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Investigate the role of fungal communities associated with a historical manuscript from the 17th century in biodegradation

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Herein, the deteriorating aspects of a historical manuscript (papers and leather bookbinding) dated back to the 17th century were evaluated with reference to the role of the associated fungal communities. The deterioration signs were investigated using visual assessment, SEM, ATR-FTIR, XRD, color changes, and pH values compared with control. Data showed that the most deterioration aspects were represented by the dust, dirt, erosion, stains, bores, weakness, missed parts, decreasing in paper crystallinity, shifting of wavenumbers of cellulosic band, and changing of color and pH. The dependent-culturable technique showed that thirteen fungal strains were associated with historical manuscript and identified using traditional and molecular methods as *Aspergillus niger* (three isolates), *A. fumigatus* (two isolates), *A. quadrilineatus* (three isolates), *Penicillium citrinum* (two isolates), and *P. chrysogenium* (three isolates). These fungal strains showed high efficacy to secretion various hydrolytic enzymes including cellulase, amylase, gelatinase, and pectinase which play a critical role in biodeterioration.

npj Materials Degradation (2022)6:88 ; https://doi.org/10.1038/s41529-022-00296-4

INTRODUCTION

Historical manuscripts, books, and cultural heritages are considered the great national wealth of all countries worldwide. These materials in archives, museums, and libraries suffer from various degradation aspects caused by microbial attacks or chemical and physical degradations[1]. Environmental conditions, storage, chemical composition, and physical properties of manuscripts are considered the main factors that can detect the quantity and quality of microbial colonization[2,3]. The microbial attack, especially by fungi, can be responsible for the damage of organic components of papers and leather binding of the historical manuscript[4].

Fungi play an important role in the biodeterioration of cultural heritage such as historical paper, and leather bookbinding, due to their massive enzymatic activity and ability to grow at low values of nutrient source[5–7]. Different types of fungi were isolated from historical paper manuscripts such as *Chaetomium* spp., *Penicillium* spp., *Aspergillus* spp., *Eurotium* spp., *Paecilomyces* spp., *Stachybotrys* spp., *Myrothecium* spp., and *Trichoderma* spp.[8,9]. Fungi can be producing collagenases (proteases) enzymes that have a major role in the degradation of collagen through its hydrolytic activity[10]. Also, it can be producing different types of cellulase enzymes that decompose the main components of paper[8]. Besides these enzymes, amylase, xylanase, and gelatinase enzymes have a critical role in the biodegradation of paper constituents[9]. The production of extracellular enzymes, as well as the hyphae due to fungal growth, can exert mechanical pressure on the paper and cause weakness[11]. It should be mentioned that acids either produced by fungi or obtained from air pollutants, play an important role in the degradation process of the historical manuscript[12,13].

In the last, the researchers focused on the degradation of archeological manuscript caused by chemical, physical, and mechanical methods and neglected the degradation caused by microorganisms, especially fungi. Therefore, it should be noted that the study of fungi and their efficacy in producing decomposing enzymes are very important factors for conservators to know the conditions of preservation of paper manuscripts and leather bookbindings within libraries and archives. It is also an important step that precedes the conservation treatment to give the possibility for a conservator to determine the appropriate materials and method of treatment which will be used[14,15].

This study aims to estimate the deterioration aspects of a historical manuscript dated back to 1663 AD and deposited at Al-Azhar Library, Cairo, Egypt with reference to the role of the fungal community isolated from the historical manuscript (paper and leather bookbinding) in biodeterioration development. To achieve this goal, different analysis tools including visual observation, Scanning Electron Microscopy (SEM), Attenuated Total Reflectance Fourier Transform Infrared (ATR-FTIR), X-Ray Diffraction (XRD), color changes, and pH values were used to investigate the deterioration aspects compared with a control (Whatman paper No. 1 for paper and Vegetable-tanned goatskin for leather). Also, isolation and identification of various fungal strains associated with deteriorated historical paper and leather bookbinding by traditional and molecular methods were achieved. The role of these fungal strains in the production of various extracellular hydrolytic enzymes including cellulase, amylase, pectinase, and gelatinase was assessed.

RESULTS AND DISCUSSION

Evaluation of deterioration aspects

The obtained results showed that there were some aspects of deterioration that appeared on the surface of leather binding and paper sheets of the manuscript under study. These aspects could be due to individual or combination factors. Moisture, temperature, and sunlight often play critical roles in the degradation of leather and paper as well as change their characteristics. Air pollutants (such as sulfur dioxide, nitrogen oxides, etc., and their transformation into acids), as well as dust also play a major role in the degradation process of the components of the studied

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manuscript. It can be added that the deterioration by microorganisms, especially fungi, is present in the various components of the manuscript with the assistance of physical and chemical factors in addition to other factors. These factors alone or in combination affected all the characteristics that were conducted in this study. As a result of the various deterioration factors mentioned above, the mechanism of deterioration occurs by oxidation, hydrolysis, or by their combination. Moreover, the deterioration aspects are investigated by visual observations, SEM, ATR-FTIR, XRD, color index, and pH value as shown below.

Visual assessment

The leather binding of the historical manuscript understudy suffered from various deterioration aspects as shown in Fig. 1. The common aspects are hardness and loss of flexibility, erosion of the tanning material, loss of different parts, the presence of dust, and stains that are derived from different sources, i.e. pollution or contamination with fungi. Moreover, the presence of some labels that stick by pressure-sensitive tape can be considered one of the main deterioration aspects of the leather binding. Also, color heterogeneity (some parts are dark and others are less dark) and shrinkage were noticed.

It can be concluded that the most deterioration aspects mentioned above can be attributed to the uncontrol surrounding environmental conditions. Compatible with the obtained results, Larsen showed that the most deterioration in the vegetable-tanned leather is due to an uncontrolled environment[16]. He also reported that the mechanisms of deterioration of vegetable-tanned leathers are due to the hydrolytic and/or oxidative damage. The hydrolytic damage can be attributed to which the acidic air pollution with SO_2 and NO_2, whereas the oxidative damage is due to the different factors such as heat, light, and oxidative contaminants[16]. Carsote et al. proved that the fluctuation between relative humidity (RH) and temperatures leads to the shrinkage of vegetable-tanned leather fibers[17].

Leathers are an organic material that contains many nutrients enhancing the growth of microorganisms. Besides, the chemical and structural nature of the substrate as well as environmental conditions is significant parameters affecting the quantity and quality of microbial colonization that impairs esthetic and functional properties. In the current study, the preservation conditions especially RH (60%) and temperature (27 °C) are suitable for enhancing the growth of fungi, actinomycetes, and bacteria that are considered a major biodeterioration sources of leathers as reported previously[18]. The attack of leathers by different microbial species is controlled by different factors such as RH, temperature, nutrients existing on the materials, light, moisture content, pH, osmotic pressure, surface properties, and the concentrations of O_2 and CO_2 in the surrounding environment[19].

On the other hand, the papers of the historical manuscript under study suffer from various deterioration aspects. Among these aspects, is the presence of bores of different sizes in different areas of paper sheets, which may be because of insects. Weakness of the paper sheets missed parts, especially in the corners, some stains, tear, deformation of the surface, yellowing of the papers, and brittleness were also observed (Fig. 1). The discoloration of paper can be attributed to the formation of chromophores from the degradation of one or more components of paper[20]. During the oxidation or photooxidation of papers formed from pure cellulose such as cotton fibers, carbonyl, and carboxylic groups are formed from the hydroxyl groups on the anhydrous-glucose units. The formation of both aldehyde and ketone groups on carbon atoms C-2 and C-3 in the anhydrous-glucose units are responsible for the yellowing of cellulose during aging[20]. The yellowing is often one of the early signs of the aging and deterioration of the papers. Depending on the nature of the papers and conditions of storage, the yellow color may eventually turn to brown and hence the paper become brittle as it reaches the later stages of aging[21].

As a result of the high use of the library and archival documents, the wear and tear (as a form of physical or mechanical degradation) are accumulated, leading to the damage of paper and becoming unfit for use. Papers with low polymerization degree (DP) have a low fold endurance and low tensile strength and hence become brittle and difficult to handle as reported previously[22]. Therefore, it could be assumed that there is an association between DP and the occurrence of physical changes associated with mechanical properties over a given number of challenges (e.g. tear, loose sheets, missing pieces, and folds that result in breaks).

Interestingly, the color change of papers can be also attributed to contamination by microorganisms as reported previously. In our recent study, fungi and bacteria isolated from archeological papers dating back to the 17th century were responsible for producing red-brownish foxing spots[23]. The poor aeration conditions, RH, and temperature encourages the growth of colored mold strains such as *Epicoccum* sp. and *Monoascus* sp. as well as actinobacteria that produce purple spots[5,24].

Scanning Electron Microscope (SEM)

The surface morphology, as well as the colonization of microbial strains to leather binding and historical papers, was investigated using SEM analysis. As shown in Fig. 2A, the surface morphology of the vegetable-tanned leather (control) was smooth, and the grouping of coarse, and fine follicles were also easily recognized. In contrast, the grouping of the coarse follicles of the historical vegetable-tanned leather sample was distorted and not easily recognized (Fig. 2B). Moreover, the surface of the historical leather sample was coarse and non-homogeneous. It was clear that the surface of the historical sample suffered from deterioration compared with the control. These deterioration symptoms can be attributed to the action of microbes, the environmental condition, or the handling of visitors or workers with the manuscript which lead to the destruction of the surface in some areas[25]. In the current study, the penetration, accumulation, and adhering of dust with the leather fibers were also noticed, which lead to the disappearance of the grain surface pattern in a large space of the surface. Ebsen et al. mentioned that the deterioration of historical leather surfaces may be due to the effect of soil contents during its deposition in the burial environment, handling during its life, and after excavation; dirt; folding, etc[26]. They also said that the degree of surface deterioration can prevent the identification of animal types, because of the loss of the grain surface pattern features.

On the other hand, Fig. 2C showed that the Whatman fibers are twisted, convoluted ribbon-like structures with a somewhat furrowed surface randomly overlapped over each other and appear as strong and broad. These features are mainly for cotton fibers as mentioned before[27]. By comparing the fibers of historical papers with the control, can be concluded that it is identical to cotton fibers (Fig. 2D). Also, the presence of microbial growth distributed among the cotton fibers as short rods (bacteria) or globes shape (fungal growth) were noticed as reported previously[28]. Moreover, some features of the fibrous structure of the cotton fibers in the historical papers were lost in some areas. This is possibly due to improper handling of the paper pages during reading. It was also noted that the width of each fiber was less than the width of the control fiber sample. The weakness of the fibers was also noticed. The surface of the paper also shows that the manuscript suffers from severe damage, which may be due to poor storage.

Fig. 1 Some deterioration aspects that were noticed in the papers and leather binding of a historical manuscript under study dated back to the 17th century deposited at Al-Azhar Library, Cairo, Egypt.

Attenuated Total Reflectance-Fourier Transform Infrared (ATR-FTIR)

ATR-FTIR analysis became a vital tool in the conservation field. It gave more details about the chemical stability of paper components. The changes in the chemical structure of the historical paper compared to the cotton fibers (Whatman paper) can be identified by using Fourier transform infrared (FTIR) spectroscopy. FTIR was used to identify the functional groups of the investigated material, which is shown by the intensity of peak absorbance[29].

As shown the collagen in control and historical leather samples is characterized at the wavenumber 3294 and 2921 cm^{-1} for the control sample, and the band at 3276 cm^{-1} and the band at 2921 cm^{-1} for the historical vegetable-tanned leather (Fig. 3A). The mentioned bands are referred to the amide A, and amide B, which are associated with the stretching of peptide N-H groups involved in inter-chain hydrogen bonding. The absorbance intensity at the mentioned bands was approximately the same. The Amide I band appeared at 1633 cm^{-1} for the vegetable-tanned leather sample (control), and at 1631 cm^{-1} for the historical leather sample. The absorbance intensity for the control and historical samples was approximately the same (0.089). The amide I at the band mentioned above is primarily caused by the stretching vibrations of the peptide carbonyl group (C=O) coupled weakly with C-N stretching and N-H bending[30]. It is sensitive to local order and its exact position is determined by the backbone conformation and the hydrogen-bonding pattern within the protein molecule. The amide II band at 1546 and 1543 cm^{-1} for the control and historical vegetable-tanned leather sample respectively was associated with N-H bending and C-N stretching vibration. The intensity of the last two bands was approximately the same. The amide III bands at 1237 and 1238 cm^{-1} for the control and historical samples respectively were associated with N-H in-plane bending and CH_2 wagging vibration of glycine backbone and proline side chain. The intensity of the amide III bands was approximately the same. The results proved that the collagen bands of historical vegetable-tanned leather shifted to a lower value compared to the vegetable-tanned leather (control). This may be due to the oxidation of the hydrolysis process resulting from a combination of deterioration factors in surrounding environmental conditions (physical, chemical, and biological factors) as mentioned above.

On the other hand, the significant difference between the Whatman paper (control sample) and the historical sample was

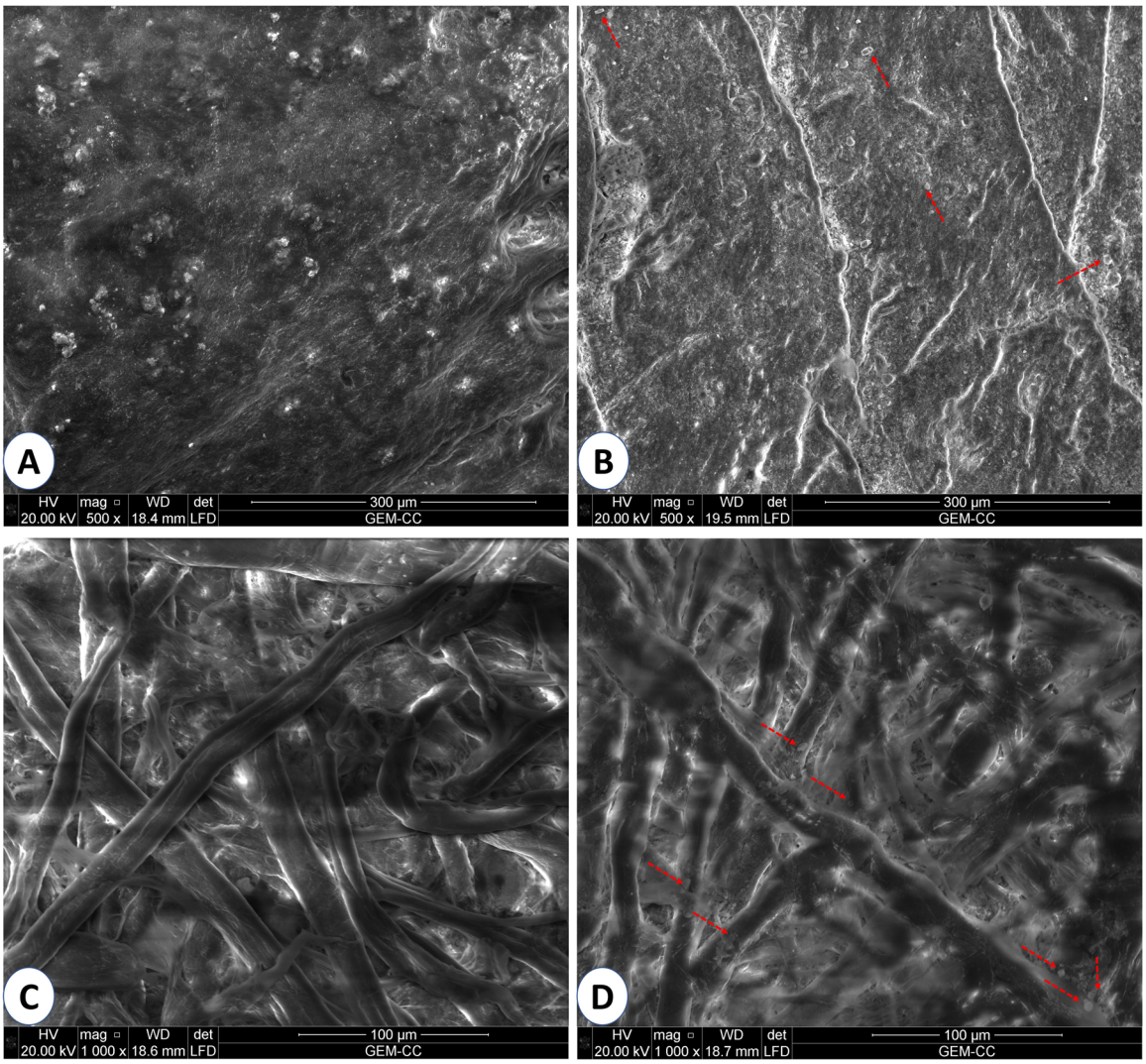

Fig. 2 SEM for historical leather binding and papers compared with control samples. A Is a leather sample (control), **B** is the historical leather, **C** is Whatman No. 1 (control), and **D** is the historical paper. The arrows refer to microbial growth.

shown (Fig .3B). The cellulose paper appeared at the wavenumber region from 3000–3700 cm^{-1}. The distinct regions of cellulose structure, which belong to O-H vibrations appeared at the wavenumber at 3332.39 and 3290.69 cm^{-1} for the Whatman paper (control), and at 3332.78 and 3291.21 cm^{-1} for the historical paper sample. It was noticed an increase in the wavenumbers and the absorbance intensity of the historical sample. The wavenumber at the regions from 2700 to 3000 cm^{-1} belongs to C-H stretching vibrations. The distinct band of C-H appeared at 2899.88 cm^{-1} for the control sample, and at 2898.20 cm^{-1} for the historical sample, which its absorbance intensity increased compared to the control sample. The band approximately at 1700 cm^{-1} for the historical sample mentioned to C = O stretching vibration of carbonyl group, which may be due to the oxidation process of historical sample. The bands at 1644.62 and 1643.63 cm^{-1} for the control and historical samples respectively also referred to H-O-H deformation vibration, and also referred to O-H in-plane bending vibration of adsorbed water which was broad in the control sample compared to the historical sample, which was stronger than the control, indicated the loss of more water. The bands at the wavenumbers between 1248 to 1580 cm^{-1} referred to C-O-H and CH_2 bending vibrations. The absorbance bands at these wavenumbers of the historical sample increased compared to the control. The results also showed that the bands at wavenumbers between 900 to 1160 cm^{-1} referred to various C-O stretching, C-O-C, and C-C-O bending vibrations. The wavenumbers and absorbance intensities at these bands increased in the historical sample compared to the control sample.

There are functional groups that are related to cellulose structure. The band at 1427 cm^{-1} referred to H-C-H and O-C-H in-plane bending vibrations. The band at 1360 and 1369 cm^{-1} for the control and historical samples referred to C-H deformation vibration. The band at 897 and 896 cm^{-1} for control and historical samples respectively belongs to C-O-C, C-C-O, and C-C-H deformation modes and stretching vibration. The band approximately at 662 cm^{-1} referred to the C-OH of plane bending mode. The absorbance intensities bands at wavenumbers mentioned above increased than the control. It can be said that the increase in the band intensities of the historical sample indicated cellulose oxidation and hydrolysis processes. The oxidation or hydrolysis processes may due to the effect of physical and chemical factors mentioned above on the manuscript studied. These results were confirmed by Munajad et al., who proved that heat aging can lead to the loss of adsorbed water of cellulose fibers by the oxidation process, and the deterioration increased with time[31]. Also, the band at wavenumber in the range of 1400 and 1800 cm^{-1} signifies the hydrolysis and oxidation of the paper cellulose[32].

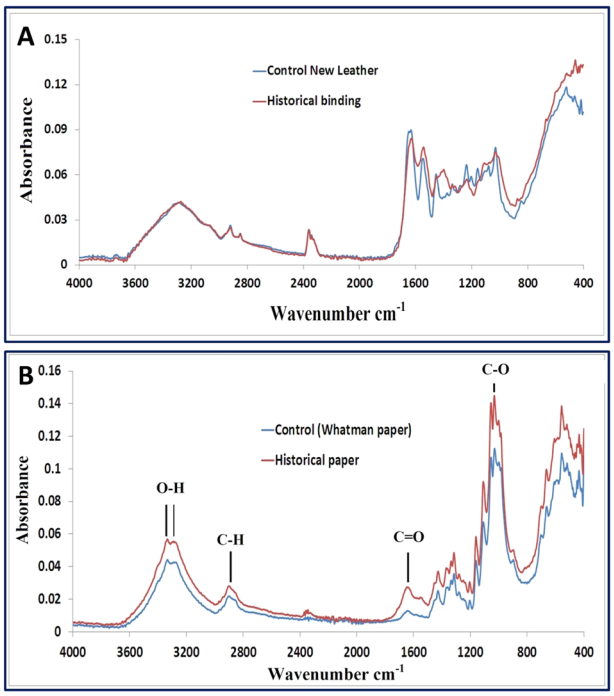

Fig. 3 ART-FTIR chart of historical leather and papers compared to control. A Is the ART-FTIR of historical and control leather, **B** is the ART-FTIR of historical paper and Whatman paper (control).

X-Ray Diffraction analysis (XRD)

It is known that native cellulose is partially crystalline. The cellulose molecules are arranged in fibrils within the fiber cell wall which have both amorphous and crystalline regions[33]. Data presented in Fig. 4 showed that the crystallinity of the Whatman paper sample was 81.68%, whereas the crystallinity of the historical paper sample was 47.54%. In general, the crystallinity degree of Whatman is high because it contains >95% cellulose content[33]. The obtained data revealed a significant decrease in the crystallinity of the historical paper as compared with the control. This decrease can be attributed to the effect of various deterioration factors such as the continuous rising in temperature and the decrease in moisture content, which leads to the destruction and damage of the amorphous areas of cellulose. Also, the presence of microorganisms helps the papers to cake and fossils[34,35]. The obtained results were compatible with those stated that the crystallization of cellulose decreased in the dry environment, or when water was removed from the cellulose material[36]. Sandy et al. reported that the crystallinity of cellulose can increase due to increasing the moisture and acid hydrolysis[33].

Measurement of color change

The color change of the historical manuscript (leather binding and papers) was assessed according to the chromatic scale CIELAB (Fig. 5A, B). As shown, the lightness (*L**) of vegetable-tanned leather (control) was 79.15, but for the historical leather binding was 38.56 (Fig. 5A). The decrease in lighting in the historical sample was 51%. The *a** value for both the control and historical leather was redder. The *a** value of the historical sample increased with percentages of 71% compared to the control sample. The results also showed that the *b** value was more yellow. The *b** value of the historical sample increased with percentages of 58% than the vegetable-tanned leather sample. There are also strong changes in the total color difference of the historical sample (48.28) compared to the control. The heat and aging time is considered the main reason for the change in the color values (L, a, and b values) and total color difference (ΔE)[37,38]. The increase of ΔE >3 is considered to be a very strong color change as reported previously[39]. In the current study, the ΔE of the leather binding was 48 which indicated that the historical manuscript understudy suffered from a strong change in the total color difference.

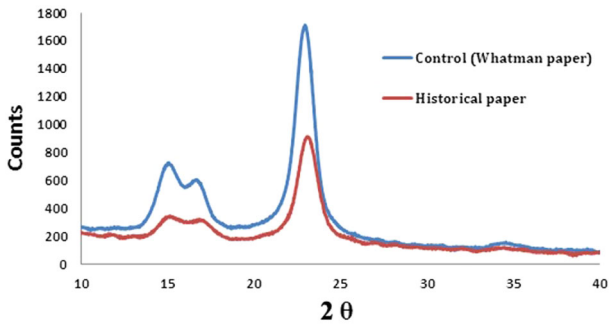

Fig. 4 Measurment of the crystallinity values. X-ray diffraction analysis for detect the crystallinity degree of a historical paper compared to a control (Whatman No. 1).

Data represented in Fig. 5B showed that the lightness (*L** value) of the control sample (Whatman paper) was 94.68, and it decreased to 68.9 for the historical paper sample. The reduction in the *L** value of the historical sample was 27%. The *a** value of the historical sample increased more than the control sample and was redder. The increase in the historical sample was 92 compared to the control sample. The *b** value of the control sample was 3.9 and for the historical sample was 32.2. This indicated that the historical sample was more yellow and increased with percentages of 88% more than the control sample. The results showed that there was a strong change in the total color difference in the historical sample (39.91). Matsuo et al. proved that the color changes during natural aging could be mainly explained as mild thermal oxidation[40]. Moreover, the heat aging process changed the color values and total color value of the paper artifacts as reported previously[41]. The aging process leads to the darkness of a color, which increased with increasing the aging time[42].

According to the obtained results, high the total color difference (ΔE) observed for the historical paper was 39.91 and this value was too high compared to the control Whatman paper. The color changes could be due to the production of pigments and organic acids by fungi. Moreover, the microbial attack also causes multi-colored spots and white films[6]. Interestingly, Mohie et al. reported that the highest color change (ΔE) was recorded for paper inoculated with fungal strain *Aspergillus niger*[15].

Measurement of pH value

The measurement of pH value was extremely important for evaluating the state of preservation of historical paper and leather binding. It indicates the chemical stability of the historical manuscript components towards natural aging as well as environmental deterioration factors[25].

The results graphically in Fig. 6 showed that the pH value of control (Whatman paper) was 7.5, whereas this value was decreased to an acidic level (5.5) for the historical paper, which confirmed that the historical manuscript suffers from several deterioration aspects. The decrease in the pH value of paper may be due to one or more of the following factors[43]:

- Preservation conditions: the uncontrolled preservation conditions can be changing the pH value. In the current study, the historical manuscript was preserved in uncontrolled conditions.
- Precipitation of air pollutants such as SO_2 and NO_2 as a result of high temperature and high RH leads to the formation of acids, ultimately degrading cellulose chains. The acid

hydrolysis reaction leads to the cracking of cellulose chains into short chains and the formation of glucose and the continues in this reaction causing decomposition and degradation of paper.
- The natural aging of cellulose can lead to the generation of acids, such as formic acid, lactic acid, and oxalic acid.
- The acidity of paper can be increased due to the decomposition of paper constituents, especially if they are stored in conditions that are not overly warm or humid.

On the other hand, the pH value of the vegetable-tanned leather sample was 4.1, and the historical leather binding sample was 3.2. It was clear that there is a strong decrease in the pH value of historical leather. The reduction in the pH value of historical leather may be due to the pollutants in the surrounding environment, the manufacturing process of the leather by adding excess acids during the tanning process, or by enzymes or acids produced by microorganisms that infected the manuscript understudy.

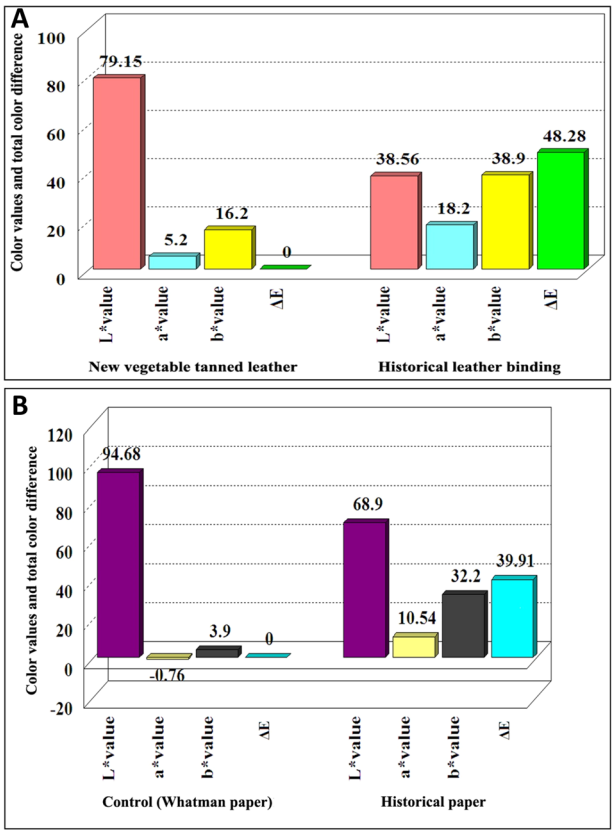

Fig. 5 Measurement of color changes for a historical manuscript under study. A is color change values of historical leather compared with control, **B** is the color change values of historical paper compared with Whatman No. 1 (control).

Fungal isolations and identifications

The fungal communities associated with the deteriorated historical manuscripts (leather binding and papers) was isolated. Thirteen fungal isolates designated as AZ.1 – AZ.13 were isolated from deteriorated collected samples and subjected to the traditional identification based on cultural and microscopic examination. Data revealed that the obtained isolates were belonging to the division Ascomycota. Six fungal isolates (AZ.1 – AZ.6) were isolated from leather binding whereas the remaining isolates (AZ.7 – AZ.13) were obtained from deteriorated papers (Table 1, Fig. 7). The traditional identification reveals that the fungal isolates AZ-1, AZ.4, and AZ.11 were identified as *Aspergillus niger*, whereas isolates AZ.2, AZ.3, and AZ.6 were identified as *A. quadrilineatus*. The fungal isolates AZ.5 and AZ.7 were identified as *A. fumigatus*. The *Penicillium* spp. isolated from deteriorated papers belonging to *P. citrinum* for isolate AZ.8 and AZ.9, whereas isolates AZ.10, AZ.12, and AZ.13 were identified as *P. chrysogenium*.

According to the obtained data, the most common fungal species associated with the historical manuscript were *Aspergillus* spp. with percentages of 61.5% of the total obtained strains followed by *Penicillium* spp. with percentages of 38.5%. Among *Aspergillus* spp., the *A. niger* and *A. quadrilineatus* were represented by equal percentages (37.5%) followed by *A. fumigatus* which is represented by 25%. Interestingly, *P. chrysogenium* was the most common *Penicillium* spp. isolated from the historical manuscript with percentages of 60% (from the total of *Penicillium* spp.) followed by *P. citrinum* with percentages of 40%. The traditional fungal identification showed that all fungal strains associated with leather binding were *Aspergillus* spp. whereas the most common fungal strains obtained from deteriorated historical papers were belonging to *Penicillium* spp. (Table 1). Compatible with the current study, 20 fungal isolates were obtained from old manuscripts dated back to

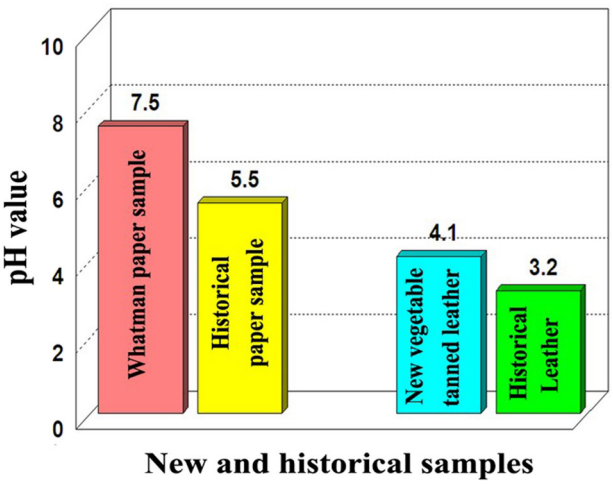

Fig. 6 Measurement of the pH values. The pH values of the papers and leather binding of a historical manuscript compared to control.

Table 1. Isolation and traditional identification of fungal communities associated with the historical manuscript (papers and leather bookbinding).

Fungal code	Source of isolation	Identified as.	Fungal code	Source of isolation	Identified as.
AZ.1	Leather bookbinding	*Aspergillus niger*	AZ.8	Paper	*Penicillium citrinum*
AZ.2	Leather bookbinding	*Aspergillus quadrilineatus*	AZ.9	Paper	*Penicillium citrinum*
AZ.3	Leather bookbinding	*Aspergillus quadrilineatus*	AZ.10	Paper	*Penicillium chrysogenium*
AZ.4	Leather bookbinding	*Aspergillus niger*	AZ.11	Paper	*Aspergillus niger*
AZ.5	Leather bookbinding	*Aspergillus fumigatus*	AZ.12	Paper	*Penicillium chrysogenium*
AZ.6	Leather bookbinding	*Aspergillus quadrilineatus*	AZ.13	Paper	*Penicillium chrysogenium*
AZ.7	Paper	*Aspergillus fumigatus*			

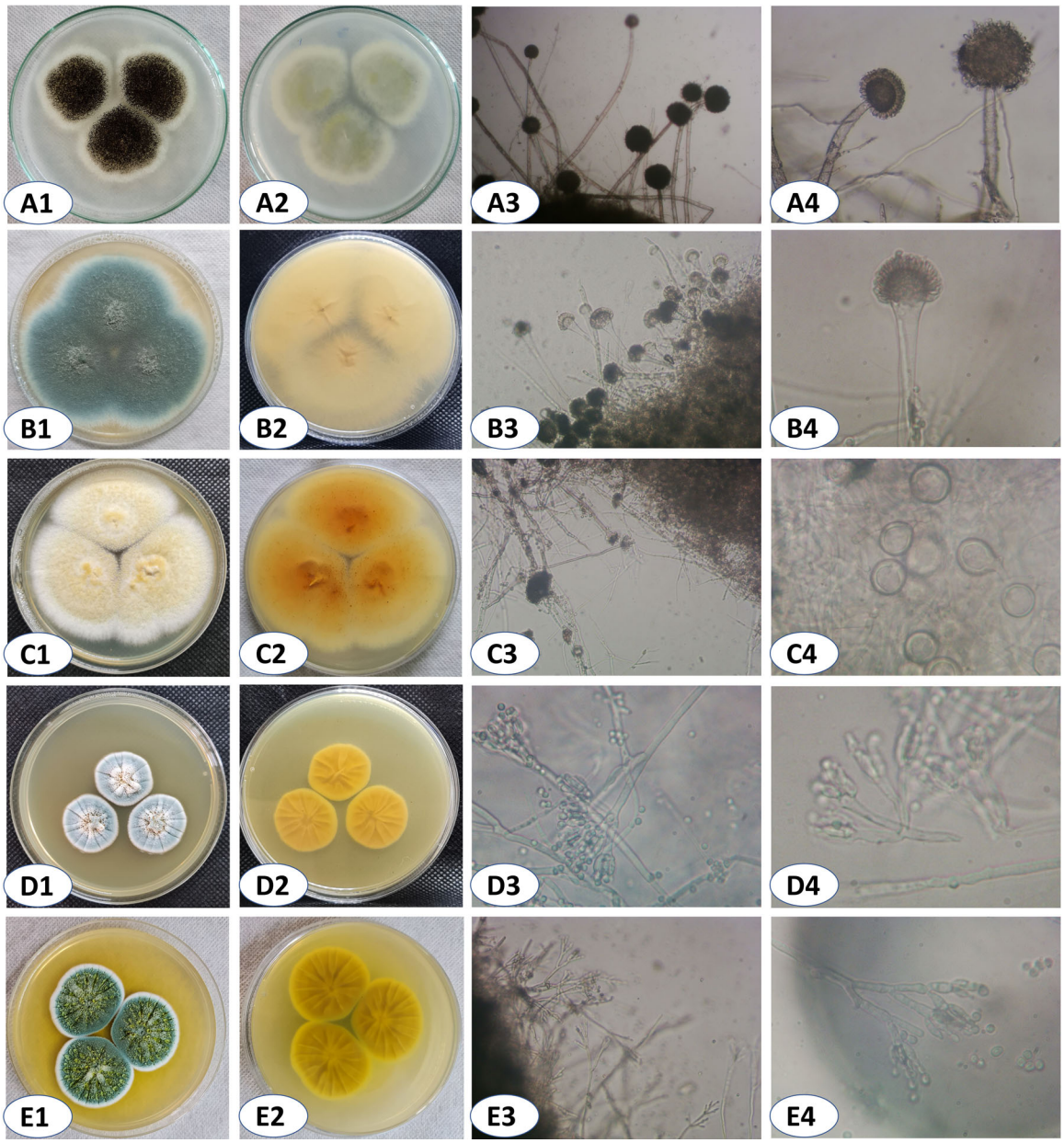

Fig. 7 Morphological and microscopic examination of various fungal strains associated with a historical manuscript (papers and leather bookbinding growing on CYA media). A Is *A. niger*, **B** is *A. fumigatus;* **C** is *A. quadrilineatus;* **D** is *P. citrinum*, and **E** is *P. chrysogenium*. 1 is a fungal colony; 2 is a reverse colony; 3 and 4 are bright filed microscopes ($X = 800$).

the 19th century. These fungal isolates were subjected to morphological and cultural identification which revealed that the most common fungal isolates were belonging to *Aspergillus* spp. with percentages of 45% followed by *Penicillium* spp. with percentages of 35%, *Eurotium* sp. with a percent of 5%, and *Mycelia sterilia* (does not form a distinguishing structure to identify the genus level) with percentages of 15%[44]. On the other hand, the fungal strains isolated from a historical manuscript dated back to the 17th century were identified using traditional methods such as *A. niger* and *A. flavus* associated with historical papers, and *A. niger, A. terreus*, and *A. flavus* associated with leather bookbinding[45]. Six fungal isolates were obtained from the leather binding of a historical manuscript dated back to the 18th century and identified using traditional methods such as *A. tamarii, A. fumigates, Cladosporium cladosporioides, Fusarium poae, Eurotium chevalieri*, and *Wallemia sebi*[46]. The fungal community associated with 79 deteriorated manuscripts collected from Astan Quds library, Iran were belonging to *Aspergillus* spp. and *Penicillium* spp. based on morphological and cultural characteristics[47].

The molecular identification based on amplification and sequencing of ITS genes were used to confirm traditional identifications. Therefore, we select one isolate from each genus to identify using the molecular technique. The fungal isolates designated as AZ.4, AZ.5, AZ.6, AZ.8, and AZ.10 confirmed their identification using ITS sequence analysis. The ITS sequence analysis showed that the selected fungal isolates were similarly to *A. niger* (closest accession number: LC195003), *A. fumigatus* (closest accession number: NR121481), *A. quadrilineatus* (closest accession number: NR131289), *P. citrinum* (closest accession number: NR121224), and *P. chrysogenium* (closest accession number: NR077145) with similarity percentages of 99.09, 99.34, 99.09, 98.77, and 99.27% respectively. Therefore, the five selected fungal strains were identified as *A. niger* AZ.4, *A. fumigatus* AZ.5, *A. quadrilineatus* AZ.6, *P. citrinum* AZ.8, and *P. chrysogenium* AZ.10

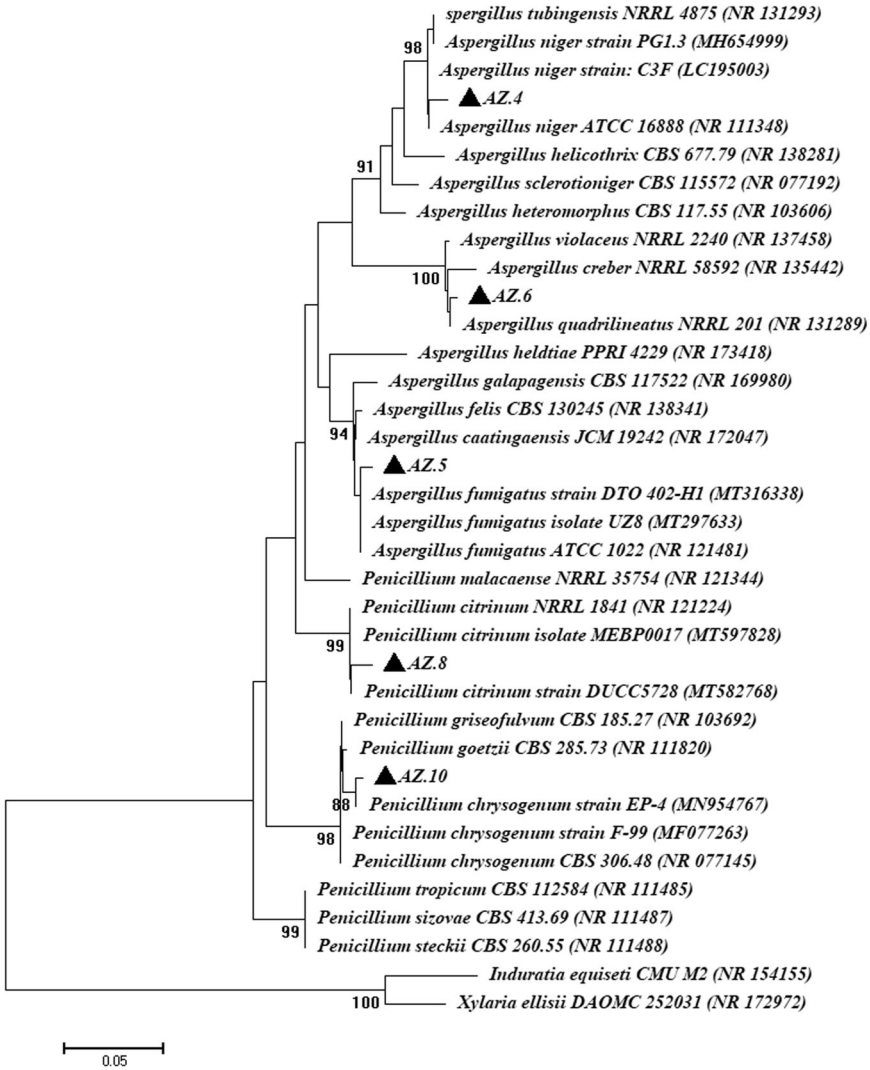

Fig. 8 Phylogenetic tree of five selected fungal strains based on ITS sequences analysis compared with NCBI reference sequences. MEGA 6 was used to achieve the analysis, which used the neighbor-joining approach with a bootstrap value (1000 replicates).

(Fig. 8). The obtained sequences were deposited in GenBank under accession numbers ON527887, ON527888, ON527889, ON527890, and ON527891 for AZ.4, AZ.5, AZ.6, AZ.8, and AZ.10 respectively.

The isolation and identification of the various fungal communities in the libraries, museums, and archives not only to investigate their role in biodeteriorations but also due to their serious effects on the health of workers and visitors. Ferrándiz-Pulido et al. reported that several fungi that colonize historical papers can be considered a source of human diseases such as allergy, dermal infections, respiratory diseases, and phaeohyphomycosis[48]. Several fungal strains belonging to genera of *Cladosporium, Rhizopus, Aspergillus, Penicillium*, and *Scopulariopsis* exist at a high concentration in different libraries and museums and counting the main sources of allergic respiratory infections[23,49]. Various *Aspergillus* strains such as *A. niger, A. flavus, A. terreus, A. ustus, A. alliaceus, A. udagawae, A. quadrilineatus*, and *A. lentulus* are causing a wide range of diseases such as otomycosis, cutaneous inflammatory, endocarditis, and aspergillosis[50].

Enzyme activity

Fungi are considered the main degrading agents for historical manuscripts through colonizing the fibers of the paper and producing hydrolytic enzymes such as cellulase, amylase, xylanase, and pectinase, or through producing acids or pigments that have a role in degradations[51,52]. In the paper industry, besides the organic components (mainly cellulose), other additives such as proteins, polysaccharides, gelatin, starch flour, and various synthetic compounds are used to reduce the spread of ink and increase the linkage between fibers[53]. Fungi can be decomposing the organic materials as well as other additives by secretion of extracellular hydrolytic enzymes ultimately weakening and altering the structure of these materials[54]. In the current study, the activity of culturable fungal strains to secrete various hydrolytic enzymes including cellulase, amylase, pectinase, and gelatinase were investigated. Data analysis showed that the thirteen fungal strains have the capacity to release cellulase, amylase, and gelatinase with the various degree, whereas the fungal strain *A. niger* AZ.6 (isolated from leather binding) and other fungal strains isolated from historical paper have the capacity to secrete pectinase enzyme (Fig. 9). Data analysis showed that the highest cellulase activity was recorded for fungal strain AZ.13 with an inhibition zone of 33.3 ± 1.2 mm followed by fungal strains AZ.10, AZ.11, and AZ.12 with inhibition zones of 19.0 ± 3.6, 18.0 ± 3.4, and 21.3 ± 4.6 mm respectively (Fig. 9A). Interestingly, the cellulase activity between fungal strains AZ.1 − AZ.8 was not significant ($p < 0.001$) with inhibition zones ranging between 7.3 ± 1.5 mm to

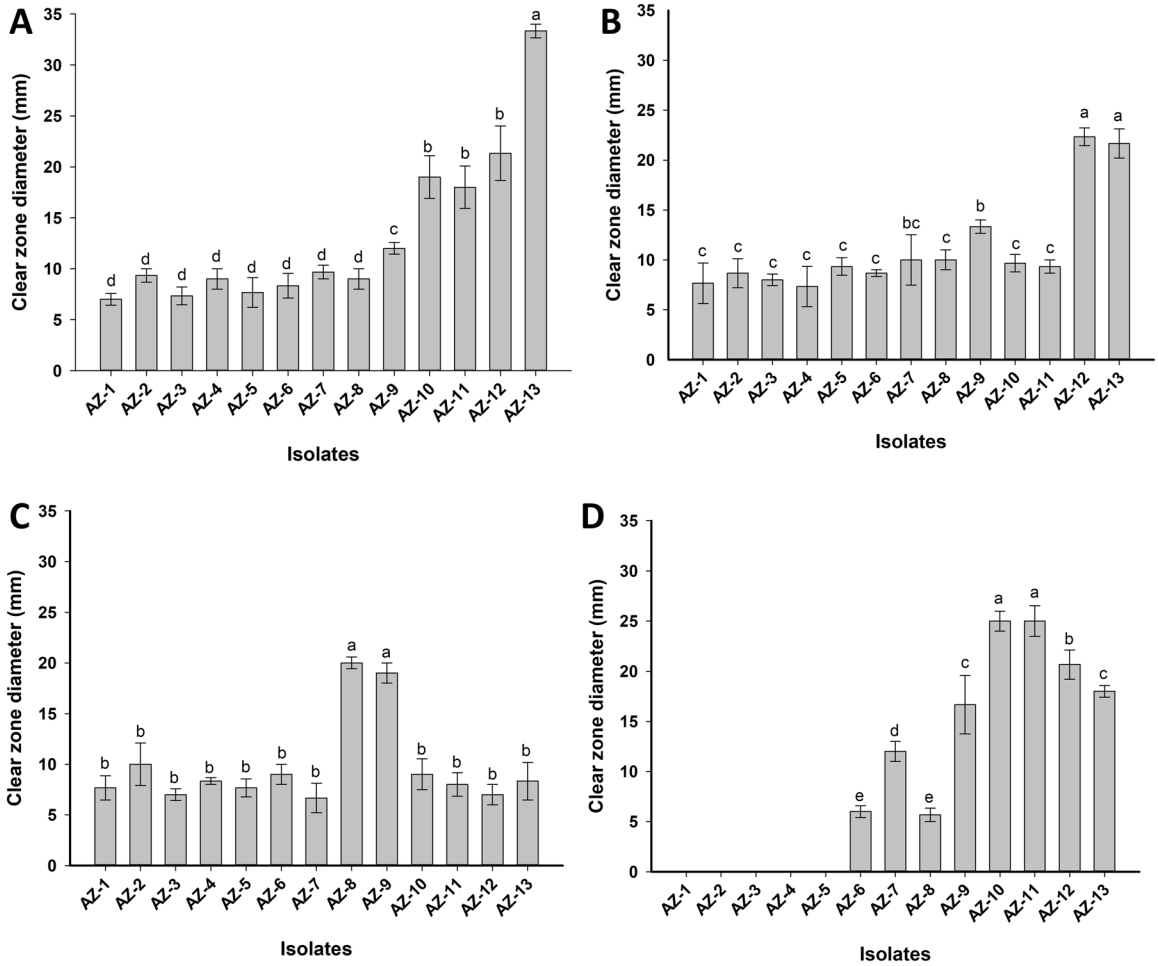

Fig. 9 The enzymatic activity of different fungal strains isolated from a historical manuscript (paper and leather bookbinding). A Is cellulase enzyme, **B** is amylase enzyme, **C** is gelatinase enzyme, and **D** is pectinase enzyme.

9.7 ± 1.6 mm. In our recent studies, *P. chrysogenium* and *A. niger* were isolated from a historical manuscript dated back to the 17th and 18th centuries and selected as the highest cellulase producers[23,55]. Out of 31 fungal strains isolated from a historical manuscript collected from Medina of Fez, only nine fungal strains showed cellulase activity[51]. After the colonization of papers by fungi, the hyphae penetrate the paper fibers causing physical changes besides the accumulation of fungal metabolic products in the fibers[9].

Analysis of variance showed that the highest amylase activity was recorded for fungal strains AZ.12 and AZ.13 with clear zones of 22.3 ± 1.5 and 21.7 ± 2.5 mm respectively, followed by fungal strains AZ.7 and AZ.9 with clear zones of 10.0 ± 4.4 and 13.3 ± 1.2 mm respectively (Fig. 9B). Moreover, all fungal strains have the activity to secrete gelatinase enzymes with the highest clear zones of 20.0 ± 1.0 and 19.0 ± 1.7 mm for fungal strains AZ.8 and AZ.9 respectively (Fig. 9C). Unfortunately, the fungal strains AZ-1 to AZ.5 do not exhibit any pectinase activity, whereas the isolates fungal isolates AZ.10 and AZ.11 were the highest pectinase producers with clear zones of 25.0 ± 1.7 and 25.0 ± 2.6 respectively (Fig. 9D).

These hydrolytic enzymes degrade macromolecules into small units such as cellulase and amylase enzymes that degrade cellulose and starch into glucose monomers. The pectinase and gelatinase have the capacity to degrade various proteins such as fibroin, collagen, and keratin that enter the silk, wool, and parchment industry respectively[56]. The obtained results imply the activity of fungal strains associated with a historical manuscript (leather binding and paper) in the production of a wide range of enzymes, which is important to understand the role of these strains in the biodegradation of the manuscript under study. Under adequate climate conditions especially relative humidity and temperature, the fungal species attacks the surfaces of archeological manuscripts and grow and reproduction with high rates allows to penetrate their components to cellulosic fibers and converted it to the caked shape[5,28]. In the current study, the climate conditions (60% RH and the temperature does not exceed 27 °C) encouraged the growth and adaptation of fungi and it was suitable habitat for the secretion of various active metabolites such as enzymes and acids as reported previously[51]. Besides the efficacy of isolated fungal strains in producing a variety of enzymes, it is characterized by their ability to the secretion of acidic metabolites as reported previously. These acidic secretions are responsible for the acid hydrolysis of historical papers. In preservation sites such as museums and libraries, due to fungal hyphae penetrations, it causes significant losses because of enzymatic degradation, acid corrosion, and mechanical degradations[57]. Papers and their constituents are considered appropriate carbon sources for the growth of various fungi with the prospect of pigment secretions especially with uncontrolled environmental conditions (RH and temperature). These pigments can cover the fungal spores or release them on the surface of paper which leads to discoloration[58]. Borrego et al. reported that the fungal strains *A. terreus, A. niger, A. versicolor, A. ustus, Cladosporium* sp., *P. commune, P. chrysogenum*, and *P. citrinum* have the efficacy to decreasing the pH value up to 4 through secretion of acidic metabolites[13].

The biodegradation caused by various fungal strains can be passed through four stages, contamination, germination, development, and degradation of the materials[59]. The contamination can arise from the air, touching the contaminated materials, transmission by vectors such as arthropods, or hyphal fragments that can germinate forming a colony[60]. Under appropriate conditions for growth, the hyphae can germinate and develop into mature strains that produce metabolites that enhance biodegradation. As a result of these degradations, the mechanical strength of paper is lost or weakened. Also, esthetic alterations due to the secretion of pigments can be caused, leading to losses of materials, difficulty in readability, and pieces of information. This study recommends the necessity of moving the studied manuscript from its current poor condition place to another controlled place from the conservation point of view (controlling in temperature, relative humidity, light, air pollution, microorganisms, etc.). Inspect reserve rooms and manuscripts at regular intervals. Train personnel to inspect reserve rooms and manuscripts regularly for mold, dust, insects, and rodents. The study also recommends the necessity of conducting various treatment processes for the manuscript, such as deacidification, disinfection, consolidation, etc., before transferring the manuscript to its controlled environment.

METHODS

Historical manuscript used

The historical manuscript named "**Kashf al-Ramz 'an Khabaya al-Kanz**" dated back to 1074 AH, 1663 AD and preserved in Al-Azhar Library, Cairo, Egypt was selected for isolation of fungal strains. This historical manuscript was written in Arabic language using carbon ink, containing two volumes, each one has a size (cm^3) of 32 × 20 x 8 (length x width x height) and is composed of 570 paper sheets with a brown leather binding with gilded geometric decoration in the center and the corners. The second volume was selected for the current study due to the presence of high deterioration aspects. The preservation conditions were as follows: temperature, 27 °C; relative humidity, 60 %; and the manuscript was stored on iron shelves of iron cabinets that were tightly closed in without an air condition system. Therefore, the ventilation in the preservation site is poor. The deterioration aspects were observed in the leather binding and paper sheets. It can be said that the manuscript selected for the current study was susceptible to fungal deterioration due to the preservation conditions mentioned above. In addition, this manuscript, before it came to its current storage place, it was preserved in the Al-Azhar Historical Library in the Al-Azhar Mosque. The contents of this library were exposed to harsh environmental factors that enhanced the emergence of many deterioration aspects, especially fungal attack. These factors were the exposure of the walls of the Al-Azhar Historical Library to poor sanitation in the vicinity of the Al-Azhar Mosque, the high level of groundwater, poor ventilation resulting from storage in uncontrolled closed rooms, poor handling by researchers, high humidity, and air pollution in Al-Azhar area. All the previous factors had negative impacts on the quality of the manuscript before it was transferred to the current library.

Preparation of paper samples (control)

Whatman paper No. 1 (24 cm in diameter) made by Whatman Company in England was used as a control to be compared with historical paper. The main aim is to assess whether the comparison illustrates patterns relative to various characteristics between the control and archeological samples. A cylindrical radius approx. (12 cm) was prepared according to Abdel-Nasser et al.[43].

Preparation of vegetable-tanned leather samples (control)

Vegetable-tanned (mimosa) goatskin samples were prepared according to Abdel-Maksoud[12]. The vegetable-tanned leather samples were used as a control to be compared with the leather binding of the historical manuscript in order to show the differences between the properties of the two samples obtained under different conditions.

Visual assessment

Visual estimation with the naked eye and using a digital camera (Samsung camera 38MP, f/2.2 lens slot) is an important step to describe the deterioration aspects of the historical manuscript under study (paper and leather binding)[25,61,62].

Scanning Electron Microscopy (SEM)

The investigation of the surface morphology of historical paper and leather binding was done using SEM (Quanta 3D 200i made by FEI, accelerated voltage of 20.00 kV, and a magnification range of 250 to 2000X)[63]. The SEM analysis was done in the Grand Egyptian Museum - Conservation Center (GEM.CC), Cairo, Egypt.

ATR-FTIR

FT-IR analysis was achieved to study the chemical change in the historical leather and paper compared to control samples[64]. The leather and paper samples were analyzed using Attenuated Total Reflectance Fourier Transform Infrared (ATR-FTIR) spectra on a Bruker Vertex 70 Platinum ATR scale with crystal diamonds in the range 4000–400 cm^{-1}, and resolution 4 at the Archeological Research and Preservation Centre—Supreme Council of Antiquities—Ministry of Tourism and Antiquities, Egypt.

X-Ray Diffraction Analysis (XRD)

The cellulose crystallinity of historical paper compared with control (Whatman No. 1) was measured using XRD analysis (Panalytical X, Pert pro-PW 3040/60—Scan axis: Gonio—Anode material: Cu—Generator settings: 40 mA, 45 kV- Goniometer radius: 240 mm) at the Grand Egyptian Museum—Conservation Center (GEM.CC), Egypt. The crystallinity index was calculated according to the following equation[65]:

$$I_{crys} = \frac{I_{002} - I_{am}}{I_{002}} \times 100 \quad (1)$$

Where I_{Crys} is the crystallization index, I_{002} is the intensity at a 2θ value of 22.6°, and I_{am} is the intensity at a 2θ value of 19°.

Measurement of color change

The color change and total color difference values of historic leather and paper samples were analyzed using the CIELAB system (portable spectrophotometer by Hunter Lab-Reston Virginia USA). The CIE system has one channel to detect the lightness (L*) value and two channels, one to measure the color change from red to green (a*) and the second to measure the color change from yellow to blue (b*). The total color difference (DE) was measured according to the following equation[66,67]:

$$\Delta E = \sqrt{(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2} \quad (2)$$

Measurement of pH value

The pH values of the historical leather compared with control samples were determined according to Wouters et al.[68] with slight modification. The leather samples were soaked in deionized water for ~6 h. to allow the ions to transfer into the solution followed by measuring the pH values using waterproof pH testers AD11 and

AD12. The calibration of the testers was between 2 and 7, at 21–22 °C. Moreover, the pH values of historical paper compared with control (Whatman No. 1) were evaluated using an Adwa AD 1030 pH/mV and temperature scale with a flat electrode. The pH measurements were performed by placing the flat electrode onto the surface of paper samples[69].

Fungal isolation

The different fungal species that inhabitant the historical manuscript was isolated either by using sterilized cotton swabs that were pressed firmly over the deteriorated part or by collecting deteriorated fragments. Either collected swabs or fragments were inoculated in the Czapeck Yeast Extract broth (CYB) media (containing g L^{-1}: sucrose, 30; $NaNO_3$, 2; KH_2PO_4, 1; KCl, 0.5; $MgSO_4.7H_2O$, 0.5; $FeSO_4.7H_2O$, 0.002; yeast extract, 5; Distilled H_2O, 1 L) supplemented with 500 mg L^{-1} chloramphenicol (to suppress the bacterial growth) and incubated under shaking conditions (150 rpm) at 27 ± 2 °C for 48 h[70]. The purpose of this step is to enhance the fungal growth that inhabitant the historical paper before growing in solid media. At the end of the incubation period, ~100 µL from the previous inoculated enrichment broth media was streaked on Czapeck Yeast Extract Agar (CYA) plate using a sterilized glass spatula and incubated at 27 ± 2 °C. The inoculated plates were checked daily to observe the fungal growth which was reinoculated into a new plate for further purifications. After that, the collected purified fungal species were inoculated into a CYA slant and preserved in a refrigerator for further study.

Fungal identification

The collected fungal species were undergone primary identification based on the cultural and microscopic examination according to standard keys for *Aspergillus* spp.[71,72] and *Penicillium* spp.[73,74]. The molecular identification based on the amplification and sequencing of the ITS gene was achieved to confirm the primary identification. The fungal genomic DNA was extracted based on Gene Jet Plant genomic DNA purification Kit (Thermo) protocol. The ITS gene region was amplified using PCR (polymerase chain reaction) with extracted DNA as a template and ITS primers [ITS1 (5′-TCCGTAGGTGAACCTGCGG-3′) and ITS3 (5′-TCCTCCGCTTATTGATATGC-3′)][75]. The PCR mixture (50 µL) contained Maxima Hot Start PCR Master Mix (Thermo), 0.5 µM of each primer, and 1 µL of extracted genomic DNA. The PCR protocol was achieved using a DNA Engine Thermal Cycler by Sigma Scientific Services Company (Cairo, Egypt) under the following conditions 94 °C for 3 min, followed by 30 cycles of 94 °C for a half minute, 55 °C for a half minute, and 72 °C for 1 min, followed by a final extension performed at 72 °C for 10 min. The commercial sequencing was conducted using ABI 3730 × 1 DNA sequencer at GATC Company (Germany). The obtained ITS sequence was compared with those deposited in the GenBank database using the NCBI BLAST program, and the phylogenetic tree was conducted by bootstrap analysis (1000 repeats).

Extracellular enzyme activity

The efficacy of the obtained fungal strains in producing different extracellular enzymes, including amylase, pectinase, cellulase, and gelatinase was assessed. Each purified fungal strain was inoculated into mineral salt agar media (MSA) (containing g L^{-1}: KCl, 5; $NaNO_3$, 6; $MgSO_4.7H_2O$, 0.5; KH_2PO_4, 1.5; $ZnSO_4$, 0.01; $FeSO_4$, 0.01; Agar, 15, Dis. H_2O, 1 L) supplemented with a specific substrate. The results were recorded as a diameter of a clear zone (mm) which is calculated by subtracting the diameter of fungal growth from the diameter of all clear zone. The activity of amylase, pectinase, cellulase, and gelatinase enzymes was estimated by inoculating the fungal strain on the MSA media supplemented with 1% soluble starch, pectin, carboxymethyl cellulose, and gelatin respectively and incubated for 96 h at 25 ± 2 °C.

The results were recorded after flooding the inoculated plates with iodine solution to examine the activity of amylase, pectinase, and cellulase[76,77], whereas the acidic mercuric chloride was used to examine the gelatinase activity[78]. The experiment was achieved in triplicates.

Statistical analysis

All the obtained data are represented as the means of three independent replicates. Moreover, the collected data were analyzed using ANOVA analysis by a statistical package SPSS v17. The mean difference comparison between the treatments was analyzed by the Tukey HSD test at $P < 0.05$.

DATA AVAILABILITY

The data presented in this article is available upon request to the authors.

Received: 26 May 2022; Accepted: 12 October 2022;
Published online: 08 November 2022

REFERENCES

1. Pinzari, F. & Gutarowska, B. In *Microorganisms in the Deterioration and Preservation of Cultural Heritage* (Edith Joseph ed), 2021, 79–113 (Springer International Publishing, 2021).
2. Gaylarde, C. C. Influence of environment on microbial colonization of historic stone buildings with emphasis on cyanobacteria. *Heritage* **3**, 1469–1482 (2020).
3. Franco-Castillo, I., Hierro, L., de la Fuente, J. M., Seral-Ascaso, A. & Mitchell, S. G. Perspectives for antimicrobial nanomaterials in cultural heritage conservation. *Chem.* **7**, 629–669 (2021).
4. Nitiu, D. S., Mallo, A. C. & Saparrat, M. C. N. Fungal melanins that deteriorate paper cultural heritage: an overview. *Mycologia* **112**, 859–870 (2020).
5. Sterflinger, K. & Piñar, G. Microbial deterioration of cultural heritage and works of art—tilting at windmills? *Appl. Microbiol. Biotechnol.* **97**, 9637–9646 (2013).
6. Saada, N. S., Abdel-Maksoud, G., Abd El-Aziz, M. S. & Youssef, A. M. Evaluation and utilization of lemongrass oil nanoemulsion for disinfection of documentary heritage based on parchment. *Biocatal. Agric. Biotechnol.* **29**, 101839 (2020).
7. Saada, N. S., Abdel-Maksoud, G., Abd El-Aziz, M. S. & Youssef, A. M. Green synthesis of silver nanoparticles, characterization, and use for sustainable preservation of historical parchment against microbial biodegradation. *Biocatal. Agric. Biotechnol.* **32**, 101948 (2021).
8. Koul, B. & Upadhyay, H. In *Fungi and their Role in Sustainable Development: Current Perspectives* (eds Gehlot, P. & Singh, J.) 597–615 (Springer, Singapore, 2018).
9. Pinheiro, A. & Sequeira, S. *Reference Module in Life Sciences* (Elsevier, 2020).
10. Solanki, P., Putatunda, C., Kumar, A., Bhatia, R. & Walia, A. Microbial proteases: ubiquitous enzymes with innumerable uses. *3 Biotech* **11**, 428–428 (2021).
11. Kirtzel, J. et al. Organic acids, siderophores, enzymes and mechanical pressure for black slate bioweathering with the basidiomycete *Schizophyllum commune*. *Environ. Microbiol.* **22**, 1535–1546 (2020).
12. Abdel-Maksoud, G. Evaluation of wax or oil/fungicide formulations for preservation of vegetable-tanned leather artifacts. *J. Soc. Leather Technol. Chem.* **90**, 58–67 (2006).
13. Borrego, S., Lavin, P., Perdomo, I., Gómez de Saravia, S. & Guiamet, P. Determination of indoor air quality in archives and biodeterioration of the documentary heritage. *ISRN microbiol.* **2012**, 680598 (2012).
14. Palermo, A. M., Gentile, A. & Pellegrino, G. Documentary heritage: fungal deterioration in compact discs. *Herit. Sci.* **9**, 133 (2021).
15. Mohi, M. A., Ismail, S., Hassan, A., Tawfik, A. M. & Mohamed, W. Assessment of the applicability of cellulolytic enzyme in disassembling of caked papers. *Egypt. J. Chem.* **65**, 581–591 (2022).
16. Larsen, R. Experiments and observations in the study of environmental impact on historical vegetable tanned leathers. *Thermochim. Acta* **365**, 85–99 (2000).
17. Carsote, C. et al. *ICAMS—5th International Conference on Advanced Materials and Systems* (ICAMS, 2014).
18. Abdel-Maksoud, G., Tharwat, N. A. & Gad, H. The role of fungi isolated from historical vegetable-tanned leather on the degradation of peptides and amino acids. *J. Soc. Leather Technol. Chem.* **98**, 1–9 (2014).
19. Valentin, N. Microbial contamination and insect infestation in organic materials. *Coalition* **6**, 2–5 (2003).

20. Carter, H. A. The chemistry of paper preservation: part 2. The yellowing of paper and conservation bleaching. *J. Chem. Educ.* **73**, 1068 (1996).
21. Mosca Conte, A. et al. Role of cellulose oxidation in the yellowing of ancient paper. *Phys. Rev. Lett.* **108**, 158301 (2012).
22. Strlič, M. et al. Damage function for historic paper. Part II: wear and tear. *Herit. Sci.* **3**, 1–11 (2015).
23. Fouda, A. et al. Eco-friendly approach utilizing green synthesized nanoparticles for paper conservation against microbes involved in biodeterioration of archaeological manuscript. *Int. Biodeterior. Biodegr.* **142**, 160–169 (2019).
24. Pinar, G., Piombino-Mascali, D., Maixner, F., Zink, A. & Sterflinger, K. Microbial survey of the mummies from the Capuchin Catacombs of Palermo, Italy: biodeterioration risk and contamination of the indoor air. *FEMS microbiol. ecol.* **86**, 341–356 (2013).
25. Helmi, F. M., Wahba, W. N., Brania, A., Abdelnasser, M. & Elkobasy, M. Characterization of a historical leather cover of manuscript 'Ensaan Elaauon fe sert Elameen Elmaamon', Al-Azhar Library, Eygpt. *Adv. Res. Conserv. Sci.* **2**, 40–52 (2021).
26. Ebsen, J. A., Haase, K., Larsen, R., Sommer, D. V. P. & Brandt, L. Ø. Identifying archaeological leather—discussing the potential of grain pattern analysis and zooarchaeology by mass spectrometry (ZooMS) through a case study involving medieval shoe parts from Denmark. *J. Cult. Herit.* **39**, 21–31 (2019).
27. Goynes, W. R. & Harris, J. A. Structural characterization of grafted cotton fibers by microscopy. *J. Polym. Sci. Part C: Polym. Symp.* **37**, 277–289 (1972).
28. Michaelsen, A., Piñar, G. & Pinzari, F. Molecular and microscopical investigation of the microflora inhabiting a deteriorated Italian manuscript dated from the thirteenth century. *Microb. ecol.* **60**, 69–80 (2010).
29. Abdel-Maksoud, G., Sobh, R. A. & Tarek, A. Evaluation of MMI/acrylate nanocomposite with hydroxyapatite as a novel paste for gap filling of archaeological bones. *J. Cult. Herit.* **57**, 194–204 (2022).
30. Sebestyén, Z. et al. Characterization of historical leather bookbindings by various thermal methods (TG/MS, Py-GC/MS, and micro-DSC) and FTIR-ATR spectroscopy. *J. Anal. Appl. Pyrolysis* **162**, 105428 (2022).
31. Munajad, A., Subroto, C. & Suwarno. Fourier Transform Infrared (FTIR) spectroscopy analysis of transformer paper in mineral oil-paper composite insulation under accelerated thermal aging. *Energies.* **11**, 364 (2018).
32. Łojewska, J., Miśkowiec, P., Łojewski, T. & Proniewicz, L. M. Cellulose oxidative and hydrolytic degradation: In situ FTIR approach. *Polym. Degrad. Stab.* **88**, 512–520 (2005).
33. Sandy, M., Manning, A. & Bollet, F. Changes in the crystallinity of cellulose in response to changes in relative humidity and acid treatment. *Restaurator* **31**, 1–18 (2010).
34. Boukir, A., Fellak, S. & Doumenq, P. Structural characterization of Argania spinosa Moroccan wooden artifacts during natural degradation progress using infrared spectroscopy (ATR-FTIR) and X-Ray diffraction (XRD). *Heliyon* **5**, e02477 (2019).
35. Samsudin, N. A. et al. Effect of temperature on synthesis of cellulose nanoparticles via ionic liquid hydrolysis process. *J. Mol. Liq.* **308**, 113030 (2020).
36. Ioelovich, M. Adjustment of hydrophobic properties of cellulose materials. *Polymers* **13**, 1241 (2021).
37. Abdel-Maksoud, G. *15th World Conference on Nondestructive Testing, Rome (Italy) October* (ICDNT, 2000).
38. Abdel-Maksoud, G. & Marcinkowska, E. Changes in some properties of aged and historical parchment. *Restaurator* **21**, 138–157 (2000).
39. Axelsson, K. M., Larsen, R., Sommer, D. V. P. & Melin, R. Degradation of collagen in parchment under the influence of heat-induced oxidation: Preliminary study of changes at macroscopic, microscopic, and molecular levels. *Stud. Conserv.* **61**, 46–57 (2016).
40. Matsuo, M. et al. Aging of wood: Analysis of color changes during natural aging and heat treatment. *Holzforschung* **65**, 361–368 (2011).
41. Rushdy, A., Noshy, W., Youssef, A. & Kamel, S. Influence of bleaching materials on mechanical and morphological properties for paper conservation. *Egypt. J. Chem.* **60**, 893–903 (2017).
42. Amanatiadis, S., Apostolidis, G. & Karagiannis, G. Consistent characterization of color degradation due to artificial aging procedures at popular pigments of Byzantine iconography. *Minerals* **11**, 782 (2021).
43. Abdel-Nasser, M. et al. Evaluation of the efficiency of nanoparticles for increasing α-amylase enzyme activity for removing starch stain from paper artifacts. *J. Cult. Herit.* **53**, 14–23 (2022).
44. Oetari, A., Natalius, A., Komalasari, D., Susetyo-Salim, T. & Sjamsuridzal, W. Fungal deterioration of old manuscripts of European paper origin. *AIP Conf. Proc.* **2023**, 020156 (2018).
45. Hassan, R. R. Analytical study of a manuscript,'Tafsir Al khazin'-the seventeenth century AD. *Curr. Sci. Int.* **4**, 196–207 (2015).
46. Mansour, M., Hassan, R. & Salem, M. Characterization of historical bookbinding leather by FTIR, SEM-EDX and investigation of fungal species isolated from the leather. *Egypt. J. Archaeol. Restor. Stud.* **7**, 1–10 (2017).
47. Shamsian, A., Fata, A., Mohajeri, M. & Ghazvini, K. Fungal contaminations in historical manuscripts at Astan Quds museum library, Mashhad, Iran. *Int. J. Agric. Biol.* **8**, 420–422 (2006).
48. Ferrándiz-Pulido, C. et al. Cutaneous infections by dematiaceous opportunistic fungi: diagnosis and management in 11 solid organ transplant recipients. *Mycoses* **62**, 121–127 (2019).
49. Mesquita, N. et al. Fungal diversity in ancient documents. A case study on the Archive of the University of Coimbra. *Int. Biodeterior. Biodegrad.* **63**, 626–629 (2009).
50. Chadiesh, N. et al. *Aspergillus quadrilineatus* infection in an elderly debilitated patient. *J. Acad. Clin. Microbiol.* **19**, 62 (2017).
51. El Bergadi, F., Laachari, F., Elabed, S., Mohammed, I. H. & Ibnsouda, S. K. Cellulolytic potential and filter paper activity of fungi isolated from ancients manuscripts from the Medina of Fez. *Ann. Microbiol.* **64**, 815–822 (2014).
52. Fouda, A. et al. The efficacy of Silver Nitrate ($AgNO_3$) as a coating agent to protect paper against high deteriorating microbes. *Catalysts* **11**, 310 (2021).
53. Hubbe, M. & Cindy, B. Handmade paper: a review of its history, craft, and science. *BioResources* **4**, 1736–1792 (2009).
54. Sabatini, L., Sisti, M. & Campana, R. Evaluation of fungal community involved in the bioderioration process of wooden artworks and canvases in Montefeltro area (Marche, Italy). *Microbiol. res.* **207**, 203–210 (2018).
55. Fouda, A. et al. Monitoring the effect of biosynthesized nanoparticles against biodeterioration of cellulose-based materials by *Aspergillus niger*. *Cellulose* **26**, 6583–6597 (2019).
56. Caneva, G. et al. In *Cultural Heritage and Aerobiology: Methods and Measurement Techniques for Biodeterioration Monitoring* (eds Mandrioli, P., Caneva, G. & Sabbioni, C.) 3–29 (Springer, Netherlands, 2003).
57. Oetari, A. et al. Occurrence of fungi on deteriorated old dluwang manuscripts from Indonesia. *Int. Biodeterior. Biodegrad.* **114**, 94–103 (2016).
58. Sequeira, S., Cabrita, E. J. & Macedo, M. F. Antifungals on paper conservation: An overview. *Int. Biodeterior. Biodegrad.* **74**, 67–86 (2012).
59. Gallo, F., Pasquariello, G. & Valenti, P. In *Cultural Heritage and Aerobiology: Methods and Measurement Techniques for Biodeterioration Monitoring* (eds Mandrioli, P., Caneva, G. & Sabbioni, C.) 175–193 (Springer, Netherlands, 2003).
60. Trovão, J. et al. Can arthropods act as vectors of fungal dispersion in heritage collections? A case study on the archive of the University of Coimbra, Portugal. *Int. Biodeterior. Biodegrad.* **79**, 49–55 (2013).
61. Abdel-Maksoud, G. Analytical techniques used for the evaluation of a 19th century quranic manuscript conditions. *Measurement* **44**, 1606–1617 (2011).
62. El-Naggar, M. E., Gaballah, S., Abdel-Maksoud, G., El-Sayed, H. S. & Youssef, A. M. Preparation of bactericidal zinc oxide nanoparticles loaded carboxymethyl cellulose/polyethylene glycol cryogel for gap filling of archaeological bones. *J. Mater. Res. Technol.* **20**, 114–127 (2022).
63. Ismail, Y., Abdrabou, A. & Abdallah, M. A non-destructive analytical study and the conservation processes of Pharaoh Tutankhamun's painted boat model. *Int. J. Conserv. Sci.* **7**, 15–28 (2016).
64. Abdel-Maksoud, G. & Khattab, R. Ananlytical and conservation techniques for general arrangement plan-screw yacht "Safa-El-Bahre" on tracing paper. *Egypt. j. archeol. restor. stud.* **11**, 115–127 (2021).
65. Abdel-Maksoud, G., Abdel-Hamied, M. & Abdelhafez, A. A. Evaluation of the condition of a Mamluk-illuminated paper manuscript at Al-Azhar Library, Egypt. *Pigm. Resin. Technol.* https://doi.org/10.1108/PRT-06-2021-0065 (2021).
66. El-Gamal, R. et al. The use of chitosan in protecting wooden artifacts from damage by mold fungi. *Electron. J. Biotechnol.* **24**, 70–78 (2016).
67. Abdel-Maksoud, G. et al. Evaluation of a mixture of castor oil and polyvinylpyrrolidone for the lubrication of dry vegetable tanned leather artifacts. *J. Soc. Leather Technol. Chem.* **106**, 20–31 (2022).
68. Wouters, J., Claeys, J., Lamens, K. & Van Bos, M. *Handbook in the Microanalysis of Parchments* (Archetype Pub. Ltd, 2002).
69. Abdel-Maksoud, G., Awad, H., Rashed, U. M. & Elnagar, K. Preliminary study for the evaluation of a pulsed coaxial plasma gun for removal of iron rust stain from bone artifacts. *J. Cult. Herit.* **55**, 128–137 (2022).
70. Rojas, J. A. et al. Isoenzyme characterization of proteases and amylases and partial purification of proteases from filamentous fungi causing biodeterioration of industrial paper. *Int. Biodeterior. Biodegrad.* **63**, 169–175 (2009).
71. Diba, K., Kordbacheh, P., Mirhendi, S., Rezaie, S. & Mahmoudi, M. Identification of *Aspergillus* species using morphological characteristics. *Pak. J. Med. Sci.* **23**, 867–872 (2007).
72. McClenny, N. Laboratory detection and identification of *Aspergillus* species by microscopic observation and culture: the traditional approach. *Med. mycol.* **43**, S125–S128 (2005).
73. Pitt, J. & Hocking, A. *Fungi and food spoilage. Blackie Academic & Professional* (New South Wales, 1997).
74. Frisvad, J. C. & Samson, R. A. Polyphasic taxonomy of *Penicillium* subgenus *Penicillium*. A guide to identification of food and air-borne terverticillate Penicillia and their mycotoxins. *Stud. Mycol.* **49**, 1–174 (2004).

75. Khalil, A. M. A. et al. Isolation and characterization of fungal endophytes isolated from medicinal plant *Ephedra pachyclada* as plant growth-promoting. *Biomolecules* **11**, 140 (2021).
76. Dai, J. et al. Production of highly active extracellular amylase and cellulase from *Bacillus subtilis* ZIM3 and a recombinant strain with a potential application in tobacco fermentation. *Front. Microbiol.* **11**, 1539 (2020).
77. Fouda, A. et al. Plant growth-promoting endophytic bacterial community inhabiting the leaves of *Pulicaria incisa* (Lam.) DC inherent to Arid regions. *Plants (Basel, Switz.)* **10**, 76 (2021).
78. Ismail, M. A. et al. Comparative study between exogenously applied plant growth hormones versus metabolites of microbial endophytes as plant growth-promoting for *Phaseolus vulgaris* L. *Cells* **10**, 1059 (2021).

ACKNOWLEDGEMENTS

Authors extend their appreciation to Botany and Microbiology Department, Faculty of Science, Al-Azhar University, Cairo, Egypt; Department of Manuscripts Conservation, Al-Azhar Al-Sharif Library, Cairo, Egypt; and Conservation Department, Faculty of Archaeology, Cairo University, Giza, Egypt for the great cooperation and supporting to achieve and publication of this research work.

AUTHOR CONTRIBUTIONS

A.F., G.A.-M. Conceptualization, supervision, manuscript administration, methodology, validation, formal analysis, investigation, data curation, writing—original draft preparation, and writing—review and editing; M.A.-N.; methodology, validation, formal analysis, investigation, data curation, writing—original draft preparation; A.M.A.K., S.E.-D.H.; validation, formal analysis, software, data curation, writing—original draft preparation.

FUNDING

Open access funding provided by The Science, Technology & Innovation Funding Authority (STDF) in cooperation with The Egyptian Knowledge Bank (EKB).

COMPETING INTERESTS

The authors declare no competing interests

ADDITIONAL INFORMATION

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