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ARTICLE

Behavior of the Cultivable Airborne Mycobiota in air-conditioned environments of three Havanan archives, Cuba

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ABSTRACT

High concentrations of environmental fungi in the archives repositories are dangerous for the documents preserved in those places and for the workers' health. The aims of this work were to evaluate the behavior of the fungal concentration and diversity in the indoor air of repositories of 3 archives located in Havana, Cuba, and to demonstrate the potential risk that these taxa represent for the documentary heritage preserved in these institutions. The indoor and outdoor environments were sampled with a biocollector. From the I/O ratios, it was evident that two of the studied archives were not contaminated, while one of them did show contamination despite having temperature and relative humidity values very similar to the other two. *Aspergillus, Penicillium* and *Cladosporium* were the predominant genera in the indoor environments. New finds for archival environments were the genera *Harposporium* and *Scolecobasidium*. The principal species classified ecologically as abundant were *C. cladosporioides* and *P. citrinum*. They are known as opportunistic pathogenic fungi. All the analyzed taxa excreted acids, the most of them degraded cellulose, starch and gelatin while about 48% excreted different pigments. But 33% of them showed the highest biodeteriogenic potential, evidencing that they are the most dangerous for the documentary collections.

1. Introduction

At present the continuous knowledge and control of the environmental conditions in archive, libraries and museums constitutes of the most important elements to take into account in the preventive conservation of the Documentary Heritage of a Nation. The prevalence of inadequate environmental conditions together with the presence of high microbial concentrations in the air of the repositories of archives and library where this heritage is conserved, has been attracting the attention of researchers and specialists in the area of the conservation of heritage property, due to the risk that this implies for both for the integrity of the preserved heritage and for the health of the staff who work

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in these institutions or who receive systematic services in them [1-3]. Specifically, fungal contamination is one of the main objects of study, since fungal spores constitute one of the most numerous bioaerosols of all the biological material that is transported by air, in addition to possessing a high biodeteriogenic and pathogenic potential [1,4-8].

The existence of high values of temperature and relative humidity in countries with a tropical climate, such as Cuba, favors the increase of dust and the concentration of fungal spores and propagules in the air, as well as their deposition over different materials, facilitating the development and proliferation of fungi. They have a powerful, versatile and adaptable metabolic machinery, which allows them to degrade a wide variety of substrates, both organic and inorganic, promoting biodeterioration of the different supports that make up artworks of heritage value [9-12]. Also, fungi are characterized by having different structures and pathogenic mechanisms, which cause specific diseases in humans [1,13,14].

Numerous studies have established a close relationship between environmental conditions, the presence of viable or non-viable propagules fungal and their incidence in triggering respiratory affectations [1,3,13], achieving associate their presence with the development of symptoms belonging to these types of pathologies and others [13,16]. For this reason, multiple research groups recommend the need to increase the frequency of systematic studies of environmental conditions in premises to assess the quality of the environments, in order of guaranteeing an environmental characterization of the same to solve problems associated with the development of pests and/or affections to the health of the personnel precociously.

Taking these aspects into account, the National Archive of the Republic of Cuba (NARC) has been investigating the environmental quality of the documental repositories not only of the institution itself but also of other archives in the country. For this reason, the aims of this work were: (1) to evaluate the behavior of the fungal concentration of the indoor air in repositories of 3 archives located in Havana, Cuba, (2) to determine the density and relative frequency of the isolated taxa in order to know their ecological and environmental impact, and (3) demonstrate the potential risk that these taxa represent for the documentary heritage preserved in these institutions.

2. Materials and Methods

2.1 Characteristics of Repositories

The study was carried out in air-conditioned repositories of three institutions that preserve documents with heritage value. They were the Map library (ML) in the National Archive of the Republic of Cuba (NARC), two premises of the same repository in the Cuban Industrial Property Office (CIPO) and two repositories of the Library of Standard (LS) belonging to the National Center for Management and Development of the Quality.

Both the CIPO and the NARC are located in the Habana Vieja municipality a few streets away from each other, while the LS is located in an adjoining municipality (municipality of Centro Habana) about 2.3 Km away from the NARC and CIPO approximately.

The ML is a large repository measuring 15.2 x 6.2 x 5 m (length x width x height) and is located on the first floor and south side of the building, has several air conditioners that maintain an annual average temperature between 23 and 26°C. This repository preserves a total of 195 lineal meters of maps, elaborated mostly in different types of papers.

The premises of the CIPO are located on the ground floor of the building and are arranged one below the other (A and B) in the form of a mezzanine built with steel and concrete beams, their dimensions are 17 x 8 x 5 m and they share the same air conditioning system with an average annual temperature that ranges between 22°C and 24°C. This institution conserves documentary funds of great value from the 18th century to the present and has a total of 1265136 documents in paper format mainly (inventions, industrial models, scientific discoveries, trademarks and other distinctive signs).

The repositories of the LS are smaller and measure 6 x 7 x 2.5 m approximately, they conserve the national norms of quality on paper support. These repositories are located on the ground floor of the building and are acclimated through a centralized climate system that works only during work hours. This repositories do not have windows and only communicate with the building itself through its access door.

2.2 Sampling and Mycological Analysis of the Air

For sampling, 11 points were selected in the CIPO (A: 6 in the premises below and B: 5 in the premises above). Also, outdoor air sample was taken from the courtyard located in the central area of the building. In the ML, 5 points were analyzed and on the roof of the building the outdoor air was analyzed while in the LS 6 points were selected in total (3 in each repository) and one outside the building (entrance) (Figure 1) . These sampling points were determined according to Sánchis (2002) [17]. All samples were taken between 10:00 am and 1:00 pm, considering the possibility of the highest concentrations of fungal propagules in the city's atmosphere [18].

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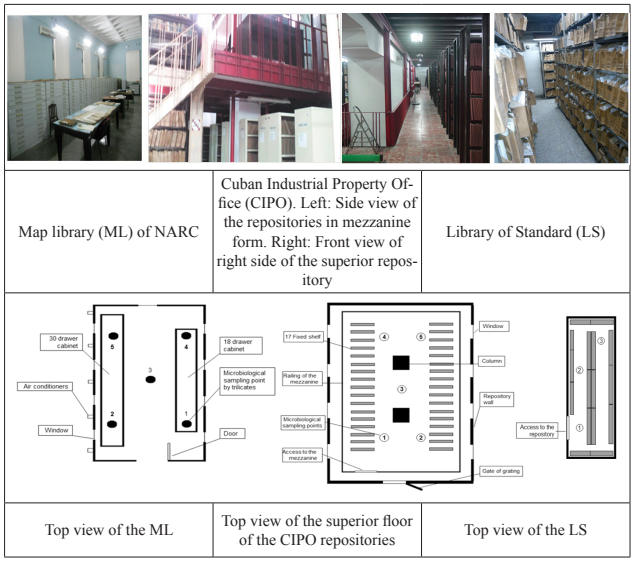

Map library (ML) of NARC	Cuban Industrial Property Office (CIPO). Left: Side view of the repositories in mezzanine form. Right: Front view of right side of the superior repository	Library of Standard (LS)
Top view of the ML	Top view of the superior floor of the CIPO repositories	Top view of the LS

Figure 1. Representative images of the studied repositories and microbiological sampling points in each analyzed repository. In Map library (ML) 5 points were analyzed, in CIPO 6 points on the floor below and 5 points on the superior floor were sampled (total = 11 points) and in LS 3 points were sampled

Culturable airborne fungi were sampled at each point by triplicate using a Super 100 SAS collector (Italy) and flow rate analyzed was 100 L/min at 1 hour intervals between replicates. The culture medium used for the isolation was Malt Agar Extract (BIOCEN, Cuba) supplemented with NaCl (7.5%) [19,20]. Once the sampling was completed, the Petri dishes were incubated at 30°C for 7 days and the isolation of the different colonies was carried out. Then, the colony count was performed and the necessary calculations of air were made in order to determine the microbial concentration expressed in colony forming units per cubic meter (CFU/m^3).

In parallel, temperature (T) and relative humidity (RH) at each sampling point were measured in situ during sampling.

2.3 Identification of the Fungal Isolates

Cultural and morphological characteristics of fungal colonies as well as conidiophores and conidia fungal structures were observed under a trinocular microscope optic with an attached digital camera (Samsung, Korea) and the identification was performed according to different manuals [21-30].

2.4 Ecological Criteria to the Environmental Taxa Isolated from the Repositories

Relative density (RD) of the fungal genera or species isolated from indoor air of each repository was conducted according to Smith (1980) [31] where:

RD = (Number of colonies of the genus or species/ Total number of colonies of all genera or species) x 100

The relative frequency (RF) determination was made according to Esquivel et al. (2003) [32] to determine the ecological category of each fungal genus or specie isolated. It was necessary to use the following formula:

RF = (Times a genus or specie is detected/Total number of sampling realized) x 100

The ecological categories are: Abundant (A) with RF = 100 – 81%; Common (C) with RF = 80 – 61%; Frequent (F) with RF = 60 – 41%; Occasional (O) with RF = 40 – 21%; Rare (R) with RF = 20 – 0. %.

2.5 Determination Semi-quantitative of the Biodegradation Potential of the Isolated Taxa

2.5.1 Determination of Enzymatic Index (EI)

To quantify the cellulolytic, amylolytic and proteolytic enzymatic index (EI), the following formula was used [5,33]:

EI = 1- Dc / Dca

Where Dc is the colony diameter and Dca is the sum of Dc and the diameter of the hydrolysis zone. Values between 0.5 and 0.59 were classified as low EI, between 0.6 and 0.69 as moderate EI, and above 0.7 as high. Each determination was made in triplicate and averages are reported.

2.5.2 Cellulolytic Enzymatic Index (CEI)

The strains were inoculated in Petri dishes containing an agar medium, the saline composition of which for one liter was: sodium nitrate 2g, potassium phosphate 1g, magnesium sulfate 0.5g, ferrous sulfate 0.01 g, chloride potassium 0.5g, yeast extract 0.5g and 20g of agar technical No. 1. As a carbon source, carboxymethyl cellulose (CMC) at 1% was added and incubated at 30°C. After seven days, a solution of Congo Red (0.05g/L) was added to each dish and was maintained by one hour, then that solution was decanted and NaCl at 1 mol/L was added for 10 min. Cellulolytic activity was evidenced by the formation of a white halo around the colony [34, 35].

2.5.3 Amylolytic Enzymatic Index (AEI)

An agar medium of saline composition similar to that used in the previous test was prepared in Petri dishes and was inoculated with each strain. Starch (1%) was added as a carbon source. After incubating for 7 days at 30°C, a Lugol reagent solution was added into each culture dish. The presence of a colorless halo around the colonies evidenced the starch hydrolysis [9, 36].

2.5.4 Proteolytic Enzymatic Index (PEI)

The strains were inoculated in dishes containing an agarized culture medium with a saline composition

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similar to that used previously, with gelatin as the carbon source (1%). The dishes were incubated at 30ºC; the test reading was performed at 7 days of incubation with the addition of the Frazier reagent. A white precipitate around the colony (halo) is indicative of the presence of non-hydrolyzed gelatin but the colorless halo revealing the gelatin hydrolysis [37].

2.5.5 Determination of the Acid Excretion

0.1 ml of a conidia suspension of each strain was inoculated into a culture broth with a saline composition similar to the medium used to determine cellulite activity. Glucose (1%) was used as the carbon source; the pH was adjusted to 7 and 0.03% of phenol red was added as indicator. The cultures were incubated at 30°C for 3 days and the pH of the broth was subsequently measured with a pH meter (Pacitronic MV 870, USA), whose precision is ± 0.2 units. The positive result was corroborated by the change in the color of the phenol red indicator (from red to yellow) and the detection of pH values less than 7 [20,36].

2.5.6 Determination of Extracellular Pigments Excretion

The strains were inoculated in tubes with slants containing an agarized culture medium with a saline composition similar to CMC medium but with dextrose as the carbon source (1%). The tubes were incubated at 30ºC during 7 days and excretion of diffusible pigments was observed in the culture medium of each tube. This determination is a modification of those reported by Borrego et al. (2010) [36]. Also, the pigments excretion in the medium with CMC was taken into account.

2.6 Statistical Analysis

The ANOVA-1 and Duncan tests were used to compare the fungal concentration obtained on the indoor of the three archives environments as well as to compare the enzymatic activities among strains. A P value smaller or equal to 0.05 was considered statistically significant.

3. Results

3.1 Fungal Concentration and Diversity Detected on Indoor Air of the Repositories

When analyzing the fungal concentrations in the indoor air of the different archives (Table 1), the significantly highest fungal concentration was detected in the LS (133.9 CFU/m^3) despite having values of T and RH similar to those obtained in the other two archives. The other repositories showed similar concentrations (CIPO with 42.7 CFU/m^3 and ML of NARC with 40.8 CFU/m^3).

Table 1. Fungal concentrations detected on the indoor and outdoor environments of the three studied archives located in Havana, Cuba

Concentrations	CIPO				Library of Standard (LS)				Map Library (ML) of NARC			
	Fungi indoor (CFU/m^3)	T (^{0}C)	HR (%)	Fungi out-door (CFU/m^3)	Fungi indoor (CFU/m^3)	T (^{0}C)	HR (%)	Fungi outdoor (CFU/m^3)	Fungi indoor (CFU/m^3)	T (^{0}C)	HR (%)	Fungi outdoor (CFU/m^3)
Maximum	80	26.2	59.9	150	280	27.0	57.9	90	70	22.9	51.4	290
Minimum	55	25.3	54.4	90	130	25.8	52.8	45	20	24.4	49.6	150
Average ± SD	42.7±26.0 **a**	25.7±0.3	56.5±1.8	103.3±40.4	133.9±72.0 **b**	26.2±0.3	56.1±1.7	53.0±24.4	40.8±20.6 **a**	23.5±0.5	50.3±0.7	208.0±51.0
I/O ratio	0.4				2.5				0.2			

Notes:

SD: Standard deviation. The determinations in CIPO were made in 11 points, in LS were made in 6 points and in the ML 5 points were analyzed by triplicate, respectively; hence the data averaged were: n = 33 (CIPO), n = 18 (LS), n = 15 (ML). a, b: Indicates significant differences according to the Duncan test ($P \leq 0.05$) on comparing the fungal concentration obtained in indoor air of the archival environments studied. I/O ratio = Indoor concentration/Outdoor concentration.

Simultaneous external air determinations in the outdoor of each archive were made with the intention to estimate the I/O ratio and to define the air quality in their environments. The I/O ratios obtained were 0.4 for CIPO, 2.5 for LS and 0.2 for ML. In this case the I/O ratio of LS was markedly higher indicative of a contaminated environment in spite of having values of T and RH similar to those that have the other two archives.

In this study a total of 12 genera of filamentous fungi and 2 non-sporing mycelia (WNSM: White Non-sporulating Septated Mycelia, PNSM: Pigmented Non-sporulating Septated Mycelia) were detected on indoor environments whilst a total of 13 genera and 2 non-sporulating mycelia were also detected in outdoor environments (Figure 2).

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From indoor environments a total of 6 taxa were isolated by the CIPO, 4 taxa by LS and 8 taxa by ML, but in all of them *Aspergillus, Cladosporium* and *Penicillium* genera as well as a white non-sporulating septated mycelium (WNSM) were detected and for these reasons were ecologically classified as abundant. *Penicillium* spp. prevailed in CIPO and in LS environments but in ML of NARC *Cladosporium* was the genus predominant. On the other hand, other genera isolated from the CIPO were *Acrodontium* and *Cylindrocarpon*; from LS the other genus was *Trichophyton* and from ML other 5 genera were isolated too (*Chrysosporium, Harposporium, Neurospora, Nigrospora, Scolecobasidium*).

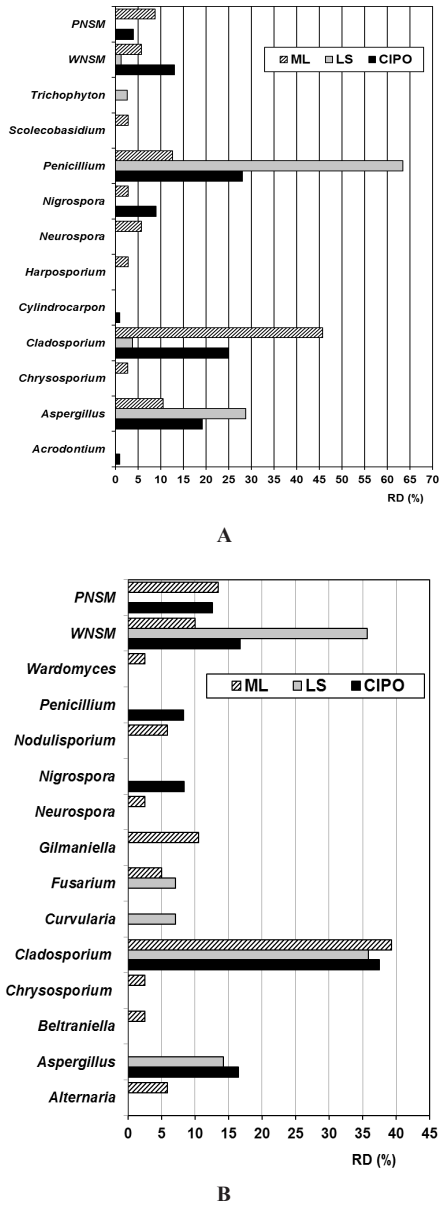

Figure 2. Relative density (RD) of the taxa detected on the indoor (A) and outdoor (B) environments of the three studied archives located in Havana, Cuba.

Note:

Mycelium, WNSM: White Non-sporulating Septated PNSM: Pigmented Non-sporulating Septated Mycelium.

The biggest diversity of taxa was detected in the indoor environment of ML with 10 of them, but 45.5% of the taxa detected were part of the repository's environment itself while the other 54.5% appear to come from abroad; in this case the highest incidence was the *Cladosporium* spp. Although in LS the number of taxa detected was markedly lower, 60% of them to come from the outdoor environment with a high incidence of the *Aspergillus* spp. while in CIPO the 100% of taxa detected indoor environments to come from outdoor with a high impact of the *Cladosporium* spp. too, but contrary to the external impact the prevalence on indoor was the *Penicillium* spp.

Although a great diversity of species was detected in general only 3 taxa were ecologically abundant (*Cladosporium cladosporioides, Penicillium citrinum* and WNSM), 9 were common taxa (*Aspergillus ochraceus, Aspergillus flavus, Aspergillus oryzae, Aspergillus versicolor, Nigrospora sphaerica, Penicillium griseofulvum, Penicillium oxalicum, Penicillium simplicissimum* and PNSM), and 31 were classified as occasional taxa (Table 2).

Table 2. Relative density (RD) of the fungal taxa detected on the indoor air of the three studied repositories as well as their relative frequency (RF) and ecological category (EC)

Taxa	CIPO	LS	ML	RF (%)	EC
	RD (%)				
Acrodontium simplex (Mangenot) de Hoog	2	0	0	33.3	O
Aspergillus athecius Raper & Fennell	0	0	2.8	33.3	O
Aspergillus candidus Link	2.7	0	0	33.3	O
Aspergillus chevalieri L. Mangin	1.0	0	0	33.3	O
Aspergillus flavipes (Bain & Sart) Thom & Church	1.0	0	0	33.3	O
Aspergillus flavus Link	2.8	0	2.8	66.7	C
Aspergillus glaucus Link (complex)	0	10.1	0	33.3	O
Aspergillus niger Tiegh.	0	1.8	0	33.3	O
Aspergillus niveus Blochwitz	1.0	0	0	33.3	O
Aspergillus ochraceus K. Wil.	0	2.6	2.8	66.7	C
Aspergillus oryzae (Ahlb.) Cahn	1.0	2.5	0	66.7	C
Aspergillus parasiticus Speare	0	2.5	0	33.3	O
Aspergillus penicilloides Spegazzini	0	6.3	0	33.3	O
Aspergillus unguis (Emile-Weil & Gaudin) Thom & Raper	5.5	0	0	33.3	O
Aspergillus versicolor (Vuill.) Tiraboschi	1.0	5.1	0	66.7	C
Aspergillus wentii Wehmer	1.0	0	0	33.3	O
Cladosporium caryigenum (Ellis & Lang)	0	0	8.5	33.3	O
Cladosporium cladosporioides (Fresen) G.A. de Vries	15.0	3.8	10.0	100	A
Cladosporium coralloides W. Yamamoto	0	0	2.8	33.3	O
Cladosporium gossypiicola Pidoplichko & Deniak	0	0	2.8	33.3	O
Cladosporium herbarum (Pers.: Fr.) Link	0	0	2.8	33.3	O
Cladosporium hillianum Bensch, Crous & U. Braun	10.0	0	0	33.3	O
Cladosporium lignicola Corda	0	0	2.8	33.3	O
Cladosporium sphaerospermum Penz.	0	0	2.8	33.3	O

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Cladosporium staurophorum (Kendrick) M. B. Ellis	0	0	5.7	33.3	O
Cladosporium tenuissimum Cooke	0	0	2.8	33.3	O
Chrysosporium sp. Corda	0	0	2.7	33.3	O
Cylindrocarpon lichenicola (C. Massal.) D. Hawksw.	1.0	0	0	33.3	O
Harposporium sp. Lohde	0	0	2.8	33.3	O
Neurospora crassa Shear & B.O. Dodge	0	0	5.7	33.3	O
Nigrospora oryzae Hudson	0	0	2.8	33.3	O
Nigrospora sphaerica (Sacc.) E. W Mason	9.0	0	2.8	66.7	C
Penicillium chrysogenum Thom	0	10.2	0	33.3	O
Penicillium citreonigrum Dierckx	2.0	0	0	33.3	O
Penicillium citrinum Thom	5.0	11.5	8.5	100	A
Penicillium commune Thom	0	3.1	0	33.3	O
Penicillium griseofulvum Dierckx	5.0	4.0	0	66.7	C
Penicillium oxalicum Currie & Thom	16.0	3.0	0	66.7	C
Penicillium simplicissimum (Oud.) Thom	0	29.6	1.0	66.7	C
Scolecobasidium sp. E.V. Abbott	0	0	2.8	33.3	O
Trichophyton sp. Malmsten	0	2.6	0	33.3	O
PNSM	4.0	0	8.4	66.7	C
WNSM	14.0	1.3	5.6	100	A

Notes:

WNSM: White Non-sporulating Septated Mycelium. PNSM: Pigmented Non-sporulating Septated Mycelium. According to Esquivel et al. (2003) [32] when RF = 100 - 81% the taxon is considered ecologically Abundant (A); 80 - 61% is Common (C); 60 - 41% is Frequent (F); 40 - 21% is Occasional (O); 20 - 0.01% as Rare (R).

In relation to the *Aspergillus* spp., it was evidenced a high variety of species, since there were 15 identified in total. Of these, 10 species were isolated in the indoor environment of CIPO, 6 in the LS environment and only 3 in the ML environment. None of them turned out to be ecologically abundant. However, 4 species were ecologically common to have been detected in two of the three archives what represents the 44.4% of all taxa that were ecologically common.

From the 9 species of *Cladosporium* spp., only *C. cladosporioides* had an important ecological representation, the rest were occasional species because they were only detected in a single archive, mainly in ML.

About the 7 species of *Penicillium* spp. 4 of them (71.4%) were ecologically important (1 was abundant and 3 were common) for the LS environment fundamentally.

3.2 Biodegradative Assays Evaluation

In relation to the degradative activities (Table 3), the majority of the taxa (93.6%) degrade in more or smaller measure the cellulose but it is of emphasizing a group of 13 taxa that showed the highest CEI (EI ≥ 0.7). They were *A. flavus 1, A. niger, A. ochraceus, Cladosporium caryigenum, Neurospora crassa, Nigrospora oryzae, P. chrysogenum, P. citrinum* 1 and 2, *P. griseofulvum, P. oxalicum* 3, *P. simplicissimum* and WNSM. It is worth highlighting in this group the predominance of *Penicillium* spp. (46.2%). In a second place for having a moderate EI, 16 strains (34%) were found with a predominance of species of the genus *Aspergillus* with a 37.5% (*A. athecius, A. flavipes, A. flavus 2, A. ochraceus 2, A. oryzae, A. versicolor*). Of the rest, 15 taxa showed low cellulose degradative activity (31.9%) and 3 did not degrade the polymer.

Table 3. Enzymatic index (EI) of the taxa isolated from the indoor air of the studied archives to assess their biodeteriogenic potential on several materials that conform the archives collections

Origin	Specie/Mycelium	Cellulolytic Activity	Amilolytic Activity	Proteolytic Activity	Acids production (pH)	Pigment Excretion *
		CEI	AEI	PEI		
CIPO	*Acrodontium simplex*	0.53 c	0.50 b	0.62 fg	5.02 hijklm	-
ML	*Aspergillus athecius*	0.60 ef	0 a	0 a	5.90 qrst	-
CIPO	*Aspergillus candidus*	0.51 b	0.62 fg	0.58 de	6.07 qrst	-
CIPO	*Aspergillus chevalieri*	0.50 b	0.65 gh	0.68 h	4.46 deg	-
CIPO	*Aspergillus flavipes*	0.60 ef	0.63 fg	0.58 de	3.72 bc	+ (yellow)
CIPO	*Aspergillus flavus* 1	0.73 j	0.74 j	0.74 j	6.22 stuv	-
ML	*Aspergillus flavus* 2	0.63 fg	0.71 ij	0.56 cd	4.62 egh	-
LS	*Aspergillus glaucus*	0 a	0 a	0 a	6.60 vw	-
CIPO	*Aspergillus niger*	0.72 ij	0.71 ij	0.73 j	5.41 nño	-
CIPO	*Aspergillus niveus*	0.59 de	0.57 d	0 a	5.30 mn	+ (yellow)
LS	*Aspergillus ochraceus* 1	0.72 ij	0.69 hi	0.74 j	5.40 nño	+ (brown)
ML	*Aspergillus ochraceus* 2	0.66 gh	0.54 c	0.57 d	6.12 rstu	+ (brown)
CIPO	*Aspergillus oryzae*	0.65 gh	0.68 h	0.71 ij	4.33 de	+ (yellow)
LS	*Aspergillus penicilloides*	0.55 cd	0.58 de	0.66 gh	6.50 uvw	-
CIPO	*Aspergillus unguis*	0.57 d	0.56 cd	0.59 de	5.82 opqrs	+ (yellow)
LS	*Aspergillus versicolor*	0.60 ef	0.68 h	0.62 fg	4.13 d	-
CIPO	*Aspergillus wentii*	0.52 bc	0.55 cd	0.53 c	4.82 ghijk	-
ML	*Cladosporium caryigenum*	0.70 hij	0 a	0 a	6.25 stuvw	+ (green olive)
ML	*Cladosporium cladosporioides*	0.66 gh	0.58 de	0.70 hij	3.34 ab	+ (brown)
ML	*Cladosporium coralloides*	0.58 de	0.55 cd	0 a	5.85 pqrs	+ (brown)
ML	*Cladosporium gossypiicola*	0.65 gh	0.68 h	0.56 cd	5.72 ñopqr	+ (green dark)
ML	*Cladosporium herbarum*	0.68 h	0.72 ij	0.62 fg	6.50 uvw	+ (green dark)
CIPO	*Cladosporium hillianum*	0.58 de	0.53 c	0.54 c	4.16 d	+ (amber dark)
ML	*Cladosporium lignicola*	0.52 bc	0.58 de	0.60 ef	6.60 vw	+ (brown)
ML	*Cladosporium sphaerospermum*	0.66 gh	0.54 c	0 a	6.30 tuvw	+ (green dark)
ML	*Cladosporium staurophorum*	0.56 cd	0.62 fg	0 a	6.30 tuvw	+ (brown)
ML	*Cladosporium tenuissimum*	0.52 bc	0.63 fg	0.54 c	6.11 qrstu	+ (brown)
ML	*Chrysosporium sp.*	0.60 ef	0.65 gh	0.56 cd	6.40 tuvw	+ (amber dark)
CIPO	*Cylindrocarpon lichenicola*	0 a	0 a	0.63 fg	3.65 bc	-
ML	*Harposporium* sp.	0.69 hi	0.65 gh	0.60 ef	3.52 abc	-
ML	*Neurospora crassa*	0.73 j	0.68 h	0.72 ij	5.10 jklmn	+ (orange clearing)
ML	*Nigrospora oryzae*	0.72 ij	0.73 j	0.71 ij	5.10 jklmn	+ (brown)
CIPO	*Nigrospora sphaerica* 1	0.55 cd	0 a	0 a	5.21 lmn	-
ML	*Nigrospora sphaerica* 2	0.64 g	0.56 cd	0.68 h	5.84 pqrs	-
LS	*Penicillium chrysogenum*	0.70 hij	0.69 hi	0.74 j	4.80 ghijk	-
CIPO	*Penicillium citreonigrum* 1	0.59 de	0.66 gh	0.71 ij	4.45 deg	-
CIPO	*Penicillium citreonigrum* 2	0.62 fg	0.54 c	0.64 g	6.01 ghijk	-
LS	*Penicillium citrinum* 1	0.72 ij	0 a	0.61 ef	5.27 lmn	+ (yellow)
ML	*Penicillium citrinum* 2	0.73 j	0.72 ij	0.70 hij	4.36 deg	+ (yellow)
LS	*Penicillium griseofulvum*	0.71 ij	0.69 hi	0.62 fg	5.15 klmn	-
CIPO	*Penicillium oxalicum* 1	0.62 fg	0.57 d	0.72 ij	3.21 a	-

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LS	*Penicillium oxalicum* 2	0.63 fg	0.52 bc	0 a	5.05 ijklmn	-
LS	*Penicillium oxalicum* 3	0.70 hij	0.68 h	0 a	4.72 eghij	-
LS	*Penicillium simplicissimum*	0.71 ij	0.68 h	0.58 de	6.60 vw	-
ML	*Scolecobasidium* sp.	0 a	0.56 cd	0.62 fg	6.64 w	-
LS	WNSM	0.72 ij	0.65 gh	0.58 de	5.35 mnñ	+ (yellow)
ML	PNSM	0.58 de	0.62 fg	0.67 h	5.19 klmn	+ (brown)

Notes:

CEI: Cellulolytic Enzymatic Index. AEI: Amilolytic Enzymatic Index. PEI: Proteolytic Enzymatic Index. Enzymatic index (EI) = 0.5 - 0.59 is low, EI = 0.6 - 0.69 is moderate, EI > 0.7 is high. +: indicates excretion of pigments. - : Indicates no excretion of pigment. Values of pH < 7 are indicative of the acids production. WNSM: White Non-sporulating Septated Mycelium. PNSM: Pigmented Non-sporulating Septated Mycelium. a - w: Different letters indicate significant differences according to Duncan test among strains in the same column ($P \leq 0.05$). *: These pigments were detected in CMC medium and a culture medium with similar composition to CMC but with dextrose as the carbon source (1%).

Regarding starch, 41 taxa (87.2%) degraded this polymer, only they did not do it with the same intensity. Six species (12.8%) showed a high AEI (*A. flavus* 1 and 2, *A. niger, Cladosporium herbarum, Nigrospora oryzae, P. citrinum* 2) while 19 taxa revealed moderate activity (40.2%), 16 showed a low degradation (34%) and 6 species did not degrade this nutrient. Likewise, 35 taxa degraded gelatin (74.5%), but 11 species stood out for showing a high PEI (*A. flavus 1, A. niger, A. ochraceus 1, A. oryzae, Cladosporium cladosporioides, Neurospora crassa, Nigrospora oryzae, P. chrysogenum, P. citreonigrum 1, P. citrinum 2, P. oxalicum 1*), which represents 23.4% of the total of taxa, while 14 strains (29.8%) degraded it moderately, 12 strains revealed low degradative power (25.5 %) and 10 did not degrade it (21.3%).

Although the acid was excreted by all the taxa, it is necessary to highlight that 14 of them (29.8%) were those that more lowered the pH of the culture medium (*A. chevalieri, A. flavipes, A. oryzae, A. versicolor, A. wentii, Cladosporium cladosporioides, Cladosporium hillianum, Cylindrocarpon lichenicola, Harposporium sp., P. chrysogenum, P. citreonigrum 1, P. citrinum 2, P. oxalicum 1 and 3*) while 21 taxa excreted different pigments (47.7%) with prevalence of the yellow, amber and brown colors.

Among these taxa 4 species were very important for documentary biodeterioration because they evidenced the highest enzymatic index related to the degradation of cellulose, starch and gelatin.

It is important accentuating that when a strain has several degradative potentialities more dangerous is for the conservation of documents; because it can use the paper components as nutritious in a vigorous way if the T the RH is already appropriate for its growth. The figure 3 shows the results in this sense. It can appreciate that 27% of taxa revealed 4 biodeteriogenic attributes while 33% of them exhibited 5 attributes; these represent a total of 60% the strains with high potentialities to degrade the majority of the paper components indicative of their high biodeteriogenic power.

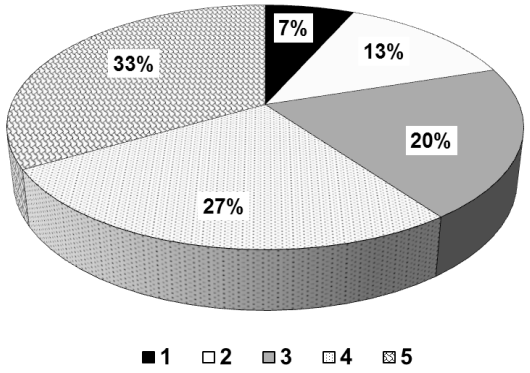

Figure 3. Behavior of the combination of different biodegradative attributes related to the enzymatic characteristics of the analyzed fungal strains detected on the indoor air of the studied archives

4. Discussion

The influence of T or RH or even of the two parameters together, on the behavior of indoor fungal concentration and its diversity has been reported by several previous studies [5-7, 38-40], however, this behavior has not been evidenced in this study where the evaluated repositories are air-conditioned and have similar average values of T and RH. Therefore, this study has shown that the high degree of air stagnation, the lack of air exchange with the outside and the existence of a high content of dust inside in some repositories were the factors that had a marked impact on the behavior of the quality of the studied environments and not the thermohygrometric values.

The environmental study of the three archives showed some differences in the obtained concentrations; in particular it was found that the LS value was significantly higher despite the values of T and RH were similar (Duncan test, $p \leq 0.05$). Despite this, the concentrations in all cases were lower than 150 CFU/m^3 which is indicative that the environments have low fungal loads according to the criteria of Roussel et al. (2012) [15]. However, the concentrations of fungi in the outdoor environments were higher than the indoor ones in the cases of CIPO and ML while for LS the opposite occurred, the outdoor concentration was lower.

Since there is still no standard in Cuba to evaluate the microbiological quality of indoor environments in archives, libraries and museums, comparisons were made with the report of French authors' mainly [15]. The results

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indicate in all cases that the environments had a low concentration (less than 170 CFU/m^3). The comparisons made with the value given by American Conference of Governmental Industrial Hygienists Guidelines (100 CFU/m^3) indicate that only the LS environment was contaminated, but the comparison with the World Health Organization Guidelines (500 CFU/m^3) [41] evidence that all environments were not contaminated. However, the climatic conditions of Cuba differ from those of France or the United States or other European countries where the environmental studies have been carried out with greater frequency, for having a humid and very warm climate; so we consider that the best way to classify the quality of an indoor environment was by analyzing the relationship between indoor and outdoor concentrations (I/O ratio) according to the recommendations made by other authors [6, 42, 43]. The obtained results show that in the case of LS the I/O ratio was markedly higher to 1 (I/O = 2.5), indicative of a contaminated environment, with little circulation of the air indoor the repositories and poor environmental quality [6, 44, 45]. On the contrary, in the case of CIPO and ML the I/O ratios were less than 1, indicating that there has been a good exchange with the outdoor environment despite the fact that the repositories are air-conditioned. In these archives, most of the fungi detected come from outdoor sources.

This environmental behavior in LS can be due to the fact that these repositories have never had air exchange with the outdoor, therefore there is air stagnation and had a high level of dust. It is very probable that the contamination existing in the dust, on the documents and on other surfaces of the repositories were kept in a process of continuous resuspension and with time those fungal propagules remain in a high concentration in the indoor environment of the repositories. However, the other two archives, while also air-conditioned, do exchange air with the outdoor environment at times, either through doors when opened or windows when facilities are cleaned which is the time when windows open to facilitate the renewal of the air inside the repositories.

It is noteworthy that in the literature refers that in the outdoor environment there must be a higher fungal concentration than the indoor one [42]. This behavior was detected in the case of CIPO and ML; however for LS the opposite happened. It is believed that this can be attributed to the fact that on that day the outdoor sampling carried out showed a high mobility of the fungal propagules due to the high existing vehicular movement that favored the formation of air and dust turbulences, preventing the propagules from sediment easily or were not readily captured by the biocollector.

In relation to environmental fungi, the most of the isolates were anamorphs of ascomycetes which is indicative of their prevalence in the indoor micobiota [19,46]. It is important to highlight that this result is characteristic of the sampling method used, since the use of culture media favors development of anamorphic phases in the fungi. Similar results were previously reported in environmental studies carried out in the NARC in acclimatized and natural ventilated repositories [19,20,38,46,47]. Regarding the predominance of the genera *Aspergillus, Cladosporium, Penicillium* and WNSM, it coincides with previous reports of results obtained in Cuban and other countries' libraries and archives [3,5,8,10,19,20,40,41,48-51]. It is reported that these genera can produce numerous conidia that can be easily dispersed by air for this reason are common on indoor environments[50]. However, other genera were also detected to a lesser extent, such as *Acrodontium, Chrysosporium, Cylindrocarpon, Nigrospora, Neurospora, Trichophyton, Harposporium* and *Scolecobasidium*, these last two genera being new finding for Cuban archive environments.

Piontelli [27] reported that *Aspergillus* genus is widely distributed in the environment throughout the world, especially in tropical and subtropical areas. Also, Leite-Jr. et al. [40] informed that *Penicillium* is a genus common in cold climates while *Aspergillus* is most common in the tropic climates and warm locations. However, according to our results, the behavior of *Penicillium* does not agree with the previous report, since it was precisely this genus that predominated in the indoor environments of CIPO and LS, an aspect that is not the first time that it occurs in environments of Cuban archives [20,47]. On the other hand, Harkawy et al. [42] and Molina and Borrego [38] indicated that is common that *Aspergillus* spp. and *Penicillium* spp. predominate in archive and library environments due to the presence of objects and documents on paper, parchment and textiles that are materials that can be biodeteriorated by species of these genera, in addition to the fact that they can be present in sedimented dust. Also, these genera are considered the first colonizers of the surfaces [3,4,8,45].

It is reported that airborne fungi detected on indoor environment usually enter a building through the ventilation, air conditioning system, doors and windows, together with the dust or they are part of the contaminants that are present on building materials [1,19,49,52]. This is one more reason that indicates the need to compare the indoor environment with the outdoor. Hence when comparing the behavior of the isolated taxa inside the archives and the outdoor environments, it was found that for CIPO the coincidence of was 100%, that is, all

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the taxa detected inside the repositories came from the outside environment, which that ratifies in good exchange between both environments. In the case of ML, a 45.5% coincidence was obtained, that is, six taxa were coincident (*Chrysosporium, Cladosporium, Neurospora*, WNSM, PNSM), evidencing the existence of exchange between both environments, although more sporadically than CIPO, while for LS the coincidence was higher (60%) since of the three taxa detected in the environment of these repositories, two may come from outdoor, despite having very little exchange between both environments. However, as this last archive has practically had no exchange with the outside, there has probably been a recirculation/resuspension of its own contamination for a long time not only of viable fungal propagules but also of VOCs and other substances (in the repositories of LS were detected high concentrations of *A. niger* on the furniture, ceiling and some documents) causing a considerable increase in the fungal concentration in the indoor environment of the few species that penetrated at any given time. Nevertheless, these results confirm what has been informed by other authors previously who expressed that most of the fungi existing in indoor environments come from outdoor sources [3,13,53].

Due to the existing conditions in LS, some constructive renovations of the premises were proposed to the administration and specialists with the intention of separating the air conditioner of the repositories from the central equipment and improving air exchange with the outdoor as much as possible, aspects that were done quickly.

Cabral [13] and Pinzari [1] reported that fungi can serve as bioindicators of air quality of indoor environments because, depending on the presence and concentration of some genera, wet environments and diseased buildings can be discovered (Sick Building Syndrome, SBS); therefore they constitute an advertisement of dangerousness environmental for health. These authors also suggest that the predominance of *Aspergillus* spp. and *Penicillium* spp. is indicative of an indoor environment with humidity in the building and evidence that the building is sick; on the contrary if *Cladosporium* is the predominant genus then the building is healthy. In fact, this genus prevailed in ML environment of NARC ratifying the good quality of this repository air in this archive.

It is highlighting that *Cladosporium* spp. and WNSM were detected in the outdoor environment of all archives and coincidentally on indoor all of them. It is known that *Cladosporium* is a very common genus of outdoor environments [48] and in particular for the Havana environment [18]; for this reason its ecological impact on indoor environments in the city was to be expected.

Of the detected species, 15 corresponded to *Aspergillus* spp. (34.9%), 10 belonged to *Cladosporium* spp. (23.3%), 6 to *Penicillium* spp. (14%) and 2 to *Nigrospora* spp. (4.7%). Of the *Aspergillus* species, many are xerophilic, as is the case of *A. penicilloides* and *A. wentii* [54, 55]; therefore they are associated with dusty environments. However, many of them and others shown in Table 2 have been detected in archive environments, libraries and museums as well as on documents [3,12,19,38,40,46,49,50,56].

Although fungi are dangerous for collections, they are also dangerous for people. Fungi are considered to be powerful sensitizers, so exposure to them can lead to allergies, even in people not prone to them. These fungi produce toxic illness, allergic, and different kinds of mycoses [14,16,57]. Among them, the species of the genus *Aspergillus* stand out, where there are some that are considered pathogenic, such as, for example, *A. flavus, A. flavipes, A. niger, A. nidulans, A. oryzae* and *A. versicolor*, only that aspergillosis it manifests itself fundamentally in immunocompromised persons [27,58-62]. In a previous paper that informed some preliminary results obtained in one of the CIPO repositories, some pathogenic attributes in isolated *Aspergillus* species were demonstrated in vitro, such as growth at 37°C, hemolysin secretion and the conidia size, indicating that the most conidia from the obtained species can penetrate until the alveoli due to their small dimensions [63]. In relation to the impact of other taxa isolated in this study on human health, it is highlighting that some species of *Cladosporium* spp. (example *C. cladosporioides, C. herbarum* and *C. sphaerospermum*), *Trichophyton* spp. and *Penicillium* spp. (example *P. citrinum, P. chrysogenum, P. commune* and *P. griseofulvum*) as well as *Cylindrocarpon lichenicola, Nigrospora sphaerica* and the non-sporulating mycelia can be pathogens [58,64].

However, there are species from other genera that can also trigger diseases in personnel. Such is the case of the study carried out by Rodríguez-Orozco et al. (2008) [65] with aimed at identifying fungal genera isolated from the nasal and pharyngeal mucosa of patients with allergic rhinitis; they detected spores of the genera *Cladosporium, Penicillium, Aspergillus, Alternaria* and *Fusarium* at the nasal level and spores of *Cladosporium, Aspergillus* and *Penicillium* at the pharyngeal level, result that supports the analysis we perform.

On the other hand, many of the fungi most commonly detected in the air of archive and libraries are capable of degrading cellulose, starch and gelatin which are substances that are part of the paper, bindings, various photographic techniques and other types of documentary

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supports [1,2,9,50,66-68]. They are characterized by secreting enzymes that degrade these polymers and as a result, they excrete organic acids and pigments [2,20,50,56,69-72]. On the other hand, if the T, RH and pH of the materials are adequate, the bioreceptivity of the paper and other documentary supports increases, triggering a vigorous growth of the fungi that facilitates the penetration of their hyphae into the materials and forms mature biofilms that mechanically damage these supports and are difficult to remove [2,56]. Therefore, determining the biodeteriogenic potentialities of environmental fungi allows us to know the potential risk to which the collections are exposed and as a result a strategy can be designed with actions that help mitigate their impact.

Regardless that a biodeteriogenic attribute under certain conditions may be sufficient to consider a microorganism harmful to a certain material [20], the meeting of several of them in the same strain without a doubt, makes it much more dangerous to be capable of inflicting damage of a different nature on the same support or affecting different materials at the same time. Of the strains evaluated, 27% showed 4 biodeteriogenic attributes, while 33% of them showed the 5 attributes, representing a total of 60% of the strains that can be highly dangerous for the documentary supports that are kept in the studied archives.

5. Conclusions

The investigation carried out in the interior environments of Havana archive repositories located relatively close to each other has revealed the environmental mycological quality. The LS environments showed the highest I/O index indicative of being contaminated due to lack of ventilation and air stagnation, despite having similar average T and RH values among the 3 archives analyzed. A total of 11 genera and two different types of non-sporulating mycelia were detected but *Aspergillus* spp., *Cladosporium* spp. and *Penicillium* spp. were the predominant genera in the three archives as well as a white non-sporulating septated mycelium. The genus *Cladosporium* predominated only in the ML environment of the NARC and together with the low I/O index obtained, it was shown that the environment of that repository had good quality while *Penicillium* spp. prevailed in the CIPO environments (environment with good quality also due to its low I/O ratio) and LS. The *C. cladosporioides* and *P. citrinum* species were dominant and therefore were ecologically classified as abundant. Among the isolates, species with a large adaptive potential were detected (such as *Aspergillus penicilloides* and *A. wentii*) that find favorable conditions for their development in this type of environment. Of the isolated species some were obtained that are risky to human health, highlighting mainly those of the genus *Aspergillus*. The biodeteriogenic potential of the isolates evidenced the ability of most to cause significant damage to materials, especially those of an organic nature. The isolates with the greatest biodeteriogenic impact were *A. flavus* 1, A. *niger, Nigrospora oryzae* and *P. citrinum* 2.

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