PHYSICO-CHEMICAL ASSESSMENT OF BIODETERIORATED AND BIODEGRADED ARCHIVAL PAPER

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Abstract

Archival preservation has always been important for mankind to transfer knowledge to the posterity. Their degradation/deterioration and preservation has been studied with a view to aid in their restoration and conservation. The present work employs analytical and microbial methods to understand the physico-chemical deterioration and degradation of a 19th century colored map and two 20th century photozincographed map. Irrespective of the era, analysis revealed that all the three samples were acidic in nature with rosins as sizing agent, were high in organic matter primarily cellulose with high moisture levels. The degraded/deteriorated regions of the paper were subjected to Fourier Transform Infrared Spectroscopy (FTIR) and Scanning Electron Microscopy (SEM) to further elucidate the type of effect. The estimates of microbial count efficiently co-related with the physico-chemical changes observed in the archival documents. It can thus be concluded that irrespective of the era biodeterioration and biodegradation of archival documents follow a similar pattern.

Keywords: Archival documents; Biodeterioration and biodegradation; Cellulose support; FTIR; SEM

Introduction

Cultural property preserved in indoor environments, such as libraries and archives, are subject to a particularly high risk of biodeterioration effects of the physical state of the artefact and, respectively, biodegradation effects of the chemical nature of the component materials [1, 2]. The two effects of historic paintings, wood, paper, glass, textiles, metals, waxes, stone, polymers and coatings by microbial action is a well documented phenomenon; no material is completely immune to microbial attack [3]. Our history is primarily stored in the form of written texts, an asset indispensable for our present and future. These historical records are our heritage to future generations [4]. Archives once lost cannot be replaced, and usually reconstruction is impossible. Archives allow us to establish communications between past and future generations [5]. Libraries and archives worldwide face the growing concern regarding the biodegradation of ancient documents and books [5]. Degradation of paper-based materials is mainly due to the alteration of cellulose caused by a number of factors, such as chemical attack due to acidic hydrolysis, oxidative agents, light, air pollution and biological attack due to the presence of microorganisms like bacteria and fungi [6-10]. Study of documentation reveals that various methods were used for manufacture of paper. Paper largely contained lignin and

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cellulosic material embedded with fillers and sizing agents of different kind. In 19th century process of photozincography was more popular for printing more copies whereas in 20th century colored printing using inks and pigments was in vogue hence differences in paper quality were expected [7].

Enzymes of microbial origin such as cellulases, proteases, etc cause structural alterations within the matrix of the paper [11-13]. These enzymes are synthesized by microorganisms which compound the frailty of paper leading to its ultimate deterioration. Additionally essential additive components such as plasticizers, adhesives, organic glues, inks, pigments and bindings are susceptible to microbial attacks [11, 12, 14] which cause extensive damage to distinguishing features of documents rendering them unusable.

India is a country with a rich history; one can trace its origins to the Stone Age [15]. India boasts of one the world’s oldest and largest collection of intact ancient manuscripts. The culture and literary heritage in India lie distributed at different institutions, libraries and private collections. These relics are becoming victims to deterioration, never to be seen again. Approximately 20-25 percent of the manuscripts surveyed is cataloged and preserved reliably [15-19]. In addition to this, India is a tropical country and the rate of deterioration is greatly affected by the environmental factors like heat, humidity, salinity due to the proximity to the sea [20]. This encourages and supports the growth of macro as well as micro organisms like insects molds, bacteria etc. Deterioration and degradation of such documents across diverse countries has been recognized in terms of the changes in their physical as well as chemical characteristics [27-31]. Many antifungal/antibacterial methods are used to prevent and/or stop biodeterioration and biodegradation. These methods can go from limiting the access to water by the fungi, to the application of chemical products in the gaseous or liquid state, or physical methods like extreme temperatures, radiation or current. A proper antifungal method for materials should have a broad activity spectrum, good chemical stability, low cost, should not be toxic to humans, and should have no adverse effects on the treated material. Several authors have measured pH of historical paper as it plays an important role to explain the mechanism of deterioration [27-29]. C. Verweris et al. [30] and G. Abdel-Maksoud [6] used chemical analysis for the estimation of hemicelluloses, lignin, and ash content. Additionally detailed methods have been utilized such as analytical techniques like Fourier transform infrared spectroscopy (FTIR) for the identification of the matrix as well as other components used in paper manuscripts [31-35]. Microbiological studies and examination of the surface morphology have also been evaluated in order to determine the degree of biodeterioration and biodegradation [35-40].

Taking into account these aspects, the objectives set for this study were to assess biodeterioration and biodegradation of archival documents belonging to the 19th and 20th century by biological as well as chemical analysis. A photozincographed map copy from the 19th century and two colored maps from the 20th century were selected for studying their degradation patterns. For characterization of the paper and to understand the degradation of these documents several non-invasive and micro-invasive techniques were used, observations were made using optical microscopy and, Scanning electron microscope (SEM). Chemical tests were carried out to identify the composition in terms of the structural matrix components and the additives used as binders or fillers for archival documents. The examination using Attenuated total reflection Fourier transform infrared spectroscopy (ATR-FTIR) complemented the chemical analysis enabling the evaluation of difference in the matrix components of the paper.
Materials and Methods

**Sample selection**

Archival materials from the 19th and 20th centuries, i.e., maps belonging to the states of Hyderabad and Mumbai, were used as a source for the study. The maps belonging to the 19th century was a photozincographed map, whereas the other two maps belonging to 20th century were colored maps. These maps were collected from a private personal collection in Mumbai. The maps were stored in open racks in an open room. These documents are fumigated using Para-dichloro benzene and Carboxide (Mixture of Carbondioxide and Ethyleneoxide = 9:1) once in 3-6 months. In order to minimize the damage caused to the text and figures of the map the paper samples were taken from the peripheral region.

**Investigation of the surface morphology by optical microscopy and SEM**

The observations using optical microscope constituted the first step in identifying the alterations in the paper structure caused due to staining, mineralization and microbial growth. A light microscope, Leica DM750 was used for the direct examination of the paper samples. A scanning electron microscope, FEI-ESEM Quanta 200, was used for the elucidation of the surface morphology of the map paper.

**Isolation of micro organisms**

Sterile cotton buds were used to swab the surface of the papers in order to isolate the microbial load, especially from the contaminated area characterized by foxed and typically discolored areas. The cotton bud was then swabbed on to Nutrient agar surface for bacteria and Sabouraud’s agar for fungi. The plates were incubated at 28°C for 2-3 days. The number colonies present on the plates were counted after the period of incubation. The colonies were screened for cellulolytic activity using Carboxy Methyl Cellulose (CMC) Congo-red agar. The bacteria which showed cellulolytic activity were subjected to preliminary identification through gram staining and biochemical characterization. The fungal isolates showing cellulolytic activity, obtained from both these techniques, were identified based on their cultural and morphological characteristics.

**Chemical analysis of paper**

**pH measurement**

The measurement of the pH of the paper during this study was in accordance with TAPPI standard T 529 om-04 [41] with required modifications. Briefly, a 200µL drop of distilled water was placed on the surface of the manuscript, and the surface combined pH electrode pressed against it. The pH values were read after being constant for 30s.

**Ash content**

The percent ash content was estimated following TAPPI standard T 211 om-02 [42]. A test specimen was ignited in a muffle furnace at 525°C. The resulting weight of ash is used to calculate the percentage ash present at 525°C on a moisture-free sample basis.

**Moisture content**

The percent moisture content was determined following the TAPPI standard T 550 om-08 [43]. The percentage of moisture in a sample was determined by drying the sample to a constant weight.

**Lignin content**

The lignin content was estimated by TAPPI standard T 222om-02 [44] for Acid-insoluble residue (AIR) and TAPPI UM 250 [45] for Acid-soluble lignin (ASL). To determine the AIR the carbohydrates in the paper pulp are hydrolyzed and solubilized by sulfuric acid; the
acid-insoluble lignin is filtered off, dried, and weighed. ASL is determined from the filtrate by spectrophotometry at 205nm.

**Cellulose content**

Alpha-, beta- and gamma-cellulose in paper pulp was estimated using TAPPI standard T 203 cm-99 [46]. The pulp was extracted consecutively with 17.5% and 9.45% sodium hydroxide solutions. The soluble fraction consisting of beta- and gamma-celluloses is determined volumetrically by oxidation with potassium dichromate, and the alpha cellulose, as in insoluble fraction, is derived by difference.

**Spot/Qualitative tests**

The qualitative analyses were based on color reactions and were carried out to identify the presence of organic and inorganic constituent. These reactions occur directly on small areas of the paper surfaces. The presence/absence of rosin glue by the Raspail test, gelatin by the Tannin test, and fillers i.e. Test for Sulfite, Sulfides, Carbonate using TAPPI standard T 421 om-97 was carried out.

**FTIR Analysis**

The FTIR spectra were obtained in Attenuated total reflection mode (ATR) using the FTIR – 460 plus, Jasco, equipped with a single reflection ATR cell and ZnSe crystal. All the spectra were acquired in the range of 4000-650cm\(^{-1}\) at a resolution of 4cm\(^{-1}\). The background measurement was recorded in order to eliminate the effect of the atmospheric carbon dioxide and water vapor.

**Validation of scaled down methods and Statistical analysis**

The physico-chemical analysis was carried out using TAPPI standards as described above. However, considering the sample quantity all the methods were validated to 1/10\(^{th}\) of the original recommendation. The scaled down method was validated using standard whatman paper no 1. All the validations were carried out in replicates. The values obtained did not show any statistical difference when the sample quantity was reduced to 1/10\(^{th}\) of recommended method. All experiments were performed in replicates. Mean and SD values are represented.

**Results and Discussions**

**Investigation of the surface morphology by Optical & SEM microscopy**

Under microscopic observation (Fig. 1) random distribution and erosion of fibers was observed on all the samples. Foreign particles present between fibers could be most likely filler material or artifacts, contaminations from stains and dust. Physical damage in form of bores, deformations and micro tears in the strata of the fibers could be attributed to macroorganisms like silverfish and other insects.

![Fig. 1. Optical Microscope images of areas from the maps showings signs of deterioration](image-url)
PHYSICO-CHEMICAL ASSESSMENT OF ARCHIVAL PAPER

SEM analysis of the three maps showed severe microbial infestation in and around the cellulose fibers (Fig. 2). The presence of organic materials possibly of microbial origin, such as fungi and bacteria, were detected on the entire surface of the paper samples. Their presence was heterogeneous, crowded in some areas and limited in others. At areas with high microbial infestation the cellulose fibers were not visible because of the biological matter. The biodiversity of microflora on the paper was confirmed from the microbial samples collected and cultured from the paper surface. The greater reflection in few of the SEM images could be suggestive of mineralization. Microbial linked mineralization is reported in degrading paper material by F. Pinzari et al. [37]. Therefore it is likely that the mineralized areas observed in the present study could be due to microbial activity. The SEM observation of the morphology of paper fibers suggests that the material comes mostly from cotton.

Isolation of microorganisms

It was observed that bacterial isolates were larger in number on the documents whereas larger number of fungi was isolated from areas which showed signs of deteriorations. The bacterial cultures isolated were more diverse with gram positive rods and cocci along with few gram negative rods/coccobacilli. Fungi isolated mainly from areas which were foxed and bleached were dominated by Aspergillus species (Table 2). The Table 1 gives the colony counts of each of the archival document. Earlier study undertaken by this lab [13] showed a similar trend where in it was observed that areas with high fungal density had invariably low bacterial count and vice versa.

![Fig. 2. SEM images of map (a) Area with high microbial infestation (b) Area with low microbial infestation](image)

<table>
<thead>
<tr>
<th>Sample</th>
<th>No. of bacterial colonies</th>
<th>No. of fungal colonies</th>
</tr>
</thead>
<tbody>
<tr>
<td>Map 1</td>
<td>12</td>
<td>9</td>
</tr>
<tr>
<td>Map 2</td>
<td>17</td>
<td>7</td>
</tr>
<tr>
<td>Map 3</td>
<td>15</td>
<td>6</td>
</tr>
</tbody>
</table>

Chemical analysis of paper

The data on various parameters of the chemical analysis are presented in Table 3. The pH measurements revealed that the archival documents studied ranged from near neutral acidic 6.1 to 5.1. When co-related with microbial load, it is observed that acidic pH supported larger
population of fungus with lower bacterial count whereas near neutral (pH = 6.1) supported larger population of bacteria with lesser number of fungi (Table 1). The differential acidity can also be attributed to the manufacturing process or due to absorption of pollutants such as sulfur dioxide and nitrogen oxides. Also, the presence of rosin, as detected by the Raspail test is known to contribute to the acidic nature of paper as rosin is acidic in nature [48].

Table 2. Fungi isolated from different areas of maps

<table>
<thead>
<tr>
<th>Sample</th>
<th>No. of fungal colonies</th>
<th>Genera of fungi</th>
</tr>
</thead>
<tbody>
<tr>
<td>Map 1</td>
<td>Foxed area (1)</td>
<td>2 Aspergillus species</td>
</tr>
<tr>
<td>Foxed area (2)</td>
<td>3</td>
<td>2 Aspergillus and 1 Penicillium species</td>
</tr>
<tr>
<td>Bleached area (3)</td>
<td>3</td>
<td>1 Aspergillus, 1 Cladosporium and 1 Penicillium species</td>
</tr>
<tr>
<td>Normal area</td>
<td>1</td>
<td>Aspergillus species</td>
</tr>
<tr>
<td>Map 2</td>
<td>Foxed area (1)</td>
<td>1 Aspergillus species</td>
</tr>
<tr>
<td>Foxed area (2)</td>
<td>3</td>
<td>2 Aspergillus and 1 Penicillium species</td>
</tr>
<tr>
<td>Bleached area (3)</td>
<td>2</td>
<td>Aspergillus species</td>
</tr>
<tr>
<td>Normal area</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Map 3</td>
<td>Foxed area (1)</td>
<td>2 Aspergillus species</td>
</tr>
<tr>
<td>Foxed area (2)</td>
<td>1</td>
<td>Aspergillus species</td>
</tr>
<tr>
<td>Bleached area (3)</td>
<td>2</td>
<td>1 Aspergillus and 1 Penicillium species</td>
</tr>
<tr>
<td>Normal area</td>
<td>1</td>
<td>Aspergillus species</td>
</tr>
</tbody>
</table>

Percentage ash content is high in map 2 and 3 as compared to map 1 which is indicative of higher inorganic content in those maps as compared to map 1 (Table 3). This is indicative of the differences in the manufacturing processes of the paper through the era. All the documents in the study showed higher moisture content ranging from 7.69 -11.9% than generally accepted for the commercial printing (2-7%) [49] which could be attributed to the environmental humidity where the maps were stored. Literature cites significant increase in the rate of paper degradation with increasing moisture content [28, 5-0, 51]. During this study the maps with higher moisture content supported higher bacterial load (Table 3).

Table 3. Chemical analysis of paper

<table>
<thead>
<tr>
<th>Sample</th>
<th>pH</th>
<th>% Ash content</th>
<th>% Moisture content</th>
<th>Lignin Content</th>
<th>Cellulose Content</th>
<th>Raspail test</th>
<th>Test for Gelatin, Sulfite, Sulfides and Carbonate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>AIR mg/g</td>
<td>ASL mg/g</td>
<td>Total: mg/g</td>
<td>Alpha %</td>
<td>Beta %</td>
</tr>
<tr>
<td>Map 1 19th century</td>
<td>5.15±0.05</td>
<td>0.71±0.01</td>
<td>7.67±0.05</td>
<td>0.34±0.05</td>
<td>3.6±0.15</td>
<td>3.94±0.35</td>
<td>80.45±0.05</td>
</tr>
<tr>
<td>Map 2 20th century</td>
<td>6.15±0.06</td>
<td>13.56±0.01</td>
<td>10.52±0.02</td>
<td>0.17±0.02</td>
<td>7.70±0.31</td>
<td>85.36±0.07</td>
<td>7.85±0.07</td>
</tr>
<tr>
<td>Map 3 20th century</td>
<td>5.80±0.01</td>
<td>14.02±0.01</td>
<td>11.76±0.15</td>
<td>0.25±0.01</td>
<td>7.61±0.29</td>
<td>85.36±0.09</td>
<td>7.88±0.13</td>
</tr>
</tbody>
</table>

The documents used in the present study were low in lignin content and high in alpha cellulose contents. The high alpha cellulose content obtained in the study was indicative of pure cellulose being used in the manufacturing of the paper. SEM imaging also supports the same in the present study (Fig 2).
Map 1 showed highest amount of beta cellulose, which is a degraded form of alpha cellulose. A higher number of fungi, isolated from map 1, seem to be the responsible factor for the cellulose degradation. Similarly, the higher acidity of the paper could also be attributed to the fungal degradation of cellullosic material. Fungi are known to degrade cellulose in archival documents and a large number of fungal spores were also seen in the SEM analysis (Fig 2).

**FTIR Analysis**

It has been already reported that the FTIR analysis, particularly in Attenuated Total Reflectance (ATR) mode appears to be promising, for preliminary analysis of the composition of ancient paper documents. FTIR-ATR analysis allows a rough classification of paper materials by examining the infrared bands not masked by cellullosic components.

General trend that appeared in the FTIR spectra of the three maps was as explained in the Table 4. The broad band in the 3600-3100cm$^{-1}$ region is attributed to OH-stretching vibration. Changes were observed in the 1300-1500cm$^{-1}$ region where the numbers of bands are due to numerous mixed vibrations rather than to characteristic group frequencies. Thus, the bands in this range are mixtures of OCH deformation vibrations, CH$\_2$ bending vibrations, CCH and COH bending vibrations. Table 4 represents IR spectra of three maps from normal as well as foxed/ bleached area. It is observed that significant shift in the wavelenghts 3366cm$^{-1}$ and 1649cm$^{-1}$ in map 2 and 3345cm$^{-1}$ in map 3 suggesting alteration in the structure of the matrix of map 2 and 3 no such deviation was observed in the spectra of map 1. Incidentally map 2 and 3 are from the same era. Vibrational spectra, presented in this work, conclude that the paper samples are almost pure cellulose. Due to the different state of degradation of cellulose (paper) differences in the intensities of the bonds (~3300, ~1600 and ~1000cm$^{-1}$) and O-H bonds (~3338cm$^{-1}$) are evident. The water absorption belonging to the C-O bond, which can be explained by hydrolysis and oxidation of cellulose. The most marked variation is the decrease in intensity of the O-H stretch hydrogen-bonded band at around 3300cm$^{-1}$. Biotic foxing is evident from the peak 1159cm$^{-1}$ present on all 3 map samples attributed to N-acetyl ester bonds, Amide II bands.

**Table 4. IR spectra and structural assignments**

<table>
<thead>
<tr>
<th>Peakposition cm$^{-1}$</th>
<th>Map 1</th>
<th>Map 2</th>
<th>Map 3</th>
<th>Peak assignment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>Foxed</td>
<td>Bleached</td>
<td>Normal</td>
<td>Foxed</td>
</tr>
<tr>
<td>1029</td>
<td>1030</td>
<td>1029</td>
<td>1029</td>
<td>1031</td>
</tr>
<tr>
<td>1053</td>
<td>1053</td>
<td>1053</td>
<td>1029</td>
<td>1031</td>
</tr>
<tr>
<td>1109</td>
<td>1108</td>
<td>1110</td>
<td>1105</td>
<td>1109</td>
</tr>
<tr>
<td>1159</td>
<td>1159</td>
<td>1159</td>
<td>1159</td>
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<tr>
<td>1317</td>
<td>1317</td>
<td>1316</td>
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<td>1647</td>
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<tr>
<td>2367</td>
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<td>2363</td>
</tr>
<tr>
<td>2900</td>
<td>2897</td>
<td>2900</td>
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</tr>
<tr>
<td>3339</td>
<td>3337</td>
<td>3338</td>
<td>3366</td>
<td>3331</td>
</tr>
</tbody>
</table>

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**Table 5.** Differences in intensities of % Transmission of peaks of different regions of 3 maps

<table>
<thead>
<tr>
<th>Peak position cm⁻¹</th>
<th>Map 1</th>
<th>Map 2</th>
<th>Map 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>~3300</td>
<td>Normal</td>
<td>88.45</td>
<td>95.32</td>
</tr>
<tr>
<td></td>
<td>Foxed</td>
<td>83.72</td>
<td>93.96</td>
</tr>
<tr>
<td></td>
<td>Bleached</td>
<td>76.81</td>
<td>88.45</td>
</tr>
<tr>
<td>~2900</td>
<td>Normal</td>
<td>83.12</td>
<td>87.96</td>
</tr>
<tr>
<td></td>
<td>Foxed</td>
<td>78.65</td>
<td>89.92</td>
</tr>
<tr>
<td></td>
<td>Normal</td>
<td>78.65</td>
<td>90.81</td>
</tr>
<tr>
<td></td>
<td>Foxed</td>
<td>83.12</td>
<td>91.47</td>
</tr>
<tr>
<td>~1600</td>
<td>Normal</td>
<td>91.86</td>
<td>89.47</td>
</tr>
<tr>
<td></td>
<td>Foxed</td>
<td>91.34</td>
<td>91.87</td>
</tr>
<tr>
<td>~1300</td>
<td>Normal</td>
<td>91.86</td>
<td>89.47</td>
</tr>
<tr>
<td></td>
<td>Foxed</td>
<td>91.34</td>
<td>91.87</td>
</tr>
<tr>
<td>~1100</td>
<td>Normal</td>
<td>86.91</td>
<td>85.29</td>
</tr>
<tr>
<td></td>
<td>Foxed</td>
<td>88.53</td>
<td>89.00</td>
</tr>
</tbody>
</table>

Figure 3 shows the IR spectra of the three regions of map 1. The IR depicts transmission peaks as a function of wave number, thus the height of peak is inversely proportionate to the concentration of the target species. Increase in transmission in the wavelength numbers characteristic of cellulosic material confirms degradation of the target species (cellulose) in the paper. A comparison of IR of the three samples indicates more or less similar pattern of degradation of map paper. Specifically, peaks representing wave numbers 1029, 1109, 1317, 1647 and 3339 cm⁻¹ are equally prominent in all the three samples indicating similar structure and similar degradation pattern.

**Conclusion**

A comparison of the three maps belonging to different centuries i.e. 19th and 20th century indicates more or less similar pattern of degradation. Chemical analysis shows that the maps have different chemical composition in terms of inorganic and organic contents; which could be due to the variations in the process of paper making. It is well documented that the paper degradation is promoted by intrinsic as well as environment factors. In addition, techniques used in the study such as FTIR, SEM confirm the involvement of intrinsic and a major role of extrinsic factors in the process of degradation. While FTIR pattern shows degradation of cellulosic components and biological signature in the form of a peak at 1159 cm⁻¹ representing amide II group, SEM depicts degrading cellulosic fibers which can be attributed to microorganisms. Biodeterioration and biodegradation cannot be completely stopped but precautions should be taken to safeguard archival documents to prevent irreversible damage.
Precaution should be taken to use agents to control damage from ultraviolet radiation, pollutant gasses, fungi and bacteria that themselves do not enhance archival degradation. To protect against biodeterioration and biodegradation archival repositories fumigate, expose to UV light archival documents as a common practice which is responsible to weaken the cellulose fibers and add to deterioration. This study highlights that documents from all eras follow the same pattern for deterioration, as environmental and extrinsic factors play a major role in deterioration. Antimicrobial protective coatings on archival documents will be an advanced procedure for preservation which will evade biodeterioration and biodegradation, and also protect from other extrinsic factors. Further studies with other paper materials are in progress for a better understanding of the abiotic factors and microbial degradation of archival documents for the preservation.

Acknowledgments

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