

Fungal spore concentrations in indoor and outdoor air in university libraries, and their variations in response to changes in meteorological variables

María Elena Báez Flores^{a*}, Pável Gaxiola Medina^a, Sylvia Páz Díaz Camacho^a,
Magdalena de Jesús Uribe Beltrán^a, María del Carmen De la Cruz Otero^a,
Ignacio Osuna Ramírez^a and Martín Ernesto Tiznado Hernández^b

^aFacultad de Ciencias Químico Biológicas, Universidad Autónoma de Sinaloa, Culiacán, México;

^bCentro de Investigación en Alimentación y Desarrollo, Hermosillo, México

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The fungal spore concentration (FSC) in the air poses a risk for human health. This work studied the FSC in university libraries and how it is affected by environmental factors. A total of 347 samples were obtained using a Microbio MB2[®] Aerosol Sampler. The wind speed (WS), cross wind (CW), temperature (T), relative humidity (HR), barometric pressure (BP) and dew point (DP) were recorded using a Kestrel[®] 4500 weather station. The median indoor/outdoor FSC was 360/1230 CFU m⁻³. FSC correlated inversely with BP, HR and DP; and positively with WS and CW; whereas T showed negative or positive correlation with FSC, depending on the region or sampling time. Eleven fungal genera were found and the dominant isolates were identified as *Aspergillus niger*, *Aspergillus tamarii* and *Aspergillus oryzae*. All fungi identified are known to be allergenic. It was concluded that environmental variables can influence the air FSC in different ways.

Keywords: indoor and outdoor air; airborne fungal spores; microbial air quality; fungi and environmental variables; bioaerosols

Introduction

Microbiological evaluation of air has attracted attention in the recent decades because the aerial microbial population has been associated with diverse detrimental effects on health including respiratory infections, allergies and immunotoxicity (Chen et al. 2011). There are numerous biological particles in the atmosphere including pollens, viruses, bacteria and fungi (Gonçalves et al. 2010). Considering that the adult man may inhale 10,000 L of air daily, the inhalation of these agents can be elevated (Soto et al. 2009). By comparison, exposure to fungal spores is higher compared to pollen grains. In addition, the smaller size of spores enables them to penetrate deeper into the bronchial tree, which may cause allergic reactions in the upper and lower respiratory tracts (Gutarowska et al. 2005). The main fungal genera known to be allergenic are *Alternaria*, *Aspergillus*, *Cladosporium* and *Penicillium* (Lizaso et al. 2003). Additionally, several studies have shown that sensitisation to fungal spores is a risk factor for asthma development (Lin & Williams 2003). In this regard, it has been demonstrated that the outdoor *Alternaria alternata* spore concentrations are significantly correlated

*Corresponding author. Email: elenabf@uas.edu.mx

with monthly frequency of asthma (Kilic et al. 2010). Moreover, *A. alternata* and *Aspergillus niger* are known to produce mycotoxins (Kalyoncu 2008), which could have carcinogenic, teratogenic, mutagenic, estrogenic, nephrotoxic and neurotoxic effects in humans (Urrego Novoa & Díaz 2006). This clearly highlights the potential negative consequences of indoor and outdoor fungal spore concentrations (FSC) on public health.

The total number of fungal spores in the air varies from 200 to 1,000,000 CFU m⁻³ (Pontón et al. 2002); their air concentration is characterised by diurnal and seasonal cycles depending on environmental conditions, availability of fresh substrates for fungal development and circadian cycles of light and darkness (Kasprzyk 2008). According to earlier studies, the microbiological quality of indoor air is controlled by microbiological composition of outdoor air and indoor air microbial sources; whereas outdoor air is very much influenced by the environment, season, weather and even time of the day (Stryjowska-Sekulska et al. 2007).

The information obtained from fungal air samples can assist in the assessment of health hazards; however, there are limitations in interpreting results obtained from bioaerosol samples since there are no laws governing indoor bioaerosol fungal levels (Shelton et al. 2002). In this regard, currently available guidelines for fungal bioaerosols issued by the American Conference of Governmental Industrial Hygienists (ACGIH 1999) and Health Canada (Nathanson 1993) are considered useful in the interpretation of fungal sampling results. The ACGIH issued a 200 CFU m⁻³ guideline; whereas in Canada, up to 150 CFU m⁻³ is acceptable if there is a mixture of species reflecting the outdoor air spores. It is generally accepted that the microbial concentration in indoor air is similar to the outdoor values. Consequently, the indoor/outdoor concentration ratio (I/O) should be close to 1 and this ratio defines the air quality, with I/O equal to or less than 1.5 representing good indoor air quality; I/O of 1.5–2.0 is considered as regular indoor air quality and I/O greater than 2 indicating poor indoor ambient conditions (Stryjowska-Sekulska et al. 2007).

Fungi usually enter a building through outdoor air intakes of heating, ventilation, or air conditioning systems; through doors and windows and as contaminants on building materials or contents (Shelton et al. 2002). Research in a number of countries demonstrated that the interior of a building may contain up to 400 fungal species (Gutarowska et al. 2005). Additionally, it has been reported that higher microbial concentrations in buildings occur at times of increased flow of people (Stryjowska-Sekulska et al. 2007; Abdulla et al. 2008). One study in a university building found a sharp increase in fungi in lecture room air and bacteria in toilets at the opening of the academic year (Stryjowska-Sekulska et al. 2007). On the other hand, in a hospital environment study, the lowest fungal concentration was found in the operating room, while the highest was recorded in the kitchen (Perdelli et al. 2006).

Due to the impact of air content on human health, the relevance of air quality studies in terms of fungal concentration in commercial and industrial buildings, housing, offices, hospitals, child care centres, schools and universities, among others, has been increasing. Based on this evidence, the presence of fungal spores in library environments could represent a health risk for users and workers. This work was aimed to investigate the concentration and type of fungal spores in university libraries, as well as the effect of environmental variables on the spore concentration in air. It is expected that the information generated in this work will allow implementation of measures to minimise the presence of fungi in libraries and as a result reduce their possible adverse effects on the health of students and workers.

Materials and methods

Sampling design

This aeromycological study was carried out in 15 libraries located in the University Campus of the Universidad Autónoma de Sinaloa. The libraries were designated with letters as follows: A: Central, B: Law School, C: Economics, D: Odontology, E: Psychology, F: Architecture, G: Biology, H: Civil Engineering, I: Physics and Mathematics, J: Chemistry, K: Philosophy, L: History, M: Humanities, N: Informatics and O: Zapata High School library (Figure 1). The university campus has an area of 353,943.59 m² and is located at 24°49'46" latitude north, 107°23'2.8" longitude west and 24°49'18.84"

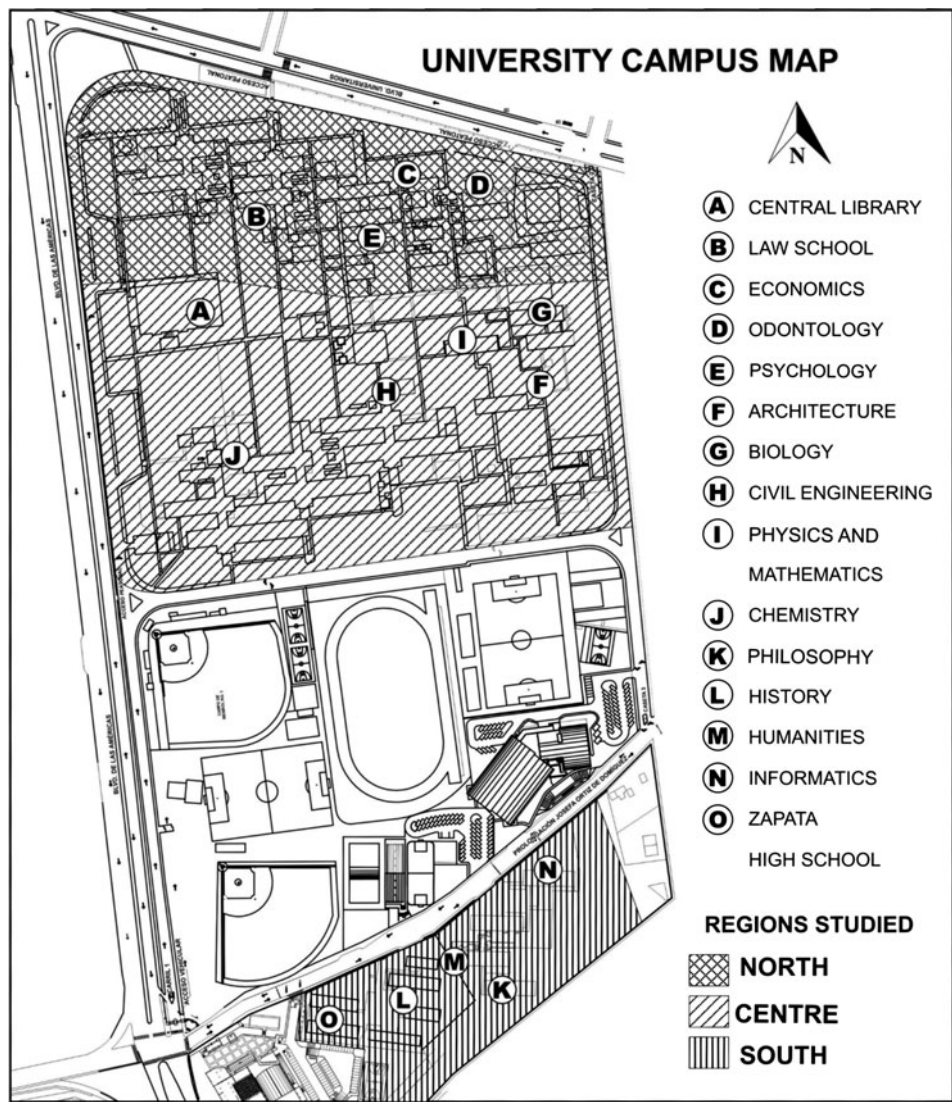


Figure 1. Map of the university campus indicating the north, centre and south regions, as well as the location of the sampled libraries in each region.

latitude north, 107°22'43.99" longitude west. This was a longitudinal, prospective, observational, descriptive and correlational study. For sampling purposes, the campus was divided in three regions: north, centre and south. Weekly from November to December 2010, a campus region was randomly selected for sampling. Within the campus region selected, three libraries were randomly selected for morning and afternoon air sampling. From January to March 2011, the sampling was carried out monthly. In all cases, the sampling in the three selected libraries was performed on the same day at two sampling times (10:00 and 17:00 h) and in triplicate (indoor and outdoor) air samples.

The prevailing environmental variables [wind speed (WS), cross wind (CW), temperature (*T*), relative humidity (RH), barometric pressure (BP) and dew point (DP)] were recorded along with the sampling time using a Kestrel® 4500 portable weather station.

Air sampling, culture and analysis

Aerial sampling was performed by the volumetric method using a single stage MicroBio MB2 (Spiral Biotech Inc. Norwood, MA, USA) air sampler and Petri dishes with potato dextrose agar as impaction medium. Based on preliminary tests, the MicroBio MB2 air flow was fixed at 100 L min⁻¹. The plates were incubated in the laboratory at 28 °C. Cultures were inspected after 4 days and periodically up to 15 days after exposure. The resultant colonies were counted and the concentration of fungal spores (CFU m⁻³) was calculated by adjusting using the positive hole correction procedure (Macher 1989), which includes the probability that more than one viable particle could be collected through a sampling hole and merged with other microorganisms at the same impaction site to produce a single colony.

Once incubation was completed, different fungal colonies in a Petri dish were separated and grown in pure cultures. After the macroscopic study, microscopic analysis was performed by staining with lactophenol blue and specimen observation under the 40X objective of a Zeiss Primo Star microscope (Carl Zeiss, Oberkochen, Germany). Morphological fungal identification was based on taxonomic keys for *Ascomycota*, *Basidiomycota*, *Chytridiomycota*, *Zygomycota* and *Deuteromycota* (Barnett & Hunter 1972; Dugan 2006). All isolated strains were identified at the genera level, but the three dominant indoor and outdoor fungi were identified at molecular level using the *internal transcribed spacers* ITS1 – ITS2 (White et al. 1990).

Molecular identification of fungi

Fungal genomic DNA was extracted following the instructions of the commercial kit ZR Fungal/Bacterial DNA Miniprep (Zymo Research Corp., Irving CA, USA). Approximately, 50 ng extracted DNA was used as a polymerase chain reaction (PCR) template to amplify the region ITS1 – 5.8S – ITS2 of the rDNA gene complex of each fungus. Illustra™ PuReTaq™ Ready-To-Go™ PCR Beads (GE Healthcare, Buckinghamshire, UK) was employed in the three PCR reactions using the ITS1/ITS4 primer set: ITS1 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS4 (5'-CGTATAGTTATTTCGCTCCT-3') (White et al. 1990) at a concentration of 50 pM in each reaction mixture. The PCR conditions were: 94 °C for 5 min followed by 35 cycles of 94 °C for 1 min, annealing at 56 °C for 1 min, extension at 72 °C for 1 min and a final extension at 72 °C for 5 min. A 5 µl aliquot of PCR product was electrophoresed on 1% agarose gel with 1× Tris-acetate-EDTA buffer. After electrophoresis, gels were stained with ethidium bromide and

photographed under ultraviolet light (LA-20E transilluminator, VWR Scientific, Buffalo Grove IL, USA).

The PCR fragments were purified (Wizard SV Gel and PCR Clean Up System, Promega) and sent to Macrogen Inc. (Seoul, Korea) for sequencing in both strand directions using the primers ITS1/ITS4. The resultant sequences were compared with the NCBI databases using the BlastN algorithm (Zhang et al. 2000) to identify the organisms.

Statistical analysis

Indoor and outdoor samples from each library were analysed separately by descriptive statistics (median, 25th, 75th, and 95th percentiles). Additionally, the results were compared by month (November, December, January, February and March), campus region (north, centre, south) and indoor versus outdoor air. The Kruskal–Wallis test (equality of populations) was used to determinate whether there were statistically significant differences in air fungal concentrations between months, regions or sampling time for both indoor and outdoor air. Due to the non-normal distribution of data, medians were used for comparisons. The statistical analysis of the data generated was based on the Chi-square test and logistic regression models. All tests were conducted with a significance level of 5% using the statistical software Stata version 11.1 (Stata Corp LP, College Station TX, USA).

Pearson's correlation analysis was conducted to evaluate the possible associations between meteorological variables and the FSC. For this analysis, WS, CW, T , RH, BP and DP were defined as independent variables, while the FSC (CFU m^{-3}) was defined as the dependent variable. In addition, a Pearson's robust regression test was carried out to measure the effect of the environmental variables on the FSC.

Results

Air sampling and overall concentrations

A total of 347 air samples from 15 libraries were analysed; 173 samples were from outdoor air and 174 from indoor air. Of the total of samples, 174 were obtained in mornings and 173 in afternoons. As the region for sampling was selected randomly, 131 samples were taken in the north, 102 in the centre and 114 in the South region. The highest global medians for air FSC were recorded in the environment of libraries L, I and E (1065, 975 and 990 CFU m^{-3} , respectively; Figure 2). It is important to note that library I is open only in the morning and the data were not collected in the afternoons. Library O had the lowest global median fungal concentration.

Indoor and outdoor fungal concentrations

In this study, 94% of outdoor air samples tested showed a higher FSC than indoor air. The median indoor FSC was 360 CFU m^{-3} (range 10–3090 CFU m^{-3}), whereas the median outdoor FSC was 1230 CFU m^{-3} (range 10–9470 CFU m^{-3}); (Figure 3, Tables 1 and 2). The ratio of the median indoor/outdoor FSC for each library was less than 1 in 97% of samplings. Overall, the median I/O ranged 0.05–1.7 in the afternoons and 0.05–1.8 in the mornings. None of the libraries had indoor air samples with an I/O higher than two.

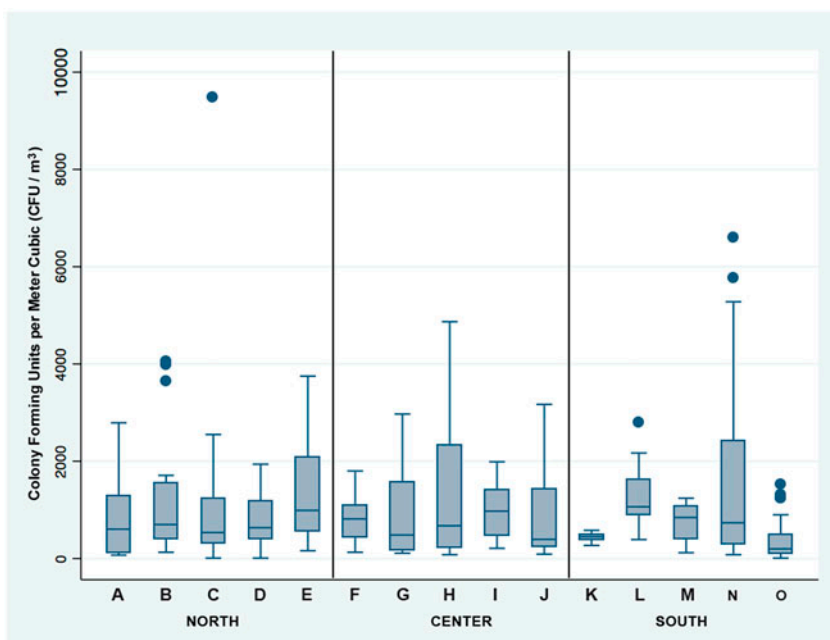


Figure 2. Descriptive analysis of the global concentration of fungal spores in libraries of the north, centre and south regions. Data from all the samplings (irrespective of the month, schedule and site of sampling) carried out in each library are included. The vertical black lines are delimiting the three regions; the top and bottom of the box plots represent the 75th and 25th quartiles, respectively; the median is the horizontal band, while the lower and upper whiskers represent the minimum and maximum values, respectively. The points above or under the boxes are outliers.

Concentration by libraries

At individual libraries, the FSC in morning indoor air ranged from 10 to 1240 CFU m⁻³; whereas in outdoor air the values ranged from 90 to 5280 CFU m⁻³ (Table 1). In the afternoon, the FSC for indoor air ranged from 50 to 3090 CFU m⁻³; whereas in outdoor air, the values ranged from 10 to 9470 CFU m⁻³ (Table 2, Figure 3). The value just mentioned was the highest FSC registered in this study and corresponded to library C. It is important to note that, of the 15 libraries studied, only library A showed a permanent indoor FSC level under the 200 CFU m⁻³ ACGIH guideline recommended by the ACGIH for indoor fungal bioaerosols (ACGIH 1999). Library G showed a permanent indoor FSC under the recommended guideline only in afternoon samples, while library M meets that recommendation only in morning samples (Figure 3, Table 1). It is important to highlight that the afternoon schedule is attended by a reduced number of students in the school to which library G belongs, whereas the morning schedule has a lower number of students in the school of library M.

Regional fungal concentrations

Among the months examined, the highest indoor FSC was observed on February in the south, whereas the highest outdoor FSC was observed on January in the north region (Tables 3–5). According to the Kruskal–Wallis test, there were no statistically

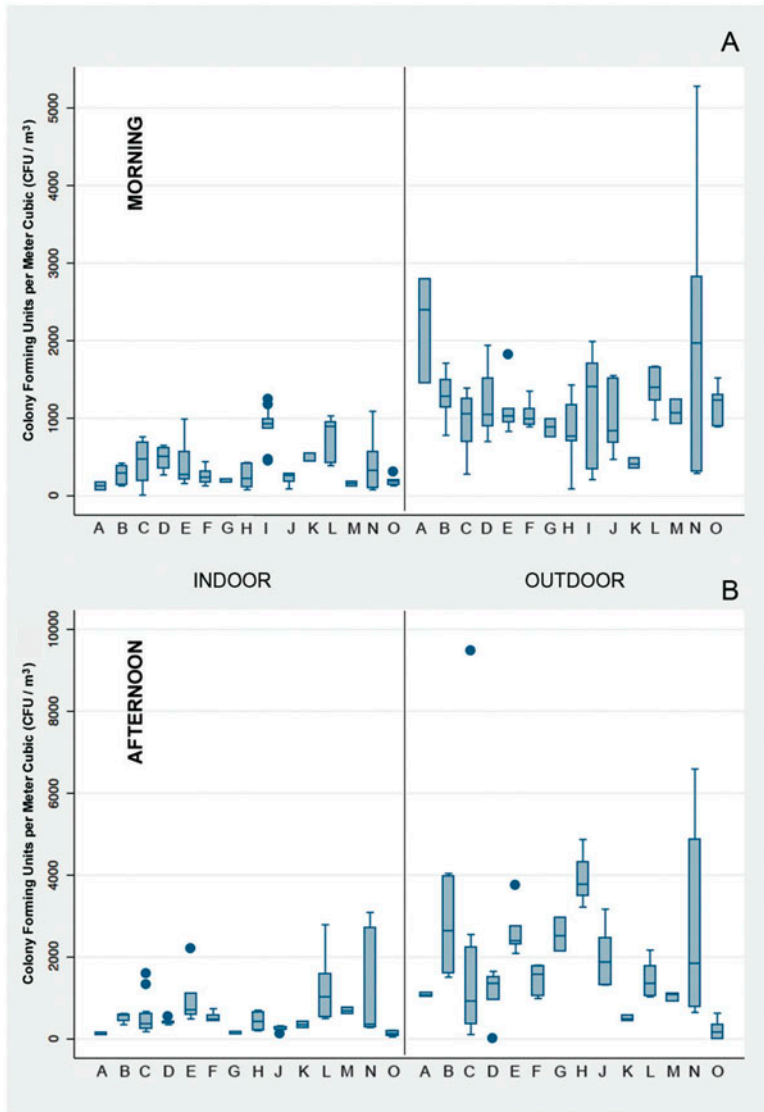


Figure 3. FSC (CFU/m³) in libraries for morning and afternoon samplings (indoor and outdoor air). Panel A: data from morning indoor and outdoor samples. Panel B: data from afternoon indoor and outdoor samples. The top and bottom of the box plots represent the 75th and 25th quartiles, respectively; the median is the horizontal band, while the lower and upper whiskers represent the minimum and maximum values, respectively. The points above or under the boxes are outliers. Library key: A: Central, B: Law School, C: Economics, D: Odontology, E: Psychology, F: Architecture, G: Biology, H: Civil Engineering, J: Chemistry, K: Philosophy, L: History, M: Humanities, N: Informatics, and O: Zapata High School library. Afternoon sampling was not carried out in library I (Physics and Mathematics library).

significant differences in indoor FSC between regions either in the mornings or afternoons ($p > 0.05$), whereas outdoor FSC was significantly different in the afternoons ($p < 0.05$). On the other hand, no significant differences were found across the three regions on comparing the spore concentrations in any month ($p > 0.05$). However, the

Table 1. FSC (CFU/m³) for indoor and outdoor morning air sampling in each library. Data in the table include the minimum and maximum values, mean, median and standard deviation.

Library	Indoor					Outdoor				
	Min [♦]	Max [♦]	Mean [♦]	Median [♦]	STDEV	Min [♦]	Max [♦]	Mean [♦]	Median [♦]	STDEV
A	70	170	123	130	50.33223	1450	2790	2213	2400	689.2266
B	130	420	278	295	122.8685	780	1710	1281	1285	328.8414
C	*10	760	424	475	264.5222	280	1390	968	1059	388.3349
D	270	650	486	510	147.8738	700	1940	1191	1050	457.0521
E	160	990	413	275	316.5228	830	1820	1130	1030	352.6471
F	130	480	321	240	132.5183	890	1780	1245	995	341.2884
G	170	210	186	180	20.81666	760	990	880	890	115.3256
H	80	430	248	225	163.1462	*90	1430	823	770	458.6357
I	450	+1240	886	930	271.0627	210	1990	1173	1410	713.3022
J	90	280	226	265	77.37355	470	1550	983	840	445.1816
K	450	550	483	450	57.73503	360	490	420	410	65.57439
L	390	1030	765	895	280.125	980	1670	1388	1400	264.4554
M	120	180	153	160	30.5505	930	1240	1080	1070	155.2417
N	80	1090	403	330	342.4179	290	5280	1957	1970	1618.207
O	130	310	193	190	64.39462	890	1520	1180	1235	244.3768

Symbols: ♦ colony forming units per cubic meter, + maximum FSC values, * minimum FSC values.

Table 2. FSC (CFU/m³) for indoor and outdoor afternoon air in each library. Data are presented as minimum and maximum values, mean, median and standard deviation.

Library	Indoor					Outdoor				
	Min [♦]	Max [♦]	Mean [♦]	Median [♦]	STDEV	Min [♦]	Max [♦]	Mean [♦]	Median [♦]	STDEV
A	100	160	126	120	30.5505	1040	1140	1083	1070	51.31601
B	350	620	530	585	106.3955	1510	4040	2738	2645	1266.135
C	180	1590	545	375	452.3767	210	+9470	1950	930	2646.802
D	350	550	420	410	68.70226	*10	1650	1141	1360	606.1161
E	490	2200	971	715	640.1068	2090	3750	2660	2400	654.0642
F	490	740	603	485	126.6228	990	1800	1283	1580	448.8132
G	110	180	140	130	36.05551	2140	2970	2543	2520	415.4917
H	200	700	436	430	245.8997	3220	4870	3908	3780	596.7719
I	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
J	120	320	238	235	68.53223	1320	3170	2006	1880	733.2848
K	270	430	350	350	80	450	580	506	490	66.58328
L	500	2790	1245	1030	904.2068	1030	2170	1460	1360	477.4097
M	610	760	686	690	75.05553	930	1120	1046	1090	102.1437
N	280	+3090	1168	360	1277.932	650	6590	2727	1850	2347.812
O	*50	210	134	130	62.27181	10	630	223	170	229.7825

Symbols: ♦ colony forming units per cubic meter, + maximum FSC values, * minimum FSC values.

Table 3. Meteorological variables recorded in north region during the outdoor and indoor aerial sampling carried out in the morning (10:00 h) and afternoon (17:00 h) during the indicated month and year. Bold type represents medians and the values within parentheses (from left to right) are the minimum and maximum, respectively. The lowest (*) and higher (+) value recorded for significantly correlated environmental variables to the FSC in the region.

Month and year of sampling																				
November 2010				December 2010				January 2011				February 2011				March 2011				
Morning		Afternoon		Morning		Afternoon		Morning		Afternoon		Morning		Afternoon		Morning		Afternoon		
Temperature (°C)																				
Indoor	25.9		27.25		24.3		26.2		24.1		25.8		24.15		24.55		23.6		25.8	
	(23.3, 26.5)		(25.9, 29.2)		(23.2, 24.6)		(24.1, 26.6)		(23.9, 24.2)		(25.6, 25.99)		(24, 24.3)		(23, 25.2)		(23.5,23.6)		(25.7, 25.9)	
Outdoor	26.3		29		22.4		25.2		22.4		25.8		23.2		26		22.4		26.1	
	(24.5, 28.9)		(27.1, 30.5)		(19.8, 24)		(20.9, 26.3)		(20.7, 22.9)		(25.7, 26.0)		(22.2, 23.8)		(23.4, 28.6)		(21.7,22.6)		(25.9,26.1)	
Relative humidity (%)																				
Indoor	44.35		41.55		50.7		49.6		54.3		40.2		59.05		41.05		44.8		39.6	
	(41.1, 62)		(36.9, 49.5)		(45.7, 53.0)		(39.4, 57.7)		(51.9, 56.4)		(39.1, 41.8)		(52.9, +65.2)		(*33.2, 48.2)		(44.4, 46.4)		(38.7, 40.3)	
Outdoor	49.85		48.7		52.4		50.5		50.1		36.3		45.5		41.8		43.4		43.4	
	(44.5, 59.5)		(37.4, 53.6)		(40.9, 62.3)		(45.1, 60.1)		(49.0, 51.3)		(34.7, 36.5)		(44.6, 47.0)		(39.9, 46.4)		(42.1, 43.8)		(42.1, 43.8)	
Barometric pressure (psi)																				
Indoor	14.705		14.65		14.73		14.68		14.72		14.68		14.72		14.67		14.71		14.65	
	(14.69, 14.73)		(14.64, 14.68)		(14.7, +14.76)		(14.67, 14.7)		(14.71,14.72)		(14.67,14.68)		(14.72, 14.73)		(14.66, 14.69)		(14.71, 14.71)		(*14.65, 14.65)	
Outdoor	14.99		14.64		14.72		14.67		14.71		14.67		14.72		14.67		14.72		14.65	
	(14.67, 14.72)		(14.62, 14.67)		(14.71,14.76)		(14.67, 14.7)		(14.71, 14.72)		(14.67,14.67)		(14.72, 14.73)		(14.66, 14.69)		(14.72, 14.72)		(14.65, 14.65)	
Wind speed (m/s)																				
Indoor	0		0		0		0		0		0		0		0		0		0	
	(0, 0.5)		(0, .4)		(0, 0)		(0, 0.5)		(0, 0)		(0, 0)		(0, 0)		(0, 0)		(0, 0)		(0, 0)	
Outdoor	0.5		0.5		0		0.3		0		0		0		0.9		0.5		1	
	(0, 0.8)		(0, 0.8)		(0, 0)		(0, 1.2)		(0, 0)		(0, 0)		(0, 0)		(0, 2.2)		(0.3, 0.6)		(0.5, 1.1)	
Cross wind																				
Indoor	0		0		0		0		0		0		0		0		0		0	
	(0, 0.5)		(0, 0.4)		(0, 0)		(0, 0.5)		(0, 0)		(0, 0)		(0, 0)		(0, 0)		(0, 0)		(0, 0)	
Outdoor	0.5		0.5		0		0.3		0		0		0		.8		0.4		0.7	
	(0, 0.8)		(0, 0.8)		(0, 0)		(0, 1.2)		(0, 0)		(0, 0)		(0, 0)		(0, 2)		(0.3, 0.5)		(0.2, 0.8)	

(Continued)

Table 3. (Continued).

Month and year of sampling										
November 2010			December 2010		January 2011		February 2011		March 2011	
	Morning	Afternoon	Morning	Afternoon	Morning	Afternoon	Morning	Afternoon	Morning	Afternoon
<i>Dew point</i>										
Indoor	12.6 (11.3, 18.6)	12.5 (11.1, 16.8)	13.1 (11.7, 14.3)	13.2 (11.7, 17.5)	14.3 (13.4, 15.0)	11.1 (10.9, 11.8)	15.65 (13.8, 17.2)	9.95 (6.8, 13.4)	10.9 (10.7, 11.4)	11 (10.8, 11.3)
Outdoor	15 (14.1, 17.6)	16.7 (14.3, 19.1)	12.4 (8.3, 13.7)	15.2 (9.2, 17.6)	11.5 (9.6, 12.3)	9.7 (9.0, 10.0)	10.75 (9.7, 11.6)	12.85 (9.0, 14.3)	9.1 (8.7, 9.5)	8 (7.8, 8.0)
<i>Spore concentration (CFU/m³)</i>										
Indoor	220 (10, 540)	375 (100, 1120)	280 (130, 990)	420 (180, 2200)	710 (670, 760)	550 (450, 670)	1285 (780,1940)	615 (350, 3090)	520 (430, 710)	1320 (360, 1590)
Outdoor	1355 (990, 2790)	1325 (110, 3750)	830 (280, 1120)	1020 (10, 2310)	1350 (1240, 1390)	1710 (1330, 9470)	380 (130, 650)	2565 (960, 4040)	1010 (1070, 900)	2520 (1970, 2550)

Notes: psi – pounds per square inch.
CFU/m³ – colony forming units per cubic meter of air.

Table 4. Meteorological variables recorded in centre region during the outdoor and indoor aerial sampling carried out in the morning (10:00 h) and afternoon (17:00 h) during the indicated month and year. Bold type represents medians and the values within parentheses are the minimum and maximum (from left to right), respectively. The lowest (*) and higher (+) value recorded for significantly correlated environmental variables to the FSC in the region.

Month and year of sampling														
November 2010			December 2010			January 2011			February 2011			March 2011		
Morning		Afternoon	Morning		Afternoon	Morning		Afternoon	Morning		Afternoon	Morning		Afternoon
Temperature (°C)														
Indoor	25.4	26.3	24	25.85	24.1	26.3	24.8	ND	24	25				
	(23.1, 28.0)	(26.0, 27.2)	(23.7, 25.3)	(25.2, 26.5)	(24.0, 24.3)	(26.2, 26.4)	(24.5, 29.7)		(23.8, 24.2)	(24.9, 25.4)				
Outdoor	25.1	27.9	23.2	25.4	24.5	23.9	25.6	ND	23.9	23.6				
	(24.1, 26.5)	(26.6, 31.4)	(19.9, 26.1)	(23.5, 27.2)	(24.0, 24.5)	(23.8, 24.3)	(25.1, 29.6)		(23.7, 24.0)	(23.5, 24.5)				
Relative humidity (%)														
Indoor	40.2	39	48.7	45.5	57.3	55.3	41.95	ND	55	43.5				
	(36.2, 51.9)	(34.3, 49.0)	(43.4, 50.3)	(44.0, 50.3)	(57.2, 61.1)	(54.4, 57.0)	(39.2, 46.7)		(55.0, 55.7)	(42.4, 43.5)				
Outdoor	53.6	47.4	52.2	52	48.6	42.3	38.4	ND	44.1	42.3				
	(46.5, +59.0)	(43.4, 49.2)	(41.6, 55.6)	(45.0, 57.4)	(48.5, 48.6)	(40.8, 43.3)	(*35.3, 40.2)		(42.6, 44.6)	(42.2, 43.0)				
Barometric pressure (psi)														
Indoor	14.69	14.65	14.72	14.68	14.71	14.67	14.71	ND	14.7	14.64				
	(14.67, 14.73)	(+14.63, 14.67)	(14.71, *14.75)	(14.67, 14.7)	(14.71, 14.71)	(14.67, 14.67)	(14.69, 14.73)		(14.7, 14.7)	(14.64, 14.65)				
Outdoor	14.69	14.64	14.71	14.69	14.71	14.68	14.70	ND	14.7	14.65				
	(14.68, 14.72)	(14.63, 14.69)	(14.7, 14.75)	(14.67, 14.7)	(14.71, 14.71)	(14.68, 14.69)	(14.68, 14.73)		(14.7, 14.7)	(14.65, 14.65)				
Wind speed (m/s)														
Indoor	0	0	0	0	0	0	0	ND	0	0				
	(0, 0.4)	(0, 0.8)	(0, 0)	(0, 0)	(0, 0)	(0, 0)	(0, 0)		(0, 0)	(0, 0)				
Outdoor	0	0	0	0.35	0	0	0	ND	0	0				
	(0, 0.5)	(0, 2)	(0, 0.9)	(0, 2.1)	(0, 0)	(0, 1.4)	(0, 0.7)		(0, 0.3)	(0, 0.8)				
Cross wind														
Indoor	0	0	0	0	0	0	0	ND	0	0				
	(0, 0.4)	(0, 0.8)	(0, 0)	(0, 0.5)	(0, 0)	(0, 0)	(0, 0)		(0, 0)	(0, 0)				
Outdoor	0	0	0	0.35	0	0	0	ND	0	0				

(Continued)

Table 4. (Continued).

	Month and year of sampling							
	November 2010		December 2010		January 2011		February 2011	
	Morning	Afternoon	Morning	Afternoon	Morning	Afternoon	Morning	Afternoon
<i>Dew point</i>	(0, 0.5)	(0, 2)	(0, 0.9)	(0, 2.1)	(0, 0)	(0, 1.4)	(0, 0.2)	(0, 0.1)
Indoor	10.9 (7.6, 17.2)	11 (9.7, 15.3)	12.4 (10.6, 13.8)	13.2 (13.0, 14.5)	15.1 (15.1, 16.3)	16.7 (16.5, 17.0)	11.85 (10.7, 15.3)	14.4 (14.2, 14.8)
Outdoor	14.9 (13.4, 17.2)	15.9 (13.2, 19.0)	12 (9.9, 13.8)	14.9 (13.8, 15.0)	13 (12.5, 13.0)	10.3 (9.8, 11.0)	10.7 (9.4, 13.7)	10.2 (10.5, 10.9)
<i>Spore concentration (Cfu/M³)</i>								
Indoor	250 (90, 440)	440 (230, 740)	250 (170, 870)	155 (110, 240)	420 (330, 430)	650 (630, 700)	975 (900, 1240)	110 (80, 120)
Outdoor	1040 (690, 1550)	1780 (990, 3170)	760 (210, 990)	1865 (1320, 2970)	710 (710, 830)	4310 (3220, 4870)	1570 (1320, 1990)	1170 (90, 1430)
								3700 (3490, 3860)

Notes: psi – pounds per square inch.
CFU/m³ – colony forming units per cubic meter of air.
ND – not determined.

Table 5. Meteorological variables recorded in south region during the outdoor and indoor aerial sampling carried out in the morning (10:00 h) and afternoon (17:00 h) during the indicated month and year. Bold type represents medians and the values within parentheses (from left to right) are the minimum and maximum, respectively. The lowest (*) and higher (+) value recorded for significantly correlated environmental variables to the FSC in the region.

	Month and year of sampling							
	November 2010		December 2010		January 2011		February 2011	
	Morning	Afternoon	Morning	Afternoon	Morning	Afternoon	Morning	Afternoon
<i>Temperature (°C)</i>								
Indoor	25.9 (23.2, 30.4)	25.7 (21.7, 27.3)	24.3 (22.6, 25.1)	25.1 (24.4, 26.2)	ND	ND	25.55 (24.8, 26.5)	25.55 (23.2, 28.2)
Outdoor	28.35 (26.5, +33.9)	28.05 (27.3, 29.6)	24.75 (23.9, 26.0)	25.8 (21.8, 26.9)	ND	ND	23.75 (23.4, 25.3)	24.8 (*20.6, 28.9)
<i>Relative humidity (%)</i>								
Indoor	43.95 (41.8, 53)	47.1 (38.7, 53.7)	44.85 (35.9, 53.6)	49.1 (39.0, 56.0)	ND	ND	46 (42.4, 49.2)	43.85 (42.4, 45.7)
Outdoor	46.75 (34.2, 53)	52.35 (45.8, 57.6)	46.7 (38.0, 60.5)	48.2 (42.2, +62.5)	ND	ND	39.55 (38.0, 42.5)	35.55 (34.7, 36.5)
<i>Barometric pressure (psi)</i>								
Indoor	14.73 (14.71, 14.74)	14.69 (14.67, 14.7)	14.755 (14.74, 14.77)	14.72 (14.68, 14.72)	ND	ND	14.745 (14.74, 14.75)	14.695 (14.67, 14.72)
Outdoor	14.72 (14.74, 14.7)	14.68 (14.66, 14.69)	14.745 (14.73, 14.76)	14.71 (14.69, 14.72)	ND	ND	14.745 (14.74, 14.75)	14.705 (14.68, 14.73)
<i>Wind speed (m/s)</i>								
Indoor	0 (0, 0.6)	0 (0, 0.5)	0 (0, 0)	0 (0, 0.5)	ND	ND	0 (0, 0)	0 (0, 0)
Outdoor	0 (0, 0.9)	0.55 (0, 1.0)	0 (*0, 0)	0 (0, 1.6)	ND	ND	1.2 (0, 2.7)	0.95 (0, +3.3)
<i>Cross wind</i>								

(Continued)

Table 5. (Continued).

	Month and year of sampling									
	November 2010		December 2010		January 2011		February 2011		March 2011	
	Morning	Afternoon	Morning	Afternoon	Morning	Afternoon	Morning	Afternoon	Morning	Afternoon
Indoor	0 (0, 0.6)	0 (0, 0.5)	0 (0, 0)	0 (0, 0)	ND	ND	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 0)
Outdoor	0 (0, 0.9)	0.55 (0, 1)	0 (0, 0)	0 (0, 1.6)	ND	ND	0.4 (0, 0.6)	0.5 (0, 1.9)	0.6 (0.4, 0.7)	1.0 (0.8, 2.2)
Dew point										
Indoor	12.75 (10.0, 19.0)	13.7 (9.6, 15.2)	11.1 (7.6, 15.0)	13.1 (11.0, 15.9)	ND	ND	13.05 (12.5, 13.6)	12.5 (9.7, 15.1)	11.3 (10.4, 11.9)	10.9 (10.6, 11)
Outdoor	15.65 (14.1, 17.6)	17.45 (14.9, +19.0)	12.5 (8.8, 17.7)	12.1 (10.4, +19.0)	ND	ND	9.65 (8.7, 9.9)	8.45 (*5.1, 12.2)	10.4 (9.2, 10.8)	9.8 (9.7, 9.9)
Spore concentration (CFU/m ³)										
Indoor	350 (130, 1090)	250 (50, 570)	275 (80, 550)	280 (70, 430)	ND	ND	225 (120, 370)	1740 (610, 3090)	920 (870, 1030)	1590 (1490, 2790)
Outdoor	1315 (890, 2120)	830 (0, 1940)	335 (290, 490)	490 (270, 780)	ND	ND	2030 (930, 5280)	2995 (930, 6590)	1470 (980, 1670)	1780 (1650, 2170)

Notes: psi – pounds per square inch.
CFU/m³ – colony forming units per cubic meter of air.
ND – not determined.

FSC varied significantly in the five sampling months ($p < 0.05$) within each region. Moreover, the indoor and outdoor air spore concentration was significantly different ($p < 0.05$) between the north and south regions during all the sampling months, with the exception of March. On the other hand, FSC significantly differed by month throughout the sampling period in the centre region. Spore concentrations were significantly different between indoor and outdoor air in the mornings and afternoons ($p < 0.05$) in the three regions studied.

Pearson's correlation analysis was conducted to investigate the possible associations between air FSC and the independent variables within the regions sampled. In indoor air from the north region, FSC had a statistically significant negative correlation with BP (correlation coefficient: -0.3050 ; $p = 0.0128$); whereas in outdoor air, there was a significant negative correlation between FSC and RH with a correlation coefficient of -0.2794 ($p = 0.0253$). The values recorded for the environmental variables in each region are shown in Tables 3–5.

In the centre region, the outdoor FSC was significantly correlated with RH and BP (correlation coefficients, -0.3474 and -0.5468 ; and p values, 0.0125 and 0.0001 , respectively); whereas in the south region, the FSC significantly correlated ($p < 0.05$) with the variables WS, T , RH and DP with correlation coefficients of 0.3166 , -0.3585 , -0.3159 and -0.4941 , respectively. No significant correlation between the FSC in indoor air was observed in these regions.

Finally, the indoor FSC showed significant correlation with the outdoor FSC in the north and south regions, with correlation coefficients of 0.3171 and 0.7035 , respectively. Comparing outdoor and indoor air, FSC were significantly higher in outdoor air ($p < 0.05$).

Additionally, the overall outdoor data were analysed with Pearson's test taking into account only the sampling time (ignoring region and month). From this analysis, the morning outdoor air spore concentration was significantly ($p = 0.01$) positively correlated with WS, CW and T (coefficients 0.55 , 0.11 and 0.24 , respectively). Similar correlation analysis of the overall outdoor data generated from afternoon sampling showed correlation coefficients of -0.39 , -0.25 and -0.42 for RH, T and DP, respectively, revealing a significant negative correlation ($p = 0.02$) between these environmental variables and the FSC in afternoon outdoor air.

To investigate the magnitude of the effect of independent variables on the outdoor FSC, Pearson's robust regression was conducted on the overall data disregarding region, month or sampling time. Increasing the WS by 1 metre per second increased the spore concentration by 1034 CFU m^{-3} . On the other hand, CW showed the opposite effect; an increase of 1 ms^{-1} in CW decreased the air FSC by 826 CFU m^{-3} . Similarly, the BP had a negative effect with an increase of 1 lb per square inch (psi) decreasing the air FSC by $10,103 \text{ CFU m}^{-3}$.

Fungi recovered

Strains of 11 fungal genera, namely: *Rhizopus*, *Fusarium*, *Bipolaris*, *Aspergillus*, *Alternaria*, *Curvularia*, *Penicillium*, *Aerobasidium*, *Stemphylium*, *Cladosporium* and *Mucor* were identified by macroscopic and microscopic morphological analysis of the fungal indoor and outdoor isolates based on taxonomic keys (Barnett & Hunter 1972; Dugan 2006). Additionally, the rDNA ITS1 – ITS2 regions of the three most frequently isolated strains denoted H1, H2 and H3 were PCR amplified and both strands sequenced; pairs of sequences (577 and 950 bp, 725 and 950 bp, 573 and 950 bp) were

obtained for each strain, respectively. By searching GenBank and performing BlastN analysis, these strains were identified as *Aspergillus tamaris* (H1), *Aspergillus oryzae* (H2) and *A. niger* (H3) with 99% similarity for each organism.

Discussion

In this study, a significantly higher FSC was found in outdoor air (Figure 3, Tables 1 and 2), especially during days with low RH. In general, the combination of low RH and elevated WS increased the concentration of spores in air. This result agrees with findings that the FSC is negatively correlated with RH and positively correlated with WS (Willocquet & Clerjeau 1998; Jones & Harrison 2004). Willocquet and Clerjeau (1998) recorded minimum WS values from 22:00 to 06:00 h, which increased during daytime reaching a maximum from 14:00 to 18:00 h. On the other hand, RH showed an opposite pattern, with low values recorded between 10:00 and 18:00 h. As a result, the authors observed the highest fungal concentrations between 10:00 and 18:00 h and the lowest from 22:00 to 06:00 h. They further suggested that high WS and low RH seem to be the main factors associated with the diurnal spore dispersal, a common behavior observed in many aerial fungi. Moreover, research in *A. alternata* reported that concentration peaks were associated with dry days, high *T* or high WS (Chen et al. 2003). The authors found that the percentage of spores trapped between 10:00 and 14:00 h generally remained high, correlating with the occurrence of high *T* and low RH. However, it was also reported that spore concentration of *Alternaria* spp. is negatively correlated with both RH and WS (Sabariego et al. 2012). In our study, the results of outdoor air generally showed significantly higher spore concentrations in the afternoon (Figure 3, Tables 1–3), when the WS was slightly higher (Tables 4 and 5) compared to morning samples. However, according to dispersion models obtained from wind tunnel experiments, the WS can also act as a dilution factor. In agreement with this, it has been shown that the number of fungal conidia dispersed decreases rapidly with increasing distance from the spore source at higher WS (Chen et al. 2003). Based on this finding, the WS could either increase or decrease the spore concentration in air.

For bacteria, a positive correlation was demonstrated between bacterial concentration and RH, WS and *T* (Chandra Mouli et al. 2005). However, the effect of RH is complicated because the concentration of bacteria in air may increase as a result of the increase or decrease in RH, since higher RH favours viability, whereas a lower RH favours the release of bacteria in greater numbers.

On the other hand, the WS did not significantly affect the FSC in indoor air in this study. It is known that both spore liberation from conidiophores and spore elevation in air require a minimum WS of 0.5 ms^{-1} (Pasanen et al. 1991; Jones & Harrison 2004; Velez Pereira et al. 2009). Based on this, the WS values recorded in the interior of libraries were insufficient to affect the air spore concentration since they were generally less than 0.5 ms^{-1} (Tables 3–5). Similarly, many WS values recorded in outdoor air in the north and centre regions were under 0.5 ms^{-1} . Consequently, the outdoor air spore concentration was significantly positively correlated with WS only in the south region ($p=0.016$), where the maximum WS value (3.3 ms^{-1}) of this study was recorded (Table 5).

It is clear that the atmospheric pressure exerts a strong influence on the concentration of microorganisms in air (Deguillaume et al. 2008). However, the results are controversial since significant positive (Burch & Levetin 2002) and negative correlation (Stennett & Beggs 2004; Segvić & Pepeljnjak 2006; Kilic et al. 2010) of this variable

with air microorganism concentrations have been reported. Based on our results, there was a negative correlation between BP and FSC in air, which is consistent with the findings from studies with *Cladosporium* (Segvić & Pepeljnjak 2006) and *Alternaria* (Kilic et al. 2010). In contrast, it has been reported that an increase in the atmospheric pressure to 43 mbar raises the algae spore concentration in air (Rosas et al. 1989). These findings could be explained by considering the size, shape and electrical charge differences between algae and fungal spores that could affect their behavior differently in air (Jones & Harrison 2004). Similar to algae, studies in fungi found that an increase in pressure induces an increment in *Cladosporium* spores in air. It was further observed that BP, hourly *T* and DP were the environmental variables that could significantly predict the total spore concentration. It was concluded that increases in BP, *T*, WS and DP were the most consistent and accurate predictors of spore presence. Additionally, the authors suggested that different weather conditions in individual days are more significant factors than the ecological differences between individual sites (Burch & Levetin 2002).

In this work, the correlation analysis of overall data by region revealed a negative correlation between the FSC in air and BP, *T*, RH and DP; and WS showed a positive correlation. On the other hand, in the analysis of the overall data by sampling time *T*, WS and CW showed a significant positive correlation with FSC in the morning, while a significant negative correlation between FSC and *T*, RH and DP was found in the afternoon. Remarkably, a significant correlation between spore concentration and WS was found only in the morning. This finding highlights the different associations between the environmental variables and the spore concentrations at different times of the day, as well as the differential influence of each variable on the spore concentration in air.

In summary, BP, RH and DP showed a significant negative correlation with FSC; *T* showed a significant negative or positive correlation (depending of the sampling time) and WS and CW showed a significant positive correlation with FSC. Beyond these results, some studies provide key evidence that the spore concentrations and the meteorological parameters show different correlation indices depending on the taxon being analysed; for example, *Alternaria* and *Cladosporium* concentrations had significant positive correlation with *T* (Sabariego et al. 2000). However, only *Alternaria* showed significant negative correlation with RH. In contrast, *Ustilago* showed significant positive correlation with RH and negative correlation with *T* and WS. It is noteworthy that these results are contrary to another study in which *Alternaria*, *Cladosporium* and *Ustilago* genera together with *Epicoccum*, *Ganoderma* and *Stemphylium* showed negative correlation with RH and positive correlation with *T* (Oliveira et al. 2009). Regarding *Aspergillus*, the predominant genera isolated in this study, it was reported that its conidia levels increase with *T* and decrease when WS is high. The analysis by species has shown that *A. niger* is more dependent on these factors than other *Aspergillus* species (Guinea et al. 2006). Moreover, the concentration of *Aspergillus* spores in the air could be negatively (Debey et al. 1995) or positively correlated with RH (Panackal et al. 2010).

Based on these data, it is important to take into account that the air analysed in this study had a mixture of different fungal spores with different representation in the fungal population; and each taxon is affected by each environmental variable differently throughout the day. Therefore, it can be expected that the FSC is difficult to predict due to the large number of interactions between the prevailing environmental conditions and fungal species at a particular moment. In this study, 11 fungal genera were identified in the air, which is a relatively low number compared with other studies in which more

than 50 taxa have been reported (Chakrabarti et al. 2012). This fact can be ascribed to differences in local weather conditions between the regions studied. Among the strains isolated, most have been reported as allergenic in several studies, especially *Aspergillus* spp. (Bowyer & Denning 2007).

Conclusions

In the indoor air of the libraries studied, there are FSC higher than those recommended by the ACGIH (200 UFC m^{-3}). However, based on the I/O ratio, the air quality in terms of fungal spore content inside the libraries was good in 97% of the samples evaluated. In addition, none of the indoor air samples showed an I/O ratio higher than 2. However, all the fungi identified are known to be allergenic and some of them are capable of producing mycotoxins. Of the variables studied, only *T* showed both significant positive and negative correlation with spore concentrations in air. BP, RH and DP showed significant negative correlation; WS and CW showed significant positive correlation. The fungi identified are present either in indoor or outdoor air, representing a health risk for people working inside or outside the libraries. To our knowledge, this is the first aeromycological study in Sinaloa, a state whose economy is based on agriculture; therefore, more studies on a larger scale are needed to profile the distribution of fungal spores in different environments (work, educational, industrial and residential). Additionally, it would be of great interest to evaluate the suitability of the currently available guidelines for the specific conditions of Sinaloa state.

Since the FSC in air has adverse effects on human health, it is important to recognise the need to establish continuous monitoring systems to create a spore occurrence and concentration calendar, such that it will be possible to predict the fungal bioaerosol behavior in a particular area based on the prevailing environmental conditions. The knowledge of location and timing, as well as the type and concentration of fungal spores will contribute to the creation of preventive programmes to avoid exposure to fungi with the goal of improving population health.

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