FUNGAL DEGRADATION OF CELLULOSIC MATERIALS USED AS SUPPORT FOR CULTURAL HERITAGE

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Abstract

A great part of the cultural heritage of humanity available in museums and libraries is stored in paper. However, this main support used from early civilization times is a biomaterial susceptible to deterioration by fungal transformation. Two fungal phenomena, cellulose degradation and synthesis of secondary metabolites, are responsible for paper deterioration. Thus, the understanding of fungal deterioration pathways is key to improve the durability of the cultural heritage in paper and develop new and adequate sustainable strategies of restoration. This review gives an approach about the current knowledge of cellulose transformation by fungi associated with paper and the mechanisms involved. Since several metabolites derived from fungi growing on paper, such as pigments, can deteriorate invaluable cultural heritage, knowledge on these metabolites is also fundamental to improve conservation strategies of historical documents.

Keywords: Biodegradation; Cellulolysis; Fungi; Pigments; Dyestuff, Cellulosic support, Preservation.

Introduction

Since ancient times, paper has been one of the most used materials to record human knowledge. Although the use of electronic copies is currently widely spread [1], most people still use and value printed materials. Therefore, preserving and maintaining printed materials is crucial for librarians and specialists in libraries and information centers [2]. Biodegradation and biodeterioration of paper-made materials is a worldwide problem that causes great damage to unique manuscripts and books stored in libraries (Fig. 1).

The basic component of paper is cellulose; however, other constituents like starch, sugar, other carbohydrates and lignin are present [3]. Thus, paper is susceptible to a wide range of biological agents, including fungi, which have a remarkable capacity of dissolving cellulose as

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it provides a satisfactory medium for mold growth [4]. On the other hand, library environments, particularly old ones, provide microorganisms with nutritional requirements in the form of old papers and paper glues. In addition, the geographical situation and weather condition influence the diversity and content of fungi in confined environments [5]. Another important aspect of the microbiological contamination by fungi in libraries and archives is related to the health of workers since some species are also potentially pathogenic, causing allergy and toxic effects [2].

Fig. 1. Biodeterioration and biodegradation effects on library material stored in a building affected by flood: different materials affected by the colonization of fungi.

Fungi deteriorate paper used as support for cultural heritage as a result of their metabolic activity upon both cellulose and non-cellulosic additives such as binders, filling, adhesives and sizing agents [3]. Indeed, they are the most commonly found agents of degradation and they can be distinguish according to their capacity to degrade the various constituents of paper into fungi with cellulolytic activity and those able to degrade adhesives and/or additive compounds [6].

The biodegradation of paper by cellulolytic fungi involves two main processes: cellulose decomposition or production of fungal colored metabolites. The aim of this review is to give an approach about the current knowledge of cellulose transformation by fungi associated with paper and their role in the esthetic degradation.

**Fungal features and their effect on biodegradation**

Fungi are a distinctive group of chemoorganotrophic eukaryotes that have a characteristic chitin cell wall and are phylogenetically circumscribed [7]. This group, with a total number of species estimated at around 1.5 million, can be classified into different groups based on phylogenetic, morphological, ecological and/or reproductive features [8, 9]. They are ubiquitous and able to colonize several substrata in terrestrial and freshwater habitats (less so in marine ones) since they present a wide range of adaptations to different environments. They play important ecological roles as saprotrophs, mutualistic symbionts, or parasites. They feed by absorption rather than by ingestion. Several fungal groups have an array of hydrolytic and oxidative exoenzymes that depolymerize the complex organic substrates on which they grow, followed by the absorption of the solubilized breakdown products; however, some of them can only use simple soluble substances such as sugars. Therefore, the ability to decompose carbon sources of increasing complexity has been associated with the taxonomic disposition of fungi [10]. While basal fungi according to the classification system from Hibbett et al. [7] which are widely considered to have a ruderal behaviour, are restricted to sugars and the simpler carbon compounds (thus called sugar fungi), the phyla Ascomycota and Basidiomycota include many representatives with the outstanding capacity of cellulose and lignin decomposition. Although most fungi are obligate aerobes, there are some facultative and obligate anaerobes such as different yeasts and those that live in the rumen of herbivores (e.g. rumen fungi), respectively.

Other parameters, such as air movement (since it can act as an additional source of spores), temperature, moisture, pH, and light, also govern fungal colonization, growth and
degradation of a substrate. In this sense, fungi are active on a substrate (e.g. in their saprotrophic role) when the environmental conditions are appropriate, i.e. when temperatures are around 25°C to 30°C, when water availability is high (relative humidity around 70-75% or higher), and when the bioreceptivity of the substrate and its physico-chemical properties are compatible with the demands of the fungi associated. Relative humidity and temperature have been reported to have a strong influence on the colonization and activity of a broad spectrum of fungi. When these factors are not appropriate, fungi decrease their degradative ability and can initiate reproductive processes, differentiate other somatic structures such as resting structures, or eventually die. If the conditions become favorable again, fungal growth will begin from the existing spores or other structures available in the substrate. However, some fungi can live under stressful conditions by using several mechanisms that allow functionality under such conditions (e.g. xerotolerant fungi).

During their life cycle, fungi can differentiate non-vegetative structures, which have distinctive features according to the taxonomic group to which they belong and are of systematic diagnosis. These structures may be involved in either sexual (i.e. nuclear fusion and meiosis) or asexual (i.e. purely mitotic nuclear division) reproduction. The sexual processes take place by the fusion of gametes (which characterize aquatic fungal groups and are associated with the presence of flagella) or gametic nuclei. They can also be associated with the differentiation of either macro - or microscopic sporophores (or fruiting bodies), which bear spores and show characteristic shapes, or with the direct generation of spores on undifferentiated structures as hyphae. Additionally, other propagules, which are typically microscopic, such as small spores produced in high numbers, can be asexually produced by fungi. These facilitate the conquest of new habitats and their substrates, and can be originated within a structure called sporangium (endogenous spores or sporangiospores) or not (exogenous spores called conidia). Also, several types of exogenous spores can be identified according to the type of ontogeny involved as well as to their features in the spore wall such as thickening and/or pigmentation. The morphology and structure of fungal spores show great variability, from unicellular to multicellular, branched or unbranched or sometimes spirally coiled, thin- or thick-walled with hyaline or pigmented walls, dry or sticky, smooth or ornamented by mucilaginous extensions, spines, folds or reticulations (Fig. 2).

Fungi are critical in the recycling of organic matter through the decomposition and the transformation of litter, wood and other residues available from plant productivity in several ecosystems as well as those from the domestic organic waste [11]. Fungi also have other beneficial effects because, for example, many species of mushrooms, morels and truffles are edible and provide a food source for humans and animals. In addition, many of them are a source for the industrial production of enzymes (including cellulases, amylases, proteases, lipases, and pectinases) and metabolites such as antibiotics, alkaloids and organic acids. Also, selected yeasts (Saccharomyces sp.) are the starting material for baking and brewing, and representatives of the genus Penicillium are used for the preparation of blue-type cheese. Besides, some fungal inoculants are used to promote plant growth. However, fungi can also have detrimental effects, causing diseases in plants of economic importance, and inducing animal and human diseases. They also attack and spoil all kinds of foodstuff, various objects like lumber and cloth, and other materials associated with buildings.

A critical aspect of fungal infection is related to the conservation of documents of heritage patrimony, which can be used by fungi as substrate and/or as a dispersion vehicle. Fungi play a considerable role in the damage of cultural heritage of paper-based and artworks of organic nature stored in museums and libraries. They can colonize these objects and deteriorate them by mechanical and chemical processes.
Fungi associated with biodegradation in several paper documents

In collections of cultural heritage as well as in libraries, paper and its derivatives are the dominant organic materials. In this environment, fungi play the most important role in biodegradation [25, 26]. Although bacteria can also degraded paper, fungi require less moisture to develop in the dry conditions that normally exist in libraries, archives or museums, being this kind of environment more favorable for the growth of fungi than bacteria [25].

Fungi causing biodegradation in paper come mainly from the air, accumulated dust and supports, being species of slow growth as well as xerophilic ones of the genera Aspergillus, Paecilomyces, Chrysosporium, Penicillium and Cladosporium belonging to the phylum Ascomycota [27]. Fungi colonize paper either by penetrating the microfibril matrix or growing superficially [13] to sustain their growth and reproduction as well as to be a vector/support for the dispersal of fungal propagules [28]. In addition, the hyphae of these cellulolytic fungi growing in the support exert a mechanical effect and generate a larger accessible microenvironment for the colonization and biofouling of non-cellulolytic microorganisms such as mucoraceous fungi [29] and several bacteria that form a biofilm [30]. This sequential process is promoted by the extracellular availability of a set of soluble organic products generated by cellulolytic fungi and by a higher retention of humidity in the attacked support.

Also, handling mold-contaminated paper objects can constitute a serious health risk, because many of these fungi can be pathogenic/toxicogenic, even when they are already dead [31, 32]. Besides, these microorganisms are widely recognized as allergenic triggers implicated in serious respiratory diseases [29]. Many fungi available in spore state in the air at high concentrations, such as those of the genus Cladosporium, can cause allergic respiratory diseases when inhaled [34, 12].

Table 1 summarizes the current knowledge of cellulose transformation by fungi associated with paper-made material of cultural heritage.
Table 1. Some fungi reported as biodeteriorating agents associated to several paper documents.

<table>
<thead>
<tr>
<th>Substrate/source of isola</th>
<th>Taxa*</th>
<th>Observations</th>
<th>Reference</th>
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<tr>
<td></td>
<td>Cladosporium*, and Penicillium†</td>
<td>The most frequent genera.</td>
<td>Mesquita et al. 2009 [12]</td>
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<tr>
<td>Wood-pulp paper</td>
<td>Chaetomium globosum*</td>
<td>The less common</td>
<td>Mesquita et al. 2009 [12]</td>
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<tr>
<td>Wood-pulp paper</td>
<td>Phlebiopsis gigantea†</td>
<td>The data available are confusing</td>
<td>Mesquita et al. 2009 [12]</td>
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<td>1920 etching</td>
<td>Taeniola (Torula) sp.*</td>
<td>Degradation associated to the presence of adhesives</td>
<td>Szczepanowska and Cavaliere, 2012 [13]</td>
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<tr>
<td>17th century paper</td>
<td>Aspergillus sclerotiorum, Cladosporium sp.* and Torula sp.*</td>
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<tr>
<td>Indoor air in an archive and one wood-pulp sample</td>
<td>Aspergillus fumigatus*, Epicoccum nigrum*</td>
<td>This fungus was the most frequent in indoor air.</td>
<td>Mesquita et al. 2009 [12]</td>
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<td>Registry book of councils from the Santa Cruz Monastery (Coimbra)</td>
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<td>In foxing spots in Leonardo da Vinci’s self-portrait</td>
<td>Eurotium halophilicum†</td>
<td>By SEM analyses</td>
<td>Piñar et al. 2015 [15]</td>
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<tr>
<td>Leonardo da Vinci’s self-portrait</td>
<td>Lichenized Ascomycota or Acremonium sp.* as the most dominant taxa</td>
<td>This is variable depending on the sampling techniques used (Swabs samples vs membrane filters, respectively).</td>
<td>Piñar et al. 2015 [16]</td>
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<td>Deteriorated 19th-century photographs, books and maps</td>
<td>Alternaria sp., Aspergillus niger, Aspergillus flavus, Cladosporium sp., Fusarium sp., Penicillium sp., Scopulariopsis sp.</td>
<td>Scopulariopsis sp. and Fusarium sp. were able to adhere to paper, using it as the sole carbon source. Also were able to attach to aged paper after incubation, develop a biofilm and cause a generalized attack on the paper</td>
<td>Lavin et al., 2016 [18]</td>
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<tr>
<td>Location</td>
<td>Microorganisms</td>
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<td>Paper arts, paper paintings, manuscripts</td>
<td>Acremonium, Alternaria, Aspergillius niger, Aspergillus sp., Aureobasidium, Botrytis, Chaetomium, Cladosporium, Curvularia lunata, Chaetomium, Mycelia sterilia, Mucor, Penicillium, Phoma, Rhizopus, Rhodotorula, Scopulariopsis, Syncephalastrum, Trichoderma, Trichosporon</td>
<td>Dominating in all sites. Many of the taxa produce various coloured superficial stains causing chromatic and aesthetic of paintings. Cellulolitic activity of Alternaria alternata, Aspergillus niger, Fusarium oxysporum, Phoma and Trichoderma were studied at different pH and temperature. Shrivastava, 2015 [4]</td>
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<td>Textile heritage objects</td>
<td>Aspergillus penicillioides</td>
<td>The biodiversity analysis with the PCR-DGGE technique revealed differences in both the number and biodiversity of fungal genotypes. Lech et al., 2015 [20]</td>
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<td>Environmental air and chemically treated books from the Brazilian Public Library Biblioteca de Maglinhos, FIOCRUZ Rio de Janeiro</td>
<td>Aspergillus versicolor, Eurotium chevalieri (teleomorph of A. chevalieri)</td>
<td>Radiosensitivity tests where applied in fungi isolated in paper and wood. A. versicolor was inactivated with doses 20 kGy. E. chevalieri was inactivated with doses 14,5 kGy. Da Silva et al, 2006 [21]</td>
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<td>Gilded wood of two altars present in Espirito Santo church – Santa Ursula Altar and Nosa Senhora da Boa Morte Altar (Évora, Portugal)</td>
<td>Cladosporium (4 strains), Mucor (1 strain), Penicillium (2 strains).</td>
<td>The predominant microbial agents were filamentous fungi as the main agents for alteration of wood. Cellulolytic and xylanolitic enzymes were detected. Rosado et al 2015 [22]</td>
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<td>Maps on textile and paper conserved in the Archivo Nacional de Cuba</td>
<td>Aspergillus flavus, A. niger, A. oryzae, A. caespitosus, A. ornatus, Penicillium citrinum, P. fellutanum, P. waksmanii</td>
<td>A. caespitosus and A. ornatus were potent degradative agents of cellulose, starch and gelatine. The authors of this paper studied the enzymatic activity and biodeteriorative capacity of the isolated strains. Molina Veloso and Borrego Alonso, 2014 [15]</td>
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<td>Dust samples from archival bindings and shelves of a repository of the Sala Alessandrina in the Archive of the State in Rome</td>
<td>Cladosporium, Penicillium, Aspergillus, Chaetomium, Alternaria</td>
<td>Cladosporium predominated in winter samples. Penicillium predominated in summer samples. The authors stand up that dust creates a microenvironment and trophic resource for the microorganisms. Maggi et al, 2000 [23]</td>
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*a, phylum Ascomycota; b, phylum Basidiomycota; c, Subphylum Mucoromycotina.
**Fungi and their ability to cause structural damage in paper**

The saprotrophic abilities of fungi cause the disintegration of the organic matter since they use cellulose as a source of energy when they colonize paper. Because the structure of cellulose is organized in both amorphous and crystalline regions, most of the fungi that grow on it are cellulolytic. The process of biodegradation by fungi involves both enzymatic and non-enzymatic mechanisms (Fig. 3). It is believed that cellulose is depolymerized through three main types of extracellular hydrolases: 1) endoglucanases, 2) exoglucanases, including cellodextrinases and cellbiohydrolases and 3) β-glucosidases [35]. Among these enzymes, endoglucanases are key in the process because they increase notoriously the number of (new) reducing and non-reducing extremes (including chain ends and oligosaccharides). Although endoglucanases are active on amorphous regions of cellulose, other proteins, such as swollenin, whose primary action is to enhance access to the hemicelluloses fraction, play a role in amorphogenesis (see below) [35, 36]. This increases the cellulose surface area by the slippage of cellulose microfibrils and thereby opens up the fibrillar matrix (creating new surfaces within the cellulose matrix) and provides the catalytic enzymes with enhanced access to the glycosidic linkages within the sugar polymers as well as reactivity of cellulose.

**Fig. 3.** Fungal degradation of cellulose. A. Schematic overview (not to scale) of non-enzymatic mechanisms involving the production of hydroxyl radicals, which disrupt cellulose, through oxygen activation in the presence of a fungal metabolite (M) and Fe^{3+} ions. Based on Gómez Toribio et al. [55], and Baldrian and Valásková [56]. B. Simplified scheme of the current view (not to scale) on the degradation of cellulose through extracellular hydrolases, which are active on amorphous parts of cellulose, and extracellular oxidoreductases, which play a role in amorphogenesis of the highly crystalline regions of cellulose and are implicated in the oxidative cleavage of the polymer. Based on Dimarogona et al. [38] and Cragg et al. [39].

In 1985, *M.P. Coughlan* [37] coined the term ‘amorphogenesis’ to suggest a possible mechanism by which the dispersion, swelling or delamination of cellulosic substrate occurred, resulting in a reduction in the degree of fibrillar aggregation and/or crystallinity, and the creation of a larger accessible surface by increasing the reactive internal surface. Consequently, amorphogenesis enhances the reactivity of the fibrous cellulosic substrates by increasing the amount of cellulose directly accessible to the enzymes [38]. Different amorphogenesis-inducing agents, including non-hydrolytic proteins such as fungal expansion-like proteins, loosenin and swollenin, have been proposed to participate in the disruption and weakening of the plant cell wall or cellulosic substrates [36]. Although considerable progress has been made to elucidate the way of action of these proteins in amorphogenesis through the disruption of hydrogen bonding between adjacent sugar chains, weakening paper and reducing its crystallinity index, the mechanism is still not well understood [35, 36]. Additionally, the knowledge on the enzyme systems used by fungi to degrade cellulose has changed dramatically in the last years [39]. There is recent evidence that different fungi degrade cellulose by means of oxidative enzymes that act complementarily to the long-known hydrolytic cellulase system [39].
In addition, non-enzymatic mechanisms can act in conjunction with processes via enzymes. Such non-enzymatic processes are associated with the activity of some Basidiomycota fungi such as the brown-rot ones. These fungi generate small molecular mass oxidants that randomly attack the substrate through quinone redox cycling and glycopeptide-based Fenton reaction [40].

Fungi that colonize damaged paper are mostly cellulolytic organisms, bearers of an array of depolymerizing enzymes; however, the microbiota associated with this substrate can also include fungi with different saprotrophic behaviours such as non-cellulolytic ones. For example, representatives of Mucorales that belong to sugar fungi are unable to use complex carbohydrates because they lack the battery of extracellular enzymes. However, they can play a role as opportunists on deteriorated paper. If spores are available and conditions to germinate are appropriate, they develop typical colonies using soluble C compounds, which are easily assimilable and derived from the cellulose depolymerization caused by the activity of extracellular enzyme systems that have been secreted by cellulolytic fungi.

In addition to cellulose and its monomers, paper can harbor other important sources of carbon like fillers, sizings or dust, which can be rich in proteins and sugars or by scavenging CO₂, combined nitrogen, and carbon-rich gases from the atmosphere. Then, under stressful conditions, fungi can develop feeding from these alternative substrata [41]. In addition, the survival strategy of the fungi that deteriorate paper under these conditions might also include the use of alternative endogenous C sources like lipids available in spores. All these limitations can lead to inconspicuous mycelial development on paper, which is associated with the production of fungal secondary metabolites rather than biomass accumulation [42].

**Fungal ability to deteriorate paper esthetically**

**Foxing**

Some natural changes in the coloration of paper can result from the physico-chemical transformations of their components (such as type-lignin aromatic compounds) due to time. Another cause of the colorimetric alterations in paper is due to fungal growth. The staining activity by fungi is considered derived from the synthesis of pigments and/or from the Maillard reaction of by-products of the fungal metabolism such as organic acids, oligosaccharides and proteic compounds that react together chemically with the material under specific conditions (at a low water activity and high temperature). This latter process forms brown products and oxidative reactions, probably resulting in melanoidin production and the consequent formation of foxing-type spots [16, 43].

Foxing spots generally appear on the paper as stains of reddish-brown, brown or yellowish color, small dimensions, and sharp or irregular edges, frequently showing fluorescence if excited with UV light [44, 45]. Foxing spots have been observed in different kinds of paper since the 16th Century. Although the mechanisms involved in the formation of these spots have been studied since 1930-1935 [45], the results are controversial and still not conclusive. A theory about the origin of foxing spots postulates the action of abiotic processes, but the nature of foxing is still under discussion. Recently, *E. Ardelean and N. Melniciuc-Pitică* [44] reported the following summary of the possible causes that generate the appearance of foxing spots: 1. Direct metal oxidation produces a distinct dark center and the migration of the soluble degradation products from the metallic center causes the surrounding discoloration. 2. Microorganism contamination is mostly airborne in origin and produces irregular yellow-brown patches. 3. Metals and fungi produce acid in the cellulose, which causes deleterious effects on papers (the foxing phenomenon has been related to a strong oxidation of the cellulose chain [46]). 4. Relative humidity increases the chemical reactivity of auto-oxidation, causing cellulotic discoloration in the wet/dry interface on the paper’s surface.

Weather foxing can appear due either to iron oxidation or to the influence of microorganisms; future studies are necessary to research if the induction of fungal siderophores (molecules secreted by the fungi under iron limitation to chelate this metal) and their interaction with iron within the paper matrix are involved in the developing of the foxing phenomenon. On the other hand, the presence of foxing spots has been also related to condensation reactions between products of cellulose oxidation with nitrogen-containing compounds, according to the
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formation of humic-type aromatic polymers (browning theory [47]). Anyway, this hypothetical mechanism can not be ruled out because, among other things, these processes can vary depending on the external conditions and the original composition of the paper. As can be seen, additional studies are required to identify the processes and conditions that contribute to the development of foxing in paper, which is probably a plethora of abiotic and biotic processes.

**Fungal pigments/dyestuff**

Many fungi that paper degradation synthesize different pigments/dyestuff, such as lipophilic ones (e.g. carotenoids), hydrophilic ones (e.g. anthraquinones) and those which are only soluble in alkaline solutions like melamins, which are an assemblage of a dark polymers highly recalcitrant [48]. These secondary metabolites, which may cause extensive color changes in the substrate, can play an array of functional roles in the fungal biology. This mechanism can work as a protector agent against environmental stress (including photo-oxidation) or against other organisms (antimicrobial action) [49, 50], or as an intermediate related to enzyme cofactors [51]. In this sense, there are fungi growing on paper that increase their pigmentation by the establishment of stressful conditions such as those generated by the irradiation with gamma radiation as control treatment on paper, as reported by S.C. Pavon Flores [52]. Despite this negative effect, it is necessary to advance in the assessment of the performance of this type of treatment for cleaning heritage documents. C. Llorente et al. [53] reported data about the nature of a dark pigment (1,8-dihydroxynaphthalene (DHN)-melanin) in agar-cultures of Cladosporium cladosporioides and mentioned that the pigmentation of its colonies was increased under the chemical stress imposed by certain fungicides. Therefore, the excretion of pigmented polymeric substances by this type of dematiaceous fungus and other relatives growing on cellulosic supports is a serious problem in the management of the biodeterioration of material of cultural heritage. Since this fungus frequently deteriorates paper and is an inhabitant in the indoor air of libraries and museums, it is a priority to study the process of melanization in this fungus and its relationship with environmental conditions.

The process of production and accumulation of pigments in deteriorated paper takes place in structures on which fungi are able to develop depending on the taxonomic group to which they belong and to their ecophysiology. Pigments can occur in spores, fruiting structures and mycelium as well as in resistance somatic structures such as the sclerotia. This can be variable depending on the physiological state of the fungus, which produce differential effects on the substrate [54].

Figure 4 (A–D) shows the colonization of abaca paper by Chaetomium globosum LPSC 259, the organization of fibrillar matrix of the attacked paper and the pigmented reproductive structures differentiated by the fungus (perithecium and spores). Although H. Szczepanowska and A.R. Cavaliere [13] suggested that the staining of several papers deteriorated by Cladosporium sp. and Chaetomium sp. was confined to the fungal elements, several studies have shown that the fungal pigments can also be secreted into the substrate and so spread far away from the origin source. H.J. Choi et al. [55] reported the production of 2-methylresorcinol, a diffusible pigment which appears to be an active antimicrobial principle, by the Helicosporeium sp. isolate (Ascomycota). Besides, pyomelanin (alkapto melanin), a watersoluble brown pigment, has been reported to be synthesized by different fungi under certain culture conditions (e.g. in the presence of aromatic amino acids as phenylalanine and tyrosine that are likely necessary for its synthesis). This type of pigment exhibits protecting properties against different kinds of stress such as those generated by reactive oxygen intermediates [56, 57].

There are species of the genus Aspergillus, including A. fumigatus, a typical fungal representative that deteriorates paper, that are able to produce pyomelanin. A. fumigatus is able to produce several melamins, including a DHN-type melanin insoluble in water, which is predominantly present in conidia, and an alternative melanin synthesized extracellularly, accumulated in the hyphae and excreted into the medium, generating darkened mycelia by the deposition of the pigment on the surface of hyphae [58-60]. On the other hand, A. Michaelsen et al. [17] postulated that the pinkish to purple-colored spots frequently found in sheets of 16th-century books are caused by migration of purple exudates of the cellulolytic fungi Aspergillus...
versicolor and A. nidulans through the pages with a felted consistency. Therefore, fungal staining can be a relevant factor that deteriorates paper and affects the choice of adequate strategies of restoration. This is compatible with results reported in experiments with bleaching agents to reduce discoloration and stains [12].

![Image](image_url)

**Fig. 4.** A-D. In-vitro degradation of abaca paper by Chaetomium globosum LPSC 259 after 1 month of incubation. A. General view of a sample of paper probe incubated axenically in the presence of sexual spores from the fungus under a 95 % moisture environment, showing several dark spots (arrow) that correspond to pigmented perithecia differentiated by the fungus. B. Fibrillar matrix of fungi-deteriorated paper showing cotton blue-stained hyphae. C. Detail of a dark perithecum with unbranched radiating hairs, associated in its basis with a mass of cotton blue-stained hyphae that are bound to the deteriorated paper. D. Mass of free pigmented ascospores generated into asci differentiated in the inside of the perithecum.

**Conclusions**

The cleaning and the control of environmental conditions where cultural heritage is stored as well as the monitoring of the indoor air quality are key procedures in the prevention of biodegradation. However, it is clear that strategies against fungal degradation of paper must still be improved to minimize the attack and staining of paper. As described in this review, the degradation of cellulose by fungi causes both detrimental effects on paper-made documents and generates various intermediate by-products, which affect the physico-chemical and nutritional quality of the deteriorated material. This precondition facilitates the colonization by non-cellulolytic fungi that can secrete other problematic metabolites. The knowledge of the biology of these fungi belonging to different ecophysiological groups is relevant for practical aspects of preservation and restoration of deteriorated and degraded paper. However, there are no concluding data about the key mechanisms and the environmental factors that control paper deterioration and degradation. In addition, the nature of chromophores synthesized by fungi when they grow on paper is unknown. Therefore, more investigations are still necessary to develop effective and sustainable processes to decrease the impact of fungal degradation on the cultural heritage.
Acknowledgments

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