

# Viable fungi in the air of indoor environments of the National Archive of the Republic of Cuba

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Received: 13 August 2015 / Accepted: 16 February 2016 / Published online: 9 March 2016  
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**Abstract** The aim of this study was to analyze the viable fungi in the air of indoor environments of the National Archive of the Republic of Cuba (NARC). The samples were taken in fifteen areas (five points for the three floors) at 2 days (rainy and dry seasons) using a biolector SAS in 3 h: 10:00 a.m., 1:00 p.m. and 4:00 p.m. The fungal concentration was indicated as colony forming units per cubic meter of air (cfu/m<sup>3</sup> of air). It was concluded that indoor air of the NARC was not polluted due to the average fungal concentration that was obtained (59 and 327 cfu/m<sup>3</sup> of air in rainy

and dry seasons, respectively). In front of the electric transformers, the fungal concentration was significantly higher than those obtained along the corridors of the lower-ground floor. A trend to the increasing of fungal concentration was observed: when the floors were increased, in dry season was higher than rainy season, where also there was biggest fungal diversity. Among the fungal genera isolated, *Aspergillus* had the highest relative frequency of appearance, and in dry season, it was isolated *Actinobacillus* sp. (Actinomycetes). Six fungal species were isolated in both samplings: *Aspergillus alliaceus*, *Aspergillus niger*, *Cladosporium cladosporioides*, *Fusarium oxysporum*, *Penicillium chrysogenum* and *Mucor racemosus*. All of fungal species isolated have high biodeteriogenic capacity, and the 61 % showed pathogenic attributes.

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**Keywords** Archive building · Fungi · Magnetic field · Microbiological quality of indoor air · Pathogenic fungi

## 1 Introduction

In the outdoor and indoor environments, there are a large number of airborne particles of different origins, shapes and sizes, which are the atmospheric aerosol (Hussein et al. 2008). Depending on the origin, this aerosol may be classified as physical (light, heat, moisture), chemical (organic or inorganic) and

biological, among them are bacteria, spores and fungal propagules, algae, viruses, protozoa and pollen grains. Among biological aerosols, the fungal propagules cause great damages to heritage of historical value because fungi possess high biodeteriogenic capacity of organic material (Borrego and Perdomo 2012). Due to this, some experts in the field of documentary heritage conservation suggest the need of systematic microbiological sampling to guarantee an environmental reference of the risk which is exposed the documents and staff in these institutions (Micali et al. 2003; Pasquarella et al. 2010). Fungal propagules are found in varieties and concentrations depending on geographic location (marine, continental, rural, industrial, urban) (Nowoisky et al. 2012) and climatic conditions (speed, temperature and relative humidity of air), the nature of the substrate (organic or inorganic substrate), season, hours of day, degree of urbanization, the culture medium for the growth, and sampling methods (Rojas and Aira 2012; Rojas et al. 2012).

On the other hand, Hussein et al. (2008) suggest that there are very few studies that allow to relate the behavior of the biological particles with the non-biological and indicate that these last ones can affect the dynamics and transportation of the first ones, like it is the case of the dust (Nowoisky et al. 2012). In that way, the presence of the magnetite in the dust particles was demonstrated (Jordanova et al. 2010; Jelenska et al. 2011; Jordanova et al. 2012). Therefore, it is possible that the microorganisms adhered to the dust can be attracted by a magnetic field such as was demonstrated with the bacteria (Jamieson et al. 2007) or the fungal spores (Shimizu et al. 2007; Kakutani et al. 2012; Takikawa et al. 2014) by an electrostatic field action. That is possible because the fungal spores are always distinguished for their electric charge (Sáenz and Gutiérrez 2003); hence, they can deposit by electrostatic deposition (Levetin 2002).

Inside of the National Archives of the Republic of Cuba (NARC), there are two electrical transformers of 1000 kVA, and in front, they can concentrate microorganisms by attraction of magnetic field generated. However, numerous studies have been made of microbiological air quality inside of repositories that retain documents of heritage value (Borrego et al. 2010; Borrego and Perdomo 2012, 2014; Borrego et al. 2012; Borrego and Molina 2014) but was not made microbiological sampling of indoor air in other locals and zone of NARC.

Therefore, the aim of this study was to analyze the viable fungi in the air of indoor environments of NARC different to repositories where the documents of heritage value are conserved, fundamentally in front to electrical transformers.

## 2 Materials and methods

### 2.1 Characterization of the sampling place

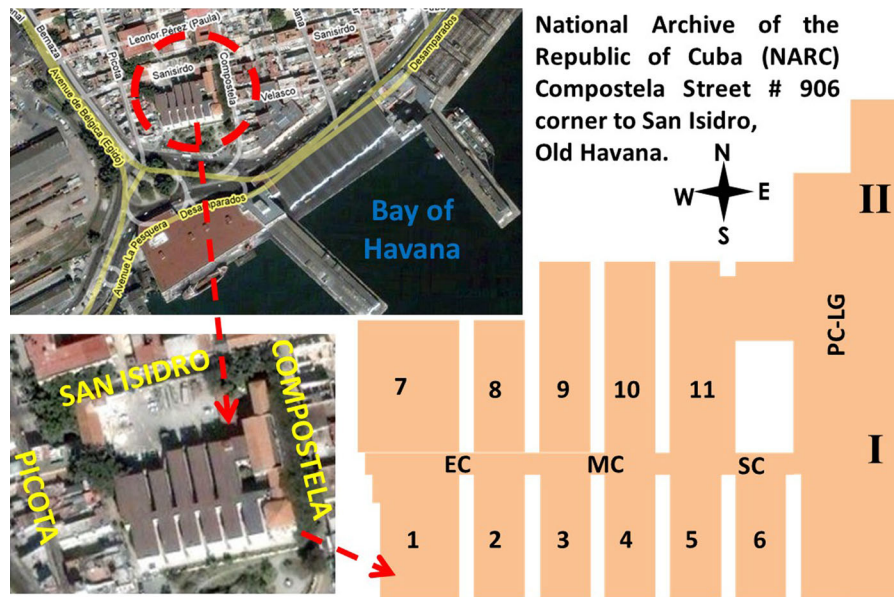
The NARC is located in old urban areas of Havana city known as Old Havana, at 174 m of Havana harbor. Hence, the environment is urban and marine. This archive building has a natural cross-ventilation system, and the most important characteristic of their design is that it was built in form of blocks (Fig. 1).

It has approximately 200 m<sup>2</sup> of surface, and it is a building of three floors: a lower-ground floor with 3 m in height and 1.5 m underground and two floors of 6 m in height, respectively; one of them is located at the street level. It has 30 repositories distributed in the three floors, in which approximately 27 km linear of documents of heritage value are preserved.

### 2.2 Description of methodology of study

The measurements of magnetic field were taken with a gaussmeter (Enertech, EmdexII, USA) of scale 1–3000 and 0.1 mG of precision. The readings were each 1 m of separation, skirting the walls and in the center of the corridors.

The microbiological air sampling was made in July 4th, 2013 (rainy season), and March 21, 2014 (dry season), on the three floors of the NARC (lower-ground floor, first floor and second floor) and in three different hours 10:00 a.m., 1:00 p.m. and 4:00 p.m., according to the similar study previously realized (Zielińska et al. 2008). The outdoor meteorological data of the two sampling days were taken by the Meteorological Station of Casablanca reports, located in front to the NARC in the opposite side of the Bay of Havana (latitude: 23.16, length: –82.35, height above sea level: 50 m). ([www.tutiempo.net/clima/CASA\\_BLANCA/783250.htm](http://www.tutiempo.net/clima/CASA_BLANCA/783250.htm)). The temperature ( $T$ ) and relative humidity (RH) of the sampling days (July 4, 2013, and March 21, 2014) are:  $T$  (°C): 27.7 and 23.4; HR (%): 79 and 71, respectively. It was no raining at both days.



**Fig. 1** Location of the satellite map, aerial view and schematic representation of NARC with the microbiological sampling zones. SC, MC and EC mean start, middle and end of the corridor, respectively. PP-LG is the principal corridor in the lower-ground floor (equivalent to lobby in the first floor and corridor in the second floor). In the lower-ground floor, the numbers 1 and 6 are laboratories and the rest are repositories of

documents; in the other two floors, numbers 1–11 are repositories too. The number one Roman (I) indicates three local: electric transformers (1000 kVA) in the lower-ground floor, room reading on the first floor and room researchers on the second floor. Number two Roman (II) indicates the Office of National Archives System on the first floor and the room computerization on the second floor

Fifteen areas distributed in the three floors were analyzed, and five points in each area were sampling as shown in Fig. 1: start corridor (SC), middle corridor (MC) and end corridor (EC) for the three floors; lower-ground (LG), the principal corridor (PC-LG) and electrical transformers (ET-LG); and first floor (1F) and second floor (2F). In addition, specifically the following locals are: the room of reading (RR) and the Office of the National Archives System (ONAS) in 1F; and room of researchers (RR) and room of computerization (RC) in 2F. All areas were sampled separated at 1 m and was resting 2 h among samplings to avoid overlap of the results. The staff (five or eight persons inside of the offices) and the visitors (never more than ten persons) were seated down and were carried out their daily activities at all time in these local during the samplings, but nobody was walking for the corridors.

The corridors have windows to the outdoor between each block (Fig. 1), but they were closed during the sampling because they are only opened for cleaning times. The doors of repositories which store heritage documents also remained closed to prevent air movement from inside the repositories to the corridors and

vice versa, and so avoid microbial cross-contamination. Only the local (ONAS, RC and RR located in the first and second floor) has air-conditioning, and other areas have room temperature because they are naturally ventilation.

One biocollector (SAS Super ISO 100/180, Italy) was used with air flow 100 L/min for 1 min (Roussel et al. 2012) with Petri dishes of 90 cm of diameter, and Malt Extract Agar supplemented with 7.5 % of sodium chloride was used to isolate fungi (Borrego and Perdomo 2012; Borrego and Molina 2014).

The Petri dishes were incubated at 30 °C for 5 days. After this time, colony count was performed and the necessary calculations per cubic meter of air were made in order to determine the microbial concentration expressed in colony forming units per cubic meter of air ( $\text{cfu}/\text{m}^3$  of air) and the degree of contamination was determined according to Roussel et al. (2012) based on four levels: low (less than  $170 \text{ cfu}/\text{m}^3$ ), moderate (between 170 and  $560 \text{ cfu}/\text{m}^3$ ), high (between 560 and  $1000 \text{ cfu}/\text{m}^3$ ) and very high (greater than  $1000 \text{ cfu}/\text{m}^3$ ).

At the moment of the microbiological determination, the temperature ( $T$ ) and the relative humidity (RH) of environments were measured with a digital thermohygrometer (Hygro-Thermometer DHT-1, China) with scales for  $T$  of 5–50 °C (0.1 °C precision) and for RH of 10–100 % (1 % precision). In the corridors, the temperature is environmental, while the locals are air-conditioned.

The fungal colonies were identified based on morphological and cultural characteristics according to mycological manuals (Barnett and Hunter 2003; Ellis 2006; Pitt 2000). The yeasts were identified according to Hoog et al. (2000) and the bacteria according to Bergey's Manual of Systematic Bacteriology (Holt 1984). From the genera identified, the ecological classification was performed according to the relative frequency of (RF), using the following equation (Esquivel et al. 2003):

$$RF = \frac{\text{Times a genus is detected}}{\text{Total number of sampling realized}} \times 100$$

From RF values to these fungal genera were given the following ecological categories: abundant (81–100 %); common (61–80 %); frequent or moderate (41–60 %); occasional (21–40 %); and rare (0.1–20 %) (Esquivel et al. 2003). An analysis of the fungal diversity of NARC indoor air was made with the predominant fungal strains of each season and their ecological categories.

### 2.2.1 Determination of enzymatic activity

The physiology of the fungal isolates was determined by the following techniques to determine enzymatic activities:

**Cellulolytic activity** The isolates were seeded in the culture medium of the following composition per liter: sodium nitrate, 2 g; dipotassium phosphate, 1 g; magnesium sulfate, 0.5 g; potassium chloride, 0.5 g; ferrous sulfate, 0.01 g; agar, 20 g; and pH 5.5. Strips of filter paper of 4.8 cm of long for 1 cm of wide (equivalent to 50 mg of filter paper) were used as a sole carbon source in one case; in the other cases, crystalline cellulose and carboxymethylcellulose (1 %) were used. Glucose (1 %) was used as a control. The cultures were incubated at 30 °C for 21 days (Borrego and Perdomo 2012; Borrego et al. 2012).

**Proteolytic activity** In this determination, the gelatin hydrolysis assays were realized. Each isolate

was inoculated by puncture inside gelatin medium in a test tube with medium composition was similar to that cellulolytic assay but without agar and 5 g/L of gelatin as sole carbon source. After 7 days of incubation at 30 °C, the tubes were then stored at 4 °C for 1 h and a gelatin hydrolysis reaction was evidenced by medium liquefaction when the tubes were inverted.

**Amylolytic activity** Each strain was streaked on a Petri dish containing a minimal medium identical to the one described above containing starch (5 g/L) as sole carbon source. After 7 days of incubation at 30 °C was added 5 mL of Lugol's reagent over each culture plate and the presence of a colorless zone around the fungal colonies was taken as an indication of the positive hydrolysis.

**Acid production** A spores suspension from each fungal isolate was seeded in a liquid minimal medium of composition identical to the one described above, but glucose at 1 % was the carbon source and the pH was adjusted to 7. Phenol red (0.001 %) was added as an indicator of change color. Cultures were incubated at the same temperature for 3 days before measuring the pH of the culture medium with potentiometer.

**Hemolytic activity** The strains isolated were seeded in Petri dishes with Czapek Dox agar supplemented with defibrinated sheep blood at 5 and 0.5 % of chloramphenicol (BIOCEN, Cuba) (Bogomolova and Kirtsideli 2009). They were incubated at 37 °C for 24–48 h. The pathogenicity was evidenced by the appearance of a halo around the colony indicating hemolysis partial if the halo is green (alpha hemolytic), total if it is colorless (beta hemolytic) and absence of hemolysis if there is no halo (hemolytic gamma) (Llop et al. 2001).

In addition, the index of activity for hemolytic exoenzymes ( $I_h$ ) was calculated according to the general equation (Bogomolova and Kirtsideli 2009).

$$I = 1 - D_c(D_c + D_h)^{-1}$$

where  $I$  is the index of activity of enzyme and  $D_c$  and  $D_h$  are the diameters of the colony and halo of hemolysis (cm), respectively.

### 2.3 Statistical analysis

The data obtained were analyzed using the statistical program Statgraphics Centurion XV. The data normal distribution was analyzed for 95 % confidence interval

( $p \leq 0.05$ ). The linear correlations between de fungal concentration and the meteorological data were applied, and the regression model was found.

An analysis of variance (ANOVA) double classification (sampling location and schedule) with the method of multiple ranges of square minimal differences (LSD) and cluster analysis was performed to find similarity between the locations of sampling using as a similarity measure the Euclidean distance with complete linkage.

### 3 Results

#### 3.1 Variation of temperature and relative humidity

Inside to the locals, there was air-conditioning which maintained similar conditions during this study ( $T = 22.6\text{--}24.2$  °C and  $RH = 52\text{--}55$  %). In relation to the average values of the  $T$  measured every of sampling days, significant statistical differences were not detected ( $p \geq 0.05$ ); however,  $RH$  showed significant statistical differences and high variability among the floors both in dry season and rainy season. The highest values of  $RH$  were obtained as the floor was higher (Fig. 2). However, it was observed that the values of  $T$  of the first floor overcame lightly to the second.

#### 3.2 Fungal contamination

The average of the fungal concentration detected in March ( $327$  cfu/m<sup>3</sup>) went 5.5 times bigger than in July ( $59$  cfu/m<sup>3</sup>) (Fig. 3a, b); nevertheless, the indoor air of the NARC has been classified of low to moderate contaminated. On the other hand, statistically significant differences were not detected when comparing the fungal concentrations detected among the three floors and to different sampling times, On the other hand, statistically significant differences were not detected when comparing the fungal concentrations detected among the three floors and to different sampling times, although it was observed a tendency to increase in concentration with the height from floor level.

The behavior of the physical parameters ( $T$  and  $RH$ ) could affect the microbiological results obtained in each of point sampled. In this respect, it can be

observed that the microbial concentration is higher in the corridors than in the first floor and in the second, and did not happen in the rest of the places.

In the PP-LG, the highest concentrations of fungi were obtained and the values continued to rise during the course of the day (until 301 and 950 cfu/m<sup>3</sup> in rainy and dry seasons, respectively), so it is classified as a contaminated area. This might be due to the fact that on both sides of this corridor there is a bathroom and a dining room, and in one of the ends, there is also a door leading outside and is used in customer public services.

Around and in front of the ET-LG, the magnetic field ranged between 0.3 and 0.6 mT, superior to the rest of the corridor. In front of the ET-LG, the fungal concentrations were significantly bigger (62–498 cfu/m<sup>3</sup>) than the obtained along the corridor of the lower ground (PP-LG, MP-LG and ET-LG: ranged between 13 and 485 cfu/m<sup>3</sup>).

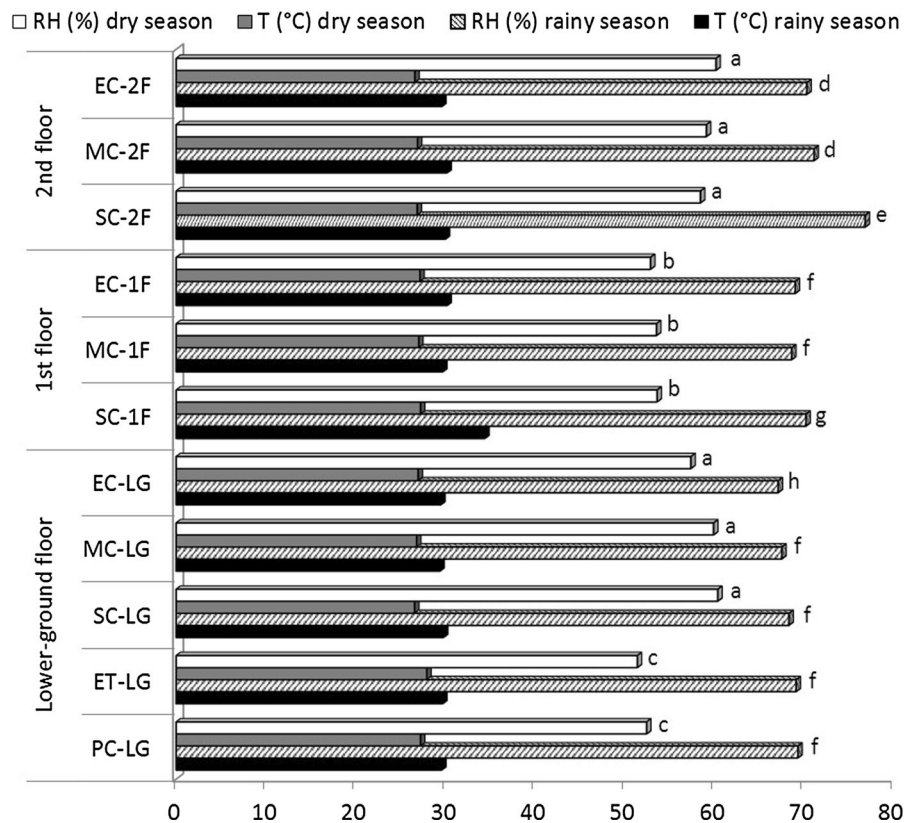
In the acclimatized locals prevailed, the trend in decreasing fungal concentration with the advancing day. A positive linear correlation ( $r_p = 0.3814$ ) was found between this variable and the  $RH$ , and the adjusted model was ( $R^2 = 0.4788$ ):

$$\text{Fungal concentration} = 723 + 3RH - 24T$$

#### 3.3 Fungal diversity

As expected, fungi belonging to the phylum Ascomycota were detected in indoor environments of NARC (Fig. 4). The biggest of fungal diversity was observed in the rainy season (26 strains, with three non-spores) in contrast with the dry season where there were 5.5 times the fungal concentration, but alone 15 strains were isolated (five non-spores and ten strains identified). There were six species that coincided in both samplings, although they varied the percentage of relative frequency of appearance (RF). The following species were highlighted *Cladosporium cladosporioides* (RF of 80 % in both samplings) and *Fusarium oxysporum* (RF of 47 % in rainy season to 60 % in dry season) that maintained their ecological categories (common and frequent, respectively), while the other four changed: rare (*Mucor racemosus* with RF of 7 %), common (*Aspergillus niger* and *Penicillium chrysogenum* with RF of 67 and 80 %, respectively) and occasional (*Aspergillus alliaceus* with RF of

**Fig. 2** Average values of temperature (*T*) and relative humidity (RH) of the NARC indoor corridors (no air-conditioning). *LG* lower-ground floor, *1F* and *2F* first and second floors, respectively, *PC* principle corridor in LG, *ET* electrical transformers in LG; *SC*, *MC* and *EC* (in each floor) start, middle and end of the corridors, respectively. Different letters on bars indicate difference statistically significant ( $p \leq 0.05$ )



40 %) during the rainy season to become abundant species (with RF of 100 %) in dry season.

For other hand, the yeasts classified as rare in the rainy season (only *Candida guilliermondii* with RF of 7 %) and they were not isolated in the dry season; instead, in their place *Exophiala castellani* and *Sporobolomyces roseus* were detected (with RF of 40 and 53 %, respectively). Along with the fungal strains isolated grew bacterial colonies of the *Actinobacillus* genus with RF of 87 % in the dry season, fundamentally isolated in the morning in ONAS and in front of the electric transformers and at noon in the corridors of the lower-ground, causing an increased concentration fungal in these areas.

In the rainy season, the strain *Aspergillus flavipes* showed 87 % of RF indicative of it is abundant specie in the environments studied of NARC. The species *C. cladosporioides* and *P. chrysogenum* were followed at the order of RF with 80 % each one and therefore were ecologically classified as commons, same as *A. niger* and *Paecilomyces variotii* (67 %, respectively), while

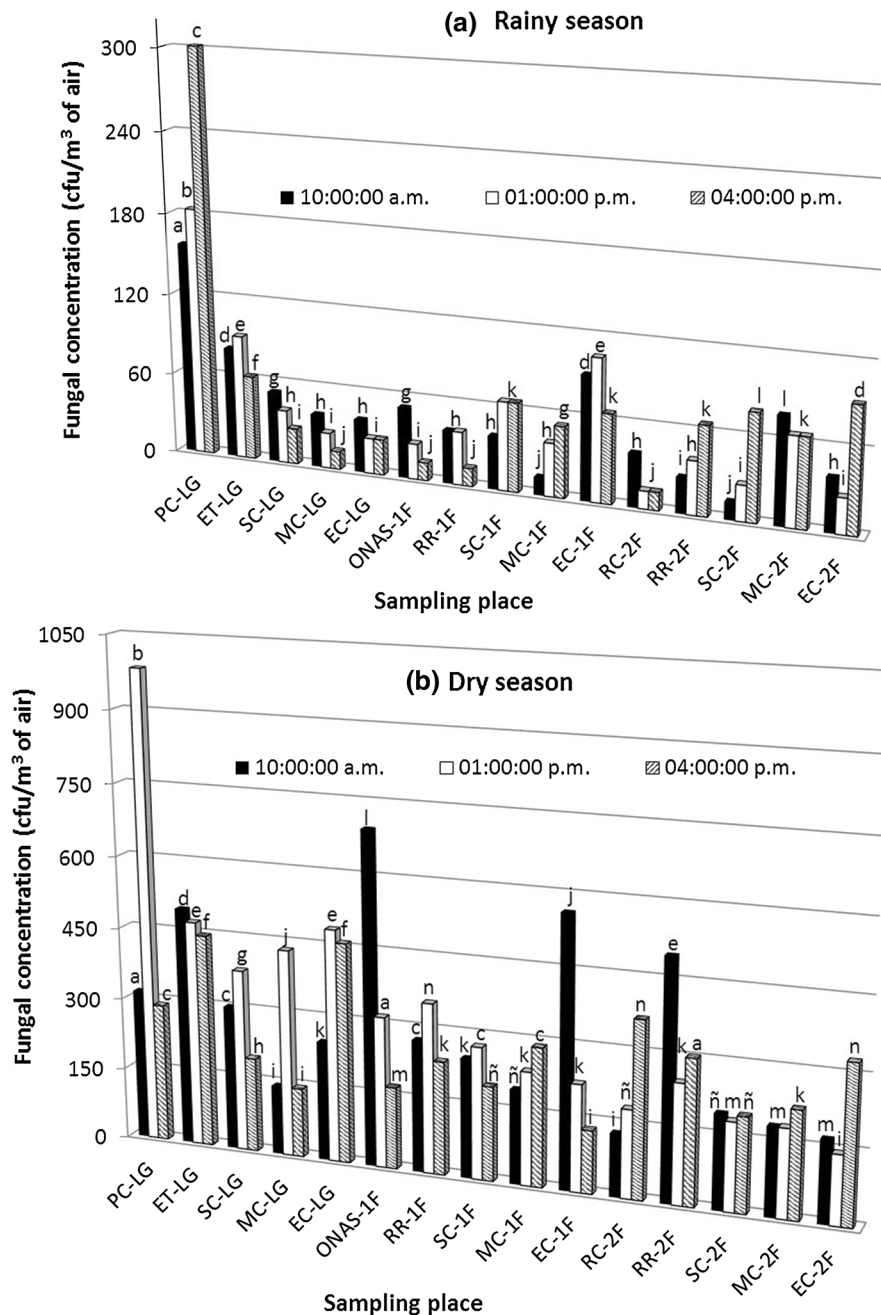
the genera *Fusarium* was frequent (strains with 47 and 53 % of RF).

Figure 5 shows that of the four areas with air-conditioning studied in the NARC in rainy season, RC was that of smaller diversity of the fungi airborne with four fungal strains, while R-Researchers and ONAS had the biggest diversity with eight fungal species. In these locals, the species of *C. cladosporioides* and *Aspergillus candidus* prevailed.

### 3.4 Microbiological quality of air

Figure 6 shows that the microbiological quality of air in corridor shad similar behavior, forming three great clusters: one in the morning (1) in the lower-ground along with SC-1F and EC-2F; another at 1:00 p.m. (2) at identical positions; and the last between 1:00 and 4:00 p.m. (2 and 3, respectively) in the first and second floor. Locals RR-1F, RC-2F and RR-2F up to 1:00 p.m. were located at the same Euclidean distance that the mentioned first cluster, showing that these

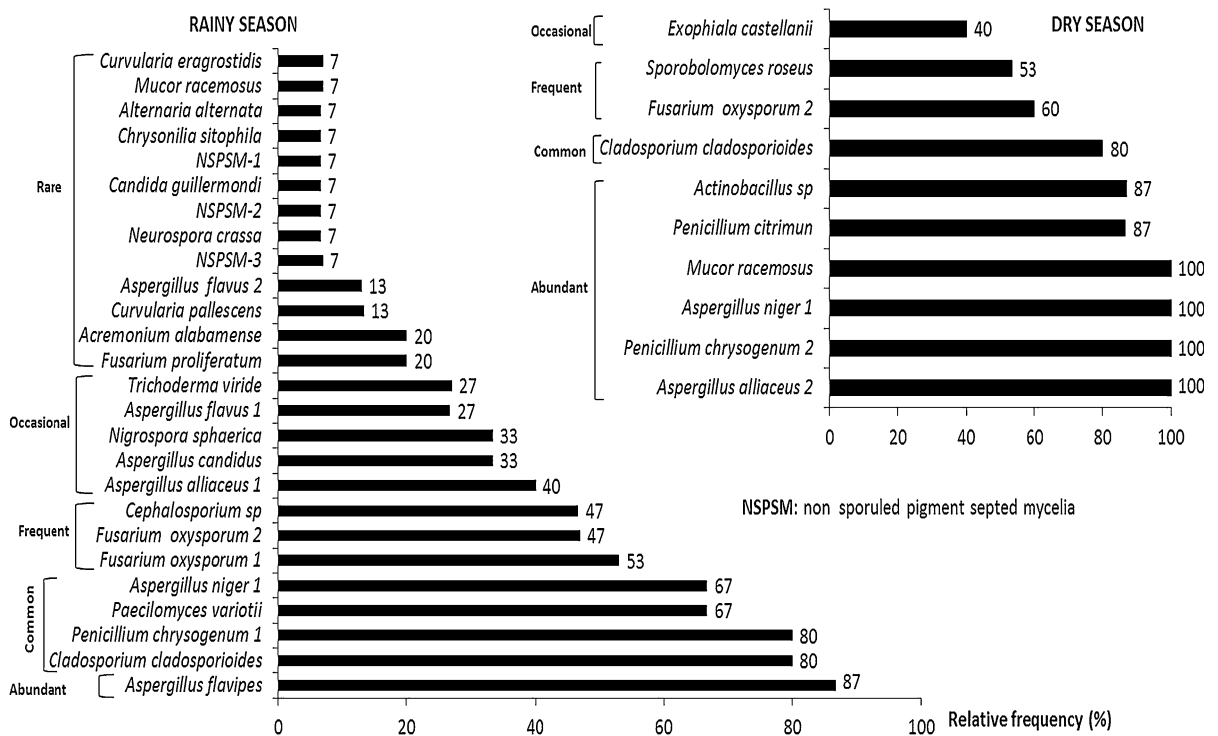
**Fig. 3** Fungal concentrations from indoor air of the various local studied at the NARC in two different months belonging to the dry and rainy seasons. Sampling performed with SAS biocollector (100 L/min for 1 min). *LG* lower-ground floor, *1F* and *2F* first and second floors, respectively, *PC* principle corridor, *ET* electrical transformers, *SC*, *MC* and *EC* (in each floor) start, middle and end of the corridors, respectively, *ONAS* Office of National Archives System, *RC* room of computerization, *RR-1F* room of reading, *RR-2F* room of researchers. Different letters on bars indicate difference statistically significant ( $p \leq 0.05$ )



local shave a microbiological quality of air similar to the corridor of lower ground at the morning.

The RR-1F since morning was localized in mentioned second cluster. That is, that since that time RR-1F was as polluted as the corridors of the lower-ground floor at 1:00 p.m. (greater movement by lunchtime) and the first and second floor at

4:00 p.m. (output of staff). Something similar happened in ONAS because since the morning was already so polluted as the first floor hallway and 1:00 p.m. as the lower-ground hall, and this pollution continued until the time of departure of workers. Then small conglomerates among the three clusters previously mentioned were formed, according to the



**Fig. 4** Ecological impact of fungal species isolated from indoor air of the different areas (offices, corridors, room reading) of the NARC

sampling hours to determine the Euclidian distances among the studied locals.

It should be noted that the fungal concentrations of 10:00 a.m. and 1:00 p.m. in the lower-ground corridor in front of the electrical transformers (ET-LG) were located in the same cluster. Last, something similar happened to the sampling carried out in PC-LG.

### 3.4.1 Enzymatic activity

Table 1 shows the fungal diversity of 26 strains isolated in rainy season in indoor of NARC with their enzymatic activities. It can be observed that the strains showed high biodeteriorative potential, due to the growth on filter paper and crystalline cellulose which evidences a marked cellulolytic activity (88 % of the strains). Contrary, the proteolytic activity was the smaller incidence, but only was detected in the 54 % of the strains isolated.

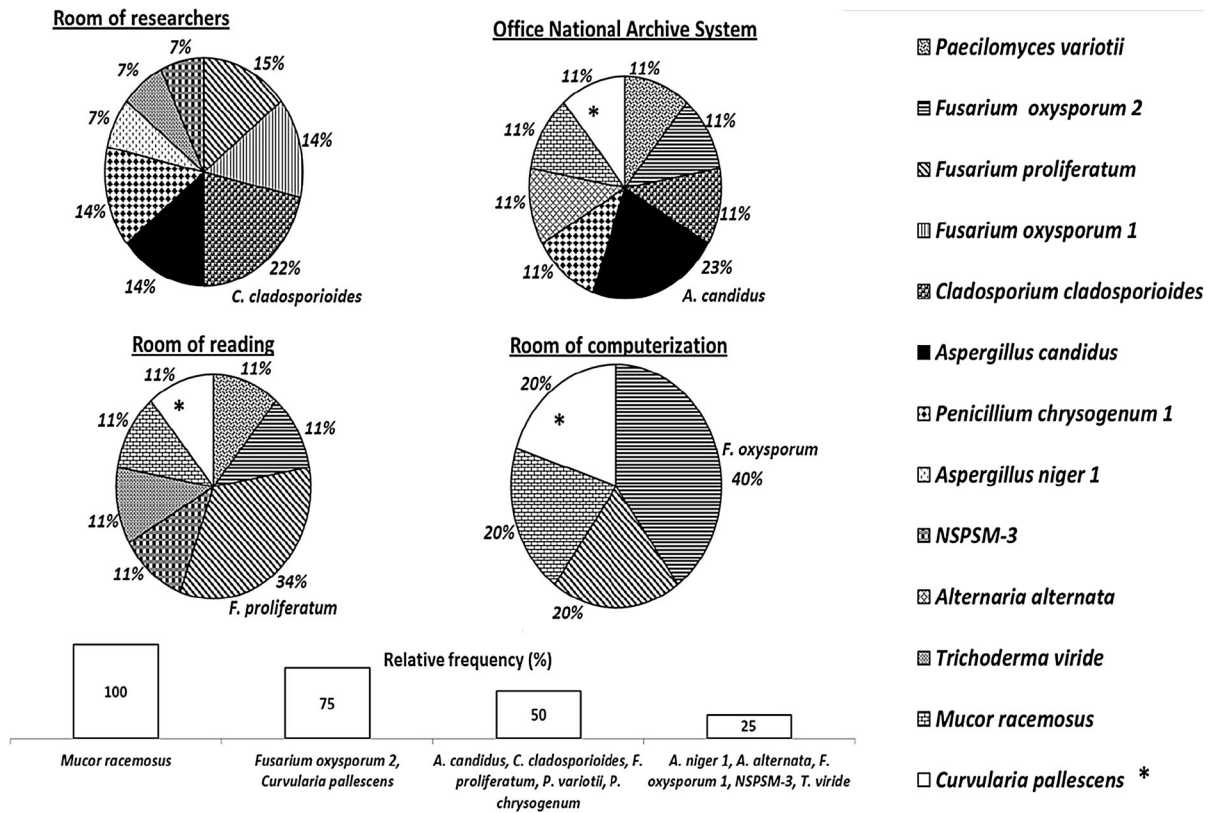
Regarding the amylolytic activity, only four strains did not degrade the starch, while the rest of them could degrade this polysaccharide (81 % of the strains). It was observed that the halo formed around most of the

colonies was greater than the size of the fungal colonies, which is indicative that the most of the species analyzed have high amylolytic activity. In that sense, the specie of *Aspergillus flavipes* formed the greatest halo and also presented proteolytic and cellulolytic activities, so can be said is the specie of largest biodeteriogenic capacity, similar to the specie of *F. oxysporum*. In addition, *A. flavipes* had 0.4 of index of hemolytic activity (Table 1, Fig. 7).

The 61 % of all strains and most of those that were isolated of the acclimatized locals showed positive results to the hemolysis test (Table 1), especially *A. flavipes* and *A. candidus* (Fig. 7a–d).

It should be noted that almost all strains showed capacity to degrade gelatin also they had high hemolytic activity (38 and 23 % of partial and total hemolysis, respectively). In the case of the *Aspergillus*, the isolated species had an ecological behavior that ranged from 13 % (specie rare) to 87 %, indicative of an abundant species. They also showed a partial hemolytic activity index ranged between 0.21 and 0.44, only surpassed by the specie *Curvularia pallescens* with 0.68 of partial hemolysis index and





**Fig. 5** Fungal diversity obtained in rainy season on indoor air of NARC in four areas that have air-conditioned and the relative frequency of the thirteen fungal isolated from them. NSPSM non-sporulated, pigmented and septated mycelia

whose environmental performance was a rare species (RF = 13 %).

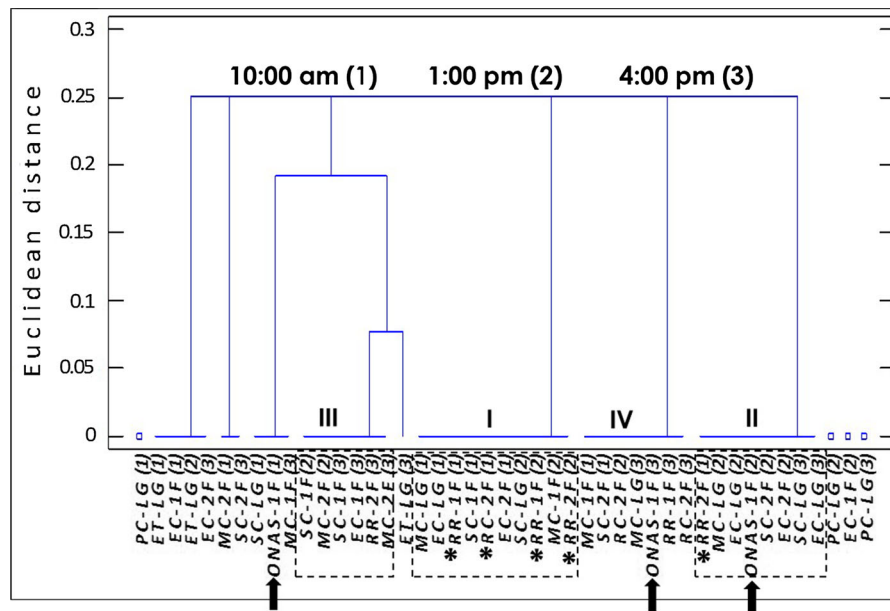
**4 Discussion**

The stability of the temperature and the behavior of the relative humidity can be explained by the construction in blocks of this building that facilitates the natural cross-ventilation of all the corridors. The above mentioned is evidenced when comparing the values of relative humidity in this study in the season of dry (March 21, exactly the day of spring equinox) regarding the rain season (July 4, to few days of the June 21 that it is summer solstice).

The sun carries out a trajectory from east to west but is inclined toward the south in the North Hemisphere, and in Cuba, the sun reaches the biggest height at noon in the summer solstice and the lowest height during the winter (incidence of the solar rays almost of 90° and

45°, respectively) (Sarmiento 2013). Therefore, the stability of the temperature in the studied areas without air-conditioning in the NARC was justified, while the smallest temperature in season of dry it due to the decrease in the incidence of the sun for the south side of this building (Fig. 1), it causes the increase in the relative humidity (Goren 2010) in the same sense of the solar movement. That is to say, there is bigger relative humidity from the start toward the end of the corridor, in the basement and in the second floor (smaller relative humidity in the first floor because the temperature is higher until the noon with the maximum height of the sun). This is reaffirmed when observing the stability of both variables in the principal corridor of the side east of the building (PP-LG and ET-LG).

It should be added that the lower-ground floor has to 1.5 m below the level of the street and the other floors are to the height of the foliage of the vegetation that are in the sides east and south of the building that can



**Fig. 6** Dendrogram allows grouping the results according to the concentration of fungi in the 90 indoor air samples made in the NARC in three stages: 10:00 (1); 13:00 (2) and 16:00 (3) and applying the Euclidean distance by the nearest neighbor method. The three clusters most significant in terms of homogeneous behavior obtained in the building air quality are identified using

the Roman numerals I, II and III displayed on figure as rectangles of dotted lines. Asterisks and arrows indicate RR-1F, RR-2F and ONAS (locals with air-conditioning) within the cluster indicating similarity in air quality between these locals and the corridors

influence in the temperature of the first floor that is also located next to the streets and the biggest movement of people in that reception area and toward the room reading (RR-1F).

According to the classification proposed by Roussel et al. (2012), the indoor environment of NARC can be considered moderate contaminated by fungi. This may be due to the constructive characteristics of the building and its natural cross-ventilation system. Similar values were obtained in library environments and repositories in Poland, where the air was classified as slightly polluted to moderately polluted (Zielińska et al. 2008). Despite that Poland possess the climate different from the Cuba, the results can be compared because they have both marine and urban environments; therefore, the similarity of the values obtained is indicative of the good sanitary conditions in indoor environments of the NARC.

The values of the physical parameters ( $T$  and RH) analyzed in this study were not statistically significant. It was noted that as the floor is higher, the highest values of RH were obtained (Fig. 2). This result is logical because the basement is located to 1.5 m below

street level, and the other floors are at the height of the foliage of vegetation. However, it was observed that the  $T$  values of the first floor slightly exceed those of the second, which could be due to its location close to the streets and the increased movement of people in the reception area and to the reading room.

The influence of the RH above 65 % in the sedimentation of the fungal propagules is known (Goren 2010; Cappitelli and Sorlini 2010). Therefore, the behavior of this variable could influence in the microbiological result obtained in each sampling point (Fig. 3). The positive linear correlation founded between these factors indicates that the microbial contamination increases lightly when RH increases also, and this result is agree with the other authors (Zielińska et al. 2008; Raisi et al. 2010). The adjusted model almost allows predicting 50 % of the behavior of microbiological air quality. The about 50 % non-explained difference by model can be due to that some locals are acclimatized and others have natural ventilation. In that sense, the microbial load was higher in the corridors of the first floor than in the second floor and did not happen so in the locals with

**Table 1** Enzymatic activity of the fungal genera isolated from indoor air of the various local studied at the National Archives of the Republic of Cuba

Specie/mycelia	Cellulolytic activity				Filter paper	Hemolytic activity				
	Glucose (control)	Carboxymethyl cellulose	Crystalline cellulose	Glucose (control)		pH	$D_c$ (cm)	$D_h$ (cm)	$I_h$	Hemolysis type
<i>Acremonium atabamense</i>	+++	+++	++	-	-	6.4	2.1	-	-	$\gamma$
<i>Alternaria alternata</i>	+++	+	-	++	++	5.2	1.5	0.5	0.25	$\alpha$
<i>Aspergillus alliaceus</i>	++	++	+	++	+++	5.4	6.3	2.7	0.30	$\alpha$
<i>Aspergillus candidus</i>	+++	++	+	++	++	4.6	5.5	3.5	0.39	$\beta$
<i>Aspergillus flavipes</i>	++++	++++	+	+++	+++	4.8	5.3	3.5	0.40	$\beta$
<i>Aspergillus flavus 1</i>	+++	+	+	±	±	4.5	6.4	2.3	0.26	$\alpha$
<i>Aspergillus flavus 2</i>	++	++	+++	+++	+++	4.8	5.0	4.0	0.44	$\beta$
<i>Aspergillus niger</i>	++	+++	+++	++	++	4.7	6.7	1.8	0.21	$\alpha$
<i>Candida guilliermondii</i>	++	++	-	-	-	4.9	1.8	1.1	0.38	$\beta$
<i>Cephalosporium sp.</i>	+++	+++	+	-	±	5.5	3.5	2.3	0.39	$\alpha$
<i>Chrysonilia sitophila</i>	+++	++	++	±	++	5.9	4.8	-	-	$\gamma$
<i>Cladosporium cladosporioides</i>	+	++	++	±	±	3.9	4.0	-	-	$\gamma$
<i>Curvularia eragrostidis</i>	++	++	++	±	±	5.8	2.8	-	-	$\gamma$
<i>Curvularia pallescens</i>	++	+++	++	+++	++	5.3	1.5	3.2	0.68	$\alpha$
<i>Fusariumoxysporum 1</i>	+++	++++	+	+	+++	4.2	3.0	1.7	0.36	$\beta$
<i>Fusariumoxysporum 2</i>	++	++	+++	+	++	4.9	3.8	2.1	0.36	$\alpha$
<i>Fusarium proliferatum</i>	+++	++	++	±	±	5.3	4.4	-	-	$\gamma$
<i>Mucor racemosus</i>	+	+	+	++	++	5.0	7.0	-	-	$\gamma$
<i>Neurospora crassa</i>	+++	++	++	+++	++	5.2	7.5	-	-	$\gamma$
<i>Nigrospora sphaerica</i>	++	+++	++	+	+	4.9	7.5	1.0	0.12	$\alpha$
<i>Paecilomyces variotii</i>	+	+	+	+	+	4.3	5.0	2.0	0.29	$\beta$
<i>Penicillium chrysogenum</i>	+++	++	++	+	+	5.1	3.0	1.0	0.25	$\alpha$
<i>Trichoderma viride</i>	+++	++	+	++	++	5.8	2.1	2.8	0.26	$\alpha$
NSPSM-1	+++	+	++	++	++	6.3	2.4	-	-	$\gamma$
NSPSM-2	++	++	++	++	++	6.0	5.3	-	-	$\gamma$

**Table 1** continued

Specie/mycelia	Cellulolytic activity			Filter paper	Amylolytic activity	Proteolytic activity	Hemolytic activity				
	Glucose (control)	Carboxymethyl cellulose	Crystalline cellulose				pH	$D_c$ (cm)	$D_h$ (cm)	$I_h$	Hemolysis type
NSPSM-3	+++	+	+++	+	-	-	5.8	4.4	-	-	$\gamma$

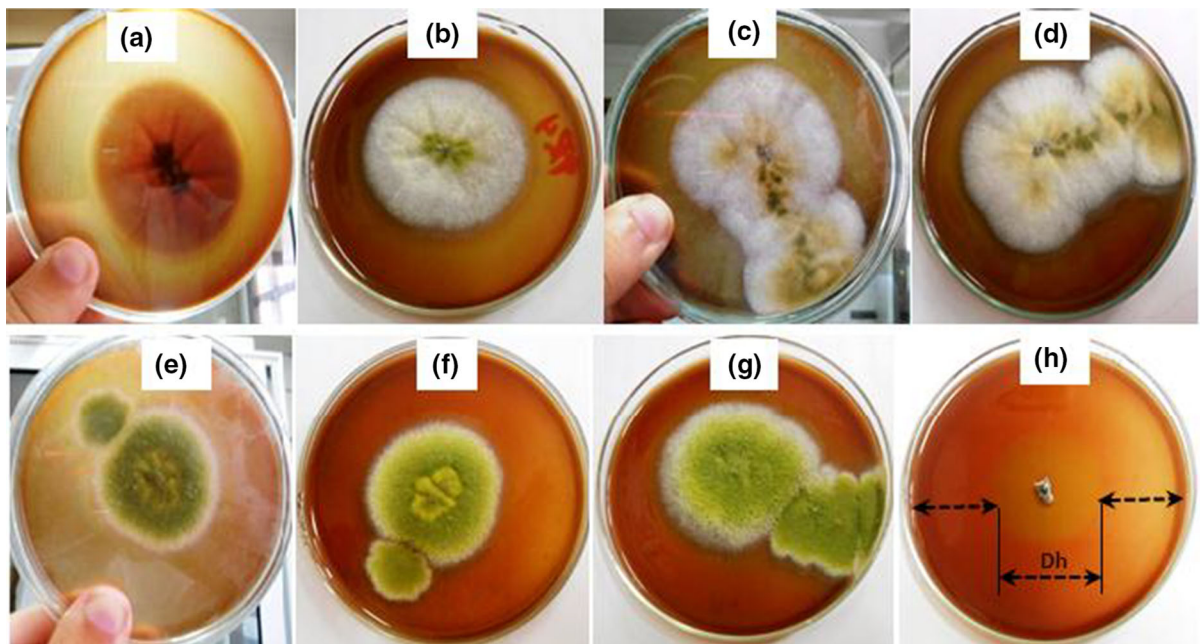
+++ Indicates very abundant growth (100 % of the saline culture medium slant and on the paper), +++ indicates abundant growth (75 % of the slant), ++ indicates moderate growth (70–50 % of the slant), + indicates poor growth (<50 % of the slant), ± indicates very poor growth, – indicates not growth or not hemolysis halo or not  $I_h$ ,  $\alpha$  the hemolysis halo is green and is indicative of the hemolysis partial,  $\beta$  halo completely colorless and is indicative of hemolysis total,  $\gamma$  there is no halo and is indicative of absence of hemolysis, NSPSM non-sporulated, pigmented and septated mycelia

the air-conditioning. This may be due to two reasons: the first is related to the movement of people during their daily activities which favored the movement of airborne suspended particles and facilitated their transportation from the interior of the premises to the corridors, and the second is linked to the fact that the spores are dried when they are captured by the biocollector with high airflows (Lee et al. 2004).

The decrease in fungal concentration with the progress of the day in the acclimatized locals is a result contrary to that observed in a similar study, in which the samples were taken from 7:00 a.m. to 1:00 p.m. and the increase in the contamination was observed with the advance of the day, although the maximum peak was detected at 11:00 a.m. (Zielinska et al. 2008). This could be explained by a possible orientation of the fungal propagules toward this area by the effect of the magnetic field that generate this device, consequently with the similar effect of electrostatic attraction of conidia informed by several authors (Shimizu et al. 2007; Kakutani et al. 2012; Takikawa et al. 2014; Anaya et al. 2015) and ratified since by the results in the principal corridor of the lower ground because in this area was detected the biggest fungal concentration during the all study.

It is important to analyze the similarity of the air quality of some local with air-conditioning regarding the corridors (Figs. 4–6). This fact is justified for RR-1F to be a local of a lot of public access, but it does not for RC-1F that is an air-conditioning area located in the last floor, which indicates that its air-conditioning system requires of cleaning. In the case of RR-2F can be due to that in the back of the local there is a library that possesses books which have dust in their surface as well as to the dirt that possesses the air-conditioning equipment, but as the ONAS is also another air-conditioning local, the climate system seems to be favoring the contamination of the local.

Therefore, it can be inferred that the air-conditioning of locals of the NARC (Fig. 5) possess high risk for the staff's health that works in the same ones due to the inadequate cleaning and maintenance of the air-conditioning devices, in accordance with the positions of several authors related to the sick building syndrome (Micali et al. 2003). On the other hand, it can be concluded that the fungal concentration in the halls has similar behavior throughout the day, which is indicative of the proper design of natural cross-ventilation in the building of NARC.



**Fig. 7** Hemolytic test in Czapek Dox agar supplemented with defibrinated sheep blood (5 %) and chloramphenicol (0.5 %) and then incubated at 37 °C for 5 days.  $\beta$ -hemolysis (total): *Aspergillus flavipes* (a, b); *Aspergillus candidus* (c, d); *A. flavus*

2 (e, f).  $\alpha$ -hemolysis (partial): *A. flavus* 1 (g).  $\beta$ -hemolysis (total): *Curvularia pallescens* (h) with high rate of  $\beta$ -hemolytic activity [ $I_h = 0.68, 1.5$  and 3.2 cm diameter of colony ( $D_c$ ) and halo ( $D_h$ ), respectively]

The presence of species of the Ascomycota phylum is predominant in marine and coastal atmospheres (Nowoisky et al. 2012) similar to the area where the NARC is located. However, it is relevant to the presence of the bacterial genus *Actinobacillus* sp. in both samplings because the used cultural medium (Malt Extract Agar with 7.5 % of sodium chloride) has the appropriate conditions for the growth of the fungi and yeasts. Therefore, it can be inferred that this bacterial species is resistant to high salt in the medium and high air flow in the SAS biocollector employed (100 L during 1 min) without causing any drying of cell. As for the low presence of yeast, Lee et al. (2004) stated that several models of SAS samplers have smaller capacity to capture this kind of microorganism than other samplers (Andersen-N6, AOC, RCS) without damaging their viability.

The ecological dominance of the genera *Aspergillus*, *Cladosporium* and *Penicillium* is informed by other authors (Rojas et al. 2008; Borrego and Perdomo 2012), since they are considered primary colonizers. The fact that *C. cladosporioides* has maintained a RF of 80 % in both surveys (with RH average between 56 and 70 %) shows that spores were captured easily by

the biocollector due to their increased size compared to the spores belonging to the genera *Aspergillus* and *Penicillium* (Rojas et al. 2008).

Both the species *Aspergillus candidus* (Fig. 7c, d) as *Curvularia pallescens* (Fig. 7h) showed complete hemolysis, and both were also isolated from two locals acclimatized (RR-1F and RC-2F). The specie *C. pallescens* showed a 75 % of RF in air-conditioned premises, which shows that it is ecologically a common species to those premises. However, in the ONAS (local with air-conditioning) only three hemolytic strains were isolated (*P. variotii*, *Aspergillus candidus* and *Alternaria alternata*). Therefore, it can be concluded that the largest genus in these locals (RR-1F and RC-2F) was *Aspergillus*, and ONAS and RR-2F are the air-conditioning locals of the most risk for human health.

The results of this study show the following: (i) it is important to conduct systematic microbiological sampling of air indoor of locals, identify the species isolated and make the appropriate tests of pathogenicity because the concentrations of fungal propagules in indoor air of locals may be low, but the pathogenicity of the isolated species can be high; (ii) it should take

into account the design of ventilation systems in the structural design of the archives buildings, because a natural cross-ventilation system can ensure the preservation of documentary heritage, since the building acts as a primary barrier that minimizes the risk to the health of staff and the users who visit these institutions. In this regard, it has been shown that in the microbiological studies indoor air only obtain low concentrations of fungi (cfu/m<sup>3</sup>) and low relative frequencies of appearance (%) of the strains are not the only criteria for determining the microbiological quality of indoor air. Therefore, some pathogenicity tests (hemolytic activity) to emit a more accurate approach are needed, since a local can have high presence of pathogenic strains with a low contamination category and (iii) it can happen that electromagnetic contamination does not exist, but the magnetic field can localize the microbiological concentration in the area around the power generator and turn it into an area of risk to health.

**Acknowledgments** The authors acknowledge the financial support received from the Assistance Program for Archives of Latin America, ADAI, from Spain (Project 064/2012).

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