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Airborne Mycobiota In Offices and Other Premises of The National Archive of The Republic of Cuba: Its Impact on The Personnel Health

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Abstract:

The mycological quality of the indoor environment has been related to the appearance of allergies and other diseases in humans due to intense and persistent exposure to biological agents. The aims of this work were to analyze the environmental mycobiota of offices and different premises of the National Archive of the Republic of Cuba, determine the fungal species isolated from the nostrils of workers exposed to environmental allergenic fungi and evaluate IgE-mediated skin sensitization. Environmental mycological sampling was made with a biocollector and using appropriate culture media to isolate fungi. The nasal mucosa of the 72 workers selected in the study was sampled with sterile swabs. A survey was applied to workers to collect necessary data and skin tests were performed with different fungal allergenic extracts. In general, the archive environment has moderate fungal contamination. Eight species belonging to filamentous fungi and two to yeasts with a predominance of the *Aspergillus* and *Penicillium* genera were isolated. In the workers nasal mycobiota, the genera *Aspergillus, Cladosporium* and *Penicillium* also prevailed. 54.2% of workers reported the incidence of more than one disease with asthma predominating. Positive skin reaction to one or more fungal extracts was evident in 12.5% of the personnel. The exposure time of workers to the more or less contaminated environment of the archive facilitates the nasal colonization of fungal species that can lead to the triggering or exacerbation of allergic conditions such as asthma and rhinitis.

Keywords: *Allergy; Archive Environment; Allergenic Fungi; Nasal Mycobiota; Occupational Hazard; Allergic Sensitization*

Introduction

The microbiological quality of indoor air has been related to the appearance of occupational illnesses, given that in certain work environments,

exposure to biological agents can be intense and persistent [1]. This phenomenon is enhanced in tropical climate zones and buildings with inefficient ventilation or air conditioning systems [2]. Several authors have established a close relationship between environmental conditions, the presence of anemophilous fungi and their incidence in the triggering of respiratory and allergic conditions in indoor environments [3-5].

It is reported that of 753 allergens officially recognized by the WHO, 16% is of fungal origin and there is sensitization to almost 80 genera [6]. Allergic responses to fungi are more directly related to spores than to other fungal propagules such as mycelium fragments or associated volatile organic compounds. Spores and remnants of fungal propagules produce allergic reactions due to high molecular weight proteins, glycoproteins, carbohydrates, and β-1,3-glucan found in the wall, membrane, and cytoplasm. The responses to each type of spore differ according to the individual and present great variability in their severity [7]. The potential for a person to inhale spores and other fungal propagules, both in outdoor and indoor environments, is high, depending largely on their environmental concentration and physical size of these bioparticles [8]. Although some studies also show that individuals with high exposure to fungi found in homes or other indoor environments, for example, workplaces, unequivocally present permanent allergic symptoms and especially in susceptible individuals [9].

The indoor environments of archives and libraries are reservoirs of fungal propagules mainly due to dust accumulated in the materials, the organic nature of the substrates, the acidity of most of the materials, incorrect air circulation on some occasions, and overcrowded conditions in repositories in others, which make these environments complex microbial ecosystems [10].

On the other hand, the nasal mucosa is a reservoir of inhaled fungi since it constitutes the main barrier against inhaled contaminants [11]. However, the relationship that may exist regarding the possible differences between the nasal fungal microbiota of healthy and allergic subjects, as well as between the presence of fungi inside the nose and the development of an allergic respiratory disease, has been little studied.

In Cuba there are few studies on the environmental microbiological quality on indoor environments and sensitization to fungi in workers even when the climatic conditions are conducive to the development and spread of fungi and there is significant sensitization of their spores in the population [12]. Most of the studies carried out have been in indoor environments of the National Archive of the Republic of Cuba (NARC), which has been studying for years the diversity and distribution of environmental fungi in repositories that preserve documents to assess their impact on Heritage Documentary conservation treasured there [13]. However, office areas and other places where staff usually work have not been widely studied since there is only one report on it [14]. Taking these aspects into account, the aims of this work were to analyze the environmental mycobiota in offices and other NARC premises, determine the fungal species isolated from the nostrils of workers exposed to environmental allergenic fungi, and evaluate IgE-mediated skin sensitization.

Materials and Methods

Study areas

NARC is located in the municipality of Old Havana, Havana, Cuba. The environment is urban and marine since it is separated by 174 m from the bay of Havana (Figures. 1a and 1b). The building was built in the period 1940 - 1944 and is designed to have natural cross ventilation (construction in the form of blocks), although two repositories that preserve special materials and some of the offices analyzed have independent air conditioning equipment, i.e. they do not have central air conditioning. It has three levels: a semi-basement 1.5 m below the ground with a height of 3 m and two floors of 6 m height respectively, one being at street level (first floor) and the other above it (second floor). It also has 30 repositories distributed over the three floors, where more than 27 linear Km of documents of patrimonial value are preserved.

The premises analyzed were: the Professional Training And Cadres Office (PTCO) and the Reading Room (RR) located on the first floor and in the north and south side of the building, respectively; the Research Office (RO) and the Computer Office (CO) which are located on the second floor and on the south and north side of the building, respectively; the Photo Library office (PLO) characterized by being a small space located next to the PL repository in the semi-basement and southern part of the building, while the Map Library (ML), a large repository-office, is located on the first floor and on the south side of the building. Two outdoor points were also sampled, one located on the south side and the other on the north side of building.

Mycological Air Sampling

Sampling was performed during the working day between 10:00 am and 1:00 pm. During the sampling of the offices and premises, the staff (2-8 People) and the users (around 10) remained seated doing their work. The number of sampling points was determined according to the criteria of Sanchis (2002) Figure 1c.

Viable bioaerosol samples were made by triplicate simultaneously taken indoor and outdoor of the building using a SAS impactor (model Super 100 TM, Italy) with an air flow of 100 L/min for 2 minutes at a height of 1.5 m from the floor and at intervals of 1 hour between replicates to ensure air recovery at the point analyzed. Petri dishes were used with two variants of culture media, Malt Extract Agar (MEA) (Biocen, Cuba) supplemented with sodium chloride (7.5%) and MEA with pH = 5. Subsequently, the dishes were incubated inverted at 30°C between 5 and 7 days, and then the colonies were counted to determine the fungal concentration expressed in colony-forming units per cubic meter of air (CFU/m^3).

To know the air quality, the indoor/outdoor (I/O) ratio was calculated and the values obtained were compared with the criteria of De Aquino Neto and Goés Siqueira (2000). These authors indicated that a value of this ratio equal to or less than 1.5 is typical of an uncontaminated environment with good ventilation, a value between 1.5 and 2 is indicative of regular environmental quality and a value greater than 2 reflects a polluted environment and with poor ventilation. Likewise, the results obtained were also compared with the criteria of Roussel et al. (2012) considering four contamination levels: low (less than 170 CFU/m^3), moderate (between 170 and 560

CFU/m^3), high (between 560 and 1000 CFU/m^3) and very high (greater than 1000 CFU/m^3).

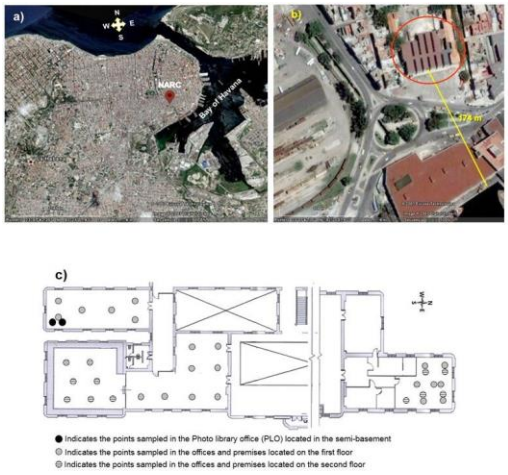

Figure 1: Satellite location of the National Archive of the Republic of Cuba (NARC) in the Havana city (A), aerial view showing its proximity to the sea (B) and schematic representation of the sampling points at the different analyzed premises (C). The premises sampled were six in total: Photo Library Office (PLO) located at the semi-basement and at the south side of the building, Map library (ML) and Reading room (RR) located in the first floor and at the south side, Professional Training and Cadres Office (PTCO) located in the first floor and at the north side of the building, Computer Office (CO) and Research office (RO) located in the second floor, at the north and the south side of the building respectively.

Ecological analysis

The relative density (RD) was calculated according to [14], where: DR = (number of colonies of a specific taxon/ total colonies of all taxa counted) x 100.

The ecological impact of the species isolated from the air was determined from the calculation of the Relative Frequency (RF) .

FR = (number of samples in which a species appears/ total samples) x 100

Where the ecological categories were classified as: abundant species (A) have an RF between 81 and 100%, common species (C) those that are between 61 and 80%, frequent or moderate species (F) those that are between 41 and 60%, occasional species (O) have an RF between 21 and 40% and rare species (R) those that are between 0.1 and 20%.

Sampling from the Nostrils

During sampling of the nasal mucosa, the worker's head was tilted backwards and a sterile swab was introduced into choanae, penetrating a minimum of 15 mm and gently rotating it to obtain a representative sample. The samples were seeded by depletion in Petri dishes with Sabouraud Agar medium with chloramphenicol (bioMérieux SA, France) and incubated at 27°C for two weeks with daily observation.

Identification of environmental fungi and isolates from nostrils

Both the colonies obtained from the air and from the personnel nostrils were isolated and purified. Those from the air were seeded on MEA and Czapek Agar (Biocen, Cuba), while those isolated from the nostrils were seeded in Potato Dextrose Agar (Merck, Germany). For the identification, observations of the cultural and morphological characteristics of each colony were made both on the front and on the back, as well as the microscopic structures (conidia, conidiophores and hyphae) making preparations in lactophenol and microcultures [16] and the criteria of different taxonomic manuals [17-20].

The isolated yeasts were sown in Cromocen CNDF medium (Biocen, Cuba) and the identification was carried out using the API 20 Caux sugar assimilation test (Auxacolor, France).

Application of a survey to the workers under study.

A study was made on 72 NARC workers who work in the handling of documents with at least six months of work exposure. Workers with less than six months of

occupational exposure, those who were carriers of diseases in a state of primary or secondary immunosuppression, pregnant women of any gestational age, and those who decided to abandon the study at any stage of it were excluded.

The studied workers were of both sex's 53 females and 19 males. The age ranged from 18 to 75 years, all residents in the metropolitan area of Havana. All participants were informed of the nature and objectives of the study and their voluntary participation was stated.

Through a direct and personal interview, a survey was carried out on each worker to collect the necessary data. Among those of greatest interest that were collected were: age, working time at NARC and in the department in which they were working, if they suffered from any chronic disease and if they suffered from any disease in the last year prior to the study.

Allergy diagnosis

To perform this test, workers with less than six months of occupational exposure, workers carrying diseases in a state of primary or secondary immunosuppression, pregnant women at any gestational age and those who decided to abandon the study at any stage of the study were excluded. To carry out the skin tests, it was taken into account that no worker was in a feverish state or in a crisis of exacerbation of allergic disease, with a known history of chronic cardiovascular, neoplastic, non-allergic pulmonary co-morbidities, with eczema, treatments antihistamines, steroids and systemic immunosuppressants at least three days before the skin test, as well as those who had local steroid treatment applied to the sites of possible punctures, and those who had weal diameters greater than 3 mm in the test-negative control.

This analysis was performed by means of clinical evaluation and Skin Prick Test (SPT) with fungal allergenic extracts at a concentration of 20,000 BU/ml of the species *Alternaria alternata, Aspergillus fumigatus, Candida albicans, Penicillium chrysogenum* and *Cladosporium herbarum*. Positive (histamine) and negative (allergen diluent) controls were used. Weal size was measured after 20 minutes, and the result was considered positive if the weal was at least 3 mm greater than the negative control.

Statistical analysis

For statistical analysis, the Statgraphics Centurion XV program was used. The comparison of averages of T, HR, of the environmental fungal concentrations and of the sensitization of different fungal species was made by means of a simple classification variance analysis (ANOVA) followed by Duncan's test ($p \leq 0.05$). Student's test was used to compare the behavior of fungal sensitization and nasal colonization between purportedly healthy personnel and those considered as patients ($p \leq 0.05$).

Results

Behaviour of environmental fungi

The air sampling of the different places analyzed showed significant differences between the fungal concentrations obtained Table 1. The lowest values were found in the PLO (65 CFU/m^3) and the ML (78 CFU/m^3) respectively, while in the rest of the offices analyzed the concentrations were higher than 350 CFU/m^3 (362 CFU/m^3 – 982 CFU/m^3).

The I/O ratios of the premises showed values for PLO, ML and CO that oscillated between 0.2 and 1.2, that is, less than 1.5, while in the other premises the ratios

were markedly higher (RR with 2.0, RO with 2.4, and PTCO with (3.3).

Regarding the T and the RH, it can be seen that the T averages in all the premises revealed significant differences ($p \geq 0.05$) with the T from the outdoor, while the RH showed significant differences between some spaces and between all the existing values in indoor environments and the RH obtained outdoors. The lowest value was obtained in the ML located on the first floor and the south side of the building and likewise the highest values were obtained in the other stores located on this same floor (PTCO and RR).

A total of 16 taxa with a predominance of the genera *Aspergillus, Cladosporium* and *Penicillium* were isolated from the environments, although other genera were also detected to a lesser extent (*Bipolaris, Curvularia, Epicoccum, Exophiala, Fusarium, Mucor, Neurospora, Nigrospora, Pestalotia, Sporobolomyces* and *Trichoderma*). In the PLO, a place located in the semi-basement and on the south side of the building, a total of 30 species were detected despite being the place with the lowest fungal concentration (65 CFU/m^3) with predominance of the species *A. niger* and *Cl. cladosporioides* followed by *P. chrysogenum, A. flavus, P. citrinum* and *Al. ricini* Figure 2. In the ML, a place located on the first floor and on the south side of the building, a total of 24 taxa were isolated, although it was another place with a low fungal concentration (78 CFU/m^3); in this case *Cl. cladosporioides* was the predominant species followed by *A. niger.*

Sampled places	Location		Concentration (CFU/m^3) ± SD	I/O ratio	T (ºC) ± SD	RH (%) ±SD
Photo library office (PLO)	South	Semi-basement floor	65 ± 12a	0.2	23.0 ± 0.2	64.2 ± 0.5 b
(ML)	South	1st floor	78 ± 23a	0.3	23.5 ± 0.5	50.3 ± 0.5 a
Reading room (RR)	South	1st floor	580 ± 111 c	2.0§	25.0 ± 2.5	70.3 ± 3.1 c
Professional training and cadres office (PTCO)	North	1st floor	982 ± 302e	3.3^	25.6 ± 3.1	70.2 ± 3.4 c
Researchers office (RO)	South	2nd floor	720 ± 1 89 d	2.4 ^	24.5 ± 2.2	63.2 ± 2.8 b
Computer office (CO)	North	2nd floor	362 ± 98b	1.2§	22.2 ± 1.2	60.2 ± 2.2b
Outdoor **	-	-	295 ± 67 b	-	31.4 ± 1.1*	75.5 ± 1.5 d

Table 1: Fungal concentrations detected in the indoor and outdoor environments of the studied premises in NARC, indoor/outdoor (I/O) ratios and thermo-hygrometric values obtained.

SD: Standard deviation. a, b, c, d, e: Indicates statistically significant differences ($p \leq 0.05$) according to Duncan's test. Similar letters indicate that there are no significant differences. *: Indicates statistically significant differences ($p \leq 0.05$) according to Duncan's test with respect to the rest of the T values.**: Indicates the average of the two sampling points in outdoor.§: Indicates a medium quality environment according De Aquino Neto and Goés Siqueira (2000).^: Indicates a polluted environment with poor air circulation according De Aquino Neto and Goés Siqueira (2000).

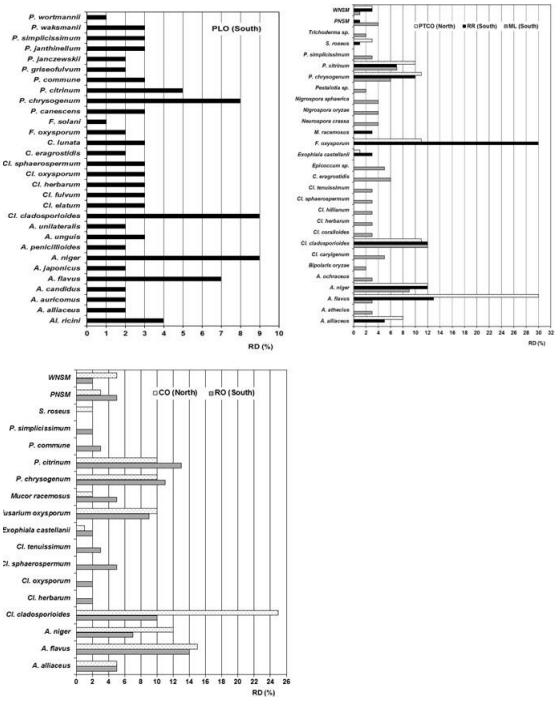

Figure 2: Relative density (RD) of the fungal species isolated form different premises studied in NARC. PLO: Photo library office located in the semi-basement of the building. ML: Map Library located in the 1st floor, PTCO: Professional training and cadres office and RR: Reading Room, are premises situated on the 1st floor of the building. RO: Research office and CO: Computer office, are premises placed in 2nd floor of the building. WNSM: white non-sporulating mycelium. PNSM: Pigmented Non-Sporulating Mycelium.

As the PTCO, RO, RR and CO premises were the ones that showed the highest concentrations, it was necessary to determine the concentration of the

predominant species in their indoor air. In PTCO, located on the 1st floor and north side of the building, the species *A. flavus* predominated (RD = 30%) with an air concentration of 295 CFU/m^3. For its part, in RR, a premises located on the 1st floor but in the south wing of the building, the prevailing species was *F. oxysporum* (RD = 30%) with a concentration of 174 CFU/m^3, followed by *A. flavus* (75 CFU/m^3), *A. niger* (70 CFU/m^3), *Cl. cladosporioides* (70 CFU/m^3) and *P. chrysogenum* (58 CFU/m^3). In the case of RO, located on the 2nd floor but on the south side, the outstanding species were *A. flavus* (RD = 14%) and *P. citrinum* (RD = 13%) with concentrations of 101 CFU/m^3 and 94 CFU/m^3 respectively, while in CO, located on the 2nd floor and in the north wing of the building, the dominant species was *Cl. cladosporioides* (RD = 25%) with a concentration of 91 CFU/m^3. It is noteworthy that in the four locations analyzed these species were maintained at concentrations higher than 50 CFU/m^3 of the 46 isolated species, 7 were ecologically classified of abundant in these environments Figure 3. Three of them belonged to the *Aspergillus genus* (*A. alliaceus, A. flavus, A. niger*), one to *Cladosporium* genus (*Cl. cladosporioides*) and two species to the *Penicillium* genus (*P. chrysogenumand P. citrinum*). *Fusarium oxysporum* was another of the species that turned out to be abundant as well as a White Not-Sporulating Mycelium (WNSM). *Exophiala castellanii, Sporobolomyces roseus* and a Pigmented Non-Sporulating Mycelium (PNSM) turned out to be common taxa. *Mucor racemosus* was classified as a frequent species, while *Cl. herbarum, Cl. sphaerospermum, C. eragrostidis and P. simplicissimum* were occasional species, the rest of the isolated species were classified as rare.

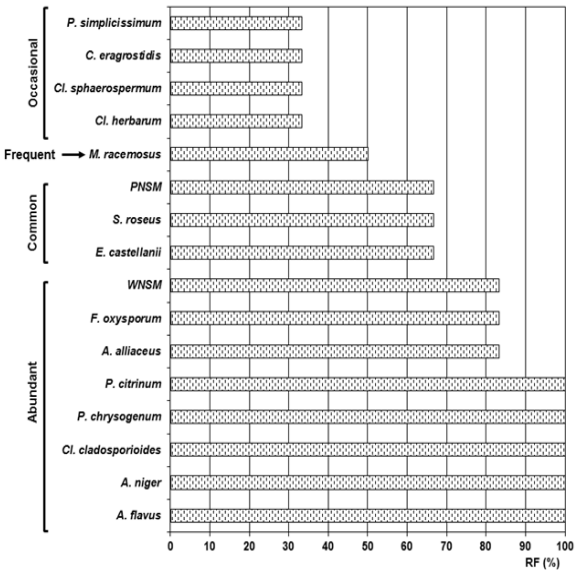

Figure 3: Ecological behavior according to the relative frequency (RF) of some of the fungal species isolated from the different premises analyzed in the NARC.WNSM: white non-sporulating mycelium. PNSM: pigmented non-sporulating mycelium.

Behavior of the fungi isolated from nostrils

The nasal secretion cultivation evidenced colonization by fungal growth in 53 workers (76.1%), of these 29 (54.7%) were reported as patients and 24 (45.3%) turned out to be purportedly healthy workers Figure 4. The predominant genus in the obtained samples was *Aspergillus* (64%) followed by *Cladosporium* (14.7%) and *Penicillium* (8%) Table 2.

Seven fungal genera were identified and it was obtained that the *Aspergillus* genus grouped 64% of the identifications, being *A. flavus* (22%) and *A. niger* (18%) the species with the greatest incidence in the nostrils. However, *A. fumigatus* was isolated from a worker's nose (1.3%), while the *Candida* genus predominated among yeasts. However, *A. alliaceus* species that was in air was not isolated from nostrils and the same happened with *P. citrinum, P. chrysogenum, F. oxysporum, M. racemosus, E. castellanii* and *S. roseus.*

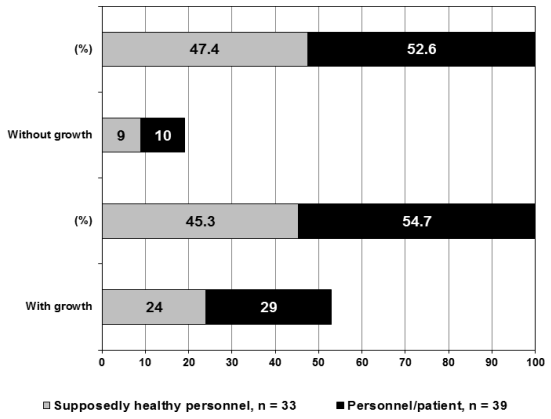

Figure 4: Behavior of the samples obtained from the nostrils in the NARC employees.

Specie	Samples, n = 72		(%)	
Filamentous fungi				
Aspergillus sp.	6	48	8.0	64.0
A. flavus	22		29.3	
A. niger	18		24.0	
A. terreus	1		1.3	
A. fumigatus	1		1.3	
Cladosporium sp.	11		14.7	
Chrysonilia sp.	1		1.3	
Mucor sp.	4		5.3	
Penicillium sp.	6		8.0	
Rhizopus sp.	1		1.3	
Yeast				
Candida sp.	1		1.3	
C. guilliermondii	1		1.3	
C. parapsilosis	2		2.7	

Table 2: Fungal species detected in the nostrils of NARC´s workers tested.

Survey analysis

In the made research, an absolute predominance of female sex (53 women for 73.6%) and only 19 men (26.4%), all with an average age of 46 years was found. They have an exposure to the workenvironment considered as important because they have remained for more than 12 years in the institution and more than 9 years in the departments, indicating the stability of workers in their positions and work areas of the total of the 72 workers evaluated, the incidence of diseases in the last year was evidenced in 39 of them (54.2%), where asthma predominated in 22 of them (56.4%), followed by chronic rhinitis in 20 (51.3%) and allergic conjunctivitis in 11 (28.3%) (Fig. 5). These diseases were more frequent in personnel who mainly performed document processing work in 8 workers (20.5%), work of offices in 7 workers (17.9%) and of conservation in 5 workers (12.8%).

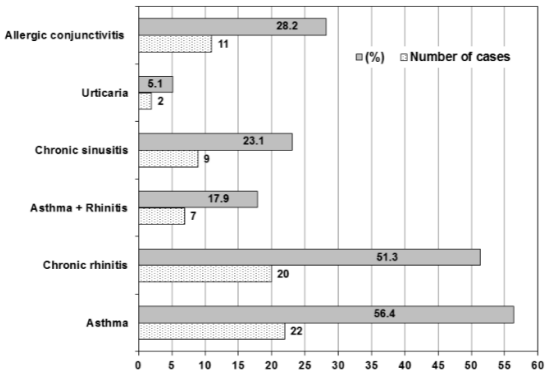

Figure 5: Report on the illnesses´ incidence in NARC employees.

Allergy diagnosis

The total studied staff, 29 (40.3%) presented positive reaction to one or more fungal extracts tested of these, 20 (69%) showed sensitization to a single extract. The sensitization in those who registered diseases in this period (39 workers, 54.2%), was positive in 24 (61.5%) and in 5 (15.5%) for allegedly healthy workers.

The sensitization to a single fungal allergen (monosensitization) was appreciated in 18 of the workers-patient before environmental fungal extracts Table 3 with a predominance of the response against *A. fumigatus* in nine workers (45%), as well as *Al. alternata* and *Cl. herbarum* in four workers (20%) respectively. Sensitization to more than one allergen was obtained through skin reaction against extracts of *Al. alternata* in six workers (31.6%), *A. fumigatus and P. chrysogenum* in five workers (26.3%), respectively, with larger wheal diameters 3 mm Table 4.

The relationship between the condition of an allegedly healthy worker (who does not have records of diseases of allergic interest) and a worker-patient can be seen in table 5. Between 74.3% and 72.7% of patient and purportedly healthy workers respectively, showed fungal colonization in the nasal exudates; however, 24 (61.5%) of the patients developed skin sensitization to environmental fungal allergens.

Skin testing (+) diameter of the weal > 3 mm	Purportedly healthy personnel,n = 33	(%)	Workers-patient, n = 39	(%)
to fungal extracts	5	15.5	24 *	61.5
Polysensitization	3	9.1	6	15.4
Monosensitization	2	6.1	18 *	46.2

Table 3: . Behavior of positive skin sensitivity according SPT in NARC workers (n = 72).

*Indicates significant differences when comparing fungal sensitization between personnel considered patients and those purportedly healthy, according Student's test ($p \leq 0.05$).

Fungal extracts 20000 UB/ml	Monosensitization		Polysensitization		Skin test (+) diameter of the weal diam eter ≥ 3 mm
	n=20	(%)	n=9	(%)	
Alternaria alternata	4b	20	6a	31.6	3.1
Aspergillus fumigatus	9a	45	5a	26.3	3.3
Candida albicans	1c	5	1b	5.3	3
Cladosporium herbarum	4b	20	2b	10.5	3.2
Penicillium chrysogenum	2c	10	5a	26.3	3.1

Table 4: Positive skin sensitivity according the SPT to various fungal species in NARC workers (n = 29).

*: Indicates significant differences whencomparing fungal sensitizationbetweenpersonnelconsidered patients and those purportedly healthy, according the Student's test ($p \leq 0.05$).

Personnel	Fungal sensitization (+)		Nasal colonization by fungi (+)	
	No.	(%)	No.	(%)
Patients, n=39	24 *	61.5	29	74.3
Purportedly healthy, n=33	5	15.1	24	72.7

Table 5. Sensitization incidence due to nasal colonization of the NARC workers caused by airborne fungi.

*: Indicates significant differences when comparing fungal sensitization between personnel considered patients and those purportedly healthy, according the Student's test ($p \leq 0.05$).

Discussion

Workers who perform tasks in any archive or library handle documents on a daily basis, primarily on paper (manuscript, printed matter, engravings, photographs, etc.) although they may also be on other media (audiovisuals, magnetic tapes, CDs, DVDs, etc.) that are still being apparently clean, they may have viable fungi on their surface, therefore they not only touch them with their hands but also spread them and breathe them when they leaf through or move these materials since the aerosolization and dispersal of their propagules (spores, hyphal fragments, other fungal fragments) is favored. Hence, the different biological agents or fragments of these (bacteria, viruses, fungi, insects, pollens and other animals) that are present in the archive environment can be inhaled by individuals [21-22] so it must be considered that workers are exposed to biological risk.

Fungi are dangerous to both people and collections. In addition, fungi are considered to be powerful sensitizers since exposure to their propagules can lead to allergies, even in people not prone to them. These fungi also produce toxic and allergic effects different from mycoses [23- 27].

The NARC preserves more than 27 linear km of documents of historical, cultural and scientific interest and several dozen are requested daily by users and researchers. This means that archivists and the rest of the personnel involved in the service are exposed to a significant number of allergens of a fungal nature [28-30]. However, other specialists such as digitize documents, restorers, researchers, etc., who do not always work providing service, are also exposed to these types of environmental allergens.

Although the majority of professional risk management entities in Cuba and in the world have carried out evaluations of specialized occupational

risk factors in various areas of companies, none have been focused on archive workers. Even if multiple studies of the behavior of environmental fungi have been carried out at the NARC, these have been directly related to the environment of the repositories that preserve documents and only on one occasion were they analyzed in common areas such as hallways, offices, reading room, etc., where the archive staff and users work most of the time [31], but a study had never been carried out that overall analyzed the behavior of environmental mycological diversity in common work areas with allergological analysis and the possibility of the fungal colonization of nostrils. Therefore, this is the first research carried out in Cuba where these three elements were taken into account: the airborne mycobiota from common work premises, the mycobiota that colonizes the nostrils and sensitization to fungal allergens in the NARC staff.

On this occasion, offices and premises were selected characterized by being closed environments that have individual air conditioning in each area, have a certain accumulation of dust derived from the handling of documents and the movement of people, with RH and variable temperatures, among other factors; all of these conditions are favourable for the existence of fungal propagules and mites in the environment, and also promote their viability and proliferation. However, air conditioning not only provides a certain thermos-hygrometric stability but also thermal comfort to personnel during the working day.

Though for some years now, multidisciplinary groups of scientists have dedicated efforts and time to quality studies of the indoor environment of premises due to the potential risk that the presence of high concentrations of fungi in these environments implies for human health and the integrity of cultural heritage, currently there is no international consensus regarding regulations that establish limit values that all own indoor environment to be classified as contaminated.

However, more and more researchers follow the criterion of comparing the concentrations of indoor environments with the outdoor environment (I/O ratio) to determine environmental quality [32-36]. This principle was taken into account in this study, although the results were also compared with the criteria [37] to evaluate the environmental quality of the studied spaces.

In relation to the environmental quality of the premises, variable results were obtained. The I/O ratios of some analyzed premises showed values ≤ 1.2 (PLO, ML and CO), indicative that they had good environmental quality [38], while in others the values were ≥2 (PTCO, RO and RR), which shows that they are contaminated and have a very low air exchange with the outdoor, demonstrating poor environmental quality [39- 41]. By following the [41] criteria, it was evident that the concentration of the *mycobiota* in three offices studied exceeded 560 CFU/m^3, which makes these places classified as highly to moderately contaminated. They showed high contamination PTCO, RO and RR, while with low contamination PLO and ML were maintained, and CO revealed a medium environmental quality. As demonstrated by the comparison of the obtained results in this study according to the two aforementioned criteria, the behavior of the environmental quality were very similar.

As is known, the RH of the air is of great importance for environmental microbial contamination [42],

especially in the case of the fungi, where it affects the level of sporulating, the aerodynamic diameter of the spores and in their sedimentation on the surfaces and even in the respiratory system of people [43]. Taking into account that the RH values in most of the workplaces were close to or greater than 60%, it can be inferred that the environmental conditions of these premises facilitated the presence of high concentrations of viable fungi in the air, which is risky for the health of the personnel who usually work in these offices. From the above, it can be inferred that the air-conditioned premises analyzed in the NARC could be risky for the health of the staff if they are not ventilated for a while at some time of the day and if the air conditioning equipment are not properly clean, since it has been reported by some authors that office air conditioning can be related to high concentrations of fungal propagules and Sick Building Syndrome (SBS) [46].

The airborne mycobiota isolated from the NARC premise and offices showed a predominance of the phylum Ascomycota [47] typical of marine and coastal environments according to [47]. The ecological predominance of the genera *Aspergillus*, *Penicillium* and *Cladosporium* coincides with that reported by other authors [48], since they are considered genera that are part of the indoor mycobiota [49] and in addition, they are primary colonizers of different supports and in particular of the paper that makes up documents [50]. It is worth highlighting the predominance of the species *A. flavus*, *F. oxysporum* and *C. cladosporioides* within the climate-controlled premises, all of them reported as allergenic and opportunistic pathogens even *A. flavus* is a species considered to have biohazard level 2.

Regarding the predominance of individual species, some authors have reported that concentrations of 50 spores/m^3 for *Alternaria*, 500 - 1500 spores/m^3 for *Cladosporium* and loads greater than 50 spores/m^3 for *Aspergillus* are potentially associated with SBS. However, [51, 52] indicated as permissible concentrations for indoor environments 50 CFU/m^3 for a single species, 150 CFU/m3 for mixtures of several fungal species and 500 CFU/m^3 only for *Cladosporium*. But, in this study it was evident that most species showed concentrations lower than or equal to 50 CFU/m^3 while a few exceeded this value. Most *Aspergillus* and *Penicillium* species showed concentrations less than or equal to 50 CFU/m^3, considered low values according to Canadian regulations. Among the species with high concentrations, *F. oxysporum* was found, which in RR showed a RD of 30% equivalent to a concentration of 122 CFU/m^3 and in RO with a RD of 9% similar to a concentration of 113.4 CFU/m^3, *A. flavus* in PTCO that revealed a DR of 30% equal to a concentration of 244 CFU/m^3, as well as *Cl. cladosporioides* in RO with a DR of 25% equivalent to a concentration of 110 CFU/m^3 and in CO with a DR of 10% that was equal to 126 CFU/m^3. Although some species showed concentrations higher than 50 CFU/m^3, it can be stated that the sites studied did not show symptoms of SBS.

However, although at low concentrations health-risk species were detected, such is the case of *A. alliaceus, A. candidus, A. japonicus, A. niger, A. penicillioides, A. unguis, Cl. cladosporioides, Cl. elatum, Cl. herbarum, Cl. oxysporum, Cl. sphaerospermum, C. lunata, E. castellanii, F. oxysporum, F. solani, M. racemosus, N. sphaerica, P. chrysogenum, P. citrinum, P. commune* and *P. griseofulvum*. However, these results were

taken into account to make some constructive adjustments within them, fundamentally related to the revitalization of the operation of the windows to facilitate their opening once a day and thereby renew the indoor air from the exchange with the outdoor air.

Regarding nasal colonization, fungi colonizing the nostrils with preponderance of the *Aspergillus* genus and in particular the species *A. flavus* and *A. niger* were evident for the first time in Cuban archive workers. This result does not coincide with other studies carried out previously, where the predominant genera were *Cladosporium* and even *Penicillium* was the dominant genus in workers of Colombian archives, although *Aspergillus* was present among the three genera with the highest percentage. The same occurred in a study made in Poland with archive and library workers, where *Penicillium* and *Aspergillus* turned out to be the predominant genera in that order.

In Cuba, where asthma affects 90.9 individuals per 1000 inhabitants of the population, the determination of the asthma prevalence in archive workers, the allergic sensitization and the identification of fungal species in the mycobiota nasal is the first study carried out in this work sector. It should be noted that the diversity of genera and species isolated from the nostrils of the NARC workers was much lower than that obtained in a previous investigation carried out by other authors, but it was very similar to that reported by [52], since 13 species were isolated corresponding to 7 genera (*Aspergillus*, *Candida, Cladosporium, Chrysonilia, Mucor, Penicillium* and *Rhizopus*). However, to a certain extent, the prevalence of the species *A. flavus* and *A. niger* in the nostrils is in correspondence with the species existing in the environment of the offices studied, and even in the PTCO *A. flavus* was the predominant species. It is known that *Aspergillus* genus is one of the main pollutants of indoor environments, capable of growing even in conditions of nutritional deficiency, and due to the small size of its spores, some species can penetrate to the alveoli; furthermore, it is responsible for approximately 70% of cases of respiratory diseases in the world.

It is important to highlight that in this study only airborne species were evaluated and the species existing in the settled dust or those that sedimented on the documents surface were not taken into account, which may also be affecting the health of the personnel by being aspirated during of documents handling despite the fact that they could be apparently clean, since it is known that during their handling the dust and fungal propagules existing in them are re- dispersed.

The level of exposure is influenced by the time spent in the archival environment, the time of operations or the tasks performed (cleaning, document restoration, etc.) and the time of contact with the documents of all of them, it was considered that the average exposure time is undoubtedly, an important element when it comes to occupational risk in this sector. Several authors have examined the association between exposure of indoor occupants to mites, mold, and other contaminants in moist or water-damaged indoor environments and respiratory symptoms (cough, wheeze, asthma symptoms, upper respiratory tract symptoms) and they have found no evidence of association. However, found sufficient evidence of a causal relationship between exposure to mold and other pollutants in humid indoor environments with the asthma. The report of 39

NARC workers (51.2%) as patients who have been diagnosed with diseases where allergic predisposition predominates becomes an important factor directly related to fungal exposure. In a study carried out by with the participation of 174 patients whose common factor was occupational exposure to documents and the archival environment, symptoms of asthma and/or allergic rhinitis were evident.

Environmental fungi have a significant allergenic capacity and in atopic subjects they can cause asthma and rhinitis. It is proposed that approximately 5% of the world's population may present symptoms of fungal allergy throughout their life (WHO 2009), hence the importance of knowing which sensitizing allergens are and establishing the relationship between exposure and the possibility of developing an allergic process. This explains the incidence of asthma as the most prevalent disease among NARC workers (22 workers) followed by chronic rhinitis and allergic conjunctivitis, and is demonstrative that the studied population has a higher prevalence of asthma than that recorded for Havana and the country (113.3 per 1,000 inhabitants), possibly due to the conditions of the indoor environments of the archives characterized by a high concentration of dust and the existence of a complex microbial ecosystem where there is a large amount of the bioparticles diverse.

In this study, of all the investigated workers, the female sex predominated, with an average age of 46 years. This trend coincided with the report by who studied a sample made up of 53 archive and library workers at the Havana University, with a predominance of female sex (85.7%) and an average age of 36 years, in whom allergic rhinitis was the most frequent disease, followed of the combination of asthma and rhinitis. This is something very common in Cuba if it is taken into account that 94.1 asthmatic patients per 1000 inhabitants are female. On the contrary, in the study carried out by where 174 workers with reports of allergic diseases were evaluated, the male sex predominated and an average age of 37.7 years with symptoms of asthma or allergic rhinitis. The common factor to take into account in all cases was work exposure to documents and the archive environment. Another important aspect is to consider the comorbidities of asthma and in particular rhinitis. Epidemiological studies have unequivocally shown that asthma and rhinitis often coexist in the same patients in all regions of the world. Most asthma patients have rhinitis (eight out of ten). However, in this study the impact of this comorbidity was not as high, as it was only evident in 51.3% of the reports related the degree of sensitization and exposure to fungal allergens with the severity of diseases such as asthma in patients sensitized to fungi. In patients diagnosed by doctors with severe persistent atopic asthma, allergic sensitization to fungi, determined by skin tests and specific serum IgE, can be detected in up to 66% of them. Fungal allergy has been associated with fatal or near-fatal asthma (life-threatening asthma), visits to hospital emergency rooms, and admissions to Intensive Care Units.

In evaluations made in the outdoor environment of Havana, it was found that the most abundant and frequent genus was Cladosporium, followed by *Aspergillus*, *Penicillium, Curvularia, Fusarium* and *Alternaria* while, in indoor environments and particularly in Cuban archives, the genera *Aspergillus*, *Cladosporium* and *Penicillium* have been the most abundant in that order. These studies that have been

carried out in Cuba allow us to compare the evident predominance of these genera as the most frequent colonizers in the nasal mycobiota. A coincidence was found with what was previously reported by regarding sensitization to the *Cladosporium genera*, followed by *Aspergillus*, *Penicillium* and *Alternaria* in last place, which shows the risk due to high exposure to environments with high concentrations of fungal spores or for a long time. This is why of the total staff number with positive skin sensitivity tests to fungal extracts, 9 workers had polysensitization to more than one fungal extract. Polysensitization could be attributed to multiple exposures, since both in the air and in the dust indoor the archives, several fungal species frequently coexist, sharing the same ecological niche in an evaluation study of fungal sensitization in schoolchildren with atopic diseases, they found *Penicillium* genus as the one that showed the most sensitization. Likewise, in the present investigation, polysensitization to *Penicillium chrysogenum, Cladosporium herbarum, Candida albicans* and *Aspergillus fumigatus* (in that order) was found more frequent than to the rest of the allergens analyzed, coinciding with similar previously reported results. Similar evaluations were carried out by to compare the presence of environmental fungi and sensitization to allergic by determining specific IgE. when determining the existence of viable allergenic fungi in a document repository of NARC, they reported prevalence of the *Aspergillus*, *Cladosporium, Penicillium* and *Alternaria* genera, referred as highly allergenic fungi, and showed that some of their spores could reach the lower respiratory tract, which accentuates the allergenic and pathogenic potential of these fungi.

The nasal entrance door for fungal spores should reflect the dominant atmospheric microbiota because the nasal mucous membrane is the first barrier againstinhaledbioaerosols. When fungal propagules are in-haled they are trapped in the nose cilia, thus putting them in direct contact with the nasal mucosa. In allergic patients the mechanical factor of the use of napkins or hand scarves for rhinitis can influence the drag of inhaled fungal particles. Contrast the fact that most respiratory allergies with associated fungal sensitization are reported for *Alternaria alternata* and yet this species was not isolated in the nasal samples of the studied personnel of NARC. This fact suggests that there should not be a direct relationship between the fungi of the nasal mucosa and the sensitization to a certain species. Situation that could be variable according to the moment in which the sample and the phenomenon of cross-reactivity are taken. Therefore, monitoring with serial crops to the same subject throughout the year could provide valuable information about these variations.

The variability of the obtained results suggests the opportunity to carry out new studies in the same individual allergic to one or more fungal species, where the monitoring of serial cultures shows the modifications of the nasal mycobiota that occur over time, the possible relationship with clinical manifestations and the evident exposure.

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