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ARTÍCULO ORIGINAL

Assessment of the Airborne Fungal Communities in Repositories of the Cuban Office of the Industrial Property: Their influence in the Documentary Heritage Conservation and the Personnel's Health

Evaluación de las comunidades de hongos aerotransportadas en repositorios de la Oficina Cubana de la Propiedad Industrial: su influencia en la conservación del Patrimonio Documental y la salud del personal

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ABSTRACT

The microbiological quality of indoor air in workplaces has been linked to the occurrence of health disorders and diseases. Archive repositories are environments with a certain level of risk, not only for the conserved documents, but also for the staff´s health, since they are reservoirs of microorganisms that under suitable environmental conditions can cause the biodeterioration of the documents and damage to health of the staff. The aim of this study was to evaluate the pathogenic and biodeteriogenic attributes of viable fungi, detected in indoor environments of two air-conditioned repositories of the Cuban Office of Industrial Property. Air samples from both indoor and outdoor environments were taken using a biocollector SAS Super 100 at 100 L/min during 1 min in Petri dishes with Malt Extract Agar supplemented with NaCl. Indoor/outdoor ratios were determined as an indicative of the environmental quality. In the selected isolated were qualitatively determined the cellulolytic, amylolytic, proteolytic activity, the excretion of pigments and organic acids (biodeteriogenic risk), as well as the spore's size, growth at 37°C and the hemolysins excretion (pathogenic risk). The obtained indoor/outdoor ratios indicated that the repositories had a good environmental quality. Seven fungal genera and two non-sporulating mycelia were detected in the air of the repositories with *Aspergillus, Cladosporium* and *Penicillium* as the predominant ones. The genera *Acrodontium* and *Cylindrocarpon* (in particular *Acrodontium simplex* and *Cylindrocarpon lichenicola*) were isolated for the first time in environments of Cuban archives. Cellulose was degraded by 73.9 % of the analyzed isolated; starch and gelatin were degraded by 71.3 % of the isolated, while 100 % of them excreted acids and only 34.8 % excreted pigments of different tonalities. The 100 % of the spores of the studied isolated can penetrate the upper respiratory tract while 71.7 % of the spores belonging to all *Aspergillus* and *Penicillium* isolated as well as those of *Cladosporium sphaerospermum* and *Acrodontium simplex* can reach the pulmonary alveoli. Only around 40

% of the evaluated isolated were able to grow at 37°C and excrete hemolysins. These results demonstrate that environmental fungi have a broad biodeteriogenic potential and some of them showed pathogenic attributes indicative that theycan be classified as opportunistic human pathogens.

Keywords: airborne fungi; air-conditioned repositories; archives; biodeterioration; indoor environments; quality of indoor environmental; pathogenicity

RESUMEN

La calidad microbiológica del aire interior en los lugares de trabajo se ha relacionado con la aparición de trastornos de salud y enfermedades. Los repositorios de archivos son entornos con cierto nivel de riesgo, no solo para los documentos conservados, sino también para la salud del personal, ya que son reservorios de microorganismos que bajo condiciones ambientales adecuadas pueden ocasionar el biodeterioro de los documentos y afecciones a la salud del personal. El objetivo de este estudio fue evaluar los atributos patogénicos y biodeteriogénicos de hongos viables, detectados en ambientes interiores de dos repositorios climatizados de la Oficina Cubana de la Propiedad Industrial. Se tomaron muestras de aire tanto de ambientes interiores como exteriores utilizando un biocolector SAS Super 100 a 100 L/min durante 1 min en placas de Petri con Agar Extracto de Malta suplementado con NaCl. Los índices interior/exterior se determinaron como indicativo de la calidad ambiental. En los aislados seleccionados se determinó cualitativamente la actividad celulolítica, amilolítica, proteolítica, la excreción de pigmentos y ácidos orgánicos (riesgo biodeteriogénico), así como el tamaño de las esporas, el crecimiento a 37°C y la excreción de hemolisinas (riesgo patogénico). Los índices interior/exterior obtenidos indicaron que los repositorios tenían una buena calidad ambiental. Se detectaron siete géneros de hongos y dos micelios no esporulados en el aire de los repositorios con Aspergillus*,* Cladosporium *y* Penicillium *como los predominantes. Los géneros* Acrodontium *y* Cylindrocarpon *(en particular* Acrodontium simplex *y* Cylindrocarpon lichenicola*) fueron aislados por primera vez en ambientes de archivos cubanos. La celulosa fue degradada por el 73,9 % de los aislados analizados; el almidón y la gelatina fueron degradados por el 71,3 % de los aislados, mientras que el 100 % de ellos excretaron ácidos y solo el 34,8 % excretó pigmentos de diferentes tonalidades. El 100 % de las esporas de los aislados estudiados pueden penetrar el tracto respiratorio superior, mientras que el 71,7 % de las esporas pertenecientes a todos los aislados de* Aspergillus *y* Penicillium *así como los de* Cladosporium sphaerospermum *y* Acrodontium simplex *pueden llegar hasta los alvéolos pulmonares; pero solo alrededor del 40 % de los aislados evaluados pudieron crecer a 37°C y excretar hemolisinas. Estos resultados demostraron que los hongos ambientales tienen un amplio potencial biodeteriogénico y algunos de ellos mostraron atributos patogénicos indicativos de que pueden ser clasificados como patógenos humanos oportunistas.*

Palabras Claves: *hongos en el aire, depósitos con aire acondicionado, archivo, biodeterioro, ambientes interiores; calidad del ambiente interior, patogenicidad*

INTRODUCCIÓN

The Cuban Office of the Industrial Property (COIP) has unique funds of records of the different modalities of Industrial Property (trademarks and other distinctive signs, inventions and industrial models) registered in Cuba from 1847 to the present. Therefore, in this institution the preserved documents make up part of the history of the technological development of the country since they have a high scientific, technological, commercial and cultural value, as well as being considered relevant in the national and international sphere.

This institution with nearly 180 years of existence is located in the Belén convent, a building from the 18th century. The building was adapted in 1990 for the COIP and most of its premises are occupied by offices and reading rooms; but its two archive repositories are physically located within two departments, the Department of Fund and the Department of Trademarks and other Distinctive signs that are always air-conditioned. In these repositories, all the information on Cuban industrial property is conserved and the oldest document dates from March 1867. However, the National Industrial Property Fund is COIP's largest fund with an approximate volume of 271604 documents on paper support and covers the entire history of innovative and commercial creativity from the 19th century to the present day. For this reason, the institution is interested that their documents with heritage value be properly preserved. Although the amount of valuable documentation conserved in the Department of Funds is greater than that kept in the Department of Trademarks (Castro and Borrego, 2018).

The paper is a material that deteriorates due to the effect of various external agents among which are fungi. In archives and libraries, fungi constitute a serious problem for the documentary preservation since they can adhere

to the documentary supports and degrade a wide variety of them (Mallo *et al*., 2017). It has been emphasized that the tropical climate can favour its development, due to the influence of temperature and relative humidity (Borrego and Perdomo, 2016; Polo *et al*., 2017), which is enhanced in environments with poor ventilation or inefficient and neglected air-conditioning systems. Filamentous fungi have great significance for the biodeterioration of cultural heritage collections, firstly because of their effective dispersion form (airborne propagules) and also because they are capable of living in a wide variety of environments supported by powerful and versatile metabolic machinery (Grbić *et al*., 2013). Some effects that fungi cause in documentary supports are enzymatic degradation of cellulose, starch and proteins, the excretion of acids and pigments as well as the discoloration, which cause mainly aesthetic damage. Furthermore, through the pressure exerted by their hyphae on the materials, alterations of a mechanical nature may occur (Mallo *et al*., 2017).

Other important properties of fungi are related to their pathogenicity (Eduard, 2009; Novohradská *et al*., 2017). Numerous studies have established a close relationship between environmental conditions, the presence offungal propagules (spores, conidia, and fungal fragment) and their possible incidence in the eventual triggering of respiratory and allergic diseases (Twaroch *et al*., 2015). Its presence in indoor environments is associated with infectious pathologies (mycosis) mediated by various virulence factors that vary depending on groups or taxa (Cabral, 2010; Guarro, 2012).

As the staff of the aforementioned COIP departments work very close to the documents during the working day, they have a high level of exposure to the same environment that documents share. Thus, it is necessary to conduct studies of characterization and monitoring of the airborne mycobiota in the repositories in order to ensure the conservation of collections and prevent possible pathologies in staff and visitors who regularly attend these places. Therefore, the aim of this study was to evaluate the pathogenic and biodeteriogenic attributes of viable fungi, detected in indoor environments of two air-conditioned repositories of the COIP.

MATERIALES Y MÉTODOS

Characterization of the repositories

The study was conducted in two repositories of the Cuban Office of the Industrial Property (COIP) located in the Habana Vieja municipality, Havana city, Cuba. Both repositories are located next to each other on the ground floor of the building. Repository 1 (R-1) is the largest of them and is situated inside of the Funds Department. Due to its dimensions, it has two storeys that were built to take advantage of the height of the premises and thus have a greater capacity for document storage. These two levels were constructed in mezzanine form using steel beams and concrete floor lined with floor slabs (A: lower level, B: upper level) (Fig. 1, I-b y I-c). Its dimensions are 17 m long x 8 m wide x 10 m high approximately. Both storeys share the same air-conditioning system made up of three air-conditioning units. This premise has a total of five windows and three doors, of which two windows and two doors are located on the left wall that faces the interior yard of the building, the other three windows are located on the right wall of the premises that faces the exterior of building. But those doors and windows are maintained closed the whole time to ensure the good air-conditioning of the space and the adequate values of T and RH for the documents conservation (Fig. 1, II-a). A grill door located in the metal grating partition that is the one that separates R -1 of the Funds Department office that precede it, is the one that constitutes the main entrance of this repository (Fig. 1, II).This space has an annual average temperature (T) varying between 24°C and 27°C and relative humidity (RH) fluctuated between 65 and 75%.

Repository 2 (R-2) is a small mezzanine that is located on the second level within the Trademark Department office (Fig. 1, I-d). Its dimensions are approximately 5 m long x 7 m wide x 5 m high. It has others air-conditioning systems that shares with the department office (Fig. 1, II-b) and is characterized by having moistures problems on some walls (Fig. 1, III). It has an annual average T that ranges between 22°C and 26°C and a RH that varies from 67 to 77 %. T and RH have been recorded for years using hygrothermographs that conservators have located on both repositories.

Sampling of airborne fungal propagules and identification of the fungal isolates

Viable fungal propagules were sampled of the air of both repositories in July 2018, month corresponding to the rainy season in Cuba. This season spans five months and runs from June 1 to November 30. A total of 11 sampling points were selected in the R-1 (6 in the level A and 5 in the level B) and 2 sampling points were selected to the R-2 according to FEDECAI-01 (2007) (Fig. 1, II). As a control, a triplicate outdoor sampling was carried out in the central yard of the building in the time interval between 11 a.m. and noon, considering working hours and a higher concentration of viable fungal propagules in Havana`s atmosphere (Almaguer and Rojas, 2013). Each point of indoor was sampled in triplicate using a SAS Super 100 biocollector (Italy) in a vertical position at one hour intervals between repetitions (approximately 10:00 am - 1:30 pm). With the biocollector 100 L of air/min was collected at a height of

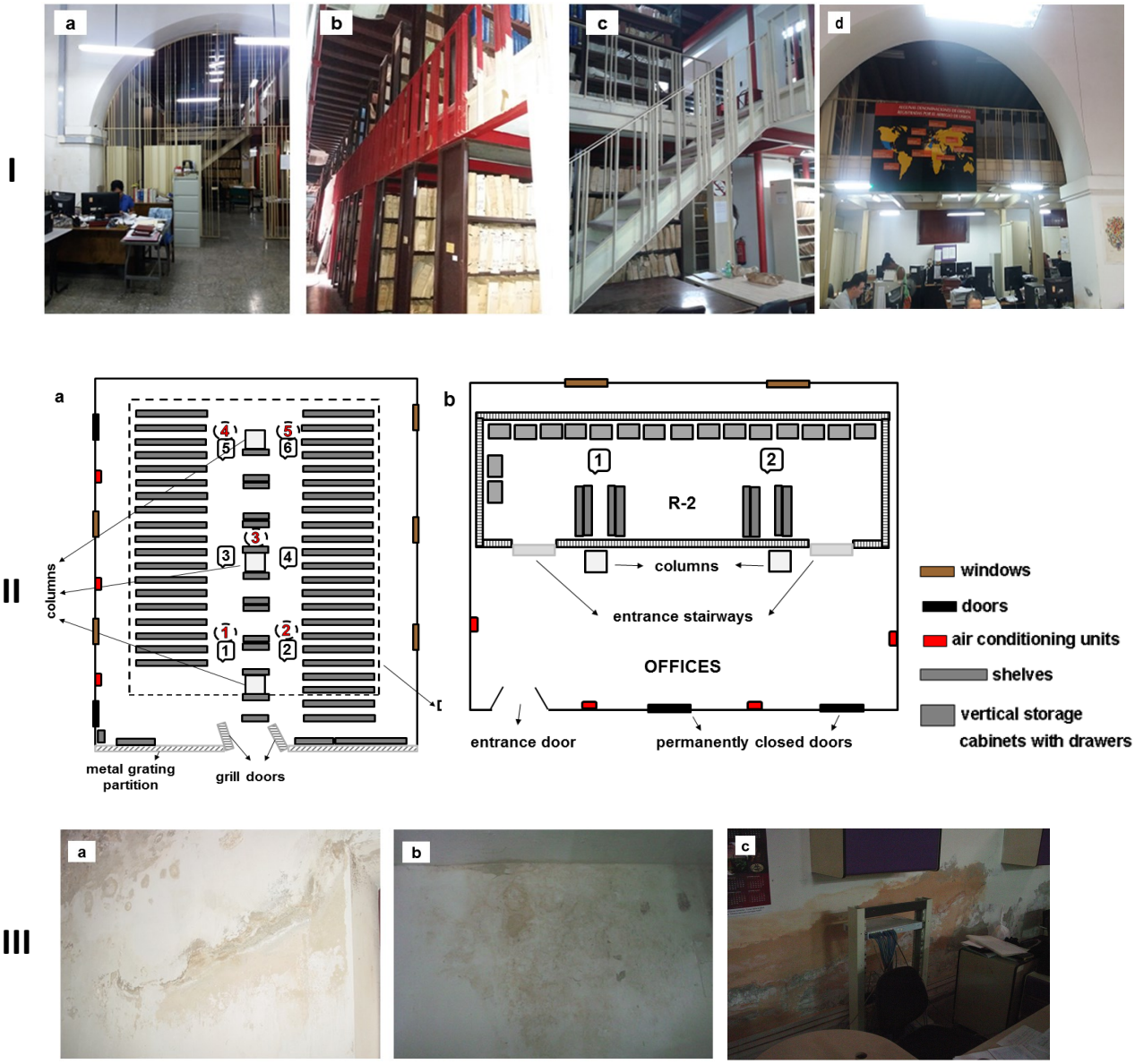

Figure 1. I -Location and characteristics of the studied repositories. a, b, c) Indicates different views of the repository 1 (R-1) within the Fund Department. Note that both storeys of the repository share the same air-conditioning units and there is no dividing door between the office (with other air conditioners) and the repository, there is only one dividing metal grill partition; therefore, air moves across this entire space. d) Indicates the Trademark Department offices on the lower level and Repository 2 (R-2) on the second level. **II** -Top view of the two repositories. a) Indicates the lower level of R-1 (Level A) showing the 6 sampling points. DL: Points with dashed lines indicate the limits of the structure located in superior level of the R-1 (mezzanine) which has another group of shelves and where 5 more points were sampled (Level B: in this case the 5 sampled points appear in red colour and within the circles with dashed lines). b) Indicates the 2 points sampled in the repository 2 (R-2). **III** -Some existing moisture problems on the walls a, b) within repository 2 (R-2) and c) in the office area located on the lower level of R-2.

Figura 1. I*-Ubicación y características de los repositorios estudiados. a, b, c) Indica diferentes vistas del repositorio 1 (R-1) dentro del Departamento de Fondo. Tenga en cuenta que ambos pisos del depósito comparten las mismas unidades de aire acondicionado y no hay una puerta divisoria entre la oficina (con otros aires acondicionados) y el depósito, sólo hay una partición de rejilla metálica divisoria; por lo tanto, el aire se mueve por todo este espacio. d) Indica las oficinas del Departamento de Marcas en el nivel inferior y el Repositorio 2 (R-2) en el segundo nivel.* ***II*** *-Vista superior de los dos repositorios. a) Indica el nivel inferior de R-1 (Nivel A) mostrando los 6 puntos de muestreo. DL: Los puntos con líneas discontinuas indican los límites de la estructura ubicada en el nivel superior de la R-1 (entrepiso) que tiene otro grupo de estantes y donde se muestrearon 5 puntos más (Nivel B: en este caso los 5 puntos muestreados aparecen en color rojo y dentro de los círculos con líneas discontinuas). b) Indica los 2 puntos muestreados en el repositorio 2 (R-2).* ***III****-Algunos problemas de humedades existentes en las paredes a, b) dentro del repositorio 2 (R-2) y c) en el área de oficinas ubicada en el nivel inferior de R-2.*

1.5 m approximately. The culture medium used was Malt Extract Agar (MEA) (BIOCEN, Cuba) supplemented with NaCl (7.5%) (Borrego and Perdomo, 2016). Subsequently, the Petri dishes were incubated inverted at 30°C for 7 days and the colonies were counted to calculate the fungal concentration per m^3 of air expressed in colony forming units per cubic meter of air (CFU/m^3) and the results were then averaged. The colonies were then isolated and purified in MEA; after growing in this medium, two copies of each colony were made in two slants of each of the culture media used for their conservation (MEA and Sabouraud Dextrose Agar) which were stored at 4°C.

The indoor/outdoor (I/O) ratio was calculated according to Stryjakowska-Sekulska *et al*. (2007) who indicated that if this relationship is equal to or less than 1.5 it is that the environments are not contaminated and there is good ventilation, if the relationship is between 1.5 and 2, then the environmental quality is regular and if it is greater than 2 it is because the environments are polluted and have poor ventilation.

Measurements of T and RH were conducted in the same points at the moment of the microbiological determination using a digital thermo hygrometer Pen TH 8709 (China).

Fungi colonies were identified according to their cultural and morphological characteristics. The characteristics of conidiophores, conidia and other fungal structures of taxonomic interest obtained in various differential culture media were also taken into account. Observations were made on a stereomicroscope (Carl Zeiss, Germany) and a clear field trinocular microscope Olympus (Japan) with a digital camera attached (Samsung, Korea). Different dichotomous keys were used as identification guides (de Hoog, 1972; Brayford, 1987; Domsch *et al*., 1980; Pitt, 2000; Klich, 2002; Barnett and Hunter, 2003; Varga *et al*., 2011 a, 2011 b; Samson *et al*., 2011; Bensch *et al*., 2012).

Relative density (RD) was calculated using the formula of Smith (1980): RD = (number of colonies of one taxon/ total number of colonies) x100.

The Sørensen's coefficient of similarity (QS) was used to compare the similarities between the composition of the taxa obtained on indoor environments and the outdoor environment (Sánchez *et al*., 2019): $QS = 2a/b + c$. Where *a* is the number of common genera detected in the two environments that are comparing, *b* the number of detected taxa only in indoor environment and *c* the number of detected taxa only in the outdoor environment.

Statistical analysis

The data obtained were processed with the statistical Statgraphics Centurion XV program. A simple variance analysis (ANOVA) was applied with multiple ranges by the method of minimum square minimal difference (LSD) to compare the obtained fungal concentrations in both indoor and outdoor environments as well as the behavior of the thermo-hygrometric parameters. The obtained T and RH values in the moment of the of R-1 and R-2 sampling, as well as the values of T and RH recorded with hygrothermographs during the previous week to the sampling and the fungal concentration data were used to determine the correlations among these parameters according to Pearson's correlation. All the values of RH and CFU/m^3 obtained in R-1 were also plotted in the Surfer 8.00 program (Golden Software, Inc.) to observe the spatial distribution of these parameters on both storeys of the repository.

Determinations of biodegradative activities and some virulence factors

After the taxonomic identification of the isolated, some repetitions of several species was observed and, therefore, a selection of 46 representative isolated (65.7 % of the total) of the identified taxa was made to carry out the different tests planned in this study. In the selection of these isolated, the number of repetitions of each species and their existence in both repositories were taken into account. In all cases the tests were performed in triplicate.

Among the biodegradation assays the cellulolytic (growth on filter paper, FP; crystalline cellulose, CC and carboxymethylcellulose, CMC), amylolytic and proteolytic activities as well as the production and excretion of pigments and acid were determined according to Borrego *et al*. (2017).

Three virulence factors were evaluated in these taxa. With the intention of analyzing the penetration of the conidia in the human respiratory tract the spore sizes were determined according to Borrego and Molina (2018); the growth at 37°C (Llop *et al*., 2001), and hemolytic activity (Bogomolova and Kirtsideli, 2009) were also evaluated.

RESULTS

Airborne fungal concentration of the repositories

The fungal concentrations in R-1 showed differences in their distribution within the repository itself, evidenced by the dispersion of the maximum and minimum values with respect to the obtained average CFU/m^3 value (Table 1). The highest fungal concentrations were detected at the entrance of both storeys of R-1 and towards the end of the premises a decrease in the CFU/m^3 was observed (Figure 2). Despite this, the comparison between fungal concentrations average detected in each level of the R-1 (A: 35 and B: 37 CFU/m^3 respectively) showed no statistically significant differences ($p \leq 0.05$); however, these values differ significantly from those obtained in the building's outdoor air, where fungal concentrations were higher (90 CFU/m^3).

Table 1. Fungal concentrations of the indoor air of the two studied repositories of COIP, indoor/outdoor (I/O) ratios and obtained thermo-hygrometric values.

Tabla 1. *Concentraciones fúngicas del aire interior de los dos repositorios de la OCPI estudiados, relaciones interiores/ exteriores (I/O) y valores termohigrométricos obtenidos.*

REPOSITORY	VALUE	CONCENTRATION (CFU/m^3) ± SD	I/O ratio	T (°C) ± SD	RH (%) ± SD
R-1 A	Max.	60			
	Min.	15			
	Average [1]	35 ± 15 a	0.4	25.3 ± 0.3 c	61.4 ± 0.5 e
R-1 B	Max.	65			
	Min.	15			
	Average [2]	37 ± 17 a	0.4	25.2 ± 0.2 c	61.5 ± 0.6 e
R-2	Max.	50			
	Min.	15			
	Average [3]	35 ± 18 a	0.4	26.5 ± 0.2 c	62.2 ± 1.1 e
Outside yard	Max.	115			
	Min.	65			
	Average [4]	90 ± 20 b	-	32.8 ± 2.0 d	70.3 ± 1.5 f

SD: Standard deviation. [1]: Represent the average of 6 points by triplicate (n = 18). [2]: Indicates the average of 5 points by triplicate (n = 15). [3]: It is the average of 2 points by triplicate (n = 6). [4]: Indicative of the average of 3 points by triplicate (n = 9). T: Temperature (n = 18, 15, 6 and 9 respectively), RH: Relative Humidity (n = 18, 15, 6 and 9 respectively). Letters a - f indicates difference statistically significant or not according to the LSD method ($p < 0.05$).

SD: Desviación estándar. [1]: Representa el promedio de 6 puntos por triplicado (n = 18). [2]: Indica el promedio de 5 puntos por triplicado (n = 15). [3]: Es el promedio de 2 puntos por triplicado (n = 6). [4]: Indicativo del promedio de 3 puntos por triplicado (n = 9). T: Temperatura (n = 18, 15, 6 y 9 respectivamente), HR: Humedad Relativa (n = 18, 15, 6 y 9 respectivamente). Letras a - f indican diferencias estadísticamente significativas o no, según el método de LSD (p < 0.05).

In the case of the R-2 the fungal concentration (35 CFU/m^3) not showed significant differences with relation to the R-1 but significant difference was obtained with the outdoor (Table 1). The indoor/outdoor (I/O) ratios showed values of 0.4 for both repositories, evidencing low ratios which are indicative of a good quality environment. However, although the value of the I/O ratio in R-2 was also low, some walls of the repository and the office located below it were observed that show humidity problems with incipient fungal development that could affect in the future the quality microbiological environment of R-2 (Fig. 1, III).

To know the incidence of T and RH on fungal load, it was necessary not only to consider the measurements made on the same sampling day (very few), but also those values monitored during the week prior to this analysis in order to have a greater number of values that allow a statistical study to be carried out. It was observed in R-1 a high positive correlation (97 %) between the fungal concentrations and RH ($r = 0.9713$, $p = 0.018$), likewise correlating of fungal concentration with T (55 %) was also positive ($r = 0.5504$, $p = 0.016$). In R-2 were not obtained any correlation among fungal concentrations, RH and T for $p \leq 0.05$ (RH and CFU/m^3, $r = 0.639$, $p = 0.067$; T and CFU/m^3, $r = 0.721$, $p = 0.091$). Because there was a greater correlation between RH and fungal load in R-1, the Surfer 8.0 software was used to exemplify the spatial distribution of these two parameters in the two floors of the repository (Fig. 2).

However, with this program it was possible to corroborate the high correlation between the two parameters ($r = 0.97429613$, $p = 0.00266666$).

A total of 70 isolated were detected on the indoor environments, of them 49 corresponded to R-1 and 21 were isolated in R-2. In the outdoor were detected a total of 30 isolated. The taxa isolated from both indoor environments were grouped as follows: in R-1 the taxa comprised six genera and two non-sporulating mycelia, in R-2 four genera and one non-sporulating mycelium were isolated, while in the outdoor environment five genera and a non-sporulating mycelium were detected (Fig. 3).

In the environment of R-1 *Cladosporium* was the predominant genus (32%) while *Penicillium* (20%) and *Aspergillus* (19%) were detected in second and third place respectively. In spite of this, *Nigrospora*, *Alternaria*, *Cylindrocarpon* and two non-sporulating mycelia (PNSM, Pigmented Non-sporulating Septated Mycelium and WNSM, White Non-sporulating Septated Mycelium) were found with lower percentages.

In R-2 *Penicillium* was the predominant genus (61%) followed by *Aspergillus* (27%) and *Cladosporium* (9.3%), although *Acrodontium* and a WNSM were other isolated taxa with a lower predominance. The taxa *Aspergillus*, *Cladosporium*, *Cylindrocarpon*, *Nigrospora*, *Penicillium* and a WNSM were isolated from the outdoor environment.

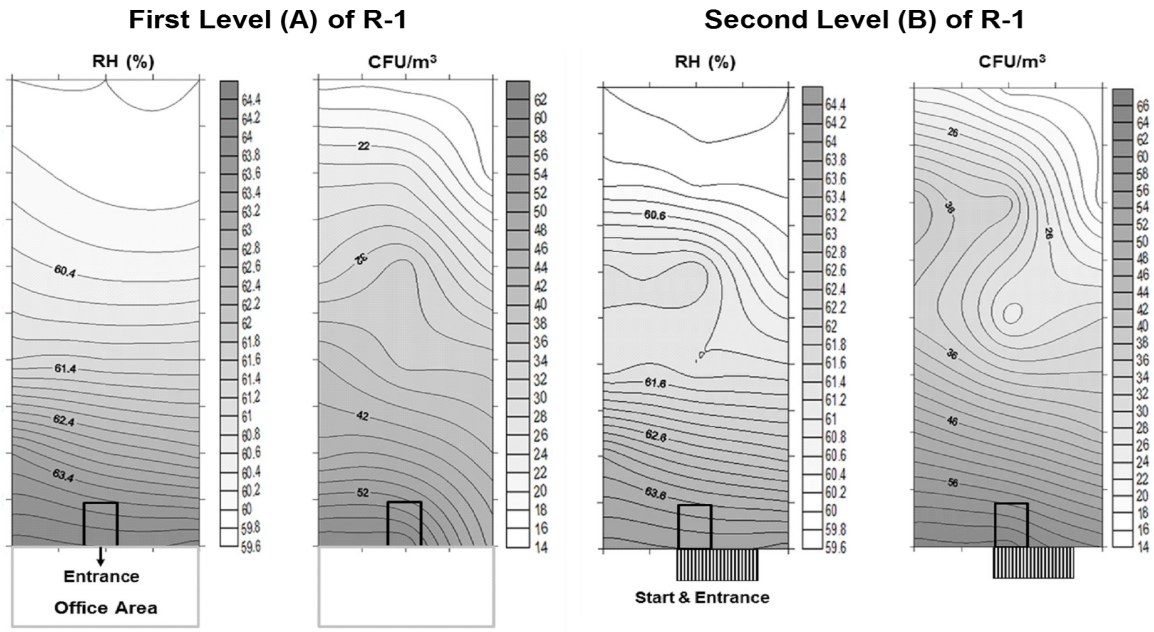

Figure 2. Contour maps obtained with Surfer 8.00 software that represents the spatial distribution of the values of relative humidity (RH, %) and fungal concentrations (CFU/m^3) detected in both levels of the repository 1 (R-1) of the COIP. A high positive correlation between these two parameters is evident (r = 0.97429613, p = 0.00266666). Higher values (darker areas) were found in the zone near to the entrance and the lowest values (clearer areas) in the background of the repository.

Figura 2. *Mapas de contorno obtenidos con el software Surfer 8.00 que representa la distribución especial de los valores de humedad relativa (HR, %) y concentraciones fúngicas (CFU/m^3) detectadas en ambos niveles del repositorio 1 (R-1) de la OCPI. Es evidente una alta correlación positiva entre estos dos parámetros (r = 0.97429613, p = 0.00266666). Se encontraron valores más altos (áreas más oscuras) en la zona cercana a la entrada y los valores más bajos (áreas más claras) en el fondo del repositorio.*

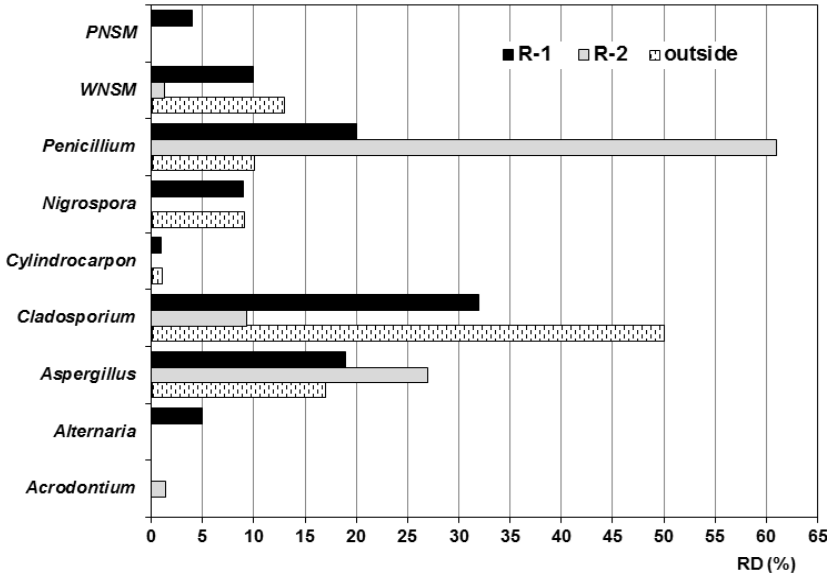

Figure 3. Relative density (RD) of the fungal genera isolated from the indoor air of both studied repositories of COIP and the outdoor air (central yard). R-1: Indicates the repository 1. R-2: Indicates the repository 2. WNSM: White Non-sporulating Septated Mycelium. PNSM: Pigmented Non-sporulating Septated Mycelium.

Figura 3. *Densidad relativa (DR) de los géneros fúngicos aislados del aire interior de ambos repositorios estudiados en OCPI y del aire exterior (patio central). R-1: Indica el repositorio 1. R-2: Indica el repositorio 2. WNSM: micelio blanco septado no esporulado. PNSM: micelio pigmentado septado no esporulado.*

Most of the taxa isolated from the indoor environments coincided with those found in the outdoor environment. As can be seen in figure 3 of the nine taxa obtained in total in R -1, six coincided with the outdoor, representing a QS of 0.9, while in R-2 of five isolated taxa, four were similar to those detected outdoor for an QS of 0.7. The predominant genus in the outdoor environment was *Cladosporium* followed by *Aspergillus* spp. and the WNSM while the other three taxa detected (*Cylindrocarpon, Nigrospora* and *Penicillium*) appear in lower percentages.

In relation to the identified species, a high diversity in the R-1 environment is evidenced (Fig. 4) since 23 taxa were detected whilst 17 taxa were found in R-2. In R-1, 10 species corresponded to *Aspergillus* spp., five belonged to *Cladosporium* spp., five to *Penicillium* spp., one to *Alternaria* spp., one to *Cylindrocarpon* spp. and one to *Nigrospora* spp. On the other hand in R-2, five species corresponded to *Aspergillus* spp., three to *Cladosporium* spp., eight to *Penicillium* spp. and one to *Acrodontium* spp.

For the *Aspergillus* genus, five species (*A. flavus, A. niger, A. oryzae, A. ungis* and *A. versicolor*) coincided in both repositories, of the genus *Cladosporium*f our species were similar (*C. cladosporioides, C. herbarum* and *C. sphaerospermum*), whilst *Penicillium* spp. only four species were found to be coincident (*P. citrinum, P. chrysogenum, P. griseofulvum* and *P. oxalicum*). All matching taxa in both repositories were ecologically abundant in those environments.

Among the predominant species in R-1, those of the genus *Cladosporium* stood out, with a prevalence of *C. cladosporioides* (23 %). However, four other species were also detected to a lesser extent (*C. herbarum, C. hillianum, C. lignicola, C. sphaerospermum*). The second genus found in this repository was *Penicillium* and the prevailing species was *P. griseofulvum* (8 %) followed by *P. citrinum* (5 %). In relation to the genus *Aspergillus* which ranked third, the species *A. niger* (5 %), *A. flavus* (4 %), *A. chevalieri* (2 %) and *A. oryzae* (2 %) predominated, while the other five species detected showed lower percentages (*A. flavipes, A. niveus, A. ungis, A. versicolor, A. wentii*).

For its part in R-2 where *Penicillium* spp. showed a considerable predominance, the species *P. citrinum* (20 %) and *P. chrysogenum* (10 %) prevailed. Within *Aspergillus* spp. which was the genus that was detected in second place, the species *A. flavus* (10 %) and *A. niger* (7 %) prevailed while the rest of the other species of this genus were detected between 5 and 2 % (*A . oryzae, A. versicolor, A. ungis*).

In general, highest prevalent taxa within R-1 were *C. cladosporioides* (23 %), WNSM (10 %), *Nigrospora sphae*rica (9 %) and *P. griseofulvum* (8 %), while in R-2 they were *P. citrinum* (20 %), *P. chrysogenum* (10 %) and *A. flavus* (10 %).

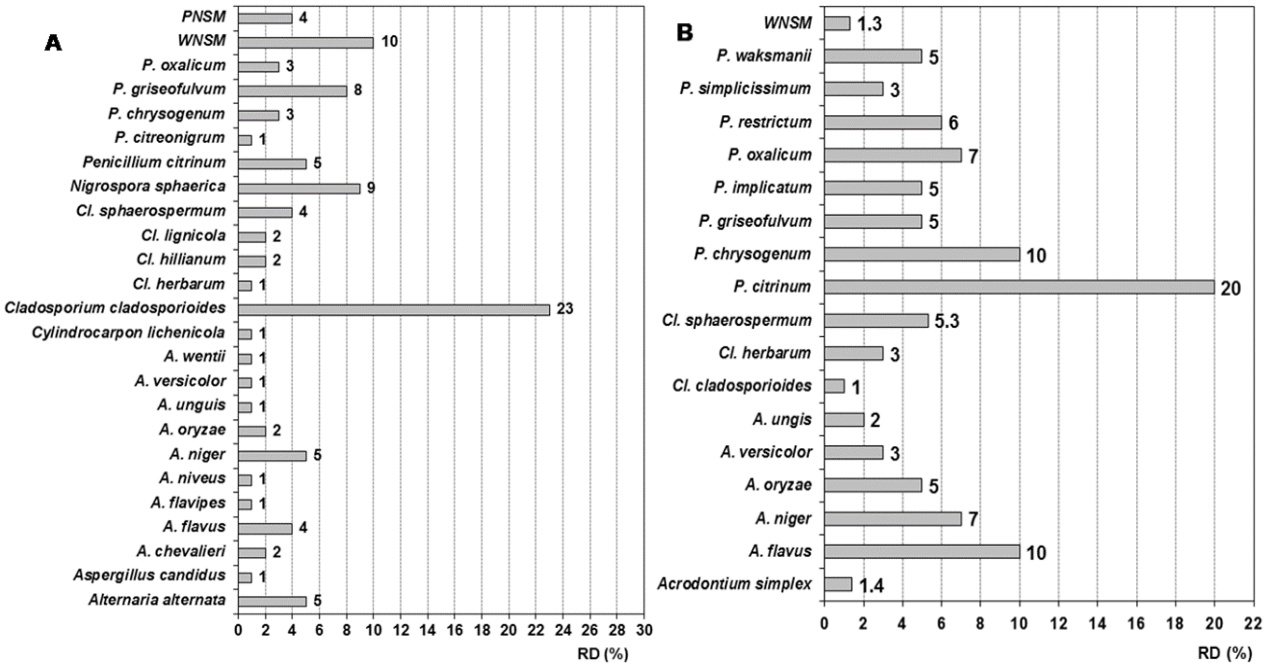

Figure 4. Relative density (RD) of species and non-sporulating mycelia detected in both repositories. A) Indicates the two levels of R-1. B) Indicates R-2. WNSM: White Non-sporulating Septated Mycelium. PNSM: Pigmented Non-sporulating Septated Mycelium.

Figura 3. *Densidad relativa (DR) de especies y micelios no esporulados detectados en ambos repositorios. A) Indica los dos niveles de R-1. B) Indica R-2. WNSM: micelio blanco septado no esporulados. PNSM: micelio pigmentado septado no esporulados.*

Evaluation of the biodegradative characteristics

The results of the biodegradative characteristics or biodeteriogenic potential can be observed in table 2. The organic acids were excreted by 100 % of those evaluated (decrease in pH in the culture medium), standing out among them 10 isolated that showed pH values lower than 4.0. They were *Alternaria alternata, A. flavipes*, the two isolated of *A. niger* (O-23 and O-24)*, A. ungis* (O-1), *Cylindrocarpon lichenicola*, two isolated of *C. cladosporioides* (O-30 and O-4), *P. oxalicum* (O-19) and WNSM (O-17).

On the other hand, 89 % of the strains grew at the expense of FP as sole carbon source (FPase activity) while good growth revealed more than 80 % of the strains on CC (composed fundamentally by α-cellulose) and CMC. Maximum growth showed the most of the *Aspergillus* and *Penicillium* strains, demonstrating that their cellulase enzymatic systems are efficient.

Among the *Aspergillus* species *A. flavus* (O-11)*, A. niger* (O-23)*, A. oryzae* (O-25)*, A. ungis* (O-21) and *A. versicolor* (O-25) exhibited the best growths in all cellulosic sources. A similar result was shown by two isolated of *P. citrinum* (O-50 and O-51)*, P. chrysogenum* (O-28)*, P. griseofulvum* (O-22), *P. implicatum* (O-14)*, P. oxalicum* (O-22), *P. simplicissimum* (O-41) and *P. waksmanii* (O-43) among *Penicillium* species as well as *C. cladosporioides* (O-4) among *Cladosporium* species.

In this study, pigment excretion was observed on the FP and the other culture media used in the determination of cellulolytic activity in 34.8 % of the evaluated isolated, highlighting the *Cladosporium* species. The pigment tonalities showed colours between yellow and the dark brown, although some strains excreted dark green pigments and in particular a pink pigment was excreted by a non-sporulating mycelium (WSNM, O-17). On the other hand, the 71.7 % of the isolated degraded the starch and gelatin.

When a fungal isolated shows several biodeteriogenic attributes, its damaging effect on documents is high as it can degrade various substances that make up the paper. In this study, 23.9 % of the isolated were positive for all five tests, 39.1 % of them were positive for four of the five tests, 12 % of the isolates were positive for three of the tests, while 8 % and 4 % of the isolates showed positivity to two or one of the analyzes performed respectively.

As can be seen, more than 60 % of the isolated showed positivity to five or four of the tests made, indicative that the most of the taxa isolated from the air representing a high risk for the documentary collections that are conserved in the studied repositories.

It highlighting isolated of the species *A. flavipes, A. niger, A. oryzae, A. unguis, C. cladosporioides, C. herbarum, C. hillianum, C. lignicola C. sphaerospermum, P. citrinum,* and *P. chrysogenum* for being able to show the five activities in a considerable way.

Evaluation of some pathogenic attributes (virulence factors)

In this study, the measurement of spores of the different isolated allowed determining their potential to penetrate the respiratory tract (Table 3).

The upper respiratory tract can be penetrated by all the spores detected. Of them only 21.7 % are retained in the upper respiratory tract because their sizes exceeds 10 µm while 78.3 % of them could pass into the rest of the respiratory tract because its dimensions are less than or equal to 5 µm. Among the spores that are retained in the first level of respiratory tract are those belonging to the isolated of *Alternaria alternata, Cylindrocarpon lichenicola* and *Nigrospora sphaerica*. The spores of these fungi can come into contact with the nasopharyngeal mucosa and trigger allergies or infections.

Acrodontium simplex and all isolated of *Aspergillus* and *Penicillium* showed conidia sizes that ranged between 1.5 and 5 µm, even as some of them tend to be rather subglobose, they showed general dimensions between 1.5 - 3.5 x 1.5 - 5.0 µm, which allows them to enter up to the alveoli depending on the position in which they enter the respiratory tract.

Conidia of seven of the nine isolated of *Cladosporium* (representing 15 % of the total isolates analyzed) belonging to four species (*C. cladosporioides, C. herbarum, C. hillianum, C. lignicola*) can penetrate the respiratory tract to the trachea, bronchi and bronchioles, while only the isolated of *C. sphaerospermum* (O-38 and O-40) could reach the alveoli.

Regarding to the growth at 37°C, it was obtained that 22 isolated (47.8 % of the total) grew at that temperature. Among them, 12 *Aspergillus* isolated (80 %) belonging to eight of the detected species of this genus stood out. Those isolated were *A. candidus* (O-36), *A. chevalieri* (O-7), *A. flavipes* (O-10), *A. flavus* (O-33 and O-11), *A. niger* (O-23 and O-24), *A. niveus* (O-8), *A. oryzae* (O-32 and O-25), *A. versicolor* (O-5 and O- 31). Even within them, six (60 %) showed exuberant growth at this temperature (O-7, O-33, O-11, O-23, O-24, O-8). *Cylindrocarpon lichenicola* was another of the species that grew at 37°C as well as nine isolated corresponding to four *Penicillium* species (60 % of the total of isolated of *Penicillium*). These were two isolated of *P. chrysogenum* (O-28 and O-22), the three isolated of *P. citrinum* (O-50, O-51 and O-12), the three isolated of *P. oxalicum* (O-19, O-29 and O-42) and *P. simplicissimum* (O-41).

Table 2. Qualitative behavior of the degradative activities in the fungal taxa isolated from the indoor air of the two studied repositories at the COIP.

Tabla 2. *Comportamiento cualitativo de las actividades degradativas en los taxones fúngicas aislados del aire interior de los dos repositorios estudiados en la OCPI.*

		CELLULOLYTIC ACTIVITY			ACID		AMILOLYTIC ACTIVITY	PROTEOLYTIC ACTIVITY
CODE	TAXA	GROWTH ON FP *	GROWTH ON CC	GROWTH ON CMC	EXCRETION (pH)	PIGMENTS EXCRETION	STARCH DEGRADATION	GELATIN DEGRADATION
O-39	*Acrodontium simplex*	+ + +	+ + +	+ + +	5.02	-	+	-
O-6	*Alternaria alternata*	+ +	±	+	3.90	+ (brown)	-	+
O-36	*Aspergillus candidus*	+	+	+	6.07	-	-	+
O-7	*Aspergillus chevalieri*	+	+	+	4.46	-	-	-
O-10	*Aspergillus flavipes*	+ +	+	+ +	3.72	-	-	+
O-33	*Aspergillus flavus*	+ + +	+	+ +	6.22	-	+	+
O-11	*Aspergillus flavus*	+ + +	+ + +	+ + +	5.13	-	+	+
O-23	*Aspergillus niger*	+ + +	+ + +	+ + +	3.12	+ (yellow)	+	+
O-24	*Aspergillus niger*	+ + +	+ +	+ +	3.35	+ (yellow)	+	+
O-8	*Aspergillus niveus*	+ +	+ +	+ + +	5.30	-	+	-
O-32	*Aspergillus oryzae*	+ +	+	+ +	4.33	-	+	+
O-25	*Aspergillus oryzae*	+ + +	+ +	+ + +	4.93	-	+	+
O-26	*Aspergillus ungis*	+ +	+	+	5.82	-	+	+
O-1	*Aspergillus ungis*	+ + +	+ + +	+ + +	3.68	-	+	+
O-5	*Aspergillus versicolor*	+ + +	+ + +	+ +	4.13	-	+	+
O-31	*Aspergillus versicolor*	+	+	+ + +	6.02	-	+	+
O-34	*Aspergillus wentii*	+	+	+ +	4.82	-	+	+
O-30	*Cladosporium cladosporioides*	+ +	+ +	+ + +	3.34	+ (brown)	+	+
O-2	*Cladosporium cladosporioides*	+ +	+ +	+ + +	5.63	+ (brown)	+	+
O-4	*Cladosporium cladosporioides*	+ + +	+ + +	+ + +	3.55	+ (brown)	+	+
O-18	*Cladosporium herbarum*	+ +	+	+ +	4.76	+ (dark green)	+	+
O-21	*Cladosporium herbarum*	+ +	+	+ +	6.50	+ (dark green)	+	+
O-3	*Cladosporium hillianum*	+ +	+	+ +	4.16	-	+	+
O-35	*Cladosporium lignicola*	±	±	±	6.60	-	-	-
O-38	*Cladosporium sphaerospermum*	+ +	+ +	+ +	5.11	+ (brown)	-	+
O-40	*Cladosporium sphaerospermum*	+	+	+	6.30	+ (brown)	+	+
O-37	*Cylindrocarpon lichenicola*	+ +	+ +	+ +	3.65	-	-	-
O-52	*Nigrospora sphaerica*	-	-	-	5.21	-	-	-
O-28	*Penicillium chrysogenum*	+ + +	+ + +	+ + +	6.56	-	+	+
O-22	*Penicillium chrysogenum*	+ +	+ +	+ +	5.79	-	+	+
O-27	*Penicillium citreonigrum*	+ +	+	+ +	4.45	-	+	-
O-50	*Penicillium citrinum*	+ + +	+ +	+ + +	6.47	+ (yellow)	+	+
O-51	*Penicillium citrinum*	+ + +	+ + +	+ + +	5.27	+ (greenish yellow)	-	+
O-12	*Penicillium citrinum*	+ +	+	+ + +	4.36	+ (greenish yellow)	+	+
O-9	*Penicillium griseofulvum*	+ +	+ +	+ + +	5.15	-	+	+
O-13	*Penicillium griseofulvum*	+ + +	+ + +	+ + +	6.54	-	+	+
O-14	*Penicillium implicatum*	+ + +	+ +	+ +	4.41	-	-	+
O-19	*Penicillium oxalicum*	+ +	+ +	+ +	3.21	-	+	+
O-29	*Penicillium oxalicum*	+ + +	+ +	+ + +	5.05	-	+	-
O-42	*Penicillium oxalicum*	+	+	+ +	4.72	-	+	-
O-15	*Penicillium restrictum*	+ + +	+ +	+ + +	5.40	-	+	-
O-41	*Penicillium simplicissimum*	+ + +	+ + +	+ + +	6.61	-	+	+
O-43	*Penicillium waksmanii*	+ + +	+ +	+ + +	5.59	+ (dark green)	+	+
O-20	PNSM	-	-	-	6.10	-	-	-
O-16	WNSM	-	-	-	5.35	+ (yellow)	-	-
O-17	WNSM	+ + +	+ + +	+ + +	3.11	+ (pink)	-	-

FP: filter paper. CC: crystalline cellulose. CMC: carboxymethylcellulose. *: Growth at the expense of FP is indicative of FPase activity, only that the assay was performed qualitatively; + + +: Indicates abundant growth (100 % of the saline culture medium slant and on the paper strip); + +: Indicates moderate growth (70 – 50 % of the slant and on the paper strip); +: Indicates poor growth (< 50 % of the slant and on the paper strip), it is also indicative of pigments presence; ±: Indicates very poor growth, or pigment production; -: Indicates NO growth and NO pigment production.

FP: papel de filtro. CC: celulosa cristalina. CMC: carboximetilcelulosa. *: Crecimiento a expensas del FP es indicativo de actividad PFasa, solo que este ensayo se realizó cualitativamente; + + +: Indica crecimiento abundante (100 % de la cuña del medio salino y sobre la tira de papel); + +: Indica crecimiento moderado (70 – 50 % de la cuña y sobre la tira de papel); +: Indica crecimiento pobre (< 50 % de la cuña o la tira de papel), también es indicativo de presencia de pigmentos; ±; Indica muy pobre crecimiento o producción de pigmentos; -: Indica NO crecimiento y NO producción de pigmentos.

Table 3. Behavior of some pathogenic attributes in the evaluated fungal isolates.

Tabla 3. *Comportamiento de algunos atributos patogénicos en los aislados fúngicos evaluados.*

CODE	TAXA	SIZE (LENGTH X WIDTH, µm)	RT	EXCRETION OF HEMOLYSINS (HEMOLYSIS TYPE)	GROWTH AT 37ºC
O-39	*Acrodontium simplex*	1.5 - 3 x 1.5 – 2.5	A, B, C	γ	-
O-6	*Alternaria alternata*	23.0 – 56.0 x 8.0 – 17.0	A	γ	-
O-36	*Aspergillus candidus*	2.5 – 4.0	A, B, C	γ	+
O-7	*Aspergillus chevalieri*	3.5 – 4.2	A, B, C	α	+ *
O-10	*Aspergillus flavipes*	2.5 – 3.0	A, B, C	γ	+
O-33	*Aspergillus flavus*	3.5 – 4.5	A, B, C	β	+ *
O-11	*Aspergillus flavus*	3.8 – 4.8	A, B, C	β	+ *
O-23	*Aspergillus niger*	3.8 – 5.0	A, B, C	β	+ *
O-24	*Aspergillus niger*	3.5 – 5.0	A, B, C	β	+ *
O-8	*Aspergillus niveus*	2.8 – 3.8	A, B, C	α	+ *
O-32	*Aspergillus oryzae*	3.5 – 4.5	A, B, C	β	+
O-25	*Aspergillus oryzae*	3.6 – 4.7	A, B, C	β	+
O-26	*Aspergillus ungis*	3.0 – 3.5	A, B, C	β[b]	-
O-1	*Aspergillus ungis*	2.5 – 3.6	A, B, C	β[b]	-
O-5	*Aspergillus versicolor*	2.0 – 3.5	A, B, C	b	+
O-31	*Aspergillus versicolor*	2.0 – 3.0	A, B, C	b	+
O-34	*Aspergillus wentii*	3.5 – 5.0	A, B, C	γ	-
O-30	*Cladosporium cladosporioides*	3.5 – 12.0 x 2.0 - 4.0	A, B	β[b]	-
O-2	*Cladosporium cladosporioides*	5.0 – 13.0 x 2.5 – 4.0	A, B	β[b]	-
O-4	*Cladosporium cladosporioides*	3.3 – 13.0 x 2.0 – 3.5	A, B	β[b]	-
O-18	*Cladosporium herbarum*	5.5 – 13.0 x 3.8 – 6.0	A, B	γ	-
O-21	*Cladosporium herbarum*	5.6 – 12.7 x 3.8 – 5.9	A, B	γ	-
O-3	*Cladosporium hillianum*	5.0 – 14.5 x 2.0 - 4.5	A, B	γ	-
O-35	*Cladosporium lignicola*	5.5 -12.5 x 3.9 – 6.0	A, B	γ	-
O-38	*Cladosporium sphaerospermum*	3.0 – 4.5	A, B, C	γ	-
O-40	*Cladosporium sphaerospermum*	3.0 – 5.0	A, B, C	γ	-
O-37	*Cylindrocarpon lichenicola* [a]	19.6 – 32.0 x 5.0	A	β	+
O-52	*Nigrospora sphaerica*	14.0 – 16.0 x 18.0 - 22.0	A	γ	-
O-28	*Penicillium chrysogenum*	2.5 – 3.5	A, B, C	γ	+
O-22	*Penicillium chrysogenum*	2.6 – 3.6	A, B, C	γ	+
O-27	*Penicillium citreonigrum*	2.0 – 2.8	A, B, C	γ	-
O-50	*Penicillium citrinum*	2.3 – 2.8	A, B, C	β	+
O-51	*Penicillium citrinum*	2.5 – 3.0	A, B, C	β	+
O-12	*Penicillium citrinum*	2.4 – 3.0	A, B, C	β	+
O-9	*Penicillium griseofulvum*	2.6 – 3.5	A, B, C	γ	-
O-13	*Penicillium griseofulvum*	3.0 – 3.5	A, B, C	γ	-
O-14	*Penicillium implicatum*	2.0 – 3.0	A, B, C	γ	-
O-19	*Penicillium oxalicum*	3.0 – 4.0	A, B, C	γ	+
O-29	*Penicillium oxalicum*	3.2 – 4.2	A, B, C	β	+
O-42	*Penicillium oxalicum*	3.5 – 4.5	A, B, C	β	+
O-15	*Penicillium restrictum*	2.0 – 3.0	A, B, C	γ	-
O-41	*Penicillium simplicissimum*	2.5 – 3.0	A, B, C	γ	+
O-43	*Penicillium waksmanii*	2.0 – 2.5	A, B, C	γ	-
O-20	PNSM	-	-	γ	-
O-16	WNSM	-	-	γ	-
O-17	WNSM	-	-	β[b]	-

[a]: Indicative that the dimensions correspond to macroconidia. RT: Respiratory tract level to which the spores can penetrate. A: Upper Respiratory Tract. B: Trachea, bronchi and bronchioles. C: Alveoli. Hemolysis type. α: Alpha hemolytic. β: Beta hemolytic. γ: Gamma hemolytic. [b]: Indicates that the excretion of hemolysins in this strain was determined at 30°C. Growth at 37°C: +: growth. -: no growth. *: Indicates exuberant growth at 37°C.

[a]: Indicativo de que las dimensiones corresponden a macroconidios. RT: Nivel del tracto respiratorio al que las esporas pueden penetrar. A: Tracto respiratorio superior. B: Tráquea, bronquios y bronquiolos. C: Alvéolos. Tipo de hemólisis. α: Alfa hemolítica. β: Beta hemolítica. γ: Gamma hemolítica. [b]: Indica que la excreción de hemolisinas en estas cepas se determinó a 30°C. Crecimiento a 37°C: +: crecimiento. -: no crecimiento. *: Indica crecimiento exuberante a 37°C.

Of the hemolysins excretion, 22 isolated (47.8 % of the total) excreted α or β hemolysins; but 20 of them excreted β hemolysin (43.5 %). Among them, eight isolated belonging to four *Aspergillus* species (*A. flavus, A. niger, A. oryzae, A. versicolor*), even the two isolated of the *A. ungis* species (O-26 and O-1) excreted β hemolysin at 30°C. Of the genus *Cladosporium*, only the three isolated belonging to the species *C. cladosporioides* (O-30, O-2 and O-4) also excreted β hemolysin at 30°C. Of the *Penicillium* genus, only 5 isolated (33.3 %) belonging to two species (*P. citrinum* and *P. oxalicum*) excreted β hemolysin. It should be noted that one of the non-sporulating mycelia (WNSM, O-17) was able to excrete β hemolysin at 30°C.

The combination in the same organism of several virulence factors is a necessary condition to classify it as potentially pathogenic, for this reason a comprehensive analysis of these attributes per isolated was carried out.

In this study, 28.3 % of the isolated showed the four pathogenic attributes, highlighting among them those of *A. flavus* (O-33 and O-11), *A. niger* (O-23 and O-24), *A. oryzae* (O-32 and O-25), *A. versicolor* (O-5 and O-31), *P. citrinum* (O-50, O-51 and O-12) and *P. oxalicum* (O-29 and O-42). With three pathogenic attributes, 6.5 % of the isolates were shown, highlighting *A. chevalieri*, *A. niveus* and *Cylindrocarpon lichenicola*.

Table 4. Fungal isolated with the highest pathogenic potentialities.

Tabla 4. *Aislados fúngicos con las mayores potencialidades patogénicas.*

ISOLATES	NUMBER/PERCENTAGE
Isolates of *Aspergillus*	15
Isolates with the 4 pathogenic attributes evaluated	8[a]
Percentage	**53.3 %**
Percentage of the total taxa	**17.4 %**
Isolates of *Penicillium*	15
Isolates with the 4 pathogenic attributes evaluated	5[a]
Percentage	**33.3 %**
Percentage of the total taxa	**10.9 %**
Isolates of other taxa	16
Isolates with the 4 pathogenic attributes evaluated	3[b]
Percentage	**18.8 %**
Percentage of the total taxa	**6.5 %**

[a]: In this analysis only β hemolysis was taken account. [b]: In this analysis α hemolysis of two isolated of *Aspergillus* (O-7 and O-8) and β hemolysis of *Cylindrocarpon lichenicola* were taken account.

[a]: En este análisis solo la β hemólisis se tuvo en cuenta.[b]: En este análisis se tuvo en cuenta la αhemólisis de dos aislados de *Aspergillus* (O-7 y O-8) y la β hemólisis de *Cylindrocarpon lichenicola.*

Two attributes were detected in 23.9% of the isolated and they corresponded to *Aspergillus candidus*, *A. flavipes*, *A. ungis* (O-26 and O-1), *C. cladosporioides* (O-30, O-2 and O-4), *P. chrysogenum* (O-28 and O-22), *P. oxalicum* (O-19) and *P. simplicissimum* (O-41). Only 34.8 % showed one pathogenic attribute that was fundamentally related to the conidia size; and in the case of the WNSM (O-17) its attribute was linked to the excretion of b hemolysin. From all this analysis, it was possible to show that the *Aspergillus* isolates were the ones that showed the greatest pathogenic potential (Table 4).

DISCUSSION

In this second study carried out in COIP repositories in July 2018, fungal concentrations slightly higher than previous results were reported (Borrego and Molina, 2020). However, the fungal concentrations obtained in this study are comparable with the values referred for Polish libraries and archives (Karbowska-Berent *et al*., 2011), the library of an Italian university (Micheluz *et al*., 2015), different Egyptian libraries (Osman *et al*., 2017) and the National Archive of Cuba (Anaya *et al*., 2016; Borrego *et al*., 2017).

Although in general the CFU/m^3 values were low in all R-1, at the entrance of both storeys of R-1 the concentrations were slightly higher. The greatest fungal load was detected in the front - left part of the repository, where precisely the final part of the office that precedes it is located. This could be due to the fact that as there is no physical barrier that prevents the movement of air between the office and the repository, air stagnation occurs in that area and with it an increase in RH and the fungal load that comes from the office where there is staff working for 8 hours. The entry of dust and fungal propagules from the outside every time the office door is opened/ closed, contamination introduced by staff and contamination generated by handling documents in the office (which facilitate aerosolization dust and bio- contaminants adhering to them) creates gradients of fungal load and RH characterized by a very high fungal concentration in that front part of the repository. Those gradients reaches a considerable height that affect the upper storey of the repository (since the same behavior was observed in both storeys of the mezzanine), and that was decreasing towards the rear - right of it.

Some authors considering that the indoor/outdoor (I/O) ratios are indicative of the air quality on indoor environments and that this criterion is decisive in classifying an environment as polluted or not (Stryjakowska-Sekulska *et al*., 2007). According to this criterion, the values of the I/O indices obtained in both repositories were 0.4,that is, less than 1, revealing environments with good quality, possibly

due to the proper functioning of the building as the main barrier for the protection of the documentary heritage from biological contamination and climatic variations in the outside environment. Also it is indicative of a good organization and hygienic conditions existing in the repositories as well as the correct and systematic maintenance of the air-conditioning units. Therefore, it is very important to maintain and control these aspects in heritage institutions. However the existence within R-2 of walls with apparent moisture damage and incipient fungal development must be highlighted that could cause increases in the environmental fungal load and with this, significant damages to both documents and the staff health. For this reason, it was proposed to make immediate constructive corrections to the building to mitigate these dangerous effects.

However, a good microbiological quality of the indoor air was obtained in the repositories, possibly due to the good functioning of the building as the main barrier for the protection of the documentary heritage from biological contamination and the climatic variations in the outdoor environment. The central yard with vegetation, on the one hand, facilitates the existence of a cooler air outside the repositories, guaranteeing that the walls of the building do not get so hot and, on the other hand, it contributes to reducing environmental bio-contamination. Also it is indicative of a good organization and adequate hygienic conditions existing in the repositories as well as a correct and systematic maintenance of the air-conditioning units. Therefore, it is very important to maintain and control these aspects in heritage institutions. However, it is necessary to carry out constructive repairs to the building quickly to eliminate some detected damage moisture in certain walls and thereby avoid outbreaks of environmental fungal contamination.

In relation to the thermo-hygrometric values detected, there was evidence that they correspond to the provisions of Resolution No. 201 (2020) for the conservation of paper documents in Cuban institutions. Several authors have reported that T and RH influence in the concentration of environmental fungi both indoors and outdoors (Górny, 2004; Pinzari, 2011). Even some authors claim that environmental RH favour the fungal growth on indoor environments (Abdel Hameed *et al*., 2012; Anaya *et al*., 2016). In order to correlate the existing effects between T, RH and CFU/m^3, the values of the measurements made in this study and T and RH values from the previous week were taken. Although the total number of measurements used in these determinations were few yet, it was possible to demonstrate the high influence that HR mainly exerts on fungal concentrations. Similar results were obtained previously by Rodriguez *et al*. (2014) in a study that made in the archive of the National Museum of Music (Cuba) for 24 weeks corresponding to the rainy season in Cuba where the amount of analyzed data by these authors were much higher to that used in the current study. The high correlation obtained indicates the strong impact that RH has on the presence of fungal propagules, their distribution, dispersion, buoyancy and viability in indoor environments such as R-1.

The high values of RH in the air-conditioned indoor environments of the archives and libraries in Cuba during the rainy season tend to generate serious fungal growth problems. In this season the average values of T was 35°C and RH ≥ 90 %, but if a tropical storm or a hurricane characterized by torrential rains for several days will pass through Havana, then the environmental RH would tend to be 100 % or oversaturated. During these rainy processes, it is extremely common for high water condensation to occur on walls, window panes, metal shelving surfaces and document wraps, so although some air-conditioning systems have humidity control, it is essential to use dehumidifiers since sometimes the automatic controls cannot reduce the environment RH to the required levels. If the repositories of an archive do not have wide walls to reduce the temperature gradient that is established by the external incidence of the Sun, the process of condensation of water on surfaces is increased because the indoor of the walls is kept warm and when colliding with the cold air generated by the air conditioned the condensation occurs. In these cases, it is essential to use dehumidifiers to help control the environment RH. As COIP has wide walls the intense heat is not penetrated into the indoor environment of the facilities, ensuring that the walls remain cool, hence, the process of condensation is not high. For this reason,when R-1 was constructively adapted, more sophisticated air-conditioning systems were not installed and when it has been required to lower the RH to the appropriate values, the dehumidifiers have been activated manually. But this study demonstrated that this way of controlling the RH in the repositories of COIP was not efficient and hence requires also of the sophisticated air-conditioning systems that help to control the environmental RH efficiently. In addition, these air-conditioning systems should be located on both sides of the repository instead of only on one side, to ensure a better and more homogeneous air circulation and prevent air stagnation and the formation of sites fungal amplification, such as those detected in this study.

Regarding to the fungal concentrations in the repositories' indoor air and the I/O ratios, the obtained results evidenced a good microbiological indoor airquality, possibly due to the proper functioning of the building as the main barrier for the protection of the documentary heritage from the biological contamination and climatic variations in the outside environment.

The central yard with vegetation on one hand facilitates the cooling of the air guaranteeing that the indoor walls of the building and the spaces remain cool and on the other hand contributes to the reduction the environmental biocontamination fundamentally. Also it is indicative of a good organization and hygienic conditions existing in the repositories as well as the correct and systematic maintenance of air-conditioning units. Therefore, it is very important to maintain and control these aspects in heritage institutions.

In relation to environmental fungi, majority of the isolates were anamorphs of ascomycetes, which is indicative of their prevalence in the indoor mycobiota (Kadaifciler, 2017; Rahmawati *et al*., 2018). It is important to highlight that this result is characteristic of the sampling method used, since the use of culture media favours development of anamorphic phases in the fungi. Therefore, if in the future these results are to be compared with others that could be obtained, it will be necessary to follow similar methodologies. It is important to note that similar results were previously reported in environmental studies conducted at National Archive of the Republic of Cuba and other Cuban archives with naturally ventilated and climate -controlled repositories (Borrego and Perdomo, 2016; Anaya *et al*., 2016; Borrego and Molina, 2020).

The prevalence of *Cladosporium* on indoor air of R-1 is coincident with previously several reports have revealed this genus as predominant on both indoor and outdoor environments (Pinzari, 2011) likewise occur in Havana atmosphere (Almaguer *et al*., 2013). Even, it is one of the fungal genera that the Sahara dust transports to Caribbean including Cuba (Sullivan *et al*., 2012). It is possible that the propagules of this fungus and many others come from the central yard with vegetation and reach the indoor environment of the repository through the existing slots in doors and windows, the ventilation/air-conditioning systems, the dust particles, adhered to clothing and footwear of employees and thus has a great impact on indoor environments. Although several species were detected, *C. hillianum* was a new find for Cuban archival environments.

Regarding to the prevalence of *Penicillium* spp. in R-2 could be because in the repository there is not a good replacement of air with the outdoor, possibly due to the effect of air conditioning inside the premises. As some walls within the entire premises show humidity and fungal development, it is possible that the air conditioning is favoring the detachment the propagules of this fungus and dispersing them in the environment of the premises itself. This can be favored and increased by the staff movement within the offices. As a sampling of the wall surface was not carried out and this could not be demonstrated, it is considered a logical assumption made according to the reports of different authors referring to *Penicillium* spp. as an indicator of moisture damage inside the facilities (Eduard, 2009; Pinzari, 2011).

Independently the predominance of *Aspergillus, Penicillium* and/or *Cladosporium* in the studied environments, these genera continue being the taxa most frequently found in indoor air of archives, libraries and museum of around the world (Micheluz *et al*., 2015; Polo *et al*., 2017; Osman *et al*., 2017). These genera have some species that are considered as primary and secondary colonizers of organic substrates, as paper, since they can grow at relatively low values of water activity ($a_w \geq 0.8 – 0.9$) (Górny, 2004; Kadaifciler, 2017) and also their spores can pass relatively easily to indoor air of the enclosures (Karbowska -Berent *et al*., 2011).

In this study, a high percentage of a non-sporulating white mycelium (WNSM) was also detected. These kinds of mycelium were also obtained in other studies in environments of Cuban archives and museums (Rodriguez *et al*., 2014; Borrego and Perdomo, 2016; Borrego and Molina, 2019; Borrego and Molina, 2020) as well as of other countries (Osman *et al*., 2017; Kadaifciler, 2017; Rahmawati *et al*., 2018). Among the genera of fungi isolated in the indoor environments studied, it should be noted that the genera *Acrodontium* and *Cylindrocarpon* showed low and unrepresentative detection percentages in those environments; however, they were genera detected for the first time in Cuban archives environments. On the other hand, everything seems to indicate that these two genera and the non-sporulating mycelia come from the outside environment.

The existence of *Cylindrocarpon* spp. in the indoor environment of R-1 is evidently consequence of the outdoor environment, in particular from the central yard where it was also detected probably for the existent vegetation in that place while *Acrodontium* spp. could have reached R-2 in the same way or it could have been introduced by the personnel working in previous days; maybe for that reason, on the day of sampling, *Acrodontium* was not isolated of the outdoor environment.

Acrodontium spp. has species that have been isolated of soil, fruits and leaf debris. It is also known that some species are phytopathogenic and others can degrade keratinous substrates, paper and wood; in particular, *Acrodontium simplex* is a plant pathogen mainly of banana (Hwang and Chen, 1986). *Cylindrocarpon* spp. contains 35 species, is a common inhabitant of the soil, both in temperate and tropical regions, and is found in the residues of plants and decaying plant material and some species are plant and human pathogens, as is the case with *Cylindrocarpon lichenicola* (Irek *et al*., 2017).

Although the *Acrodontium simplex* and *Cylindrocarpon lichenicola* species were isolated in this study at low RD, they constitute the first reports for Cuban archives environments.

In the evaluation of the biodeteriogenic potential of the air isolates, results were obtained indicating that most of them have metabolic potentialities to degrade the cellulosic sources studied, as well as starch, gelatin and excrete acids. While a considerable percentage was able to excrete pigments.These results evidenced of the cellulose degradation, the main component of paper (El Bergadi *et al*., 2014). In particular the growth on FP is known as FPase activity (the paper is composed by pure cellulose, but is a mixture of α and β cellulose) because activates the "cellulase" enzyme system completely (formed by endoglucanases, exoglucanases and β-glucosidases enzymes) able to partially or wholly break down cellulose into cellobiose and glucose (Rodriguez *et al*., 2014; Mallo *et al*., 2017; Osman *et al*., 2017) evidencing that the most of the isolated in particular those belonging to the genera *Aspergillus* and *Penicillium,* could degrade cellulose with varying intensity. In previous reports, it has been mentioned that species of the *Aspergillus* and *Penicillium* genera isolated from the archives and libraries environments are good cellulose degraders (El Bergadi *et al*., 2014; Savković *et al*., 2019).

The excreted organic acids by all analyzed isolated not only cause the acid hydrolysis of the β-1-4-glucosidic bonds (acid hydrolysis) but also increase the cellulase efficiency in their degradation activity (Mallo *et al*., 2017). Also these acids form chelating agents with mineral cations, favoring the biodeterioration process (Rodriguez *et al*., 2014). On the other hand, the acids facilitate the colonization of other acidophilic fungi propitiating ecological successions and the formation of potent biofilms that accelerate the materials deterioration until destroying them completely (El Bergadi *et al*., 2014).

Pigments excretion is a complex phenomenon and is the most frequent alteration caused by filamentous fungi in paper, an aspect that is also evidenced in foxing spots (Piñar *et al*., 2015; Sequeira *et al*., 2019). They cause an irreversible aesthetic damage, and it is one of the most serious problems caused by fungal contamination (Polo *et al*., 2017; Sequeira *et al*., 2019). In previous studies were reported the different tonalities of pigments excreted by the some species of *Aspergillus* and *Penicillium* (Rodriguez *et al*., 2014; Sequeira *et al*., 2019; Borrego and Molina, 2020). In this study different tonalities of pigments with prevalence of the yellow and brown colour were excreted by the isolates of these genera and other as *Cladosporium* spp.

Starch and gelatin, two of the common components in bindings in old manuscripts, books and photos, were assimilated by most of the obtained isolates coinciding with the results of other studies conducted in archives and libraries (El Bergadi *et al*., 2014; Osman *et al*., 2017; Borrego and Molina, 2020).

In general, all fungal isolates detected in this study have certain nutritional and environmental requirements that favour their growth and the substrates colonization. Therefore, it is important to pay special attention to the environmental conditions that can favour their development, which, unlike the presence of their propagules, can be controlled. Fungi are mostly mesophilic (live at temperatures between 22 – 30°C), acidophilus (pH 4 - 6) and grow well at relative humidity above 70 %. Only if the temperature, humidity conditions (water activity) and acidity in the substrate are favorable, the fungal spores sedimented on the documents can germinate and grow abundantly. The main limiting factor that determines the development of fungi on these materials is the water although some xerophilic/halophilic fungi have been associated with these materials (Micheluz *et al*., 2015).

A microorganism can be considered risky for documental conservation, if it presents at least one biodeteriogenic attribute that under the right conditions compromises the documentary collection (Borrego *et al*., 2017). Therefore, the number of attributes that gather a same strain is indicative of its biodeteriogenic potential. In this study was demonstrated that the majority of the evaluated isolated were positive to four or five of the physiological tests analyzed indicating to be high risk agents for the documentary collections; similar results were previously reported (Borrego *et al*., 2017; Borrego and Molina, 2020).

Regarding to the pathogenicity of environmental fungi, it has been reported that fungal fragments, spores and conidia are potent allergens (Górny, 2004; Green *et al*., 2006). However, in the triggering of mycosis in the respiratory tract, the size of the conidia plays an important role (de Hoog *et al*., 2000; Twaroch *et al*., 2015). However, the individual ability of a microorganism to cause disease depends on virulence factors or pathogenic attributes (Brunke *et al*., 2016; Novohradská *et al*., 2017). In filamentous fungi, these pathogenic attributes are diverse and vary according to the group, genus or species (Crameri *et al*., 2014). But it is important to emphasize that the ability to cause damage is not a property only of the fungus as a microorganism, but it is essential that there is an interaction with a susceptible person. So much so that generally when people are immunodeficient or have immune disorders, respiratory fungal diseases such as allergies or deep mycoses in the lower respiratory tract can appear (Novohradská *et al*., 2017).

On the other hand, prolonged exposure to relatively high concentrations of fungal propagules could affect individuals (Llop *et al*., 2001). For this reason, when personnel are exposed for long periods of time to environments that may have high concentrations of fungi or when they constantly work with documents that may be infected, they run the risk of suffering not only allergies but also superficial mycoses and even deep mycoses of the respiratory tract. Hence the importance of evaluating the pathogenic potential of isolated environmental fungi since it gives a measure of the risk to which the personnel in the archive are exposed.

Aspergillus spp. is one of the predominant fungal genera in both studied environments and it is known that the pathogenic species of *Aspergillus* can cause a wide spectrum of affectations in humans ranging from allergies to fungal infections (mycoses) that can be superficial and deep, including the deep invasive aspergillosis (Green *et al*., 2006; Hedayati *et al*., 2007); some of the species that have a considerable impact on human health are *A. flavus* and *A. niger* (Novohradská *et al*., 2017). However, de Hoog *et al*. (2000) reported a greater number of *Aspergillus* species with health risks including the majority of the species detected in this study.

The affectations to the human organism by inhalation of conidia is not a unique condition of *A. fumigatus* since other species such as *A. flavus* (conidia size: 3.5 - 4.8μm) and *A. niger* (conidia size: 3.5 - 5 μm) can also affect the organism using the nasal cavity and the respiratory tract as an entrance door (Klich, 2006; McCormick *et al.*, 2010). Hussain *et al*. (2011) described the areas of the human respiratory tract that could be affected by the different particles sizes and although alone the bioparticles ≤ 5 μm the can penetrate the pulmonary lobules other bigger particles can be caught in upper zone of the lungs or in the middle respiratory tract. For this reason, the conidia size of the analyzed isolates that belong to the different genera detected in the environment of both repositories was measured. In this study, most of the fungal isolates showed conidia so small that they can reach the pulmonary alveoli. Within them were found all the isolated of *Aspergillus* and *Penicillium* as well as those of *C. sphaerospermum* and *Acrodontium simplex*. In relation to *Aspergillus* and *Penicillium* similar results were previously reported (Richardson *et al*. 2019).

In addition to the conidia size, other characteristics are included among the virulence factors and within them growth ability at 37°C stands out, secretion of hydrolytic enzymes such as proteases, phospholipases, lipases and hemolysins, as well as the existence of structures that allow them to adhere to tissues, the production of melanin and others (Brunke *et al*., 2016).

For these reasons, the analysis of other pathogenic attributes was carried out in the 46 selected isolated. Growth ability at 37°C is one of the necessary and essential conditions for a microorganism to be considered as a potential pathogen (McCormick *et al*., 2010). Hence, this parameter was evaluated as a principal condition in the isolated fungal analyzed.

It should be highlighted that about half of the studied isolated grew at 37°C, with emphasis on the *Aspergillus* isolates and especially those belonging to species *A. flavus, A. niger* and *A. oryzae,* which were detected in this study and have been cited as causing human mycosis (Klich, 2006), especially *A. flavus*, which is also one of the main causes of human bronchial aspergillosis and pulmonary infections in immunocompromised individuals (Hedeyati *et al*., 2007); similarly occur with *P. citrinum* (de Hoog *et al*., 2000). Hedayati *et al.* (2007) reported that 37°C is the optimal temperature for the growth of *A. flavus*, although this species can grow in a temperature range between 12° C and 48°C as well as *A. niger*, which are, together with *A. fumigatus*, the main causal agents of aspergilloma and invasive aspergillosis.

Besides, the isolates corresponding to the species *A. flavus, A. niger, A. oryzae, A. versicolor, Cylindrocarpon lichenicola, P. citrinum* and *P. oxalicum* detected in this study also excreted β hemolysin while *A. chevalieri* and *A. niveus* excreted α hemolysin at 37°C. It is not known that hemolysins are pore-forming exotoxins that recognize specific structural sites on the surface of red blood cells (Nayak *et al*., 2013). These are excreted into the extracellular medium where they can provoke the lysis of the erythrocyte (β hemolysis) causing anemia and anoxia in the host organism (Bogomolova and Kirtsideli, 2009). It has been recognized like one of the main functions of these enzymes in the infection process the release of iron, important enzymatic cofactor required in many metabolic processes of the infectious agent (Nayak *et al*., 2013). On the other hand, Vesper and Vesper (2004) reported a list of hemolysin-producing fungal species, including some of the species detected in this study as is the case of *A. flavus, A. niveus, A. niger, A. versicolor, P. citrinum* and *P. oxalicum.*

In studies conducted by Nayak *et al.* (2011), several *Aspergillus* species showed hemolytic activity but highlighted *A. flavus, A. niger, A. oryzae, A. versicolor* while in another study conducted by Masaphy and Ezra (2016) three *Aspergillus* species (*A. flavus, A. fumigatus, A. niger*) with hemolytic activity were also reported. On the other hand, *P. chrysogenum* has also been reported as a producer of β hemolysin (Nayak *et al*., 2011). There are few reports that mention the species *C. cladosporioides* producing hemolysin (Vesper and Vesper, 2004; Nayak *et al*., 2011; Oliveira and Caramalho, 2014); but this is the second time that an

isolate of *C. cladosporioides* has been detected in the environment of a Cuban archive that has β hemolytic activity, the first finding was reported by Borrego *et al*. (2020).

These results emphasize the need to perform microbiological studies periodically and to know the fungal variability (including their physiological and pathogenic characteristics) that may exist between apparently similar indoor environments.

Although the pathogenic attributes studied *in vitro* were few, the results indicate that personnel should not confide on environmental fungi just because they remain in the air as they represent a potential health risk, therefore it is important for personnel to protect themselves during the working day using suitable face masks fundamentally.

Based on these results, some suggestions were made to the COIP administration. These were fundamentally related to the need to separate the R-1 environments from the air in the offices with the intention of reducing cross-contamination between both environments as much as possible. It was also suggested to install other more sophisticated air-conditioning systems on both sides of R-1 and continue to maintain good organization and cleanliness of documents and repositories, carry out systematic maintenance of the new climate equipment installed in R - 1 and those existing in R-2 as well as the mandatory use of personal protection means, with an emphasis on the use of appropriate face masks, to minimize the risk of inhalation of fungal propagules due to their aerosolization during the handling of documents for a long time.

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