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REVIEW ARTICLE



Fungi in archives, libraries, and museums: a review on paper conservation and human health

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ABSTRACT

The action of fungi on books, documents, maps, and works of art on paper can result in inestimable cultural losses. Plus, some of the fungi present in paper documents, surfaces and air from archives, libraries and museums are also a threat to human health. This work aims to review the literature on the most important and frequent microfungal populations found in paper-based collections all over the world, and correlate these data with human health risks. A total of 71 studies, dating between 1997 and 2018 were reviewed and organized. From 27 different countries, 207 fungal genera and 580 species were reported. *Chaetomium* sp. and *Fusarium* sp. were found to be special contaminants in the air of archives and have been associated with paper biodeterioration. The most common fungi reported (e.g. *Penicillium*, *Aspergillus*, and *Alternaria* species) have an impact on paper conservation but can also cause adverse human health effects. The most frequent fungal species retrieved from discoloured paper materials are discussed in greater detail. Considerations on methods of identification and quantification of fungal contamination are also presented. Finally, the authors acknowledge an urgent need for standardizing research in this area and further studies are proposed.

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Fungi; archives; libraries; paper conservation; human health

Introduction

Important artistic works and documents are made of paper and, therefore, susceptible to chemical, physical, and biological damage. Biodeterioration of paper by fungi is one of the most important causes of damage occurring on library and archives collections (Figure 1). Some fungi involved in the biodeterioration of paper may also be dangerous to library/archival professionals and users as fungal metabolites can trigger allergic responses.

The last review on fungi isolated from library materials was performed by Zyska in 1997. In it, data were collected from very diverse origins as it was not just focussed on paper but on panoply of materials one can find in museums, archives, or libraries. Now, 22 years later, numerous researches regarding fungi on paper collections were made, new identification methods were applied and some fungal load limits, for paper conservation and human health, were proposed. Therefore, at this time several questions need to be answered: what is the present scenario in terms of fungal contamination in

archives? Do air contamination studies relate to surface studies? Are the values similar between countries? What are the most common genera/species and can they induce damage to the collections and human health? Can we really talk about fungal load limits? Are the molecular biology protocols now being put to use bringing new light into the subject? And what are the major gaps in this research field?

This review intends to coordinate the data retrieved from isolated studies, dated from 1997 to 2018, and analyse it in order to progress towards answering the above questions.


Methodology of the review process

Literature research was performed to collect studies on the analysis of the mycoflora present in the air and surfaces of archives and libraries or discoloured paper from cultural heritage objects. Papers or book chapters were identified through searches using Science Direct,

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Figure 1. Fungal outbreak as a result of leaking water from the roof of a depository room (AHU-DGLAB, Portugal, 2005).

Scopus, ISI Web of Knowledge, Google Scholar, Google Books, and ResearchGate, with various combinations of the following search terms: fungi, microorganisms, mould, paper, archive, library, air, museum, cultural heritage, books, documents, health, and exposure. For air analysis, both active and passive methods were considered. The contamination of the surfaces considered the analysis of shelves, reading tables, bindings, and floors of the facilities holding paper-based collections.

The analysis of fungi from discoloured paper considered paper objects not only from libraries and archives but also from other cultural institutions holding different types of objects besides paper-based ones. Only samples taken from paper areas showing evidence of fungal colonization were considered, as the objective was to characterize the mycoflora eventually responsible for paper biodeterioration.

Only studies comprised between 1997 (after Zyska 1997) and 2018 were considered for this review, resulting in the compilation and analysis of 71 articles.

Fungal species reported in archives and libraries

Since Zyska (1997), a sharp increase in the number and quality of studies on biodeterioration of paper cultural

heritage has been noted. Gathering nearly all the available data inevitably results in a vast display of information that is placed, with all necessary detail, as [Supplementary Table S1](#). [Table S1](#) presents the identified fungal species collected from air samples and surfaces in facilities holding paper collections, as well as from paper documents/artworks exhibiting fungal biodeterioration evidence, along with the countries where those studies were performed. The adopted nomenclature considered the species' current names according to the Mycobank Database (www.mycobank.org).

From [Figure 2](#) one can spot the countries most committed to the study of fungal contamination in archives and libraries: Italy, Poland, Portugal, and Cuba present the highest number of published studies on the subject and they all have focussed on all the three sample types considered. However, given that the performed evaluations are very diverse regarding methodologies and sample treatment/analysis, it is not possible to infer directly on the intrinsic fungal flora diversity. This problem points to the need to find common ground and to establish experimental guidelines for the study of fungal contamination in archives.

In order to facilitate the analysis of the most commonly identified fungal species/genera in [Table S1](#),

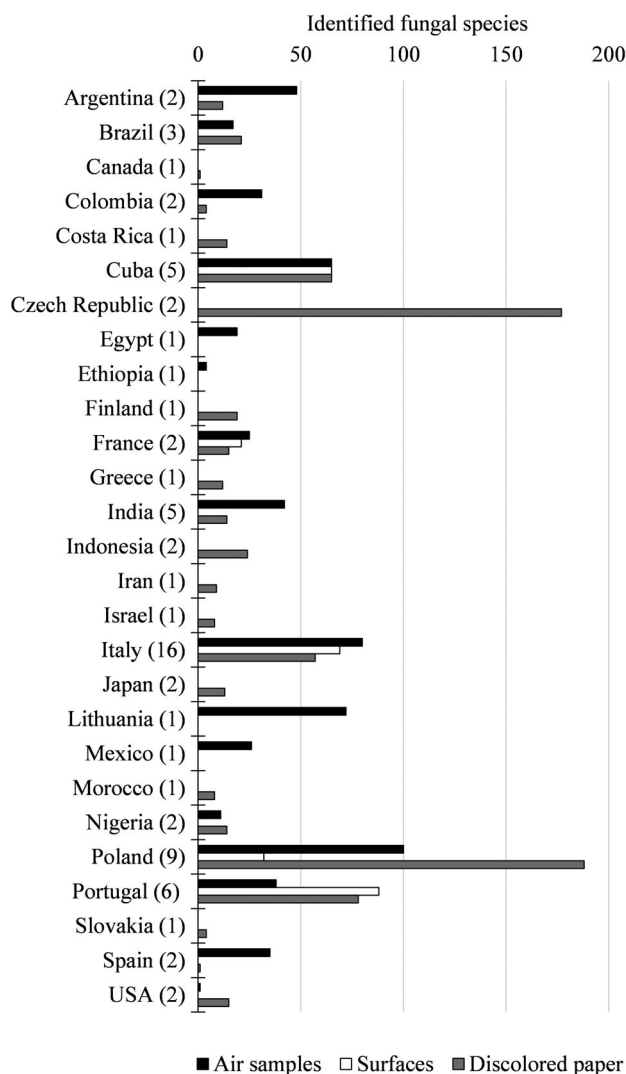


Figure 2. The present review examined data from 27 different countries. In brackets are the number of studies analysed and also shown is the diversity encountered per country and per sample type (air, surfaces, and discoloured paper).

Figure 3 presents the fungi identified in more than three case studies.

Air contamination – qualitative analysis

The vast majority of environmental studies performed in the last 20 years focuses on the fungal population present in the air of the studied facilities. *Cladosporium*, *Aspergillus*, and *Penicillium* species are almost ubiquitous in air samples and can produce numerous conidia that can be easily dispersed by air (Abrusci et al. 2005). Many of the fungi most commonly encountered in the air of archives and libraries are capable of degrading cellulose: *Aspergillus* spp., *Chaetomium* spp., *Trichoderma* spp., and *Penicillium* spp. are some of them. Comparing with other environments, *Chaetomium* sp., *Fusarium* sp.,

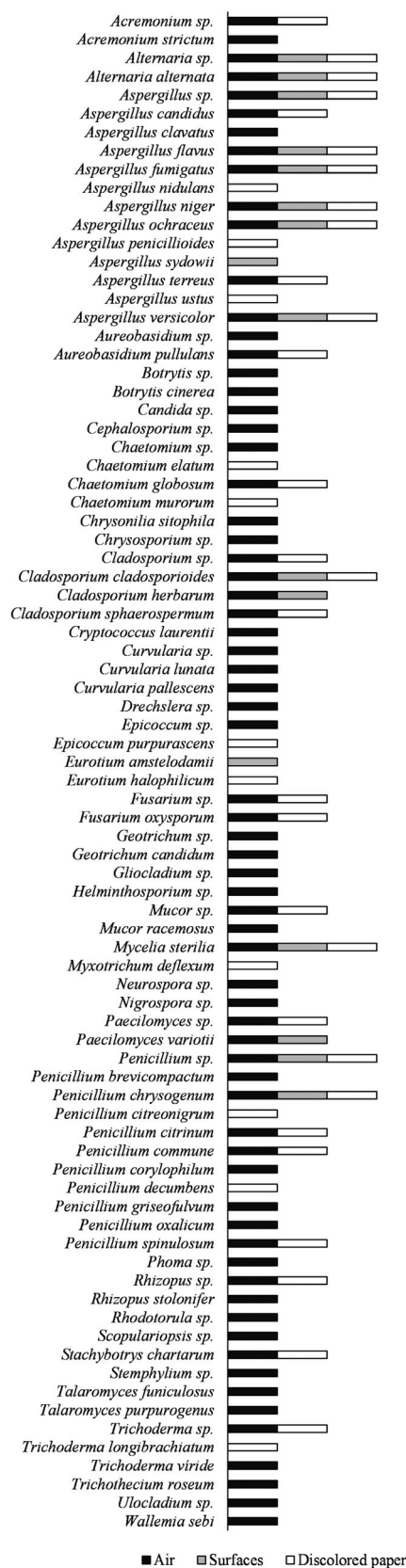


Figure 3. Fungal species isolated from air, surfaces or discoloured paper from the reviewed literature. Each bar (black, grey, or white) represents three or more studies from which these species were identified.

and *Geotrichum* sp. were found to be special contaminants in the air of archives and the first two have been associated with paper biodeterioration (Caneva et al. 2003; Corte et al. 2003; Lourenço et al. 2005; da Silva et al. 2006). *Trichoderma* species are not considered common elsewhere and can be especially relevant in paper conservation as this genus is well known for its vast production of cellulases and hemicellulases (Pandey et al. 2015). Both *Trichoderma viride* and *Trichoderma longibrachiatum* were frequently identified in paper or air in the revised literature, as shown in Figure 3.

The fungi that most often cause allergic symptoms belong to the genera *Alternaria*, *Cladosporium*, *Aspergillus*, *Candida*, *Penicillium*, *Mucor*, *Fusarium*, and *Rhizopus* (Micalli et al. 2003). At least 600 species of fungi are in contact with humans and less than 50 are frequently identified and described in epidemiologic studies on indoor environments (Khan and Karuppayil 2012). Allergenicity tests conducted on an array of commonly encountered indoor fungi indicate that *Aspergillus versicolour*, *Aspergillus niger*, and *Penicillium citrinum* can induce allergic reactions (Khan et al. 2009). Potentially pathogenic/toxigenic species (Valentin 2007; NT-SCE-02 2009) *Alternaria alternata*, *Aspergillus glaucus*, *A. niger*, *Aspergillus fumigatus*, and *A. versicolour*, *Chaetomium globosum*, *Cladosporium herbarum*, *Penicillium brevicompactum*, and *Stachybotrys atra* have all been identified in air samples taken in archives and/or libraries (Table S1). Valentin (2010) and the Portuguese Ordinance Law 353-A/2013 also consider *Aspergillus flavus*, *Aspergillus ochraceus*, and *A. Aspergillus terreus*, *Trichoderma viride* and *Rhizopus nigricans* potentially toxigenic, which are also present in the reviewed literature (Table S1).

In the work performed by Zielinska-Jankiewicz et al. (2008), 12 species were regarded as potentially pathogenic for humans: 8 of them displayed allergic properties and 11 displayed toxic properties. From the results obtained by their survey, Lugauskas and Kristaponis (2004) stress that the allergenic properties of the fungi detected in libraries, and their potential to produce, accumulate and release volatile toxic secondary metabolites into the library surroundings might pose a health risk to library workers. Both *Chaetomium* spp. and *Stachybotrys* spp. (present as most common in Figure 3) have produced various secondary metabolites in pure culture. *C. globosum* can produce chaetoglobosins and chetomin whereas other *Chaetomium* species can, on certain occasions, produce sterigmatocystin, a potent carcinogen. *Stachybotrys chartarum* can liberate trichothecenes and atranones, considered responsible for mycotoxicoses in humans (Bennett and Klich 2003).

Aspergillus species, abundant in the air samples presented in Table S1 and accounted as very frequent in archives around the world (see Figure 3) are also producers of mycotoxins. *In vitro* experiments show that metabolite and mycotoxins production is influenced by the media used, the incubation temperature, and the water activity (Nielsen 2003). This also explains why mycotoxins are not always detected even in the presence of mycotoxin-producing fungi. Moulds are likely to generate different metabolites when they grow on building materials and toxin production is affected by the biologic neighbours in mixed cultures (Nielsen 2003). Secondary metabolites and mycotoxins are species-specific which makes it very important to identify isolates to the species level. Also, mixtures of metabolites can see their effects added and potentiated (Nielsen 2003).

Fungi retrieved from surfaces and its relevance in fungal contamination studies

Very few studies present an evaluation of the surfaces where books and documents are actually kept and of the bindings that come in contact with the staff's/readers' hands. The studies performed in these settings, which are both quantitative and qualitative, are referred to in Table S1, third column – Isolation from surfaces (e.g. shelves, reading tables, and bindings).

As already mentioned, the last formal review regarding fungi in archives and libraries, performed by Zyska (1997), recollects data from very diverse origins. In fact, it is not just focussed on books but on panoply of materials one can find in museums, archives, or libraries. It does have the great advantage of presenting data from both air and surface samples which can be essential to truly know the fungal communities present in those locations. Some spores, given their characteristics, tend to aggregate and are not easily airborne which makes it difficult to find them in air samples although they can be present in the environment (Duchaine and Mériaux 2001). This could be deemed of lesser importance if this was not the case for *Stachybotrys chartarum*, the fungi most associated with Sick Building Syndrome (Bennett and Klich 2003). In the archival settings analysed in this review, this particular fungus can be considered common and is found in both the air and deteriorated paper samples (see Figure 3).

Sampling surfaces is also the best choice when looking for dermatophytes. These are fungi capable of degrading keratin. None of the three genera (*Microsporum*, *Trichophyton*, and *Epidermophyton*) was found in

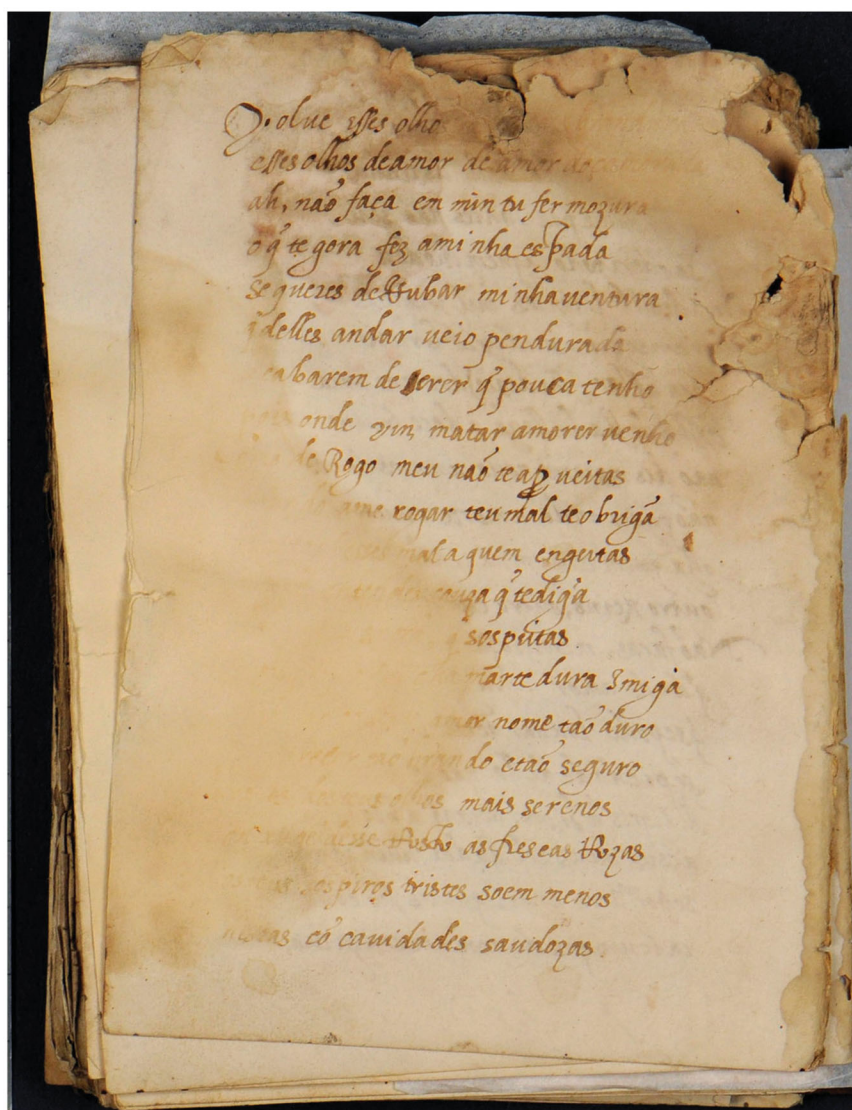


Figure 4. Detail of manuscript song book (sixteenth century, private collection) showing fungal biodeterioration.

previous studies, but *Trichophyton rubrum* and other keratinophilic fungi were isolated in a University library (Jain 2000). It is important for library and archival workers and users to be aware of this problem so that adequate care is taken when handling these contaminated surfaces.

Also Valentin (2007) quantitatively compared air and surface analyses and concluded that cellulose objects require longer exposure time in an improved climate to achieve a significant decrease in microbial growth. This adds to the importance of surface sampling cellulosic materials as these are a more reliable measuring tool in the event of altered environmental conditions.

Of the few studies performed on the analysis of surfaces (Table S1, third column), in Maggi et al. (2000) the most represented genera were *Aspergillus* (*A. fumigatus* included), *Cladosporium* sp., *Penicillium* sp., *Chaetomium* sp., and *Alternaria* sp. An average of 10 colony forming

units (CFU) was determined per sampled area (24 cm²) (≈4000 CFU/m²). The study performed by Zielinska-Jankiewicz et al. (2008) included both air and surface samples. *Cladosporium* and *Penicillium* were the most prevalent genera and the contamination levels were 800–1000 CFU/m². At the Jasna Góra monastery library, Harkawy et al. (2011) found the maximum concentration obtained per surfaced area to be 10 000 CFU/m². Levels such as the mentioned 10 000 CFU/m² were also found (and crossed) in Pinheiro (2014). In both Harkawy et al. (2011) and Zielinska-Jankiewicz et al. (2008), air samples resulted in a higher fungal diversity than surface samples, but in Pinheiro (Pinheiro 2014; Pinheiro 2015) surface samples presented a more diverse array of fungal genera/species than the one obtained in air samples. This result is in accordance to other settings (gymnasiums, elderly care centres, for instance) where the number of different species/genera in surfaces

Table 1. The most commonly identified fungal species in discoloured paper/book materials – identification methods and main characteristics.

Fungal species	Isolation from altered paper/book materials		Characteristics				
	Identification	References	Colouration/ colourants	Enzymes	Growth conditions		
					a_w	T (°C)	
<i>Aspergillus flavus</i> Link	C	Nol et al. (2001); Zotti et al. (2008); Bankole et al. (2010); Borrego et al. (2012); Castillo et al. (2016)	Yellow, green, orange	Cellulases (-/+ +++) Amylases (+++/++++) Gelatinases (+) Proteases (+)	>0.78–0.80	10–48 (33)	Das et al. (1997); Gopinath et al. (2005); Janda et al. (2009); Pitt and Hocking (2009); Samson et al. (2010); Saleem and Ebrahim (2014); Anaya et al. (2016); Oetari et al. (2016); Okpalanozie et al. (2018)
	C + DNA	Oetari et al. (2016); Okpalanozie et al. (2018); Teixeira et al. (2018)					
<i>Aspergillus fumigatus</i> Fresen.	C	Das et al. (1997); Nol et al. (2001); Lourenço et al. (2005); Bankole (2010)	Turquoise-grey, dark green	Cellulases (+/+ +++) Amylases (++++) Gelatinases (+) Lipases (++) Proteases	>0.82–0.94	12–55 (37–42)	Pasanen et al. (1991); Das et al. (1997); Tepsic et al. (1997); Samson et al. (2000); Chang et al. (2004); Gopinath et al. (2005); Janda et al. (2009); Pitt and Hocking (2009); Oyeleke et al. (2010); Low et al. (2011); Saleem and Ebrahim (2014)
	DNA	Di Bonaventura et al. (2003); Kraková et al. (2012)					
<i>Aspergillus niger</i> Tiegh.	C + DNA	Mesquita et al. (2009); Castillo et al. (2016); Oetari et al. (2016)					
	C	Das et al. (1997); Nol et al. (2001); Adelantado et al. (2005); Lourenço et al. (2005); da Silva et al. (2006); Michaelsen et al. (2009); Bankole (2010); Borrego et al. (2012)	Black/dark brown (melanin and hexahydroxyl pentacyclic quinoid colorants)	Cellulases (-/+ +++) Amylases (-/+ +++) Gelatinases (++) Proteases (+)	>0.77 (0.97)	6–47 (30–37)	Fogarty and Kelly (1979); Das et al. (1997); Samson et al. (2000); Parra and Magan (2004); Gopinath et al. (2005); Janda et al. (2009); Jorgensen et al. (2011); Borrego et al. (2012); Saleem and Ebrahim (2014); Bergadi et al. (2014); Anaya et al. (2016); Oetari et al. (2016); Okpalanozie et al. (2018)
<i>Aspergillus terreus</i> Thom	C + DNA	Bergadi et al. (2014); Oetari et al. (2016); Pietrzak et al. (2017); Coronado-Ruiz et al. (2018); Kraková et al. (2018); Okpalanozie et al. (2018); Teixeira et al. (2018)					
	C	Fabbri et al. (1997); Ricelli et al. (1999); Nol et al. (2001); Zerek (2003)	Yellow-brown	Cellulases (++++) Amylases (-/+ +++) Gelatinases (+)	>0.78	11–48 (35–40)	Samson et al. (2000); Pitt and Hocking (2009); Shahriarour et al. (2011); Saleem and Ebrahim (2014)
<i>Aspergillus versicolor</i> (Vuill.) Tiraboschi	DNA	Michaelsen et al. (2010); Pinheiro (2014)					
	C	Hyvärinen et al. (2002); Zerek (2003); Lourenço et al. (2005); da Silva et al. (2006)	White, yellow, orange, green, flesh, pink, greyish green	Cellulases (+/+ +++) Amylases (+/+ +++) Gelatinases (++++) Lipases (+)	>0.78 (0.90–0.95)	5–39 (25–27)	Reiss (1978); Samson et al. (2000); Pitt and Hocking (2009); Samson et al. (2010); Adan and Samson (2011); Borrego et al. (2012); Saleem and Ebrahim (2014); Oetari et al. (2016)
<i>Chaetomium globosum</i> Kunze	DNA	Michaelsen et al. (2009); Michaelsen et al. (2010); Pinheiro (2014); Okpalanozie et al. (2018)					
	C + DNA	Mesquita et al. (2009); Sato et al. (2014); Oetari et al. (2016); Coronado-Ruiz et al. (2018); Kraková et al. (2018); Teixeira et al. (2018)					
<i>Chaetomium globosum</i> Kunze	C	Das et al. (1997); Szczepanowska and Cavaliere (2000); Corte et al. (2003); Lourenço et al. (2005)	Dark brown, chrome, slightly yellowish or purplish tinge	Cellulases (+/+ +++) Amylases (++) Proteases	>0.90	4–38 (25)	Florenzano (1949); Lakshmikant (1990); Das et al. (1997); Skeist (2007); Sharma and Shukla (2008); Pitt and Hocking (2009); Abdel-Azeem et al. (2016); Saleem and Ebrahim (2014); Andersen et al. (2016)
	C + DNA	Rakotonirainy et al. (2007) Sequeira et al. (2019); Mesquita et al. (2009); Pietrzak et al. (2017); Coronado-Ruiz et al. (2018)					

(continued)

Table 1. Continued.

Fungal species	Isolation from altered paper/book materials		Characteristics				
	Identification	References	Colouration/colourants	Enzymes	Growth conditions a _w T (°C)	References	
<i>Cladosporium cladosporioides</i> (Fresen.) G.A. de Vries	C	da Silva et al. (2006); Zotti et al. (2008)	Olive, bluish grey or black	Cellulases (-/+ +++) Amylases (-/+ +++) Lipases (-/+ +) Proteases (-/+)	>0.86 (0.985)	-4–32 (25)	Sautour et al. (2002); Gopinath et al. (2005); Pitt and Hocking (2009); Borrego et al. (2012); Saleem and Ebrahim (2014); Anaya et al. (2016); Coronado-Ruiz et al. (2018)
	C + DNA	Mesquita et al. (2009); Michaelsen et al. (2009); Coronado-Ruiz et al. (2018)					
<i>Epicoecium nigrum</i> Link	C	Corte et al. (2003)	Orange, brown, red, pink, yellow, green, black	Cellulases (+/+ +++) Amylases (+/+ +++) Proteases (-)	>0.86–0.90	4–45 (20–28)	Samson et al. (2000); Pitt and Hocking (2009); Fávoro et al. (2011)
	DNA	Michaelsen et al. (2009); Pinheiro (2014)					
<i>Penicillium chrysogenum</i> Thom	C + DNA	Karakasidou et al. (2018); Teixeira et al. (2018)	Dull blue, greyish turquoise, dull green blue–green, yellow	Cellulases (+/+ +++) Amylases (+/+ +++) Proteases (-/+ +++) Proteases (-/+)	>0.78–0.81 (0.985)	-4–37 (23–25)	Samson et al. (2000); Sautour et al. (2002); Pitt and Hocking (2009); Chinedu et al. (2011); Saleem and Ebrahim (2014); Bergadi et al. (2014); Anaya et al. (2016); Andersen et al. (2016)
	C	Fabbri et al. (1997); Ricelli et al. (1999); Corte et al. (2003); Michaelsen et al. (2009); Borrego et al. (2012)					
<i>Penicillium citrinum</i> Thom	DNA	Di Bonaventura et al. (2003); Michaelsen et al. (2010); Karakasidou et al. (2018)	Greyish Orange, greyish turquoise; bright yellow	Cellulases (-/+ +++) Amylases (+/+ +++) Proteases (+) Lipases (+)	>0.80–0.84	5–37 (26–30)	Samson et al. (2000); Gopinath et al. (2005); Pitt and Hocking (2009); Borrego et al. (2012); Saleem and Ebrahim (2014); Okpalanozie et al. (2018)
	C + DNA	Sequeira et al. (2019); Mesquita et al. (2009); Michaelsen et al. (2009); Sato et al. (2014); Sterflinger and Engel (2014); Bergadi et al. (2014); Pietrzak et al. (2017); Coronado-Ruiz et al. (2018); Kraková et al. (2018); Teixeira et al. (2018)					
<i>Penicillium commune</i> Thom	C	Corte et al. (2003); Lourenço et al. (2005); da Silva et al. (2006); Borrego et al. (2012)	Greyish turquoise to dull green conidia	Cellulases (+/+ +++) Amylases (+) Proteases (+) Cellulases (+)	>0.83	? –30 (25)	Samson et al. (2000); Pitt and Hocking (2009); Borrego et al. (2012); Bergadi et al. (2014)
	C + DNA	Rakotonirainy et al. (2007)					
<i>Penicillium spinulosum</i> Thom	C	Sequeira et al. (2019); Oetari et al. (2016); Karakasidou et al. (2018); Okpalanozie et al. (2018)	Mycelium white; pinkish or pale Brown; Orange Brown or red brown	Cellulases (+) Cellulases (+)	>0.80	22–25	Ruegger and Tauk-Tormisielo (2004); Pitt and Hocking (2009)
	C + DNA	Sequeira et al. (2017); Kraková et al. (2018)					
<i>Stachybotrys chartarum</i> (Ehrens.) S. Hughes	C	Das et al. (1997); Fabbri et al. (1997); Ricelli et al. (1999); Castillo et al. (2016)	Black, grey, brown, olivaceous brown	Cellulases (-/+ +++) Amylases (+)	>0.91–0.94	2–40 (23–27)	Samson et al. (2000); Flannigan et al. (2011); Saleem and Ebrahim (2014); Sato et al. (2014); Andersen et al. (2016)
	C + DNA	Sequeira et al. (2019); Sato et al. (2014)					

a_w: water activity; C: classical morphological characterisation; DNA: culture independent molecular biology; C + DNA: culture dependent molecular biology. Enzymatic potential is assigned with (++++) high; (+++) medium; (++) low; or (-) negative levels. Minimal and maximal water activity (a_w) and temperature (T) values for growth are presented, with optimum values in brackets.

doubles the one found in air samples (Viegas et al. 2011; Viegas et al. 2014). The level of contamination is naturally related to dust and organic matter accumulation and one can argue that this can be prevented with maintenance. However, because archives and libraries are usually large premises with little room (and staff) to spare, proper maintenance can be a daunting task to perform regularly. Having this in consideration it is probably important to consider the use of personal protection when working *in situ*.

Fungi retrieved from discoloured paper – more than suspects?

Fungi are considered serious degrading agents of paper documents and works of art (Fabbri et al. 1997). Several studies have focussed on the effects fungi can have on our written heritage which can include pigment's and ink's discolouration (Florian and Manning 2000; Pinzari et al. 2006; Mesquita et al. 2009), chemical deterioration (Canhoto et al. 2004; Pinzari et al. 2010), formation of coloured stains, including foxing (Szczepanowska and Cavaliere 2003), and also physical and mechanical stress (Ponce-Jimenez et al. 2002; Caneva et al. 2003; Sterflinger 2010) (Figure 4). Some fungi can produce cellulolytic enzymes which break the cellulose polymer into smaller units, weakening the paper structure (Zyska 2002; Adamo et al. 2003; da Silva et al. 2006). Also, acidic metabolites can be excreted by these microorganisms, promoting acid hydrolysis in the paper fibres (Ponce-Jimenez et al. 2002). The release of all these extracellular enzymes and/or pigments acts chemically on the substrate.

The correlation between a given change in a document (a stain, for instance) and the presence of a fungal species has been the subject of several studies (Table S1, last column). Most of these studies have achieved a positive identification, but the doubt remains on many of them as to whether the identified fungus is the actual culprit for the (usually chromatic) alteration. It is also important to notice that only the most recent studies make use of molecular biology protocols for fungal identification, the majority of them in association with conventional culturing methods.

Apart from the cases where visible colonies are noticed, most of the damage associated with fungal causes appears in the form of chromatic changes in the paper. This effect may last long after the fungus has been active. From discoloured samples, with no visible fungal development, fungal growth is usually very difficultly attained and molecular biology protocols play a pivotal role in determining the possible culprit.

Due to their importance in paper conservation/restoration, the most frequently identified fungal species (identified to the species level and present in more than five studies) from discoloured paper and/or book materials taken from Table S1 are presented in greater detail in Table 1.

All these frequently identified fungi possess cellulolytic activity with varying degrees depending on the species and strain (Table 1). Besides cellulases, most species produce other exoenzymes that degrade paper composing or binding materials, like amylases (starch decomposition) and proteases (gelatin or leather decomposition). Also, they can excrete coloured metabolites with several different hues, having the potential to disfigure the documents and artworks they dwell in. The great majority of these fungal species are already able to develop at water activity levels near 0.80, which qualifies them as xerotolerants. Regarding temperature, the optimum growth levels are circa 20–25 °C, a common interval for human comfort in libraries, archives and museums.

Fungi can excrete acidic compounds to alter the pH of the substrate according to their development needs. Fungal growth of species such as *Aspergillus niger*, *A. terreus*, *A. ustus*, and *A. versicolour*, *Cladosporium cladosporioides*, *Penicillium chrysogenum*, *P. citrinum*, and *P. commune* have shown the ability to greatly decrease the pH (up to 4 pH units) of saline solutions initially buffered to pH 7 (Borrego et al. 2012). *A. niger* is also capable of reducing a pH 8 buffered iron gall ink growth medium to pH 1.7 (Messner et al. 1988). This reveals a significant production of acidic metabolites by the most common fungal species found on paper materials. As paper is highly susceptible to acidic hydrolysis, such acid production represents an important biodeterioration potential. Sequeira et al. (2017) show the acidification and severe loss of folding endurance caused by the metabolites left by *A. niger* on paper in the long term.

Table 1 also highlights the methods used to achieve the identification of the deterioration potential culprits. We can see an increasing trend towards the use of molecular biology identification methods on the most recent studies where culture-dependent DNA analysis is the most used methodology.

Fungi like *Chaetomium murorum*, *Myxotrichum deflexum*, and *Trichoderma longibrachiatum* (and these are just the ones mentioned as most common) have only been identified in these sometimes very challenging samples through molecular biology (Table S1). However, the introduction of DNA analyses has also brought some problems in terms of data handling and

processing. A joint collaboration was proposed by Katja Sterflinger and working groups at the IBBS Meetings in Manchester and Coimbra (Sterflinger et al. 2018), as recent advances in the identification methods (such as Next Generation Sequencing) have come to add (too much?) information and it is important that we do not lose focus on what is important for conservation: what is causing the problem, what are the deterioration mechanisms and how do we prevent and, ideally, revert them. Although still in a very incipient phase, the joint collaboration is aimed at defining gold-standard procedures (from collection to data analysis) in order to be able to advance in the understanding of biodeterioration. A database of the most commonly encountered microorganisms on cultural heritage damaged artefacts is the first step in this endeavour, and this review – on the very specific setting of archives and paper collections – could be an important contribution to this initial stage.

Impact of fungal load on human health and paper preservation. Can it be assessed?

Some fungi involved in the deterioration of paper may also be dangerous to library/archival professionals and users (Bennett and Klich 2003; Mesquita et al. 2009). Fungal spores can cause allergic skin and respiratory symptoms that may appear continuously or with seasonal rhythm. Skin contact with mould-infested substrates and inhalation of spore-borne toxins are also important sources of exposure (Bennett and Klich 2003). Mycotoxins, even in low concentrations, cause gastrointestinal disorders, damages to the haemopoietic and genital system, and non-specific symptoms (asthenia and nausea) similar to those connected with sick building syndrome (Micalli et al. 2003). According to some authors, the most common malaises reported by staff working in libraries, archives, or book containing premises are dermatitis, rhinitis, allergies, and asthma (Valentin 2007). During the last 20 years, very few studies evaluated the health risk resulting from exposure to this particular environment or from the use

of paper materials. Wiszniewska et al. (2009), concluded that 30% of museum employees were sensitized to at least one of the fungal allergens tested and that the prevalence of allergic symptoms among the subjects was relatively high and frequently related to specific sensitization. The most frequent symptoms reported by the examined subjects were: conjunctivitis (68.5%), rhinitis (66%), skin symptoms (54%), chronic cough (26%), and dyspnoea (28%). These symptoms are often related to the fungal load present in the air of the facilities holding paper-based collections. However, quantitative analysis of fungal load in the air of archives and libraries was performed by just a few authors (Table 2).

The mentioned studies used the impact method (air suction and immediate impact on an agar Petri dish) with contamination determined as CFU/m³, but other methods and data presentations are possible, sometimes with completely different results (Molina-Veloso and Borrego-Alonso 2017). Such non-volumetric air sampling methods should only be used as preliminary or when only qualitative information is sought (Samson et al. 2000).

Some of the available proposals regarding fungal load indoor air limits (considering multiple building types) for health-risk are presented in Table 3. When considering human health, the data obtained from the air contamination studies presented in Table 2 reveals higher than recommended values by most of the existing guidelines on indoor air quality (Table 3). Despite the fact that environments containing fungal counts between 1 and 1000 CFU/m³ are considered low contaminated environments (according to (Nevailanen and Hyvarinen 2015)) one must take into account the fact that “total” levels of airborne microbial particles are normally between 10 and 100 times higher when using other than culture-based evaluation methods (Lignell et al. 2008; Nevailanen and Hyvarinen 2015) and this is probably the cause why, even in low mould-contaminated indoor settings, there are occupational diseases such as asthma, fungal allergy, hypersensitivity pneumonitis, and other health outcomes (Nevailanen and Hyvarinen 2015).

Table 2. Fungal contamination in indoor air determined for archives and libraries in different countries, analysed by the impact method.

Fungal concentration (CFU/m ³)	Archive or library	References
From 100 to 500	Library of the Lithuanian Academy; Lithuania	Lugauskas and Krikstaponis (2004)
From 300 to 800	Martynas Mažvydas National Library of Lithuania, Lithuania	Lugauskas and Krikstaponis (2004)
Average values between 180 and 2300	Library and Archive Storage, Poland	Zielińska-Jankiewicz et al. (2008)
From 200 to circa 1500	Historical Archive of Ca' Granda; Italy	Cappitelli et al. (2009)
From 2 to 200	Arquivo Distrital de Évora; Arquivo Histórico Ultramarino; Instituto de Habitação e Reabilitação Urbana; Torre do Tombo	Pinheiro (2014); Pinheiro (2015)
From 10 to 180	Archivo Nacional de Cuba	Molina-Veloso and Borrego-Alonso (2017)

Table 3. Fungal load indoor air limits for conservation and health-risk proposed by distinct authors in different countries and organisations.

Fungal load limits and guidelines for Human Health (CFU/m ³)		References
>100 is a sign of internal contamination		Ohgke et al. (1987); Hurts et al. (1997)
<150		World Health Organisation (Goyer et al. 2001)
<200		Yang et al. (1993); Etkin (1994)
<500		Reynolds et al. (1990)
2000 – very high contamination and a health threat		Klánová (2000); Zielińska-Jankiewicz et al. (2008)
<25 – Very low contamination		Hayleeyesus and Manaye (2014)
25–100 – Low contamination		
100–500 – Intermediate contamination		
500–2000 – High contamination		
>2000 Very high contamination		

Fungal load limits and guidelines for Conservation (CFU/m ³)	Country	References
100	France	Capderou and Flieder (1999)
120		Parchas (2009)
150	Italy	MIBAC (2001)
200	Poland	Karbowska-Berent et al. (2011)
50 for particular species		Cieplik, 1997 (in Harkawy et al. (2011))
150 for a mixture of several species		
500 for common airborne fungal contaminants		
0–25 no expected problems;	Netherlands (The Dutch National Archives)	Brokerhof et al. (2007)
25–100 possible presence of source, further testing needed;		
100–1000 source present		
>1000 active mould growth		

High-level exposure to airborne viable fungi (10^6 CFUs/m³) was determined as the cause in a case of organic dust toxic syndrome in a museum staff handling mouldy books (Kolstad et al. 2002). According to Zielińska-Jankiewicz et al. (2008), some of the archive workers who participated in a survey conducted by Schata in 1995 reported various skin, eye, and respiratory symptoms which could have been associated with occupational exposure to moulds. It was estimated that about one-third of archive workers might have developed an allergy to moulds, which is about twice as high as in the general population. The workers taking part in the survey performed by Krake et al. in 1999 (Zielińska-Jankiewicz et al. 2008), reported respiratory and sinus-related symptoms which could have been associated with workplace exposure to moulds. In that study, mycological microflora belonging mostly to the *Penicillium*, *Cladosporium*, *Aspergillus*, *Alternaria*, and *Tritirachium* genera was detected in levels that ranged from 200 to 450 CFU/m³, depending on the facility, but for the *Tritirachium* species levels of approximately 800 CFU/m³ were measured.

Attempts have been made to determine the minimum amounts of CFU necessary to induce a negative reaction. This has not been an easy task since individual's variability alone is enough to impair the result from exposure to a given fungus or amount of fungi. Health-risk levels suggested in the literature vary greatly from author to author and throughout the years. Being a

much more recent discipline, no globally accepted international standard for paper conservation has yet been established (Cappitelli et al. 2010), either regarding the species or the number of fungi that can be considered dangerous when present in an archival environment. However, some countries have designed their own national guidelines and some countries have set them informally (Brokerhof et al. 2007; Harkawy et al. 2011).

Table 3 also presents the guidelines proposed for fungal load in distinct countries for paper conservation. All the studies presented in Table 2 indicate contaminated environments, according to the lowest level established by the Dutch National Archives guidelines (Table 3).

The measured CFU/m³ variation is very high between countries (Table 2), within the same country and within the same facility at different seasons. Given this variability and the specificity of different fungal species regarding their toxic and deteriorating potential, it is probably too difficult (not to say misleading) to define an international maximum value of fungal load for conservation, instead, these values should be kept as low as possible. Studying the features of outside air, the least contaminated location and comparing them with the locations depicting a higher value should be a valuable aid in adopting the necessary practices to reduce fungal contamination. Periodic assessments, as well as a qualitative characterization of the fungal

communities present, is also extremely important as it allows early detection of a shift in population – either in terms of the species or present amounts. Periodic surface analysis – and determining a desirable contamination level (after regular cleaning, for instance) – can also help determine whether or not to increase maintenance efforts.

The proposed values for maximum loads of fungi presented in Table 3 can be quite varied, do not depend on the fungal species, and the reasons behind a given value are not always well explained. Geographic differences should also be considered since a country's climatic characteristics are intimately related to the fungal flora. As in other settings, there are no proposed limits for surface contamination (CFU/m²). It would be of great interest to determine these limits since it is the surface of books and their condition that we intend to see preserved. As happens with the limits proposed for human health, the theoretical and experimental bases for the proposed numbers are sometimes difficult to ascertain. The extreme variability in the mentioned values may also be interpreted as a pointer to the need for identifying the species present in a given environment.

Conclusions

This review gathers studies from across the globe, showing us the fungal communities encountered so far in samples taken from altered paper samples, surfaces, and in the air of archives and libraries. A high number of different species were identified for the following genera: *Penicillium* (61 species), *Aspergillus* (44 species), *Cladosporium* (19 species), *Chaetomium* (16 species), and *Trichoderma* (11 species).

The most common genera found in the air of archives resemble the outdoor fungal flora as well as common contaminants of other unrelated settings. However, fungi like *Fusarium* sp., *Chaetomium* sp., and *Geotrichum* sp. display higher than usual frequencies in the air from archives and libraries. Many of the fungi most commonly encountered inside these places are capable of degrading cellulose. When obtained fungal loads are displayed against the existing guidelines and guideline proposals, some sampled locations cross the highest level defined by the Dutch Guidelines, of 1000 CFU/m³. The disparity obtained on CFU/m³ reinforces the need for further studies, namely the identification of the contaminating species, a measure of great importance not only for conservation purposes but also for human health concerns.

The air is not the only source of contamination. Surfaces are a resting site for fungal particles and often translate a wider picture in terms of fungal diversity. The study of these surfaces reveals the communities from which the biodeterioration agents emerge and thrive. Another type of surface considered in this review is the specific location where discolouration occurred and fungal biodeterioration is considered as a possible cause. For the most relevant fungal elements encountered in these specific locations, the methods of identification are also presented.

Regarding fungal identification, determining the microfungi agents responsible for paper biodeterioration has not been an easy task and has led to confusion and never-ending lists of possible culprits. The identification of a fungal genus or species on a document does not necessarily mean this fungus is the actual cause. To overcome this issue, evidence suggests the need for various and simultaneous methods to be employed since no single method developed so far gives us the full picture. Also, to be able to make the most out of every research project, to achieve comparable results and truly advance in the field, a joint effort is being made to define standard operating procedures and create an environment where different backgrounds and expertise come together to address this important and complex field. Several initial measures are to be taken. The first step is the creation and management of a databank with information related to what, where and how microorganisms were isolated from cultural heritage artefacts. Reviewing all the available data is, hence, of pivotal importance and we hope, as participants of this initiative, to ease the way into the world of paper biodeterioration by fungal agents.




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