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An environmental assessment of biodeterioration in document repositories

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Experiments were designed (1) to investigate the bioadhesion, biofilm formation, foxing, and micropitting in documentary collections, (2) to assess the risk of biodeterioration, (3) to investigate the environmental microbial concentration, and (4) to study the influence of environmental factors in biodeterioration of documentary heritage in three archives. The importance of this work in the field of biodeterioration of documentary heritage was verified by bioadhesion and biofilm formation by microorganisms isolated from the collections under study. *Bacillus* sp. and *Scopulariopsis* sp. isolated from paper books showed considerable evidence of attacking the paper structure and of pigment production, constituting a hazard to the loss of documentary heritage.

Keywords: bioadhesion; micropitting; documentary heritage; environmental air quality; foxing; microorganisms

Introduction

Archives and libraries are part of the cultural heritage of a nation, safeguarding the memory of people (UNESCO 1982). In the repositories of documentary heritage, maintenance conditions of temperature and relative humidity must be strict because oscillations allow the development of microorganisms that can cause physico-chemical and biological damage (Villalba et al. 2004). Biodeterioration of materials of cultural heritage is an unwanted and irreversible change in the physico-chemical and mechanical properties of the material, caused by the action of different microorganisms. Disturbances occur depending on the components of the material and environmental conditions. The factors that influence colonization and biodeterioration are climatic and environmental such as sunlight, shadows, rain, temperature, inorganic and organic pollutants, surface receptivity, and the nature of the materials (Urzi & Krumbein 1994; Valentín 2003; Allsopp et al. 2004; Gaylarde et al. 2008; Piñar et al. 2013). A large number of particles of different origin, size and shape can be found in the air in indoor and outdoor environments, forming the atmospheric aerosol (Mandrioli 2002).

Water activity (A_w), the moisture content of a material, is one of the most important factors in microbial growth. Many species of fungi and bacteria begin developing as a function of moisture content on the surface of an object, adhering and developing biofilms. Biofilms are aggregates of microorganisms encased in a matrix of extracellular polymeric substances (EPS). These EPS consist of a mixture of polysaccharides, proteins, nucleic acids, and other highly hydrated biopolymers. They keep biofilm organisms together and allow their attachment to substrata (Gaylarde & Videla 1987; Characklis & Marshall 1990; Little et al. 2006; Flemming & Wingender 2010). Many of the collections on display in the archives and libraries are of an organic nature, characterized by their high hygroscopicity. This implies a significant increase in the moisture content of the support (eg papyrus, photographic paper, and parchment), especially when the objects are exposed to insufficient ventilation and a relative humidity of >65%.

When conditions for the conservation of materials are unsuitable (relative humidity, temperature), these are exposed to the development of microorganisms (Valentín 2004). Fungi are the most harmful microorganisms to paper as they have the ability to grow at lower A_w than bacteria. Research indicates that the internal moisture content of the paper must be at least 10% for fungal growth to occur (Borrego et al. 2010; Pinzari et al. 2010).

Fungi, like many bacteria, produce spots of different colours on materials, which have been associated with a process called foxing (Arai 2000; De Paolis & Lippi 2008; Zotti et al. 2011). Foxing results in the release and excretion of pigments and acids, causing the appearance of brown-yellow dots. While their chemical or biological origin is discussed, damage can be initiated by microorganisms on paper. Several fungal species have been isolated and characterized from paper affected by foxing

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(Arai 2000; De Paolis & Lippi 2008). These microorganisms have an extensive enzymatic pool that allows them to use paper as a sole carbon source (Villalba et al. 2004). Additives used in paper manufacture, such as starch, provide another excellent nutrient source for microorganisms that can biodegrade cellulose-based materials (Desjardins & Beaulieu 2003).

Many of the bacteria that are present in these kinds of organic substrata (eg paper, photographic paper, parchment) grow using very low concentrations of nutrients and allow the development of micro-niches for the growth of other microorganisms (Koestler et al. 1988).

The aims of this work were: (1) to investigate the bioadhesion, foxing, biofilm formation and micropitting in documentary collections, (2) to assess the risk of biodeterioration of documentary heritage, (3) to investigate the environmental microbial concentration, and (4) to study the influence of environmental factors in biodeterioration of materials stored in three archives: (1) the Archive of Historical and Cartographic Research Department from the Geodesy Direction, the Ministry of Infrastructure Buenos Aires Province (AHCR), (2) the Historical Archive of the Museum of La Plata (HAMLP), and (3) the Archive of Notaries of Buenos Aires Province (AN) located in La Plata city, Buenos Aires Province, Argentina.

Materials and methods

Characterization of the archives and environmental parameters

The temperature (*T*) and relative humidity (RH%) were measured in the repositories, at the moment of microbiological sampling. Measurements were performed using a digital thermo hygrometer HOBO H08-004-02. Standard reference points were (Bell & Faye 1980): T maximum and minimum 22 °C and 15 °C, respectively; RH% 65 and 45 respectively.

Environmental microbiological sampling and isolation of microorganisms

Microbiological sampling was carried out by the sedimentation method as described by Omeliansky (Bogomolova & Kirtsideli 2009; Borrego et al. 2010). Hence, Petri dishes containing YGC Agar® (yeast extract, glucose, and chloramphenicol) for the isolation of moulds and yeasts, and nutrient agar for bacteria were placed open at ~ 2 m above the ground and exposed for 30 min at five different points with triplicates at each of the repositories. Afterwards, the plates with YGC were incubated for 7 days at 28 ± 2 °C, and those containing nutrient agar were kept at 28 ± 2 °C for 72 h (Guiamet et al. 2011).

The colony-forming unit (CFU) number per cubic meter of air (CFU m^{-3}) was estimated according to Omeliansky's formula:

$$N = 5a \times 10^4 \ (bt)^{-1}$$

where N = microbial CFU m^{-3} of indoor air, a = number of colonies per Petri dish, b = the dish surface, in cm^2, and t = the exposure time, in minutes.

Relative microbial distribution was carried out according to Smith (1980) where: relative distribution = (number of colonies of the genus or species/total number of colonies of all genera or species) × 100.

Selection of documentary heritage

Several nineteenth-century photographs, books and maps were examined with a stereoscopic microscope (NIKON SWZ-10). The following were selected for further study: three photographs, two photographic papers (P1 and P2), and one glass slide (P3) from HAMLP; one book (B1) and one map (M1) both made of paper from AHCR and two notarial acts (affidavit), both made of paper (NA1a, NA1b and NA2) from AN.

Microbiological sampling of documentary heritage and isolation of microorganisms

Samples of 1 cm^2 from the surface of each document were taken using sterile cotton swabs (Pinzari et al. 2010). The cotton swabs were submerged in 1 ml of sterile distilled water, and decimal dilutions were performed in saline solution. Suitable dilutions of each sample were inoculated onto nutrient agar on Petri dishes to isolate the total bacteria. They were incubated at 28 ± 2 °C for 72 h, and subsequently colonies were counted by spread plate (Madigan et al. 2009). Amylolytic and proteolytic bacteria were counted in differential culture media (starch agar and Frazier gelatin agar, respectively) and their percentages were determined in relation to the total number of aerobic bacteria (Borrego et al. 2012). Acid-producing bacteria were enumerated in broth for total acidifying bacteria (Guiamet et al. 2011). To count fungal colonies, suitable dilutions of each sample from different materials were inoculated onto YGC agar and incubated at 28 ± 2 °C for 7 days prior to counting colonies (Madigan et al. 2009). The CFU number was determined after 7, 14, 21, and 28 days.

Identification of the microorganisms isolated from air and documents

The cultural and morphological characteristics of fungal colonies were observed, and the identification was performed according to different manuals (Barnet & Hunter 1987; Pitt 1988; Klich & Pitt 1994). Bacteria were typified according to Gram staining and biochemical tests

(Sneath et al. 2000). *Bacillus* sp. were identified by molecular techniques (16S rRNA gene), with NCBI accession number EU184084 (Guiamet et al. 2011, 2012).

Laboratory assays: biofilm formation and foxing tests

Biofilm formation and pigment production by fungi and bacteria were demonstrated by laboratory assays. Prior to these assays, isolated fungal strains were seeded on a solid medium slant, the composition of which was sodium nitrate 2 g; dipotassium phosphate 1 g; magnesium sulphate 0.5 g; potassium chloride 0.5 g; yeast extract 0.5 g; ferrous sulphate 0.01 g; agar 20 g per 1 l; pH = 5.5. A strip of aged sterilized filter paper 4.8 cm long and 1 cm wide (72 h at 105 °C, corresponding to 25 years of aging) (Browning 1969), was used as the sole carbon source. The control was the same medium with the addition of 1% glucose. The inocula were 10^7 conidia ml^{-1} in saline solution counted with a Petroff-Hausser camera. Cultures were incubated at 28 ± 2 °C for 21 days (Rautela & Cowling 1966; Borrego et al. 2010). The long-term viability of fungi using paper as sole carbon source was tested by subcultures in the solid mineral medium described above.

Cultures of *Bacillus* sp. were incubated at 28 ± 2 °C over 7 days in the same solid mineral medium as specified above. Strains that showed better growth and bioadhesion using paper as the sole carbon source were selected for studies on foxing simulation. Laboratory tests were performed in triplicate (Figure 1) with aged 0.5 cm diameter discs, sterilized by dry heat (72 °C). The formation of foxing spots on the paper discs was evaluated visually and by a stereoscopic microscope.

Monitoring bioadhesion and biofim formation by microscopic techniques

Samples from original documents were observed by scanning electron microscopy (SEM).

Paper discs of laboratory bioadhesion assays with different selected strains from documents were monitored. Bioadhesion to paper and biodeterioration were observed by SEM (Jeol 6,360 LV). Samples were kept in a closed chamber with pure ethanol for 24 h and metalized with Au/Pd prior to observation.

Results and discussion

Environmental parameters and indoor air quality

According to the Omeliansky scale for evaluating the degree of air contamination (Bogomolova & Kirtsideli 2009), AHCR is considered to be highly contaminated. The fungal and bacterial concentrations in this archive ranged between 640 and 2,720 CFU m^{-3} (Figure 2).

Figure 1. *Scopulariopsis* sp. growing on filter paper as sole carbon source (P) and control (C) with 1% glucose.

In the HAMLP, both fungal and bacterial concentrations were low and varied between 60 and 200 CFU m^{-3} (Figure 2); according to the Omeliansky scale, it is considered a non-contaminated environment. The AN showed the greatest total microbial concentration (14,400 CFU m^{-3}), and the greatest fungal concentration was detected here (7,667 CFU m^{-3}). This environment is considered highly contaminated (Figure 2).

When analyzing fungal and bacterial concentrations in the air of archives, in spite of having similar values of temperature and relative humidity, a wide variation was observed (Figures 2, 3 and Table 1).

The prevailing fungal genera isolated from indoor air were *Aspergillus* and *Penicillium*, although *Cladosporium, Curvularia, Alternaria, Fusarium,* and *Scopulariopsis* were also observed. Similar results were obtained by other authors from archive environments (Borrego et al. 2010; Gutarowska 2010; Valentín 2010; Guiamet et al. 2011; Borrego & Perdomo 2012). Some of these genera are able to cause respiratory disorders, primarily for personnel handling such materials daily (Gost et al. 2003; Cavallera & Asbasti 2006).

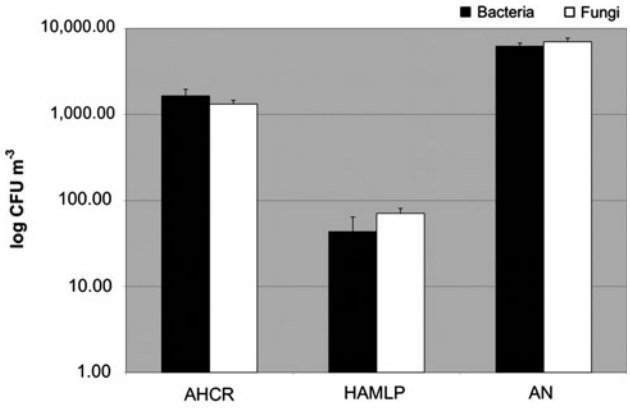

Figure 2. Microbial contamination in the air of the studied archives.

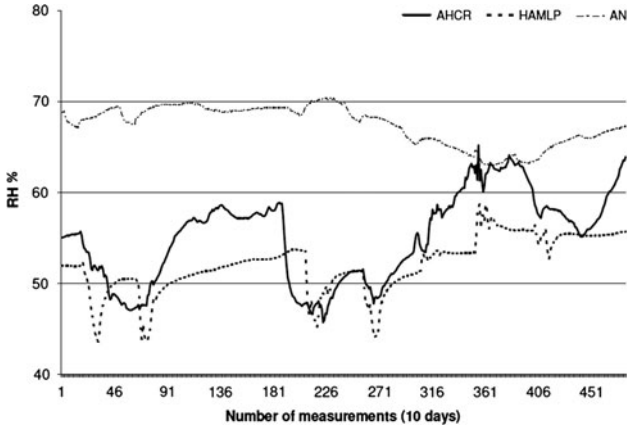

Figure 3. RH measures in repositories (June 2011) of AHCR, HAMLP and AN; $n = 481$ (measurement over 10 days).

Table 1. Measurements of environmental parameters.

Archive	Temperature	RH %
AHCR	18.7 ± 1.6	50.1 ± 5.2
HAMLP	19.7 ± 1.4	50.6 ± 5.1
AN	18.5 ± 1.9	68.6 ± 1.7

At the AHCR, *Aspergillus* spp. prevailed (60%), at the AN, *Aspergillus* spp., and *Penicillium* spp. were isolated with the same relative distribution (30%), whereas at the HAMLP, there were only colonies of *Penicillium* spp. (100%). *Scopulariopsis* spp. were only found at the archives of AHCR and AN (8 and 9%, respectively).

In relation to environmental bacteria (cocci and rod shaped), the predominance of Gram-positive bacteria was observed (HAMLP: 100%; AHCR: 88%; AN: 84%). In all the archives, *Bacillus* spp. were predominant, according to similar results obtained previously (Borrego et al. 2010; Guiamet et al. 2011). Other Gram-positive genera such as *Staphylococcus* and *Streptococcus* could be found, and within Gram-negative bacteria, strains of *Serratia* spp., *Pseudomonas* spp., *Serratia marcescens*, and *Enterobacter agglomerans* were identified. Genera such as *Bacillus*, *Serratia*, *Staphylococcus*, *Streptococcus*, and *Streptomyces* have been isolated by other authors in archive and museum indoor environments (Pangallo et al. 2007; Valentín, 2010; Borrego & Perdomo 2012). The genera *Staphylococcus* and *Streptococcus* isolated in these archives can be a significant health risk (Valentín, 2010).

When comparing the environments of the archives, it can be observed that HAMLP is the only uncontaminated archive (150 CFU m^{-3}), while the others showed higher contamination values. This could be due to the fact that these archives do not have a ventilation system that facilitates water absorption by conidia and their further sedimentation, previously explained by Reponen et al. (2001). The HAMLP is located in a wooded area, far from the center and with low pollution. In turn, occupational activity is lower in the HAMLP than in the other two archives, in which there are more personnel and, therefore, greater air convection. However, the AHCR and AN archives are located in the town center of La Plata, characterized by high traffic and dust pollution; the ventilation systems do not have suitable or sanitary conditions. Fungal concentrations were lower than 300 CFU m^{-3} only at the HAMLP, and at the AHCR and the AN they were significantly higher, reaching a value of 7,000 CFU m^{-3}.

Regarding hygrometric measurements, the average temperatures were similar in the three archives, while the RH was similar for HAMLP and AHCR, and markedly superior to that at AN (Table 1). RH is one of the most important factors for microbial growth because it determines the water available for spore germination and growth (Valentín 2003). When the RH increases in indoor environments, and there is no ventilation for long periods of time, the conidia could be deposited on documents more quickly, grow to form hyphae, and deteriorate the documentary heritage.

In AN, the greatest total microbial load of air was found, relative to the average RH of that archive, which is located on the edge of the paper conservation area and outside it, reaching mouldiness areas (RH above 65%). While HAMLP and AHCR have similar RH, in the AHCR archive higher peaks of RH were measured, showing a greater microbial load present in the air (Figures 2 and 3). The detection of airborne bacteria and fungi, which can be considered merely as transient microorganisms and not those deteriorating the paper, highlights the necessity of clarifying their role in the biodeterioration process (Schabereiter-Gurtner et al. 2001; Lopez Mirás et al. 2013).

Table 2. Type of microorganism isolated from the different documents.

Type of microorganism and/or morphological characteristics	B1	M1	P1	P2	P3	NA1a	NA1b	NA2
Aspergillus spp.	+	+	+	−	−	+	+	+
Penicillium spp.	−	−	+	−	+	+	+	−
Cladosporium spp.	−	−	−	−	−	−	−	+
Alternaria spp.	−	+	−	−	−	−	−	−
Scopulariopsis spp.	−	+	−	−	−	+	−	−
Non-sporing isolated	−	−	−	−	+	−	−	−
Bacillus spp.	−	+	+	+	+	+	+	+
Clostridium spp.	−	−	+	−	−	−	−	−
Gram-positive cocci	−	−	+	+	+	−	−	+
Gram-negative cocci	−	−	−	−	−	−	−	−
Non sporulated Gram-positive bacilli	−	+	−	−	+	−	−	−
Gram-negative bacilli	−	−	+	−	−	−	−	−

Table 3. Microbial counts from documents.

Document	Material	Location	Bacteria (CFU cm^{-2})	Fungi (CFU cm^{-2})
Book 1 (B1)	Paper	AHCR	2	1
Map 1 (M1)	Paper	AHCR	20	3
Photograph 1 (P1)	Photographic paper	HAMLP	2.2×10^3	1.0×10^2
Photograph 2 (P2)	Photographic paper	HAMLP	3.7×10^4	−
Photograph 3 (P3)	Glass slide	HAMLP	3.0×10^4	1.0×10^3
Notarial act 1a (NA1a)	Paper	AN	1.3×10^3	1.4×10^3
Notarial act 1b (NA1b)	Paper	AN	4×10^2	5×10^2
Notarial act 2 (NA2)	Paper	AN	1.14×10^6	2×10^4

Bioadhesion in documentary heritage

Microbial adhesion to documentary substrata showed viable bacteria and fungi were isolated from the documents (Table 2). Microbial counts obtained in different documents (Table 3) showed a bacterial predominance regardless of the type of documentary support (paper, photographic paper, and glass slides). This trend was similar to that obtained in the air from the repositories.

SEM micrographs (Figure 4) showed bioadhesion, biofilm formation, and EPS on book paper (B1). It is worth mentioning that bacterial and fungal counts on documents preserved at the AHCR were significantly lower than on the rest of the documents analyzed, regardless of the support material (Table 3).

Proteolytic and amylolytic activities, and production of acids were detected in all bacterial strains isolated from most of the documents (Table 4). The greatest concentrations of proteolytic bacteria were isolated from photographic supports (P1, P2, and P3). Also, acidifying bacteria were isolated from the map (M1), P1, and notarial act (NA1a, NA1b, and NA2). *Clostridium* spp. were detected in P1 and in NA2. As regards bacteria isolated from documents, it was observed that 80% were Gram-positive, such as *Bacillus* spp., and only 20% Gram-negative bacteria were found.

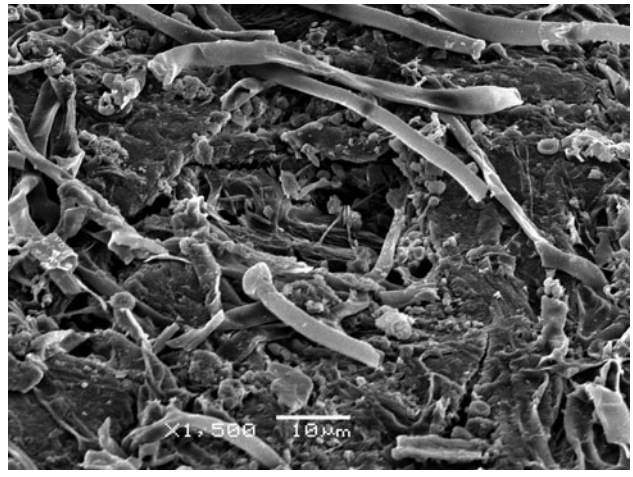

Figure 4. SEM image of book paper (B1) from an AHCR sample showing fungal structures (hyphae and conidia) (1500×). Bar = 10 μm.

Aspergillus niger and *A. flavus* were isolated from most of the documents. Colonies of *Penicillium* spp. from P3 and P4, *Mycelia sterilia* (non-sporing) from P3, and *Scopulariopsis* spp. from M1 and NA1a and *Alternaria* spp. from M1 were isolated. *Penicillium* sp.,

Table 4. Prevalence of bacteria isolated from documents exhibiting different physiological characteristics.

Document	TAB (CFU cm^{-2})	AB	PB	Acidifying B	Sulphite RB (*Clostridium* sp.)
B1	2	−	−	−	−
M1	20	−	3	+	−
P1	2.2×10^3	3.4×10^3	2.3×10^3	+	+
P2	3.0×10^4	1.0×10	3.7×10^4	−	−
P3	3.0×10^4	2.5×10^4	3.0×10^4	−	−
NA1a	1.3×10^3	−	20	+	−
NA1b	4.0×10^2	3	3	+	−
NA2	1.1×10^6	3×10^3	1.1×10^3	+	+

TAB: Total aerobic bacteria determined on Nutrient Agar; AB: amylolytic bacteria determined on Starch Agar; PB: proteolytic bacteria determined on Frazier Gelatin Agar; Acidifying B: acidifying bacteria determined on broth for total acidifying bacteria; Sulphite RB: sulphite-reducing bacteria determined on reinforced *Clostridium* medium (DRCM).

Aspergillus sp., *Alternari* sp., and *Scopulariopsis* sp. found in the documents are considered to be strongly cellulolytic and, therefore, capable of colonizing pure cellulose (Krogh et al. 2004; Borrego et al. 2010; Guiamet et al. 2011; Borrego & Perdomo 2012). *Scopulariopsis* sp., isolated from M1, showed bioadhesion and a capacity for biofilm formation. The attack on cellulose fibers was observed (Figure 5). Much of the material stored in three archives had fox-like reddish-brown colour spots, type 'foxing' (Figure 6).

It is known that the majority of the fungal genera isolated from the air and from archive documents, libraries and museums exhibit cellulolytic, proteolytic, and/or amylolytic activities, producing acids, excreting different pigments onto the substrata (paper), and contributing to the formation of biofilms, which accelerate the deterioration of the different substrata (Florian 2004; Abrusci et al. 2007; Borrego et al. 2010; Guiamet et al. 2011; Pinzari et al. 2011). As regards documentary heritage studies, in Figure 4 particulate matter (dirt) and fungal conidia interspersed with the cellulose fibers of the material can be observed. The highest bioadhesion values corresponded to Gram-positive bacteria, with a predominance of the genera *Bacillus* and *Streptomyces* able to excrete hydrolytic enzymes, such as proteases and amylases (Strzelczyk et al. 1990; Aktuganov et al. 2007) that can degrade proteins and carbohydrates, respectively. *Bacillus* spp. can degrade a wide range of substrata owing to their physiological characteristics (Claus & Berkeley 1986), and many species produce endospores that are highly resistant to extreme environmental conditions, antibiotics, disinfectants, and other chemicals. Spores of this genus are also easily spreadable.

It was reported that during paper production, damage is caused by a wide variety of microorganisms (Flemming et al. 2013), and during the manufacturing process of photographic paper, *Bacillus* spp. can pollute the gelatin of the emulsion (Stickley 1986). More recently, it has been demonstrated that many bacteria can colonize the gelatin

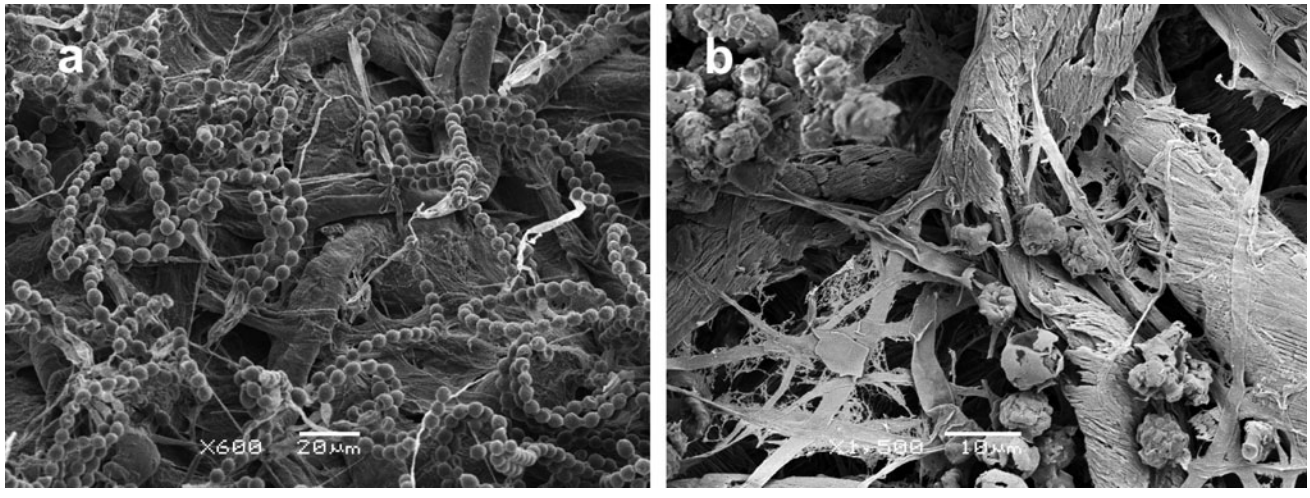

Figure 5. SEM image of: (a) Biofilm formation by *Scopulariopsis* sp. on aged paper (600×). Bar = 20 µm. (b) Detail showing deteriorated cellulose fibers (1500×). Bar = 10 µm.

Figure 6. (a) Foxing spots on a map (M1) from AHCR; (b) notarial act (NA1) from AN showing microbial attack; (c) photograph (P2) from HAMLP with fungal spots on the surface.

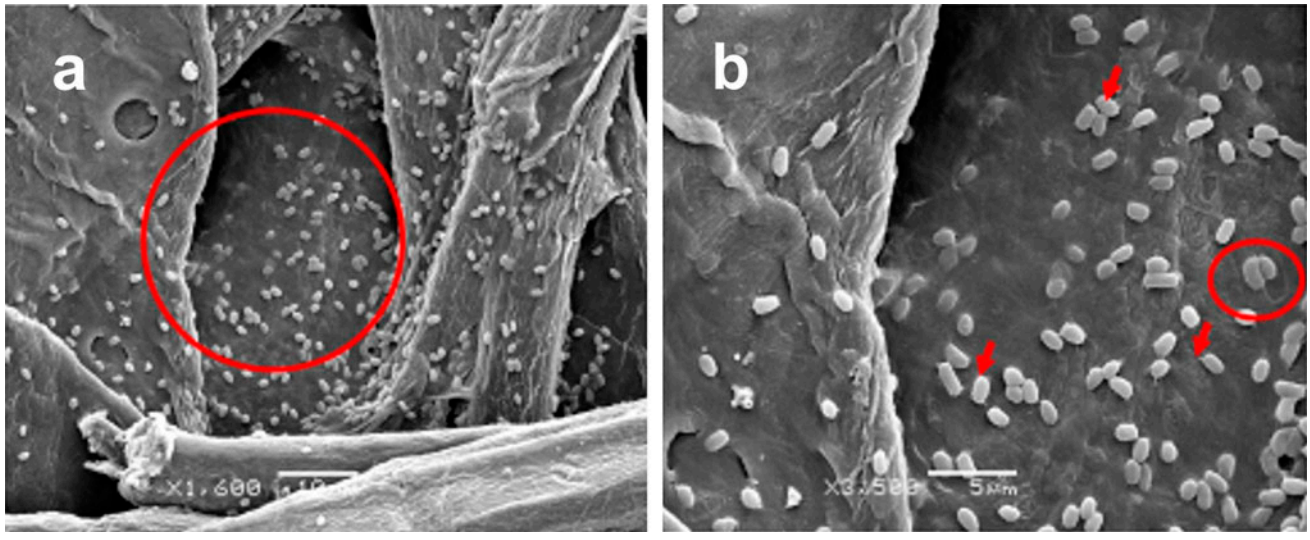

Figure 7. (a) SEM image of *Bacillus* sp. adhered to the cellulose fibers of paper after incubation for 48 h (1600×). Bar = 10 µm. (b) In more detail (3500×). Bar = 5 µm. EPS can be observed (rows) and micropitting (2.5 µm) on the paper due to microbial attack (circle).

during the manufacturing process of photographic paper. Nonsporulated bacteria from different species of the genera *Salmonella*, *Kluyvera*, *Pseudomonas*, *Enterococcus*, *Streptococcus*, and *Staphylococcus* can liquefy the gelatin of photographic paper (De Clerck & De Vos 2002).

Biofilm formation: laboratory assays

Biofilm formation by *Scopulariopsis* sp. on filter paper and microbial activity were corroborated by the appearance of different colors, and rusty red and irregular-shaped areas (Figure 1). Degraded cellulose fibers (general attack) and EPS on the paper were observed in SEM images (Figure 5).

Biofilm formation by *Bacillus* sp. can be observed in Figure 7. EPS and the microbiological attack resulted in the formation of micropits (2–3 µm in diameter) on the cellulose fibers. *Bacillus* sp. and *Scopulariopsis* sp. adhered to paper, produced EPS, and grew using paper as the sole carbon source. The viability of *Scopulariopsis* sp. was verified for more than 18 months without renewal of the culture medium. Production of pigments by this fungal strain, with consequent aesthetic damage, caused by such pigments, was demonstrated.

Conclusion

Bacillus spp. and *Scopulariopsis* sp. isolated from paper showed different attacks on cellulose fibers. Micropits were observed in the case of *Bacillus* sp., whereas *Scopulariopsis* sp. showed a general attack. Data obtained from this research work provide evidence of the prevalence of microorganisms in indoor air environments and on documents preserved in archives. Microorganisms were able to form biofilms and produce pigments and acids, with consequent aesthetic and structural damage, constituting a hazard for the documentary heritage.

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