SEM-EDX microanalysis of selected areas from the studied samples

Elements (%)	**Samples**		
	Mohamed Ali palace		**Baron Empain Palace**
	1	**2**	**3**
Na	——	1.04	——
Mg	5.23	1.8	0.54
Al	0.8	1.37	0.41
Si	2.14	3.54	1.28
P	5.53	2.8	3.74
S	2.67	2.12	7.49
Cl	1.76	1.71	1.57
K	4.65	4.53	7.05
Ca	23.79	10.64	24.17
Fe	——	2.36	6.48

The wood samples that were collected from Baron Empain palace (sample no. 3 and 4) were also covered with bat excretions. The SEM images of sample no. 3 revealed the effect of the gesso layer on the wood structure. Severe erosion, the loss of the middle lamella, and the disintegration of the wood cells could be seen. In addition, the SEM images illustrate the presence of numerous sustained cracks and fractures across the cells that were present along the wood grain (Figs. 3a and b).

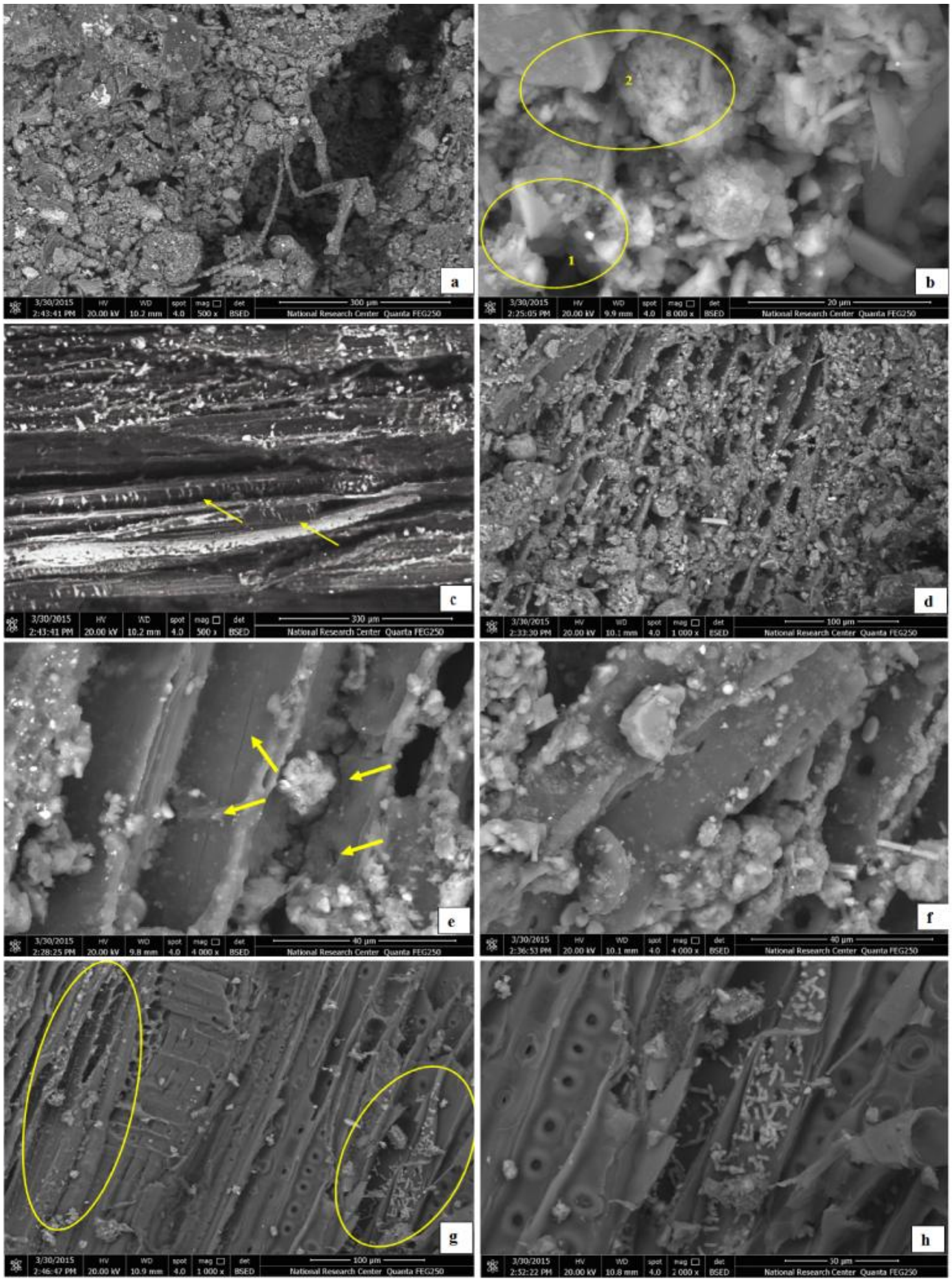

Fig. 2. SEM images of longitudinal section of sample no. 2: **a** - shows the bats guano layer which contains remains of the insects especially the antenna and legs; **b** - illustrate the bats guano layer that is covered the wood surface and the areas that were analyzed with EDX; **c** - shows clearly the microscopic scratches on the wood cells, as well as the fractures and separation along the wood structure; **d** - illustrate the precipitation of bats' guano on and in the wood cells. Also, loss and separations along the cell walls appeared; **e** - shows erosion and loss of cell walls in some regions. Added to, a serious of separations occurred along the cells and within the cell wall layers. Also, cracks and fractures appeared clearly on the cell walls especially beneath the bat's guano; **f** - shows breakdown of wood tissue in some regions;
g - shows cell walls erosion in the wood tissue due to bacterial infection; **h** - Detail of the previous image shows the bacteria growing on the cell walls.

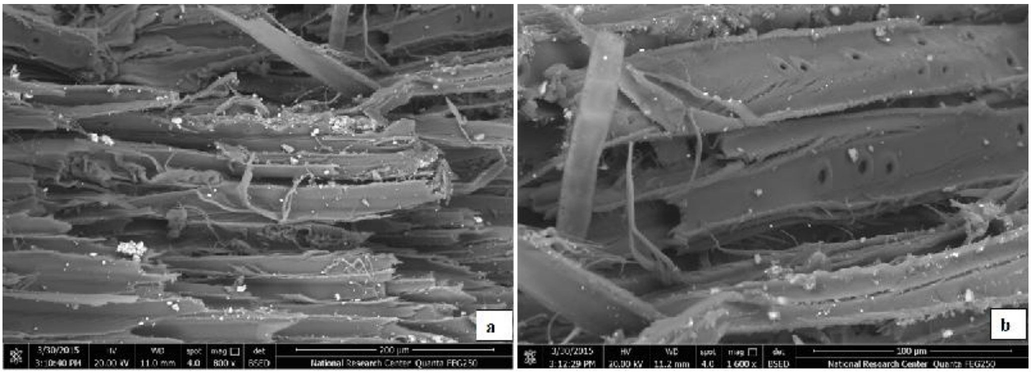

Fig. 3: SEM images of longitudinal section of sample no. 3; **a -** shows the wood surface beneath the gesso layer that suffer of loss in the middle lamella region which causes separations between cells. Also, erosion of the cells is evident and caused breakdown in the wood anatomical structure; **b -** showing weakening and disintegration of the wood cells and a series of separations along the cells. Besides, serious cracks, fissures and fractures through the cell walls

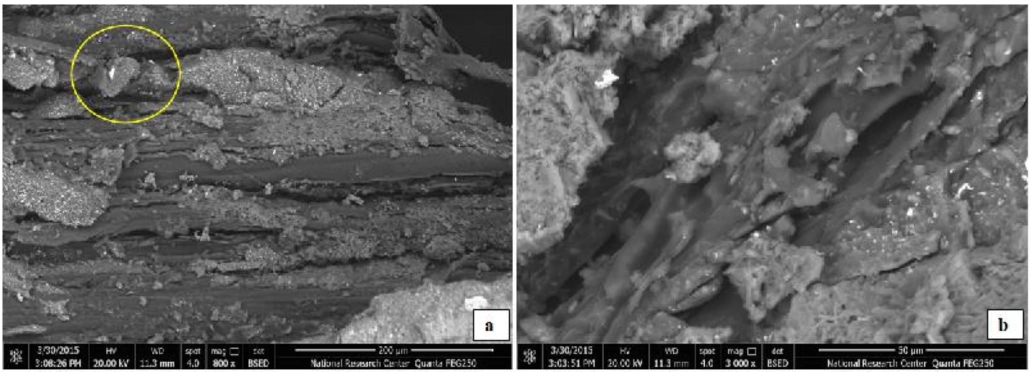

Fig. 4: SEM images of longitudinal section of sample no. 4: **a -** shows deterioration of the wood cells due to bats' excretions. Erosion and separations along the cells are obvious. Besides, loss and cracks through the cell walls. Also, this spot was analyzed with EDX; **b -** shows degradation of the cells that lay directly beneath bats' excretions.

Wood sample no. 4 showed separation along the cell walls, which led to large voids between cells and the detachment of adjacent cells due to the complete loss of the middle lamellae region. Deep fractures can be observed within the cell wall. The wood tissue appeared to suffer from deformation and collapsing in some regions (Figs. 6a and b). EDX analysis was performed on an area of bat excretions that accumulated on the wood surface (Table 2).

X-ray Diffraction (XRD)

Composition of the gesso layers

The XRD results showed that the gesso layer that covered the wood surface in both buildings was composed primarily of calcium carbonate - calcite (Fig. 5).

Cellulose Crystalline Index

The different percentages of the crystalline index of the control wood samples and the samples affected by gesso layers and bat excretions were determined using XRD. The results revealed a drastic decrease in the crystalline index of the samples that were covered with bat guano and blood, as presented in Table 3.

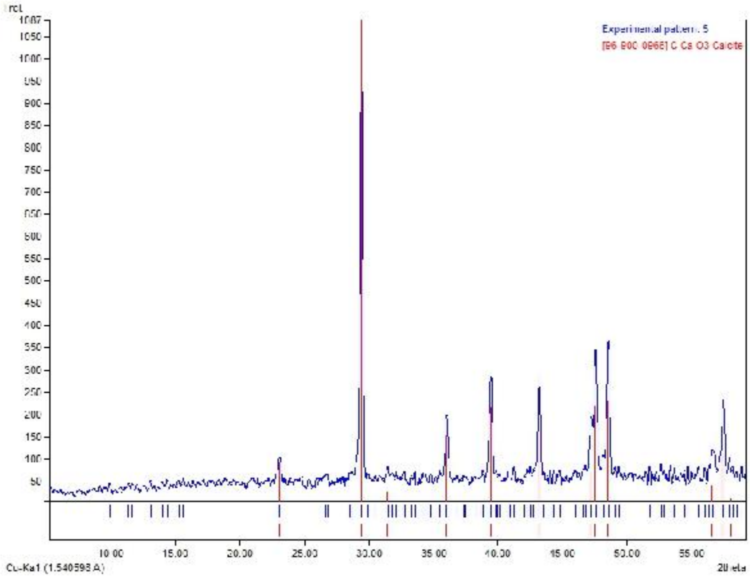

Fig. 5. XRD patterns of the studied samples illustrate that calcite is the main component of gesso layer covering their surfaces.

Table 3. Crystalline index of the wood samples

Samples		Crystalline area		Amorphous area		Crystallinity Index (%)
		2ϴ	Counts	2ϴ	Counts	
Mohamed Ali palace	Control sample	**18.1 º**	**1.2**	**22.6º**	**3.5**	**65.7**
	Wood beneath gesso layer	**18.3 º**	**2.3**	**22.7º**	**3.5**	**34.3**
	Wood beneath bat blood	**18.1 º**	**2.1**	**22.6º**	**3.2**	**34.4**
	Wood beneath bat guano	**18.2 º**	**2.0**	**22.7º**	**2.9**	**31.0**
Baron Empain palace	Control sample	**18.1 º**	**2.0**	**22.6º**	**3.8**	**47.4**
	Wood beneath gesso layer	**18 º**	**1.3**	**22.5º**	**2.1**	**38.1**
	Wood beneath bat blood	**18.2 º**	**1.0**	**22.6º**	**1.7**	**41.1**
	Wood beneath bat guano	**18.3 º**	**1.1**	**22.8º**	**1.7**	**35.3**

Fourier Transform Infrared Spectroscopy (FTIR)

The infrared spectra of the surface of the control samples show the same basic structure as all wood samples [13-15], a very strong, broad O-H stretching absorption centered at approximately 3429 and 3405cm^{-1}. In addition, the absorption arising from C-H stretching vibrations was centered at approximately 2926cm^{-1}. The absorption at 1640 and 1642cm^{-1} that was attributable to the C=O stretching band is relatively strong, as is the band at 1511 and 1510cm^{-1}, which is caused by the presence of aromatic groups in lignin.

The most significant feature in the spectrum of the wood samples stained with bat blood in comparison to the spectrum of the control is a sharp decrease in the O-H intensity at 3429 and 3405cm^{-1}, which was accompanied by a slight decrease in the intensity of the C-O stretching band at 1057cm^{-1}. These findings are attributed to the oxidation of functional groups of the glucopyranose rings in cellulose. In addition, a significant increase in carbonyl absorption was observed, together with a dramatic decrease in the intensity of the lignin bands, which disappeared as a result of degradation. In addition, the appearance of a new C≡N functional group due to pyrrole was attributed to the bat blood.

The spectrum of the wood samples stained with bat guano resembles the spectrum of the samples exposed to the preparation layer's (gesso) effect. Both spectra show a slight increase in hydroxyl absorption and a significant increase in the intensity of carbonyl absorption. Dramatically, the aromatic group of lignin completely disappeared due to degradation.
Additionally, a qualitative increase in amorphous cellulose content, which corresponds to the band at 1426 and 1427cm^{-1}, can be assumed in all affected samples [Figs. 6a and b].

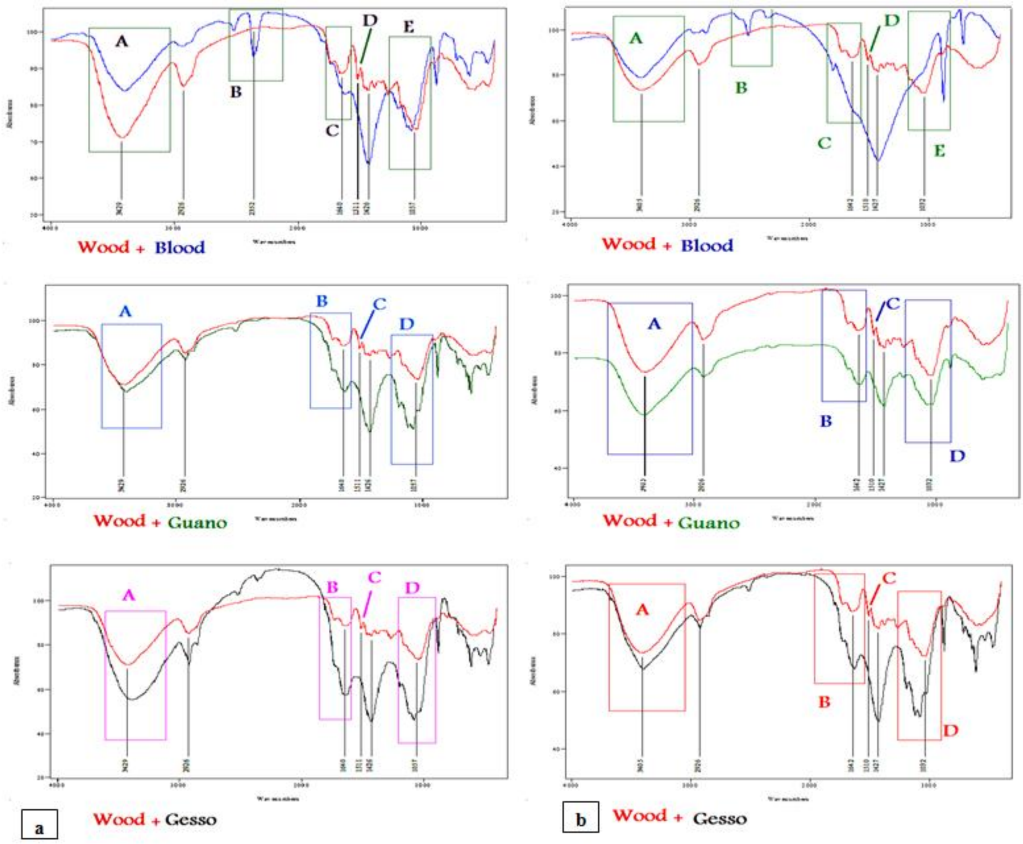

Fig. 6. FTIR spectra of wood samples: **a -** from Mohamed Ali's palace showing the alkaline effect of gesso layer and bats' guano on lignin bands which decreased in intensity; **b -** from Baron Empain's palace showing the alkaline effect of gesso layer and bats' guano on lignin bands which decreased in intensity.

Microbiology study

The SEM examination showed the presence of bacterial attack in the wood samples, and the microbial study identified three kinds of bacteria, two of which were Gram-positive (i.e., *Pediococcus dextrinicus* and *Pediococcus halophilus*) and one of which was Gram-negative (i.e., *Methylococcus luteus*).

Discussion

Wood samples were collected from two different buildings that were located in different places in Egypt and inhabited with bats. The wooden elements in these buildings were identified as the same kind of wood (*P. halepensis L.*),which was covered with a calcite-based preparation layer and subjected to the same kind of deterioration, such as weathering, mechanical deterioration, and chemical deterioration due to the preparation layer (gesso) and the accumulation of bat excretions.

To understand the characteristics of the archaeological wood stained with bat excretions in greater detail, it is essential to begin with an understanding of the structure of this wood and the associated decay processes. As demonstrated in a recent study, the calcite-based gesso layer degrades lignin more readily than carbohydrates in the wood structure [6]. In addition, the fact that the lignin content of softwoods (25-35%) is higher than those of hardwoods (18-25%) [16], which explains the SEM results that revealed a general weakening of the wood structure beneath the calcite-based gesso layers and disintegration and separations along the fibril orientation of the cell walls due to the loss of lignin in the middle lamella and cell wall layers. Fissures and erosion were also observed within the cell wall. The chemical attack of the calcite caused the defibration of the entire wood surface. In addition, wood exhibits had variable behaviors under different loads and stresses [7].

SEM results from samples stained with bat excretions clearly showed the presence of insect remains due to the food habits of the bats (i.e., most species of bats typically eat insects) [17, 18], as there are no signs of insect attack in the wood structure. In addition, EDX analysis of spots with bat guano layer revealed a substance rich in minerals. However, the studied areas had lower levels of sodium (Na), aluminum (Al), chlorine (Cl) and iron (Fe). Considerable amounts of magnesium (Mg), silicon (Si), sulfur (S), phosphorus (P), and potassium (K) were recorded. In addition, calcium was the dominant element in the studied areas. The elemental composition of these areas indicates the presence of some salts related to the chemical composition of bat guano [19], as well as dust particles accumulated on the surface.

Because bat guano contains billions of bacteria, one of which is rich in ammonia, as discovered in a previous study [20], this could explain the bacterial attack within the wood tissue. Additionally, we observed a strong resemblance between the decay patterns that resulted from bat excretions and those that resulted from the calcite-based gesso layer, as the bat guano and blood also have an alkaline effect on the wood structure.

The CI of cellulose was used to interpret the changes in cellulose structure that were caused by the gesso layer and the bat excretions. The observed decrease in the CI cannot be attributed to weathering because weathering degrades the amorphous phases of the cellulose and consequently enriches the relative crystalline content [13, 2]. The depolymerization of cellulose may have occurred due to the ability of the bacteria to secrete some acids, as proved by other researchers, who demonstrated that some bacteria degrade carbohydrates preferentially [21] and affect the crystalline structure of cellulose microfibrils [22]. In addition, the reduction of the crystalline index may be related to the mechanical deterioration caused by loads and stresses, as the weakest part of the ultrastructure of wood is the bonds between parallel strands of microfibrils, as demonstrated by Borgin 1971 [23].

As shown in the FTIR spectra of the affected wood, no differences in chemical attack patterns were observed between the wood samples collected from Mohamed Ali palace and the wood samples collected from Baron Empain palace. The results suggest that the calcite-based gesso layer and the bat excretions, accompanied by the weathering effects of light and water, caused lignin to be broken down severely. Moreover, the observed increase in carbonyl groups was attributed to the oxidation of functional groups of the glucopyranose rings in cellulose and phenolic compounds in lignin to carbonyl groups in wood samples stained with bat blood. This finding indicates that bat urine may be present with the bat blood, resulting in the degradation of both cellulose and lignin.

Conclusion

Collectively, the findings of this study shed light on the role of bat excretions in the deterioration of wooden elements in bat-inhabited historical buildings. According to the results of this study, wood covered with a calcite-based preparation layer undergoes chemical deterioration due to the alkaline effect of calcium carbonate, which degrades lignin more readily than carbohydrates. In addition, the results indicated that bat blood and guano have an alkaline effect that breaks down lignin as much as calcite-based gesso. Furthermore, the fact that the bat guano contains billions of bacteria might reduce the CI of cellulose. In addition, this study suggests that the observed decrease in the CI of cellulose may also be caused by mechanical deterioration resulting from loads and stresses. Moreover, the bats can protect the wood from insect attack, as insects are considered the main source of food for bats.

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