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Fungal assessment on storerooms indoor environment in the National Museum of Fine Arts, Cuba

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Abstract

The artworks that are preserved in museums can be damaged by fungi when the conditions of temperature and humidity are adequate to accelerate their growth. This is increased in Cuba by the climatic conditions of the country, characterized by high temperature and relative humidity during the all year. These fungi do not only cause the artworks biodeterioration but also affectations in the personnel's health. The aims of this work were to characterize the mycobiota in two microenvironments of the National Museum of Fine Arts, Cuba (1: three interior environments of repositories or storerooms and 2: six surfaces of artworks) and analyze its relationship with the conservation of artworks. Environmental samples were taken using a SAS Super 100 biocolector, while samples from the artworks surfaces were taken using sterile cotton swabs. Appropriate selective culture media were used to isolate fungi. The concentrations obtained in the indoor environments indicate that one storeroom was contaminated, while the other two were not. Fourteen filamentous genera, one genus of yeast, and two fungal mycelia were detected in the air of the storerooms, while four filamentous genera and one genus of yeast were obtained from the artworks studied. *Aspergillus* spp. was the predominant genus in the two microenvironments. Six species of the *Aspergillus* genus (*A. glaucus*, *A. flavipes*, *A. niveus*, *A. proliferans*, *A. conjunctus*, *A. restrictus*, *A. janus*) and three genera (*Botryoderma*, *Hansfordia*, *Nodulisporium*) detected in the repositories air as well as four isolated genera of artworks (*Olpitrichum*, *Verticillium*, *Engyodontium*, *Tritirachium*) are new findings for Cuban museums.

Keywords Artworks · Environmental quality in museum storerooms · Indoor environments in museum · Fungi · Museum

Introduction

The Cultural Heritage preserved in museums, galleries, archives and libraries is affected by numerous factors that cause their deterioration and unwanted alterations in the physical and chemical properties of the constituent materials, putting at risk of loss of values and enjoyment of the cultural heritage for present and future generations (Rodríguez 2016).

Among the agents of deterioration that can damage heritage assets are living organisms that use these materials as nutrients or substrates for their growth and development, causing serious effects on their chemical composition and alterations of their physical-mechanical structure, as well as provoking esthetic damages that affect the correct reading of their message. There

are many living organisms responsible for the biodeterioration of artworks, but filamentous fungi stand out among them because of their high degradative power of materials and frequency of appearance, especially in countries with humid tropical climates, typical of Cuba (Rojas et al. 2012; Borrego and Perdomo 2016). These microorganisms are heterotrophic and can colonize numerous substrates because they have a wide physiological diversity and have different mechanisms of dispersion of their spores, especially through the air (Pinzari 2011). This allows them to settle on the surface of heritage assets and under favorable environmental conditions can be developed widely colonizing these substrates and forming biofilms that cause the deterioration of cultural heritage (Pinzari 2011).

The majority of fungi need a high relative humidity and temperature to grow and develop; its development is enhanced in microclimates caused by condensation. But some fungal species are able to live at low water activities for that are classified as xerophilic fungi; they are perfectly adapted to indoor environments and thrive in dusty environments, with lack of ventilation or low water retention in the hygroscopic materials which are characterized by a very low water activity

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facilitating the colonized the xerophilic species (Pinzari 2011; Borrego et al. 2017). They can be found in the indoor air of archives, libraries, and museums where much organic materials exist. Dust is a good source for these fungi to feed and grow; these conditions intensify fungal contamination (Borrego et al. 2017; Skóra et al. 2015).

It is known than some filamentous fungal species of *Aspergillus*, *Penicillium*, *Cladosporium*, *Fusarium*, *Alternaria*, *Chaetomium*, *Ulocladium*, and *Trichoderma* have been commonly isolated from the artworks and the storeroom's indoor air of the heritage institutions in all world (Niesler et al. 2010; Rojas et al. 2012; Grbić et al. 2013; Ortiz et al. 2014; Skóra et al. 2015; Di Carlo et al. 2016; Rodríguez 2016; Okpalanozie et al. 2018). Viable fungi isolated from artworks conserved in an indoor environment in most cases come from the own indoor air. The outdoor air that penetrates through the door and windows as well as the shoes, the clothes, and the own body of the workers and visitors are the main ways of entrance of the fungal propagules to an interior environment, such as the storerooms of the museums (Niesler et al. 2010; Grbić et al. 2013); although incorrectly operating air-conditioning system may also be a source of fungal propagules (Grbić et al. 2013). For these reason is very important to made sampling of those two ecological microenvironments because surfaces analysis complements microbiological characterization of the air and vice versa.

The National Museum of Fine Arts (NMFA) is located in the Havana, Cuba. The museum has two building one of them it is called building of Universal art (1) and the other building of Cuban Art (2) for the collections that are conserved and exhibited in them. Building 1 is located 100 m from the Capitol of Havana, an important construction that marks the zero kilometer in Cuba; and building 2 is located approximately 300 m from building 1 towards the sea. In the Universal art building (1) are conserved artworks in different support as wood, textile, parchment, stone, crystal, metal, paper, etc. Due to flaws in its climate systems, some storerooms were affected by fungal infestations and for this reason the museum authorities requested an environmental study from the National Archive of the Republic of Cuba.

For these reasons, the aims of this research were to characterize the mycobiota in two micro-environments of the National Museum of Fine Arts, Cuba (NMFA) (1: three indoor environments of storerooms and 2: the surfaces of six artworks) and to analyze their relation on artworks conservation.

Materials and methods

Repositories and artworks analyzed

The study was carried out in three storerooms located in the basement of the building 1 of the museum. These repositories are characterized to be tightly closed spaces and to be acclimatized by means of a central system of air conditioning with the help of water to maintain the T between 20 and 25 °C and the RH below 65% with the exception of R1 that possesses two air-conditioning equipments independent of the climate system. But for approximately 6 months the central air conditioning system and the two air conditioning equipments of R1 had been broken, so during that time the collections were maintained with values of T and RH higher than 30 °C and 75%.

Three wooden sculptures conserved in R1, 1 colored wooden seat (installation) conserved in R2 and 2 easels painting (oil painting on canvas) preserved in R3 were selected to analyses.

Mycological sampling

The microbiological air sampling was made in 8 points selected and distributed in the following way: 4 points in the repository of Wooden sculptures (R1) and 2 points in the storerooms of Installations (R2) and Easel paintings (R3) respectively. All samples were taken between 10 and 12 h, considering the working hours and greater concentration of fungal propagules in the atmosphere of the Havana city (Almaguer and Rojas 2013). Each point was sampled by triplicate using a biocollector SAS Super 100 (Italy) at intervals of 1 h between replicates. With the impactor air sampler 100 L of air for 1 min was collected at a height of 1.5 m approximately.

For another hand 2 cm^2 of each artwork surface was sampled with sterile cotton swabs humidified in sterile saline solution (Rojas et al. 2012; Grbić et al. 2013; Skóra et al. 2015). The swabs were then immersed in 1 ml of sterile saline solution. The samples were thoroughly shaken and serial dilutions were made. Each dilution selected was inoculated (0.1 ml) on Petri dishes containing culture medium. The culture medium used in both assays was Malt Extract Agar (BIOCEN, Cuba) supplemented with NaCl (7.5%) (Rojas et al. 2012; Borrego and Perdomo 2016; Borrego et al. 2017) facilitates the growth of halophilic fungi and some xerophilic species; also, it is used to limit the colonies growth of Mucorales. Subsequently, the Petri dishes were incubated inverted at 30 °C for 7 days and the different colonies were isolated. After this time, colony count was performed and the fungal concentration in the air was expressed as CFU/m^3 of air while the obtained concentration on the artwork surfaces was reported in CFU/cm^2.

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

For the identification of fungal isolates cultural and morphological characteristics of the colonies as well as the conidiophores and conidia structures were observed in a stereomicroscope (× 14) and a clear field trinocular microscope (Olympus, Japan) at × 40 and × 100 attached to a digital

camera (Samsung, Korea), and different mycological manuals were used (Ellis 1976; Domsch et al. 1980; Klich and Pitt 1988; Barnett and Hunter 1998).

Ecological approaches

Relative Density (RD) of fungal genera isolated from indoor air of each repository was conducted according to Smith (1980) where:

RD = (number of colonies of one taxon/total number of colonies) x100

Relative Frequency (RF) of the fungal species detected on indoor environments as well as the genera and species isolated from artworks was determined according to Esquivel et al. (2003) where:

RF = (times a genus is detected/total number of sampling realized) x 100

The ecological categories 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%.

Statistical analysis

The data obtained of fungal concentrations were processed with the statistical Statgraphics Centurion XV program. A simple variance analysis (ANOVA) was applied with Duncan test to compare the obtained fungal propagule concentrations in the three indoor environments. The fungal concentration data obtained in R1 were plotted in Surfer 8.00 (Golden Software, Inc.).

Results and discussion

Behavior of environmental fungi

When this study was carried out, the air conditioning system of the museum was recently repaired after having been broken for more than 6 months, promoting high values of T and RH in all its repositories. The lack of adequate air conditioning to guarantee the appropriate T and HR and good air circulation as well as the increase of dirt on the works and the contamination introduced by the workers in the clothes and footwear every time they entered these storerooms, they were propitiating the conditions that favored the viability of the environmental fungi and their accelerated and exuberant growth on the pieces of art. Although R1 had an independent air conditioning system because it had two splits, these devices were defective, particularly the one on the right side of the storeroom that was not cooling well. On the other hand, the smell of mold was strongly perceived at the entrance of all the storerooms, indicative of

the presence of volatile organic compounds (VOC) that were excreted by fungi. This not only favored acidification of materials and air but also contributed to an abundant fungal growth. These odors were markedly strong in R1, where the highest values of RH were detected.

The average of the environmental fungal concentrations obtained inside the three repositories were different from each other, in fact the highest concentration was detected in R1 (125.6 CFU/m^3) followed by R2 (43.3 CFU/m^3) and the lowest was in R3 (23.3 CFU/m^3). Also, the concentration obtained in the external environment was 87.5 CFU/m^3 (Table 1). However, the values were lower than expected despite the fact that R1 showed a high dispersion of the concentrations favoring a very high standard deviation. This large dispersion of fungal concentration is attributable to the existence of areas with high fungal concentrations known as "amplification sites" days (Pinzari 2011). These sites are produced as a result of poor air circulation that causes empty spaces where the condensation of water occurs rapidly favoring the increase of the RH; therefore the accumulation of fungal propagules and even their deposition and growth on the artworks is stimulated in these zones. Figure 1, which is a map of contours, shows a large amplification site on the right side of this repository due to insufficient air circulation that caused an air stagnation zones.

As in Cuba there is no standard that allows establishing parameters of environmental microbiological quality for cultural heritage institutions, the values obtained in this study were compared with those proposed by various authors in different regions of the world (Nevalainen and Morawska 2009; Roussel et al. 2012) in particular with those established by Culture Ministry of Italy for conservation of indoor cultural heritage (Micheluz et al. 2015) that indicate fungal concentrations of 150 CFU/m^3 as the maximum permissible for a museum environment to be of high quality. In this comparison it was demonstrated that the environments of the NMFA repositories were not contaminated.

However some authors considering that the indoor/outdoor concentration ratio (I/O) is indicative of the air quality on indoor environments, inclusive some of them consider that this criterion is decisive in the classification of an environment in contaminated or not (Stryjakowska-Sekulska et al. 2007; Cabral 2010). The I/O ratios of the repositories were calculated and the obtained values were 1.8 in R1, 0.5 in R2, and 0.3 in R3. In the case of R1 the value was higher than 1.5 revealing a contaminated environment while R2 and R3 shows values lower than 1.0 indicative of no-contaminated environments according to Stryjakowska-Sekulska et al. (2007).

The average values of T ranged between 24.5 and 25.3 °C (Table 1), which were considered appropriate to preserve the existing materials in the repositories studied (Environmental Guidelines 2013). As for the RH, the R1 showed the highest value (68.3%) while in R2 it was close to 61% and R3 almost 59%. Although the RH values were lower than 70%, they

Table 1 Fungal **c**oncentrations detected on the indoor air of three repositories of the National Museum of Fine Arts (NMFA), Cuba

Concentrations	Wooden sculptures (R1)				Installation (R2)				Easel painting (R3)			
	Fungi (CFU/m³)	T (°C)	RH (%)	I/O	Fungi (CFU/m³)	T (°C)	RH (%)	I/O	Fungi (CFU/m³)	T (°C)	RH (%)	I/O
Maximum	760	24.8	69.8		50	24.6	60.7		40	25.3		
Minimum	10	24.3	66.0		40	24.4	60.4		10	25.1		
Average	155.6 ± 240.5[a]	24.5 ± 0.2	68.3 ± 1.6	1.8	43.3 ± 5.8[b]	25.3 ± 0.2	60.7 ± 0.2	0.5	23.3 ± 5.3[b]	25.2 ± 0.1	58.6 ± 0.5	0.3

The fungal determination was made in 4 points in R1 and 2 points in R2 and R3 by triplicate, respectively and the data averaged ($n = 12$ in R1, $n = 6$ in R2 and R3). The fungal concentration in outdoor was $87.5 ± 8.4$ CFU/m³ . I/O, indoor/outdoor ratios. [a, b] Indicate significant differences according to the Duncan test ($p \leq 0.05$) on comparing the average of the fungal propagule concentrations

were considered very high to conserve these materials, since it has been reported that the most convenient RH is $50 ± 5\%$ to protect hygroscopic materials against biological deterioration (Valentín et al. 2010; Environmental Guidelines 2013). Indeed, when air RH reaches values above 60%, a lot of fungal species can germinate and grow (Grabek-Lejko et al. 2017). Although the T showed a positive correlation with the environmental fungal concentration ($r = 0.49–0.59$, $p \leq 0.05$), the correlation of the RH was considerably higher ($r = 0.96–0.98$, $p \leq 0.05$) in particular for R1; therefore, environmental RH has played a more significant role in maintaining the viability of fungal propagules in the environment. In previous studies, the high correlation between T and RH with the concentration of fungal environmental propagules in museums has been reported (Skóra et al. 2015). Likewise, the positive effect of environmental T and RH on fungal growth was also reported by Okpalanozie et al. (2018).

Germination of spores and development of fungal colonies is determined by the chemical composition of the material itself and by the environmental factors like RH, T, and gases composition; and the level of initial contamination of the material (Grabek-Lejko et al. 2017). The fungal growth is increased by these factors, but water is the most important ingredient for the germination, growth, and development on indoor bacterial and fungal bioaerosols. Relationships between indoor bioaerosols and moisture content have been established in several studies (Pinzari 2011). The most favorable conditions for microbial growth vary depending on the species, but few species can live in a material with 65% water activity ($a_w = 0.65$) (Sterflinger and Pinzari 2012). It has been reported that hygroscopic materials (paper, textiles, leather, wood, etc.) are the most susceptible to microbial growth (Pinzari 2011). Therefore, it is very important to record the RH of the repositories daily.

Fig. 1 Contour maps obtained with the software Surfer 8.00 from the values of fungal concentration (UFC/m³) detected in wooden sculptures repository (R1). Note that on the right side of the repository the highest fungal concentrations were detected, which is indicative of the existence of an amplification site
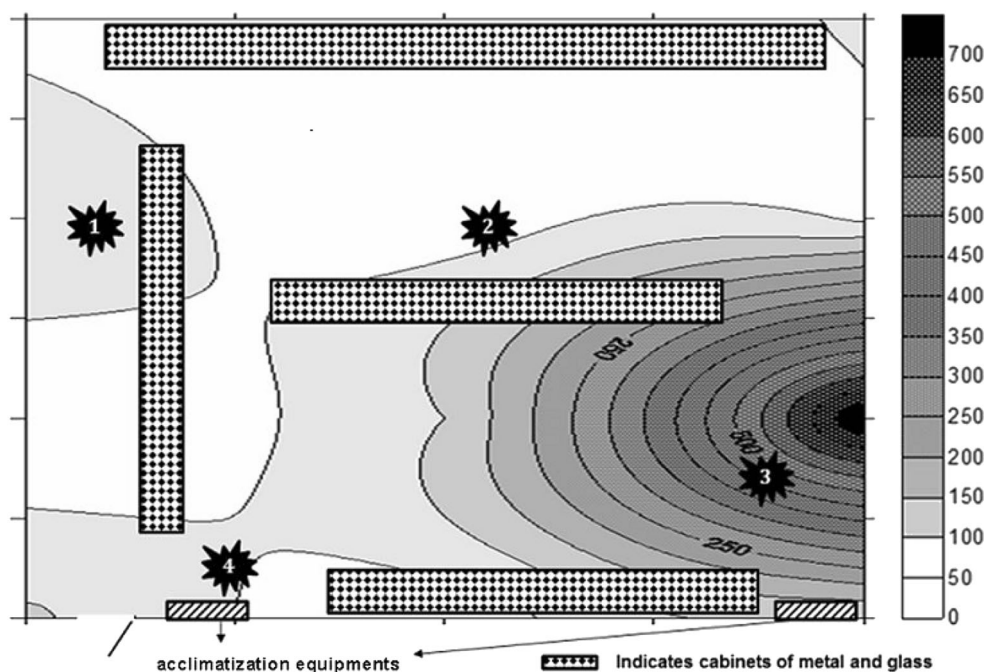
A total of 9 taxa (7 of filamentous fungi and 2 of yeast) and a non-sporulated mycelium were isolated from the indoor air of the repositories (Fig. 2). Concentrations and diversity of filamentous fungi were usually higher than yeasts. The genera *Aspergillus* Link and *Botryoderma* Papendorf and Upadhyay were isolated in the three repositories of the museum, while *Tritirachium* spp. Limer was detected in two repositories (R1 and R2); however, representatives of the genera *Penicillium* Link, *Cladosporium* Link, *Hansfordia* S. Hughes, *Nodulisporium* Preuss, *Candida* Berkhout, *Rhodotorula* Harrison, and a white non-sporulated septate mycelium (WNSM) were detected in a single repository. However, R1 was the repository that showed the highest fungal diversity, since species of 6 genera (*Aspergillus*, *Botryoderma*, *Penicillium*, *Hansfordia*, *Tritirachium,* and *Candida*) and a WNSM were isolated. On the other hand, *Aspergillus* was the predominant genus in all of them; similar result was previously obtained by other authors (Rojas et al. 2012; Gutarowska et al. 2012; Grbić et al. 2013; Okpalanozie et al. 2018). *Cladosporium* spp. and *Penicillium* spp. were detected in the air but their presence was not significant, which differs from other results (Gutarowska et al. 2012; Rojas et al. 2012; Okpalanozie et al. 2018; Paiva de Carvalho et al. 2018).

Although *Botryoderma* was one of the genera detected in all the repositories studied, it was obtained with an RD less than 15% in all cases. This is a fungus from the soil (Demirel et al. 2005) and could have been found in the outdoor environment at some point (although it was not detected at the time of the study), and since the museum is surrounded by parks with vegetation, it managed to penetrate inside the repositories through the air. *Tritirachium* was another genus isolated in two repositories with an RD of 15.9% in R1 and 7.1% in R2. Their natural habitat is soil and decaying plant material; it has been commonly isolated from paper, jute, textiles, adhesives, gypsum board, and air. Also the genus is an insect pathogen. Health effects include reports of corneal ulcers and a case of otomycosis (EMLab 2018a). This genus was previously reported in museum environments in Cuba (Rojas et al. 2012). *Hansfordia* spp. is an endophytic fungus isolated from plant materials and it was isolated previously on documents (Okpalanozie et al. 2018). Also *Nodulisporium* spp. is an endophytic genus isolated from plants (Nair and Padmavathy 2014) and affects human health either by provoking severe allergic states or infections in the human brain (Umabala et al. 2001).

The genera *Botryoderma*, *Hansfordia,* and *Nodulisporium* were detected for the first time on indoor environments of a museum in Cuba.

Regarding the WNSM detected, it is necessary to emphasize that these mycelia are organisms that have not been able to sporulate in the culture conditions provided routinely in a laboratory. Most of them never sporulated in culture media (sterile mycelia). Some representatives of non-sporulated colonies are common fungi (e.g., *Cladosporium*, *Alternaria*, even *Aspergillus*); frequently non-sporulated colonies are produced by basidiomycetes (mushrooms) too, which usually do not produce fruiting structures on lab media. These mycelia may

Fig. 2 Relative densities (RD) of the fungal genera detected on the indoor and outdoor air of the three studied artwork repositories in the NMFA. PNSM: Indicate Pigmented Non-sporing Septate Mycelium. WNSM: Indicate White Non-sporing Septate Mycelium
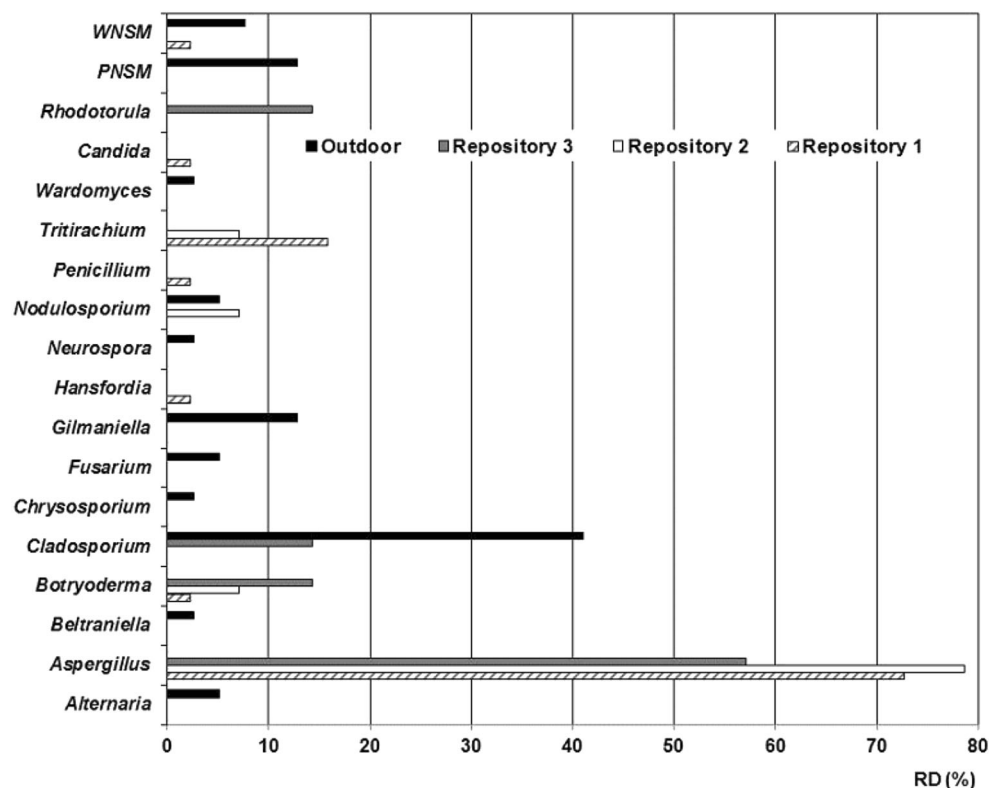
appear as colorless or pigmented (brown), septate (with cross-walls), or non-septate but in conclusion their identification is not possible without sporulation; it is known that they growth on a variety of substrates (EMLab 2018b). These kinds of mycelia were detected previously on indoor environments of a museum (Grbić et al. 2013) and Cuban archives (Borrego and Perdomo 2016; Borrego et al. 2017; Li et al. 2016).

Yeasts of the genera *Candida* and *Rhodotorula* have been previously detected in museum environments (Gutarowska et al. 2012; Skóra et al. 2015); even *Rhodotorula* spp. is associated with high affinity to osmotic environments (Sterflinger and Pinzari 2012). Although the yeasts do not represent a high risk for works of art, if they are for human health. It is known that some species of *Candida* spp. they are pathogenic (De Hoog et al. 2000) and in the case of *Rhodotorula* spp. it is known that some species are part of the normal biota of human skin and throat; however during the last two decades this genus has emerged as an opportunistic etiologic agent, particularly in immunocompromised persons (Tuon and Costa 2008).

According to Cabral (2010) and Pinzari (2011) fungi can serve as bioindicators of air quality because depending on the presence and concentration of some fungal genera you can discover wet environments and sick buildings (Sick Building Syndrome, SBS), therefore they constitute a advertisement of dangerousness environmental for health. These authors suggest that the predominance of *Aspergillus* spp. and *Penicillium* spp. is indicative of an indoor environment with humidity in the building and evidence that the building is sick; also the volatile organic compounds (VOCs) that can be produced and excreted by these genera, confirm the existence of SBS (Ishizaka et al. 2019). On the contrary if *Cladosporium* is the predominant genus then the building is healthy. Other authors indicate that for individual fungi, the threshold concentrations for evoking allergic symptoms have been estimated as 100 of *Alternaria* spores per cubic meter air and 3000 *Cladosporium* spores per cubic meter air, while *Aspergillus* spore concentrations above 50 CFU/m^3 have been potentially associated with a higher prevalence of sick-building syndrome (Chen et al. 2010; Borrego et al. 2017). According to these criteria, the storerooms studied turned out to have sick environments due to the significant and almost absolute predominance of the genus *Aspergillus*. It is noteworthy that *Cladosporium* spp. it was only detected in R3 at an RD of 14.3% while *Aspergillus* spp. was detected at an RD of 57.1%, that is, its prevalence was almost 4 times (3.99) higher than that of *Cladosporium* spp. In the other two repositories, not even the *Cladosporium* genus was isolated.

Twenty-one species of filamentous fungi, two yeasts, and the WNSM were also identified on the indoor environments (Fig. 3). The species with the highest prevalence were *Aspergillus glaucus* Link and *Botryoderma* sp. Papendorf and Upadhyay and were considered ecologically abundant. Common species were found to be *Aspergillus flavus* Link

(detected in R2 and R3), *Aspergillus ustus* (Bain.) Thom & Church (isolated in R1 and R2), *Aspergillus versicolor* (Vuill.) Tiraboschi (detected in R1 and R2) and *Tritirachium oryzae* (Vincens) of Hoog (isolated in R1 and R2); the rest of the species (ei, *A. candidus* Link, *A. conjunctus* Kwon & Fennell, *A. chevarieli* Mangin, *A. flavipes* (Bain. & Sart.) Thom & Church, *A. fumigatus* Fresen, *A. janus* Raper & Thom, *A. niveus* Blochwitz, *A. ochraceus* Wilhelm, *A. proliferans* G. Smith, *A. restrictus* G. Smith, *Cladosporium cladosporioides* (Fresen.) G.A de Vries, *Hansfordia* sp. S. Hughes, *Nudulisporium* sp. Preuss, *Penicillium chrysogenum* Thom, *Engyodontium album* (Limber), *Candida* sp. Berkhout, *Rhodotorula* sp. Harrison) were ecologically casual. It should be noted that contrary to what was obtained in other environments of Cuban museums, *A. niger* was not isolated from the air of any storeroom in the NMFA (Rojas et al. 2012). Although *A. flavus* was classified as a common species because it was detected in two of the three environments analyzed, it is noteworthy that in previous reports this species was isolated from the environment of several Cuban museums (Rojas et al. 2012). On the other hand, *A. ustus*, *A. versicolor*, *A. chevalieri*, *A. candidus*, *A. fumigatus*, *A. ochraceus*, *C. cladosporioides,* and *P. chrysogenum* were also previously isolated from the environment of Cuban museums and archives (Rojas et al. 2012; Borrego et al. 2017) and some of them were reported in Portugal museum environments with wooden artworks (Paiva de Carvalho et al. 2018). However, the species *A. glaucus*, *A. flavipes*, *A. niveus*, *A. proliferans*, *A. conjunctus*, *A. restrictus,* and *A. janus* have never before been reported in Cuban museums, hence they are new findings for Cuba.

Within the genus *Aspergillus*, the species *A. chevalieri*, *A. flavipes*, *A. flavus*, *A. fumigatus*, *A. glaucus*, *A. janus*, *A. niveus*, *A. ochraceus*, *A. restrictus*, *A. ustus,* and *A. versicolor* have a considerable impact on human health (De Hoog et al. 2000). Also, it has been reported that *Penicillium chrysogenum* and *Tritirachium oryzae* can also cause problems to human health (De Hoog et al. 2000). It has been possible to confirm that 66.7% of the species detected are pathogenic or emerging opportunistic pathogens, which represents a high risk to the health of the museum's workers.

In the outdoor environment of the museum, 9 genera were detected (*Alternaria* Nees, *Beltraniella* Subram, *Cladosporium*, *Chrysosporium* Corda, *Fusarium* Link, *Gilmaniella* Barron, *Neurospora* Shear & BO Dodge, *Nodulisporium*, *Wardomyces* Brooks and Hansford) and 2 non-sporulated mycelia (one pigmented and one hyaline) (Fig. 2). As it is observed, there is a marked predominance of the *Cladosporium* genus. Similar results were report previously (Cabral 2010) and likewise occur in Havana city atmosphere (Almaguer and Rojas 2013). Even, *Cladosporium* it is one of the fungal genera that the Sahara dust transports to

Fig. 3 Ecological impact of fungal species detected on the indoor air of the studied artwork storerooms in the NMFA. Single asterisk indicates that these species have the greater clinical interest (De Hoog et al. 2000). Double asterisks indicate that the former name was *Tritirachium album*
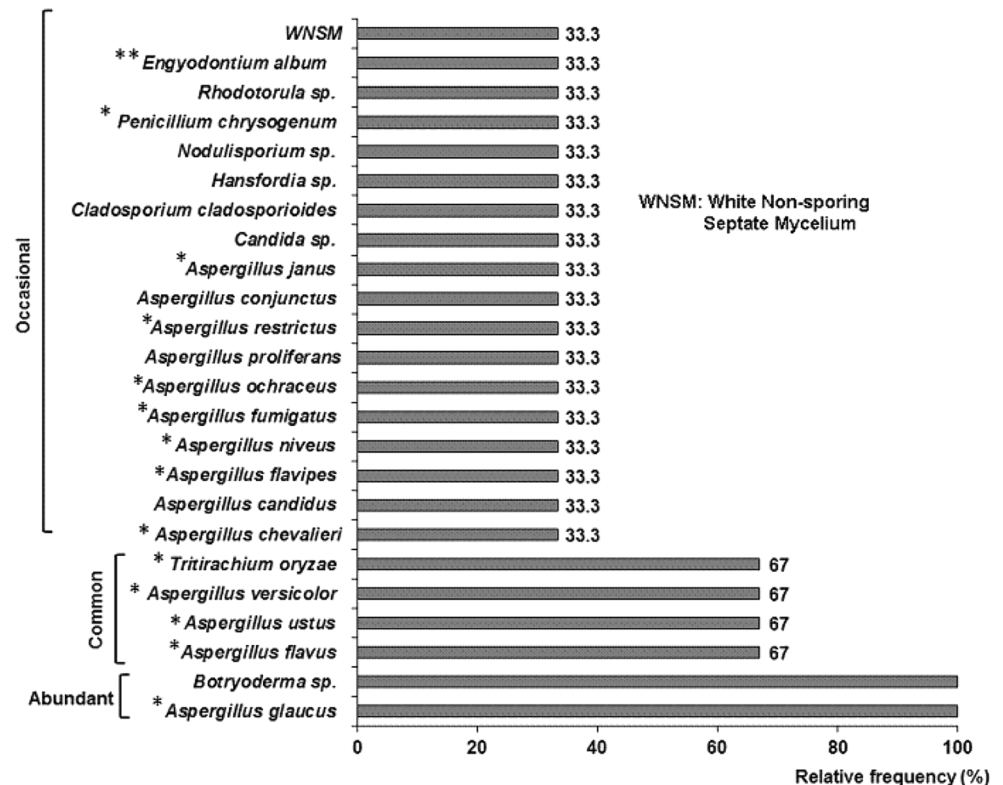
Caribbean, including Cuba (Sullivan et al. 2012). It was rare not to detect *Aspergillus* spp. and *Penicillium* spp. in this study, because they turn out to be very common genera in the Havana environment (Rojas et al. 2012; Almaguer and Rojas 2013). The genera *Alternaria*, *Fusarium,* and *Nodulisporium* have also been previously isolated from the environment of Havana (Almaguer and Rojas 2013).

Of all the isolated genera, they were coincident both on indoor environment of repositories as in outdoor, *Cladosporium* and *Nodulisporium* as well as the WNSM. This represents a level of coincidence of 27.3%, that is, low (< 50%). Although *Cladosporium cladosporioides* was predominant outside, in the indoor environment of the repositories it was an ecologically rare species and was only isolated in the R3 environment. Therefore, these findings confirm the fact that the impact of the outdoor environment inside the repositories is low and indicative of poor air exchange between the two environments.

Fungal behavior on the artworks

The main drawback of the museum's repositories at the time of this study was the lack of ventilation or air circulation, that is, there was no adequate distribution of the air flow with internal/entrance and evacuation through filters that favored the "dilution" of harmful VOCs and will avoid the deposition of dust, which has associated spores and propagules of microorganisms (Valentín et al. 2010). It should be noted that although at the time of sampling the air conditioning system was working, time ago the climate equipment was broken for many months, so even though the thermo-hygrometric values were not excessively high, they could continue to favor the fungal growth that it had occurred started months ago with conditions of HR much higher and close to the optimum values for the development of most fungi (≥ 65%). On the other hand, the ventilation lack, the poor air circulation and surface temperature lack of homogeneity can produce water condensation points and local micro-climates with higher water availability than in the rest of an indoor environment. These circumstances are favorable to some fungal species; as a result these are able to proliferate on the artworks with relative easiness. A fungal colony can grow up to 4 mm/day and an outburst of fungi can contaminate a whole collection within just a few days (Sterflinger and Pinzari 2012).

In reviewing the artworks that would be sampled, it was observed with the naked eye that they had a large amount of dust deposited on their surface and an abundant fungal growth indicative of the high degree of colonization of the materials. However, all the art pieces preserved in R1 had a very abundant fungal growth (Fig. 4). In the figure it can be seen that all the pieces have a strong fungal biofilm adhered to them. Likewise, the oil paintings on canvas that are preserved in R3 also showed an abundant fungal growth in the form of colonies of active fungi and of a broad and deep biofilm in the support (Fig. 5), an aspect that was confirmed during the sampling.

WOODEN SCULPTURES

Fig. 4 Wooden sculptures conserved in Sculpture repository (R1). Note that all of them are contaminated with abundant fungal colonies and biofilms

From the artworks, a total of 5 genera and 1 a yeast were isolated. In almost all the artworks studied, the concentrations of colony forming units per cm^2 (CFU/cm^2) were very high and they oscillated between 1 CFU/cm^2 and 2.5×10^7 CFU/cm^2 which showed that the pieces were highly contaminated (Table 2). On the wooden sculptures the fungal concentrations varied between 5×10^2 and 1.8×10^7 CFU/cm^2. *Aspergillus* was the predominant genus; although *Engyodontium* de Hoog, *Olpitrichum* G.F. Atkinson, and *Tritirachium* were the other genera isolated. The species *A. janus* and *A. versicolor* were detected at the highest concentrations. Similarly on the easel painting *Aspergillus* was the predominant genus with concentrations that oscillated between 1 ×

10^2 CFU/cm^2 and 2.5×10^7 CFU/cm^2 followed by *Tritirachium*, *Verticillium* Nees, and *Candida*. In this occasion *A. flavus*, *A. fumigatus*, *A. niger,* and *A. proliferans* were the species detected with higher impact on the artworks analyzed. On the artwork called "installation" only *A. flavus* specie was detected.

An ecological analysis evidenced that *Aspergillus* was an abundant genus, *Tritirachium* was a common genus, *Olpitrichum* was occasional and *Verticillium*, *Engyodontium* and *Candida* were genera rare.

Olpitrichum genus is a cosmopolitan fungus and its niches are varied and being found in plant litter, seeds, rotten areca nut, wood, soil, and parasitic on other fungi (Li et al. 2016;

OIL PAINTINGS ON CANVAS

Fig. 5 Oil paintings on canvas conserved in the Easel painting repository (R3) of the museum with an abundant fungal growth. Notice that the left paint has grizzly stains indicative of a wide fungal biofilm on the painting surface. The paint of the right has a great quantity of white points indicative of the fungal colonies active which are degrading the paint layer

Table 2 Concentrations of the fungal species isolated from the studied artworks

Repository	Concentration on the art pieces (UFC/cm^2)	Specie	Concentration on the art pieces (UFC/cm^2)	Specie
Wooden sculptures	1.2×10^6	*Aspergillus janus* Raper & Thom 1	4×10^3	*Aspergillus ustus* (Bain.) Thom & Church
	3×10^3	*Aspergillus janus* Raper & Thom 2	1.2×10^4	*Engyodontium album* (Limber) de Hoog
	6×10^2	*Aspergillus janus* Raper & Thom 3	2×10^4	*Olpitrichum* sp. G.F. Atkinson
	1.8×10^7	*Aspergillus versicolor* (Vuillemin) Tiraboschi	5×10^2	*Tritirachium oryzae* (Vincens) de Hoog 1
Easel painting	2×10^3	*Aspergillus flavus* Link 1	1	*Candida* sp. Berkhout
	2.5×10^7	*Aspergillus fumigatus* Fresen	7×10^3	*Tritirachium oryzae* (Vincens) de Hoog 2
	1×10^2	*Aspergillus niger* van Tieghem	7×10^3	*Verticillium* sp. Nees
	5×10^4	*Aspergillus proliferans* G. Smith	–	–
Installation	3×10^3	*Aspergillus flavus* Link 2	–	–

Schultes et al. 2017). For its part, *Verticillium* is a genus that includes saprophytic and parasitic species of higher plants, insects, nematodes, eggs of mollusks, and other fungi (Ashraf et al. 2012) and it has lignocellulolytic activity (Ortiz and Uribe 2010). It should be noted that the genera *Olpitrichum* and *Verticillium* were isolated only from artworks and not from the air; and no previous report was found that refers to the isolation of these fungal genera in cultural heritage institutions. However, *Engyodontium* spp. was previously isolated from wooden structures in heritage buildings in Chile (Ortiz et al. 2014). These three genera have been detected for the first time in artworks in a Cuban museum.

The art pieces S1, S2, and EP1 were the most contaminated, because on them grew and developed three different fungal genera. However, the genus *Aspergillus* was isolated from all of them, which represents a common finding. In addition to the wooden sculptures S1 and S2, the genera *Olpitrichum* and *Tritirachium* were also isolated. This last genus has never before been isolated from pieces of Cuban museums, so it turns out to be the first report for Cuba.

As for the predominance of *Aspergillus* spp. colonizing wooden sculptures, other authors had reported it (Rojas et al. 2012; Grbić et al. 2013; Rodríguez 2016; Paiva de Carvalho et al. 2018). The fact that *Aspergillus* spp. was the one that

Table 3 Fungal species detected in each analyzed artwork and their ecological impact

Specie	Analyzed artworks						RF (%)	EC
	S1	S2	S3	I1	EP1	EP2		
Aspergillus flavus Link*					X	X	33.3	O
Aspergillus fumigatus Fresen*					X		16.7	R
Aspergillus janus Raper & Thom*	X	X	X	X		X	83.3	A
Aspergillus niger van Tieghem*					X	X	33.3	O
Aspergillus proliferans G. Smith						X	16.7	R
Aspergillus versicolor (Vuill.) Tiraboschi*	X	X	X	X		X	83.3	A
Aspergillus ustus (Bain.) Thom & Church*			X				16.7	R
Candida sp. Berkhout**					X		16.7	R
Olpitrichum sp. G.F. Atkinson	X	X					33.3	O
Engyodontium album (Limber) de Hoog		X					16.7	R
Tritirachium oryzae (Vincens) de Hoog*	X			X		X	50.0	F
Verticillium sp. Nees					X		16.7	R

*Indicates that that these species have the greater clinical interest (De Hoog et al. 2000)

**Indicates that this genus has some species pathogens (De Hoog et al. 2000)

RF relative frequency, *EC* ecological category, *A* abundant with RF = 100–81%, *C* common with RF = 80–61%, *F* frequent with RF = 60–41%, *O* occasional with RF = 40–21%, *R* rare with RF = 20–0%

predominated in the colonization of the works, evidence that the environmental RH of the repositories was high for a long time, because it is stated that this genus turns out to be a primary colonizer when the RH is higher than 68% since this humidity favors their propagules deposition when the air velocity is very low (< 0.5 m/s) (Rojas et al. 2012).

Table 3 shows the species detected on the different artworks analyzed. It is evident that the species *A. janus* and *A. versicolor* were isolated from almost all art pieces and therefore were predominant species, hence they are classified ecologically as abundant; *Tritirachium oryzae* was classified as a frequent species since it was detected in 3 pieces. Occasional species were *A. flavus*, *A. niger,* and *Olpitrichum* sp. which were isolated from two artworks while *A. fumigatus*, *A. proliferans*, *A. ustus*, *Candida* sp., *Engyodontium album* (old name *Tritirachium album*) and *Verticillium* sp. were rare species. It is also observed that the art pieces EP2 (oil painting on canvas) and I1 (painted wooden chair) were the ones with the greatest fungal diversity, since 5 fungal species of them were isolated, while in the other pieces only 3 or 4 species were isolated. From the five species detected in EP2, 4 belong to the genus *Aspergillus* so ecologically this genus is the one that predominantly colonizes this piece. Among them *A. janus*, *A. niger,* and *A. versicolor* are dangerous to health since they are powerful allergens and opportunistic pathogens (De Hoog et al. 2000). The fifth species detected in EP2 was *T. oryzae* and also represents a risk to human health (De Hoog et al. 2000). Similarly, it happened with piece I1 where 4 of the 5 species belong to *Aspergillus* (*A. flavus*, *A. janus*, *A. niger,* and *A. versicolor*) the fifth species was also *T. oryzae*. It should be noted that these two artworks, although found in different repositories, share similarity in 4 of the 5 species isolated from them (*A. janus*, *A. niger*, *A. versicolor,* and *T. oryzae*).

The species *A. flavus*, *A. niger,* and *A. versicolor* were previously isolated from wooden sculptures (Grbić et al. 2013) and from other wooden art objects (Niesler et al. 2010; Gutarowska et al. 2012). In Cuba, these species were isolated from wooden art objects (Rojas et al. 2012; Rodríguez 2016) and it was even reported that *A. flavus* was associated with brown rot of valued woods (Rojas et al. 2012). However, no strain of *A. flavus* was isolated from the wood sculptures (S1, S2, and S3), but one strain was isolated from a painted wooden chair (I1) as was an *A. niger* strain. As for the species *A. versicolor*, 4 strains were isolated from different wooden art objects (S1, S2, S3, I1).

On the other hand, *A. flavus*, *A. fumigatus,* and *A. niger* grew on top of the pictorial layer of two oil painting on canvas (EP1 and EP2) and went deep into it, because during the sampling it was detected to the touch that the biofilm was not located only on the surface of the painting. It is known that the species *A. flavus* was isolated in Cuba from oil paintings (Rodríguez 2016), but no previous reports were found

mentioning *A. fumigatus* among the species that can biodegrade oil paintings. However, references have been found indicating that *Aspergillus* spp. and in particular the species *A. flavus*, *A. fumigatus,* and *A. niger* can degrade fats because they possess lipolytic enzymes that are secreted (Gopinath et al. 2005; Alfonseca and Serrat 2018), it is even suggested that hydrolysis of fats leads to the acidification of the medium (in this case the paint), which together with the low water activity characteristic of solid materials rich in fats, creates an environment conducive to the preponderant development of filamentous fungi with respect to other microbial groups. In this way, the natural environment (oil painting) acts as a selective medium (Nwuche and Ogbonna 2011). This could explain the existence of large and deep fungal biofilms on the pictorial layer in the studied paintings, in particular of EP1. In addition, it demonstrates the impact that these species have on the biodeterioration of oil paintings on canvas.

The species *A. versicolor* was also isolated from the oil painting EP2; however, no reference was found to indicate this behavior previously, but surely its growth on this type of work was justified by its lipolytic activity (Gopinath et al. 2005). In the same way, it occurs with *Candida* spp. (Alfonseca and Serrat 2018).

Relation of the mycobiota detected on indoor air of the repositories and on the artworks

During the last decades, a strong correlation between aerobiology and the biological deterioration of works of art has been affirmed, due to the fact that air is the main vehicle for the dispersion of microorganisms (Pinzari 2011; Sterflinger and Pinzari 2012). In general, similarities have been reported between isolated microorganisms in the air and isolated ones from degraded artworks (archive, library, wall paintings, museum collections, etc.). Therefore, in many cases, a direct effect was inferred (Pinzari 2011; Di Carlo et al. 2016). Hence, the monitoring of microbial contamination both on the surface of the artworks and in the surrounding air represents the basis for an adequate conservation strategy, in order to gather information to identify a specific hazard that should lead to a rapid intervention that provides an adequate conservative action (Di Carlo et al. 2016).

Of a total of 46 strains isolated inside the museum, 31 were obtained from the air of the repositories and 15 from artworks. However, a 19% similarity between the species isolated from the air and from artworks was obtained (Table 4) and only 2.1% of the strains showed different behavior, this was the case of *Candida* sp. that was detected in the air of R1 while it was isolated from an oil painting that is preserved in R3. The species *A. flavus* was isolated from the air of R2 and R3 and from artworks preserved in these repositories. *Aspergillus fumigatus* was isolated both from the air of R1 and from a sculpture that is preserved there. Specie *A. janus* was isolated

Table 4 Similar fungal species detected on the indoor air of three repositories as well as on the analyzed artworks and their ecological impact

Specie	Air			Sculptures	Installation	Easel painting	RF (%)	EC
	R1	R2	R3					
Aspergillus flavus Link	–	X	X	–	X	X	66.7	C
Aspergillus fumigatus Fresen	X	–	–	X	–	–	33.3	O
Aspergillus janus Raper & Thom	–	–	X	–	X	X	50.0	F
Aspergillus proliferans G. Smith	–	X	–	–	X	X	50.0	F
Aspergillus ustus (Bain.) Thom & Church	X	X	–	X	–	–	50.0	F
Aspergillus versicolor (Vuill.) Tiraboschi	X	X	–	X	–	X	66.7	C
Candida sp. Berkhout	X	–	–	–	–	X	33.3	O
Nodulisporium sp. Preuss	–	X	X	–	–	X	50.0	F
Engyodontium album (Limber) de Hoog	X	–	–	X	–	–	33.3	O
Tritirachium oryzae (Vincens) de Hoog	X	X	–	X	–	X	66.7	C

from: the air of the R3 repository, one artwork conserved in this repository and a piece preserved in R2. Similarly occurs with *A. proliferans* that was detected in the air of R2 and a piece preserved in this same repository, but was also isolated from a piece preserved in R3. A strain of *A. ustus* was isolated from the air of the repositories R1 and R2 and nevertheless was only detected in an artwork preserved in R1. *Aspergillus versicolor* was isolated from both R1 and R2 but was detected in a sculpture that is preserved in R1 and in an oil painting that is preserved in R3. *Nodulisporium* sp. not only was it isolated in the air of R2 and R3 but also of a piece preserved in R3 and *Engyodontium album* was only detected in the air of R1 and in a sculpture that is conserved in that repository. Although *Tritirachium oryzae* was isolated from the air of R1, R2, and R3 was only detected in one piece preserved in R3.

It is interesting to highlight that the species *A. janus* and *A. versicolor* were isolated both from pieces that were kept in a same repository as well as pieces conserved in different repositories. This shows that cross infestation of the air occurred towards the works of art and vice versa, and that the transfer of some species from one repository to another through the air conditioning was favored. Likewise, it was evidenced that both microenvironments (air and artworks) are ecologically dominated by the species *A. flavus*, *A. versicolor,* and *T. oryzae*.

The species *A. candidus* was detected in the air of R1 but was not isolated from any of the wooden sculptures that are conserved there. However, it was previously detected in Cuba on wood objects of heritage value (Rojas et al. 2012).

As a final result of this investigation, a report was delivered to the curators and the museum administration that included a group of measures that included as a first action the cleaning and disinfection of both artworks and storerooms (walls, ceilings, and floors). The use of dehumidifiers for the control of environmental RH was also suggested. Among the first measures proposed was highlighted, the imperative need to use personal protective equipment to avoid not only the triggering of allergic processes, but also other possible pathologies caused not only by the fungi detected but also by dust mites. Taking into account that at this time the benzalkonium chloride was the only disinfectant that the museum had, its use was suggested at 10% for the disinfection of walls, ceilings, doors, and floors of the storerooms and at 5% for the different artworks, since in a study carried out in previous years by our research team with natural and chemical biocides, we had obtained good results with this product at these concentrations against environmental fungi. This report was also good for museum administrators because they were able to design a short, medium, and long term strategy with the intention of curbing the imminent deterioration of their collections. This strategy included aspects related to environmental management, constructive improvements of storerooms and the air-conditioning system; also, the possibility of adapting another space for storerooms with an independent air-conditioning system and with natural cross ventilation was an alternative to take into account. This option was designed with the purpose of being used to ventilate the storerooms mainly in disaster situations (hurricanes) where it is essential to keep the Havana city without electricity for several hours or days.

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