

Trichocomaceae: biodiversity of *Aspergillus* spp and *Penicillium* spp residing in libraries

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Abstract

Introduction: Atmospheric air is the most common vehicle for the dispersion of fungi. Fungi belonging to the genera *Aspergillus* and *Penicillium* are cosmopolitan and are classified in the family Trichocomaceae. Species of the genera are commonly found in soil, decaying organic materials, animal feed, stored grains, and other materials. This study aimed to determine the taxonomic diversity of airborne fungi of the genera *Aspergillus* and *Penicillium* residing in the dust of library environments to contribute to current knowledge of these characteristic genera.

Methodology: Three libraries in the city of Cuiabá, State of Mato Grosso, Brazil, were selected as the study areas. A total of 168 samples were collected at randomized sites within each library in areas containing journals, archives, in study rooms, and in collection storage areas in two different periods, the dry season (n = 42) and the rainy season (n = 42). Samples were collected by exposing Petri dishes containing Sabouraud agar with chloramphenicol to the environmental air. Additional samples were collected with sterile swabs which were rubbed over the surface of randomly chosen books on the shelves; the swabs were subsequently incubated in the laboratory.

Results and conclusion: The genus *Aspergillus* was highlighted as one of the principal airborne fungi present in indoor environments. *Aspergillus* spp was identified in 1,277 (89.6%) samples and *Penicillium* spp in 148 (10.4%). The dry period exhibited a greater number of isolates of the two taxons.

Key words: Trichocomaceae; *Aspergillus*; *Penicillium*; library; anemophilous

J Infect Dev Ctries 2011; 2012; 6(10):734-743.

(Received 12 May 2011 – Accepted 29 August 2011)

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Introduction

Atmospheric air is the most common means of dispersion among fungi, not only for the spores, but also for fragments of vegetative mycelium that become viable parts of these organisms during the process of aerial dispersion [1,2,3].

Anemophilous fungi, which possess dispersion mechanisms, have the ability to colonize different habitats and substrates in a unique way: exposing their fungal propagules and their metabolites, especially in indoor environments, such as offices, schools, hospitals and homes, they can survive large temperature fluctuations, low humidity, and large variations in pH and oxygen concentration [2-4].

Fungi belonging to the genera *Aspergillus* and *Penicillium* are cosmopolitan and generalist representatives of anamorph fungi of ascomycetes classified in the family Trichocomaceae, Eurotiales Order, with over 182 and 225 species, respectively

[5,6]. Species listed in the genera *Aspergillus* and *Penicillium* are commonly found in soil, decaying organic materials, animal feed, stored grains, and other materials [5,7,8].

Species of the genus *Aspergillus* are of great economic importance because of their biochemical properties of producing enzymes that are used in industry. However, certain species can produce secondary metabolites, called mycotoxins, which are highly hazardous to human and animal health. The biochemical properties of the genus *Penicillium* have been widely studied since their discovery in 1929, with the advent of penicillin [9].

Aspergillus species are more common in warm climates and several thermotolerant species exist [8,10,11], while *Penicillium* species are more common in regions where low temperatures prevail. Many *Penicillium* species are psychotrophic and capable of damaging foods at refrigerated

temperatures [10-12]. The two genera possess xerophilic species, but *Aspergillus* species are less tolerant of water activity than those of the genus *Penicillium* [9,13].

Fungi are considered one of the main spoilage agents of library archives, since the storage environment contains high concentrations of organic matter. This finding is often favored by the absence of adequate ventilation of collections [13,14].

Libraries could be considered important reservoirs in the etiology of respiratory diseases and as potential habitats for opportunistic agents responsible for different clinical manifestations of fungal infections, given the accumulation of book dust and the large quantities of airborne fungi as environmental polluters. Thus this study aimed to determine the taxonomic diversity of airborne fungi of the genera *Aspergillus* and *Penicillium* residing in the dust of library environments, determining the presence of pathogenic organisms and environmental contaminants and contributing to current knowledge regarding the ascomycota trichocomacea of these characteristic genera and their relation with seasonality in these environments.

Methodology

Three libraries in the city of Cuiaba, State of Mato Grosso, Brazil, were selected as the study areas. The climate is characteristic of the semiarid region, with a mean annual rainfall of 1469.4 mm and average annual temperature of 24°C to 26°C. Despite this inequality, the region is well supplied with rain and seasonality is typically tropical, with maximal temperatures in summer and minimal in winter. Over 70% of the total rainfall accumulated during the year falls from November to March. The winters are excessively dry because rainfall is very rare. The study areas are surrounded by open arboreal vegetation.

Samples were collected at randomized sites within each library, including 168 samples in areas containing journals, archives, in study rooms and in collection storage areas in two different periods: two collections during the dry season (July-August 2009) and two during the rainy season (January-February 2010), with an interval of 15 to 20 days between collections in the same period. Of these samples, 84 were collected by exposing Petri dishes (passive sedimentation technique) containing Sabouraud agar with chloramphenicol (100 mg/L) [15,16] to the environment. The Petri dishes were opened inside the room for about 30 minutes at a height of 1.2 m, in

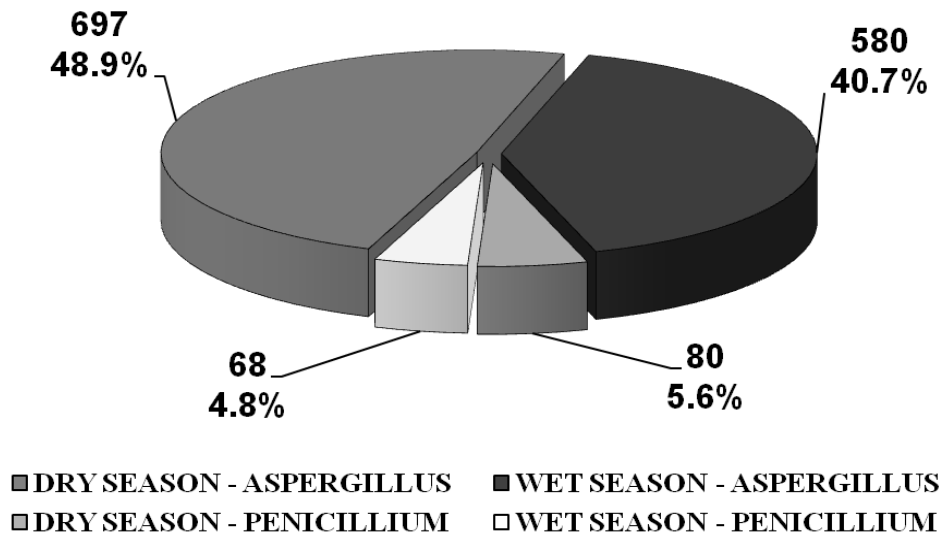
accordance with the procedure described by Gambale [17]. Additional samples were collected with the aid of sterile swabs moistened in sterile 0.20% saline and rubbed over the surface of randomly chosen books that were on the shelves. The swabs were then immediately inserted into flasks containing sterile saline for posterior incubation in the laboratory in a BOD chamber at 27°C. These techniques were chosen to compare the activity of airborne (suspended particles) and immobile fungal mycoflora (particles deposited on books, journals, *etc.*). The temperature and humidity of the library literary units were recorded using a digital hygrometer.

Following collection, the samples were sent to the Mycology Investigation Laboratory of the Faculty of Medical Sciences at the Federal University of Mato Grosso (*Faculdade de Medicina/Universidade Federal de Mato Grosso, FM/UFMT*). Colony growth was monitored for three to five days. Following this period, the presence and identification of fungal colonies were determined by macro and micromorphology. Direct isolation from the fungal culture media was performed later to confirm identification. Following purification, the samples were transferred to specific media (Czapeck and Malt agars) for observation of macroscopic structures. The agar culture media were inoculated with suspensions of conidia in solid agar [6,18] at three equidistant points on the Petri dish, forming the vertices of a triangle. The cultures were incubated at 25°C and observed after seven days of incubation [12]. To observe the microscopic structures of filamentous fungi, the Riddell technique was used [16] with microculture on slides stained with lactophenol cotton blue. Identification of species of the genera *Aspergillus* and *Penicillium* was achieved by standardized methodologies [6,8,12,13,18-21].

Results

In total 1,425 fungal isolates were obtained. They represented seven genera and 50 different species; moreover, *Aspergillus* spp was identified in 1,277 (89.6%) samples and *Penicillium* spp in 148 (10.4%). The dry period exhibited a greater number of isolates of the two taxons, with 697 (48.9%) and 580 (40.7%) *Aspergillus* spp. and 80(5.6%) and 68 (4.8%) *Penicillium* spp. identified during the dry and wet seasons, respectively (Figure 1).

Figure 1. Isolates of the genera *Aspergillus* and *Penicillium* in the library units in the dry (2009) and wet season (2010) in the city of Cuiabá, MT, Brazil



Among the species isolated, 488 (34.2%) were identified in Library A, 551 (38.7%) in Library B, and 386 (27.1%) in Library C (Tables 1 and 2).

In this work, the total number of isolates of *Penicillium* spp was 148 (11.5%) of the sample total; moreover, species classification of the subgenera identified from the literary environments of the libraries were as follows: subgenus *Penicillium* (118, 79.7%); subgenus *Furcatum* (16, 10.8%); *Aspergilloides* (10, 6.8%) and subgenus *Biverticillium* (4, 2.7%) (Table 2).

Discussion

In study area the average annual rainfall is 1,300 mm to 1,450 mm, with maximum intensity in January, February and March. The mean maximum temperature reaches 25°C to 35°C, but the absolute maximum can reach 40°C in the warmer months on rainy days. In these two periods, rainfall corresponding to 136.6 mm to 141.8 mm and 209.9 mm to 118.2 mm respectively, was detected, with accumulation of 278.4 mm during 2009 (July and August) and 328.1 mm during 2010 (January and February).

These meteorological data confirm that the climate of Cuiabá is Cwa, according to the climatic classification of Köppen-Geiger created in 1928 [22].

A Cwa climate presents subtropical characteristics, with a dry winter and a rainy summer.

This climatic condition favors fungal development and growth in environments where the concentration of organic matter is diverse.

Analysis of the results obtained in this work clearly demonstrates that the library environment and the collections therein undoubtedly house a rich and diverse source of fungal microorganisms capable of inducing allergic reactions, atopic sensitization, and potential fungal infections of various etiologies, and therefore make an important contribution to the existing relevant literature [2,4,14,17].

Numerous studies have been conducted that describe the air quality of environments [23-25], although many of these reported the taxonomic classification of microorganisms only up to fungal genus, without species identification. In this work, the genus *Aspergillus* was highlighted as one of the principal airborne fungi present in indoor environments.

Species of the genus *Aspergillus* were more frequently identified in both climatic periods, while *Penicillium* species presented a low homogeneity among the four collections performed. Regarding this fact, it should be emphasized that for certain species, the occurrence of a high incidence of fungal diversity often requires specific habitats, as well as a high frequency of essential elements, such as humidity [5,9,13,26].

Table 1. Frequency of species of the genus *Aspergillus* (anamorph and teleomorph) isolated from library units in the dry (2009) and wet season (2010) in the city of Cuiabá, MT, Brazil

SUBGENUS/SECTION/SPECIE	Library /CFUs				%
	La	Lb	Lc	total	
SUBGENUS ASPERGILLUS					
Section Aspergillus					
<i>Eurotium amstelodami</i> (= <i>A. hollandicus</i>)	2	0	0	2	0.2
<i>Eurotium chevalieri</i> (= <i>A. equitis</i>)	2	0	2	4	0.3
SUBGENUS FUMIGATI					
Section Fumigati					
<i>Neosartorya fischeri</i> (= <i>A. fischerianus</i>)	2	0	0	2	0.2
<i>Aspergillus fumigatus</i>	28	47	9	84	6,6
SUBGENUS ORNATI					
Section Ornati					
<i>Aspergillus paradoxus</i>	4	4	0	8	0.6
<i>Scleroclaista ornata</i> (= <i>A. ornatulus</i>)	1	0	1	2	0.2
SUBGENUS CLAVATI					
Section Clavati					
<i>Aspergillus clavatus</i>	3	16	2	21	1.6
SUBGENUS NIDULANTES					
Section Nidulantes					
<i>Emericella nidulans</i> (= <i>A. nidulans</i>)	0	2	5	7	0.5
Section Versicolores					
<i>Aspergillus coespitosus</i>	1	0	2	3	0.2
<i>Aspergillus sidowii</i>	4	2	0	6	0.5
<i>Aspergillus versicolor</i>	5	0	2	7	0.5
Section Usti					
<i>Aspergillus puniceus</i>	0	2	2	4	0.3
Section Terrei					
<i>Aspergillus terreus</i>	25	3	13	41	3.2
Section Flavipedes					
<i>Aspergillus flavipes</i>	7	12	0	19	1.5
<i>Aspergillus niveus</i>	0	1	1	2	0.2
SUBGENUS CIRCUNDATI					
Section Flavi					
<i>Aspergillus flavus</i>	73	99	80	252	19.7
<i>Aspergillus parasiticus</i>	38	17	11	66	5.2
<i>Aspergillus sojae</i>	5	8	0	13	1.0
<i>Aspergillus tamari</i>	1	11	4	16	1.3
Section Nigri					
<i>Aspergillus acidus</i> (= <i>A. foetidus</i>)	5	10	9	24	1.9
<i>Aspergillus carbonarius</i>	17	17	9	43	3.4
<i>Aspergillus helicotrix</i>	2	1	0	3	0.2
<i>Aspergillus japonicus</i> var. <i>aculeatus</i>	21	22	21	64	5.0
<i>Aspergillus japonicus</i> var. <i>japonicus</i>	45	24	27	96	7.5
<i>Aspergillus niger</i> var. <i>awamori</i>	9	18	3	30	2.3
<i>Aspergillus niger</i> var. <i>niger</i>	117	147	122	386	30.2
Section Circundati					
<i>Aspergillus alliaceus</i>	2	0	0	2	0.2
<i>Aspergillus melleus</i>	2	0	0	2	0.2
<i>Aspergillus ochraceus</i>	20	21	2	43	3.4
<i>Aspergillus ostianus</i>	3	0	3	6	0.5
<i>Aspergillus sclerotiorum</i>	2	0	4	6	0.5
<i>Aspergillus sparsi</i>	0	4	2	6	0.5
Section Candidi					
<i>Aspergillus candidus</i>	5	0	2	7	0.5
Total	451	488	338	1277	100

LA = Library A; LB = Library B; LC = Library C.

Fungi naturally occur under oligotrophic conditions or nutrient stress. In the presence of chemicals and metals, these elements can influence and contribute to reducing the abundance of certain species, compromising their diversity and further contributing to the physical-chemical changes by making them more or less toxic [27,28,29]. In contrast, the percentage of *Aspergillus* spp isolation could be justified by the characteristic of tolerance to high temperatures and this factor likely contributes to the high frequency of this genus in these environments [9,10,12].

In the United States, Shelton *et al.* [30] evaluated the presence of airborne fungi in 1,717 buildings from 1996 to 1998, including hospitals, homes, schools and industries. The American researchers determined *Aspergillus versicolor* as the predominant species, with a high frequency of isolation, followed by *A. flavus*, *A. fumigatus* and *A. niger*. The similarity of these species in relation to species abundance can be observed in this study, while the taxa obtained in this study are part of the cosmopolitan mycota present in virtually all terrestrial environments.

Regarding *Aspergillus* species: *A. niger* (386, 30.2%) was isolated in all four collections and was the most frequently isolated species, followed by *A. flavus* (252, 19.7%), an aspergillosis agent and potential producer of aflatoxin and *A. fumigatus* (84, 6.6%), which is undoubtedly the most important pathogenic species of *Aspergillus* yet described belonging to the section Fumigati. *A. versicolor* was isolated in Library A and Library C (7, 0.5%) (Table 1). Regarding *Penicillium* species, *P. digitatum* (37, 25.0%) and *P. expansum* (19, 12.8%) were the most frequently isolated, both identified in all three libraries, followed by *P. griseofulvum* (19, 12.8%) (Table 2).

Klich [6] reported that all species of *Aspergillus* form filamentous colonies exhibiting different characteristics and present more than a hundred species that are identified according to morphological characteristics which are currently divided into six subgenera: *Aspergillus*, *Fumigati*, *Ornati*, *Clavati*, *Nidulantes* and *Circundati*. In this work, while the species classification of the total number of isolates corresponded to 89.6% *Aspergillus* spp, all the subgenera identified were isolated in literary environments of the libraries evaluated: subgenus *Aspergillus* (6, 0.5%); subgenus *Fumigati* (86, 6.7%); subgenus *Ornati* (10, 0.8%); subgenus *Clavati* (21,

1.6%); subgenus *Nidulantes* (89, 7.0%) and subgenus *Circundati* (1065, 83.4%).

Again according to Klich [6], some species have a sexual form characterized by the presence of cleistothecia, asci and ascospores. In these cases, the fungus is classified as a teleomorph and as belonging to one of the eleven genera: *Dichlaena*, *Chaesartorya*, *Emericella*, *Eurotium*, *Fennellia*, *Hemicarpenales*, *Neopetromyces*, *Neosartorya*, *Petromyces*, *Sclerocleista* and *Warcupiella*. Among the genera of *Aspergillus* spp identified in this study, the teleomorph forms of *Eurotium* (6, 0.6%), *Neosartorya* (2, 0.2%), *Sclerocleista* (2, 0.2%) and *Emericella* (7, 0.5%) were isolated within the anamorphic genus *Aspergillus* and its subgenres (Table 1).

The *Eurotium* species identified in this study (*E. chevalieri* and *E. amstelodami*) use grains and cereals as their main substrate, like all the other *Aspergillus* identified. All species of *Eurotium* are xerophilic and the majority possess both anamorph and teleomorph forms of reproduction [7]. *Eurotium chevalieri* is a very common fungal species, especially in hot regions [12].

Domsch [8] reported that members of the group *A. ochraceus* (section *Circundati*) are widely distributed in nature and have been isolated in many parts of the world. These species are common in the microflora of decaying vegetation; moreover, the authors describe *A. ochraceus*, *A. melleus* and *A. sclerotiorum* as active representatives, especially in tropical and subtropical areas. In this study, all these species were identified and *A. ochraceus* was the most frequently isolated (43, 3.4%), occurring in all three libraries, followed by *A. sclerotiorum*, *A. ostianus* and *A. sparsi* (6, 0.5%), respectively. Other representatives of the section *Circundati* were also identified, including *A. melleus* (2, 0.2%) and *A. alliaceus* (2, 0.2%) (Table 1).

It is known that the fungi denominated “Black *Aspergilli*” include species that produce mycotoxins and this group was the most representative in this study. *A. niger* was the most frequently identified species (646, 50.5%) and was subdivided into its variations *A. niger* var. *niger* (386, 30.2%) and *A. niger* var. *awamori* (= *A. awamori*; 30, 2.3%), contributing to the records of the isolates. Other representatives of the section *Nigri* included *A. japonicus* var. *japonicus* (= *A. japonicus*; 96, 7.5%); *A. japonicus* var. *aculeatus* (= *A. aculeatus*; 64, 5.0%); *A. acidus* (= *A. foetidus*; 24, 1.9%);

Table 2. Frequency of species of the genus *Penicillium* isolated from library units in the dry (2009) and wet season (2010) in the city of Cuiabá, MT, Brazil

SUBGENUS/SECTION/SPECIES	Library / CFUs			Total	%
	LA	LB	LC		
SUBGENUS ASPERGILLOIDES					
<u>Section Aspergilloides</u>					
<i>Penicillium glabrum</i>	2	0	3	5	3.4
<i>Penicillium spinulosum</i>	5	0	0	5	3.4
SUBGENUS PENICILLIUM					
<u>Section Penicillium</u>					
<i>Penicillium camembertii</i>	1	0	0	1	0.7
<i>Penicillium commune</i>	1	2	0	3	2.0
<i>Penicillium chrysogenum</i>	0	5	6	11	7.4
<i>Penicillium digitatum</i>	11	15	11	37	25.0
<i>Penicillium expansum</i>	5	8	6	19	12.8
<i>Penicillium griseofulvum</i>	0	12	7	19	12.8
<i>Penicillium italicum</i>	5	8	0	13	8.8
<i>Penicillium restrictum</i>	4	0	0	4	2.7
<i>Penicillium sclerotiorum</i>	0	5	4	9	6.0
<i>Penicillium solitum</i>	0	0	2	2	1.4
SUBGENUS BIVERTICILLIUM					
<u>Section Simplicium</u>					
<i>Penicillium verruculosum</i>	2	2	0	4	2.7
SUBGENUS FURCATUM					
<u>Section Furcatum</u>					
<i>Penicillium simplicissimum</i>	0	0	4	4	2.7
<i>Penicillium citrinum</i>	0	4	0	4	2.7
<u>Section Eupenicillium</u>					
<i>Eupenicillium ochrosalmoneum</i>	0	1	5	6	4.1
<u>Section Lapidosa</u>					
<i>Eupenicillium cinnamupurpureum</i>	1	1	0	2	1.4
TOTAL	37	63	48	148	100

LA = Library A; LB = Library B; LC = Library C.

A. carbonarius (43, 3.4%), mentioned above; and *A. helicotrix* (3, 0.2%) (Table 1).

The biology and ecology of species of *Penicillium* and other activities produced by these fungi can be observed in several studies [3,8,18,29]. These authors reported that many species of *Penicillium* produce mycotoxins. The importance of these toxic compounds varies widely and is governed by both the biology and ecology of the species in question and by the intrinsic toxicity of the compound itself. Taking as examples species cited by these authors, *P. citreonigrum* and *P. islandicum* produce potent toxins, but since both species are rare in nature, the toxins are not important. Although *P. simplicissimum* is widely distributed and produces a potent toxin, this species is rarely found outside the soil. *P. crustosum* is very common and produces a potent thermogenic mycotoxin, though fortunately, the toxin is only produced in very high water activity. Another example is *P. verruculosum*, a species unknown in the tropics, which is widespread in the cereals sector in cold climates; consequently, mycotoxin production by this species causes severe intoxication. A review of 74 published studies conducted on soil [26] observed that the fungi of the genus *Penicillium* are among the main constituents of the mycoflora and include certain pathogenic species affecting fruits (*P. digitatum*, *P. expansum* and *P. italicum*), which were isolated in this work, participating in the mycoflora of the study sites with 37 (25.0%), 19 (12.8%) and 13 (8.8%) colonies isolated, respectively (Table 2).

Among the species considered rare in relation to the environment studied, observation revealed that *Penicillium* species considered less abundant were *P. camembertii*, with only 1 (0.7%) recorded isolation, and *P. commune* (3, 2.0%) and *P. solitum* (2, 1.4%) isolations. Two other organisms registered limited presence within the library environments surveyed, *P. simplicissimum* (4, 2.7%) and *P. verruculosum* (4, 2.7%), both reported by the authors mentioned above.

Research conducted by Horn and Peterson [31] indicates that *Eupenicillium ochrosalmoneum* and *E. cinnamopurpureum* are conidial parasites of *Aspergillus* sections Nigri and Flavi isolated from the soil. The work conducted by these researchers on their series raised this hypothesis, when assessing colonization of *Aspergillus* section Flavi on peanuts, suggesting a degree of specificity among these species. *Eupenicillium ochrosalmoneum* (= *P. ochrosalmoneum*) was isolated from two of the libraries studied, totaling 6 (4.1%) isolates and

Eupenicillium cinnamopurpureum (= *Penicillium cinnamopurpureum*) 2 (1.4%) (Table 2).

Aspergillus flavus is frequently detected in environments and its heightened importance is due to its ability to produce mycotoxins, the most abundant known as aflatoxins. Although the environments showed a greater frequency of *A. parasiticus* compared to *A. flavus* [32], the latter seems to be dominant, suggesting greater species aggressivity [10,12,33]. Other species are also mycotoxins producers, including *A. nomius* (not isolated) and *A. flavus* (252, 19.7%), *A. parasiticus* (66, 5.2%) and *A. tamaritii* (16, 1.3%), all isolated in this study. Additionally, the study isolated one of the so-called “*Koji molds*”, which include *A. oryzae* (not isolated) and *A. sojae* (13, 1.0%); these two species are recognized by the U.S. Food and Drug Administration as species that are safe to use in industrial production of food products and as not presenting a history of toxin production [34].

Regarding the recommended methodology of direct culture used for quantitative characterization, it should be emphasized that it presents certain limitations compared to current molecular techniques [35,36]; however, the great advantage of this type of methodology is that, in relation to qualitative characterization, direct culture greatly enables the isolation of fungal spores, even those in small quantities.

Besides *Aspergillus* and *Penicillium*, the airborne fungi identified in these studies were characterized as *Cladosporium* spp, *Rhizopus* spp, *Mucor* spp, *Acremonium* spp and *Fusarium* spp, demonstrating that the presence of anemophilous fungi is easy to detect, regardless of the activity. Pitt [10] discussed the importance of *Aspergillus flavus* as an aflatoxin producer and the causative agent of human deaths in parts of Africa and Asia, and the impact of ochratoxins, produced by *Penicillium verruculosum*, on human and animal health in Europe. The species reported by Pitt were isolated in Cuiabá-MT-Brazil, confirming the marked importance of these organisms due to their production of potent mycotoxins. The spores of these two taxa should also be mentioned, because according to Richardson and Warnock [40], *Penicillium* spores are likely to be found in all environments dispersed through the air, thus presenting not only wide distribution, but the most common contamination agent. According to Kern and Blevins [15], the danger of inhaling *Penicillium* spp spores lies in their ability to produce penicilliosis in debilitated individuals, characterized

by lung disease that can spread through the blood vessels, affecting the cerebrospinal fluid, kidneys and endocardium, making it a fatal agent. Regarding the toxicity of *Aspergillus* species, Denning [41] affirmed that they infect humans causing aspergillosis, a fungal disease that manifests as an allergic bronchopulmonary clinical state that can become invasive and systemic, affecting the upper airways, meninges, brain, heart, liver and bones.

Aspergilloses are commonly caused by the fumigatus, flavus and niger groups of *Aspergillus*. Other groups rarely act as agents of pulmonary disease, but it is assumed that any species can cause hypersensitivity reactions [42-44]. According to Londero and Guadalupe-Cortés [43], *Aspergillus* species isolated from patients with pulmonary aspergillosis include *A. amstelodami*, *A. candidus*, *A. carneus*, *A. fischeri*, *A. flavus*, *A. fumigatus*, *A. glaucus*, *A. niger*, *A. niveus*, *A. phialiseptus*, *A. restrictus*, *A. sydowii*, *A. terreus* and *A. versicolor*.

With the exception of *A. glaucus*, *A. carneus*, *A. phialiseptus* and *A. restrictus*, all the species described by Londero and Guadalupe-Cortés were also isolated in this study, showing the relevance and importance of identifying these species within the literary environment, as well as determining their potential effect on allergic and pulmonary reactions.

The role of fungi in allergy has been frequently overlooked. Undoubtedly, species of *Aspergillus* and *Penicillium* can be allergenic, but minimal information exists regarding the importance of certain species. In this paper, species of *Aspergillus* (89.6%) and *Penicillium* (10.4%) were isolated, organisms considered to be causative agents of respiratory tract infections in immunocompromised and immunodepressed individuals [10,37,38,39].

Some studies [45-47] reported that the main factor in fungal growth is the moisture available in the substrate, which is measured as relative humidity on the surface where fungal growth occurs, usually expressed as water activity (a_w).

Other authors [48-50] reported the effects of temperature and humidity on fungal growth and recommended the use of alternative technologies, including ventilation, to achieve the environmental conditions that diminish the level moisture in buildings, thus preventing biodeterioration of collections and documents. Furthermore, while investigating biological deterioration in books, Nyuksha [51] discovered that inside libraries, fungal spores of the group *A. flavus* germinate in paper at a relative humidity of 60% to 80%, even when the

content moisture of the substrate rarely achieves 8%. *A. flavus* is characterized by its dormant state viability and, under conditions of increased temperature and osmotic pressure, it grows at a temperature of 45 to 48°C.

In this study, *A. flavus* was detected in all three library environments evaluated, 73 (5.7%) in Library A, 99 (7.7%) in Library B and 80 (6.3%) in Library C. These values confirm the direct association of this microorganism with the collections and that it is a highly active biological agent in these environments.

Fungi of the genera *Aspergillus* and *Penicillium* are generally the most frequently isolated microorganisms. These genera can cause deterioration of stored products in numerous areas; therefore, they are economically and ecologically important. The importance of these microorganisms extends to the health risks they represent; they are omnipresent and their presence as contamination agents in libraries and literary collections studied is predictable.

The hot, humid climate of the central-western region of Brazil provides climatic conditions that favor fungal growth. Routine cleaning measures in these environments and improved aeration of the sites studied may contribute to improving the air and reducing the spread of both the vegetative forms of fungi and fungal spores. It can also be inferred that the species isolated in this work constitute a risk for the acquisition of fungal infections. The presence of aflatoxigenic and ochratoxigenic species highlights the need to adopt conservation practices and hygiene in these environments. The lack of a standardized methodology for regular maintenance at these sites may be a source of spore inhalation, responsible for the onset of respiratory diseases in users of these sites, thus justifying routine mycotoxicological monitoring at these literary units, aimed at reducing the impact of these biodeteriogenic organisms.

Acknowledgements

Financial support for this study was provided by FAPEMAT – Fundação de Amparo à Pesquisa no Estado de Mato Grosso [Foundation for the Support of Science of the State of Mato Grosso].

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Conflict of interests: No conflict of interests is declared.