

MICROBIAL DEGRADATION STUDY OF HISTORICAL BOOKS
 "BOOK DESCRIPTION DE L' EGYPTE ANTIQUITIES TOME II EGYPT 30": A CASE
 STUDY

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Abstract:

The present study represents both a case study about microbial degradation by microorganisms that can cause damage to old manuscripts as well as health threats to the librarians. Most of the microbiological damage started in poor environmental conditions of storage and display. The study aims to characterize the microbes that cause microbial deterioration of the historical books. Microbial swabs were taken from the manuscript under study, and the isolated organisms were identified using the sequencing of rRNA of the ITS region of the rDNA gene at Solgent company, South Korea. The results showed three bacterial species: *Micrococcus luteus* (MH450098), *Microbacterium schleiferi* (NR044936T) and *Bacillus subtilis* (NR112629T), as well as: two fungal species *Aspergillus ustus* (NR134245T) and *Aspergillus chevalieri* (NR135340T). The genus *Aspergillus ustus* was the dominant genus isolates. It was followed by *Aspergillus chevalieri*. The biological activities of the isolated microorganisms were studied and showed the highest activities cellulolytic and proteolytic activities observed by *Aspergillus chevalieri*. Modern devices were used to study the infected and the uninfected parts of the same manuscript by taking some of the falling pieces from the manuscript, such as (light microscopes, SEM with EDXS, and FTIR). Surface observation obtained by light microscopy showed microbial infiltration on infected paper, significant changes detected by SEM in some elements decreased the percentage, and the other element percentage increased. Moreover the other elements disappeared. The chemical changes inside the paper structure resulted from the degradation of large organic compounds (chemical functional groups) by the action of extra-cellular enzymes secreted by deteriorating fungal species, converting these compounds into smaller ones using the FTIR.

1. Introduction

In the 14th century, the manufacturing of paper was established in Europe. It passed through several production stages, starting with the hand-made paper from rags (cotton or linen), with the assistant of lime and even wooden paper, which was made of wood fibers because wood contains the basic component of paper, namely, cellulose [1].

A large portion of our cultural heritage is within the illustrated paper-pain-tings, sketches and drawings on paper, etc. These paintings and manuscripts are not life forms, but they are a mark and a symbol of life, thus, they are vibrant. If we focus attention on the surrounding environment, it is full of germs, and our paintings do not

have any defense systems like the alert immune system of our own bodies to fight against these germs [2]. Paper is specially made from cellulose fibers, sizing material (e.g., Rosin utilized with aluminum sulfate), adhesive (different gums and mucilage), fillers, and loadings. Cellulose fibers including paper are suitable for writing (i.e., for holding, and absorbing inks and colors). Furthermore, cellulose fibers used in manuscripts are susceptible to biological attacks by microorganisms, and insects. Microbial deterioration includes bacteria, actinomycetes, and fungi [3]. In addition to natural causes, such as climate, light, fungi, and insects, several human factors cause damage to the paper and add materials, including ink and hand coloring with pigments or dyes. Deterioration of paper materials result from the degradation of cellulose by many factors, such as a chemical attack by acidic hydrolysis, oxidative agent, light, pollution, and biological attack. Furthermore, the presence of microbiological contamination with fungi and bacteria can cause significant damage to old manuscripts or hazard to those working in archives or libraries. Microbial growth on objects of cultural heritage often causes a severe aesthetical spoiling owing to colony formation and fungal pigments [4]. Bacteria and fungi were isolated from an infected manuscript dated back to the 8th century H. kept at Al-Azhar library in Cairo, Egypt. The manuscript was made of cellulose fibers. Three bacterial species were characterized, namely *Bacillus subtilis*, *Bacillus megatherium*, and *Streptomyces sp.* Also, five fungal species, i.e., *Aspergillus niger*, *Aspergillus flavus*, *Penicillium citrinum*, *Alternaria alternata*, and *Acremonium kiliense* were isolated [5]. Museum materials, such as paper, textiles, and wood are also attacked by fungi, causing extreme deterioration [6-8]. Fungi and bacteria cause severe damage to historic materials resulting from secreting organic acids and specific enzymes, such as cellulase, protease, and ligninase [9]. In recent decades, the dogma has changed and it's now generally agreed

that fungi and bacteria do not only cause serious aesthetical destruction of paintings, costumes, ceramics, mummies, books, and manuscripts but also inhabit and penetrate the materials, leading to material loss, owing to acid corrosion, enzymatic degradation, and physical alteration. Microbial development poses two serious risks: Metabolic processes produce substance such as pigments, enzymes (cellulases and proteases), organic acids, chelating agents, and other biochemical substances [10]. The presence of microorganisms and toxins pose risks to conservation personnel and visitors. In the past, air relative humidity was considered as the main indicator of deterioration risk and was usually measured in combination with temperature. Recently, water activity has been used as a measure of how efficiently water present can take part in a chemical and physical reaction [11]. WHO studied the deterioration of rare books and identified isolated microorganisms, including *Aspergillus niger*, *Aspergillus flavus*, *Aspergillus oryzae*, *Penicillium citrinum*, *Fusarium flocciferum*, *G+ve bacilli*, *G+ve short bacilli*, and *G+ve cocci*. The degradation of documents made from parchment, which is principally composed of collagen, maybe a complex process, involving chemical oxidative deterioration of amino acid chains and hydrolytic cleavage of the peptide structure. Microorganisms can hydrolyze collagen fibers and other protein-based materials, but can also modify the inorganic components or produce pigments and organic acids, discoloring the parchment. Bacteria displaying proteolytic activities play a serious role in the deterioration of ancient documents and books made from parchment. Species belonging to the genera, such as *Bacillus*, *Staphylo coccus*, *Pseudomonas*, *Virgibacillus*, and *Micromonospora* are isolated from decaying parchments [12]. The historic book paper can be studied using instrumental techniques, such as light microscopes, FTIR, and SEM-EDXS that allowed the production of images of the sample surfaces at very high magnifica-

tion, and at the same time the determination of the major elemental components of the sample [13], and illustrating the chemical changes to the paper structure resulted from the degradation of large organic compounds (chemical functional groups) by the action of extracellular enzymes secreted by deteriorating fungal species that convert these compounds into smaller ones using the FTIR [14]. The use of the Scanning Electron Microscope for the study of the structure of the paper provided a lot of information not only about the morphological changes of old paper but also about the elemental composition of the different layers that were used to cover and decorate paper in the past [15].

2. Materials and Methods

2.1. Place of work

The study was carried out in the Microbiology Laboratory, Conservation Center, the Grand Egyptian Museum, Egypt.

2.2. Collection of swabs

Swabs were obtained from a rare book from the parliament of the Arab Republic of Egypt "*Book Description de l' Egypt Antiquities-Tome II Egypt 30*". Description of Egypt book has scientific, historical, and cultural significance. Napoleon invaded Egypt in 1798; he brought an entourage of over 160 scholars and scientists, refe-

rred to as the French Commission on the sciences and arts of Egypt. These experts undertook an intensive survey of the country's archeology, topography, and natural history. A soldier who was a part of the expedition found the famous Rosetta Stone, which the French linguist and scholar Jean-François Champollion (1790-1832) later used to reveal many of the mysteries that had long veiled the ancient Egyptian language. In 1802, Napoleon authorized the publication of the commission's findings in a monumental, multi-volume work that included plates, maps, scholarly essays, and a detailed index [16]. Most of the book pages suffer from severe microbial infections, as manifested in the presence of clear microbial lesions in the form of brown spots combined in many book pages to cover the entire pages in brown color [17], fig. (1). Microbial swabs were cultured on plates of cellulose media (Sodium nitrate 2-3 g, Potassium chloride 0.5 g, Di-potassium hydrogen phosphate 1g, Magnesium sulphate 0.5 g, Cellulose 10 g, agar agar 15 g. , and tap water 1000 m), protein media (Sodium nitrate 3 g , Di-Potassium hydrogen phosphate 1 g, Magnesium sulphate 0.5 g, potassium chloride 0.5 g, gelatin 10 g, agar agar 15 m, and tap water 1000m), and nutrient agar media (peptone 5 g, Beef ext-ract 5 g, Sodium chloride 3 g, agar 15 g, Distilled water 1000 m, agar agar 15 g) [18].

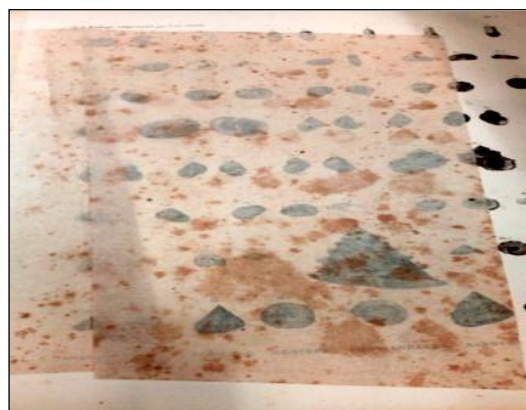


Figure (1) Show microbial deterioration on historic book's papers

2.3. Isolation and purification

The growths present in the Petri dishes were taken separately and cultured on the

same previous media to obtain the microorganisms in pure forms to complete the

steps of characterization. Microbial slides were made for all isolates and examined under a light microscope to prepare the special photo of each microorganism [19].

2.4. Characterization and identification

Microbial colonies that grew on the incubated plates were subculture into fresh separate sterile cellulose agar and nutrient agar plates and incubated to obtain pure cultures of causative microorganisms. The purified isolates were kept in slants and stored for characterization [20]. The identification of all microbial isolates was carried out at the Laboratory of Microbiology, Grand Egyptian Museum, Cairo using the sequencing of the rRNA gene at Solgent Company, South Korea.

2.4.1. Identification of isolated bacteria

Bacterial identification was performed with the molecular approach: bacterial isolates were cultured in sterile test tubes containing 10 ml of nutrient broth medium [21]. Cultures were incubated at 28 °C for 48 hours. A small amount of bacterial culture was scraped by sterile spatula suspended in 100 µl sterile distilled water in 2 ml sterile vials and boiled at 100° C for 15 minutes. Bacterial DNA was extracted and isolated, utilizing a SolGent purification bead. Before sequencing, the ribosomal rRNA gene was amplified using the polymerase chain reaction (PCR) technique in which two universal bacterial primers 27 F (forward) and 1492 R (reverse) were incorporated in the reaction mixture. Primers used for gene amplification have the following composition: 27F (5'AGAGTTTGA TCMTGGCTCAG), 1492R (5'TACGGYT ACCTTGTTACGACTT). The purified PCR products (amplicons) were reconfirmed employing a size nucleotide marker (100 base pairs) by electrophoresis on 1% agarose gel. Then, these beads were elated and sequenced with the incorporation of dideoxynucleotides (DD NTPs) within the reaction mixture. Each sample was

sequenced within the sense and antisense directions using an equivalent primer [22]. Sequences were further analyzed using the basic local alignment search tool (BLAST) from the National Center of Biotechnology Information (NCBI) website. Phylogenetic analysis of sequences was done using the Meg Align (DNA Star) software version 5.05. The identification of bacterial isolates was done by the sequencing of the rRNA gene at Solgent Company, South Korea.

2.4.2. Identification of isolated fungal

Fungal isolates were cultivated in Petri plates containing 20 ml of Czapek's yeast extract agar with 20 % sucrose (CY20S) medium at 28 °C for 7 days [23]. A little amount of fungal culture was scraped by sterile spatula suspended in 100 µl sterile distilled water in 2 ml sterile vials and boiled at 100 °C for a quarter hour. Fungal DNA was extracted and isolated using a SolGent purification bead. Before sequencing, the ribosomal rRNA gene was amplified using the polymerase chain reaction (PCR) technique during which two universal fungal primers were sequenced with ITS1 and ITS4 primers [24]. They were incorporated within the reaction mixture. Primers used for gene amplification have the subsequent composition: ITS1 (5'-TCCGTAGGTGAACCTGCGG3') ITS4 (5'-TCC TCCGCTTATTGATATGC-3') for fungi. The purified PCR products (amplicons) were reconfirmed employing a size nucleotide marker (100 base pairs) by electrophoresis on 1% agarose gel. The amplicons were sequenced with the incorporation of dideoxynucleotides (DoNTPs) within the reaction mixture. Sequences were further analyzed using the (BLAST) from (NCBI) website. Phylogenetic analysis of sequences was through with the assistance of Meg Align (DNA Star) software version 5.05. The identification of fungal isolates was done by the sequencing of the rRNA gene at Solgent Company, South Korea.

2.5. Determination of cellulase and protease activity of the isolated microorganisms

2.5.1. Enzyme production

Production was carried out in 250 ml conical flasks. Each contains 100 ml of the production medium (Dox's minerals) for the production of fungal cellulase and protease enzymes. The main source of carbon (sucrose) was replaced with 10 g of cellulose and 10 g of gelatin, respectively and nutrient minerals for the production of bacterial enzymes. Flasks were sterilized at 121°C for 15 min. After cooling, they were inoculated with 2 ml of standard inoculum of each isolate. The inoculated flasks were incubated at 28-30 °C for seven days to fungi and one day to bacterial isolates. At the end of the incubation period, the liquid cultures were centrifuged at 3000 RPM for 15 minutes. The supernatant was taken for the determination of enzyme (cellulase and protease) activity as described below.

2.5.2. Biochemical determination of the enzyme activity

Cup plate clearing zone technique (CCZ) was used for assaying the activities of cellulase and protease enzymes. The procedure was carried out by pouring 20 ml aliquots of the detection medium into a sterile Petri dish and allowing them to solidify [25]. A sterile cork borer (15 mm diameter) was used to make three cups in each plate, and 0.1 ml of the supernatant (cell-free enzyme) of each isolate was placed into the three cups. Plates were incubated at 30 °C for 24 h after which plates were flooded with a Lugol's iodine solution to assay cellulase and with an acid mercuric chloride solution for protease assay. Enzyme activities were compared based on the diameter (mm) of the clear zone.

2.6. Analysis of infected and uninfected parts of the same paper manuscript

The properties of book papers can be analyzed by using modern techniques, such as Light Microscope (LM), Scanning

Electron Micro-scope (SEM) and Fourier Transform Infrared (FTIR).

2.6.1. Surface features

Light Microscopy (Grand Egyptian Museum) was used at 400 magnifications to examine the infected and uninfected parts of the same manuscript by taking some of the worn-out and falling pieces from the manuscript.

2.6.2. Chemical characteristics

The chemical properties of the infected and uninfected parts book papers were determined by FTIR and SEM, as follow: **A)** FTIR (*Fourier Transform Infrared Shimadzo-Prestige 21*) at the Grand Egyptian Museum, changes in the shape and linkage of the structure of paper were determined using the FTIR apparatus to examine and determine the functional groups of the compounds according to wavelength and absorbance capacity. **B)** SEM (*SEM Model Quanta 250 FEG; Field Emission Gun*) attached with EDX Unit (*Energy Dispersive X-ray Analyses*) at the Central Lab, the Egyptian Mineral Resources Authority, Ministry of Petroleum, with accelerating voltage 30 Kv, magnification 14-x up to 10⁶ was used to analyze specimen elements at magnification power ranging from 500-10000-x.

3. Results

3.1. Identification of isolated microbial

The resulting microbial colonies were subjected to preliminary characterization of the type of organism as mentioned previously. The following genera were identified: *Aspergillus*, *Bacillus*, and *Micrococcus*, tab. (1). The results showed that *Aspergillus ustus* was the dominant genus in ten swabs having 66,6 % of the total fungal isolates, followed by *Aspergillus chevalieri* having 33,3 % of the total fungal isolates. From the results obtained, tabs (2 & 3), the bacterial isolates were identified according to the molecular approach by 16S rRNA sequencing analysis compared to closely related strains accessed from the Gen Bank, as *Micrococcus luteus*, *Microbacterium schleiferi*, and *Bacillus*

subtilis. Also, the fungal isolates were identified according to the molecular approach by the ITS region of rDNA sequencing

analysis compared to closely related strains accessed from the Gen Bank, as *Aspergillus ustus* and *Aspergillus chevalieri*, fig. (2).

Table (1) Isolated microorganisms on different media (*cellulose, protein, and nutrient agar*)

Swab No.	Media used		
	Cellulose agar	Protein agar	Nutrient agar
1	<i>Aspergillus ustus</i>	-	<i>Microbacterium schleiferi</i>
2	<i>Aspergillus ustus</i>	<i>Aspergillus ustus</i>	<i>Micrococcus luteus</i>
3	<i>Aspergillus chevalieri</i>	-	<i>Micrococcus luteus</i>
4	<i>Aspergillus chevalieri</i>	-	<i>Bacillus subtilis</i>
5	<i>Aspergillus ustus</i>	<i>Aspergillus chevalieri</i>	<i>Micrococcus luteus</i>
6	<i>Aspergillus ustus</i>	-	<i>Bacillus subtilis</i>
7	-	-	<i>Micrococcus luteus</i>
8	<i>Aspergillus chevalieri</i>	<i>Aspergillus ustus</i>	<i>Microbacterium schleiferi</i>
9	<i>Aspergillus ustus</i>	-	<i>Micrococcus luteus</i>
10	<i>Aspergillus ustus</i>	-	<i>Bacillus subtilis</i>

Table (2) Identification of bacterial isolates obtained from archaeological materials by 16S rRNA sequencing analysis compared with closely related strains accessed from the Gen Bank

Sample No.	Identification	Closely related microbial strains accessed from Gen Bank		
		Strain No.	Accession No.	Similarity
1	<i>Micrococcus luteus</i>	MB5	MH450098	99.71%
2	<i>Microbacterium schleiferi</i>	DSM 20489	NR044936T	99.35%
3	<i>Bacillus subtilis</i>	NBRC 13719	NR112629T	100%

Table (3) Identification of fungal isolates obtained from archaeological materials by ITS region of rDNA sequencing analysis compared with closely related strains accessed from the Gen Bank

Sample No.	Identification	Closely related microbial strains accessed from Gen Bank		
		Strain No	Accession No.	Similarity
8	<i>Aspergillus ustus</i>	NRRL73	NR134245T	99.50%
9	<i>Aspergillus chevalieri</i>	NRRL 78	NR135340T	99.81%

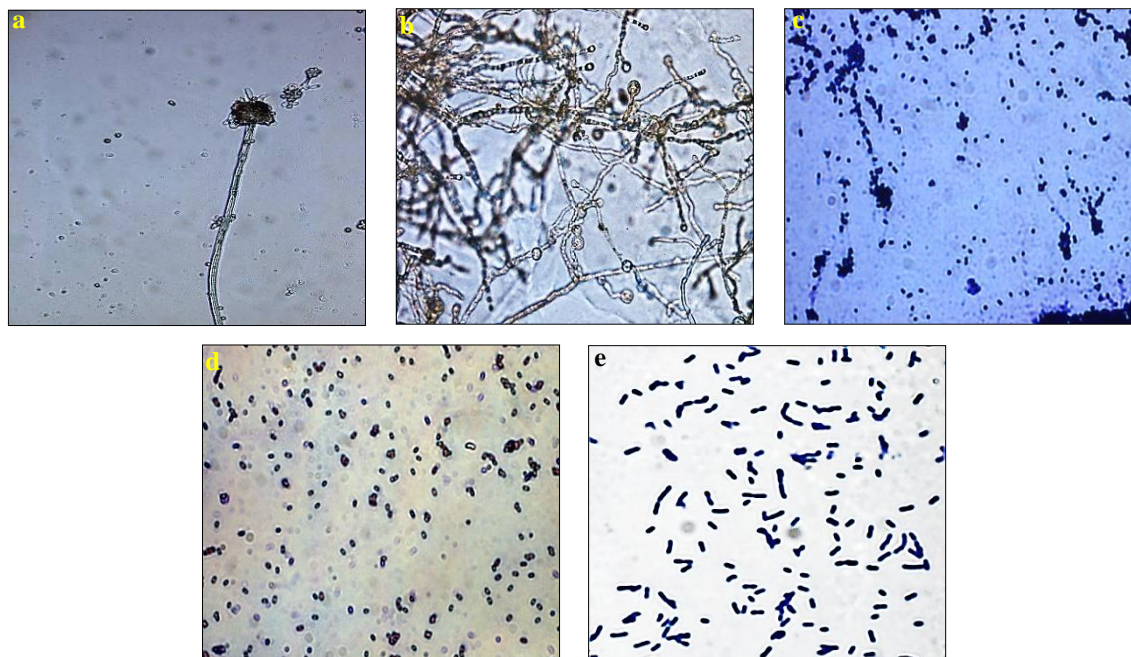


Figure (2) Shows photos of isolated microorganisms; **a.** *Asp. ustus*, **b.** *Asp. chevalieri*, **c.** *Micrococcus luteus*, **d.** *Microbacterium schleiferi*, **e.** *Bacillus subtilis*

3.2. Determination of cellulases and protease produced by the isolated microorganisms by the cup plate technique

Data obtained, fig. (3) indicated that the tested microorganisms proved that they varied in the ability to produce the cellulases and protease enzymes, thus they vary in the degree of decomposing protein

and cellulose. The tabulated data showed that the highest activities cellulolytic and proteolytic activities were observed by *Aspergillus chevalieri*.

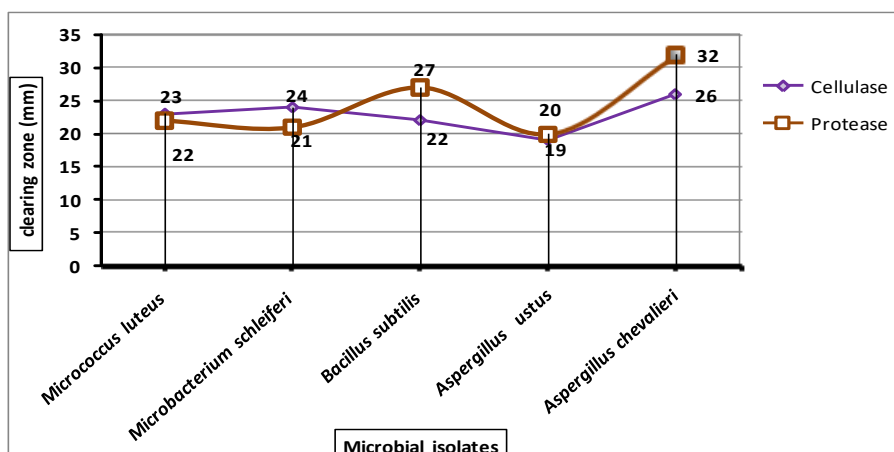


Figure (3) Shows diameters of clearing the zone (mm) produced by the isolated microorganisms using the cup plate technique.

3.3. Morphological and chemical characteristics of infected and uninfected book papers

The morphology and chemical properties of infected and uninfected parts of the same manuscript by were determined by taking some of the worn-out and falling pieces from the manuscript by the LM, FTIR, and SEM. The features observation obtained by light microscopy, at 400-x showed microbial infiltration on infected paper, as well as black and red spots and weakness, figs. (4-a, b), tab. (4), figs. (5-a, b). The results showed that significant changes were detected in carbon and chloride, which increased from 41.76 to 52.13% and from 0.80 to 1.87%, these changes occurred as a result of some organisms secreting enzymes and organic pigments. Moreover, potassium and calcium decreased from 0.49 to 0.39 % and from 10.19 to 9.82 %. Sodium and sulfur ions disappeared in the deteriorated paper. Micrographs of infected and uninfected book papers are shown in figs. (6-a, b). By determining the chemical characteristics of a paper manuscript by FTIR equipment, it could be shown that there was a net change in dipole moment

during the vibration of the molecule or the functional group (specific groups of atoms or bonds among molecules, indicating the characteristic chemical reactions of these molecules) for infrared activity leading to the absorption of infrared radiation. Figure (7) illustrates that the chemical changes inside the paper structure resulted from the degradation of large organic compounds (which have a certain chemical functional groups) by the action of extracellular enzymes secreted by deteriorating fungal species, converting these compounds it into smaller ones (having other chemical functional groups) and disappearance of other chemical groups. The following bands were obtained: 1) Change in the shape of (N=H) stretch linkage is typical protein bands assigned to the amide functions of the peptide groups at wave number area (1560-1640 cm^{-1}) 2) Change in the shape of (C=C) bending linkage movement at wave number area (1636-1508 cm^{-1}) 3) Change in the shape of (OH) bending linkage movement at wave number

area (1300-1500 cm^{-1}) 4) Change in the shape of (C-H) bending linkage movement at wave number area (1370-1426 cm^{-1})

5) Change in the shape of (C-O-C) Ester stretch linkage movement at wave number area (1160 cm^{-1}).

Table (4) Chemical analysis of elements of infected and uninfected book papers.

Element	Wt. % before	Wt. % after
C	41.58	52.13
O	45.76	43.26
Na	0.41	-
Cl	0.80	1.87
K	0.49	0.39
Ca	10.19	9.82
Fe	0.77	-
Total	100.00	100.00

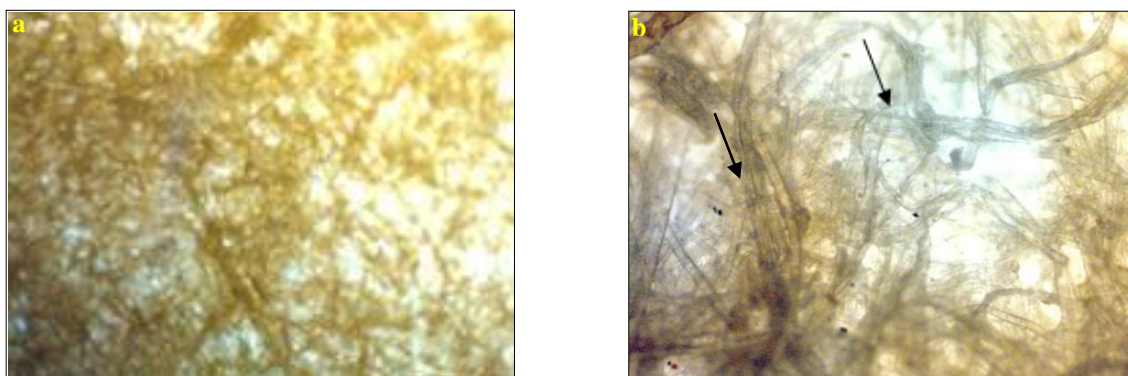


Figure (4) Shows **a.** light microscopy of uninfected book paper at 400-x, **b.** microscopy of infected paper at 400-x showing microbial growth and brown spots on the surface of the book paper.

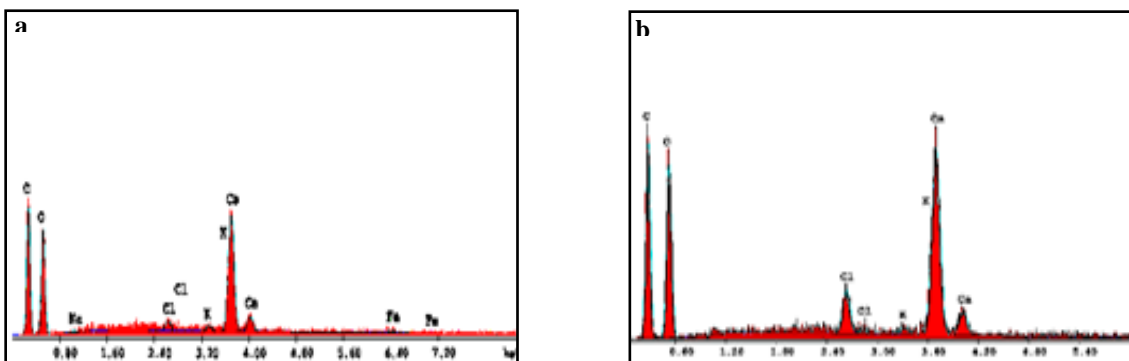


Figure (5) Shows **a.** peaks of major element component of uninfected book paper, **b.** peaks of major element component of infected paper.

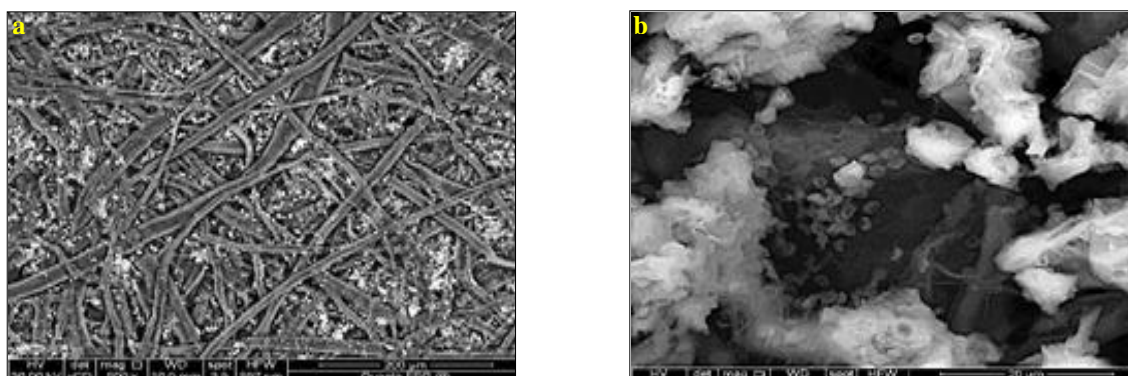


Figure (6) Shows **a.** SEM micrograph of uninfected book paper, 500-x, **b.** SEM micrograph of infected book paper shows that microbial growth on the surface, 2400-x.

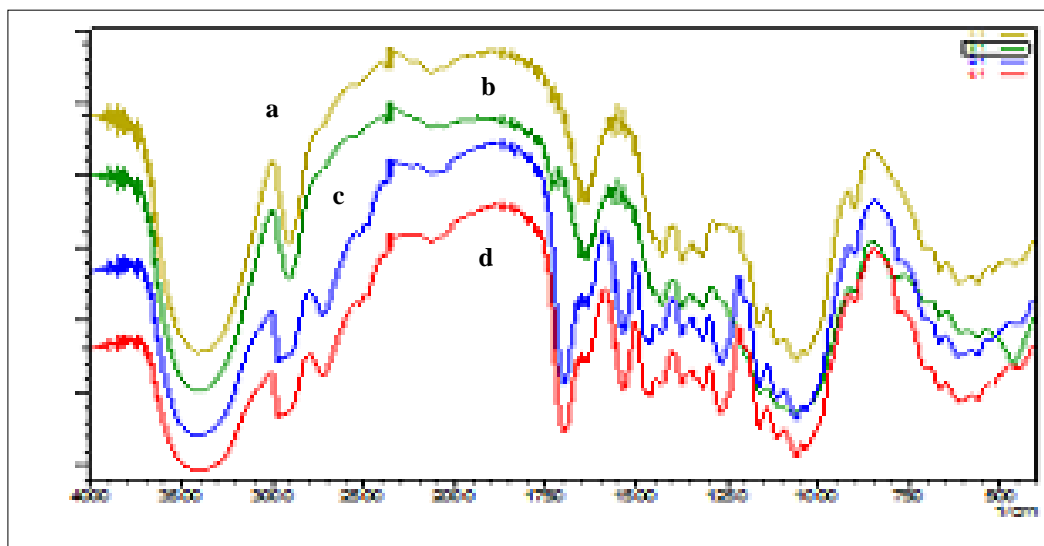


Figure (7) Shows FTIR of an infected and uninfected paper manuscript (from up to down) blue color to uninfected the other colors (red, black, and green) for infected parts.

4. Discussion

Swabs were used to inoculate cellulose and protein-containing solidified media, as well as nutrient agar medium. The observed microbes were picked up and maintained on the proper media. Then, they were subjected to molecular identification as *Micrococcus luteus*, *Microbacterium schleiferi*, *Bacillus subtilis*, *Aspergillus ustus*, and *Aspergillus chevalieri*. The results obtained from the identification of microorganisms agree with those obtained by Naji, et al. [26]. Whose isolate microorganisms, which were identified as *Aspergillus niger*, *Aspergillus flavus*, *Aspergillus oryzae*, *Penicillium citrinum*, *Fusarium flocciferum*, *G+ve bacilli*, and *G+ve short bacilli* from rare books. Also, the same species of fungi were isolated from museum materials, such as paper, textiles and wood [27]. The isolated microorganisms were tested to produce the cellulose and protease enzymes. The tabulated data showed that the highest activities. Cellulolytic and proteolytic activities were observed by *Aspergillus chevalieri*. These results were in agreement with Pangallo, et al. [28] who found that the genus *Penicillium* and *Aspergillus* exhibit at the relevant cellulolytic activity. In addition, Maggi et al. [29] found that *Aspergillus* was higher in cellulose production, and *Cladosporium*

herbarium was the highest in protease activity based on the zone area. The results obtained by the light microscope at 400-x, showed microbial infiltration on infected paper, black and red spots, and weaknesses. In the results of the SEM-EDX, figs. (5-a & 6-a), significant changes were detected in some elemental ratios. Where some of them decreased, others increased and others disappeared, e.g. carbon varying from 41.76% to 52.13% and increasing calcium's percentage from 0.80% to 1.87%. Furthermore, disappearing sodium and sulfur ions in a deteriorated paper, tab. (4), figs. (5-b & 6-b). It could be said that the microbial growth on the object's surface can alter these surfaces due to their metabolism. This process finally leads to generating organic acids (oxalic and citric) that have chelating properties by weakening the metal-oxygen bond, increasing the solubility of some metals, and forming complexes with the mineral cations present on the surface matrix [30,31]. Finally, it could be asserted that the chemical changes within the paper structure due to microbial infection lead to the degradation of organic compounds into tiny compounds (which have other chemical-specific groups). Also, the disappearance of atoms or bonds specific groups within molecules was

responsible for the characteristic chemical reactions of those molecules. In considering the different functional groups in the sample without infection against the infected sample, fig. (7) the following bands were obtained: **1.** Change in the shape of (N=H) stretch linkage was typical protein bands assigned to the amide functions of the peptide groups at wave number area was (1560-1640 cm^{-1}). **2.** Change in the shape of (C=C) bending linkage movement at wave number area was (1636-1508 cm^{-1}). **3.** Change in the shape of (OH) bending linkage movement at wave number area was (1300-1500 cm^{-1}). **4.** Change in the shape of (C-H) bending linkage movement at wave number area was (1370-1426 cm^{-1}). **5.** Change in the shape of (C-O-C) Ester stretch linkage movement at wave number area was (1160 cm^{-1}). Similar findings were reported by other studies that attenuated total reflectance Fourier transform infrared (ATR-FTIR) spectroscopy was used to detect microbial metabolic products on carbonate mineral surfaces [32,33].

5. Conclusions

Microbiological contamination with fungi and bacteria can cause a significant destroy to old manuscripts or health risks to those working in archives or libraries. The isolated microorganisms were characterized according to the molecular approach. The following genera were identified by the sequencing of rRNA and ITS region of the rDNA gene at Solgent Company, South Korea. Micrococcus luteus (MH450 098), Microbacterium schleiferi (NR 044936T), Bacillus subtilis (NR112629T), Aspergillus ustus (NR134245T), and Aspergillus chevalieri (NR1 35340T). These microorganisms have the ability to destroy old books when stored under poor environmental conditions. The result showed the highest activities cellulolytic and proteolytic activities observed by Aspergillus chevalieri. Modern devices were used to study infected and uninfected parts of the same manuscript by taking some of the worn-out and falling pieces from the manuscript, such as (LM, SEM-EDXS and FTIR). Surface features obtained by light microscopy, at 400-x magnification, showed microbial infiltration on infected paper. Significant changes were detected by SEM-EDXS,

where some elements decreased, others increased and others disappeared. The chemical changes inside the paper structure resulted from the degradation of large organic compounds (chemical functional groups) by the action of extracellular enzymes secreted by deteriorating fungal species, converting these compounds into smaller ones using the FTIR.

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