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Evaluation of the Chemical and Microbiological Conditions of a Manuscript and a Deed from the 18th Century

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Abstract

Evaluation of an 18th century manuscript and a deed was carried out using different analytical methods to find out the composition of the papers and to discuss their deterioration state. Visual analysis, microorganism identification, pH determination and chemical analysis have been carried out. The morphology of the surface of the manuscript, the deed and the type of leather used in the manuscript's binding were identified by scanning electron microscopy (SEM), X-ray diffraction (XRD), and Infrared spectroscopy (FTIR) techniques. Chemical tests were carried out to identify the ash content, type of pigment, and the pigment binder used. The microbiological test results revealed the presence of Aspergillus sp. fungi on the leather binding and Penicillium sp. on both the manuscript and the deed. The SEM-EDAX results confirmed that the ink used in the deed was found to be carbon-iron gall combination, while the type of ink in the manuscript is only carbon. The results also showed that gold shell is the pigment used for the paper gilding. The spot test, using phoroglucinol die, indicated the presence of cotton fibers in addition to linen fibers. pH test showed that both the manuscript and the leather binding were acidic, indicating the deterioration of the leather and papers.

Keywords: Restoration; Deterioration; Manuscript; Deed.

1. Introduction

Historical manuscripts are precious cultural heritages that carry valuable history over the ages. This makes the preservation of such materials a crucial matter. They are found in Egypt in different places; such as museums, store houses and, libraries like the Bibliotheca Alexandrina. Historical papers, like other materials, undergo degradation through time. The deterioration of these materials can be chemical, physical, or biological [1]. Chemical deterioration is caused by air pollutants, humidity, heat, and light, especially ultraviolet radiation found in sunlight [2]. The biological deterioration of manuscripts is caused by the microbes present in the storage environment. These microbes are either bacteria or fungi which have the ability to feed on the paper components especially cellulose and lignin [3]. It is reported that these microbes have the ability to excrete endo-gluconase and β-glucosidase enzymes which are the main cause of cellulose content degradation [4]. In order to find out a proper method of preservation of historical manuscripts, it is preferred to start with an assessment of the manuscripts and of the paper condition and to determine the aspects of deterioration. Recently, many analytical methods have been used for the assessment of paper and leather binding of manuscripts [5-15]. These methods include chemical analysis of cellulose and ash content, infrared, scanning microscopy, and diffraction techniques.

The purpose of this work is to specify the materials used in a manuscript and a deed stored in the

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Bibliotheca Alexandrina, and to determine the deterioration aspects of these papers.

2. Material and Methods

For the purpose of finding out the components of the materials used in the papers and to explain the aspects of deterioration, pH, SEM, EDAX, XRD, FTIR techniques were used, in addition to chemical methods of analysis.

2.1 Visual Inspection

Inspection by the critical eye was carried out to find the aspects of deterioration apparent on the manuscript and deed papers and on the manuscript's leather binding. This procedure is useful because the causes of decay and mechanism of action can be easily determined. This method can be used to decide the techniques to be applied for identifying the condition of the papers.

2.2 Isolation and identification of fungi

In order to know the type of microorganism that causes the biological deterioration, we used a sterile swab to wipe the surface of the leather binding and the papers to separate the fungi and/or bacteria, especially in the contaminated areas. The separation was done after the wiping process. The fungi and bacteria were isolated by wiping the swabs on Sabouraud dextrose agar CM0041 culture medium at 25 ^{o}C for 5 days. The glassware used in this research was calibrated and has traceability to SI. The isolation and identification processes of the fungi were carried out at microbiological laboratories in Bibiliotica Alexandria, Egypt.

2.3 Acidity measurements of the papers and leather

The acidity of the manuscript and the deed, in addition to the leather binding, were studied as reported by Wouters et al. [16]. Samples weighing 0.01 gram were taken mechanically from loose-fitting fibers; near the damaged area of the papers and leather surfaces. The samples were divided into tiny pieces. The pH was measured after six hours of suspension in pure distilled water to allow the ions to migrate into the solution. The acidity was measured using QC pH meter HANNA instrument with pH 210 electrode. The pH meter was calibrated by immersion of the pH electrode in distilled water at the beginning, followed by immersion in standard Hanna buffer solutions ranged from 4 to 10. The pH electrode was rinsed with distilled water after each measurement.

2.4 Surface analysis by SEM and EDAX

A scanning electron microscope, JEOL-T 330 microscope fitted with a Tracor Northern Microanalysis Addressor Electrodes, sputter-coated with a thin gold film before SEM was carried out (magnification 1500x at 20 kV), and was used for the analysis of the morphology of the surfaces of the manuscript and the deed papers and the leather binding. SEM of samples was done at the Electron Microscope Unit, Faculty of Science, Alexandria University, Egypt. The obtained results from EDAX were automatically normalized to 100%.

2.5 XRD-EDAX analysis of ink and pigments

The ink samples and gold gilding were analyzed by X-ray diffraction using a Compact X-ray Diffractometer System (Bruker- Germany- D2-Philips-PW3710/31) Diffract meter. A 40 KV beam energy was adjusted. The analysis was conducted at the X-ray diffraction analysis Lab., Fac. of science, Alex. University, Egypt. It should be mentioned that all the decimal points obtained from EDAX were effective. EDAX were performed at the Electron Microscope Lab., Central Lab., Alex. University, Egypt.

2.6 Determining the type of leather used for the manuscript binding

Surface examination by a scanning electron microscope was performed to determine the type of leather used for the binding. The dimensions of the used samples were 2 to 4 mm, and were hanged on a stub, with the hair follicles opening towards the stub as noticed by the microscope. The samples were hanged on a stub and coated with fine gold. The thickness of the sample was 20 nm. Haines procedure [17] was taken as a reference for comparison with the identified type of leather used for the binding.

2.7 Identification of paper composition by Infrared

Infrared analysis was used to identify the composition of the paper of the deed and the manuscript. Milligrams of the papers were taken from the blank and the inked areas, and were measured by FT-IR. The range used in this analysis is 400 to 4000 cm^{-1}. The examination was done by using an infrared Bruker instrument. The analysis was performed at the

QC Lab., Drug Research Center, Faculty of Pharmacy, Pharos University, Egypt.

2.8 Chemical analysis of the manuscript and the deed's papers

2.8.1 Determination of ash amount

The amount of ash was calculated by burning a weighed sample in a muffle furnace in a porcelain crucible for 30 min at 400 ºC, then continuously for 45 min at 850 ºC. The amount of ash was estimated according to the reported method [18]. The amount of ash was calculated using the following equation:

Ash % = [A / B] x100

Where A is the mass of ash after burning; B is the mass of dry paper before burning. The muffle furnace was calibrated at the working temperatures using thermocouple that has traceability ITS 90 (International Temperature Scale).

2.8.2 Determination of α-cellulose amount

The amount of cellulose was obtained according to the reported method [19]. 10 mL 10 % NaOH solution added to 0.1 gram of both papers. The mixture was kept to swell on papers for 5 minutes at 20 ºC, and pressed for 5 min. with glass rod. The suspension was carefully mixed for about 1 min and kept covered at 20 ºC. After 35 minutes, 100 mL of distilled water was added, and then filtration in a sintered crucible took place. 10% solution of acetic acid was added to the residue, followed by distilled water. The temperature used was 20 ºC and kept constant during the whole experiment. The amount of α-cellulose was calculated by gravimetric methods after dryness in an oven at 105 ºC for 6 hrs. The amount of α-cellulose is calculated using the following equation:

α-cellulose = [A / B]x100

Where A is the mass of dry treated sample; B is the mass of dry sample.

3. Results and Discussion

3.1 Visual examination

The visual examination of the manuscript, its leather binding, and the deed, used in this study is very important. It can give an idea about the deterioration state of the papers and leather. The aspects of the deterioration can be seen in the photographs in Fig. 1. We can notice from the photographs in Fig. 1 the deterioration of the papers and leather; such as holes that may have been caused by insects, fungal spots, hardness all over the papers, erosion of the tanning leather, missing parts, salt crystallization and local damages and lost parts at the bends with creases. These aspects of deterioration could be a result of bad storage methods that do not conform to the international standards that must be taken into consideration in store houses and museums, in addition to the climate conditions in the environment of the storage area. It can be noticed that the deterioration state of the manuscript is more pronounced than that of the deed; which may indicate that the storage conditions in Bibliotheca Alexandrina are much better than those in the Municipal Library of Alexandria where the manuscript was conserved for many years before being transferred to the Bibliotheca.

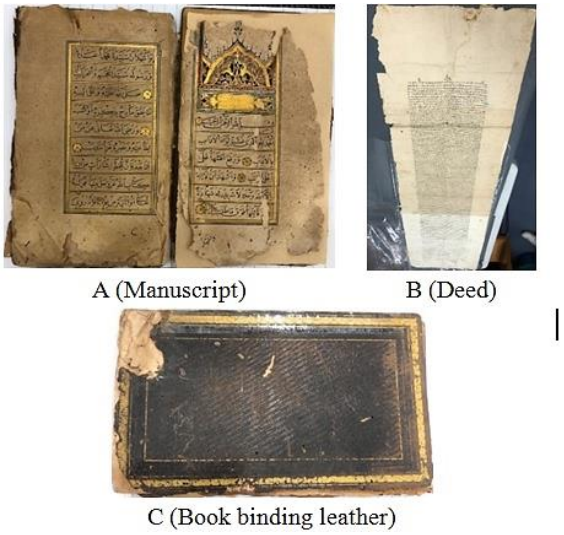

Fig. 1 Deterioration characteristics found on: A) manuscript; B) deed; C) bookbinding leather.

3.2 Identification of fungi

Among all microorganisms, fungi are the chief cause of ruining paper and leather. It is worth noting that fungi are the main cause for the decomposition of either cellulose or protein in paper and leather [20, 21]. Old manuscripts and leather are mainly organic materials and are liable to biological deterioration routes; resulting in the loss of appealing properties and degradation of the art [22]. The results of microbial tests indicated that Aspergillus sp. fungi is overriding on the leather binding, while Penicillium sp. is the foremost fungi on both the manuscript and the deed.

3.3 pH measurements

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The pH of the leather was found to be 5.7, while the pH of the paper of both the manuscript and the deed were 4.62 – 4.86. It is clear that the leather and the papers were acidic. The acidity might be caused by the presence of acidic salts that accumulated on the papers and leather, in addition to the gases present in the atmosphere due to air pollution. In Alexandria, where all the materials in this study are stored, both nitrogen oxides and sulfur oxides gases, which are the preliminary chemicals that form the acid rain, are present in very high amounts due to the existence of the industrial area in West Alexandria. Acidic rain is known to be aggressive to paper and leather fibers causing their deterioration through time due to exposure. The salts accumulated on paper and leather are due to the salt spray of the coastal environment. It is not surprising to express the degradation caused by the higher values of relative humidity of the storage area which is 55 – 60 % at least. The relative humidity in addition to the acidic rain, result in the acidic hydrolysis process lead to breaking down long chain fibers into small chains. These factors cause softening of leather after long time exposure to these farm full conditions and hence causing degradation of leather and paper [23]. Other harmful factors besides humidity and acid rains are O_2, CO_2 and O_3 gases in the environment which cause oxidation of materials and are accountable for the chemical corrosion of papers and leather [24]. It is worth telling that these acidic conditions are suitable for fungi growth on paper and leather.

3.4 Surface morphology of papers and leather

The investigation of the surface morphology of the papers and binding and of the deed was done by scanning microscopy technique as represented in Fig. 2. The SEM analysis is done for samples from different places on the deed, the manuscript's paper and its leather binding. The SEM micrographs show total deformation of the leather and both papers. The SEM micrographs of the leather bookbinding (A) show the erosion and destruction which appeared as bores of fibers at many areas, as well as the presence of fungi, in addition to the overall deterioration state. The SEM micrographs of the leather binding are compared to the SEM of control samples of: buffalo, cow, goat and sheep which were commonly used as source of leather in that era [25]. Unfortunately, the type of animal skin could not be determined due to the degree of deterioration of the leather binding. SEM micrographs of both the manuscript and the deed showed the presence of cotton and linen fibers. The micrographs also showed some contaminants and dust deposits on the surface of both papers, in addition to damaged areas caused by insects. The accumulation of fungi on the surface of both papers is clear. In order to get an idea about the degradation of the samples, EDAX analysis was carried out to show the mineral deposits on the samples (Table 1). EDAX examination of the samples showed the presence of trace amount of minerals such as aluminium and silicon in the manuscript; aluminium, sodium, potassium, calcium and iron in the deed; aluminum, silicon, potassium, magnesium, sulfur and chlorine on the bookbinding leather.

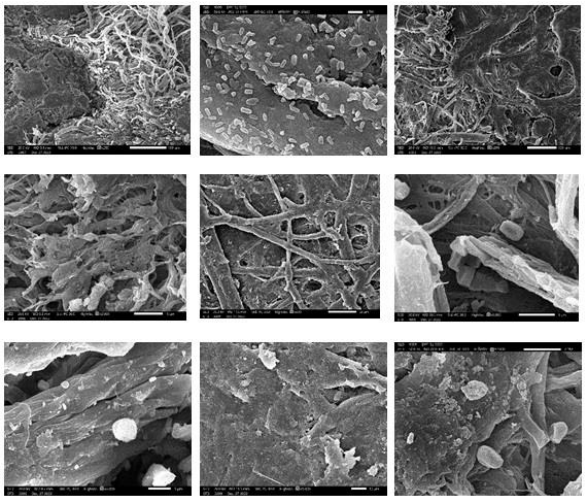

Fig. 2 Surface morphology by SEM of: A) bookbinding leather; B) deed; and C) manuscript.

Table 1 EDAX analysis of the leather bookbinding, the manuscript and the deed.

Element (mass %)	C	N	O	Na	Mg	Al
Leather bookbinding	40.16	11.55	42.64	0.41	0.37	0.45
Deed	40.23	-	56.28	-	-	1.26
Manuscript	43.65	5.11	50.14	-	-	0.95
Element (mass %)	Si	S	Cl	K	Ca	Fe
Leather bookbinding	0.6	0.66	0.56	0.28	2.32	-
Deed	1.09	-	-	0.14	0.24	0.76
Manuscript	0.14	-	-	-	-	-

The existence of these elements is elucidated by taking into consideration the fact that these elements were found in specific fillers, in addition to sizing

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materials and impurities in these fillers; thus the contamination might be from either the manufacturing process or from nearby environmental conditions. The existence of sodium is explained by the sodium chloride present in the salt spray of coastal environment. The presence of calcium may approve the presence of calcium carbonate results from the reaction between calcium hydroxides (lime) residues and carbon dioxide from atmosphere. The existence of aluminum, potassium and sulfur may be from the alum salt added to gelatine throughout the sizing process. The presence of silicon is due to the dust particles on the apparent surface [26, 27].

The data revealed the state of the bad storage of the materials of this study, which was reflected by the presence of minerals on the materials, especially the bookbinding leather. The data also indicated the exposition of materials to clay minerals and sand from the surrounding environment, salt spray from coastal environment and polluting gases from the atmosphere. Accumulation of these salts and minerals lead to the deterioration of the leather and papers, and to the decay of the materials.

3.5 Analysis of the ink and paper gilding

The type of ink used for writing on the manuscript and deed and the paper gilding were identified by EDAX analysis. Figure 3 represents the EDAX analysis charts for ink and paper gilding. Carbon ink resulted from the incomplete combustion of carbohydrates. It was used for writing on valued manuscripts in the past. Iron gall ink was widely used in writing manuscripts and deeds. A mixture of the two types of ink was used for writing historical manuscripts. EDAX analysis showed that the paper gilding was from the gold shell. It was prepared from powdered gold put off in gum and was applied by brush before writing on the manuscript.

EDAX investigation showed that the ink used in the deed was a blend of carbon and iron gall. This fact is observed from the higher percentage of carbon in the presence of ink on paper sample than in absence of ink and the presence of iron in the inked areas only. The fact that iron gall ink is present only in the manuscript is confirmed by the absence of iron in the EDAX analysis of the manuscript, while the carbon is used in both the deed and manuscript. The other elements presented in the charts might be impurities from the gum used as suspension for the ink [7].

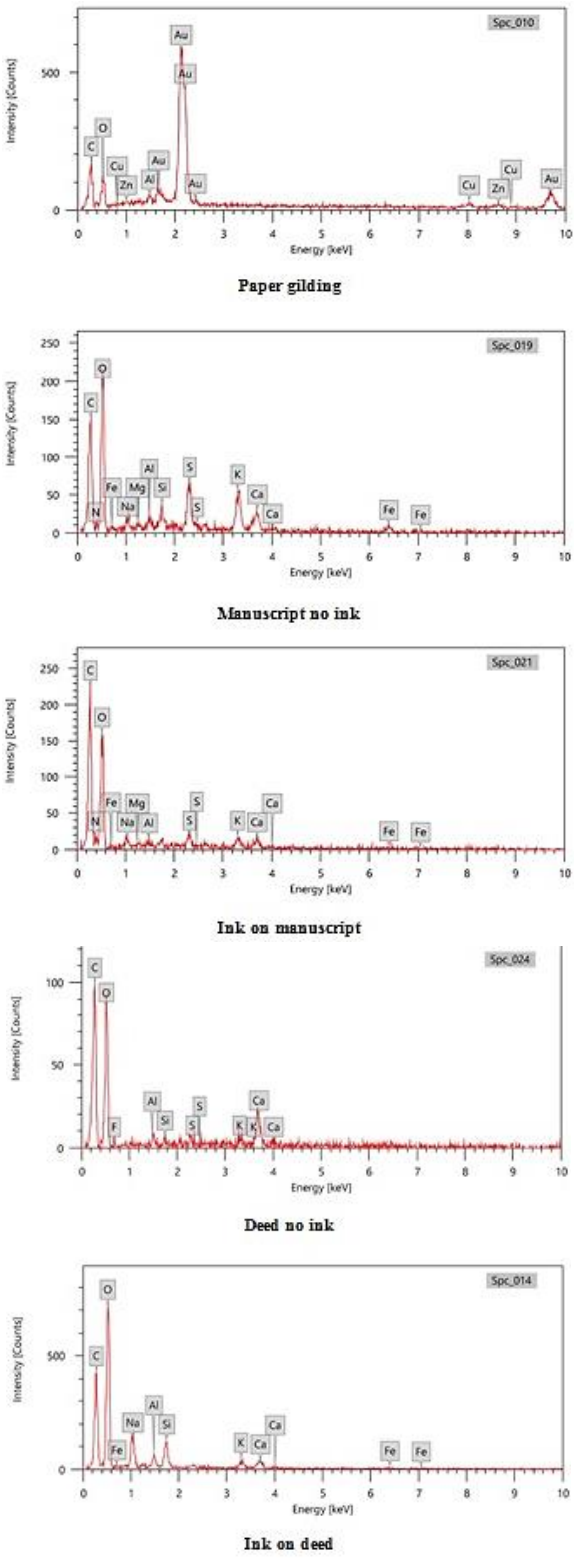

Fig. 3 EDAX study of ink and paper gilding.

3.6 Chemical analysis of the manuscript and the deed papers and determination of dye.

The chemical analysis of the manuscript and the deed is shown in Table 2.

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Table 2. Chemical study of the manuscript and the deed papers

Composition, %	α-Cellulose	Ash
Manuscript	71.4 %	1.51 %
Deed	58.1 %	1.01 %

The results showed a high percentage composition of α-cellulose, 71.4 % and 58.1 % for the manuscript and the deed, respectively. A low percentage composition of ash was found to be 1.51 % and 1.09 % for the manuscript and the deed, respectively.

The lignin content in the papers was determined by micro chemical spot testing by using phoroglucinol indicator. The spot testing indicated the absence of lignin dye in both the manuscript and the deed. Fibers identification has been carried out by using optical microscopy and scanning microscopy SEM Fig. 4 and 5. Cotton fibers are characterized by ribbon-like twists and birefringence, while linen fibers have crosshatches or nodes [28, 29].

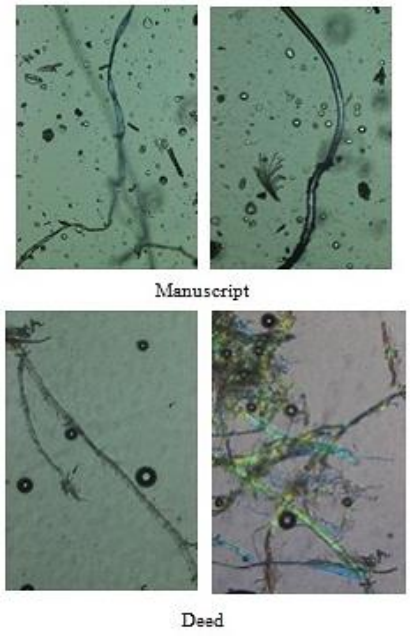

Fig. 4 Optical microscopy displaying the cotton and linen fibers: a) deed; b) manuscript.

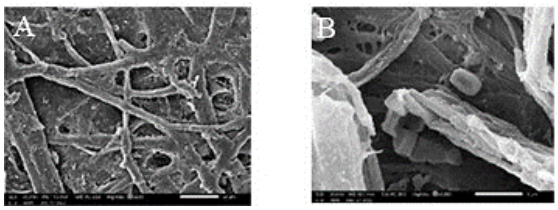

Fig. 5 SEM photographs displaying the cotton and linen fibers: a) deed; b) manuscript.

The optical microscopy and SEM testing showed the presence of cotton and linen fibers and the absence of wood fibers in both the manuscript and the deed. It is known that papers made of wood fibers are very weak compared to those made of cotton and linen fibers [30]. This indicates the resistance of the used papers to environmental conditions.

3.7 Infrared Spectroscopy analysis

Infrared spectroscopy analysis is carried out to get an idea about the composition of the papers used in this study. Figure 6 represents FTIR spectrum for both the manuscript and the deed, and the inked area and paper gilding in the papers. The results are compared with the IR spectrum of the cellulose paper in the literature. Cellulose has characteristic peaks at 3391 cm^{-1} for O-H bond, 2906 cm^{-1} for C-H bond, 1760 cm^{-1} for carbonyl group and 1061 cm^{-1} for C-O bond.

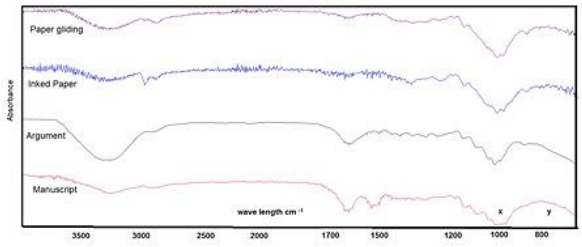

Fig. 6 FTIR spectrum for: Manuscript; Deed; Inked paper and paper gilding.

The spectrum showed peaks similar to those in the cellulose paper IR regions, indicating that the papers are formed mainly from cellulose. A similar study was done to assess the condition of a 19th century Quranic manuscript. The results showed the presence of fungi on the papers similar to that found in our work. The study showed that the type of ink used was also a mixture of carbon and iron gall which were commonly used in the time. The results showed that cotton fibers have been used as raw material for the paper used [19]. Another study aimed to evaluate the deterioration of a historical book from the 14th century in the Arabic Language Academy, Cairo University. The findings demonstrated a variety of deterioration signs such as: colour change, weakness and erosion. The historical paper had a lower level of cellulose. The microbial study showed the presence of Penicillium chrysogenum, P. citrinum, Aspergillus ustus, A. terreus, A. chinensis, Paecilomyces sp., and Induratia sp. on the historical manuscript. These fungal strains produced several hydrolytic enzymes with high

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activity, such as cellulase, amylase, gelatinase, and pectinase, which play a key role in biodegradation [31].

4. Conclusions

The studied manuscript's paper and leather binding and the deed suffer from a high degree of deterioration caused by the poor storage conditions and the environmental pollution in the store houses. Aspects of deterioration were investigated by visual inspection and surface morphological study SEM.

The microbiological study revealed the presence of Asperggillus sp. and Penicillium sp. fungi accumulated from the environmental conditions.

The study showed the presence of inorganic contaminants from the environmental depositions, in addition to contaminants during the manufacturing process of the papers.

Acidic conditions of the papers and bookbinding leather were due to the acidic salts deposited from the environment. The golden colour was extracted from gold shell.

The SEM-EDAX results confirmed that the ink used in the deed is a combination of carbon and iron gall. While the ink used in the manuscript is carbon only. The optical microscopy and SEM showed that the fibers used in manufacturing the papers are cotton and linen fibers.

The results obtained from this study shed the light on the negative effects of poor storage conditions that may lead to the deposition of salts on papers and dust particles which, are harmful to the materials used, in addition to microbial contamination.

It is advised that storage houses should be located away from coastal environments rich in these salts and minerals, which lead to the deterioration of the leather and papers and cause the decay of the materials.

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Declarations:

Authors Contribution
Prof. M. Helal: Assigning the point of research, analysis of data, and revision of manuscript.
Prof. M. Soliman: Assigning the point of research, revision of manuscript.
Ass. Prof. O. Abdullatef: Sharing experimental work, analysis of data, writing the manuscript.
Dr. H. El-Menshawy: Practical work, data analysis, revision of the manuscript.

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Data Availability
The data is available upon request.

Conflict of interest
Submission of this manuscript implies that the work described has not been published before; the manuscript is being submitted exclusively for the Int. J. Mat. Tech. Inovation; it is not under consideration for publication anywhere else; and its publication has been approved by all the co-authors.

Ethical Approval
Not Applicable.

Consent for Participate
Participants of the research are fully informed about publication in your journal.

Consent for Publication
All participants in the research agree to my authorization to publish.

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