Indoor-outdoor fungal-aerosols ratios of domestic homes in Merida, Mexico

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ABSTRACT
The aim of this study was to establish the relation between indoor and outdoor fungal aerosols in domestic homes in Merida, Yucatan, Mexico. Air samples were collected, using the 6-stage Andersen impactor, inside and outside thirty domestic homes. The humidity and the temperature are factors that affect the development of fungi. The relative humidity varied from 32 to 93% outdoors and from 40 to 70% indoors; while the temperature varied from 14 to 34°C and from 18 to 29°C, respectively. The relative humidity during the monitored period was close to the ideal conditions for the development and growth of fungal spores. Sixteen fungal species were isolated from these samples. The main species found were *Cladosporium spp., Fusarium spp., Acremonium spp., Alternaria spp.,* and *Bipolaris spp*. *Cladosporium* was present inside 53% of the homes studied and 70% of their outdoors. The second most common fungal species was *Fusarium*, present in 43% of the indoors and 14% of the outdoors. It was found that indoor and outdoor fungal concentrations ranged from 264 to 17788 CFU/m^3 and from 123 to 5771 CFU/m^3, respectively. Breathable fungal particles amounted to approximately 79-98% of the total fungal concentrations. The percentage of breathable fungi is generally not dependent on the type of home (1 or 2 stories) or their orientation. Most of the indoor-outdoor ratios were close to one. Higher indoor concentration levels in the I/O ratio observed for airborne fungi in four houses were associated with indoor sources and the ratios lower than 1 were related with higher penetration of fungal spores from outdoor to indoor environment.

***Keywords**: Bioaerosols, bacteria, fungi, indoor air, mould problem.*

Relación interior-exterior de esporas fúngicas en viviendas en Mérida, México

RESUMEN
El objetivo de este estudio fue establecer la relación interior/exterior de aerosoles fúngicos en viviendas de la ciudad de Mérida, Yucatán, México. Las muestras de aire fueron recolectadas usando el impactor Andersen de 6 etapas, dentro y fuera de 30 viviendas. La humedad relativa del aire y la temperatura son factores que afectan el desarrollo de hongos. La humedad relativa en las viviendas muestreadas varió en un rango entre 32 a 93% en exteriores y de 40 a 70% en interiores, mientras la temperatura varió de 14 a 34 °C y 18 a 20°C, respectivamente. La humedad relativa durante el periodo de monitoreo estuvo cercana a las condiciones ideales para el crecimiento y desarrollo de hongos. Dieciséis géneros fúngicos fueron aislados de estas muestras. Los principales géneros encontrados fueron *Cladosporium spp., Fusarium spp., Acremonium spp., Alternaria spp.* y *Bipolaris spp*. El género *Cladosporium* se encontró en un 53% en el interior de las viviendas y en un 70% en el exterior. El segundo género fúngico más común fue *Fusarium* que se presentó en un 43% en interior y en un 14% en exterior. Las concentraciones totales de esporas fúngicas en interior y exterior variaron en un rango de 264 a 17788 UFC/m^3 y de 123 a 5771 UFC/m^3, respectivamente. La fracción fúngica respirable se mantuvo entre un 79 y 98% de la concentración total de esporas.

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El porcentaje de esporas respirables de manera general no fue dependiente del tipo de vivienda (1 ó 2 niveles) ni de su orientación. La mediana de la relación interior-exterior estuvo cercana a uno. Los niveles superiores a uno observados en cuatro viviendas fueron asociados con fuentes de contaminación interior y las relaciones con niveles inferiores a uno encontradas se asociaron con la penetración de esporas fúngicas del exterior al ambiente interior.

Palabras clave: *Bioaerosoles, bacterias, hongos, aire interior, problemas de humedad*

INTRODUCTION

Amongst the different kinds of bioaerosols, fungi represent a heterogeneous group, which plays an important role in human pathology (Carrera *et al*. 2001). These microorganisms can affect human health in a variety of ways. Possible reactions generally fall into one of three groups: allergic reactions (sensitization and immune responses, i.e., asthma, allergic rhinitis, or hypersensitivity pneumonitis), infections (growth of the fungal infections, e.g., aspergillosis), and toxic responses (Gorny *et al.* 2002). Epidemiological investigations have shown that these diseases are often associated with exposure to large concentrations of airborne microbes (Carrera *et al*. 2001). However, infectious diseases caused by inhalation of different fungi depend, not only on the number of spores inhaled, but also on the site of their deposition in the respiratory system. Because the deposition site of particles is directly related to the aerodynamic diameter of the particle (fungal spore), particles smaller than 5 μm, the so-called breathable fraction, are able to penetrate into the alveoli and can lead to allergic alveolitis and other serious illnesses (Pastuszka *et al.* 2000).

Generally, outdoor air is the dominant source of indoor fungi. The fungal spores enter a building through outdoor-air intakes, i.e., the air conditioning system, through doors and windows and by means of contaminated building materials and contents (Shelton *et al.* 2002). If elevated moisture conditions exist indoors for a sufficient time period, fungal growth and sporulation may occur. Pasanen *et al.* (1991) found that temperature of 21–30ºC and relative humidity of 75-92% could induce a rapid fungal germination and growth. Therefore, it is considered that bio-aerosols are much easier to grow in areas with humid and warm climate.

The city of Merida in Yucatan, Mexico is characterized by a humid and warm climate with an annual mean temperature of 27ºC and a relative humidity of 80%. The aim of this study was to establish the relation between indoor and outdoor fungal aerosols in domestic homes in Merida, to guide future determination of criteria for assessing indoor air quality in regions with this climate.

MATERIALS AND METHODS

Selection of sampling site. Measurements were carried out during February 2008 in houses constructed a few years earlier (1 to 3 years before the research), in a residential neighbourhood located at the north section of Merida city in Yucatan, Mexico. Thirty houses with and without mould problems were selected on the basis of the interest shown by the residents to participate in the study. Three groups of sampling sites were formed, the first group consisted of houses reported with the presence of visible mould problems in different locations (e.g., on the walls, ceilings, floors, furniture and clothes). The second group was composed by houses where the growth of fungi had only affected clothes, shoes and furniture, but had not been observed in the structure of the house, and had a smell of mould inside them. Lastly, the third group or control group was formed by houses where the residents reported smell of mould, but they had not reported any visible mould problems. All homes presented the same type of windows and building materials; however, the homes had different histories of previous moisture damage, types of construction (1 or 2 stories) and/or orientations (see Table 1).

Measurement of indoor and outdoor fungal aerosols. Air samples were collected using the 6-stage Andersen impactor with aerodynamic cut-size diameters by level of 7, 4.7, 3.4, 2.1, 1.1 and 0.65 μm. Samples were taken inside and outside of domestic homes. Fungal spores were collected on Petri-dishes with Malt extract agar (MEA) with 0.1 g/L of novobiocin to inhibit bacterial growth. The sampling period was 4-min, drawing air at a flow rate of 28.3 L/min (corresponding to a velocity of 24 m/s). During sampling, the temperature and relative humidity were monitored and variability of these environmental factors was observed during the 5 following days.

Sample analysis. After air sampling, the Petri-dishes were incubated for 3 days at room temperature. The total concentration was calculated by dividing the volume of air sampled into the total number of colonies observed on the plate and was reported as colony forming units per cubic meter of air (CFU/m^3) using positive hole correction. The species of fungi,

were identified based upon their micro- and macro-morphological characteristics, using standard taxonomic keys (De Hoog *et al*. 2000; Dugan 2006; Barnett and Hunter 1998).

Table 1. Sampled houses and its corresponding characteristics.

	TYPE OF HOUSE		
	Mould problems on structure and personal property (Group 1)	Mould problems only on personal property (Group 2)	Absence of mould problems. Occasional smell of mould (Group 3)
House Number (HNN)	H3 – IH, SN, OS, NAC, WP H4 – IH, SN, OS, NAC, WP H5 – IH, NS, OS, AC, WP H6 – IH, EW, OS, AC, WP H8 – IH, EW, OS, NAC, WP H9 – IH, SN, OS, NAC, WP H10 – IH, NS, OS, AC, WP H11 – UH, NS, OS, NAC, WP H12 – IH, SN, OS, NAC, WP H15 – UH, SN, OS, NAC, WP H17 – IH, EW, OS, NAC, WP H21 – IH, NS, OS, NAC, WP H22 – IH, SN, OS, NAC, WP H24 – IH, SN, OS, NAC, WP H25 – IH, SN, OS, NAC, WP	H19 – IH, NS, TS, NAC, WP H20 – IH, WE, OS, NAC, WP H23 – IH, EW, OS, NAC, WP H27 – IH, SN, OS, NAC, WP	H1 – IH, EW, OS, AC, NWP H2 – UH, NS, OS, NAC, WP H7 – UH, NS, OS, NAC, WP H13 – IH, NS, OS, NAC, WP H14 – IH, NS, OS, NAC, WP H16 – UH, NS, OS, NAC, WP H18 – UH, SN, OS, NAC, WP H26 – IH, EW, TS, AC, WP H28 – IH, SN, TS, AC, WP H29 – IH, NS, OS, NAC, WP H30 – IH, NS, OS, NAC, WP
REMARKS:	IH – Inhabited UH – Uninhabited NS – Oriented with front facing North and back towards South SN – Oriented with front facing South and back towards North EW – Oriented with front facing East and back towards West WE – Oriented with front facing West and back towards East OS – One storey TS – Two storey AC – with air conditioning system NAC – without air conditioning system WP – Roof with waterproof coating NWP – Roof without waterproof coating		

RESULTS AND DISCUSSION

The concentrations of fungal aerosols in the indoor and outdoor environment for the three groups of homes studied are shown in Table 2. It was found that indoor and outdoor fungal concentrations ranged from 264 to 17788 CFU/m^3 and from 123 to 5771 CFU/m^3, respectively. The results showed that, in the three groups the concentrations of fungi were similar and depended strongly on sampled environment. The increase of outdoor concentrations resulted in a corresponding increase of indoor concentrations in most houses. However, indoor fungal concentrations were an order of magnitude higher than those outdoors.

In homes selected for groups one and three, the geometric mean of the concentration of fungal aerosol indoor and outdoor were near 2000 CFU/m^3 and were distinctively higher than the geometric mean concentrations of airborne fungi in group two, which were approximately 1060 CFU/m^3 indoor and 642 CFU/m^3 outdoor.

Oddly, the maximal concentration of fungal aerosol reached almost 17700 CFU/m^3 and was measured inside a house of group three, where the residents did not report any visible mould problem. The indoor concentration of this house could have been influenced by the construction activities in close proximity to the sampling point.

Breathable fungal particles amounted to approximately 79-98% of the total fungi concentration. The percentage of breathable fungi was generally not dependent of home groups (1-3), sampled environment (indoor/outdoor), and the type of construction (1 or 2 stories) or their orientation.

Table 2. Fungal aerosol in indoor and outdoor environment in Merida, Mexico.

		Indoor				Outdoor			
		Min	Max	Geom. Mean	Median	Min	Max	Geom. Mean	Median
Group one (n = 15)									
Total fungi	(CFU/m^3)	714	9242	2003	2247	714	3348	1628	1533
Breathable fungi [a]	(CFU/m^3)	678	9128	1916	2163	670	3260	1549	1366
Breathable fraction	(%)	92	99	96	95	89	98	95	96
Group two (n = 4)									
Total fungi	(CFU/m^3)	564	2062	1060	1049	123	1630	642	943
Breathable fungi [a]	(CFU/m^3)	546	1991	1016	996	97	1498	567	855
Breathable fraction	(%)	94	97	96	97	79	98	88	89
Group three (n = 11)									
Total fungi	(CFU/m^3)	264	17788	2145	1908	493	5771	2019	2053
Breathable fungi [a]	(CFU/m^3)	247	17272	2014	1823	476	5533	1922	1974
Breathable fraction	(%)	91	97	94	93	90	97	95	96

[a] Viable, fungal particles with aerodynamic diameter < 5 μm. Min: minimum; Max: maximum; Geom. Mean: Geometrical mean. n is number of domestic homes where fungi were analyzed.

There are some international proposals for an upper limit of the normal indoor concentration of airborne fungi, but due to the particular climate of the region, it can be expected a different "normal range" of fungal aerosols in Merida homes. The fungal aerosol concentrations in Merida, Mexico were similar to those from other reports of cities with comparable climate (fungal spores geometric mean of near 1000 CFU/m^3) i.e., Yokohama, Japan (Chih-Shan and Yu-Mei 1993), Taiwan (Pei-Chih *et al*. 2000), Korea (Lee and Jo 2006); but significantly higher (from 1.2 to 1000 times) to those reported from others cities with cold climate, i.e., Upper Silesia, Poland (Pastuszka *et al*. 2000), Santa Fe, Argentina (Basilico *et al*. 2007) and diverse cities of the United States (Tsai and Macher 2005).

The indoor/outdoor ratios (I/O) for fungal aerosols are shown in Table 3.

Table 3. Indoor/outdoor ratios for fungal aerosols calculated in domestic homes situated in Merida, Mexico.

	Indoor/ Outdoor			
	Min	Max	Geom. Mean	Median
Group one (n = 15)	0.51	7.39	1.23	1.04
Group two (n = 4)	0.76	7.52	1.65	1.14
Group three (n = 11)	0.16	3.29	1.06	1.24

Min: minimum; Max: maximum; Geom. Mean: Geometrical mean. n is number of domestic homes where fungi were analyzed.

As it is shown in Table 3, although the minimum and maximum values of the I/O ratios obtained for houses in the third group differ from the other two groups, which have very similar minimum and maximum values, the median value is very close to the ones obtained for the other two groups.

The average I/O ratios for total fungal spores determined for each house sampled are presented in Figure 1. The I/O ratios for fungal spores were different amongst houses but not different amongst groups given that median values of the ratios obtained for each were close to 1. This value indicates a balance between the particles found inside with those found outside the houses. Higher indoor concentration levels in the I/O ratio observed for airborne fungi in four houses are associated with indoor sources and the ratios lower than 1 are related with higher penetration of fungal spores from outdoor to indoor environment.

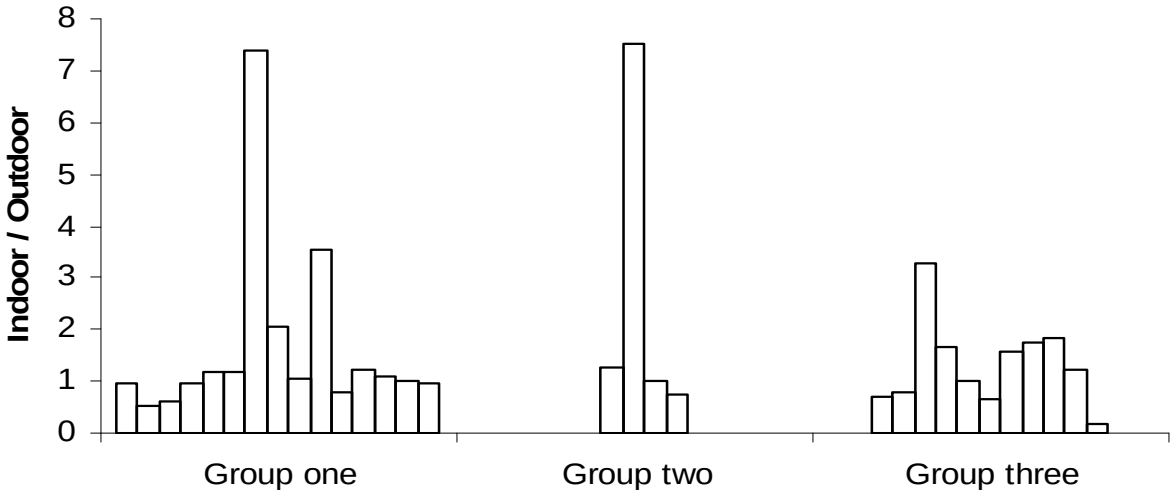

Figure 1. Indoor/outdoor ratios for total fungal spores in each home calculated for the three groups.

The humidity and the temperature are factors that affect the development of fungi. Therefore, these factors were monitored during 5 days, and the results showed that, relative humidity varied from 32 to 93 % outdoors and from 40 to 70% indoors; the temperature, from 14 to 34°C and from 18 to 29°C, respectively. The relative humidity and temperature during monitoring periods were near the ideal conditions (relative humidity of 75-92% and temperature of 21–30 ºC) for the development and growth of fungal spores.

An important fact of the microbial pollution in mouldy houses is that their occupants may be exposed to higher concentration of fungal spores than residents of healthy homes, which could increase their risk of developing asthma or different respiratory symptoms. However, asthma and allergic rhinitis may be caused not only by the quantity of mould exposure but also by the contact with some specific genera. Therefore, it is necessary to assess the identification of fungal spores found indoors and outdoors domestic environments.

Figure 2 shows the fungi genera found. Fifteen known fungal species were isolated, twelve were found outdoors and fourteen indoors. The main species found were *Cladosporium spp., Curvulalria spp., Fusarium spp., Penicillium spp.* and *Acremonium spp*. The *Cladosporium* was present in 53% of the indoors studied and 70% of the outdoors. The second most common fungal species found was *Fusarium*, present in 43% of indoors and 37% of outdoors sampled.

Other fungi species as *Alternaria spp., Bipolaris spp.* and *Aspergillus spp.,* were present in approximately 20% of the homes. Also, *Absidia spp., Chrysosporium spp., Geotrichum spp., Mucor spp., Rhizopus spp., Scopulariopsis spp.* and *Trichophyton spp.* were found, but in fewer occasions (less than 10%).

The *Aspergillu*s, present in some of the houses studied, is used by several countries as an indicator of the quality of indoors air due to pathologic characteristics of some of its species.

Further research is required to examine the effects that the presence of fungal spores found may cause and how exposures to them may be affecting related health problems. However, the fungal spores, *Cladosporium, Alternaria, Aspergillus,* and *Penicillium,* frequently isolated from chronic rhinosinusitis patients (Shin *et al*. 2007), are considered to be the most allergenic fungi in environmental air (Gomez de Ana *et al*. 2007).

The fungal species identified in this work are comparable to those published about countries with comparable climate; in these, members of the *Cladosporium* genus were found predominantly, i.e. Lee and Jo (2006) in Korea, Pastuszka *et al.* (2000) in Poland, Pei-Chih *et al*., (2000) in Taiwan and Takahashi (1997) in Japan.

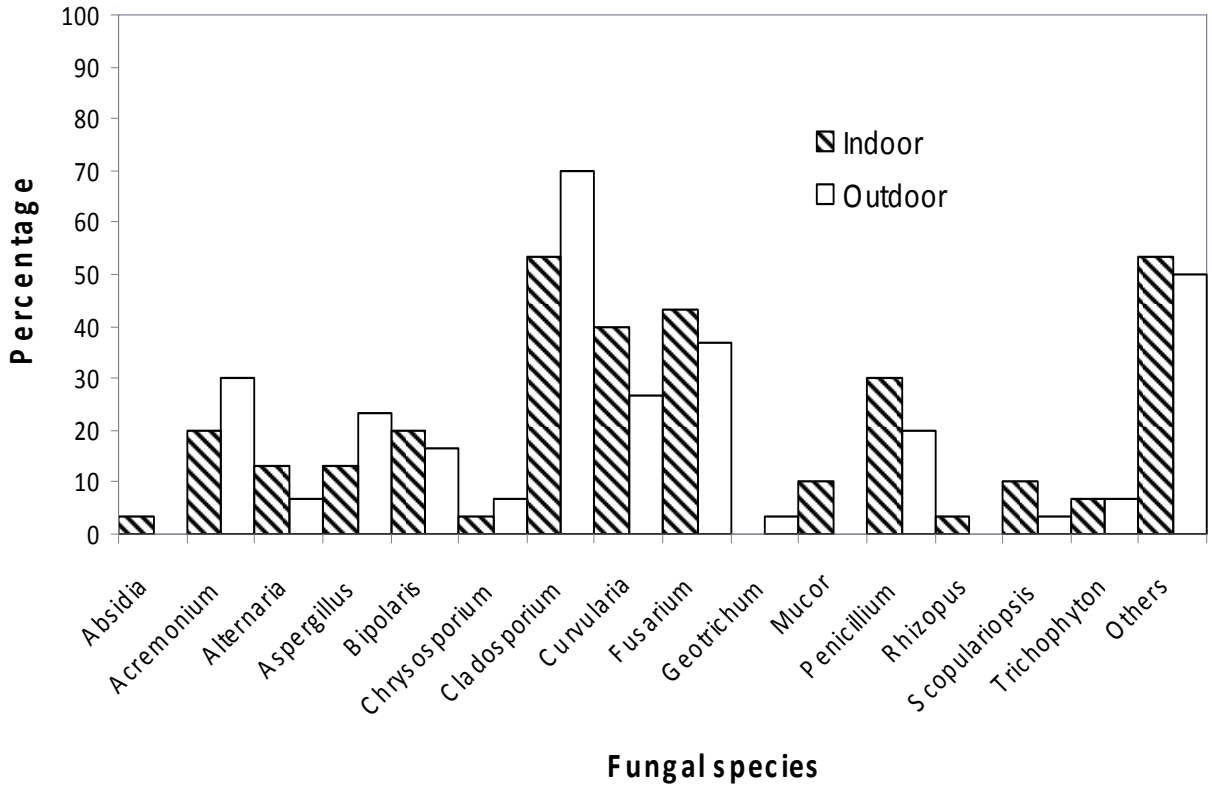

Figure 2. Viable fungi identified in 30 houses (indoor and outdoor environment). Percentage of presence in all houses sampled versus fungal species

SUMMARY AND CONCLUSIONS

The typical concentration of fungal spores in air, inside and outside domestic homes in Merida during February, was 1000 CFU/m^3. The concentrations obtained for the three groups of houses sampled were similar and were dependent on sampled environment (indoor or outdoor air). Most of the indoor/outdoor ratios were close to 1 and not dependent of group of homes. Breathable fungal particles were found in higher levels than any others (between 79 to 98% of the total). The concentrations observed, as well as the fungal species identified in the different houses, seem to be a threat to residents of all the groups of houses studied given that they are related to diverse health pathologies.

The bioaerosol concentrations in Merida, Mexico are similar to those from other reports of cities with comparable climate, but significantly higher than those reported from others cities with colder climate. In indoor and outdoor air the predominating genus was *Cladosporium spp.* which is frequently associated to diverse pathologies. Further research is recommended to examine the effects of these exposures on related health problems.

The presented data of fungal spores quantified and identified indoors and outdoors domestic houses can help to determine some criteria for assessing indoor air quality in Merida. Furthermore, the data can be incorporated into existing models to quantify the penetration of biological particles into indoor environments from outdoors. However, for this, it is necessary to determine the seasonal variability of the airborne fungi as well as the environmental factors that can influence their growth.

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