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AIRBORNE MYCOBIOTA ISOLATED FROM AN ARCHIVE IN HAVANA, CUBA

MICROBIOTA AEROTRANSPORTADA AISLADA DE UN ARCHIVO EN LA HABANA,
CUBA

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ABSTRACT

The goals of this research were to determine fungal diversity and air quality in a repository belonging to the Cuban Industrial Property Office and to evaluate the behavior of *Aspergillus* and *Penicillium* species over time. Indoor and outdoor sampling was conducted with a biocollector during both rainy and dry seasons in Cuba, and the indoor/outdoor (I/O) ratio was determined. In the first sampling (rainy season), the average fungal concentration was 28.4 CFU/m³ and the I/O ratio was 0.81, while in the second sampling (dry season), the average fungal concentration was significantly higher (47.7 CFU/m³), and the I/O ratio was 0.64, indicating good air quality in both cases. Nevertheless, concentration values increased at various sampled points, indicating areas of amplification of fungal concentration. Numerous genera and non-sporulated mycelia were detected, with *Penicillium* predominating in the first isolate and non-sporulated mycelia in the second. Some species of *Aspergillus* and *Penicillium* showed very high I/E (≥ 2), indicating that they are contaminants in this environment; among these, *A. oryzae*, *A. brasiliensis*, *A. unguis*, *P. citrinum*, *P. sclerotiorum* and *P. janczewskii* stood out, highlighting the risk they could represent for both staff health and conservation of the documentary heritage stored in this repository.

KEYWORDS | airborne mycobiota, air quality, archive, fungal diversity, indoor environment

RESUMEN

Los objetivos de esta investigación fueron determinar la diversidad fúngica y la calidad del aire en un repositorio de la Oficina Cubana de la Propiedad Industrial y evaluar el comportamiento de las especies de *Aspergillus* y *Penicillium* a lo largo del tiempo. Los muestreos interiores y exteriores se realizaron con un biocollector en las dos estaciones del año existentes en Cuba (temporada lluviosa y temporada de escasas lluvias) y se determinó la relación interior/exterior (I/E). En el primer muestreo (temporada lluviosa), la concentración fúngica promedio fue de 28,4 UFC/m³ y la relación I/E fue de 0,81, mientras que en el segundo muestreo (temporada de escasas lluvias) la concentración fúngica promedio fue significativamente mayor (47,7 UFC/m³), y la relación I/E fue de 0,64, lo que denota en ambos casos un ambiente de buena calidad. A pesar de esto, las concentraciones revelaron aumentos en diferentes puntos muestreados, lo que indica la existencia de zonas de amplificación de la concentración fúngica. Se detectó una gran cantidad de géneros y micelios no esporulados, pero *Penicillium* predominó en el primer aislamiento y un micelio no esporulado en el segundo. Algunas especies de *Aspergillus* y *Penicillium* mostraron una I/E muy alta (≥ 2), lo que indica que son contaminantes de este ambiente, entre ellas destacaron *A. oryzae*, *A. brasiliensis*, *A. unguis*, *P. citrinum*, *P. sclerotiorum* y *P. janczewskii*, destacando el riesgo que podrían representar para la salud del personal y la conservación del patrimonio documental almacenado en este repositorio.

PALABRAS CLAVE | micobiota aerotransportada, calidad del aire, archivo, diversidad fúngica, ambiente interior

INTRODUCTION

Fungi comprise great biological diversity, in agreement with their wide distribution in nature. Their propagules are a constant component of the air as constituents of bioaerosols, with concentrations and compositions that fluctuate according to the complex interaction between biological and environmental factors, including geographic location, air pollution, climatic conditions, human activity and local sources of vegetation, among others (Grinn-Gofron & Bosiacka, 2015). The most frequent fungal genera in air are *Cladosporium*, *Penicillium*, *Aspergillus* and *Alternaria*, which occur in high concentrations independently of latitude and altitude above sea level (Ramos & Meza, 2017). Likewise, these genera are also the most frequent in indoor environments such as archive repositories, libraries and museums (Borrego, 2023; Camargo *et al.*, 2024). Due to their physiological adaptations and versatile metabolic machinery supported by enzymes and other metabolites, they can grow on different substrates, causing undesirable changes in their properties (Sánchez *et al.*, 2024).

Fungi represent the most significant biological agents involved in the biodegradation of cultural heritage materials. This is so because, although bacteria also take part in such processes, fungi require less humidity to develop and thus the typical environmental conditions of archive repositories, libraries and museums, are more favorable for the growth of fungi than bacteria (Mallo *et al.*, 2017; Camargo *et al.*, 2024). Fungi are capable of remaining in the environment for long periods without causing major damage, but with favorable environmental conditions, *i.e.* temperature (T) above 25°C, relative humidity (RH) greater than 65%, high water availability in materials (water activity (a_w) greater than 70%), low ventilation and air circulation, and bioreceptivity, as well as with substrates with physicochemical properties compatible with fungal nutritional demands, they can cause negative effects on materials, colonizing and accelerating their biodeterioration (Mallo *et al.*, 2020; Camargo *et al.*, 2024; Iliopoulou *et al.*, 2024).

Xerotolerant and xerophilous species are able to develop in low environmental RH conditions (Sánchez *et al.*, 2024; Stratigaki *et al.*, 2024). In addition, with global T and RH values increasing due to climate change, this has direct effects on heritage

materials, including documents on any medium, as well as on biological activity, particularly of fungi (Awad *et al.*, 2020; Mallo *et al.*, 2020).

It is also extremely important to keep in mind that when handling contaminated or dusty documents, it is possible for staff to inhale high concentrations of environmental fungal propagules, which may constitute a serious risk to their health, since many of these fungi are pathogenic/toxigenic, even if they are not viable (Viegas *et al.*, 2022). Furthermore, these microorganisms are widely recognized as allergenic triggers involved in severe respiratory diseases (Viegas *et al.*, 2017; Alvarez *et al.*, 2020; Herrera *et al.*, 2021; Borrego *et al.*, 2024) and can cause superficial and deep infections or mycoses as well as other diseases (Iyalla, 2017; Egbuta *et al.*, 2017). In this context, the goals of this work were to determine fungal diversity and air quality in a repository belonging to the Cuban Industrial Property Office (CIPO), as well as to assess the behavior of *Aspergillus* and *Penicillium* species over time.

MATERIALS AND METHODS

Characteristics of the repository

The study was conducted in the Fund repository of the Cuban Office of Industrial Property (CIPO), an institution located in Havana, Cuba. The CIPO protects highly valuable documentary collections (records of inventions, industrial models, scientific discoveries, brands and other distinctive signs) and holds 1265136 copies, mostly on paper, with the oldest dating to the 18th century.

The repository is built on two levels in the form of a mezzanine (Figure 1a, 1b), located on the ground floor of the property and accessed through a preceding office. It is quite large, 17 m long x 8 m wide x 10 m high, and has five windows. It contains three units of air conditioning systems located on the left side of the repository at almost 7 m high.

Environmental microbiological sampling

We sampled a total of 12 points (Sánchez, 2002) distributed as follows: five points from the upper part of the double repository (R1), six points from the lower part of the same repository (R2) (Figure 2) and one point from the outdoor courtyard (Figure 1c).



Figure 1. Characteristics of the collections repository at CIPO. a and b: Different views of the indoor area of the repository, illustrating the height of the premises, which is the reason for the shelves being located on two levels built as a mezzanine for efficient use of space. c: View of the courtyard, indicating the outdoor sampling point.

Figura 1. Características del repositorio de Fondos en la OCPI. a y b: Diferentes vistas del interior del repositorio, donde se aprecia la gran altura del local, por lo que las estanterías se ubicaron en dos plantas que se construyeron en forma de entresuelo para optimizar el uso del espacio. c: Vista del patio, indicando el punto de muestreo al aire libre (Fuente: elaboración propia).

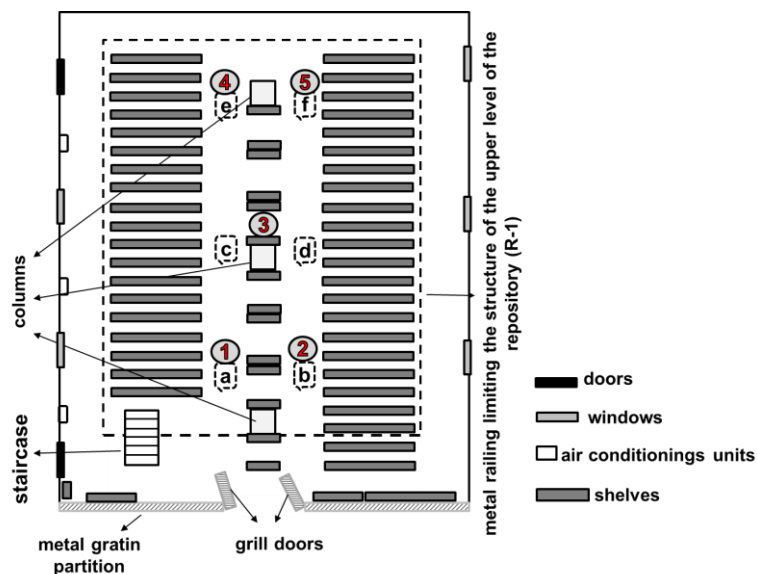


Figure 2. Top view of the repository studied. The five numbers enclosed in solid-line circles indicate the sampling points at the top of the repository (R1); the six letters enclosed in dashed lines indicate the points analyzed at the bottom (R2). In addition to these 11 indoor sample points, one outdoor sample was located in the center of the courtyard.

Figura 2. Vista superior del repositorio estudiado. Los cinco números, encerrados en círculos con líneas continuas, indican los puntos de muestreo en la parte superior del repositorio (R1) y las seis letras, encerradas en figuras con líneas discontinuas, representan los puntos analizados en la parte inferior (R2). En total, se identificaron 11 puntos interiores y uno exterior ubicado en el centro del patio (Fuente: elaboración propia).

The first microbiological sampling was done on May 23, 2023, a month corresponding to the dry season in Cuba; the repository remained heated throughout this period. The second sampling was done on October 9, 2023, within the rainy season, and in this case the repository was heated until 11:00 a.m. and the air conditioning equipment was turned on until 1:00 p.m.

For both isolates, air samples were taken by triplicate from each sampling point, using a portable SAS type biocollector (Super 100 TM, Italy) at one-hour intervals between replicates and using an airflow of 100 L of air per minute. Petri dishes with Malt Extract agar (MEA) supplemented with NaCl (7.5%) (Borrego *et al.*, 2017) and MEA at pH = 5 were used (Borrego *et al.*, 2022a). The culture medium MEA supplemented with NaCl facilitates the growth of halophilic fungi and some xerophilic species; furthermore, it reduces the growth of Mucorales colonies and prevents growth of most environmental bacteria. Both culture media were used to isolate the greatest fungal diversity. Once the samples were collected, the sealed and inverted dishes were transported to the laboratory of the National Archive of the Republic of Cuba (NARC) located very close to CIPO, and incubated in inverted position for 7 days at 30°C. Subsequently, the fungal colonies grown in the culture media were counted and the colony-forming units by m³ of air (CFU/m³) were determined following the air sampler manual. Then the colonies were isolated, purified and conserved in MEA slants at 4°C.

Determination of environmental mycological quality

To assess air quality, indoor/outdoor ratio (I/O) was calculated according to De Aquino Neto & Goes Siqueira (2000). According to these authors, for countries in tropical climates, a value of 1.5 or less for this ratio is typical of well-ventilated, non-contaminated environments, while values between 1.5 and 2 are indicative of average environmental quality, and values greater than 2 reflect poorly ventilated, contaminated environments.

Thermohygrometric analysis

T and HR determinations were made with a digital thermo-hygrometer (Pen TH 8709, China) in triplicate at the same microbiological sampling points.

Taxonomic identification

We recorded cultural and morphological characteristics of the isolated colonies observed under stereomicroscope (20X). The microscopic examination of structures was performed using an optical microscope (Olympus, Japan) connected to a digital camera (Samsung, Korea) and the observations were made under objectives of 10X, 40X and 100X (with immersion oil). Fungal structures of taxonomic value were observed from preparations made using lactophenol and/or lactophenol-fuchsin for observation of hyaline structures (Klich & Pitt, 1994). The characteristics of the structures examined were compared to the manuals by Barnett & Hunter (1996) and Domsch *et al.* (1980) for genus identification. For the identification of *Aspergillus* species we followed the criteria of Klich & Pitt (1994), while for *Penicillium* we followed Pitt (2000).

Ecological determinations

Relative Density (RD) of taxa isolated from indoor air samples of each repository was calculated according to Smith (1980) where: $RD = (\text{number of colonies of one species} / \text{total number of colonies}) \times 100$.

Relative Frequency (RF) of the *Aspergillus* and *Penicillium* species detected in this study and in previous research was determined according to Esquivel *et al.* (2003) where: $RF = (\text{number of times a species was detected} / \text{total number of samples analyzed}) \times 100$.

The ecological categories are classified as: Abundant (A) when $RF = 100-81\%$, Common (C) with $RF = 80-61\%$, Frequent (F) when $RF = 60-41\%$, Occasional (O) with $RF = 40-21\%$, Rare (R) with $RF = 20-0\%$.

Statistical analysis

Statistical analysis of the data was made using the Startgraphic Centurion XV program. We used the Student test to compare indoor RH between samples, RH of indoor vs outdoor in the second sampling, the mean indoor concentrations between the samples and the mean outdoor concentrations between the samples.

Pearson's correlation was used to assess the relationship between fungal concentration and thermo-hygrometric parameters. The mean values obtained were compared by means of a multiple analysis using the minimum significant difference method (LSD) for

($p \leq 0.05$). In addition, the values of these three parameters were plotted in radial graphics to visualize their behavior and analyze the possible existence of areas of amplification of fungal concentration. To corroborate the presence of these areas in the repository, the values of fungal concentration obtained in the 1st sampling (done only for this sampling as an example) and on both floors were also plotted using the Surfer 8.00 program (Golden Software, Inc.) to visualize their spatial distribution.

RESULTS

Behavior of thermohygrometric variables, concentrations of indoor airborne fungi and indoor environmental quality in the repository

In the 1st sampling carried out during the dry season, the mean values of T and RH in repository indoor were 29°C and 64.2% respectively; in contrast, outdoor values were 30.8°C and 59% (Table 1). The 2nd sampling carried out in the rainy season was characterized by mean T and RH values of 29.2°C and 76.4% respectively, while outdoor values.were 30°C and 86% respectively. The indoor T values on the premises were stable regardless of the season in which the sampling was carried out and did not differ from outdoor T values. Contrastingly, RH showed different behavior, with significant differences ($p \leq 0.05$) between the indoor and outdoor RH values in both samplings.

Parameter (mean values)	1st sampling (Dry season)	2nd sampling (Rainy season)
T (°C) indoor	29.0	29.2
T (°C) outdoor	30.8	30.0
RH (%) indoor	64.2	76.4 a
RH (%) outdoor	59.0	6.0 b
Indoor concentration (CFU/m3)	28.4	47.7 c
R1: Indoor concentration of the upper part of the repository (CFU/m3)	31.1	44.4
R2: Indoor concentration of the lower part of the repository (CFU/m3)	33.8	50.0
Outdoor concentration (CFU/m3)	35.0	75.0 d
I/O ratio	0.81	0.64

a, b, c, d: Indicates statistical differences according to the Student test ($p \leq 0.5$) when comparing indoor RH between samples, indoor vs outdoor RH of the 2nd sampling, mean indoor concentrations between samples, and mean outdoor concentrations between samples.

Table 1. Parameters determined in the samplings carried out in the CIPO repository.

Tabla 1. *Parámetros determinados en los muestreos realizados en el repositorio de la OCPI.*

In the 1st sampling, the mean fungal concentration in the indoor repository was low compared to outdoor values, although the difference was not statistically significant, resulting in an I/O ratio of 0.81, while in the 2nd sampling the indoor fungal concentration was significantly higher (47.7 CFU/m³) than that obtained in the 1st sampling (28.4 CFU/m³), but significantly lower than the outdoor concentration (75 CFU/m³); this resulted in an I/O ratio of 0.64. When fungal concentrations were compared between the two floors of the repository, we found slightly higher values in the lower level, although the differences were not statistically significant ($p \leq 0.05$).

The correlation between T and RH was negative for both samplings (52% in the first sampling and 60% in the second) ($r = -0.5234$, $p = 0.0005$ in the first sampling; $r = -0.6005$, $p = 0.001$ in the second sampling), indicating that as T increases, RH decreases. However, there was no correlation between these thermohygrometric variables and fungal concentration.

The graphic behavior of these parameters according to the points sampled on each floor of the repository (Figure 3), showed that both T and HR were highly stable regardless of the time of year analyzed. Nevertheless, the fungal concentrations showed increases at different points depending on the sampling. In the 1st sampling (dry season) slight increases were detected at points 1 (42 CFU/m³) and 2 (36 CFU/m³) of the upper part of the repository, located at the entrance and on the left and right side respectively, while the increases were more marked at points (d) (50 CFU/m³) and (f) (61.7 CFU/m³) of the lower part of the repository, both located on the right side, as corroborated in Figure 4. In the 2nd sampling (rainy season) increases were observed for the values of points 1 (56 CFU/m³) and 4 (62.5 CFU/m³), both located at the top of the repository and on the left side, one at the beginning and another at the end of the space, as well as at points (b) (62 CFU/m³) and (e) (61.7 CFU/m³) located at the bottom of the repository, but in this case, point (b) is located on the right side of the room, while point (e) is located on the left side.

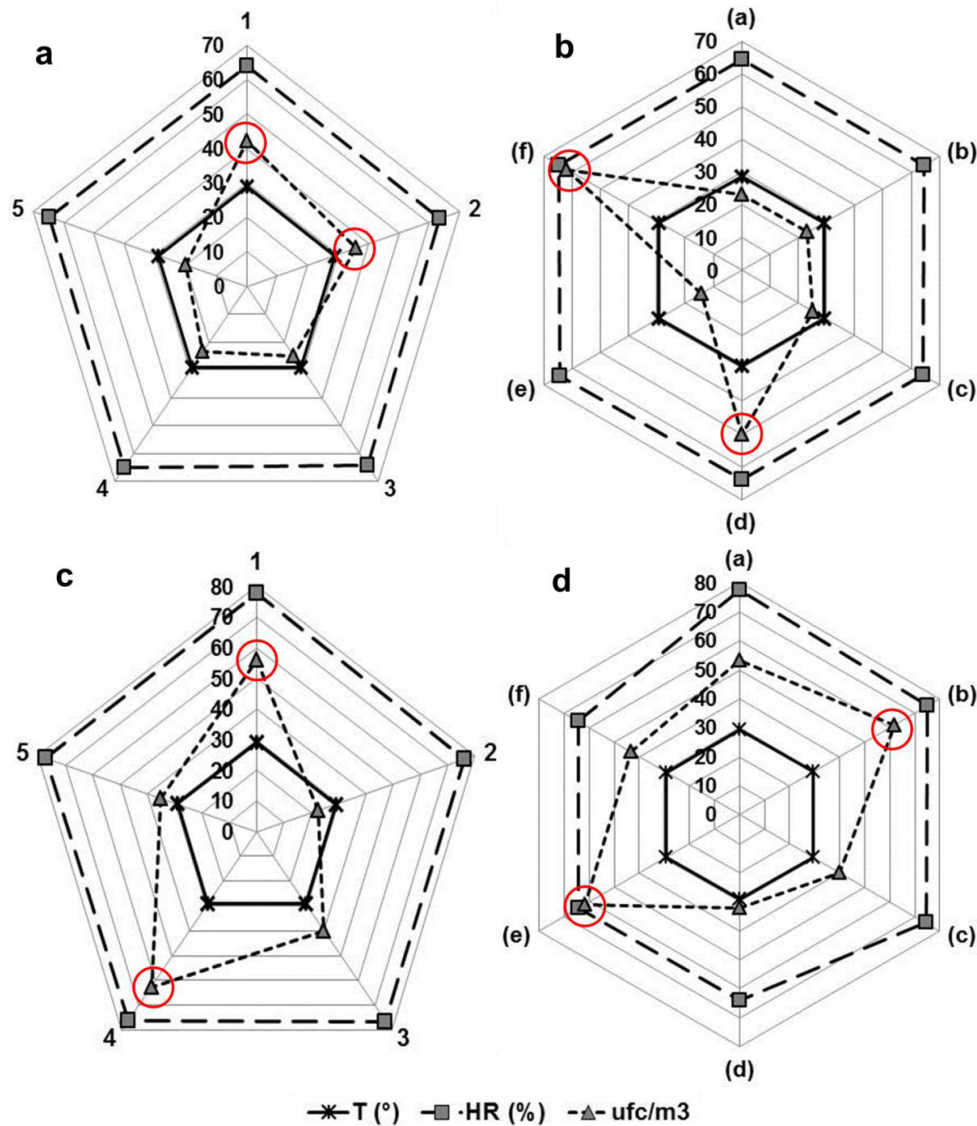


Figure 3. Behavior of T, RH and fungal concentration at each sampled point for both isolates. a: Refers to the top of the repository (R1) in the 1st sampling (dry season). b: Refers to the bottom of the repository (R2) in the 1st sampling. c: Represents R1 in the 2nd sampling (rainy season). d: Refers to R2 in the 2nd sampling. Note that in the 1st sampling there are increases in fungal concentration at points 1 and 2 at R1 and at points (d) and (f) at R2, while in the 2nd sampling the increases were detected at points 1 and 4 at R1 and at points (b) and (e) at R2.

Figura 3. Comportamiento de la T, la HR y la concentración fúngica en cada punto muestreado para ambos aislados. a: Se refiere a la parte superior del repositorio (R1) en el 1er muestreo (temporada de poca lluvia). b: Se refiere a la parte inferior del repositorio (R2) en el 1er muestreo. c: Representa R1 en el 2do muestreo (temporada de lluvias). d: Se refiere a R2 en el 2do muestreo. Nótese que en el 1er muestreo hay aumentos en la concentración fúngica en los puntos 1 y 2 en R1 y en los puntos (d) y (f) en R2, mientras que en el 2do muestreo los aumentos se detectaron en los puntos 1 y 4 en R1 y en los puntos (b) y (e) en R2 (Fuente: elaboración propia).

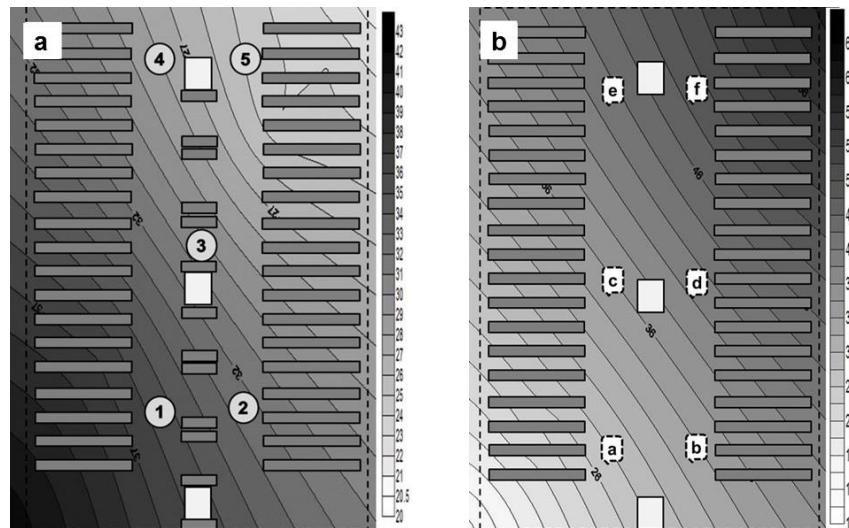


Figure 4. Example of the surface representation of fungal concentrations obtained in the 1st sampling carried out in the CIPO repository. a: Behavior in the upper part of the repository (R1). b: Behavior in the lower part of the repository (R2). Note in figure a that the points 1 and 2 have the highest concentrations, while in figure b the points (b) and (e) are among those with the highest concentrations.

Figura 4. Ejemplo de la representación superficial de las concentraciones fúngicas obtenidas en el 1er muestreo realizado en el depósito de Fondos de la OCPI. a: Indica el comportamiento en la parte superior del depósito (R1). b: Se refiere al comportamiento en la parte inferior del depósito (R2). Obsérvese en la figura a que los puntos 1 y 2 presentan las concentraciones más altas, mientras que en la figura b, los puntos (b) y (e) se encuentran entre los de mayor concentración (Fuente: elaboración propia).

Diversity of fungal genera detected in the air of the repository

In the isolates, a predominance of Ascomycota genera was detected (Figure 5). In total, seven genera of filamentous fungi and one white non-sporulated mycelium (WNSM) were isolated in the 1st sampling. Of these, the genus *Penicillium* Link showed marked predominance (DR = 28,4%), followed by the genera *Aspergillus* P. Micheli ex Haller (17,6%), *Gliocladium* Corda (15,3%), *Cladosporium* Link (11,4%), *Cunninghamella* Matr (7.5%), *Paecilomyces* Bainier (7%), and *Nigrospora* Zimm (3%) (Figure 5a). However, only four genera were isolated from the outdoor air, with the predominant one being also *Penicillium* (32%), followed by *Aspergillus* (28%), *Gliocladium* (24%) and *Cladosporium* (16%).

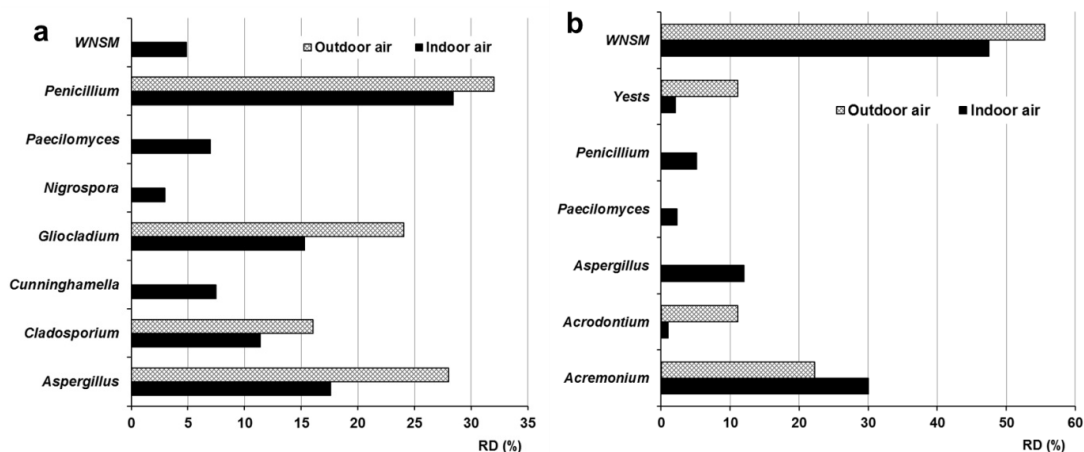


Figure 5. Relative density (RD) of the fungi isolated in the samplings. a: First sampling (dry season). b: Second sampling (rainy season). WNSM: White non-sporulated mycelium.

Figura 5. Densidad relativa (DR) de los hongos aislados en los muestreos realizados. a: Se refiere al 1er muestreo (temporada de pocas lluvias). b: Indica el 2do muestreo (temporada de lluvias). WNSM: Micelio blanco no esporulado (Fuente: elaboración propia).

In the 2nd sampling, five genera were found in the air, but with marked predominance of a white non-sporulated mycelium (WNSM) (47.5%), followed by the genera *Acremonium* Link (30%) and *Aspergillus* (12%) (Figure 5b). In addition, the genera *Acrodontium* De Hoog, *Paecilomyces* and *Penicillium* were detected with $DR \leq 5.1\%$. It should be noted that in this isolate, we also detected yeast (2.1%). Only two genera were isolated from the outdoor air (*Acremonium*, *Acrodontium*), as well as yeasts and WNSM, of which the latter also prevailed (55.6%).

Diversity and concentration behavior of *Aspergillus* and *Penicillium* species isolated from the air of the studied repository

Considering that *Penicillium* and *Aspergillus* were the predominant genera in the 1st isolate and that they were also detected in the second sample, although with lower concentrations, we furthered the identification to the species level. As shown in Table 2, six species were identified within the genus *Penicillium*, with *P. sclerotiorum* being predominant in both indoor and outdoor samples (indoor concentration 160 CFU/m³, outdoor concentration 30 CFU/m³). In the indoor sample this species was followed by *P. janczewskii* (150 CFU/m³) and *P. citrinum* (110 CFU/m³). At much lower concentrations we detected the species *P. olsonii* (30 CFU/m³) as well as *P. corylophilum* and *P. variabile* with 10 CFU/m³ respectively. In addition to *P.*

sclerotiorum, three other species (*P. citrinum*, *P. olsonii* and *P. variable*) were found outdoors at concentrations that varied between 10 and 30 CFU/m³, that is, much lower than those obtained in the indoor samples. When analyzing their I/O ratios, *P. citrinum* showed the highest value (11.0) followed by *P. sclerotiorum* (5.3); these turned out to be very high values. Seven species of *Aspergillus* were isolated; the predominant one was *A. oryzae* (110 CFU/m³) followed by *A. auricomus* (70 CFU/m³). The other species (*A. aculeatus*, *A. alliaceus*, *A. brasiliensis*, *A. caespitosus* and *A. unguis*) were found at concentrations • 50 CFU/m³. Only three species (*A. auricomus*, *A. brasiliensis* and *A. unguis*) were isolated from the outdoor air at concentrations ranging from 10 to 40 CFU/m³, which were also lower than those detected in the indoor air. The I/O ratios were highest for *A. brasiliensis* (3.0) followed by *A. unguis* (2.0), which was also high.

Species	Indoor air	Outdoor air	I=I/O
	CFU/m ³		
<i>P. citrinum</i> Thom *	110	10	11.0
<i>P. corylophilum</i> Dierckx	10	0	-
<i>P. janczewskii</i> K.M. Zalesky	150	0	-
<i>P. olsonii</i> Bainier & Sartory	30	20	1.5
<i>P. sclerotiorum</i> J.F.H. Beyma	160	30	5.3
<i>P. variable</i> Sopp	10	10	1.0
<i>A. aculeatus</i> (Iizuka) Al-Musallam *	10	0	-
<i>A. alliaceus</i> Thom & Church *	50	0	-
<i>A. auricomus</i> (Guég.) Saito	70	40	1.8
<i>A. brasiliensis</i> Varga, Frisvad & Samson	30	10	3.0
<i>A. caespitosus</i> Raper & Thom	40	0	-
<i>A. oryzae</i> (Ahlb.) Cahncon *	110	0	-
<i>A. unguis</i> (Émile-Weill & L. Gaudin) Dodge *	40	20	2.0

*: Indicates that these species are pathogenic according to De Hoog *et al.* (2000).

Table 2. Concentration of *Penicillium* and *Aspergillus* species detected in the first isolate and their I/O ratios.

Tabla 2. Concentración de especies de *Penicillium* y *Aspergillus* detectadas en el 1er aislamiento y sus relaciones I/E.

In the 2nd sampling, eight species of the genus *Aspergillus* and a single species of *Penicillium* were isolated from indoor air (Table 3). The *Aspergillus* species with highest concentration was *A. caespitosus* (380 CFU/m³) followed by *A. tamarii* (110 CFU/m³) and *A. japonicus* (80 CFU/m³). The rest of the species (*A. aculeatus*, *A. candidus*, *A. terreus*, *A. unguis* and *A. wentii*) showed concentrations • 50 CFU/m³. The only species of *Penicillium* detected was *P. fellutanum* at a concentration of 30 CFU/m³.

Species	Concentration (CFU/m3)
<i>A. aculeatus</i> (Iizuka) Al-Musallam *	30
<i>A. candidus</i> Link *	20
<i>A. caespitosus</i> Raper & Thom	380
<i>A. japonicus</i> Saito *	80
<i>A. tamarii</i> Kita *	110
<i>A. terreus</i> Thom *	20
<i>A. unguis</i> (Emile-Weil & Gaudin) Thom & Raper *	10
<i>A. wentii</i> Wehmer **	50
<i>P. fellutanum</i> Biourge	30

*: Indicates that these species are pathogenic according to De Hoog *et al.* (2000).

** : Pathogenic species according to Halsey *et al.* (2011).

Table 3. Concentration of *Aspergillus* and *Penicillium* species detected in indoor air in the 2nd sampling.

Tabla 3. Concentración de especies de *Aspergillus* y *Penicillium* detectadas en el aire interior en el 2do muestreo.

Ecological behavior of *Aspergillus* and *Penicillium* species isolated from the air of this repository throughout studies carried out in different years

When analyzing the behavior of the *Aspergillus* and *Penicillium* species in these two isolates with respect to previous reports, we observed that *A. unguis* was the only abundant species, since it had been isolated from the air of this repository in all the environmental analyses carried out (Table 4). In addition, four other species were found to be common in all these works, namely *A. candidus*, *A. oryzae*, *A. wentii*, and *P. citrinum*. Two *Aspergillus* species (*A. aculeatus* and *A. caespitosus*) were classified as abundant for this study, while nine species were frequent (*A. alliaceus*, *A. auricomus*, *A. brasiliensis*, *A. candidus*, *A. japonicus*, *A. oryzae*, *A. tamarii*, *A. terreus*, *A. wentii*), and the seven *Penicillium* species detected in these samplings were also categorized as frequent (*P. citrinum*, *P. corylophilum*, *P. fellutanum*, *P. janczewskii*, *P. sclerotiorum*, *P. olsonii*, *P. variable*).

Species	In the present study				In previous studies			
	1st sampling	2nd sampling	RF (%)	EC	Molina <i>et al.</i> (2014)	Borrego <i>et al.</i> (2021)	RF (%)	EC
<i>A. alliaceus</i>	x	-	50	F	-	-	-	-
<i>A. aculeatus</i>	x	x	100	A	-	-	-	-
<i>A. auricomus</i>	x	-	50	F	-	-	-	-
<i>A. brasiliensis</i>	x	-	50	F	-	-	-	-
<i>A. caespitosus</i>	x	x	100	A	-	-	-	-
<i>A. candidus</i>	-	x	50	F	x	x	75	C
<i>A. japonicus</i>	-	x	50	F	-	-	-	-
<i>A. oryzae</i>	x	-	50	F	x	x	75	C
<i>A. tamarii</i>	-	x	50	F	-	-	-	-
<i>A. terreus</i>	-	x	50	F	-	-	-	-
<i>A. unguis</i>	x	x	100	A	x	x	100	A
<i>A. wentii</i>	-	x	50	F	x	x	75	C
<i>P. citrinum</i>	x	-	50	F	x	x	75	C
<i>P. corylophilum</i>	x	-	50	F	-	-	-	-
<i>P. fellutanum</i>	-	x	50	F	-	-	-	-
<i>P. janczewskii</i>	x	-	50	F	-	-	-	-
<i>P. sclerotiorum</i>	x	-	50	F	-	-	-	-
<i>P. olsonii</i>	x	-	50	F	-	-	-	-
<i>P. variable</i>	x	-	50	F	-	-	-	-

*: Indicates that these species are pathogenic according to De Hoog *et al.* (2000).

Table 4. Ecological behavior of the *Aspergillus* and *Penicillium* species isolated in this study compared to previous determinations.

Tabla 4. Comportamiento ecológico de las especies de *Aspergillus* y *Penicillium* aisladas en este estudio en comparación con determinaciones previas.

DISCUSSION

Thermohygrometric values, fungal concentrations and I/O ratios obtained in the two samplings

In the 1st sampling performed in the CIPO Funds repository, the indoor T value was high, which could be due to the fact that the climate control equipment only worked from 8:00 am to 11:00 am and from 1:00 pm to 5:00 pm; thus, when the sampling was carried out, the repository had not been sufficiently cooled yet and, in addition, the equipment had to be turned off shortly after, preventing proper cooling of the premises. With respect to RH, the mean value was slightly higher in the indoor repository than for the outdoor site, possibly due to the water condensation resulting from the climate control equipment being turned off. However, these parameters did not show higher values because the windows located in the upper part of the premises

facing the indoor yard of the building were kept open in the evenings and nights to facilitate ventilation. The T values of the 2nd sampling were very similar to those of the 1st sampling, indicating that the behavior of this parameter is closely related to the cooling speed provided by the air conditioning equipment. However, two days before the 2nd sampling was carried out, it rained heavily even into the early morning, which together with the indoor water condensation due to the irregular operation of the air conditioning equipment, favored significantly higher RH values ($p \leq 0.05$) than those obtained in the 1st sampling.

The fact that the air conditioning equipment is not kept running continuously throughout the year contradicts the guidelines established in Resolution No. 201 of CITMA (2020) for Cuba; furthermore, fluctuations in T and RH are known to contribute to accelerated deterioration of paper documents since paper is a hygroscopic material (Abdel-Maksoud *et al.*, 2022).

The existence of negative correlation between T and RH, as well as the fact that these meteorological variables showed no correlation with fungal concentrations, agrees with the result of a previous study (Torres *et al.*, 2022). The graphical analysis of the behavior of T, RH and fungal concentration at each of the sampling points revealed non-significant increases in fungal concentration at some of them. This could be due to the presence of areas of fungal concentration amplification, in which an accumulation of fungal propagules occurs as a consequence of inadequate circulation of air within the repository that causes stagnation in those areas. In previous studies, areas of fungal concentration amplification had been detected in this repository, but according to those works, these areas were located fundamentally at the entrance of spaces R1 and R2 (Borrego *et al.*, 2021; Torres *et al.*, 2022). In contrast, in this case, these areas corresponded not only to some points at the entrance of the repository, but also to others located on the right side and at the far end of the premises, indicating that air circulation is not adequate in all areas of this space, due to the incorrect location of the air conditioning equipment positioned on only one side of the premises (left), as well as to the fact that they are not functioning permanently. This situation could contribute not only to the increase of fungal propagules in these areas but also to their deposition onto the materials, stimulating fungal growth on them and the degradation of their

components, which would lead to accelerated biodeterioration of the documents kept in these areas. Similarly, the non-significant differences ($p \leq 0.05$) in the fungal concentrations detected between the two floors of the repository are probably due to the location of the climate control equipment and the opening during the evenings and nights of the windows situated in the upper part of the premises, elements that facilitate air circulation in that area.

The fungal concentrations detected in both samplings were lower than those reported in a previous study where the values ranged between 88.2 CFU/m³ and 133.3 CFU/m³ (Torres *et al.*, 2022), although other previous references mention concentrations as low (18 and 35 CFU/m³) as those obtained in the present study (Molina *et al.*, 2014; Borrego *et al.*, 2021). The fact that the I/O ratio was less than 1 in both cases is indicative of an environment with good mycological quality, in agreement with the criteria of several authors (De Aquino & Goés Siqueira, 2000; Awad *et al.*, 2020; Pyrri *et al.*, 2020). Furthermore, this appears to be a characteristic of this repository, since previous studies also detected a ratio lower than 1 (Borrego *et al.*, 2021; Torres *et al.*, 2022). However, it is necessary to maintain systematic monitoring of the thermohygrometric parameters, the fungal concentration and the documents statekept in this repository in order to prevent the onset of fungal outbreaks on these materials in time, because this type of infestation could easily occur given the large size of the site and the issues with air circulation.

Fungi detected

The fact that most of the fungal genera isolated from both samplings belong to the phylum Ascomycota agrees with previous references for archive and library environments in Cuba and elsewhere (Li *et al.*, 2022; Borrego *et al.*, 2021; 2022a; Branysova *et al.*, 2024; Derksen *et al.*, 2024). In previous samplings of this same repository, *Penicillium* was the predominant genus followed by *Cladosporium* and *Aspergillus* (Molina *et al.*, 2014; Borrego *et al.*, 2020; 2021). In contrast, in a later study, *Aspergillus* was the prevailing genus followed by a non-sporulated mycelium, and by the genera *Penicillium* and *Cladosporium* (Torres *et al.*, 2022). In any case, *Aspergillus*, *Cladosporium* and *Penicillium* have been among the main genera in the air of this repository, coinciding with reports from other archives and libraries in both Cuba

(Borrego *et al.*, 2020; 2022a, b, c; Rodríguez & Borrego, 2023) and other countries (El Jaddaoui *et al.*, 2023; Branysova *et al.*, 2024; Derksen *et al.*, 2024; Iliopoulou *et al.*, 2024). The genus *Cladosporium*, detected only in the 1st sampling, is one of the most frequent fungal genera in archives both in Cuban and worldwide, since it adapts well to different conditions, including variable temperatures and relative humidity, which allows it to thrive indoors and outdoors (Borrego & Molina, 2014; Hernández-Velandia & Lizarazo-Forero, 2015). *Gliocladium* is a rarely detected genus in the air of Cuban archives. It was only isolated once, years ago in the NARC (Borrego, 2023) and afterward it had not been detected in any other archive, library or museum in the country. However, it has been isolated from the air of museums, archives and libraries in other countries in low proportions (Hernández-Velandia & Lizarazo-Forero, 2015; Moctezuma *et al.*, 2015; Pyrri *et al.*, 2020; Silva *et al.*, 2021).

Representatives of the genera *Cunninghamella*, *Nigrospora* and *Paecilomyces* were found to a lesser extent. *Cunninghamella* is rather rare in the indoor air of archives, libraries and museums, but it had been previously detected in the indoor air of a Cuban library and museums located in Havana (Rojas *et al.*, 2012), in the air of a Mexican library (Moctezuma *et al.*, 2015), in Brazilian libraries (Leite-Jr *et al.*, 2018), and recently in the indoor air of Colombian libraries (Camargo *et al.*, 2023; 2024); it has also been found on documents kept in an Italian museum (Montemartini-Corte *et al.*, 2003). Although this genus was not isolated from the outdoor air on this occasion, it has been reported for the Havana atmosphere (Díaz *et al.*, 2020), which indicates that its detection in the indoor sample may have come from outdoors. *Nigrospora* had been previously detected in this repository (Molina *et al.*, 2014; Borrego *et al.*, 2021); it has been isolated from the environment of several Cuban archives (Borrego & Molina, 2018; Borrego *et al.*, 2020; 2022a, c) and from archives and libraries in other countries (Leite-Jr *et al.*, 2018; Pyrri *et al.*, 2020; Silva *et al.*, 2021). It was also previously isolated from the outdoor air, which would justify its presence indoors (Sánchez *et al.*, 2019).

The presence of *Paecilomyces* in the air of this repository had been previously informed at very low proportion (Torres *et al.*, 2022). This genus has been reported at low concentrations in archive and library environments in both Cuba (Rojas *et al.*, 2012;

Borrego & Molina, 2018; Borrego *et al.*, 2022a) and other countries (Pyrri *et al.*, 2020; Camargo *et al.*, 2023; 2024; Iliopoulou *et al.*, 2024).

The fact that *Penicillium* was predominant in the outdoor environment in the first sampling could have influenced its predominance within the repository, and the same could be the case with the other three genera detected (*Aspergillus*, *Cladosporium*, *Gliocladium*). This points to a marked influence of the outdoors on the indoor environment, despite the fact that this repository is air-conditioned during part of the working day, as previously mentioned (Awad *et al.*, 2020; Borrego *et al.*, 2022a; El Jaddaoui *et al.*, 2023).

The environmental fungal behavior of the 2nd sampling was different from that of the 1st, because although the genera *Aspergillus* and *Penicillium* were isolated, they were not predominant; on the other hand, *Cladosporium* was not detected; but a white non-sporulated mycelium prevailed, which had been found on the first occasion at a lower proportion. In any case, this mycelium had been previously reported in this environment at low levels (Molina *et al.*, 2014; Borrego & Molina, 2020; Borrego *et al.*, 2021; Torres *et al.*, 2022).

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It is possible that the genera *Aspergillus*, *Cladosporium* and *Penicillium* were not isolated from the outdoor air because it had been raining for two days before sampling, even until dawn on that same day, and this could have considerably cleaned the atmosphere of their propagules (Sabariego *et al.*, 2004; Castro e Silva *et al.*, 2020), eliminating these genera and enhancing the presence of others that require higher humidity, such as *Acremonium*. The wet spores of *Acremonium* have been reported to disperse by water droplets and wind, which requires high humidity since its a_w is 0.90 to 0.98 (Mould Compendium Website, 2024). However, this genus was detected at considerably high proportion (RD = 33.9%) in the indoor air despite not being very common in archive and library environments, although it has sometimes been detected at low concentrations (Leite-Jr *et al.*, 2018; Pinheiro *et al.*, 2019; Pyrri *et al.*, 2020; Iliopoulou *et al.*, 2024). *Acremonium* was found abundantly in outdoor air (RD = 22.2%) and its presence has been previously mentioned for the atmosphere of Havana (Almaguer & Rojas-Flores, 2013).

The genus *Acrodontium* had been previously detected in the air of this repository, also in low percentages, i.e. less than 2% (Molina *et al.*, 2014; Borrego & Molina, 2020; Borrego *et al.*, 2021). Its presence indoors could be due to its occurrence in the outdoor air (RD greater than 10%), in contrast with a previous report in which it was not detected outdoors (Borrego *et al.*, 2021). Although it is also a rare genus, there are reports of its detection in the indoor air of Greek libraries at very low concentrations (Pyrri *et al.*, 2020; Iliopoulou *et al.*, 2024).

Regarding yeasts, several genera have been isolated from archive and library environments both in Cuba and in other countries (Moctezuma *et al.*, 2015; Leite-Jr *et al.*, 2018; Borrego *et al.*, 2022a, b; Borrego, 2023; Rodríguez & Borrego, 2023; Camargo *et al.*, 2023; 2024; Branysova *et al.*, 2024; Derksen *et al.*, 2024); yeasts were isolated also in a previous study of the air of the same CIPO repository (Torres *et al.*, 2022). Non-sporulated mycelia have also been detected in the indoor environments of other Cuban archives located in different regions (Borrego *et al.*, 2022a, b, c; Borrego, 2023; Rodríguez & Borrego, 2023; Borrego *et al.*, 2024) and were previously detected in this repository at low concentrations (Molina *et al.*, 2014; Borrego *et al.*, 2021; Torres *et al.*, 2022).

Concentrations of *Aspergillus* and *Penicillium* species obtained in indoor air and their I/O ratios

Given that Cuba has no national regulations to determine whether a species is an environmental contaminant, we followed criteria from other countries, despite having different climates. Thus, Portugal has established a maximum fungal concentration of 500 CFU/m³, but toxigenic species such as *A. flavus*, *A. fumigatus*, *A. versicolor* and others should not exceed more than 12 CFU/m³, and uncommon species should not exceed 150 CFU/m³ (Pinheiro *et al.*, 2012; Pinheiro, 2014). Likewise, Canada has established that the concentration of each species should not exceed 50 CFU/m³ as long as these species do not correspond to the genera *Cladosporium* or *Alternaria* (Guild & MacDonald, 2004). Therefore, in this study, we considered those species with concentrations equal to or greater than 50 CFU/m³ and with I/O ratios equal to or greater than 2 (De Aquino Neto & Goés Siqueira, 2000).

The comparison of indoor and outdoor concentrations of *Aspergillus* and *Penicillium* species obtained in the 1st sampling, shows that some of them not only had high indoor concentrations, that is, above 50 CFU/m³, but also their I/O ratios were very high. Among them, eight species stood out for both their concentrations and their I/O ratios. Of these, *P. sclerotiorum* predominated with the highest concentration (160 CFU/m³) and with an I/O of 5.3, while *P. citrinum* despite having a lower concentration (110 CFU/m³) showed the highest I/O ratio, namely 11. These species were followed by *P. janczewskii* (150 CFU/m³), *A. oryzae* (110 CFU/m³), *A. brasiliensis* (30 CFU/m³, I/O = 3), and *A. unguis* (40 CFU/m³, I/O = 2).

In the 2nd sampling only three species of *Aspergillus* exceeded the concentration of 50 CFU/m³ (*A. caespitosus*, *A. japonicus* and *A. tamarii*). This shows that even though the environment of the storage facility turned out to be uncontaminated, there were amplification zones that promoted high concentrations of species that may be harmful both for the preserved documents and for staff health. Hence, it is not enough to simply determine the environmental quality through I/O ratios based on total fungal concentrations, but it is essential to take into account the concentrations and I/O ratios of species belonging to the most harmful genera, such as *Aspergillus* and *Penicillium*, to truly define the environmental microbial quality of an archive facility. According to this criterion, the environment of this repository is highly contaminated with species of *Aspergillus* and *Penicillium*, and therefore it is necessary to take immediate measures related to improving air circulation in order to prevent outbreaks of abundant fungal growth on documents, as well as to prevent health hazards to staff. Continuous monitoring of this environment is also essential to assess the variability and concentration of these species over time.

Beyond this, the present study provided knowledge of the fungal diversity of the repository, since the detection of species such as *P. sclerotiorum* and *P. olsonii* turned out to be new records for the indoor air of Cuban archives and even for the environment of the CIPO, while *P. citrinum*, *P. corylophilum*, *P. janczewskii* and *P. variable* had been detected in the air of several Cuban archives including the CIPO (Borrego *et al.*, 2017; 2020; Borrego & Molina, 2020; Borrego *et al.*, 2021; 2022a, b, c; Borrego, 2023; Borrego *et al.*, 2024).

The species *P. citrinum*, *P. corylophilum*, and *P. janczewskii* had been previously isolated from the atmosphere of Havana (Almaguer *et al.*, 2021), and in addition, in this study *P. citrinum* was detected outdoors, showing that the source of these species is the outdoor air. On the other hand, the discovery of *P. fellutanum* in the 2nd air sampling is particularly significant, given that it is the first time that it has been reported in this location. This fact underlines the need for continuous sampling in archives, since the existence of less common species can influence the conservation of documentary heritage. The presence of *P. fellutanum* in the atmosphere of Havana (Almaguer *et al.*, 2021) indicated that this species could have come from outside.

Regarding the species of *Aspergillus*, members of the Flavi section are known to be tolerant of adverse conditions, such as low RH, which may be a key factor for their prevalence and may have facilitated the high concentration of *A. oryzae* in the indoor air of this repository (Abdel-Maksoud *et al.*, 2022). Likewise, this is one of the species that has been most frequently isolated in Cuba, both in indoor archive environments (Rodríguez, 2016a; Borrego *et al.*, 2017; 2022a, b, c; Borrego, 2023) and in the Havana atmosphere (Almaguer *et al.*, 2021), although in this study this species was not detected in the outdoor air. *Aspergillus auricomus*, despite being identified for the first time in this repository, had been previously isolated at NARC and in other archives of the country (Molina & Borrego, 2014; Rodríguez, 2016b; Borrego *et al.*, 2017; 2022b; Rodríguez & Borrego, 2023). The species *Aspergillus unguis* was detected in both samplings. The results of this study agree with a previous one in which similar values were obtained in this repository for this species (Borrego *et al.*, 2021). The species *A. caespitosus* (belonging to the Versicolores section) detected in the 2nd sampling, is known for its ability to grow in a variety of environmental conditions as well as for its resistance to adverse conditions, which could explain its prevalence in indoor environments. Although the species *A. japonicus* and *A. tamaraii* have been previously isolated from the air in Cuban archives (Borrego & Molina, 2014; Molina & Borrego, 2014a; Borrego *et al.*, 2017; 2022b), these two species, along with *Aspergillus aculeatus*, are not among the most frequently found in archives, libraries and museums in other countries since few authors refer to them (Sobral *et al.*, 2017; Leite-Jr *et al.*, 2018).

The similarity of species of these two genera between the two samplings when compared with previous studies shows that the species *A. unguis* appears in all the isolates, and it may thus be considered as a species specific to this repository. Likewise, the existence of four other species classified as common (*A. candidus*, *A. oryzae*, *A. wentii*, *P. citrinum*) and representing a 21.1% coincidence, suggests a certain stability in the fungal community of the indoor air of this repository. The persistence of these species may be related to their ecological characteristics, such as the ability to form resistance structures and their adaptability to changing conditions (Hanf, 2019; Torres-Hernández, 2021).

Furthermore, considering these factors, a suggestion has been made to the management of this institution to make urgent modifications to the air conditioning system of the repository, to ensure efficient air circulation and thereby reducing the risk of deterioration of stored documents and preventing staff health problems.

CONCLUSIONS

- Although the I/O ratios obtained in both isolations showed that the environment of the CIPO Fund repository is not contaminated, we detected species of the genera *Aspergillus* and *Penicillium* contaminating the air of this repository. This, together with the existence of areas of fungal concentrations amplification could cause health problems for staff due to the increase in the concentration of dangerous species. Therefore, this study demonstrates that in order to determine the environmental quality of a archive repository, it is not enough to assess the I/O ratio depending on the total fungal concentrations, but it is essential to take into account the concentrations and the I/O ratios of the species that are most harmful for health, such as those of genera *Aspergillus* and *Penicillium*. According to this criterion, the environment of the Fund repository of CIPO is highly contaminated with species of these genera, so it is indispensable to take immediate measures for the improvement of air circulation to prevent outbreaks of abundant fungal growth over documents and also damage to staff health.

- Four species of the genus *Aspergillus* were discovered (*A. aculeatus*, *A. auricomus*, *A. brasiliensis* and *A. caespitosus*) that represent new findings for the air of this CIPO repository, while two species (*P. sclerotiorum*, *P. olsonii*) were new records for the air of Cuban archives.

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