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ARTICLE

# Pollution of Airborne Fungi in Naturally Ventilated Repositories of the Provincial Historical Archive of Santiago de Cuba (Cuba)

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ABSTRACT

Environmental fungi can damage the documentary heritage conserved in archives and affect the personnel's health if their concentrations, thermo-hygrometric parameters and ventilation conditions are not adequate, problems that can be accentuated by Climate Change. The aims of this work were to identify and to characterize the airborne fungal pollution of naturally ventilated repositories in the Provincial Historical Archive of Santiago de Cuba and predict the risk that these fungi pose to the staff's health. Indoor air of three repositories of this archive and the outdoor air were sampled in an occasion every time in 2015, 2016 and 2017 using a SAS sampler. The obtained fungal concentrations varied from 135.6 CFU/$m^3$ to 421.1 CFU/$m^3$ and the indoor/outdoor ratios fluctuated from 0.7 to 4.2, evidencing a variable environmental quality over time, but in the third sampling the repositories environments showed good quality. *Aspergillus* and *Cladosporium* were the predominant genera in these environments. *A. flavus* was a prevailed species in indoor air, while *A. niger* and *Cl. cladosporioides* were the species that showed the greatest similarities with the outdoor air. *Coremiella* and *Talaromyces* genera as well as the species *Aspergillus uvarum*, *Alternaria ricini* and *Cladosporium staurophorum* were the first findings for environments of Cuban archives. Xerophilic species (*A. flavus, A. niger, A. ochraceus, A. ustus*) indicators of moisture problems in the repositories were detected; they are also opportunistic pathogens and toxigenic species but their concentrations were higher than the recommended, demonstrating the potential risk to which the archive personnel is exposed in a circumstantial way.

## 1. Introduction

Among the actions that are usually performed in the archives, libraries and museums of the world to conserve the Documentary Heritage, are the monitoring of temperature (T) and relative humidity (RH), environmental parameters of great importance since are involved in the chemical processes that occur during the aging of substrates, inks

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and pigments. The luminous intensity and in particular the UV radiation intensity, the chemical pollution levels in the repositories air, etc. are also measured. However, in relation to environmental microbial contamination in Heritage Institutions, there is no established internationally standard for the collections conservation, only the Italian Ministry of Cultural Heritage proposed limit values of ≤ 150 CFU/$m^3$ for fungi and between 100 CFU/$m^3$-120 CFU/$m^3$ for bacteria or the collections should be subjected to disinfection [1]. For this reason, there are groups of scientists that perform specific studies to assess the environmental microbial diversity and distribution, particularly of fungi, and have established some regulations in their countries.

The Cuba geographical location and the existence of high values of T and RH favor the increase of dust and the fungal propagules (spores, hyphae and fragments of spores and hyphae) concentration in the air, as well as their settlement over different materials, facilitating the fungal development and proliferation. On the other hand, it is known that papers and other documentary supports (microforms, films, audiovisuals, etc.) are infected during the manufacturing process [2] and that these microorganisms, particularly fungi, can trigger the materials biodeterioration when environmental conditions are optimal for their growth and propagation since they use all the components of the documentary supports as nutrients. They have powerful, versatile and adaptable metabolic machinery, which allows them to degrade a great diversity of substrates, both of organic and inorganic origin, favoring the biodeterioration of the different materials stored in archives repositories, libraries and museums [3-5].

Fungi are characterized by having different structures and pathogenicity mechanisms, which cause specific diseases in humans [6]. They behave as allergens triggering numerous allergenic and respiratory diseases [7], some of them can even lyse the blood erythrocytes to obtain the iron necessary for growth during infectious processes [8], they also facilitate tissue damage and affect the immune system mechanisms in human [9]. In addition, they have a high degradation capacity of the organic matter existing in documents of heritage interest and in other substrates such as wood, textile, leather, etc. [10-12]. They can also excrete organic acids, which, when reacting with the paper, acidify and deteriorate it, accelerating the degradation processes caused by the fungi themselves [10,13], as well as excrete pigments that irreversibly and aesthetically affect the documents [5]. These effects are greater in countries with a tropical climate, such as Cuba, due to the influence of high T and RH values that favor the proliferation of fungi, sometimes triggering pests that are difficult to control and eliminate.

Likewise, these effects can be enhanced by Climate Change (CC), since environmental conditions are suitable for increasing the fungal load in the air due to the increase in air transport of fungal propagules from long distances in the planet [14,15]; this will also accelerate the proliferation of fungi and their metabolic recrudescence, which will further facilitate the increase in the environmental fungal load, the efficient degradation of natural materials cellulosic (due to damage to plants and soils that constitute their natural reservoirs), as well as exacerbation of its virulence and pathogenicity causing emerging diseases, all as adaptation and survival mechanisms to adverse environmental conditions [16]. In fact, it has been reported that CC will increase the chronic phenomena of allergies and invasive mycoses because the human immune system will also be affected by climatic stress [17]. Therefore, the possibility of acquiring these diseases will be exacerbated in indoor environments where people spends more than 90% of his time, be it at home, work, school or even doing recreational and social activities [16]. Although studies are being made to monitor a group of factors that will vary with the CC and that will affect the Cultural Heritage of Humanity preserved indoors environments [18], these investigations are still insufficient.

Several authors have established a close relationship among environmental conditions, the presence of airborne fungal propagules and in the settled dustborne, as well as the existence of viable fungi over the stored artworks and documents with the possibility of triggering the biodeterioration of these materials [4,19], respiratory disorders in humans [7,20], and other symptoms belonging to different types of pathologies [6,21]. Hence, multiple research groups recommend the need to increase the frequency of systematic studies in the indoor premises to assess the quality of the environments, in order to guarantee an environmental characterization of the same that allow the early solution of problems associated with the fungal pest development and their effects on the human health.

The indoor environments of archives and libraries are a reservoir of airborne fungal propagules mainly due to the abundance of dust, the heterogeneity of the substrates with a predominance of those of an organic nature and the conditions of document overcrowding in the repositories, which is why they constitute complex ecosystems. The National Archive of the Republic of Cuba (NARC), as the governing entity of the Network of Historical Archives in the country, has been conducting research on the environmental mycobiota in several Cuban Historical Archives, among which is the Provincial Historical Archive of Santiago de Cuba (PHA SC). Hence the aims of this work were to identify and to characterize the airborne fungal pollution of the naturally ventilated repositories of the

PHA SC and predict the risk that these fungi pose to the staff's health.

## 2. Materials and Methods

### 2.1 Characteristics of the Archive Building and the Studied Repositories

The PHA SC is located in one of the eastern provinces of Cuba (20°1'19.524" N and 75°49'52.823" W), at 877.6 km (by road) from Havana city, country capital (Figure 1A). The archive is located in a centric area of the Santiago de Cuba city surrounded by avenues with high vehicular and pedestrian traffic.

The building that occupies the archive fulfilled different functions in the past. Firstly, it was the church of Santa Catalina, which in 1515 was built with mud walls and a guano roof. It was in that humble church that Diego Velásquez celebrated the first mass during the founding ceremony of the Villa. On April 15, 1522, by the bull of Pope Adriano VI, the church assumed the rank of cathedral, when the headquarters of the diocese of Baracoa was transferred to Santiago de Cuba. Later, at the beginning of the 19th century, the Provincial Prison of Oriente was built there. The building is characterized by having a symmetrical neoclassical architecture made up of two floors, built with materials of the time based on earth, limestone, clay and masonry. Given the function for which the property was built, the walls are excessively thick with small and very high windows. On the other hand, the roof is funnel-shaped, with creole tiles and has a system of channels and downspouts for collecting rainwater that accumulates in the cistern located in the central courtyard (Figure 1B). It has wide interior corridors and seven galleries that have been transformed into repositories for documents.

In 1997 - 1998, the Historian Office of the Santiago de Cuba city undertook the restoration work of the property and, among other repairs that were performed, covered the walls with cement. As this material is not compatible with the wall stone and as a result of the humidity existing in them, the cement began to detach from the walls in mid-2014, leaving part of the stone exposed and increasing the dust level in the repositories that falls on the documents constantly (Figures 1C and 1D).

For the study, three of the five repositories of the PHA SC were selected; especially, three located on the ground floor of the building and around the central courtyard that has the cistern. All these repositories are naturally ventilated. Repository 1 (R-1) coincides with the current repository 3 of the archive, it has two small and very tall windows (located close to 4 m high), it is located in the northwest side of the building and it measures (length × width × height) 8.2 m × 5.5 m × 6 m. Repository 2 (R-2) coincides with repository 4, it has only one window (installed at the same height), it is located on the north side and it measures 2 m × 5 m × 6 m, while repository 3 (R-3) coincides with repository 5, it has two windows similar to R-1, it is located on the northeast side and its dimensions are 11.2 m × 5.2 m × 6 m (Figure 1E).

### 2.2 Indoor Microclimate Monitoring

T and RH were recorded continuously from the first days of 2015 and until the end of 2017 using a thermo-hygrograph per repository that was placed in the center of each one of them. Weekly the recorded data was taken to an Excel page to determine the average values and the standard deviations by day, week, month and year.

### 2.3 Sampling of Airborne Fungal Propagules

The samplings were performed in different months of three years. The first two samplings were performed in months belonging to the rainy season in Cuba, and specifically they were made on September 1, 2015 and June 9, 2016. The third isolation was carried out on February 22, 2017, month corresponding to the season of little rain. The number of points to be sampled was determined according to Sánchis [22], which indicate a simple method based on the cube root of the premises volume. According to this criterion, a total of 8 points were sampled, 3 in R-1 and R-3 as well as 2 points in R-2 (Figure 1F). Also, a point outdoor in the courtyard center was sampled.

The samples were taken between approximately 10:00 am and 1:30 pm, considering the working hours. Each selected point (indoor and outdoor) was sampled by triplicate with a SAS biocollector (Super 100$^{TM}$, Italy) in vertical position at intervals of one hour between replicates. Airflow of 100 L of air in 1 min was used and the biocollector was located at 1.5 m of height approximately. Two variants of the culture medium were used to guarantee the greatest possible fungal diversity and were Malt Extract Agar (MEA) (Biocen, Cuba) at pH 5 [23] and MEA supplemented with NaCl (7.5%) [12,19]. Later, the dishes were transported sealed and invert to the laboratory in NARC (Havana), where they were incubated at 30 ºC for 7 days after removing the seal. The colonies were counted to calculate the fungal concentration per $m^3$ of air expressed in colony forming units ($CFU/m^3$) according to air sampler's manual. Then the colonies were isolated, purified and conserved in slants of MEA at 4 ºC.

Along with taking microbiological samples, T and RH were measured in the same points with a Pen TH 8709 digital thermo-hygrometer (China).

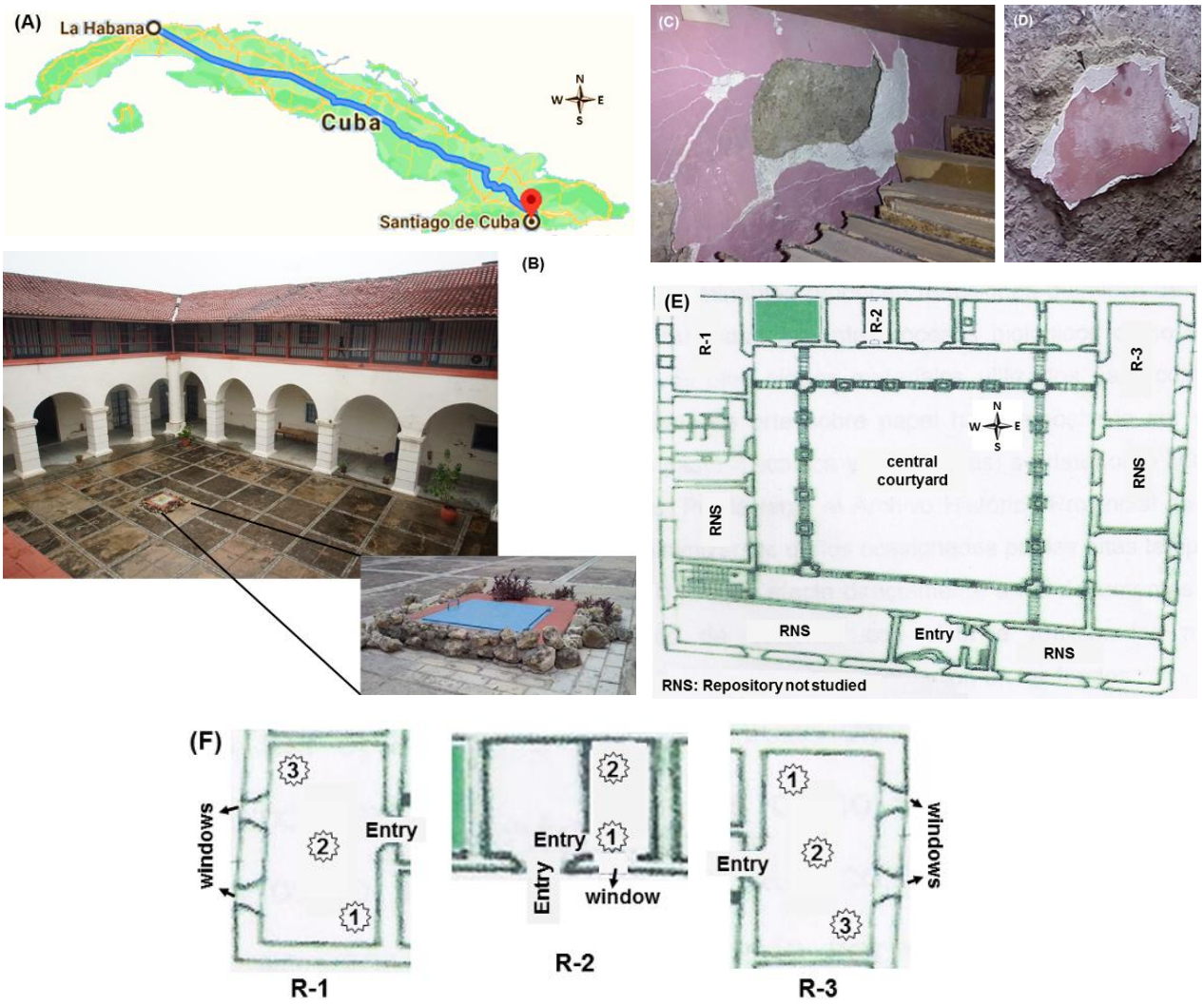


**Figure 1.** (A) Location of the Provincial Historic Archive of Santiago de Cuba (PHA SC) in Cuba map (20°1'19.524" N and 75°49'52.823" W). (B) General view of the building indoor with the central courtyard and the cistern. (C) Mortar layer with which the repositories walls were covered during the last building restoration (1997-1998) that by not allowing the water to escape from the wall, favors the presence of efflorescence and the detachment of the concrete plaster, generating dust in the environments and over the documents. (D) Walls particular characteristics in R-1. (E) Building ground floor sketch where the analyzed repositories are located. (F) Sampling points in each of the studied repositories.

## 2.4 Identification of the Fungal Isolates

Macroscopic observations of morphological characteristics of each colony, both front and back were made with the naked eye and using a stereomicroscope (14X). Through a clear field trinocular microscope (Olympus, Japan) at 40X (dry) and 100X (with oil immersion) connected to a digital camera (Samsung, Korea), conidia, conidiophores and other fungal structures of taxonomic value were observed from preparations made with lactophenol or lactophenol with cotton blue (for hyaline structures) or microcultures. For taxonomic identification some manuals and keys were consulted. To locate the isolates in genera the criteria of Barnett and Hunter [24] and Domsch *et al.* [25] were followed.

For the identification of *Aspergillus* and *Penicillium* species different procedures were followed [26-31]. In the identification of *Cladosporium* species other keys were used [32-35]. The MycoBank website was also consulted.

## 2.5 Ecological Criteria of the Taxa Isolated in the Environments

The Relative density (RD) analysis was performed according to Smith [36], where:

RD = (number of colonies of a specific taxa/total number of colonies of all taxa counted) × 100.

Relative frequency (RF) was calculated according to

Esquivel *et al*. [37], where:

RF = (number of times a genus or species is detected/ total number of samplings realized) × 100

According to RF five ecological categories were established: Abundant taxa (A) were those that had a RF = 100% - 81%, Common taxa (C) had a RF = 80% - 61%, Frequent taxa (F) had a RF = 60% - 41%, Occasional taxa (O) had a RF = 40% - 21% and Rare taxa (R) had a RF = 20% - 0.1% [19].

Sørensen's coefficient of similarity (QS) was used to compare the taxa obtained in the indoor air of each repository with those obtained in the outdoor air [23].

$$QS = 2a/b + c$$

where *a* is the number of common genera detected in the two environments that are comparing, *b* the number of detected genera only in a sample and *c* the number of detected genera only in the other sample.

The QS values ranged between 0 - 1. A value equal to 0 indicates that the obtained taxa in both compared environments were completely different and a value equal to 1 indicates that taxa were identical [38].

### 2.6 Statistical Analysis

The data obtained were analyzed using the statistical program the Statgraphics Centurion XV. Normal distribution of data was analyzed for 95% confidence interval ($p \leq 0.05$). Pearson's correlation was used to determine the relationship between fungal concentration and thermo-hygrometric parameters. To compare the averages of T and RH obtained in 2015, 2016 and 2017, as well as the fungal concentrations averages obtained per year and per repository, a simple classification analysis of variance (ANOVA) was used followed by the Fisher LSD test (Least Significant Difference). Also a Duncan test was used to determine the differences among fungal concentrations obtained in the repositories per each sampling.

## 3. Results

### 3.1 Thermo-hygrometric Parameters Behavior and Concentration of Airborne Fungi in the Repositories

The average values of T and RH obtained in the three studied repositories during the year 2015 were 30 ºC and 75%, respectively, in 2016 they were 28 ºC and 80% and in 2017 they were 29 ºC and 72%; this made the general average values of T and RH during the three years of study were 29.6 ºC and 77%, respectively. However, the T showed great stability in 2015 with values close to 30 ºC, a behavior that was not similar in 2016 and 2017. In these years values lower than 29.6 ºC were detected during the months of March to May 2016 and from January to April 2017, the remaining months showed fluctuations in T that were in the order of approximately 29.6 ± 1 ºC (Figure 2). Regarding RH, the behavior was less stable and the variations were more marked. In 2015, although the values remained above 70%, the oscillations were between 72% and 75%. In 2016 the values were more unstable and higher, since most of the year the values oscillated between 77% and 87%. In 2017, the RH trend was lower than the previous year and the values fluctuated between 71% and 73%, even in March an average of 69% was obtained (the lowest).

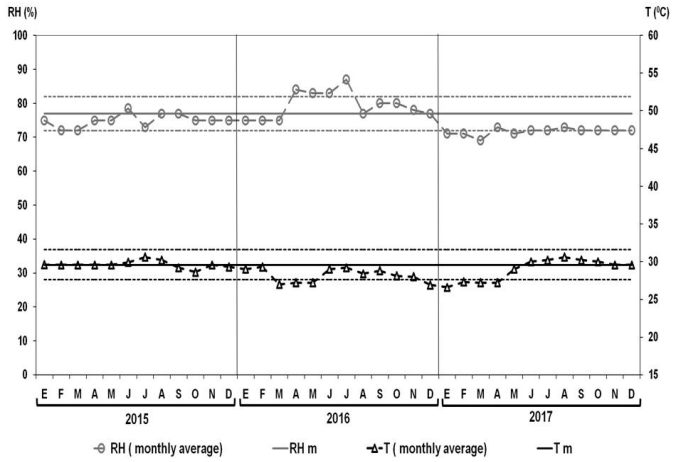


**Figure 2.** T and RH behavior during the 3 years of study in the analyzed three repositories of the AHP SC. The recorded T and RH values comprise average of all the readings made in the three repositories per month. The first microbiological sampling was performed on September 1, 2015 (month included in the rainy season), the second sampling was carried out on June 9, 2016 (another month belonging to the rainy season) and the third isolation was made on February 22, 2017 (month belonging to the season of little rainy). General averages of T and RH for the 3 years were 29.6 ± 2.5 ºC and 77.3 ± 5.2%, respectively.

In the statistical analysis of the obtained thermo-hygrometric parameters during the studied years, no statistically significant differences were found for both T and RH within each year, which indicates stability of these parameters. However, there were significant differences of these variables between the years, with a decrease in T and an increase in RH over time (negative linear correlation, p = –0.3595) (Figures 3A and 3B).

At the time of each sampling, the T average values oscillated between 33.1 ºC and 27.8 ºC, while those of RH fluctuated between 80.7% and 48.6%. The first two sampling were performed in months that corresponded to the rainy season in Cuba (June to November) and the RH values were higher than 64%, even in the first sampling (2015) they were higher than 74%. R-1 showed slightly higher RH values (79.7% to 80.7%) than the other two repositories (74.4% to 78.3%), possibly due not only to the existing environmental humidity on those days but also to marked moisture problems in the building that was evidenced in the deterioration of some repositories walls, with a higher incidence in R-1 (Figures 1C and 1D).

About the obtained fungal concentrations, it could be seen that in 2015 the highest concentrations were detected (Figure 3C) and that R-1 in the first sampling was the most contaminated repository of the three sampling (Figure 3D). There were also statistically significant differences in the fungal concentrations obtained in 2015 compared to 2016 (p = 0.0356) and between the R-1 and R-3 repositories (p = 0.0248).

Table 1 shows the average values of the fungal concentrations obtained in each repository and in each sampling. As can be seen, R-1 showed the highest concentration (421.1 CFU/m$^3$) in the first sampling (2015) followed by R-2 (229.1 CFU/m$^3$) and R-3 (147.8 CFU/m$^3$). In the second isolation (2016) the values were more homogeneous with each other. Contrary to what happened in the previous isolation, R-1 was the repository that showed the lowest concentration (135.6 CFU/m$^3$), followed by R-3 (153.7 CFU/m$^3$) and R-2 (176.7 CFU/m$^3$). In the third isolation (2017), R-2 again showed the highest concentration (298.9 CFU/m$^3$) followed by R-1 (268.9 CFU/m$^3$) and R-3 (162.2 CFU/m$^3$). Also, there were statistically significant differences of R-1 among the three samplings, while R-2 and R-3 did not show significant differences between the samplings. Fungal concentrations obtained in these years did not show any correlation with T and RH for $p \leq 0.05$ (RH, p = 0.5270; T, p = 0.1862).

The environmental mycological quality of the repositories in 2015 was very bad in general (Table 1). R-1 with an I/O ratio of 4.2 and R-2 with an I/O ratio of 2.3 stood out for having poor environmental quality and for being poorly ventilated environments with little air circulation, only R-3 showed a regular environmental quality (I/O = 1.5). Though, the environmental quality improved in the following isolates because in the second isolation (2016) the repositories showed a regular quality (I/O = 1.5 - 2), while already in the third isolation (2017) the environmental quality was good (I/O < 1.5).

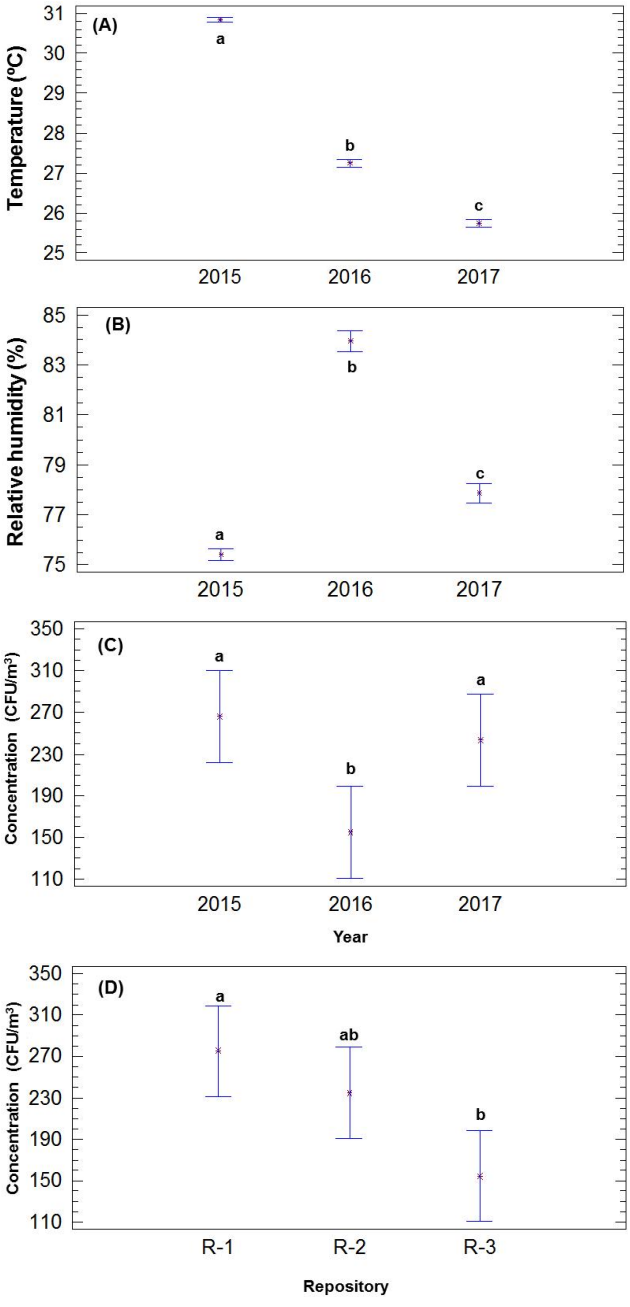


**Figure 3.** Statistical behavior (LSD) of the recorded T (A) and RH (B) in the analyzed repositories during the three years of study as well as the fungal concentrations per year (C) and per studied repository (D). a, b, c: Indicates statistically significant differences ($p \leq 0.05$). Similar letters designate that there are no significant differences.

**Table 1.** Fungal concentrations in the studied repositories and the indoor/outdoor ratio (I/O) obtained in each of them.

| Isolation | R-1 | | R-2 | | R-3 | | Outdoor |
|---|---|---|---|---|---|---|---|
| | (CFU/m$^3$) ± SD | I = I/O | (CFU/m$^3$) ± SD | I = I/O | (CFU/m$^3$) ± SD | I = I/O | (CFU/m$^3$) |
| First (2015) | 421.1 ± 328.2 a | 4.2 | 229.1 ± 197.4 d | 2.3 | 147.8 ± 36.7 e | 1.5 | 100.0 |
| Second (2016) | 135.6 ± 27.9 b | 1.5 | 176.7 ± 97.8 d | 1.9 | 153.7 ± 30.3 e | 1.7 | 90.0 |
| Third (2017) | 268.9 ± 132.7 c | 1.2 | 298.9 ± 124.9 d | 1.4 | 162.2 ± 95.5 e | 0.7 | 220.0 |

SD: Standard deviation. a, b, c, d, e: Indicates statistically significant differences ($p \leq 0.05$) of the fungal concentrations among the samples according to Duncan's test. Similar letters signpost that there are no significant differences. I ≤ 1.5: Non-contaminated environment with good ventilation. I = 1.5 – 2: Environment of regular quality. I > 2: Contaminated environment and with poor ventilation [59].

### 3.2 Diversity and Distribution of the Airborne Fungi

According to the culture method used, the anamorphic genera of the phylum Ascomycota (including yeasts) predominated in the air of the PHA SC repositories. Mytosporic fungi prevailed among them, evidencing the hyphomycetes *Alternaria*, *Aspergillus*, *Candida*, *Chrysonilia*, *Cladosporium*, *Coremiella*, *Curvularia*, *Fusarium*, *Penicillium*, *Talaromyces*, *Torula*, *Tritiriachium* and *Zygosporium*. A genus belonging to coelomycetes (*Pestalotia*), one corresponding to ascomycetes (*Eurotium*) and two types of non-sporulating mycelia (WNSM: white non-sporulating mycelium and PNSM: pigmented non-sporulating mycelium) were also detected in minority (Table 2).

In the first and third sampling 8 taxa were isolated, while in the second isolation 12 taxa were obtained. The predominant genera both in outdoor and indoor environments were *Aspergillus* and *Cladosporium*, which were obtained in all the samplings hence they turned out to be

**Table 2.** Ecological behavior of the fungal taxa detected in the analyzed environments in the different samplings.

| Taxa | R-1 | | | R-2 | | | R-3 | | | RF (%) | EC | Outdoor | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1st | 2nd | 3rd | 1st | 2nd | 3rd | 1st | 2nd | 3rd | | | 1st | 2nd | 3rd |
| | RD (%) | | | | | | | | | | | RD (%) | | |
| *Alternaria* Nees | - | - | 8.6 | 6.3 | 4.0 | - | - | - | - | 33.3 | O | - | - | - |
| *Aspergillus* P. Micheli ex Link | 27.7 | 71.4 | 37.0 | 56.2 | 40.0 | 8.4 | 56.3 | 66.6 | 58.8 | 100 | A | 30.0 | 11.1 | 4.5 |
| *Candida* Berkhout | 2.2 | - | - | - | - | - | - | - | - | 11.1 | R | - | - | - |
| *Chrysonilia* Arx. | - | - | 8.6 | - | - | - | - | - | - | 11.1 | R | 5.0 | - | - |
| *Chrysosporium* Corda | - | - | - | - | - | - | - | - | - | 0 | - | - | 55.6 | - |
| *Cladosporium* Link | 29.7 | 17.9 | 17.2 | 18.7 | 44.0 | 20.7 | 12.7 | 2.4 | 26.4 | 100 | A | 40.0 | 11.1 | 32.7 |
| *Coremiella* Bubák & Krieg. | 19.1 | - | - | - | - | - | - | - | - | 11.1 | R | 5.0 | - | - |
| *Curvularia* Boedijn | - | - | - | - | 4.0 | - | - | - | - | 11.1 | R | - | - | - |
| *Eupenicillium* F. Ludw. | - | - | - | - | - | - | - | - | - | 0 | - | - | 11.1 | - |
| *Eurotium* Link | - | - | - | - | - | - | - | - | 2.9 | 11.1 | R | - | - | - |
| *Fusarium* Link | 3.2 | - | 8.6 | 9.4 | 8.0 | 4.2 | - | - | - | 55.6 | F | 15.0 | 11.1 | 13.6 |
| *Neurospora* Shear & B.O. Dodge | - | - | - | - | - | - | - | - | - | 0 | - | - | - | 9.1 |
| *Penicillium* Link | 18.1 | 10.7 | 20.0 | - | - | 8.3 | 14.1 | 26.2 | 8.8 | 77.8 | C | - | - | 31.0 |
| *Pestalotia* De Not. | - | - | - | - | - | 8.3 | - | - | - | 11.1 | R | - | - | - |
| *Talaromyces* C.R. Benj. | - | - | - | - | - | 29.2 | 2.8 | 4.8 | - | 33.3 | O | - | - | - |
| *Tritiriachium* Limber | - | - | - | - | - | 4.2 | - | - | - | 11.1 | R | - | - | - |
| *Torula* Pers. | - | - | - | - | - | - | 1.4 | - | - | 11.1 | R | - | - | - |
| *Zygosporium* Mont. | - | - | - | - | - | - | 2.8 | - | - | 11.1 | R | 5.0 | - | 9.1 |
| Other Yeasts | - | - | - | 9.4 | - | - | - | - | - | 11.1 | R | - | - | - |
| WNSM | - | - | - | - | - | 12.5 | 9.9 | - | 3.1 | 33.3 | O | - | - | - |
| PNSM | - | - | - | - | - | 4.2 | - | - | - | 11.1 | R | - | - | - |

RD: Relative density. RF: Relative frequency. The ecological categories (EC) are classified as: Abundant (A) with RF = 100%-81%, Common (C) with RF = 80%-61%, Frequent (F) with RF = 60%-41%, Occasional (O) with RF = 40%-21%, Rare (R) with RF = 20%-0%. WNSM: white non-sporulating mycelium and PNSM: pigmented non-sporulating mycelium.

ecologically abundant (Table 2). *Penicillium* genus was also isolated, which due to being detected in the three samplings (but not in all the repositories) was classified as common, *Fusarium* that was isolated in the first and second sampling (although not in all the repositories) turned out to be a frequent genus; *Alternaria*, *Talaromyces* and a non-sporulating mycelium (WNSM) which, due to having been detected in two samplings, were classified as occasional. Likewise, *Candida*, *Chrysonilia*, and *Coremiella* were isolated in the first sampling, *Curvularia*, *Pestalotia*, *Tritiriachium* and other non-sporulating mycelium (PNSM) in the second sampling, *Eurotium*, *Torula* and *Zygosporium* in the third sampling and all of them were classified as rare. The yeasts that were isolated in 2016 from air of R-1 were classified as rare.

Sørensen similarity coefficient was used to analyze the similarity of detected genera in the indoor air of the repositories as well as between these and those isolated from the outdoor air. This coefficient showed values that varied between 0.3 to 0.5, i.e., the values were not high, and rather they were low to medium, indicating that there was a certain exchange of taxa between the environments during the years of study (Table 3).

Regarding the isolated species, 2 corresponded to the *Alternaria* genus, 17 belonged to *Aspergillus*, 5 were from the *Cladosporium*, 5 from *Penicillium* and only 1 species was obtained from the remaining genera. The analysis of all taxa detected in the indoor of R-1, R-2 and R-3 as well as the outdoor air during the three samplings showed the exchange of species among these environments manifesting with certain similarities within the wide diversity (Figure 4). The *Alternaria* species isolated were *Al. alternata* (Fr.) Keissl (R-3) and *Al. ricini* (Joshi) Hansford (R-1 and R-2), but as this last species was detected on two occasions was categorized as an occasional (RF = 22.2%), and constitutes the first record for Cuban archives environments. Of the *Fusarium* genus, the species *F. xylaroides* Steyaert was the predominant one (detected in R-1, 1st sampling and in R-3, 1st and 2nd samplings with FR = 33.3%) and also constituted the first report for Cuban archive environments.

Within *Aspergillus* species, *A. flavus* Link dominated (RF = 77.8%), which due to having been isolated on seven occasions (three times on R-1 and twice on both R-2 and R-3), was considered as common from these environments, while *A. niger* Tiegh. and *A. parasiticus* Speare were detected in second place (four times, equivalent to RF = 44.4%) and hence were classified as frequent species. In third place were *A. oryzae* (Ahlburg) Cohn and *A. versicolor* (Vuill.) Tirab, which turned out to be occasional (RF = 33.3%). Two other species were detected with lower frequencies (RF = 22.2%) and they also turned out to be occasional (*A. chevaliery* (L. Mangin) Thom & Church and *A. ochraceus* K. Wilh.), while another 10 were classified as rare species: *A. auricomus* (Guegen) Saito, *A. cervinus* Massee, *A. glaucus* Link, *A. japonicus* Saito, *A. nidulans* (Eidam) G. Winter, *A. penicilloides* Speg., *A. tamarii* Kita, *A. terreus* Thom, *A. ustus* (Bainier) Thom & Church and *A. uvarum* G. Perrone, Varga & Kozak. From *Cladosporium* genus, *Cl. sphaerospermum* Penz prevailed followed by *Cl. cladosporioides* (Fresen.) G.A. de Vries that were classified as common (RF = 77.8%) and frequent (RF = 44.4%), respectively; the other species (*Cl. oxysporum* Berk. & Curt., *Cl. staurophorum* Kendrick and *Cl. tenuissimum* Cooke) were obtained in minority hence turned out to be rare (RF = 11.1%). Although *Cl. staurophorum* was only isolated in R-2 in 2015, this species constitutes the first record for Cuban archives environments. From *Penicillium* genus, *P. citrinum* Thom dominated followed by *P. brevicompactum* Dierckx, hence they were

**Table 3.** Sørensen similarity coefficient (QS) values obtained by comparing the common genera detected in the repositories environments as well as between these and those isolated in the outdoor environment.

| | R-1 | | | R-2 | | | R-3 | | |
|---|---|---|---|---|---|---|---|---|---|
| | **$QS_1$** | **$QS_2$** | **$QS_3$** | **$QS_4$** | **$QS_5$** | **$QS_6$** | **$QS_7$** | **$QS_8$** | **$QS_9$** |
| **Outdoor** | 0.4 | 0.4 | 0.4 | 0.3 | 0.4 | 0.4 | 0.3 | 0.3 | 0.4 |
| | **$QS_{10}$** | **$QS_{11}$** | **$QS_{12}$** | - | - | - | **$QS_{13}$** | **$QS_{14}$** | **$QS_{15}$** |
| **R-2** | 0.4 | 0.5 | 0.4 | - | - | - | 0.3 | 0.3 | 0.4 |
| | - | - | - | - | - | - | **$QS_{16}$** | **$QS_{17}$** | **$QS_{18}$** |
| **R-1** | - | - | - | - | - | - | 0.4 | 0.3 | 0.4 |

$QS_1$, $QS_2$, $QS_3$: Indicate the similarities between R-1 and Outdoor in the three samplings (2015, 2016, 2017). $QS_4$, $QS_5$, $QS_6$: Indicate the similarities between R-2 and Outdoor in the three samplings. $QS_7$, $QS_8$, $QS_9$: Indicate the similarities between R-3 and Outdoor in the three samplings. $QS_{10}$, $QS_{11}$, $QS_{12}$: Indicate the similarities between R-1 and R-2 in the three samplings. $QS_{13}$, $QS_{14}$, $QS_{15}$: Indicate the similarities between R-2 and R-3 in the three samplings. $QS_{16}$, $QS_{17}$, $QS_{18}$: Indicate the similarities between R-1 and R-3 in the three samplings.

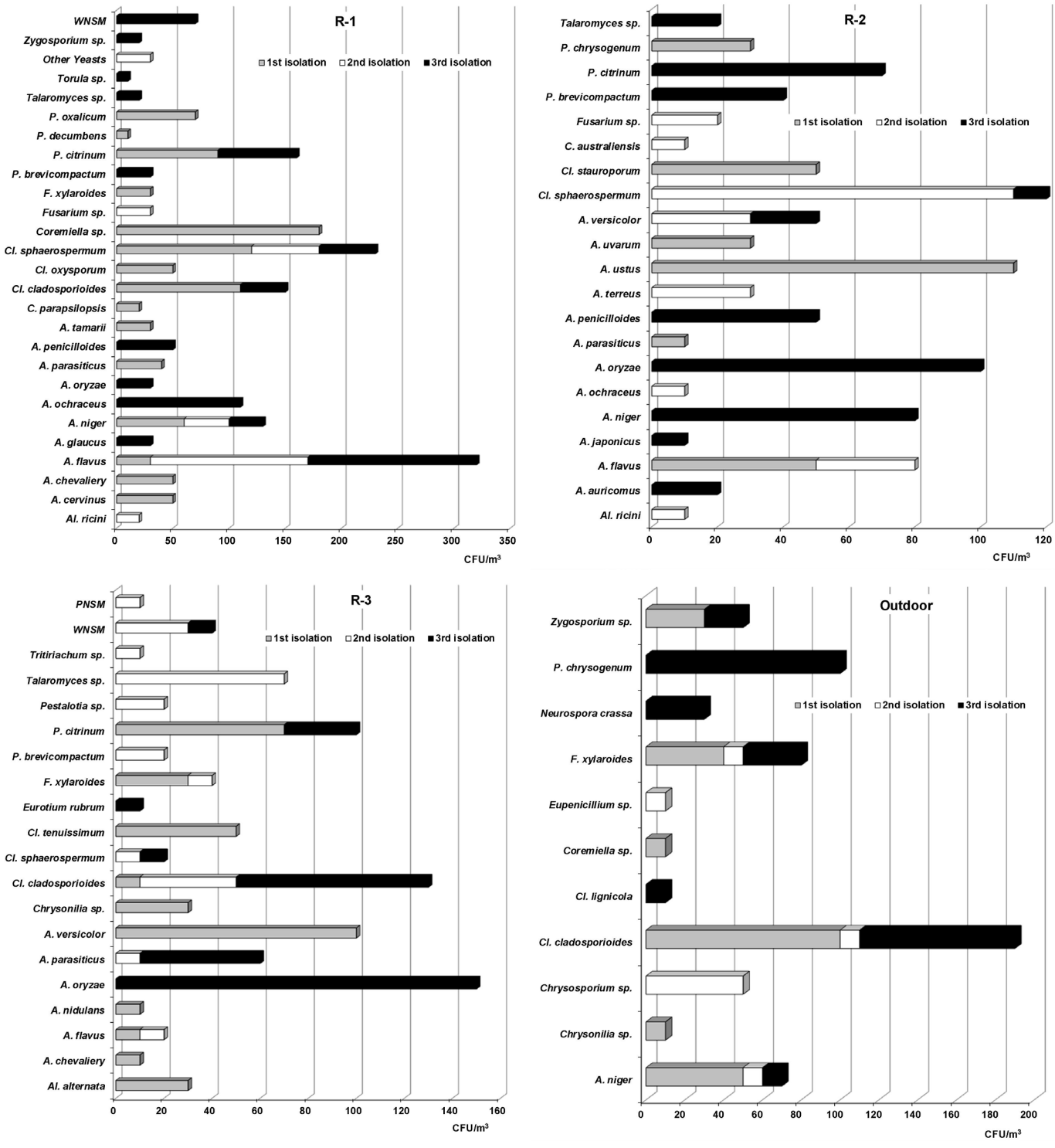


**Figure 4.** Species concentrations (CFU/m$^3$) detected in the indoor air of studied repositories by sampling and in the outdoor air.

classified as frequent (RF = 55.6%) and occasional (RF = 22.2%), respectively. The other three detected species were classified as rare (Table 4).

As *Aspergillus* has toxigenic species, the concentrations of all the detected species in the studied indoor environments were taken into account, and in the same way the concentrations of all *Cladosporium* and *Penicillium* species were also analyzed (Table 4). Some species of *Aspergillus* were detected with concentrations ≥ 50 CFU/m$^3$, distinguishing *A. flavus*, *A. niger*, *A. oryzae* and *A. versicolor*, while the other species were detected at concentrations between 10 CFU/m$^3$ and 40 CFU/m$^3$. *Aspergillus flavus* dominated with concentrations that ranged between 10 CFU/m$^3$ to 150 CFU/m$^3$, being R-1 in the second and third sampling where the highest concentrations were obtained (140 CFU/m$^3$ and 150 CFU/m$^3$, respectively); *Aspergillus ochraceus* was also detected in R-1 in the third isolation with a high concentration (110 CFU/m$^3$). In R-2, *A. ustus* were isolated in the first sampling and *A. oryzae* in the third isolation with high concentrations (110 CFU/m$^3$ and 100 CFU/m$^3$, respectively).

In general, the obtained concentrations sum from the different *Cladosporium* and *Penicillium* species together by isolation were markedly less than 500 CFU/m$^3$. R-1, which was the most contaminated repository in the first sampling, showed only 342 CFU/m$^3$ of the total species belonging to these two genera, in the other repositories the concentrations ranged between 60 CFU/m$^3$ and 150 CFU/m$^3$.

**Table 4.** Concentrations (CFU/m$^3$) and ecological behavior of the different species of *Aspergillus* spp., *Cladosporium* spp. and *Penicillium* spp. isolated in the repositories' air per sampling.

| Specie | 1st isolation | | | 2nd isolation | | | 3rd isolation | | | RF (%) | EC |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | R-1 | R-2 | R-3 | R-1 | R-2 | R-3 | R-1 | R-2 | R-3 | | |
| *A. auricomus* (Guegen) Saito | - | - | - | - | - | - | - | 20 | - | 11.1 | R |
| *A. cervinus* Massee | 50 | - | - | - | - | - | - | - | - | 11.1 | R |
| *A. chevaliery* (L. Mangin) Thom & Church * | 50 | - | 10 | - | - | - | - | - | - | 22.2 | O |
| *A. flavus* Link * | 30 | 50 | 10 | 140 | 30 | 10 | 150 | - | - | 77.8 | C |
| *A. glaucus* Link * | - | - | 30 | - | - | - | - | - | - | 11.1 | R |
| *A. japonicus* Saito * | - | - | - | - | - | - | - | 10 | - | 11.1 | R |
| *A. niger* Tiegh. * | 60 | - | - | 40 | - | - | 30 | 80 | - | 44.4 | F |
| *A. nidulans* (Eidam) G. Winter * | - | - | 10 | - | - | - | - | - | - | 11.1 | R |
| *A. ochraceus* K. Wilh. * | - | - | - | - | 10 | - | 110 | - | - | 22.2 | O |
| *A. oryzae* (Ahlb.) Cohn * | - | - | - | - | - | - | 30 | 100 | 150 | 33.3 | O |
| *A. parasiticus* Speare | 40 | 10 | - | - | - | 10 | - | - | 50 | 44.4 | F |
| *A. penicilloides* Speg. | - | - | - | - | - | - | - | 50 | - | 11.1 | R |
| *A. tamarii* Kita * | 30 | - | - | - | - | - | - | - | - | 11.1 | R |
| *A. terreus* Thom * | - | - | - | - | 30 | - | - | - | - | 11.1 | R |
| *A. ustus* (Bainier) Thom & Church * | - | 110 | - | - | - | - | - | - | - | 11.1 | R |
| *A. uvarum* G. Perrone, Varga & Kozak.* | - | 30 | - | - | - | - | - | - | - | 11.1 | R |
| *A. versicolor* (Vuill.) Tirab. * | - | - | 100 | - | 30 | - | - | 20 | - | 33.3 | O |
| *Cl. cladosporioides* (Fresen.) G.A. de Vries* | 110 | 40 | 10 | - | - | 40 | - | - | 80 | 44.4 | F |
| *Cl. oxysporum* Berk. & Curt. * | 50 | - | - | - | - | - | - | - | - | 11.1 | R |
| *Cl. sphaerospermum* Penz. * | 120 | - | - | 60 | 110 | 10 | 50 | 10 | 10 | 77.8 | C |
| *Cl. staurophorum* Kendrick | - | 50 | - | - | - | - | - | - | - | 11.1 | R |
| *Cl. tenuissimum* Cooke, Grevillea | - | - | 50 | - | - | - | - | - | - | 11.1 | R |
| *P. brevicompactum* Dierckx * | - | - | - | - | - | 20 | 30 | 40 | - | 22.2 | O |
| *P. citrinum* Thom * | 90 | - | 70 | - | - | - | 70 | 70 | 30 | 55.6 | F |
| *P. chrysogenum* Thom * | - | 30 | - | - | - | - | - | - | - | 11.1 | R |
| *P. decumbens* Thom * | 10 | - | - | - | - | - | - | - | - | 11.1 | R |
| *P. oxalicum* Currie & Thom | 70 | - | - | - | - | - | - | - | - | 11.1 | R |

*: Species reported as pathogens according to de Hoog *et al.* [75]. RF: Relative frequency. The ecological categories (EC) are classified as: Abundant (A) with RF = 100%-81%, Common (C) with RF = 80%-61%, Frequent (F) with RF = 60%-41%, Occasional (O) with RF = 40%-21%, Rare (R) with RF = 20%-0%.

The indoor/outdoor ratios (I/O) of the 6 species that were common in both environments (*A. niger*, *Chrysonilia* sp., *Cl. cladosporioides*, *Coremiella* sp., *F. xylaroides*, *Zygosporium* sp.) showed in general that, there was a low contamination level in the repositories indoor environments by them (I/O ≤ 1.5), with the exception of *A. niger* (R-2, 3rd isolate), *Chrysonilia* sp. (R-3, 1st isolation) and *Coremiella* sp. (R-1, 1st isolation), which presented I/O ratios of 4.2, 1.7 and 3.8, respectively, indicating the presence of an internal source of contamination of these species in the air (Table 5). R-1 in the first isolation was the repository that presented the largest number of common species (4) and within them *Coremiella* sp. which turned out to be predominant in that environment (RD = 19.1%), although in later isolates this species was never detected again. The remaining repositories showed one to three common species with a low degree of contamination by them. Only *A. niger* and *Cl. cladosporioides* were the species that showed the greatest similarities with the outdoor air. In fact, *A. niger* was detected in R-1 in all isolates, indicating that it is a typical species of this environment. *Cladosporium cladosporioides* was a species that turned out to be common in R-1 and R-3, but with ratios that did not indicate environmental contamination.

**Table 5.** Indoor/outdoor (I/O) ratios of the common species detected in each repository and in the outdoor environment by isolation performed.

| Taxa | First isolation | | | Second isolation | | | Third isolation | | |
|---|---|---|---|---|---|---|---|---|---|
| | R-1 | R-2 | R-3 | R-1 | R-2 | R-3 | R-1 | R-2 | R-3 |
| *A. niger* | 0.2 | - | - | 1.1 | - | - | 0.9 | 4.2 | - |
| *Chrysonilia* sp. | - | - | 1.7 | - | - | - | - | - | - |
| *Cl. cladosporioides* | 0.3 | - | 0.1 | - | - | 1.5 | 0.2 | - | 0.3 |
| *Coremiella* sp. | 3.8 | - | - | - | - | - | - | - | - |
| *F. xylaroides* | 0.2 | - | 0.6 | - | - | 0.4 | - | - | - |
| *Zygosporium* sp. | - | - | - | - | - | - | 0.3 | - | - |

I = I/O ratio. I ≤ 1.5: Non-contaminated environment with good ventilation. I = 1.5 - 2: Environment of regular quality. I > 2: Contaminated environment and poor ventilation [59].

## 4. Discussion

Although some research has been performed in Cuba on the environmental mycobiota of archives, libraries and museums, these have focused on the western region of the country, mainly in the Capital (Havana) because the largest number of these institutions are located in this region, hence this is the first investigation to be carried out in an archive of country eastern region.

To have a representativeness of the fungal concentrations and diversity in the environments of the repositories of this archive, the samplings were performed in different seasons and years. Cuba is characterized by having only two annual seasons that are a season of little rains or winter (December - April) and the rainy season or summer (May - November) that coincides with the period in which hurricanes occur in the Caribbean region (June 1 to 30 November) and that Cuba is affected by at least one of them every year. Although the eastern region of the country is the least affected by these meteorological phenomena, it is the most disturbed by earthquakes and in particular the province of Santiago de Cuba. Earthquakes occur almost constantly there, which in most cases are imperceptible by man, but are perceived by the sensors that the National Center for Seismological Research has located throughout the province. It was precisely intended to perform a sampling at the beginning of 2016 (rainy season) and it had to be postponed several months because on January 17, 2016, an earthquake was recorded south of the Santiago de Cuba bay that had 1500 aftershocks in 50 days, 37 of them perceptible not only in the Santiago de Cuba province but also in neighboring provinces [39]. Hence, the sampling for that year was performed on June 9.

In addition, the location of the province between the sea to the south and the high mountains of the Sierra Maestra to the north, provide it with high T and RH throughout the year [40], for that reason the thermo-hygrometric parameters were taken into account in the investigation.

Microclimatic conditions are known to play an important role in microbial proliferation [41,42]. The thermo-hygrometric parameters favor the microbial colonization of the substrates that make up the artworks [15]. Environmental RH directly determines the moisture content in materials, promotes sedimentation of suspended airborne fungal propagules, as well as proliferation and growth of fungi on surfaces [1,16]. The interactions of existing microorganisms in the bioaerosols, in particular fungal propagules, with microclimatic conditions and environmental chemical pollutants create alterations in the structures of the Cultural Heritage, including the documentary heritage, favoring the fungal attack of the substrates [3]. Therefore, T and RH behavior was evaluated in the repositories studied.

The T and RH in the repositories showed a normal distribution during the three years of study. Those thermo-hygrometric parameters can be considered high (29.6 ± 2.5 °C and 77.3 ± 5.2%, respectively), since the RH must be ≤ 65% to reduce the deposition of fungal propagules and the T must be ≤ 25 °C to minimize the microbial growth [43]. However, high RH values can negatively influence sporulation and

the growth of fungal spores that settle on materials [44]. So, the microclimatic conditions of the studied repositories in the PHA SC were not suitable for the development of fungi. Although natural ventilation does not achieve to lower the T and RH in the repositories to those recommended values, it does manage to keep them stable. For this reason, this type of ventilation is recommended in Cuba to guarantee thermo-hygrometric stability in archival repositories that do not store special materials, since it allows dealing with the daily variations of these parameters and the disasters that involve water, such as intense rains and hurricanes, as well as to face the climatic variations that CC is causing in the country [45].

Although the studied archive building has a cistern under the central courtyard that favors the considerable increase in humidity in the land that rises to the repositories by capillarity through the walls, no values higher than 85% or statistically significant differences were found of the RH in the repositories within each year, indicative of the stability of this parameter, possibly due to the beneficial role played by natural ventilation [1]. The detected differences in the thermo-hygrometric parameters among the years, with a decrease in T and an increase in RH with respect to time could be due to the raininess levels or drought of each season analyzed and to the construction conditions of the building that led to high moisture in the walls and floors of the repositories. This construction, like the majority of the buildings destined to conserve documentary collections in Cuba, was not conceived for this purpose, so there is a whole group of aspects related to the construction materials, the conception and distribution of the premises that are not they meet some of the most elementary construction standards required for an archive building. This corroborates the fact that the structure type of a building will determine the risk, the deterioration kind and the problems associated with the management of the indoor environment [15].

The quantification of the airborne mycobiota in the archive repositories constitutes, without a doubt, an indicator of the environmental microbiological quality that has a direct influence on the conservation of the documentary heritage and on the quality of life of the personnel. Air pollution in these places, mainly by filamentous fungi (aeromycobiota), is considered one of the greatest threats to health [7,46]. Because of this, many specialists dedicated to the cultural heritage conservation suggest systematic aerobiological sampling in archive and library environments. With them, an environmental reference of the risk to which the heritage value documents and the personnel who work with them are exposed is guaranteed [4,47].

Although the fungal concentrations detected in the repositories air did not exceed 500 CFU/$m^3$, they considered intermediate [48] or moderate [49], R-1 being the most contaminated repository during the three years studied. However, the concentrations obtained in this research were similar to those reported in previous studies performed in archives and libraries in other countries [41,47,50] and in Cuba both in naturally ventilated and air-conditioned environments [12,51-53]. Likewise, these concentrations turned out to be low in relation to others obtained in foreign archives [48,54,55].

It was evidenced that there was no correlation of the fungal concentration with the T and RH, indicative of the thermo-hygrometric stability in the repositories that favored the fungal load steadiness, demonstrating once again the importance of natural ventilation. Contrary behaviors were previously reported [3,41,51,52,54,55].

Although it is suggested that the fungal concentration of the outdoor environment is generally higher than that of the indoor environment, being a modulator of the fungal concentration in indoor environments [56,57] because it levels the quality of these environments, it has been reported that the I/O ratio is indicator of the emission of microorganisms and, therefore, defines the environmental microbiological quality. If this I/O ratio is $\leq 1$, the outdoor environment is the main source of bioparticles emission to the indoor environments [3,58], if it oscillates between 1.5 and 2 the environment has a regular quality, while if it is $\geq 2$, the indoor sources are responsible for environmental pollution, in addition to indicating poor ventilation or poor air circulation indoors [59]. According to these criteria, variations were observed from one sampling to another. In the first sampling R-1 and R-2 revealed I/O ratios typical of a poor environmental quality suggesting the existence of internal sources of fungal contamination that could induce or accelerate the degradative activity and the documents deterioration; in the second sampling all the repositories showed a regular environmental quality, while in the third sampling all repositories showed values lower than 1.5 indicative of a correct ventilation and air circulation in the repositories and consequently a normal exchange with the outdoor air [59], which prevented the formation of amplification zones of the fungal load in the indoors. A similar result was obtained in the PHA of Pinar del Rio (PHA PR) [52].

These behaviors in the environmental quality indicated that the conservators were not carrying out a correct management of the natural cross ventilation in the repositories at the beginning of the study and that after the first results and the suggestions offered to them, was possible to improve the environmental quality in repositories, demonstrating that the natural cross ventilation system turned out to be efficient to control the of fungal propagules load

within the monitored spaces despite the walls moisture. However, the high ratios obtained in R-1 and R-2 in the first isolation as well as in R-2 and R-3 in the second sampling could also be due to the fact that the external fungal loads were very low at those moments. It should be noted that the first two samplings were performed days after it had rained a lot in Santiago de Cuba city and is very likely that the outdoor environment was little polluted as a result of the washing that the rain causes into the atmosphere, since by dragging the suspended bioparticles in the air, their concentrations decrease [60]. Similar results were obtained in previous studies in the NARC and the PHA PR, respectively [52,60].

Regarding the taxa detected a preponderance of anamorphs of the phylum Ascomycota was evidenced, a question that had been previously reported [50-53,55-57] and this may be due to the fact that in the Caribbean region, representatives of this phylum have been detected in high concentrations in the outdoor air [14] and particularly in Cuba [62]. A total of 18 taxa were identified in the archive environments with a prevalence of the genera *Aspergillus*, *Cladosporium* and *Penicillium*, a result that was expected and agrees with others previously obtained in Cuba [11,12,19,51-53]. In earlier studies with similarity of culture methods, comparable amounts of taxa were referred [19,44,50,51,63].

In relation to the prevalence of *Aspergillus*, *Cladosporium* and *Penicillium*, everything seems to indicate that they were among the genera existing in the outdoor environment and penetrated to the indoor environments of the repositories at some time; although these genera are considered part of the indoor environments mycobiota [42,64] and particularly of archives and libraries due to the large amount of materials of an organic nature that are conserved in these institutions that serve as nutrients [1]. It has also been reported that humidity in indoor spaces, whether in the air or in walls and ceilings, is a factor that promotes the germination of spores and the harboring of fungal propagules belonging to *Penicillium* and *Aspergillus* genera [65]. Perhaps the fact that there was moisture on the studied repositories walls has contributed to the fact that these genera have remained viable and have also been detected of majority.

The three predominant genera are characterized by having a cosmopolitan distribution and include a large number of species that form small and dry spores (dry walled spores) that can be easily dispersed by the effect of air [21]. These spores can be quite numerous in indoor air that they can be easily inhaled [21,42,54]. They can also deposit on materials if the RH is high [15] and grow on paper, leather, textiles and other substrates, forming extensive biofilms. The fungal biofilms on documents compromise the structural cohesion of paper and book binding, irreversibly deteriorating them [57]. These fungi can degrade the substrates due to the excretion of hydrolytic enzymes, cause staining on the documentary materials due to the excretion of secondary metabolites and acidify the substrates causing irrevocable damages [5,49,51,56].

It should be noted that for the first time the genus *Coremiella* is detected in the indoor environment of a Cuban archive repository. This genus characterized by growing on submerged and terrestrial plant remains and by having species that have been isolated from the outdoor environment of several countries with different climates types [66]. It is probable that this fungal genus entered the of R-1 air from the outside, and was possibly isolated due to the high concentration that existed at the time of sampling in the repository air, since it was not detected in any of the other studied repositories or in any other sampling.

It is also important to note that for the first time the *Talaromyces* genus (teleomorph of *Penicillium*) was detected in the environment of a Cuban archive repository. It had previously been isolated from documents preserved in the NARC [11,20,66], but never from the air of an archive repository. However, it was isolated from the air in a Turkish archive [63] and several Brazilian libraries [54] as well as of Greek documents [68], Italians [69], Czechs and Poles [70]. It is suspected that the presence of *Talaromyces* in indoor environment of repositories could be influenced by the existence of species of this genus over documents, or their propagules could have entered the indoor air at some time from the outside, since *Eupenicillium* F. Ludw was detected in outdoor air of PHA SC, and is also a teleomorph of *Penicillium*. In relation to the genus *Tritiriachum*, it is not very common to find it in the environment of archives, libraries and museums, although it was previously isolated in the environment of a Cuban museum [71] and on artworks surface in two Cuban museums [70,71].

*Zygosporium* genus has been isolated in repositories environments of the NARC [20], which is located in the western region of the country, where there are also reports of being isolated from outdoor air [73]. This genus was isolated of the outdoor air in the first and third sampling performed in PHA SC, indicating that this genus is part of the country's aerial mycobiota and that it penetrated at some time into the indoor environment of the documents' repositories analyzed.

The species number detected in the studied indoor environments was slightly higher than those discovered in the outdoor environment, suggesting the existence of an internal source of contamination that could be risky for the documents kept in these repositories. However, contrary behaviors were obtained in previous studies [4,57]. The

exchange between the studied environments was low to moderate. Sørensen's coefficient of similarity showed that most of the values were less than 0.5, indicative of a low to moderate interchange between environments. Likewise, the I/O ratio for most of the common species were equal to or less than 1.5, showing that these species came from outdoor air and did not represent important pollutants in premises indoor. Only *A. niger* showed an I/O ratio of 4.8 (in R-2, third isolation) and *Coremiella* sp. (in R-1, first sampling) yielded a I/O ratio of 3.8, that were, very high, but since they were detected only once (even *Coremiella* sp. was never isolated again), it could be indicative of specific events.

Fungi, not yet viable, can be harmful to the personnel's health that work in archives, libraries and museums, since their propagules and mycotoxins can be inhaled as well as the volatile organic compounds that they excrete [6,7,14,42,46], it has been suggested that to define the mycological quality of an indoor environment not only must take into account the total fungal concentration but also the concentration of the isolated species [1,74]. In addition, analyzing the existence of "indicator species" and their concentrations are of great value for indoor environmental quality studies, hence the selection of those species that can be used as markers of improper indoor conditions should be performed by studying the ecology of the microbial communities in indoor environments [1].

Yang and Li [64] reported that persistently high RH in places with poor ventilation allows hygroscopic materials to increase water activity ($a_w$) to a level that favors the growth of xerophilic fungi. They also stated that xerophilic species limit their growth if the RH is less than 75% or greater than 98%. Although the genera *Chaetomium*, *Stachybotrys* and *Ulocladium* are indicators of high humidity or water damage in buildings [1,42,64], some *Aspergillus* species, such as *A. versicolor*, are also indicators of humidity in buildings. Likewise, species considered xerophilic can also be indicators of moisture problems in the premises; such is the case of *A. flavus, A. niger, A. nidulans, A. ochraceus, A. terreus* and *A. ustus* [64]. According to these criteria, it could be affirmed that the *Aspergillus* species mentioned above and that were detected in the studied environments, were indicators of moisture problems in the building of PHA SC despite their xerophilic condition.

It should be noted that the *Aspergillus uvarum* pathogenic species according to de Hoog *et al*. [75], constitutes the first record for the environment of a Cuban archive. Although its ecological impact was low, is a species that was recently isolated in the outdoor environment of Havana [23] indicating its existence and dispersion in the outdoor air of the all country.

It has been mentioned in some countries that an indoor environment of acceptable quality should have concentrations between 100 CFU/m$^3$ and 500 CFU/m$^3$ of various non-pathogenic fungal species and that for toxigenic species (*Stachybotrys chartarum*, various species of *Aspergillus*, *Fusarium* and *Penicillium*) the concentrations should be less than 12 CFU/m$^3$ [43], while for other countries these concentrations must be up to 50 CFU/m$^3$ [47]. However, among the *Aspergillus* species isolated in the repositories, *A. flavus* stood out first, as it had been detected on five occasions at concentrations between 30 CFU/m$^3$ and 150 CFU/m$^3$ followed by *A. niger*, which was isolated on four occasions at concentrations that ranged from 30 CFU/m$^3$ and 80 CFU/m$^3$. The other isolated species showed concentrations $\geq$ 100 CFU/m$^3$ in some repository, such was the case of *A. ochraceus, A. oryzae, A. ustus* and *A. versicolor*. Although these findings were specific, they could be signs that in the repositories there were conditions for a significant amplification of dangerous species both for documents and for the personnel's health; hence, if adequate conservation measures are not taken and the correct management of natural ventilation is maintained in the repositories, there is a risk that the documentation begins to be damaged quickly and progressively. These conditions could affect the staff health, but the fact that employees do not work constantly in the repositories and that when they have to take out or save documents in them they use personal protective equipment, avoids negative effects on their health.

The two aforementioned behaviors are justified by the existing moisture problems in the building, and in particular in the repositories wall, that favors the detachment of concrete from the stone walls and increases the dust level within these premises, a situation that can contribute to a significant increase in environmental fungal diversity. Therefore, it was proposed to perform further studies that allow comparing the behaviors of the airborne mycobiota, that of dust and that of documents surface.

The *Aspergillus* genus is characterized by having toxigenic (*A. flavus, A. ochraceus, A. terreus, A. versicolor*) and pathogenic species (examples: *A. chevaliery, A. flavus, A. japonicus, A. niger*, etc.) [6,9,75-77]; however, a large number of them were isolated from the air of the studied repositories to a greater or lesser extent, evidencing the risk they cause to the staff's health. There are species that can grow on materials with low $a_w$ because they are xerophilic or xerotolerant [78], it should be noted that a xerophilic species are capable of growing at $a_w \leq 0.85$. Though xerotolerance is not related to the species pathogenicity, these two conditions have coincided in some of them [75].

Of these species, some are considered within Biosafety Level (BSL or BL) 1 such is the case of some *Cladosporium* species that rarely cause infections in humans [78], so they are considered opportunistic pathogens [79], while others can be included in BSL-2 or BSL-3 [78].

Among the species detected in the studied repositories environment, 15 were found to be xerotolerant and were *A. flavus, A. niger, A. ochraceus, A. oryzae, A. penicillioides, A. tamarii, A. terreus, A. ustus, A. versicolor, Cl. cladosporioides, Cl. sphaerospermum, P. chrysogenum, P. citrinum, P. oxalicum*, and *Talaromyces* sp. Of these, only *A. flavus* is cataloged with BSL-2 for being a pathogenic species [76-78], while remaining of the 14 species were categorized with BSL-1 [78] despite being opportunistic pathogens [9,75,78]; but the frequency of health damage by *A. flavus* is higher than the rest of the species detected [76]. Although *A. niger* was isolated to a lesser extent and at lower concentrations than *A. flavus*, it should be noted that this species in addition to being xerotolerant is also thermotolerant [80], which could have a significant impact on health in situations of important of the environmental temperature increases as can happen in the case of CC. This evidences the risk to which personnel are exposed in a circumstantial way, since *A. flavus* was the dominant species within the *Aspergillus* genus.

If one takes into account that with CC many characteristics of environmental fungi will be modified or enhanced [17] then it is important to take these aspects into account to establish mitigation and resilience strategies in Cuban archives with a view to preserving both their documentary heritage such as the staff's health.

It should be noted that these results allowed the administration of the PHA SC to make efforts in the search for another more adequate building that allows it to better preserve its documentary collections.

At present, this process has already been resolved with the help of the provincial government and they are focused on the culmination of the constructive adjustments to soon start the movement of documents. This will not only improve the conservation conditions of the collections but also the environmental quality of the staff work areas.

## 5. Conclusions

This paper summarizes four key aspects obtained from the study of the naturally ventilated repositories in the PHA SC related to 1) the diversity of airborne fungi isolated from the indoor of these environments, 2) the environmental mycological quality of the repositories studied, 3) the isolated species behavior and 4) the potential risk that these environments represent for the personnel´s health. The conclusions are potted below:

- This first study performed in environments of different repositories of the PHA SC showed that the mycological quality of these environments varied over time as a consequence of the lack of systematicity in the correct management of the natural ventilation by conservators. Despite this, it was evidenced that the species number detected was slightly higher than those discovered in the outdoor environment, suggesting the existence of an internal source of contamination that could be risky for the documents kept in these repositories. Sørensen's coefficient of similarity indicated a moderate interchange between the indoor and outdoor environments. In addition, the I/O ratios for most of the common species proved that these species came from outdoor air and did not represent important pollutants from premise indoor.
- *Aspergillus* and *Cladosporium* were found to be predominant genera and ecologically abundant, while *Penicillium* was found to be a common genus.
- The *Coremiella* and *Talaromyces* genera, as well as the species *Aspergillus uvarum*, *Alternaria ricini*, *Cladosporium staurophorum* and *Fusarium xylaroides*, turned out to be new findings for the Cuban archives, despite the fact that most of these taxa did not show a significant ecological impact.
- Xerophilic species such as *A. flavus, A. niger, A. nidulans, A. ochraceus, A. terreus* and *A. ustus* were indicators of moisture problems in the repositories, evidencing the potential risk that this situation represents for the conservation of the documentary heritage that this archive treasures.
- Some species that have been reported as opportunistic and toxigenic pathogens (*A. flavus, A. niger, A. ochraceus, A. oryzae, A. ustus, A. versicolor*) were detected in concentrations higher than those recommended. It was also shown that most of the isolated environmental species correspond to the Biosafety Level (BSL) 1, and that only *A. flavus* is cataloged with BSL-2 for being a pathogenic species. These aspects, together with the exposure level of the personnel during the working day to the studied environments, showing that there is a potential risk to health and that the personnel is circumstantially exposed to biological risk.

## Author Contributions

Sofia Borrego directed the project, conceptualized the research and the methodology to be used, participated in the analysis of results and data, in the writing, review and

editing of the manuscript, as well as in the final version approval of the manuscript to submit.

Alian Molina contributed to the experiments design, performed all the mycological experiments, participated in the data analysis, wrote the first version of the manuscript, and gave final approval of the version to be submitted.

Yuneisis Bonne participated in the experiments design, calibrated and weekly placed the continuous measurement instruments of T and RH in the analyzed repositories, weekly extracted the data of T and RH from the instruments and placed them in the database created for this purpose, facilitated the sketch of the PHA SC, participated in the realization of some figures and in the reviewed of the written manuscript.

Anyilena González participated in the experiments design, performed the statistical processing of the T and RH data and their graphing, participated in the revision of the written manuscript.

Lidiersy Méndez participated in the experiments design, monitored the building constructive conditions and performed the daily management of the repositories, participated in the creation of some tables and figures and in the critical review of the significant intellectual content, and gave the final approval of the version to submit.

## Conflict of Interest

The authors declare that they have no competing financial interests or personal relationships that could have negatively influenced the work reported in this document. They also declare that they have no conflict of interest.

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