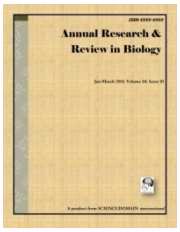


Annual Research & Review in Biology
4(24): 3915-3931, 2014

SCIENCEDOMAIN *international*
www.sciencedomain.org

Microbial Characterization of Contaminating Cells on Scientific Collections in a Specialized Library

Antonio Carlos Augusto Da Costa[1,2*], Fernanda Do Nascimento Corrêa[2], Lucia Alves Da Silva Lino[1], Eloisa Helena Pinto De Almeida[1], Ana Lucia Chaves De Oliveira[3], Márcia Teresa Soares Lutterbach[3]

[1]Museum of Astronomy and Related Sciences/CDA, R. Gal. Bruce 586, S. Cristóvão, Rio de Janeiro, Brazil.
[2]Universidade do Estado do Rio de Janeiro/PPG-EQ, R. S. Fco. Xavier 524, Maracanã, Rio de Janeiro, Brazil.
[3]Instituto Nacional de Tecnologia/LABIO, Av. Venezuela 82, Praçaa Mauá, Rio de Janeiro, Brazil.

Authors' contributions

This work was carried out in collaboration between all authors. Authors ACADC and FDNC designed the study, performed the literature searches, performed the microbiological culturing, wrote the protocol for classical microbiology, and wrote the first draft of the manuscript. Authors LADSL and EHPDA were responsible for the selection of books to be treated. Authors ALCDO and MTSL performed all molecular biology tests and their protocols. All authors read and approved the final manuscript.

Original Research Article

Received 13th May 2014
Accepted 3rd July 2014
Published 15th July 2014

ABSTRACT

Aims: Microbial characterization of contaminants on Scientific Collections in a Specialized Library.
Study Design: Selection and segregation of books from special collections, followed by an investigation of the fungal contamination through molecular biology.
Place and Duration of Study: Museu de Astronomia e Ciências Afins, located in Rio de Janeiro, Brazil, during seven months.
Methodology: Several books with a clear fungal contamination on their surface and several air samples from distinct locations in the library were included. Classical

**Corresponding author: Email: antoniocosta@mast.br;*

microbiological identification, molecular biology techniques and chemical treatment after flooding and cleaning of surfaces and books were performed.
Results: Results indicated that mainly cosmopolitan species were found in the environment and on the surface of selected books. Most species presented potential cellulolytic action and are toxin producers. Some unusual species were found such as *Periconia*, *Hypocrea* and *Pestalotiopsis*. Due to this unusual occurrence concentrations higher than 200mg/L were necessary to decontaminate the area and surfaces of the library after flooding.
Conclusion: The rapid action of the staff to solve the problems associated with the flooding in the scientific library of the Museum of Astronomy and Related Sciences in Rio de Janeiro, proved to be essential to minimize higher damage that could occur on rare books and special collections. The cooperative work involving Chemistry, Classical Microbiology, Molecular Biology and Preventive Conservation proved that the integrated practices among professionals of several areas were essential to ensure a free public access to documentation and books of interest for the scientific society. The multidisciplinary work involving Chemistry, Biology and Molecular Biology led to important conclusions about the presence of cosmopolitan fungi in the air and in selected books, their DNA characterization and their resistance to a fungistatic compound.

Keywords: Ubiquitous molds; biodeterioration; DNA identification; special book collections.

1. INTRODUCTION

Cellulosic materials are amenable to fungal deterioration, particularly paper constituents of archival materials which are slowly consumed by fungi and bacteria [1-3]. Depending on the environment different fungal genera can be found, but, irrespective of the humidity and temperature conditions, some cosmopolitan typical fungi are found. Aríngoli et al. [4] found predominantly the genera *Cladosporium* (58.9%) and *Alternaria* (8.7%), followed by *Epicoccum* (5.7%), *Fusarium* (5.4%), *Curvularia* (3.5%), *Acremonium* (1.3%), *Drechslera* (1.3%), *Penicillium* (1.3%), and *Aspergillus* (1.1%), which, together with yeasts (3.7%), represent 90.9% of total mycobiota in an Argentinean city. Accordingly, Rintala et al. [5] also confirm that Based on numerous cultivation studies, *Penicillium*, *Aspergillus*, *Cladosporium*, and about 20 other fungi are usually isolated from house dust.

Montanari et al. [6] studied the deterioration of library materials by some fungi, stating that this process has important economic and cultural consequences. The work of the authors is focused on movable shelves due to its wide use for the conservation of library materials, and due to the appearance of a single species mould infection already reported by the literature. Contamination was commonly characterized by white spots of mycelium, measuring 0.5–1.0 cm in diameter and observed on volume binding, especially those of leather, parchment or textile. In that work the authors concluded that the species belong to the species *Eurotium halophilicum* and *Aspergillus halophilicum*.

In order to study the presence of cultivable fungi and bacteria in the air and dust in 5 Polish libraries and archives, Karbowska-Berent et al. [7] observed that in all sites the total concentrations of cells ranged from 100 to 1000 CFU/m^3, with a predominating presence of *Staphylococcus* spp. and *Micrococcus* spp., followed by filamentous fungi *Penicillium, Trichothecium laxicephalum* and *Alternaria tenuis*. The main conclusion of the authors is related to procedures also adopted in the present case study in the Brazilian library: a

marked reduction of fungal and bacterial species was observed, after proper removal and mechanical cleaning of the storeroom, thus contributing for the hygienic quality of the space.

With the same purpose, Li and Yang [8], also presented an extensive review on fungal contamination as a major contributor to sick building syndrome. Authors provide a large list of fungal genera reported as allergenic, emphasizing the need for a control in environmental conditions, particularly those related to temperature and relative humidity. Main genera included: *Aspergillus, Amanita, Boletus, Cladosporium,* and *Claviceps.*

Based on the ubiquitous nature of molds in archives, libraries and museums a fungal index to detect critical conditions for fungal growth was developed in the storerooms of historical buildings in Higashiomi, Japan. Each microclimate was divided into levels A, B or C, depending on the index values, <1.8, 1.8–18 or >18, respectively. If a room maintains level A continuously, the room is free of contamination. For level B, fungal contamination might occur, and for level C, fungal contamination is high, and countermeasures should be taken promptly. The systematic use of fungal indices can provide practical information for conservation being an useful tool for integrated pest management in libraries, archives and museums [9].

A possible remediation of fungal contamination is usually costly and there is a risk of further damage due to the degraded materials [10]. In this case, the earlier the detection of fungal growth is possible, the lower the need for invasive treatments to remediate fungal contamination. Konkol et al. [11-12] used β-N-acetylhexosaminidase activity for fungal detection on a series of cultural heritage materials. The particular importance of the method was the fluorescence generated by small quantities of fungi quickly detected at an early stage of growth, associated to a sensitivity of the assay and practical use on paper for rapid remediation practices.

During restoration activities, evidence for the existence of fungal contamination in a ceiling decoration, was confirmed by classical culturing methods and HPLC to separate DNA from several species [13]. The investigators reported the first documented case of *Serpula lacrymans* in cultural heritage and the need to keep water activity levels under control to prevent fungal regrowth.

Several alternatives are being tested against fungal proliferation in cultural heritage objects and documents. Most of the alternatives rely on the use of chemical products, usually effective against fungi, but, sometimes deleterious to the objects tested, depending on their chemical structure. For instance, the antifungal activity of benzalkonium chloride was tested against fungal species isolated from stone and wooden substrata of cultural heritage objects [14]. Results indicated that effective benzalkonium chloride concentration ranged from 0.1 (fungistatic) to 4.0 (fungicidal) µL/mL, against *Aspergillus niger* and *Aspergillus ochraceus*.

In an attempt to replace the use of aggressive chemical agents for lighter ones Neves et al. [15] tried different mixtures of paraben and alcohol to decrease the action of *Cladosporium* and *Penicillium* on paper. The authors observed that the minimum required concentration of the agents were 1% propyl paraben in 85% ethanolic solution. However, authors also observed a slight increase in yellowing of the paper due to the application of the solution.

Alternatively, LeBouf et al. [16] tested models to predict mold growth, as a function of microbial volatile organic compounds. Their strategy implements a pattern recognition program that can be used with chemical sampling in built environments to predict the

presence of mold growth. Another possible alternative for mold control is the use of chlorine dioxide as fumigant, in the atmosphere and on moldy books, as proposed by some authors [17-18].

Thus, the objective of the present work was to present a methodology for the screening of fungal contamination in the atmosphere, shelves and books from a scientific library, with further identification of the main contaminating agents through classical microbiological and molecular biology techniques to correlate the results with possible cellulolytic activity, cosmopolitan occurrence and resistance against a commercial biocide.

2. MATERIALS AND METHODS

2.1 Description of the Area

In 2013, on April 2nd., the staff from the Paper Conservation and Restauration Laboratory (Lapel) from the Museum of Astronomy and Related Sciences detected that some publications stored in the library of the Museum, close to the Brasiliana Collection were partially damaged on some leather pieces and some other visibly contaminated with molds. The total area of the library is 105m^2, without air-conditioning, naturally ventilated. During an entire year average temperature is 25±3ºC and the average relative humidity is 67±15%. The following and immediate step was to proceed to a complete survey of the collections, particularly in the Brasiliana and Documentos Brasileiros Collections, in order to detect the amount of books damaged and the source of the chemical and/or biological degradation. The whole staff from the laboratory was called in order to start the necessary procedures to detect and solve the problem. Some glass windows in the library presented white dots on their surface, clearly indicating that it was not just clear water. These dots were present in several other windows in the library, close to the bookshelves. It was observed that the chemical damage on the leather cover of some books was related to the presence of whitewash from the ceiling of the library. Due to a strong storm during that period rainwater accumulated in the roof of the building increasing the pressure towards the library, partially solubilizing the whitewash used for its painting. However, it was not the main problem observed; due to this initial chemical damage, the increasing level of humidity opened space for microbial contamination, corroborating the fact that increasing humidity levels, highly contributed to this phenomenon.

To resolve this problem the staff from the laboratory decided to sequentially number the books in the shelves in order to start, immediately, the sanitization of the space and physical removal and chemical treatment of the contaminated pieces from the library collections. This selection lasted three consecutive days with a total staff of seven specialized technicians, including conservators, librarians and biochemists. After a suitable selection of the contaminated pieces, in the second week after the flood, some books were transferred to another room, in order to be chemically treated.

2.2 Microbiological Monitoring of the Air and Plating Conditions

According to Horner et al. [19] there is no single sampling method that is specific for mold growth and robust to reliably quantify mold growth. The microbiological characterization of the microflora in the air of the library was done by sedimentation on Petri dishes containing sterile Sabouraud Dextrose Agar supplemented with chloramphenicol solution. Samples were collected every cubic meter of the space, one meter above the ground of the library, for

2 hours. Particularly, some book shelves and books were monitored, where fungal contamination was clearly present. In that case, sterile swabs were used on the surface of selected objects and books. The same culture medium was used for fungal growth. After swabbing, the contaminated swabs were placed inside 9.0mL of saline water, followed by decimal dilutions, inoculating 0.1mL of the dilutions on Petri dishes containing Sabouraud Dextrose Agar. Petri dishes were then placed inside a temperature controlled chamber at 25ºC for 21 days. Molds grown on the dishes were isolated in selective culture media and stored in mineral oil [20-21].

2.3 Microbial Identification by Molecular Biology

After isolation of various fungi, micro cultivation techniques were done, followed by incubation at 25ºC from 7 to 14 days, depending on the specific genus. After growth, optical microscopic observations, according to standard protocols [22].

The identification of the molds was initially performed through macroscopic and microscopic observations. The phylogenetic identification was performed by molecular biology techniques, where cell lysis was performed during three cycles of freezing/defrosting, followed by DNA extraction with a commercial kit.

The gene that codes ITS region was amplified using a group of primers ITS5 (5'-GGAAGTAAAAGTCGTAACAAGG-3', forward) and ITS4 (5'TCCTCCGCTTATTGATATGC-3', reverse). PCR products were purified using a specific kit followed by sequencing. Identification keys were based on standard procedures [22-23].

2.4 Fungal DNA Extraction

After growth in specific media, pellets of the molds were removed from each dish and transferred into 15mL Falcon conical tubes. The tubes were placed in a cylinder containing liquid nitrogen for 5 minutes, and left stand in a hot water bath at 60ºC for 10 minutes, followed by crushing. This procedure was repeated three times. After this procedure, DNA extraction was performed with the help of the commercial kit Ultra Clean Soil Isolation (MO BIO Laboratories) according to instructions provided by the seller. DNA was eluted in 30mL of the solution provided in the kit and quantified through spectrophotometric methods (Nano Drop ND-1000, Thermo Scientific, Waltham, USA).

2.5 Amplification of ITS Region by Polymerase Chain Reaction (PCR)

Polymerase chain reactions were performed using the kit Top Taq Master Mix (Qiagen) in a total volume of 50µL, containing 0.5µM of each primer. The primers used were ITS-5 (sense, 5`-GGAAGTAAAAGTCGTAACAAGG-3`) and ITS-4 (anti-sense, 5`-TCCTCCGCTTATTGATAT GC-3`). PCR reactions were performed in a PCR thermocycler System 9700 (Applied Biosystems). The amplification product was observed through an eletrophoretic run in 1% agarose gel in TE 1X buffer.

2.6 Sequencing

DNA fragments of the isolated molds were sequenced using the kit Big Dye Terminator (Applied Biosystems, version 3.1) in an automatic sequencer ABI 3130 (Applied Biosystems) with 4 capillaries 50 cm each. The primers ITS-% (sense) and ITS-4 (anti-sense) were used

to sequence the region around 600pb and the primers Sadir (sense) and S17 (anti-sense) were used to sequence the region 1500pb. The concentrations of the primers were 3.2 pmol.

A fragment of 600 pairs of base corresponding to the gene ITS was amplified from the genomic DNA using the primers ITS5 (5'-GGAAGTAAAAGTCGTAACAAGG-3', forward) and ITS4 (5'-TCCTCCGCTTATTGATATGC-3', reverse) in the thermo cycler Gene Amp PCR System 9700 (Applied Biosystems). Each reaction included 25µL of TopTaq Master Mix Kit (PCR Master Mix, Qiagen), 0,5µM of each primer, 5µL of the extracted DNA added to water, in a total volume of 50µL. A first denaturation was performed at 94ºC for 4 minutes followed by 30 cycles in the following conditions: 94^{o}C for 30 seconds, 50ºC for 30 seconds and 72ºC for 30 seconds. The experiment was ended at 72ºC for 10 minutes. The amplicons were purified using the kid Wizard® SV Gel and PCR Clean-Up System (Promega) followed by proper sequencing [24-25].

The extension products were purified with the kit Big Dye XTerminatior purification, to remove nucleotides and primers not incorporated. After purification, the products were electronically injected in the sequencer. The chromatograms obtained from the sequencing were subjected to Chromas Lite software, version 2.01 and Bioedit software in order to evaluate the quality of the sequences. The sequences validated by the programs were paired to the sequences from the Genbank DNA Bank [26]. To validate the sequences the Basic Alignment Search Tool (BLAST) was used. Just fragments with similarity levels over 98% were considered.

2.7 Chemical Treatment of Selected Books Contaminated with Molds

After identification of the species found as contaminating agents, a chemical treatment was performed in order to detect the susceptibility of the chemical agent Preventol (Ortophenilphenol) to act as fungistat on the cultures of the molds isolated after the flood in the library and proper identification. The product has a very low solubility in water and a high solubility in ethanol and isopropanol. The minimum inhibitory concentration of the product, in proper nutrient medium, is dependent on the species type, particularly for molds (mg/L): *Alternaria* (100-200), *Aspergillus* (50-200), *Aureobasidium* (35), *Chaetomium* (50-100), *Cladosporium* (40-60), *Mucor* (200), *Penicillium* (35-100), *Rhizopus* (200) and *Trichoderma* (75). As this minimum inhibitory concentration of the product ranges from 35 to 200mg/L, depending on the genus of the fungus, it was adopted the highest concentration suggested, 200mg/L of Preventol dissolved in ethanol, sprayed on the surface of the culture medium before inoculation of each species in Sabouraud Dextrose Agar. However, one of our purposes was to sanitize the surface of some selected books and shelves. To reach this goal a concentration of 400mg/L of the product was also tested, in order to reach effects stronger than minimum inhibition, but complete elimination of the species. The choice for this second concentration was also made, because the fungal susceptibility to the product was not described for all molds identified. For each fungal genera and ortophenilphenol concentration chosen experiments in this set were performed in triplicate.

3. RESULTS

Fig. 1 presents an overview on the total amount of books transferred to the laboratory for chemical and physical treatment.

From Fig. 1, a total of 250 books were separated for treatment in the laboratory, most of them with problems associated to the presence of active molds. The chemical damage promoted by the attack of whitewash in the leather cover had to be solved with the partial restauration of the cover, a problem solved by another team of specialists.

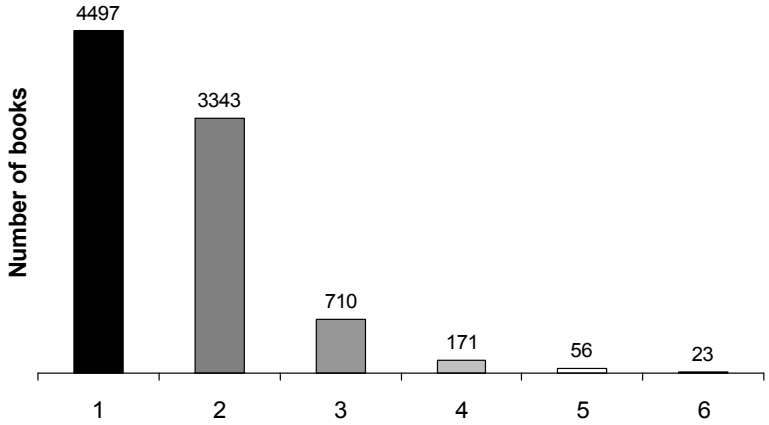


Fig. 1. Statistics of the selection and treatment of the books in the library after flooding: (1) Total books in the library; (2) Books without apparent damage; (3) Books that were physically cleaned do remove solid particles and dust; (4) Books clearly contaminated with active molds; (5) Books damaged by whitewash in their leather cover (chemical damage); (6) Books contaminated with active molds and also presenting chemical damage

Our concern about the microbiological contamination of the books was mainly related to the importance of the two bibliographical collections affected: *Brasiliana* and *Os Pensadores*. The first one is of particular importance due to its broad scope, covering History, Anthropology, Political Sciences and Geography, Sociology and Linguistics, Economy and Natural Sciences, with contributions of national and foreign writers that describe important European expeditions to Brazil during the XIX Century. *Os Pensadores* collection is a unique collection in Brazil that worried about the publication of the most influential writers in the occidental knowledge. Both of them constitute huge collections, just to mention the rare *Brasiliana* collection with 357 printed volumes in its complete and rare collection in Brazil.

Among the fungal species isolated as a result of this research, some deserve particular attention due to their possible cellulolytic action on books. The most important genera characterized by molecular biology will be briefly discussed, looking for evidence of cellulolytic activity, although their cosmopolitan origin. The main fungal genera found in books and in the air, after flooding in the library, are presented in Fig. 2. *Cladosporium cladosporioides, Pestalotiopsis* sp*., Hamigera paravellanea, Aspergillus flavus, Trichoderma viride, Aspergillus niger, Hypocrea lixii, Periconia macrospinosa* and *Trichoderma longibrachiatum.* After reaction through PCR in the ITS region of the isolated samples, the

migration through 1% agarose gel was performed, in order to observe the size of the amplicons generated. Images of gel eletrophoresis obtained are presented in Figs. 3 and 4.

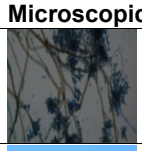
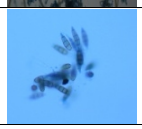
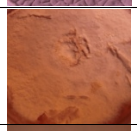
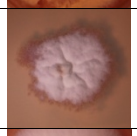
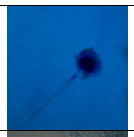
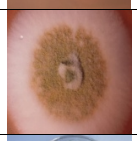
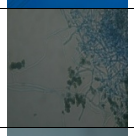
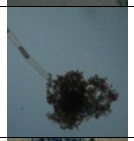
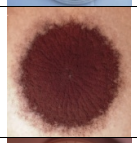
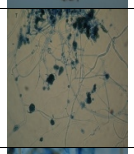
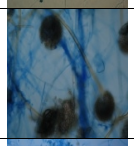
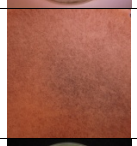
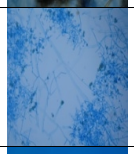
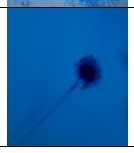
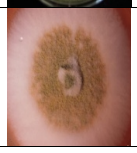
Sample	Classification	Microscopic observation	Petri dish growth
Central area of the library (Table)	*Cladosporium cladosporioides*		
	Pestalotiopsis sp.		
	Hamigera paravellanea	Not available	
	Aspergillus flavus		
Brasiliana Collection, Volumes 297-299	*Trichoderma viride*		
Shelf 16A Position 2	*Aspergillus niger*		
Shelves 13A Position 1, 18B Positions 1 & 2	*Hypocrea lixii*		
Os Pensadores Collection	*Periconia macrospinosa*		
	Trichoderma longibrachiatum		
	Aspergillus flavus		

Fig. 2. Molds in the air and special collections of books from a library in a scientific museum

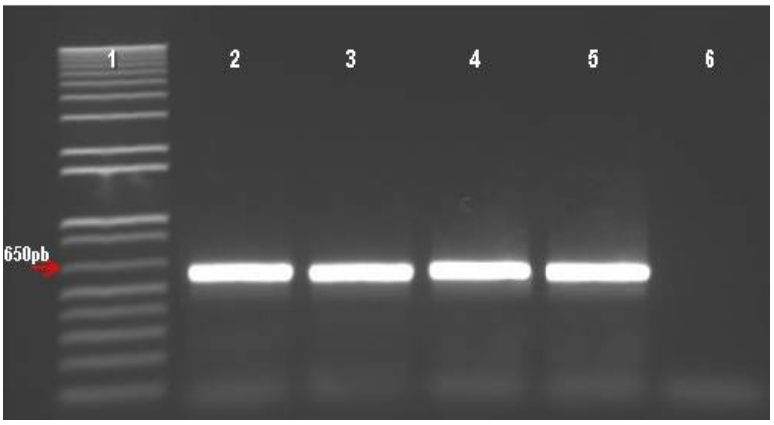


Fig. 3. Agarose gel 1% GelRed® (Invitrogen). Molecular weight standard 1Kb (1). Each code corresponds to na amplified sample, further identified as: (2) *Cladosporium cladosporioides*, (3) *Pestalotiopsis* sp, (4) *Hamigera paravellanea*, (5) *Aspergillus niger*, (6) PCR negative control

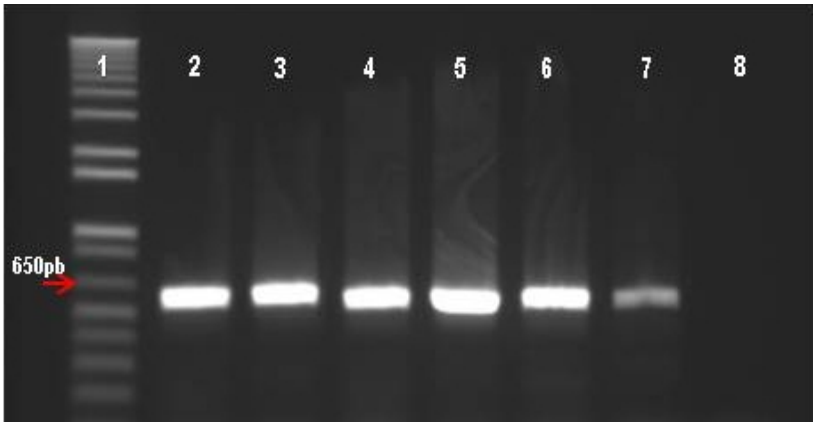


Fig. 4. Agarose gel 1% GelRed® (Invitrogen). Molecular weight standard 1 Kb (1). Each code corresponds to an amplified sample, further identified as: (2) *Aspergillus flavus*, (3) *Aspergillus flavus*, (4) *Trichoderma viride*, (5) *Hypocrea lixii*, (6) *Periconia* sp, (7) *Trichoderma longibrachiatum*, (8) PCR negative control

Regarding the chemical treatment performed on selected books contaminated with molds, it could be observed that the chemical treatment performed with the biocide (ortophenilphenol) at the reported inhibitory concentration of 200mg/L in ethanol and also in the double concentrated solution, are presented. It can be observed that ortophenilphenol at the concentration of 200 mg/L was not completely effective against all fungal species, as seen in Table 1. At this lower concentration, as suggested by the producer as a high concentration for cosmopolitan fungal species, only *Hamigera paravellanea, Aspergillus flavus* and *Hypocrea lixii* were effectively affected by the presence of the biocide. Even though species such as *Pestalotiopsis* sp., *Trichoderma viride, Aspergillus niger* and *Periconia macrospinosa* were affected by the presence of the biocide, the results were not conclusive, as observed for the triplicate results reported. However, when it was decided to use a much higher concentration of ortophenilphenol, in fact the double as suggested, proved to be

efficient against the growth of all species isolated from the library. These results indicate that the type of microbes present in the air and surfaces of the library are quite resistant to biocide treatment with ortophenilphenol, as confirmed by the trial treatment performed.

Table 1. Results from the treatment of the molds on nutrient agar with ortophenilphenol in two distinct concentrations

Fungal species	Ortophenilphenol concentration (mg/L)	
	200	400
Aspergillus flavus	- - -	- - -
Aspergillus niger	- - +	- - -
Cladosporium cladosporioides	+ + +	- - -
Hamigera paravellanea	- - -	- - -
Hypocrea lixii	- - -	- - -
Periconia macrospinosa	- + +	- - -
Pestalotiopsis sp.	- + -	- - -
Trichoderma longibrachiatum	+ - -	- - -
Trichoderma viride	- + +	- - -

+ (Positive) and – (Negative) growth in each Petri dish in triplicate). The number of (+) and (-) symbols corresponds to the number of positive or negative results of growth of isolated fungal species

4. DISCUSSION

In relation to the chemical damage observed in some pieces of the *Brasiliana* and *Os Pensadores* collections, it is supposed that the chemical wash performed by the wash water drawn from the ceiling of the library during a strong storm in the region was responsible for the damage on the leather cover of some books. The chemical damages observed in the books opened the opportunity to investigate the further susceptibility of books and flooded areas for fungal contamination. In fact, the chemical damage observed was the first indication that a biological attack could occur.

The central area seemed to be the most strongly affected space in the library. In that area it were found most of the fungal species: *Cladosporium cladosporioides, Pestalotiopsis* sp*., Hamigera paravellanea and Aspergillus flavus.* With the exception of *Aspergillus flavus*, that also was detected in *Os Pensadores* collection, the remaining species were not found on the books, indicating that they were probably present in the air of the library, and not inside the shelves, where special collections were stored.

The obtained results were in agreement with the results obtained by Sato et al. [27] who isolated several fungal species from paper-based documents, soaked with seawater from the tsunami followed the earthquake in Japan at 2011. Although their results indicated a high sodium chloride tolerance, the same genera were found. Their biodeteriorating potential was confirmed by the presence of black and red-spotted alterations in the documents, associated to the presence of cosmopolitan fungi. The remaining fungi found included: *Trichoderma viride, Aspergillus niger, Hypocrea lixii, Periconia macrospinosa and Trichoderma longibrachiatum*. An interesting point to be discussed is the lack of cross-contamination inside the library; only one fungal species was found in more than one space or object in the library, with the remaining species isolately found. This is an indication that although it is known that the library is deficient in automated humidity and temperature control, the natural ventilation in the space provides enough air quality to prevent fungal proliferation. On the other hand, when flooding took place in the library, these conditions were no longer kept,

giving place to mold growth. However, as soon as the original conditions were restored, it could be observed that cross-contamination was not observed, even after flooding. Further, some particular discussions about the genera found will be presented, in order to envisage an estimation of the potential cellulolytic action of the fungi on selected books and wooden shelves.

Cladosporium is an allergenic mold, usually associated to humans. It is commonly found as a dark brown culture easily grown in ceilings, bathrooms and water-soaked window frames. *Cladosporium* species produce a large number of spores that easily disseminates through the air when any disturbance occurs, being then inhaled causing pulmonary problems to humans and to some animal species [28]. This genus is one of the largest ones, characterized by a coronate scar structure. The authors examined a high number of isolates from the species *Cladosporium cladosporioides* based on DNA sequencing of the nuclear ribosomal RNA gene operon. As a result, 22 species were described on the basis of phylogenetic characters and morphological distinctions. According to Stepalska et al. [29] *Cladosporium* spores are predominant in the air, with changing concentrations depending on the season of the year.

In a study conducted by Foladi et al. [10] an evaluation of fungal presence in archives from a northern province of Iran, with a particular focus on *Stachybotrys chartarum*, was performed. The samples were collected from 20 archives of offices and controls using a single-stage impactor containing malt extract agar and cellulose agar. Surface samples were also collected by a cotton swab on different areas of archives.

In indoor air of archives, *Cladosporium* spp (25.1%), *Aspergillus* spp (22.9%) and *Penicillum* spp (22.9%) and *Stachybotrys chartarum* (7.9%) had the most frequencies. *Cladosporium* spp had the highest total CFU concentration in indoor air of archive samples (1227/m^3). *Stachybotrys chartarum* was recovered from surface collected samples of 4 archives of offices. Out of the 22 rooms of archives, 45.4%, 45.4% and 9.1% had concentration level <170 CFU/m^3,>170<560 CFU/m^3 and>560<1000 CFU/m^3, respectively. In the Photographic Library of the National Archive of the Republic of Cuba and also in the Historical Archive of the Museum of La Plata, the presence of fungal species with cellulolytic, proteolytic and amylolytic acitivities were investigated in order to evaluate their biodeterioration potential [30]. The methods used were the same as the ones used in the present work, based on sedimentation of particles on Petri dishes containing suitable culture media for the growth of aerial species. The authors concluded that the predominating fungal genus in the National Archive was *Cladosporium* and in the Museum of La Plata was *Penicillium*, thus confirming the ubiquitous nature of both fungi, in accordance to the present work.

Guo [31] widely studied the genus *Pestalotiopsis*, indicanting that some species are plant pathogens, and endophytes are novel metabolites producers. The authors states that a precise identification of species is still needed. Morphological characteristics of the genus showed the presence of conidia (5 cell appendages), an average size of conidium equal to 25-30µm x 4-5µm and olivaceous in shape. Usually present 2-3 apical appendages, located in the top, 10-20µm length, not knobbed. All those characteristics can not be easily identified in the results presented in Table 1, however, little information about the genus is available in the literature.

Seergiva et al. [32] described the occurrence of *Pestalotiopsis* genus in grapevines associated to several other related genera. According to the work of Schwartz [33] *Pestalotiopsis microspora* was identified by the typical five-celled spores, with three dark

center cells surrounded by two outer hyaline cells. Spores are also characterized by one basal and with three to four apical appendages. The occurrence of the species was tested in frequency of *Pestalotiopsis microspora* was examined in 62 plants across *T. taxifolia.* The fungus was isolated on 56 (90%) plants on both twig (85%) and needle (88%) tissue.

According to Peterson et al. [34] the genus *Hamigera* evolved from *Talaromyces* species that make asci singly instead of in organized chains. Formerly, just two species were known: *Hamigera avellanea* and *Hamigera striata*, and just in 2010 the new species *Hamigera paravellanea.* According to the United States Departament of Agriculture [35] the fungal species *Hamigera avellanea* and *Hamigera striata* were initially isolated from spoiled blueberries. This fungus presents a vast difference in appearance, consequently needs DNA sequence comparisons.

Khan and Karuppayil [36] reviewed the role and the ubiquitous nature of fungi in indoor environments, alerting for their action as allergenic and potential for infections and toxicity. According to the authors the predominating species found in most environments are from the same genera as found in the present work, indicating their ubiquitous presence, irrespective of the region.

Based on information provided by the Broad Institute [37], no other fungal genus contains species that are so harmful and beneficial to humans as the genus *Aspergillus*, beyond the fact that a huge number of *Aspergillus* species are of biomedical and industrial concern. For example, *A. nidulans* is a fungal species widely used for genetics and cell biology, *A. niger* is exploited by the industry for citric acid production, and *A. oryzae* involved in fermentation processes of several beverages and sauces. In opposition, *A. flavus* is a pathogen that produces aflatoxin, and several others are important opportunistic pathogens. The deep distinct characteristics presented by each of this growing set of *Aspergillus* species make *Aspergillus* a model clade to solve fundamental questions in functional and comparative genomics.

The Encyclopedia of Infectious Diseases [38] indicates that common pathogen species of Aspergillus include the species: *Aspergillus fumigatus, Aspergillus flavus*, *A. niger, A. amstelodami, A. avenaceus, A. candidus, A. carneus, A. caesiellus, A. clavatus, A. glaucus, A. granulosus, A. nidulans, A. oryzae, A. quadrilineatus, A. restrictus, A. sydowi, A. terreus, A. ustus, A. versicolor*. Those species are widely found in the environment, particularly in dust particles, soil, cereal grains and manure. The most common way of transmission is through spore inhalation. Some species causes the diseases aspergilosis, pulmonary allergies and onicomycosis. It is important to emphasize that diseases caused by the several *Aspergillus* spp are directly related to immunodeficiency.

According to Ruaudrew et al. [39], the occurrence of *Aspergillus* moulds was directly associated to the presence of the toxic aflatoxin in several dried foods. In fact, all food samples presented a high level of *Aspergillus* genera.

Guiamet et al. [40] investigated biofouling and biodeterioration of photos and maps from the Historical Archive of the Museum of La Plata in Argentine and also in repositories of the National Archive of Cuba Republic, in order to characterize and isolate molds and bacteria from the environment, through air sampling through sedimentation techniques and swabbering on maps and photos samples. The formation of biofilms were monitored by scanning electron microscopy, with a clear observation of *Aspergillus*, *Cladosporium* and *Penicillium* as predominating genera. The authors observed that the molds isolated

degraded cellulose and produced pigments and acids, thus compromising the long-term permanence of the documents.

Trichoderma spp. is a fungus usually present in agricultural soils and decaying wood. *Trichoderma* species use to grow tropically toward hyphae of other fungal species degrading the cell wall of the target fungi. It is very commom in potatoes, chilli, tomatoes, cucumbers, orchards and vineyards [41]. The International Code of Botanical Nomenclature adopted from January 2013 that only one official name will be allowed for each pleomorphic fungus. This is particularly interesting for a proper differentiation between *Hypocrea* and *Trichoderma*.

Druzhinina and Kubicek [42] relates that *Trichoderma/Hypocrea* is a fungal genus typical from soil-borne or wood-decaying origin, with important enzyme and biocontrol agents producers. They can be pathogens as well as opportunistic pathogens of immunocompromised humans. Species identification is particularly difficult through traditional methods, being necessary a combination of morphological, physiological and genetic tests for a confirmation at species level.

Oliveira [43] describes that *Periconia* sp. was described by Tode (1791) ex Fries (1821) and presents some uncommon synonym: *Sporocybe* Fries, 1825; *Sporodum* Corda, 1836; *Trichocefhalum* Costantn, 1887; *Harpocefhalum* Atkinson, 1897 e *Berkeleyna* Kuntze O., 1898 (Ellis, 1971). The genus is typically represented by 183 species, 12 varieties and 2 *formae speciales* described in the Index Fungorum. Most *Periconia* sp. species are known by its pathogenic nature as a function of its produced toxins. They are widely found in tropical and subtropical regions as well as in lakes and oceans in temperate areas. They are also widely distributed in vegetable substrates, particularly sugarcane. In Brazil, the main *Periconia* species found associated to plants are: *Periconia atra* Corda, *P. byssoides* Pers., *P. cookei* E. W. Manson & M. B. Ellis, *P. heveae* J. A. Stev. & Imle, *P. laxa* Bat. & Peres, *P. manihoticola* (Vincens) Viégas, *P. minutissima* Corda, *P. sacchari* J. R. Johnst., *P. sidae* Bat. & J. L. Bezerra e *P. stemonitis* (Pers.) Pers. (Embrapa Cenargem, 2010). The genus *Periconia* presents brownish isolated tiny colonies. The immersed mycelium sometimes presents aerial parts and stromes are commonly present.

It is commom that when a fungal contamination occurs in archives or libraries, the disinfection of the contaminated are take place by an aerial disinfection of the storerooms, once emptied of their contents. In the work conductred by Rakotonirainy et al. [44], thermal fogging with an alkyl dimethylbenzyl ammonium chloride solution has been used for the cleansing of the atmosphere contaminated by fungi. This is effective in inhibiting the spores suspended in the air but only has a weak and limited action on those species deposited on surfaces. The authors showed that a solution of thiabendazole ((thiazolyl-4)-2 benzimidazole) applied at 10% by thermal fogging, at 5mL/m^3, makes it possible the effective sanitation of the atmosphere and also present an effective action on the spores deposited on surfaces. It is important to mention that, at this concentration the chemical does not damage paper or cause any visible degradation on painted surfaces and metal shelves.

Rakotonirainy and Lavédrine [45] studied an alternative way to eliminate mould species usually found and library and archival materials. Instead of using classical alcohol preparations and disinfecting solutions, researchers decided to use essential oils. That way, the inhibitory action of vapour phase of oils of armoise, clove, boldo, eucalyptus, ravensare, lavender, tea tree, thuya, wormseed were tested against fungal species. Results indicated

that the action of linalool is fungistatic but not fungicidal at the concentrations and conditions tested. The potential use of linalool as an alternative to chemical fungicide to disinfect mouldy documents can be hard to assess in a large scale, but can be useful as a complimentary treatment to control indoor environment. The use of the oil seemed not to affect brightness of two types of paper and also did not affect the polymerization of cellulose. On the other hand, the action of the product on paper reduced its pH.

5. CONCLUSION

The small number of books impregnated with fungal colonies (around 4% of total books in the library) presented a high diversity of cosmopolitan fungi and some uncommon species for this kind of environment.

Fungal species isolated and characterized were limited to the central area of the library and specific shelves where special collections were placed.

Not necessarily fungal species isolated from air samples contaminated the special collections, indicating the lack of cross-contamination in the library.

The use of molecular biology techniques for the identification of fungal species proved to be an useful tool to explain the fungistatic action of ortophenilphenol in concentrations higher than the ones specified for type fungal species.

The rapid action of a multidisciplinary staff, proved to be essential to minimize higher damage that could occur on rare books and special collections in the flooding situation.

ETHICAL APPROVAL

Authors have obtained all necessary ethical approval from the institutions involved to present the names of the books from the special collections in the Museu de Astronomia e Ciências Afins. This confirms either that this study is not against the public interest, or that the release of information is allowed by legislation.

This work is part of the D. Sc. thesis of Fernanda do Nascimento Corrêa at PPG-EQ/UERJ.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Reis-Menezes AA, Gambale W, Giudice MC, Shirakawa MA. Accelerated testing of mold growth on traditional and recycled book paper. Int. Biodet. Biodegr. 2011;65:423-428.
2. Ortiz R, Navarrete H, Navarrete J, Párraga M, Carrasco I, De La Vega E, Ortiz M, Herrera P, Blanchette RA. Deterioration, decay and identification of fungi isolated from wooden structures at the Humberstone and Santa Laura saltpeter works: A world heritage site in Chile. Int. Biodet. Biodegr. 2014;86:309-316.

3. Koziróg A, Otlewska A, Piotrowska M, Rajkowska K, Nowicka-Krawczy kP, Hachulka M, Wolski GJ, Gutarowska B, Kunicka-Styczynska A, Libudzisz Zakowska Z, Zydzik-Bialek A. Colonising organisms as a biodegradation factor affecting historical wood materials at the former concentration camp of Auschwitz II – Birkenau. Int. Biodet. Biodegr. 2014;86:171-178.
4. Aríngoli EE, Basílico MLZ, Altahus RL, Basílico JC. Multivariate analysis of fungal associations in the indoor air of Argentinean houses. Int. Biodet. Biodegr. 2008;62:281-286.
5. Rintala H, Pitkaranta M, Taubel M. Microbial communities associated with house dust. Adv. Appl. Microbiol. 2012;78:75-120.
6. Montanari M, Melloni V, Pinzari F, Innocenti G. Fungal biodeterioration of historical library materials stored in Compactus movable shelves. Int. Biodet. Biodegr. 2012 ;75;83-88.
7. Karbowska-Berent J, Górny RL, Strzelczyk AB, Wlazlo A. Airborne and dust borne microorganisms in selected Polish libraries and archives. Build and Env. 2011;46:1872-1879.
8. Li DW, Yang CS. Fungal contamination as a major contributor to sick building syndrome. Adv. Appl. Microbiol. 2004;55:31-112.
9. Abe K, Murata T. A prevention strategy against fungal attack for the conservation of cultural assets using a fungal index. Int. Biodet. Biodegr. 2014;88:91-96.
10. Sequeira S, Cabrita EJ, Macedo MF. Antifungals on paper conservation: An overview. Int. Biodet. Biodegr. 2012;74:67-86.
11. Konkol N, McNamara CJ, Mitchell R. Fluorometric detection and estimation of fungal biomass on cultural heritage materials. J. Microbiol. Meth. 2010;80:178-182.
12. Konkol NR, McNamara CJ, Hellman E, Mitchell R. Early detection of fungal biomass on library materials. J. Cult. Heritage. 2012;13:115-119.
13. Pinheiro AC, Oliveira BP, Veríssimo C, Brandão JC, Rosado L, Jurado V, Macedo MF. Identification of a fungal community on gilded Wood carved heritage. J. Cult. Herit. 2013;14:76-81.
14. Stupar M, Grbić ML, Džamić A, Unković N, Ristić M, Jelikić A, Vukojević J. Antifulgal activity of selected essential oils and biocide benzalkonium chloride against the fungi isolated from cultural heritage objects. South Afr. J. Bot. 2014;93:118-124.
15. Neves ER, Schafer S, Philips A, Canejo J, Macedo MF. Antifungal effect of different methyl and propyl paraben mixtures on the treatment of paper biodeterioration. Int. Biodet. Biodegr. 2009;63:267-272.
16. Le Bouf RF, Schuckers SA, Rossner A. Preliminary assessment of a model to predict mold contamination based on microbial volatile organic compound profiles. Sci. Tot. Environ. 2010;408:3648-3653.
17. Weaver-Meyers PL, Stolt WA, Kowaleski B. Controlling mold on library materials with chlorine dioxide: An eight-year case study. The J. Acad. Librarianship. 1998;24:455-458.
18. Southwell KL. The use of chlorine dioxide as a mold treatment and its effect on paper acidity: A case study. The J. Acad. Librarianship. 2002;28:400-405.
19. Horner WE, Barnes C, Codina R, Levetin E. Guide for interpreting reports from inspections/investigations of indoor mold. J. Allergy and Clin. Immun. 2008;121:592-597.
20. Da Costa ACA, Lino LAS, Hannesch O. Total microbial populations in air-conditioned spaces of a scientific museum. J. Bioproc. Biotech. 2011;1:1-7.

21. Foladi S, Hedayat MT, Shokohi T, Mayahi S. Study on fungi in archives of offices, with a particular focus on *Stachybotrys chartarum*. J. Med. Mycol. 2013;23:242-246.
22. Barnett HL, Hunter BB. Illustrated genera of imperfecti fungi. Burgess Publishing Company, 3rd. Ed. 1972;4-49.
23. Ellis MB. Dematiaceous Hyphomycetes. CABI Publising. 2001;13-23.
24. Galvão M, Lutterbach MTS. Application of the qPCR technique for SRB quantification in samples from the oil and gas industries. In: Applications of molecular microbiological methods. Skovhus TS, Caffrey SM and Hubert CRJ (eds.), Caister Academic Press. 2014;215:69-77.
25. White TJ, Bruns T, Lee S, Taylor JW. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: PCR Protocols: A Guide to Methods and Applications, eds. Innis MA, Gelfand DH, Sninsky JJ, White TJ. Academic Press, Inc., New York. 1990;315-322.
26. NCBI; 2014. Available at www.ncbi.nlm.nih.gov, access on April 2014.
27. Sato Y, Aoki M, Kigawa R. Microbial deterioration of tsunami-affected paper-based objects: A case study. Int. Biodet. Biodegr. 2014;88:142-149.
28. Bensch K, Groenewald JZ, Dijksterhuis J, Starink-Willemse M, et al. Species and ecological diversity within the *Cladosporium cladosporioide*s complex (*Davidiellaceae*, *Capnodiales*). Studies Mycol. 2010;67:1-94.
29. Stepalska D, Harmata K, Kasprzyk I, Myszkowska D, et al. Occurrence of airborne Cladosporium and Alternaria spores in Southern and Central Poland in 1995–1996. Aerobiologia. 1999;15:39-47.
30. Borrego S, Guiamet P, Saravia SG, Batistini P, et al. The quality of air at archives and the biodeterioration of photographs. Int. Biodet. Biodegr. 2010;64:139-145.
31. Guo, L.D., 2011. Available:http://www.dnabarcodes2011.org/documents/presentations/122/taxsessions a/fungi1/940-Guo.pdf.
32. Sergeeva V, Priest M, Nair NG. Species of *Pestalotiopsis* and related genera occurring on grapevines in Australia. Austral. Plant Pathol. 2005;34:255-258.
33. Schwartz MW. Occurrence of *Pestalotiopsis microspora* on *Torreya taxifolia* Plant Dis. 1996;80:600-606.
34. Peterson SW, Jurjevic Z, Bills GF, Stichigel AM, et al. Genus *Hamigera,* six new species and multilocus DNA sequence based phylogeny. Mycologia. 2010;102:847-864.
35. USDA; 2014. Available:http://www.ncaur.usda.gov/research/publications/publications.htm?seq_no_115=245044.
36. Khan AAH, Karuppayil SM. Fungal pollution in indoor environments and its management. Saudi J. Biol. Sci. 2012;19:405-426.
37. Broad Institute; 2014. Available:http://www.broadinstitute.org/annotation/genome/aspergillus_group/MultiHome.html.
38. Infectopedia; 2014. Available at: http://infectopedia.com/fungos/aspergillus.
39. Ruadrew S, Craft J, Aidoo K. Occurrence of toxigenic *Aspergillus* spp. and aflatoxins in selected food commodities of Asian origin sourced in the West of Scotland. Food Chem. Toxicol. 2013;55:653-658.
40. Guiamet P, Borrego S, Lavin P, Perdomo I, et al. Biofouling and biodeterioration in materials stored at the Historical Archive of the Museum of La Plata, Argentine and at the National Archive of the Republic of Cuba. Colloids Surf B: Biointerfaces. 2011;85:229-234.

41. TradeIndia Corp; 2014. Available at: http://www.tradeindia.com/fp31012/Biofungicide-Trichoderma-viride-harzianum.html.
42. Druzhinina I, Kubicek P. Species concepts and biodiversity in *Trichoderma* and *Hypocrea*: from aggregate species to species clusters? J. Zhejiang Univ. Sci. B. 2005;6:100-112.
43. Oliveira FC. Aspectos gerais e morfológicos do fungo *Periconia* sp; 2014. Available at: http://fitopatologia1.blogspot.com.br/2010/07/aspectos-gerais-e-morfologicos-do fungo_9126.html.
44. Rakotonirainy MS, Fohrer F, Flieder F. Research on fugicides for aerial disinfection by thermal fogging in libraries and archives. Int. Biodet. Biodegr. 1999;44:133-139.
45. Rakotonirainy MS, Lavédrine B. Screening for antifungal activity of essential oils and related compounds to control biocontamination in libraries and archives storage areas. Int. Biodet. Biodegr. 2005;55:141-147.

© 2014 Costa et al.; This is an Open Access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/3.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history.php?iid=582&id=32&aid=5335