

Aerobiological investigations inside repositories of the National Archive of the Republic of Cuba

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Abstract The objectives of this study were to evaluate the microbial prevalence inside six repositories of the National Archive of the Republic of Cuba in 2 months of the year and to examine some of the physiological features of fungi isolated in order to evaluate their potential for biodeterioration. The microbiological sampling was conducted in February and September using a slit impactor as air sampler. Appropriate selective culture media were used to isolate fungi and bacteria. Temperature and relative humidity were measured during the samplings. The cellulolytic activity and the production of acids and pigments of the fungi isolated were qualitatively determined. Total viable microbiota and bacteria concentrations were greater in February while the fungal concentration was higher in September. *Aspergillus*, *Cladosporium*, *Penicillium*, *Curvularia* and *Alternaria* were the predominant fungal genera in February while *Cladosporium* prevailed in September, although *Fusarium*, *Mucor* and *Neurospora* genera were also isolated in this month. The fungi isolated were capable of degrading cellulose and excreting pigments and acids. The Gram-positive bacteria group prevailed in the air and *Corynebacterium*, *Streptomyces*, *Bacillus*, *Streptococcus*, *Staphylococcus*,

Enterobacter and *Serratia* were some of the genera identified.

Keywords Archive · Bacteria · Biodeterioration · Fungi · Indoor aerobiology · Indoor environment

1 Introduction

Archives and libraries are keepers of expressions of human thought, from the most ancient documents written on papyrus, parchment, paper, etc. to the new photographic and electronic supports. These materials of plant, animal or synthetic origin are subject to deterioration by physical agents (light, heat, moisture), chemical agents (atmospheric pollution), and biological ones (bacteria, fungi and insects) (Maggi et al. 2000). It is known that microorganisms are potent agents of deterioration and they can be carried by airborne particles (Florian 2004) which can eventually settle in the dust.

Dust particle components vary as to quantity and quality depending on the location of the building, the activities taking place inside, season of the year and the conservation condition of books, documents and objects (Maggi et al. 2000). Dust can represent a source of nutrients for some insects and fungi and it creates a microenvironment on the surfaces of collections, preventing normal air flow over them which

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facilitate the absorption of significant humidity by surface areas, thus leading to plague proliferation (Florian 2004). Likewise, microorganisms can be transported by dust particles towards repositories through ventilation systems and visitors too (Nevalainen and Morawaska 2009).

Under appropriate temperature and humidity values, air microbiota may coexist with the documentary collections and humans in a specific ecosystem without causing noticeable damages. However, an increase of these thermohygrometric variables, facilitates the growth of microorganisms that could cause negative effects not only on collections, due to accelerated biodeterioration (Pinzari et al. 2006) but also on the health of humans (Toivola et al. 2002). Hence the great interest of specialists regarding the knowledge of the levels of microorganisms in the air of repositories, namely in tropical climate countries whose climate conditions favor the increase of dust and the concentration of fungal spores in the air, as well as their sedimentation on the substrates, facilitating their development (Aira et al. 2002; Borrego et al. 2008, 2010a, b; Guimet et al. 2011).

Taking into account these aspects, the objectives of this study was to evaluate the microbial prevalence inside six repositories of the National Archive of the Republic of Cuba (NARC) in 2 months of the year and to examine some of the physiological features of fungi isolated in order to evaluate their potential for biodeterioration.

2 Materials and methods

2.1 Description of the repositories of the archive

The NARC is located in old urban areas of Havana City known as Old Havana, near Havana harbour. The construction of the archive was finished in 1944 and an appropriate design of building for the tropics according to the epoch was used, because it has a natural passive ventilation system that guarantees the preservation of documents. The repositories have holes that cross over the outer walls and they are protected by metallic meshes. These holes were made at different heights (1.5 and 4.5 m, approximately) to facilitate the inflow of outside air into the repositories and to secure a good ventilation. The identification numbers of the

repositories are: 3 and 8 (premises of the lower-ground floor), 13 and 18 (premises of the first floor), 24 and 28 (premises of the second floor). The repositories are characterized by their large size, and their dimensions (length, width and height) are the following: repository 3: $15.2 \times 6.2 \times 2.5$ m, repositories 13 and 24: $15.2 \times 6.2 \times 5$ m, repository 8: $25.3 \times 6.2 \times 2.5$ m, repositories 18 and 28: $25.3 \times 6.2 \times 5$ m. The study of microbes in the air and the thermohygrometric conditions were conducted in six repositories randomly selected and distributed across the three floors of the building and on both sides of it (north and south).

2.2 Thermohygrometric sampling

Temperature and relative humidity are daily measured in all the repositories of the NARC since many years ago. However, at the moment of the microbiological determination, a digital thermohygrometer (Model 8705, Bresciani, Italy) was used to measure the temperature (T) and relative humidity (RH) inside these repositories at each point of sampling.

2.3 Microbiological sampling of air

Sampling was done in two different months of the year 2007, in February (winter month or dry season) and in September (month of summer or rainy season), using the Chirana Aeroscope (an air sampler by impaction with slot II and 30 l min^{-1}) placed at a height of 1.5 m. On February 20 and September 18 five points per repository were analyzed by triplicate at the same day using a diagonal method according to FEDECAI-01 (2007) for a total of 60 samples tested in the year (Fig. 1).

Petri dishes with malt agar (Merck) supplemented with NaCl (7.5%) (Rojas et al. 2002) were used for isolating fungi and nutrient agar for bacteria. The malt agar plates were incubated for 7 days at 28°C and the nutrient agar plates were incubated at 32°C for 72 h. After this time, colony count was performed and the necessary calculations per m^3 of air were made in order to determine the microbial concentration expressed in colony forming units (CFU m^{-3}).

Relative microbial density was conducted according to Smith (1980) where:

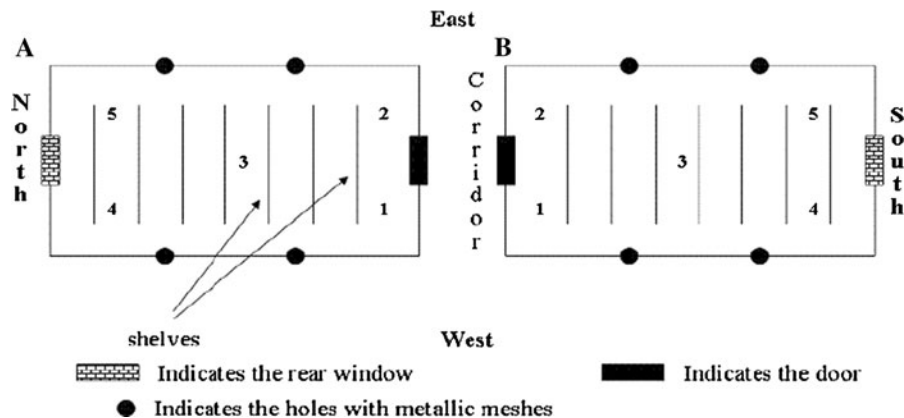


Fig. 1 Microbiological sampling points in the repositories according to FEDECAI-01 (2007). Also, the T and RH were tested. In the position **A** or north side is the repositories numbers 3, 13, 24 and in the position **B** or south side is the numbers 8, 18, 28

Relative density

$$= \frac{\text{Number of colonies of the genus or species}}{\text{Total number of colonies of all genera or species}} \times 100$$

2.4 Identification of microbial isolates

In the case of fungal colonies, their cultural and morphological characteristics were observed and the identification was performed according to Barnett and Hunter (1987), Ellis (1971, 1976), Klich and Pitt (1994), as well as to website published information (Pitt 2000; *Aspergillus* Image Bank 2006). Bacteria were grouped on the basis of their Gram staining: the biochemical tests described in Bergey's Manual of Systematic Bacteriology (Holt 1984, 1986) were used for the identification of bacteria.

2.5 Qualitative determination of the cellulolytic capacity and the production of pigments by fungi

The fungi isolated were seeded in slants with a saline culture medium of the following composition per liter: sodium nitrate, 2 g; dipotassium phosphate, 1 g; magnesium sulphate, 0.5 g; potassium chloride, 0.5 g; ferrous sulphate, 0.01 g; agar 20 g; and pH 5.5. In one case, a strip of filter paper (4.8 cm of long for 1 cm of wide, equivalent to 50 mg of filter paper) was used as a sole carbon source, and in the other, crystalline cellulose (1%) was used. The cultures were incubated at 28°C during 21 days (Borrego et al. 2010a, b).

2.6 Determination of the production of acid by fungi

A suspension of spores from each fungus isolated was seeded in a minimal liquid medium of identical composition to the one above, but with glucose at 1% as carbon source and the pH was adjusted to 7. Cultures were incubated at 28°C for 3 days before measuring the pH of the culture medium.

2.7 Statistical analysis

The Student test was used to evaluate differences in the fungal, bacterial and microbial concentration of the air in 2 months studied as well as the relative density of *Aspergillus* genus between winter and summer in each repository. The ANOVA-1 and Duncan tests were used to compare the concentration of fungi, bacteria and total microorganisms among floors of the building and to compare the relative distribution of the species of *Aspergillus*, *Cladosporium* and *Penicillium* identified in each repository. Results with $P \leq 0.05$ were considered statistically significant.

3 Results

The trends of the microclimate of the repositories examined are described in Figs. 2 and 3 and show that the thermohygrometric values during the months from January to April were lower than those in other months of the year. This stage coincides with the dry season

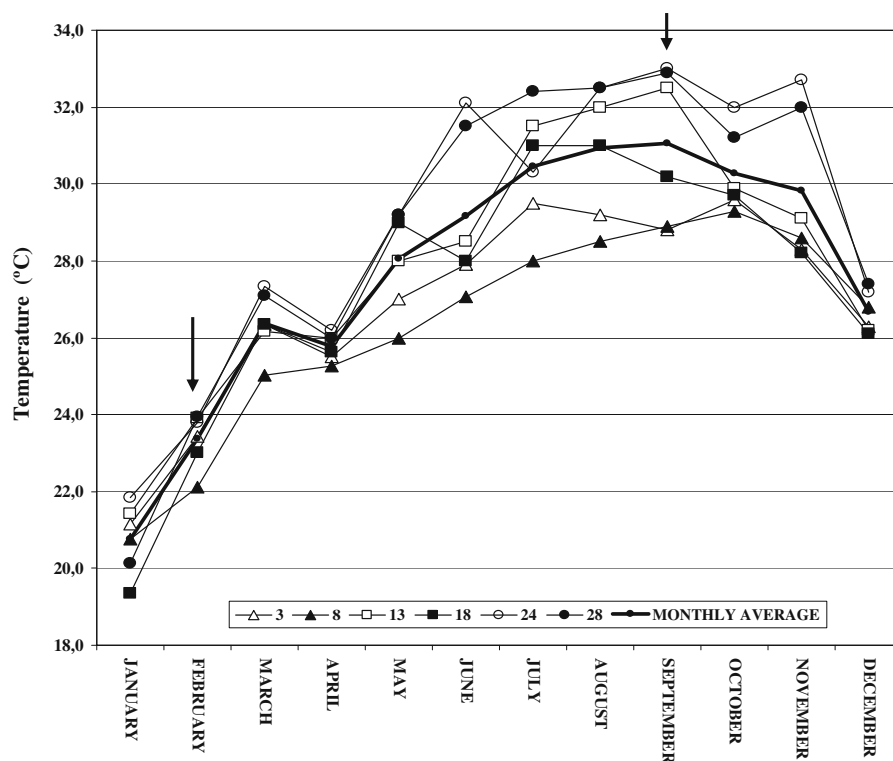


Fig. 2 Monthly average of T registered in 1 year (2007) for each repository analyzed of the National Archive of the Republic of Cuba. The arrows show the months studied. T_{median} in February = 23.4°C and T_{median} in September = 31.1°C

and it is considered acceptable for a country of tropical climate. However, from May until the end of November is the season of hot weather, heavy rain and hurricanes during which the T and RH are significantly increased. Table 1 shows the average values of thermohygrometric variables of all repositories studied during winter and summer months and the average values of these variables according to the position of them in the building (north or south side).

The first microbiological sampling was performed in February (winter month) and at that time, the mean values of T and RH for all repositories were 23.4°C and 68%, respectively. The second analysis was made in September (month of intense heat) and once more, the average values of T and the humidity values were very high ($T_{\text{median}} = 31.1^{\circ}\text{C}$, $\text{RH}_{\text{median}} = 76.6\%$) (Figs. 2, 3). The data concerning total concentrations of fungi and bacteria obtained show that in February the concentration of fungi was 320.8 CFU m^{-3} while that of bacteria was $1,495.8 \text{ CFU m}^{-3}$. In September, the concentration of fungi (649.3 CFU m^{-3}) was significantly higher than that detected in February

while the bacteria were significantly lower in number ($1,038.9 \text{ CFU m}^{-3}$), hence the total microbial concentration obtained in September does not differ significantly from that obtained in February (Table 2). In Table 3 is shown the concentration of fungi, bacteria and total microorganisms per repository located in different sides of the building during the 2 months studied and it can be noticed that the highest concentrations of fungi and bacteria were observed in the repositories on the north side of the building. It is also noted that regardless of the location of the deposits in the building, the fungal concentration was significantly higher in September in all repositories, while the bacterial concentrations were significantly higher in February in all repositories.

In Table 4 it is noted that regardless the month, the concentration of fungi, bacteria and microorganisms obtained per floor differ significantly and the trend is increasing according to the height with the exception of fungal concentrations obtained in the basement, which were significantly higher (314 or 800 CFU m^{-3}) than those of the first floor (180 or 450 CFU m^{-3}) although,

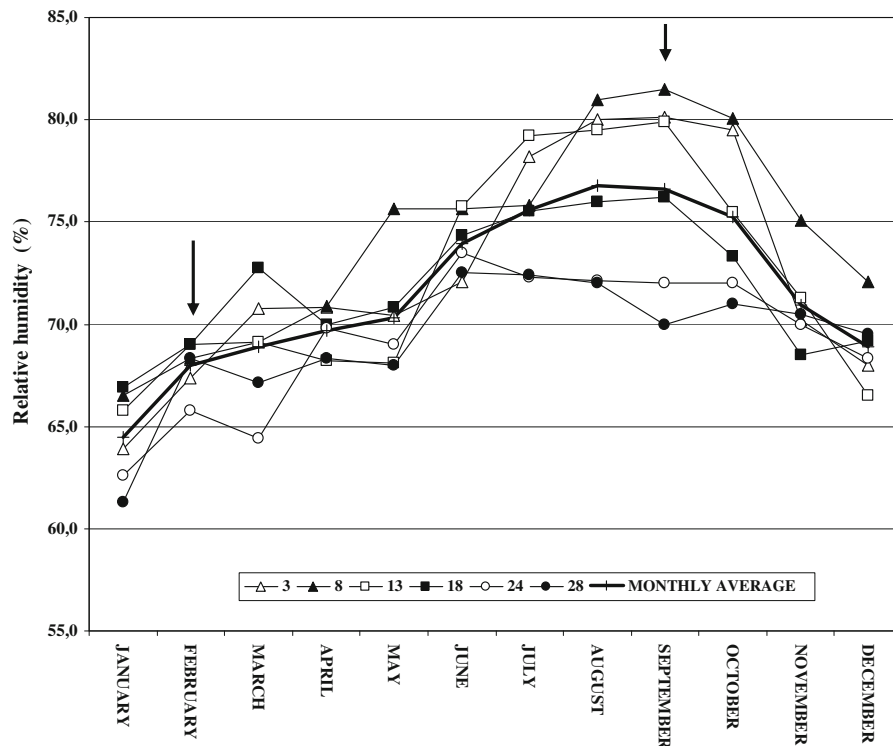


Fig. 3 Monthly average of RH registered in 1 year (2007) for each repository analyzed of the National Archive of the Republic of Cuba. The arrows show the months studied. RH_{median} in February = 68% and RH_{median} in September = 76.6%

Table 1 Microclimatic conditions of the repositories during the period studied

Thermic and hygrometric variables in the repositories	Winter		Summer	
	Lower values	Upper values	Lower values	Upper values
Average value of T for all repositories	19.3	27.3	28.2	30.9
Average value of RH for all repositories	61.3	68.9	68.0	81.5
Average value of T of repositories of north side	21.2	27.3	28.3	31.3
Average value of RH of repositories of north side	62.6	70.8	66.5	80.1
Average value of T of repositories of south side	19.3	27.1	28.8	31.1
Average value of RH of repositories of south side	61.3	72.7	68.0	81.5

the humidity is not much higher than the values obtained in the other floors, probably due to the low ventilation. In order to compare fungi and bacteria regarding the 2 months it appears that the concentrations of fungi were significantly lower in February, while those of bacteria were higher.

In this study a total of 300 colonies of fungi and 2,200 colonies of bacteria were isolated. Altogether, 8 genera of fungi were isolated and identified in the indoor air all the environments, but the relative density of these genera differed between 2 months studied. In

February, regardless the side of the building analyzed, *Aspergillus*, *Cladosporium* and *Penicillium* predominated over other genera (Table 5). Particularly, in February the concentration of *Aspergillus* species ranged from 67.03 to 97.5 CFU m⁻³ (Fig. 4). In September, *Cladosporium* was not only the predominant genus but it also prevailed in the repositories located in the north side of the building and it was followed by *Aspergillus* while *Penicillium* was only isolated in the repository 28 and *Curvularia* in the repository 13; also *Alternaria* was only detected in the

Table 2 Median values of air microbial concentration, T and RH at the repositories during February (drought month) and September (rainy month)

	February			September		
	Conc. (CFU m ⁻³)	T _{median} (°C)	RH _{median} (%)	Conc. (CFU m ⁻³)	T _{median} (°C)	RH _{median} (%)
Fungi	320.8 ^c	23.4 ^b	68.9 ^b	649.3 ^c	31.1 ^b	72.3 ^b
Bacteria	1,495.8 ^d			1,038.9 ^d		
Microorganisms total ^a	1,816.6			1,688.2		

^a Indicates the sum of the median concentration of fungi and bacteria of all repositories

^b Represents the median of all the measurements performed in the repositories (60 measurements)

^{c, d} Indicates significant differences according to the Student test ($P \leq 0.05$) on comparing the fungi and bacteria concentration between February and September. The microbial determination was made in 5 point by triplicate of six repositories and the data averaged ($n = 60$)

repositories of the north side of the building and while *Mucor* and *Neurospora* genera were isolated in all the repositories only in this month and they were among the most abundant ones in the repositories 8, 18 and 28 (Table 5).

The relative density of *Aspergillus*, *Cladosporium* and *Penicillium* species at the repositories during February is shown in Table 6. *A. niger*, *Cladosporium cladosporioides* and *P. chrysogenum* were the most abundant species in all repositories followed by *Aspergillus flavus*, *C. oxysporum* and *P. commune*. *Aspergillus clavatus* was isolated only from repositories located in the north side of building whereas *A. flavipes* was identified only in the first and second floor like *Cladosporium fulvum* strain.

The physiological characterization of fungal isolates revealed that most of them grew at the expense of filter paper as sole carbon source, whereas nearly 38% grew moderately (growth in 70–50% of the slant of culture medium) on crystalline cellulose (α -cellulose) (Table 7). Likewise, most of the strains excreted pigments on paper and all of them produced acids.

With respect to the bacterial groups isolated from the air, Gram-positive bacteria (between cocci and bacilli) were the most abundant ones at both months in all the repositories (Table 8). The Gram-positive sporulated bacilli completely correspond to the genus *Bacillus*. This genus was the most abundant Gram-positive bacilli isolated in repositories located in the basement and first floor. The percentages obtained were equivalent to concentration ranging from 296.4 to 340.2 CFU m⁻³ in February while *Streptomyces* genus was detected in different percentages (1–8%) in

the repositories located at the north side of the building with a tendency to increase in September and also with the height of the building.

On the basis of biochemical tests, *Staphylococcus*, *Streptococcus*, *Corynebacterium*, *Bacillus* and *Streptomyces* genera were isolated among Gram-positive bacteria while *Serratia* and *Enterobacter* were identified among Gram-negative bacteria.

4 Discussion

Water is a key ingredient for the germination, growth, and development of indoor bacterial and fungal bioaerosols. Several studies have established relationships between indoor bioaerosols and moisture content (Green et al. 2003). There are maximal and minimal moisture threshold conditions for microorganisms to grow on different materials. The most favorable conditions for microbial growth vary depending on the species, but few can live under the equilibrium of RH of a material 65% (water activity, $a_w = 0.65$) (Pasenen et al. 2000). It has been reported that hygroscopic materials (paper, textiles, leather, etc.) are the most susceptible to microbial growth (Green et al. 2003), for these reasons it is very important to record daily the RH of the repositories.

In Cuba, the T and RH are high, but the ventilation plays an important role in slowing down microbial growth. Therefore, in this study these hygrometric variables were taken into account in their relation to the concentration and variability of the microbiota of the repositories, which represents a potential risk of

Table 3 Median values of air microbial concentration (CFU m⁻³) in the indoor air at each repository analyzed of the National Archive of the Republic of Cuba

Type of microorganisms	North side		South side		North side		South side		North side		South side	
	Repository 3		Repository 8		Repository 13		Repository 18		Repository 24		Repository 28	
	February	September	February	September	February	September	February	September	February	September	February	September
Fungi	204 ^A	550 ^{di, *}	110 ^B	250 ^{b, *}	120 ^C	300 ^{c, *}	60 ^D	150 ^{d, *}	250 ^E	350 ^{e, *}	147 ^F	170 ^{f, *}
Bacteria	425 ^{G, *}	220 ^g	188 ^H	160 ^h	750 ^{i, *}	405 ⁱ	360 ^{j, *}	245 ^j	1,600 ^{K, *}	720 ^k	1,108 ^{L, *}	388 ^l
Microorganisms total	629	770	298	410	870	705	420	395	1,850	1,070	1,255	558

* Indicates significant differences according to Student test ($P \leq 0.05$) on comparing the concentration of bacteria and fungi obtained in the same repository but in different month

A, B, C, D, E, F Indicates significant differences according to student test ($P \leq 0.05$) on comparing the concentration of fungi isolated in February between repositories located in the same floor (repositories 3 and 8 are located in basement, 13 and 18 are in the first floor, 24 and 28 are in the second floor)

a, b, c, d, e, f Indicates significant differences according to Student test ($P \leq 0.05$) on comparing the concentration of fungi isolated in September between repositories located in the same floor

G, H, I, J, K, L Indicates significant differences according to Student test ($P \leq 0.05$) on comparing the concentration of bacteria isolated in February between repositories located in the same floor

g, h, i, j, k, l Indicates significant differences according to Student test ($P \leq 0.05$) on comparing the concentration of bacteria isolated in September between repositories located in the same floor

Table 4 Median values of air microbial concentration in the indoor air of the repositories located in the three floors of the National Archive of the Republic of Cuba

Levels in the building	Type of microorganism	February			September		
		Conc. (CFU m ⁻³)	<i>T</i> _{median} (°C)	RH _{median} (%)	Conc. (CFU m ⁻³)	<i>T</i> _{median} (°C)	RH _{median} (%)
Basement	Fungi	314 b ^c	24.0^b	68.2^b	800 B ^c	27.8^b	75.4^b
	Bacteria	613 a ^d			380 A ^d		
	Microorganisms total ^a	927 a			1,180 A		
First floor	Fungi	180 a ^c	24.1^b	68.5^b	450 A ^c	29.1^b	73.3^b
	Bacteria	1,110 b ^d			650 B ^d		
	Microorganisms total ^a	1,290 b			1,100 A		
Second floor	Fungi	397 b ^c	24.8^b	65.9^b	520 A ^c	30.8^b	70.8^b
	Bacteria	2,708 c ^d			1,108 C ^d		
	Microorganisms total ^a	3,105 c			1,628 B		

a, b, c indicates significant differences according to the Duncan test ($P \leq 0.05$) on comparing the fungi, bacteria and total microbial concentration between the floor of building in February

A, B, C indicates significant differences according to the Duncan test ($P \leq 0.05$) on comparing the fungi, bacteria and total microbial concentration between the floor of building in September

^a Indicates the sum of the median concentration of fungi and bacteria of the two repositories of the floor

^b Represents the median of 30 determinations performed in two repositories analyzed in each floor (5 measurements by triplicate per repository)

^{c, d} Indicates significant differences according to the Student test ($P \leq 0.05$) on comparing the fungi and bacteria concentration between February and September. The microbial determination was made in 5 point by triplicate of two repositories per floor and the data averaged ($n = 30$)

biodeterioration for documents and damage to the health of staff.

Our analysis shows a higher bacterial prevalence in February (dry month), whereas the highest fungal concentration was obtained in September (rainy month). This is due to the weather in tropical countries, especially the high humidity and *T* recorded during the rainy season, which favor the development of fungi and increase the level of viable conidia in the atmosphere. However, in winter it is frequent the occurrence of strong winds as a result of the systematic arrival of cold fronts to the island, causing a significant increase in the speed of wind from the north and facilitates the inflow of bacterial cells and dust towards the building through the system of natural ventilation. But in summer, particularly from May until the end of November, the rains are intense, the hurricanes occur frequently and the humidity relative increases significantly. During this period, rains cause a systematic cleaning of the atmosphere (Dopazo et al. 2002; de la Rosa et al. 2002; Sabariego et al. 2004) and therefore the bacterial concentration in the outdoor environment

tends to decrease, whereas the fungi are favored by high humidity, although the building has natural ventilation. This effect is increased mainly in the basement of the building. However, in the repositories located on the north side of the building both fungal and bacterial concentrations were significantly higher due to northerly winds which easily penetrate into the building. However, the minimum and maximum values of *T* and RH obtained in each month ($T_{\text{median}} = 23.4^{\circ}\text{C}$, $\text{RH}_{\text{median}} = 68.9\%$ in February and $T_{\text{median}} = 31.1^{\circ}\text{C}$, $\text{RH}_{\text{median}} = 72.3\%$ in September), never came close to the recommended values for the conservation of paper, since most of the time they were higher than the $T > 19^{\circ}\text{C}$ and $\text{RH} > 60\%$ (Walker 2003). Nevertheless, the air movement is considered as a way to prevent fungal infestation (Valentín 2010).

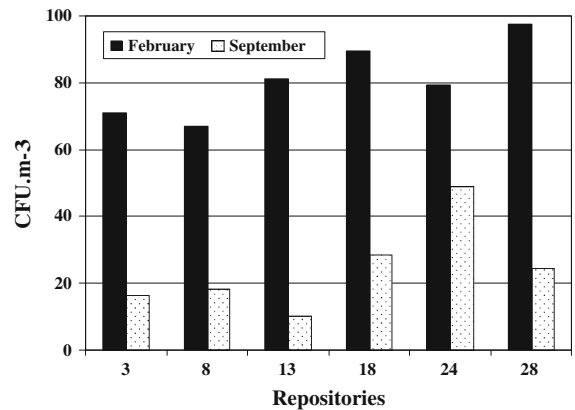
In this study, the microbial prevalence is low in comparison to studies previously performed in other repositories of the NARC (Borrego et al. 2008) in spite of having used a sedimentation method for sampling the air. In other studies carried out in indoor environments of Cuban libraries, archives and museums

Table 5 Relative density (%) of the fungal genera isolated from the air of the six repositories studied at the National Archive of the Republic of Cuba

Fungal genus	North side		South side		North side		South side	
	Repository 3		Repository 8		Repository 13		Repository 18	
	February	September	February	September	February	September	February	September
<i>Aspergillus</i>	35*	8	33*	9	40*	5	44*	14
<i>Cladosporium</i>	21	85 ^a	21	31	24	52 ^a	17	33 ^a
<i>Penicillium</i>	15	—	40	—	19	—	12	—
<i>Curvularia</i>	11	—	4	—	3	6	5	—
<i>Alternaria</i>	11	2	—	4	7	12	9	—
<i>Fusarium</i>	7	2	2	4	7	9	13	6
<i>Mucor</i>	—	2	—	25	—	9	—	10
<i>Neurospora</i>	—	1	—	27	—	7	—	37

* Indicates significant differences according to the Student test ($P \leq 0.05$) on comparing the relative distribution of *Aspergillus* genus between February and September. The microbial determination was made in five points of each repository by triplicate and the data averaged ($n = 15$)

^a Indicates significant differences according to the Student test ($P \leq 0.05$) on comparing the relative distribution of *Cladosporium* genus between February and September. The microbial determination was made in five points of each repository by triplicate and the data averaged ($n = 15$)

**Fig. 4** Concentration of *Aspergillus* genus in the repositories during 2 months studied

higher fungal concentrations were reported (Gómez et al. 1989; Rojas et al. 2002, 2008).

Despite the fact that there is not an international standard to determine whether an indoor environment is contaminated or not, it has been suggested that an environment with a microbial prevalence above 1,000 CFU m⁻³ should be considered contaminated (Nevalainen and Morawaska 2009). Thus, the microbial concentration should be lower than 1,000 CFU m⁻³. Other authors consider that microbial prevalence should not exceed 750 CFU m⁻³, above this level the environment is regarded as contaminated (Radler de Aquino and de Góes 2000) and still other authors consider that 150 CFU m⁻³ should be the lower limit for fungi (Cappitelli et al. 2009).

Predominant fungal genera in the study were *Aspergillus*, *Cladosporium* and *Penicillium*, but *Aspergillus* was more frequent in February, while in September it was *Cladosporium* particularly in the repositories located on the north side of the building. Probably because it is the side of easiest air access to the building, these conidia can easily enter from outside.

Moreover, only in September *Mucor* and *Neurospora* genera were detected in all the repositories and *Neurospora* genus was found at high concentrations in the repositories 18 and 28 (77.1 and 83.3 CFU m⁻³, respectively). Among the species of *Neurospora*, *Neurospora crassa* was the only detected and it was first isolated from the indoor environment of repositories in the NARC (Borrego et al. 2008, 2010a, b). Both genera are found in soil and can become airborne

Table 6 Relative density of *Aspergillus*, *Cladosporium* and *Penicillium* species at the repositories studied during February

Repositories	North 3	South 8	North 13	South 18	North 24	South 28
Fungal species	Relative density (%)					
<i>A. niger</i> *	16 a	9 a	17 a	9 a	18 a	21 a
<i>A. clavatus</i>	6 b	–	9 b	–	3 b	–
<i>A. flavus</i> *	8 b	6 ab	7 bc	3 b	7 b	10 b
<i>A. flavipes</i>	–	–	3 c	1 b	4 b	8 bc
<i>A. terreus</i>	4 b	4 b	2 c	2 b	3 b	3 c
<i>A. versicolor</i>	1 b	2 b	2 c	2 b	4 b	6 bc
<i>C. cladosporoides</i>	12 a	16 a	13 a	20 a	11 a	15 a
<i>C. herbarum</i>	4 b	5 bc	2 b	2 c	1 b	–
<i>C. sphaerospermum</i>	–	4 bc	–	5 bc	1 b	2 a
<i>C. oxysporum</i>	5 b	7 b	6 b	9 b	5 b	4 a
<i>C. fulvum</i>	–	–	1 b	5 bc	2 b	1 a
<i>C. elatum</i>	–	1 c	2 b	3 c	–	1 a
<i>P. chrysogenum</i>	11 a	19 a	9 a	8 a	9 a	8 a
<i>P. citrinum</i>	1 b	5 bc	–	1 b	–	2 b
<i>P. decumbens</i>	–	–	2 b	–	–	–
<i>P. griseofulvum</i>	–	3 c	1 b	–	–	–
<i>P. aurantiogroscum</i>	–	4 bc	–	–	–	–
<i>P. commune</i>	3 b	9 b	5 ab	3 b	4 b	5 ab
<i>P. digitatum</i>	–	–	2 b	–	–	1 b

* Indicates that it is one of the species of greater clinical interest (Aira et al. 2002; Hedayati et al. 2007; Person et al. 2010; Gautan et al. 2011)

a, ab, b, bc and c indicates significant differences according to the Duncan test ($P \leq 0.05$) on comparing the relative distribution of the *Aspergillus*, *Cladosporium* and *Penicillium* species

and transported by the air into the building. In particular, *Mucor* is spread by rain splashes and has an $a_w = 0.90$ – 0.94 , perhaps for this reason it appears in high concentrations in September (rainy month) (EMLab P & K 2010).

Aspergillus, *Cladosporium* and *Penicillium* have been reported among the predominant indoor fungi (Maggi et al. 2000; Nevalainen and Morawaska 2009; Mesquita et al. 2009) and have commonly been found in indoor environments of houses, archives, libraries and museums in Cuba (Valentín et al. 1997; Rojas et al. 2002, 2008; Aira et al. 2002; Borrego et al. 2008; 2010a, b; Guamet et al. 2011). Particularly, the concentrations of *Aspergillus* in February ranged from 67.03 to 97.5 CFU m^{-3} which are considered within a high concentration taking into account that Holmberg (1987) suggested that the levels of *Aspergillus* spp. should be lower than 50 CFU m^{-3} to avoid excessive exposure to bioaerosols and consequent health effects to people.

Aspergillus and *Penicillium* are considered to be primary colonizers, since both can grow at lower water activity (a_w) values ($a_w < 0.8$) while other fungal genera (e.g., *Cladosporium*, *Alternaria* and *Phoma*) require a_w values at least between 0.8 and 0.9 (Nielsen 2003). This could explain the high prevalence of *Cladosporium* ($a_w = 0.85$ – 0.88) in September ($RH_{median} = 72.3\%$) in our study. Despite the existence of a high RH, the fungal genera were detected in low concentration or not detected due to the natural ventilation of the repositories, which facilitates the movement of particles in the air and makes their settlement difficult.

All the fungal genera isolated that may affect health are known as allergens (Mesquita et al. 2009; Nevalainen and Morawaska 2009). Since many species of *Aspergillus* are pathogenic and winter climate changes in Cuba are quick and sudden, this encourages the occurrence of frequent attacks of allergies, sneezing and asthma, therefore we decided to conduct a detailed

Table 7 Qualitative cellulolytic activity, excretion of pigments and acids of the fungi isolated from the air of the repositories studied in the National Archive of the Republic of Cuba

Strains	Growth on filter paper strip	Growth on crystalline cellulose	Production of pigments ^a	pH
<i>A. niger</i>	+++	++	+	4.5
<i>A. clavatus</i>	++	+	+	4.9
<i>A. flavus</i>	+++	++	+	5.0
<i>A. flavipes</i>	++	+	–	5.2
<i>A. terreus</i>	+++	++	+	6.2
<i>A. versicolor</i>	+++	++	+	5.1
<i>C. cladosporoides</i>	+++	++	+	3.6
<i>C. herbarum</i>	+	±	+	4.7
<i>C. sphaerospermum</i>	++	++	+	5.0
<i>C. oxysporum</i>	+++	+	+	3.2
<i>C. fulvum</i>	++++	++	+	4.5
<i>C. elatum</i>	+++	++	+	3.5
<i>Penicillium chrysogenum</i>	++	+	+	5.6
<i>P. citrinum</i>	++	+	–	6.0
<i>P. decumbens</i>	++	+	–	5.8
<i>P. griseofulvum</i>	+++	++	–	4.4
<i>P. aurantiogroscum</i>	+++	+	+	5.2
<i>P. commune</i>	+++	++	+	4.5
<i>P. digitatum</i>	++	+	+	4.0
<i>Curvularia lunata</i>	+	+	+	5.5
<i>C. pallescens</i>	+	+	+	6.0
<i>C. clavata</i>	+	±	+	4.5
<i>Alternaria alternata</i>	–	–	±	4.8
<i>A. tenuissima</i>	+	±	+	5.4
<i>Fusarium</i> sp.	++	+	+	5.7
<i>Mucor</i> sp.	+++	+	+	5.3
<i>N. crassa</i>	+++	+	–	6.1

^a The production of pigments was evidenced on the filter paper strip, +++ indicates abundant growth (100% of the saline culture medium slant and on the paper), ++ indicates moderate growth (70–50% of the slant), + indicates poor growth (>50% of the slant), it is also indicative of presence of pigment, ± indicates very poor growth or production of pigment, – indicates NO growth and NO production of pigment

analysis of *Aspergillus* species. Two species of *Aspergillus* were abundant in February (winter month). *Aspergillus niger* forms colonies that stand out because of their black conidiophores; its conidia are globular, rough or have spines. It has a musty odor, and as it is a cosmopolitan fungus and can grow at a_w minimum of 0.77, it is commonly found in the environment on textiles, in soils, grains, fruits and vegetables. It may cause damage to different materials including the paper (Valentín 2010), especially in warm climates, and on water-damaged books and documents (Storey et al. 2004) as well as cause respiratory allergies and cutaneous diseases (Aira et al. 2002). *A. flavus* is characterized by its yellowish green colonies and globular conidia. It is an opportunistic pathogen, which contains allergens and produces carcinogenic aflatoxin. This

fungus needs to grow at $a_w = 0.78$ and can degrade paper (Valentín 2010). These species are associated with aspergillosis of the lungs (Hedayati et al. 2007; Person et al. 2010; Gautan et al. 2011). Also, *A. clavatus* was isolated only in the north side of the building and it has colonies of grayish or bluish green colour and has ellipsoidal conidia. It is a cosmopolitan fungi and abundant in soil and it may cause aspergillosis and lung infections (Deshpande and Gangawane 1997). These species were frequently isolated by Rojas et al. (2002) in different cultural institutions of Havana University.

Among the *Cladosporium* species identified *C. cladosporoides* prevailed. This fungus has smooth conidia; it is cosmopolitan, and it is also a saprophyte and parasite on plant and has been cited as the cause of lung, skin and nasal infections (Aira et al. 2002).

Table 8 Relative density of the different bacterial groups isolated from the air of the repositories studied at the National Archive of the Republic of Cuba

Repository	Bacterial groups	February Relative density (%)	September
3	Gram-positive cocci	33.1	37.3
	Gram-negative cocci	28.5	25.2
	Non sporulated Gram-positive bacilli	26.1 ^a	–
	Sporulated Gram-positive bacilli	–	24.0 ^b
	Gram-negative bacilli	12.3	13.5
8	Gram-negative cocci	22.9	10.5
	Sporulated Gram-positive bacilli	55.5 ^b	65.2 ^b
	Gram-negative bacilli	21.6	24.3
13	Gram-positive cocci	35.1	39.9
	Gram-negative cocci	9.3	7.0
	Non sporulated Gram-positive bacilli	28.9 ^a	27.7 ^a
	Sporulated Gram-positive bacilli	26.7 ^b	25.4 ^b
18	Gram-positive cocci	33.0	20.8
	Non sporulated Gram-positive bacilli	40.3	36.2 ^a
	Sporulated Gram-positive bacilli	26.7 ^b	27.6 ^b
	Gram-negative bacilli	–	15.4
24	Gram-positive cocci	38.2	40.1
	Non sporulated Gram-positive bacilli	41.8 ^a	36.6 ^a
	Gram-negative bacilli	20.0	23.3
28	Gram-positive cocci	41.4	45.7
	Non sporulated Gram-positive bacilli	44.8 ^a	38.5 ^a
	Gram-negative bacilli	13.8	15.8

^a Indicates that it is included in a percentage of *Streptomyces* sp strains. The levels are variable and tend to be higher in September

^b Indicates that this percentage belongs to *Bacillus* genus

Finally, *Penicillium chrysogenum* ($a_w = 0.78$) was the predominant species within the genus which is considered as the most common species of all penicillia in indoor environments and in household dust (Fernández 2005). It is characterized by its velvety and sulcate colonies with a blue-green colour and loosely branched smooth terverticillate conidiophores and smooth ellipsoidal conidia. It has been identified as an important toxigenic mold (Singh 2005) and allergen in indoor environments (Cooley et al. 1999) and as a rare causative agent of opportunistic mycosis in humans (Scott et al. 2004).

Neurospora crassa was the main species of *Neurospora* detected. In its natural environment, this species lives in tropical and sub-tropical regions. Different species have been described as causing occupational asthma (Côté et al. 1991). However, *Mucor* is found on a variety of substrates, including leftover food, soft fruits and juices. This fungus is also reported to cause opportunistic infections in severely debilitated patients (Madrigal et al. 2008).

It is known that the majority of the fungal strains isolated from the air of the repositories exhibit cellulolytic activity, produce acids, excrete different pigments and contribute to the formation of biofilms, which accelerate the deterioration of different document substrates (Fernández 2005; Borrego et al. 2010a; Guimet et al. 2011). Our results are consistent with these findings.

Our observation that Gram-positive bacteria prevailed among the bacterial groups isolated from the six repositories is consistent with other studies performed in the same type of buildings (Valentín 2010; Borrego et al. 2008, 2010a; Guimet et al. 2011). *Streptococcus* sp., *Staphylococcus* sp., *Corynebacterium* sp. and *Bacillus* sp. strains were isolated among the Gram-positive bacteria, whereas *Enterobacter* and *Serratia* were the genera isolated among the Gram-negative bacteria. All these genera have been isolated from archive environments (Valentín et al. 1997; Borrego et al. 2008, 2010a, b).

Some species of *Streptococcus*, *Staphylococcus*, *Corynebacterium* and *Bacillus* are human pathogens

(Miller et al. 2004). In particular, it has been reported that some species of *Streptomyces* are noxious to the human respiratory system (Rintala et al. 2008).

It has been reported that high concentrations of *Bacillus* species in indoor air is usually indicative of water damage or lack of building maintenance (Baxter et al. 1981). In this sense, the presence of high concentration of this bacterial genus may indicate the need for a constructive repair of the repositories and the ventilation system to which dust filters should be added. In addition, *Bacillus* and *Streptomyces* constitute a potential risk for paper support, because they can produce cellulases (Ramírez and Cocha 2003).

Finally, *Enterobacter* and *Serratia* species have been detected previously in indoor archive or library environments too (Valentín et al. 1997; Borrego et al. 2010a; Guiamet et al. 2011). The inhalation of endotoxins produced by these genera can cause irritation of the respiratory system, resulting in fever, shivers, malaise and headache. The continued exposure to this type of bacteria may lead to bronchitis and asthma (Burge and Gallup 2005).

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