

*Original article***CHARACTERIZATION OF HISTORICAL BOOKBINDING LEATHER BY FTIR,
SEM -EDX AND INVESTIGATION OF FUNGAL SPECIES ISOLATED FROM
THE LEATHER**Mansour, M.¹, Hassan, R.¹ & Salem, M.²¹*Conservation dept., Faculty of Archaeology, Cairo Univ., Cairo, Egypt.*²*Forestry and Wood Technology dept., Faculty of Agriculture, Alexandria Univ., Alex. Egypt**E-mail:* rushdyarabii@yahoo.com

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Abstract

The aim of the present study was to identify fungi that cause deterioration of historical leather in the storage room, compare quantitative compounds of elements present in historical leather and new ones, and check the ability of the isolated fungi from recently leather book to colonize new leather. The leather binding was evaluated by visual assessment, SEM, isolation and identification of fungi and Fourier Transform Infrared Spectroscopy (FTIR). Goat skin was identified as the animal skin of the bookbinding, *Cladosporium cladosporioides*, *Aspergillus tamarii*, *Eurotium chevalieri*, *Aspergillus fumigatus*, *Wallemia sebi*, and *Fusarium poae* were the most dominant species of fungi found on the leather book binding. Based on the EDX analysis of leather binding and new leather sample, some changes happened in the old leather due to bio deterioration factors and causes of changes in the elements rate.

Keywords: Leather, Fungal deterioration, Isolation and identification, FTIR, SEM, EDX.**1. Introduction**

Fungal attack is a common and severe problem in the storage rooms of museums. Fungi can damage the different organic materials. Egypt has a long history of fur and leather apparel man fracturing. According to literature, as early as the primitive society (3000 B.C.), in Badarian graves, ancient Egyptians made clothing articles like coats, aprons, and shawls with animal hides and skins [1]. In early times, ancestors processed beast hides with some simple treatments. Leather is a complex material of complex and dynamic dimensions that constantly vary with respect to the quantity and degree of interaction with each other and with those materials which are being stored

within them. The deterioration of leather collagen exposed to SO₂ and other pollutants has extensively been studied [2]. Additionally, several studies were carried out to determine the mechanical and thermal behavior of historical bookbinding leather and parchment [3,4]. The production and aging history of cultural heritage leathers are, in most cases, unknown. In a review by Sterflinger (2010) [5], fungi play a considerable role in the deterioration of cultural heritage due to their enormous enzymatic activity and their ability to inhabit and decay paintings, textiles, paper, parchment, leather, oil, casein, glue, and other materials used for historical art objects. Concerning the studies on the

microbial attack on old bookbinding leather, it was found that microbial communities overgrew in the samples depending on the kind of leather. Fungi, as microorganisms, were reported as potent pigment producing [6]. Furthermore, soluble pigments extracted from *Monascus purpureus*, *Isaria* spp., *Emericella* spp., *Fusarium* spp. and *Penicillium* spp. did not significantly alter the organoleptic properties of the leather sample [7]. Several studies reported that the proteinase, metalloproteinase, and some other enzymes produced by fungi and the microorganisms significantly changed the structure and the matrix properties of leather materials [8-10]. Additionally, fungi were reported to produce a variety of proteases enzymes that are active over a wide pH (4-11) [11] and are utilized for making the leather softer [12]. Fungi that attack tanned leather often utilize the fats in leather as a source of carbon [13]. Therefore, the isolated fungi played the most important role in the deterioration of calf leather that was totally deteriorated after 180 days of experiment [14]. Furthermore, ancient parchments can be attacked by certain species of fungi, e.g. *Cladosporium*, *Fusarium*, *Aspergillus*, *Penicillium*, *Trichodernia*..etc. Leather has some chemical composition that is similar to parchment and its susceptibility to bio-deterioration. Also, the species involved are almost the

same [15,16]. Moreover, fungal growth on the objects in a museum is caused by hyphae airborne that may penetrate the porous substrate [17]. They utilize sugars that are commonly used as fillers or free amino acids left by the tanning process between the collagen fibers and the different stained spots, loss in tensile strength. If proteins are attacked, hydrolysis of the leather could appear [16]. Leather has a pH of about 3 to 5 after tanning, which is more suitable for fungal growth [14,18,19]. Historical leathers were investigated by thermal analysis methods and the various observations are proposed as qualitative indices for assessing the age and storage conditions of leather [20]. Energy-dispersive X-ray spectroscopy (EDX) analysis yields reasonably accurate quantitative results featuring all the elements of the tested material, namely C, O, N, Na, S, Al, Si, and Cl [21]. In cultural heritage field, FTIR studies of tanning bookbinding leathers were conducted on parchment samples [22,23]. Therefore, the aim of the present study was to identify fungi that cause deterioration of historical leather in the storage room and compare the quantitative compounds of the elements of historical leather and new ones. Moreover, it aimed to check the ability of the isolated fungi from recently leather book to colonize new leather (goat skin).

2. Materials and Methods

2.1. The historical aspect of leather bookbinding

The study investigated leather binding that dates back to 1858 (the age of Mohammad Ali pasha and his family) of

a book entitled "Soeur de Valsovondre, fig. (1) (photographer real Montofome) Torisus, Abertellie

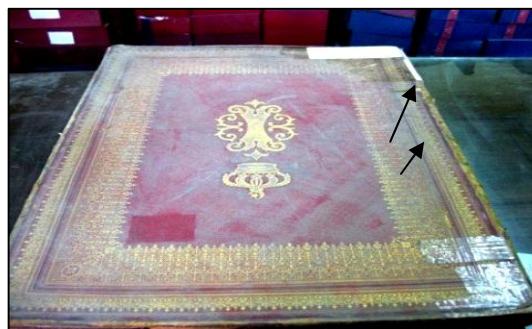


Figure (1) Shows the overview of the historical book used in the present study (The arrows refer to the infected parts)

2.2. Visual observation

This method is effective because the causes and mechanism of deterioration may be easily identifiable. The critical eye of the conservator can also determine the

2.3. FTIR

The samples were analyzed using FTIR spectrometer (Model 6100 Jasco, Japan). Each spectrum was obtained in the transmission mode with TGS detector.

2.4. Isolation and identification of fungi

Abundant superficial fungal colonies were found on the surfaces of the historical bookbinding leather during in situ observation. The isolation of fungi was directly performed in the laboratory after swabbing, as follows: fungi were isolated by rubbing the swabs gently on two different culture media namely; M40Y (400 g. sucrose, 20 g. molt extract, 5 g. yeast extract and 20 g. agar) and potato-dextrose agar (PDA) (200g potato, 20 g.

2.5. Scanning electron microscopy (SEM)

Leather surface was examined using the JEOL scanning electron microscope JXA-840A). The employed energy of the acceleration beam was 20 KV.

2.6. Fungal surface colonization

Leather samples were cut into small pieces (25×25 mm). Their surfaces were left unpolished in order to increase surface roughness and facilitate microbial colonization [27]. Test pieces were sterilized using UV light exposure for 48 hours, after autoclaving at 121°C for 6 hours and drying in an oven at 115°C for 24 hours [28]. For the preparation of spore suspensions, 10 mL. of sterilized distilled water was added to culture plates containing PDA (7-days old), and then spores were freed by the aid of a

most effective techniques of analysis which should be applied for identifying the condition of the leather book binding under study.

By using KBr method, it represented (2 mm/s) co-added scans at the spectral region ranging from 4000 to 400 cm^{-1} with a resolution of 4 cm^{-1} .

agar and 20 g. dextrose). Then, inoculated Petri dishes with fungi were incubated at $25^{\circ}\text{C} \pm 2$ for 7 days. Resultant cultures were purified using the hyphal tip and/or a single spore technique [24]. Macroscopic and microscopic characteristics of the obtained isolates, as well as color, size and morphology of the vegetative and reproductive structures were examined. Fungal isolates that speculated were identified using the taxonomic keys [25, 26].

(SEM)

JEOL attached with electron dispersive X-ray spectroscopy (EDX) was used for the elemental analysis characterization.

camel brush, and afterwards spore suspensions were individually through muslin and standardized to contain 1.2×10^6 spores/mL using a hemacytometer slide. The samples were deliberately inoculated by fungal species to be studied before and after infection. The colonization was evaluated after 90 days [29,30]. SEM model-a FEI Quanta 200 SEM FEG was used to study the colonization of the leather surface by micro-organisms and investigate changes in the surface's morphology.

3. Results

3.1. Visual observation

Through visual observation, it was noted that there were dark spots, folding of the fibers in the lower places and

3.2. FTIR

No dramatic changes were observed in the historical sample, fig. (2), but it is interesting to note that there were

general stiffness, fungal spots, weakness of the fiber, erosion of tanning material, and some missed parts.

some differences in the decrease of the relative intensity of the N-H group at 3408 cm^{-1} and disappeared of the peak at

2340 cm⁻¹ (sulfur ion stretching band). This might indicate the role of deterioration factors. In addition, the 3320 cm⁻¹ (due to N-H) and 2925.48 cm⁻¹ (C-H stretching)

bands [31] as well as at 1642.09, 1529.27 cm⁻¹ (amide bands I, II, III, correspondingly) decreased in relative intensity.

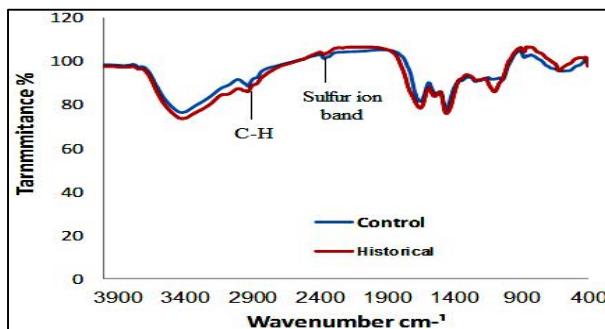


Figure (2) Shows FT-IR spectra of historical and control leather sample

3.3. Scanning electron microscopy with EDX

After the study of the leather binding's grain layer, it became clear that the type of skin used for the book-binding was goat skin. Fig. (3-a) showed the destruction and random distribution of the fiber structures erosion and many bores. There was a total deformation of the surface's morphology and the initial colonization entered the fibers structure, fig. (3-b). According to EDX analysis, tab. (1) fungi utilized a large amount of Cl in the leather (4.55 %) to heterotrophy comparative in a goat leather (52.02 %). While oxygen in historical leather rated 48.6 %, in goat leather it rated 17.69 %. Sulfur was increased from 2.78 % to 26.95 % in historical leather. Organisms need nitrogen (N) as a major element to

growth. Histo-rical leather did not contain any nitrogen but it was found in goat leather, rating 11.03 %. Fungi required some traces elements such as calcium (Ca) which was reduced from 8.89 % to 11.58 %, magnesium (Mg) was decreased from 0.75 % to 0.6%, aluminum (Al) was decreased from 0.69 % to 0.4%, silicon (Si) was reduced from 2.01 % to 1.91 %, selenium (Se) was reduced from 1.58 % to 0.5 %, and potassium (K) was increased from 3.54 % to 6.05 %. Copper (Cu) with trace amount (1.13 %) in goat leather was not found in historical leather, while the amount of zinc (Zn) was almost equal amount in historical (1.4 %) and goat (1.3 %) leathers.

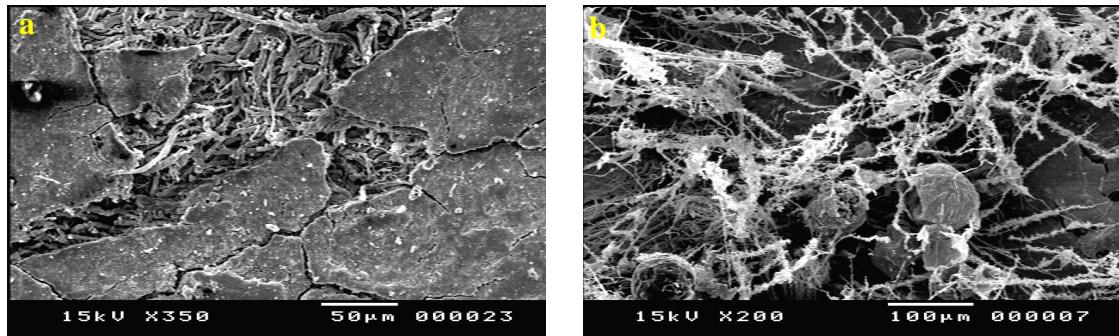


Figure (3) Shows Investigation of deteriorated of leather by SEM: missing parts of the leather & fungal colonies within the pores of the leather.

Table (1) Elemental analysis of the historical and reference leather.

Samples	Chemical composition (atomic %)											
	Mg	Al	Si	S	Cl	K	Ca	Se	Zn	O	N	Cu
Reference	0.75	0.69	2.01	2.78	52.02	3.54	11.58	1.58	1.3	17.69	11.03	1.13
Historical	0.6	0.4	1.91	26.95	4.55	6.05	8.89	0.5	1.4	48.6	-	-

3.4. The isolation and colonization of fungi

A total number of six species were identified in the ruins of the book's leather, i.e. *Cladosporium cladosporioides*, *Aspergillus tamarii*, *Eurotium chevalieri*, *Aspergillus fumigatus*, *Wallemia sebi*, and *Fusarium poae*, fig. (4). Fungi play an important role in the deterioration of leather and the isolated microorganisms from the leather samples depending on the type of leather [14]. Fungi can inhabit, alter, and degrade all types of organic and inorganic materials. However, most conservators and museum curators are not aware of this enormous deteriorative potential [5]. Additionally, leather has some chemical composition that is

similar to parchment and the ancient one can also be attacked by certain species of fungi, e.g. *Cladosporium*, *Fusarium*, *Aspergillus*, *Penicillium*, *Trichodernia*, etc. [16]. *Penicillium* and *Paecilomyces* attack tanned leather and utilize the fats in leather as a source of C [13]. Furthermore, 20 types of Indian tanned leathers were studied for their resistance/ susceptibility to microbial attack and 9 common fungi, namely *A. niger*, *A. pavus*, *A. fumiqatus*, *A. nidubns*, *A. terreus*, *A. sulphureus*, *Penicillium cyaneum*, *Paecilomyces varioti* and *Mucor* species were isolated from the various types of tanned leather [32].

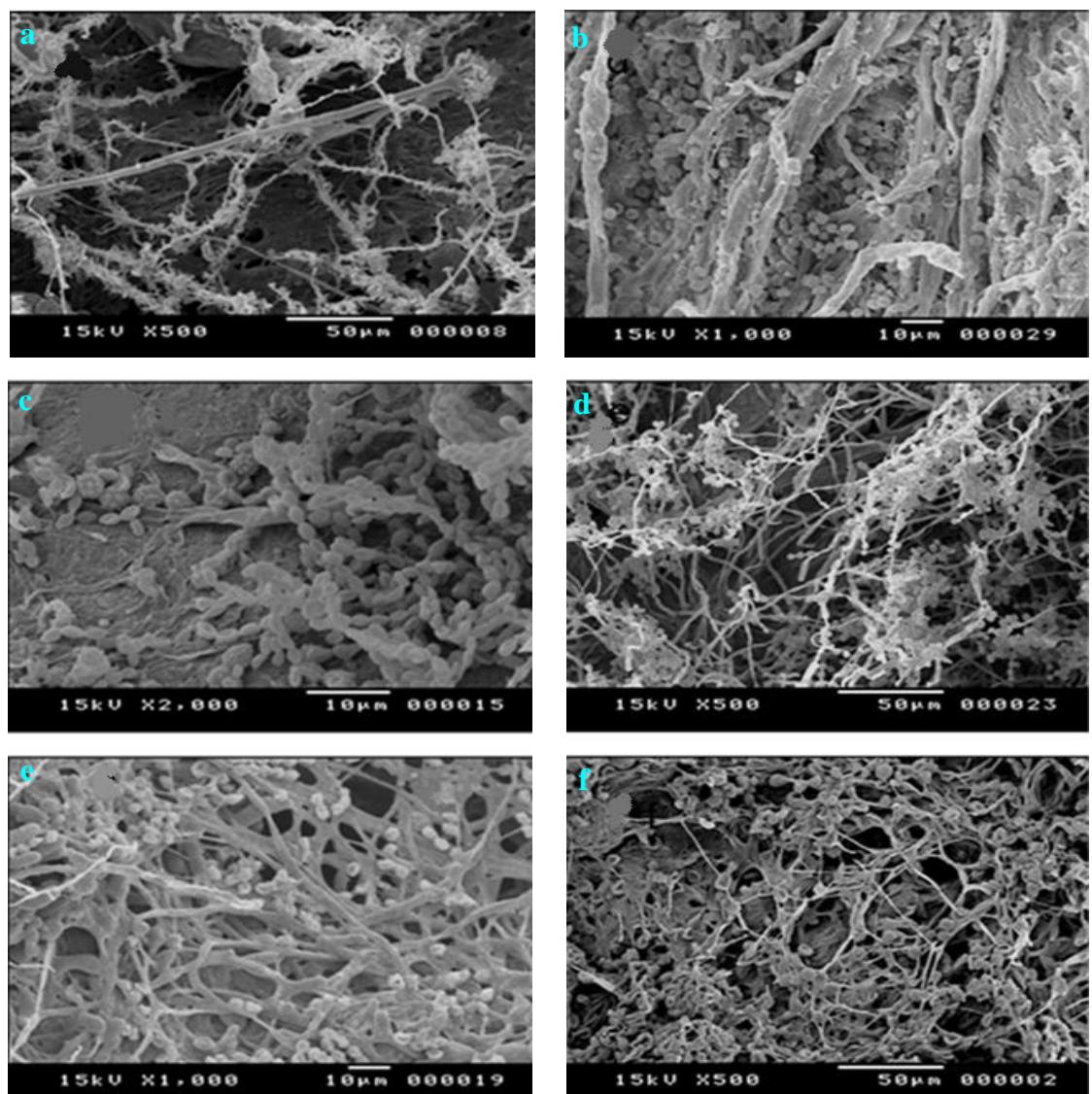


Figure (4) Shows the identified fungal taxa from the ruins of leather in the room of storage **a**. *Aspergillus tamarii*; **b**. *Cladosporium cladosporioides*, **c**. *Fusarium poae*, **d**. *Eurotium chevalieri*, **e**. *Wallemia sebi*, **f**. *Aspergillus fumigatus*

4. Discussion

Leather has a chemical composition that is similar to that of parchment and it is susceptible to bio-deterioration. Species involved are almost the same, where, a powdery patina rich in calcium characterize the areas affected by molds. EDX showed the presence of calcium in crystals [15,16]. It was concluded that the defacement of the parchment was due to both collagenolytic activity and the biotransformation of calcium-based minerals by fungi [16]. A growth factor is therefore defined as a specific organic compound required for fungi. Fungi that attack tanned leather often belong to lipolytic species and utilize the fats present in leather as a source of C [33]. With attacking proteins, different stained spots and loss in tensile strength were found by fungal deterioration, which subsequently leads to hydrolysis of the leather [18, 19, 33]. After the degradation of collagen by bacteria or chemically or physically deteriorating environment, the freed amino acids were utilized by fungi [34]. A white substance on 20th century leather bindings was reported to have elemental chemicals, such as; Ca, C and O and other one particle contained Mg, Al and Si, indicating the presence of a silicate-based material [35]. The neutralization of leather caused an abundant development of microorganisms accompanied by chemical changes. The most active organisms in the decay were *Chaetomium globosum*, *Scopulariopsis brevicaulis*, and *Streptomyces* [36]. The results obtained by EDX analysis of historical bookbinding leather and new leather sample (Goat) supported the fact that some changes happened in old leather under the bio-deterioration factors and caused changes in elements' rate. It was noted that fungi caused breaking of fibers leather. Subsequently, the leather was disintegrated. Furthermore, by checking the fibrous structure, it was found that collagen protein was literally broken and turned into dust due to the abundance of leather losses as well as there were several tears and splits in the primary

decorative leather. Keratin was the most abundant structural protein. Together with collagen in the leather and enzymes proteases like keratinases and collagenases were responsible for its degradation [20]. *Aspergillus* formed well-developed vegetative mycelia and conidial apparatus (conidiophores). It is characterized by the head-like termination of the conidiophores. The conidial heads can be observed as the conidiophore grows directly on leather with septate and hyaline hyphae. Fig. (4-a) showed the growth of *Aspergillus tamarii* and *A. fumigatus*, respectively. *Aspergillus* was related to Ascomycota have septate, mycelium and produce asci and ascospores. It can be found that almost anywhere, including soil, plant debris, wood, and both outdoor and indoor air. Some aspergilli are capable of degrading more refractory compounds such as fats, oils, chitin, and keratin [13]. Products of *Aspergillus* cell "factories" are citric, gluconic, itaconic, kojic acid and the production of citric acid by using of *A. niger* [37]. *C. cladosporioides* formed well developed vegetative mycelia and conidiophores and conidia on leather, fig. (4-b). The genus *Cladosporium* is one of the largest genera of Dematiaceous Hypocreates. It is characterized by a unique coronate scar structure, and conidia in acropetal chains and *Davidiella* teleomorphs [38,39]. The Cleistothecia of *Eurotium chevalieri* is yellow to buffy citrine and mycelium yellow to greyish yellow orange shades, fig. (4-d). *Wallemia sebi* is Filamentous hyphae branched and intertwined into compact mycelial pellets, densely interwoven mycelial masses. Hyphal tips, at the outer part of mycelial pellets, were shorter at the high salinity. SEM micrographs of *W. sebi*, fig. (4-e) showed relatively smooth and regular hyphal surfaces. The septa of the deute-romycete *W. sebi* (Fr.) v. Arx expanded pore margins surrounding a central pore and lack parent-hesomes and capable of growth over a wide range of water activity [40,41]. The fungal diversity that was restricted to xerophilic and xerotolerant species such as *Eurotium* sp.,

Aspergillus sp. or *Wallemia* sp. depended on the climate in the museum or storage rooms [5,41]. *Fusarium poae* was observed non-colored septate mycelium, globular to fusiform microconidia with zero to three septate, fig. (4-c). Fungus has white pigment, Macroconidia rose to burgundy and generally rare on hyphae and sporodochia-falcate. Leather samples were studied under the conditions of high humidity chambers. It was reported that fungi played the most important role in the deterioration of calf leather after 180 days of experiment and attacked the tanning substances [14]. Fungal pigment for leather like *M. purpureus*, *Emericella* spp. and *Penicillium* spp. pose no toxic effects [42,43] and the pigments produced by these fungi are reported to be biodegradable [44]. Fungus *C. cladosporioides* was found in keratinous substrates (leather, wool, feathers, fur, and hair) [45,46]. The fungal communities are dominated by hyphomycetes species

including *Cladosporium* spp. in moderate and humid climates [5]. Recently, *Aspergillus* and *Penicillium* were documented on the wooden substratum while *Fusarium* was isolated from the photographs in the air of the examined room [47]. Additionally, yeasts and filamentous fungi of *Aspergillus* and *Penicillium* genera were the main causes of bio-deterioration in photograph collections from Photographic Library of the National Archive of the Republic of Cuba and in the Historical Archive of the Museum La Plata [48]. Based on the measurements of the present study, the hygienic status of the museum can be determined according to the deteriorated historical bookbinder's leather (period of Mohammad Ali Pasha and his family). Subsequently, the concepts for optimizing the hygienic status and specific disaster management plans can be developed [49,50].

5. Conclusion

*Fungi play a major role in the deterioration of historical bookbinding leather in the cultural heritage. A total number of six fungi species namely, *Cladosporium cladosporioides*, *Aspergillus tamarii*, *Eurotium chevalieri*, *Aspergillus fumigatus*, *Wallemia sebi*, and *Fusarium poae* were isolated and identified from the deteriorated pieces. It was noted that they caused breaking of fibers leather. Consequently, the leather was disintegrated and collagen protein was literally broken and turned into dust due to the abundance of leather losses. In addition, there were several tears and splits in the primary decorative leather. Changes in elements and shifting in functional groups in historical leather related to the deterioration caused by fungi.*

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