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Fungal spores in four catholic churches in the metropolitan area of Monterrey, Nuevo León State, Mexico – First study

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Abstract

Introduction. About 500,000 species of fungi have been described to-date, although an estimated between 1 – 1.5 million species may occur. They have a wide distribution in nature, contributing to the decomposition of organic matter and playing a part in the biogeochemical cycles of major nutrients. A small number are considered pathogens of animals and plants. There is ample historical evidence that certain types of allergies are associated with fungi; exposure to fungal allergens occurs in both outdoor and indoor spaces. Many indoor allergens are the same as those found outside buildings, entering through windows and doors, ventilation systems, or through cracks or other fissures in the walls.

Objective. To determine the diversity and abundance of fungal spores inside four churches in the metropolitan area of Monterrey city in Mexico.

Materials and methods. The study was carried out from July 2009 – January 2010 using a Hirst type volumetric collector (Burkard Manufacturing Co Ltd).

Results. A total of 31,629 spores from 54 taxa were registered in the four churches. The building that showed the highest amount of spores was the Santa Catarina Mártir Church with 12,766 spores, followed by Cristo Rey with 7,155 and Nuestra Señora del Roble with 6,887. Regularly high concentrations of spores were recorded from 14:00 – 20:00 hours. The highest concentration value was observed at the church of Santa Catarina Mártir at 16:00 hours with 1153 spores/m^3 air.

Conclusions. The most abundant spores in the four churches studied corresponded to *Cladosporium*, the *Aspergillus*/*Penicillium* complex, *Coprinus*, *Ganoderma*, *Curvularia* and *Ustilago*.

Key words

allergy, fungal spores, diversity, México

INTRODUCTION

Many airborne fungal, actinomycetes and bacterial spores are capable of causing disease in humans and animals by direct infection; living tissue is invaded by the microbe, by toxicoses or by allergy. Respiratory allergy in humans may develop immediately, as in hay fever or asthma, or can be delayed, as in Farmer´s Lung. Potential sources of hazards airborne spores are many stored products, including hay, straw, grain, wood chips and composts [1]. Fungi live as saprophytes on organic material or as parasites (mainly plant pathogens), so the majority of fungal spores in the air outdoors come from farms, forest stands and decomposing plant matter. Fungal spores are a normal and major component of indoor as well as outdoor air [2].

Studies to determine the air quality in indoor and outdoor environments, mainly in Asia, Europe and North America, have gained increasing importance in recent decades. In Mexico, aerobiological studies are scarce compared with other countries and have been mainly focused outdoors [3, 4, 5, 6]. Among the biologically-active airborne particles, those of fungal origin are important due to the damage they are likely to cause on both relics of historical value and health (pathogenic and allergenic character), with the as air their main vehicle for dispersion [7]. Fungal allergen exposure occurs outdoors and indoors; many of the indoor allergens are the same as those found on the outside of buildings, which may enter through windows, doors, vents, cracks or fissures through walls. However, they can also be introduced by shoes. Colonization and growth of microorganisms on the surface of indoor objects can also be a major source of air pollution [8]. Generally, the concentration of microorganisms inside buildings is considerably higher than outside, and most indoor bodies are commonly found in the respiratory tract of humans [9]. Indoor species most currently identified are *Cladosporium* sp, *Aspergillus*/*Penicillium*, pink and white yeasts, *Botrytis cinerea*, *Paecillomyces variotii*, *Phoma* sp, *Aureobasidium* sp, *Alternaria alterna*, *Epicoccum purpurascens*, *Geotrichum candidum*, *Ulocladium* sp, *Trichoderma viride*, *T. harzianum* and *Mucor* sp [10].

Air quality in historic buildings and churches is of concern due to increased indoor air pollution caused by tourism, mass services, burning of incense, oil lamps and candles. The increased importance gained by indoor research is due to the presence of microorganisms in the air which may cause allergies and infections to the respiratory tract, especially in individuals sensitive to mould spores. The presented study therefore is an attempt to reveal the diversity and abundance of fungal spores and the quality of air inside churches.

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MATERIALS AND METHOD

Area of study. *The Metropolitan Area of* Monterrey (MAM) in Nuevo León State, Mexico, includes the municipalities of Monterrey, Guadalupe, San Nicolas de los Garza, Santa Catarina, San Pedro Garza Garcia, General Escobedo, Apodaca and Benito Juarez; located at the piedmont of the Sierra Madre Oriental mountains and overlooking the vast steppe of northeastern Mexico. This steppe area, in turn, covers the north and east of Nuevo Leon State, part of northeastern Coahuila State and northwestern Tamaulipas State. The MAM covers an approximate area of 1,480 km^2 located between the parallels 25°35′ and 25°50′ North latitude, and between the meridians 99°59′ and 100°30′ West longitude. Downtown is located at 25°40′ North latitude and 100°18′ West longitude at an altitude of 534 meters. Considering the slope of the terrain of the urban site, oriented west-east, the same altitude varies from 680 m in Santa Catarina to 380 m in the municipality of Benito Juarez. The MAM is located where three major physiographic provinces converge, the Sierra Madre Oriental Mountains, the Northeast Coastal Plains and the Northern Highlands. These three units determine the natural landscape that surrounds the MAM. The soils in the MAM present semi-arid climate characteristics unfavourable for its development. This extreme environmental condition with large thermal ranges and a torrential moisture regime has influenced soil genesis since late Quaternary period.

The distribution of plant communities is generally established accordingly to the pattern of relative humidity in the region. This situation is evident on the plains of the upper and lower platforms where humidity is higher in the south and southeast and decreases lower in the north and northwest. The same pattern occurs to the matorral going from caducifolious in the south to crassifolious in the north and northeast. Furthermore, the MAM is located within the subtropical high pressure zone, therefore, this latitudinal geographical position and its regional orographic conformation are conditions of climate behaviour greatly influenced by marine and continental air masses modified by the upper atmosphere circulation. The characteristic climate that prevails in the MAM, according to the Köppen classification system modified by García [11], is a dry warm steppe, extreme with erratic rains falling in late summer, which is classified as -S(h')hw (e'), although the highest rain incidence occurs in September and the lowest in January with the total annual between 300–500 mm. The average annual temperature is 22.1 °C. The prevailing winds in the region blow from northeast and southeast and become more intense in the warm half of the year.

Aeromycological study. This study was conducted during July 2009 – January 2010 in four churches in the MAM. The selected church buildings are named in Spanish: Santa Catarina Mártir, Nuestra Señora del Roble, Nuestra Señora del Perpetuo Socorro and Cristo Rey. To obtain the spores, a Hirst type volumetric collector (Burkard Manufacturing Co. Ltd.,), was placed inside each building at a height of 1 m above ground level. This collector worked continuously during 24 hours at a constant air flow of 10 liters/minute. Air entered through a hole at the top of the device and the particles impacted onto a slide coated with silicone oil, which rotated at a speed of 2 mm/hour, giving a total length of 48 mm each sampling day. Sampling was carried out during 12 days in each of the churches, i.e., 48 days total, ensuring that half of this period occurred in days of low anthropogenic activity, that is, without a massive influx of people that might affect the atmospheric fungal content, while in the other half of the sampling period a high anthropogenic activity occurred.

The collect samples were transferred to the Laboratory of Plant Anatomy and Physiology of the Universidad Autónoma de Nuevo León (UANL) and mounted in glycerol-gelatin inclusion medium. For the identification of fungal spores, the works of Kapp [12], Lacey and West [1] were used as references. The frequency occurrence of the different types of spores was calculated from the data obtained for each type of spores in relation to the records obtained from the collectors installed at the four sampling sites (churches). The daily total concentration of spores was evaluated by counting the collected spores after two longitudinal runs, and the quantities obtained were multiplied by the correction factor of 1.08 in order to express the concentration as spores per cubic meter of air (spores/m^3).

RESULTS AND DISCUSSION

During the period of study between 5 July 2009 – 18 January 2010, a total of 31,629 spores were counted in the four analyzed churches. These spores were classified within 54 types of spores, although 1,851 spores could not be identified (Tab. 1). The location that showed the highest spore counts during the study period was the church Santa Catarina Mártir with 12,766 spores, followed by Cristo Rey with 7,155 and Nuestra Señora del Roble with 6,887, while the Nuestra Señora del Perpetuo Socorro church presented the fewest spores with 4,821. Analysis of the type of spores showed that conidiospores were found at a higher percentage (81%), followed by the basidiospores with 10%, ascospores with 3% and 6% of other kinds. The prevalence of conidiospores resulted from the spore *Cladosporium* which represents 67% of total spores in the four churches. This finding coincides with Rocha Estrada et al. [6] who reported it as the most abundant spore in the MAM, representing 68% of total spores. In addition, *Cladosporium* is considered one of the most abundant moulds in most environments worldwide, and often found on windows, walls and a variety of cellulosic materials [13, 14, 15]. Besides *Cladosporium* as the most abundant spores found in the churches under study, the complex *Aspergillus/Penicillium, Coprinus, Ganoderma, Curvularia* and *Ustilago* followed in that order. Caretta [16] has mentioned that the number of indoor fungal spore types in the air depends mainly on the presence of sources of dispersion, with *Aspergillus, Penicillium, Cladosporium, Alternaria, Mucor, Rhizopus* and *Oidiodendron* appearing more frequently. Different authors have mentioned that the highest concentrations of spores are produced in spring and the most abundant indoor spores correspond to *Aspergillus, Penicillium* and *Cladosporium* [5, 17, 18]. Similar results were found in the presented study since *Cladosporium* and the complex *Aspergillus/Penicillium* were the most abundant types in the churches examined. The obtained results also coincide with those published by Petushkova and Kandyba [8], who found some species of *Aspergillus, Cladosporium, Geotrichum* and *Penicillium* inside cathedrals in Moscow,

Table 1. Type of fungal spores of four churches of MAM

Type of spores	Catarina Mártir	El Roble	Perpetuo Socorro	Cristo Rey	Total
Alternaria	53	9	21	33	116
Arthrinium		1	1	3	5
Aspergillus/Penicillium	1543	570	390	931	3434
Beltrania	1	1		1	3
Bispora	1			1	2
Bolbitius	17	53	35	71	176
Botryodiplodia	8	1	1	3	13
Cercospora	12	1	1	10	24
Chaestosporium		2	4	3	10
Chaetomium	3	2	8	1	14
Cladosporium	8739	5209	3159	3932	21039
Coprinus	692	238	347	731	2008
Curvularia	158	57	48	130	392
Dactylaria	10	9	5	48	71
Daldinia	26	6	11	15	58
Didymella	14	35	6	43	98
Didymosphaeria	22	13	13	23	70
Diplodia	6	4	1	2	14
Diplodina	22	12	2	18	54
Drechslera	10	5	2	2	19
Epicoccum	5	4	1	8	18
Exosporium	3			4	8
Fusarium	8	11	5	63	86
Ganoderma	264	111	76	144	594
Geotrichium	1		1	6	9
Helminthosporium	1	1		3	5
Hypoxylon	44	17	21	26	108
Leptosphaeria	35	9	9	28	80
Micronectriella	4		3	11	18
Mycosphaerella	1			5	6
Nectria	13	2	2	17	35
Nigrospora	36	17	23	27	103
Paecilomyces				8	8
Panaeolus	14	2	4	23	43
Periconiella	53	30	27	29	139
Phaeosphaeria	3	3	1	5	13
Pithomyces	19	2	1	3	26
Pleiochaeta	3	4	3	3	14
Pleospora	10		2	5	17
Puccinia	18	3	13	16	51
Rhizopus	14	32	40	5	92
Rhodotorula	11	4	3	6	25
Rosellinia	3	2	1		6
Serpula	68			3	71
Sordaria		4			4
Spegazzinia	1	1	1		3
Sporormia	9			15	24
Sporormiella	1		1		2
Stemphylium	6	1	2	10	19
Tilletia				2	2
Torula	31	5	4	32	73
Ulocladium	6	6	3	9	25
Ustilago	107	79	70	66	322
Venturia	31	8	16	50	105
Unidentified	604	297	432	518	1851
Total spores	12766	6887	4821	7155	31629

Russia, which are likely to produce some allergy related illness. In Mexico, fungal particles can be considered a risk factor in children causing asthma, rhinitis and allergy; however, it is difficult to establish a direct relationship between exposure to mould and respiratory symptoms, particularly if the volumetric results are not well documented [19]. The concentration of spores of *Ganoderma, Ustilago* and *Coprinus* inside the churches was considerably high, and it is important to remark that *Ganoderma* basidiospores are dominant members of aerial spores in many regions of the world and considered important allergens [20]. On the other hand, the relative abundance of spores of *Coprinus* is somehow erratic since reports on their presence are scarce. This spore type is a common saprophyte favoured by warm climate, but it is unlikely to identify their basidiospores in viable sampling studies, or even in volumetric impact studies which have not usually reported them at high concentrations [21].

The presence of *Alternaria* in the churches under study was limited in the church of Nuestra Señora del Roble, only nine spores were found, while in Santa Catarina Mártir Church a maximum average of 53 spores was found, which is in accordance with reports from several authors for this spore [15, 22] since it is more commonly found in outdoor environments, although it is likely to colonize indoors when conditions are favourable. However, another important reason for it not being found indoors is the size of their spores, since they can grow up to 20 – 40 µm [11]. However, there are reports on *Alternaria* as a major fungal spore in indoor environments [23].

The daily variation of spores in the four churches of the MAM is shown in Figure 1. There it may be noticed that in the Church of Santa Catarina Mártir the day with the highest concentration of spores occurred on Sunday, 6 September, with a total of 2,089 spores/m^3 and did not vary considerably with respect to the concentration the following day. The highest abundance during these two consecutive days may have occurred because rainfalls in September are high, thus increasing the favourable conditions for fungal growth outdoors, which are then transported to the interior environment by air currents and visitors. On the contrary, the day with the lowest concentration of spores occurred on Sunday, 5 July, with a total of 71 spores/m^3. The days with the highest and lowest concentration of spores both occurred on Sundays, which are days with four Mass services attended by a crowd, compared to the rest of the weekdays. At the Nuestra Señora del Roble Church, the day with the highest concentration of spores occurred on Monday, 7 December, with 1,686 spores/m^3 and the lowest concentration was recorded on Thursday, 16 July with 33 spores/m^3. Generally, higher concentrations of spores were recorded on samples taken on Sundays, with the exception of Thursday (17 September) and Monday (7 December which exhibited an unusually high count of spores, most likely due to parade celebrations of both Independence Day and the Anniversary of the Apparition of Our Lady of Guadalupe during the previous nights, which would explain the high concentrations of fungal spores brought indoors by the crowd. For the Church of Perpetuo Socorro the day with the highest concentration of spores occurred on Sunday November 29th with 1135 spores/m^3 while the lowest concentration was recorded on Monday, 20 July with 22 spores/m^3. The highest peaks of spores at this site occurred on

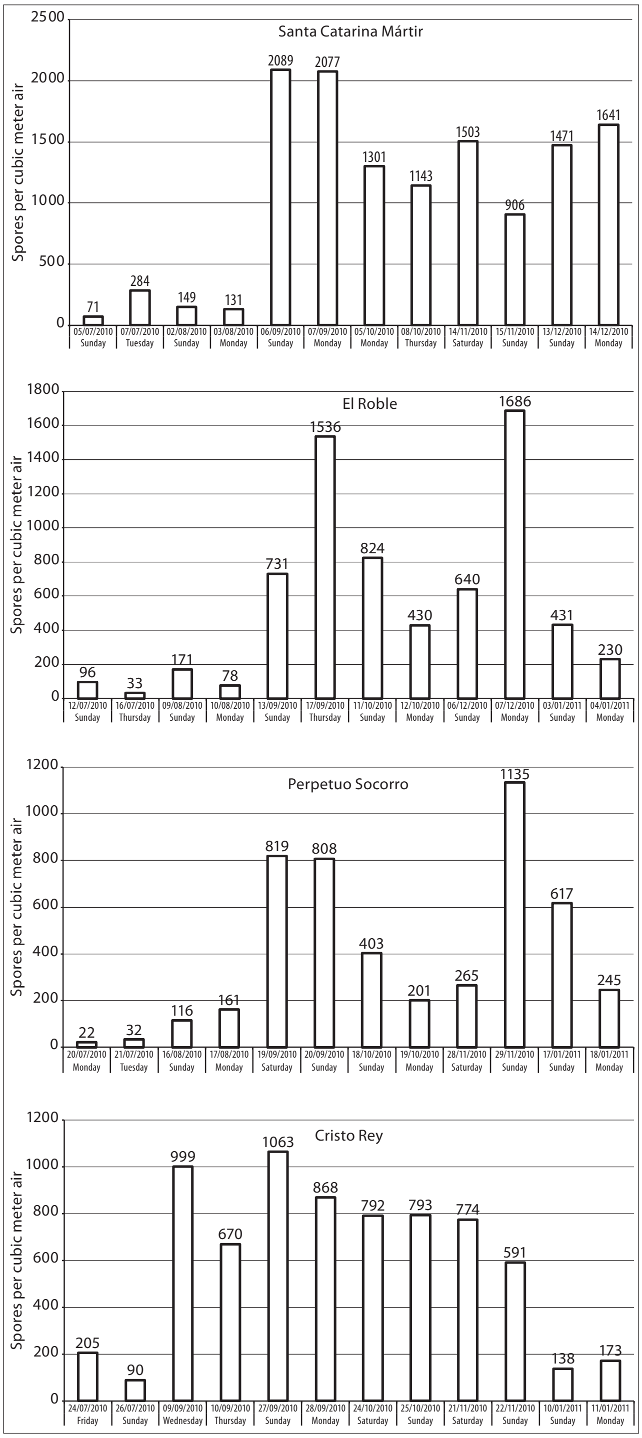

Figure 1. Daily variation of spores in four churches of MAM

Sundays and occasionally on some Saturdays, suggesting that anthropogenic activity may be the cause of this increment due to the crowd outside and inside the church, and since the doors of this particular building remain open, fungal particles may enter and contribute to the rise in the spore concentration. Similarly, the day with the highest number of collected spores at the Cristo Rey Church occurred on Sunday, 27 September with 1,063 spores/m^3, whereas there were only 90 spores/m^3 on Sunday, 26 July. The number of sampled spores remained uniform every single sampling day most likely because the surrounding environment

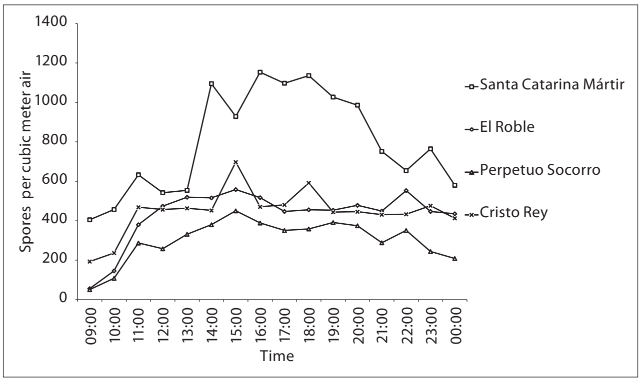

Figure 2. Hourly variations of spores in the four churches of MAM

persisted almost undisturbed throughout the weekdays. On the contrary, six Mass services and other activities are held on Sunday with crowds coming and going.

With respect to the hourly variation, it was found that the highest concentrations of spores in the churches were recorded between 14:00 – 20:00 hours (Fig. 2). The peak concentration at the church of Santa Catarina Mártir was at 16:00 hours (1,153 spores/m^3 air) while the church of Nuestra Señora del Perpetuo Socorro showed the lowest concentration at 09:00 with 51 spores/m^3 (Fig. 2). Low quantities of spores were detected in the four churches during the first two months of the study (July and August), but increased from September – January when it declined again. These records of lower and greater abundance of spores matched with the months of lower and higher relative humidity, respectively. Chakraborty et al. [24], in a comparative study of fungal spores in the air from five indoor and five outdoor environments in Burdwan, West Bengal, India, found a significant seasonal variation in spore concentration with low values in summer (March-June) and high values in the rainy season (July-October), attributing it to the fact that during the rainy season moderate temperatures and high relative humidity favoured fungal growth and sporulation, while fungi grew poorly in the high temperatures and dry conditions prevailing during the summer period. Furthermore, Aira et al. [25] mentioned that the concentration of fungal propagules were higher during spring and summer, but significantly decreased from September both indoors and outdoors.

In general, church buildings tend to be constructions with extraordinary roof elevation in order to establish the high spirit of its founding, giving more importance to an aesthetic-religious design over a functional project; therefore, churches are dominated by closed areas which are regularly lighted and ventilated in a deficient manner, especially in old buildings. Aira et al. [25] mentioned that high humidity and reduced ventilation are the main conditions favouring microbial contamination of air in indoor environments, despite a substrate always being necessary to provide nutrients to the fungus (wood, cellulose, various textiles such as curtains, carpets, etc.). Thus, poor ventilation has been a common problem in churches due to the need to regulate the temperature of the building to create a pleasant atmosphere for visitors, for health reasons, or even to prevent bio-deterioration of the artistic and monumental heritage; hence, mechanical ventilation is commonly chosen among the alternatives. In the four church buildings of this study, assisted natural ventilation and fans are generally employed, but an air conditioning system is also allowed in both the church of Nuestra Señora del Perpetuo Socorro and the church of Cristo Rey, and the use of evaporative air coolers are permitted in Santa Catarina Mártir and Nuestra Señora del Roble.

CONCLUSIONS

A total of 31,629 spores from 54 types of spores were found in the four churches studied. The church with the highest number of spores collected during the study period was the church of Santa Catarina Mártir with 12,766 spores belonging to 49 types of spores, followed by Cristo Rey church with 7,155, the church of Nuestra Señora del Roble with 6,887, while the Perpetuo Socorro church had 4,821 spores. Of the total identified fungal spores at the four churches, conidia had the highest percentage (81%), followed by the basidiospores with 10%, ascospores with 3%, and other spores 6%. The most abundant spores in the four churches were *Cladosporium*, followed by the complex *Aspergillus/ Penicillium*, *Coprinus*, *Ganoderma*, *Curvularia* and *Ustilago*. The highest concentration of spores in the churches occurred between 14:00 hours – 20:00 hours, and then decreased throughout the rest of the day.

REFERENCES

1. Lacey ME, West JS. The air spora. A manual for catching and identifying airborne biological particles. Springer, The Netherlands, 2006.
2. Durugbo EU, Kajero AO, Omoregie EI, Oyejide NE. A survey of outdoor and indoor airborne fungal spora in the Redemption City, Ogun State, south-western Nigeria. Aerobiologia 2013; 29: 201–216.
3. Rosas I, Calderon C, Ulloa M, Lacey J. Abundance of airborne *Penicillium* CFU in relation to urbanization in Mexico city. Applied and Environmental Microbiology 1993; 59 (8): 2648–2652.
4. Calderon C, Lacey J, McCartney A, Rosas I. Influence of urban climate upon distribution of airborne Deuteromycetes spore concentrations in Mexico city. Int J Biometerol. 1997; 40: 71–80.
5. Rosas I, Calderón C, Martínez L, Ulloa M, Lacey J. Indoor and outdoor airborne fungal propagule concentrations in Mexico City. Aerobiologia 1997; 13: 23–30.
6. Rocha Estrada A, Alvarado Vázquez MA, Gutiérrez Reyes R, Salcedo Martínez SM, Moreno Limón S. Variación temporal de esporas de *Alternaria*, *Cladosporium*, *Coprinus*, *Curvularia* y *Venturia* en el aire del área metropolitana de Monterrey, Nuevo León. Rev Int Contam Ambie. 2013; 29(2): 155–165 (in Spanish).
7. Sorlini C. Aerobiology: general and applied aspects on the conservation of art works. Aerobiologia 1993; 9: 109–115.
8. Petushkova J, Kandyba P. Aeromicrobiological studies in the Moscow cathedrals. Aerobiologia 1999; 15: 193–201.
9. Madigan MT, Martinko JM, Parker JB. Biología de los Microorganismos, Prentice Hall Iberia, Madrid, 1999 (in Spanish).
10. Dagmar SE. Biocontaminants in door environments. Cutter Information Corp USA, Arlington, 1994.
11. García E. Modificaciones al sistema de clasificación climática de Köppen. Instituto de Geografía. Universidad Autónoma de México, México, 2004 (in Spanish).
12. Kapp OR. Pollen and spores. Second edition. The American Association of Stratigraphic Palynologists Foundation. Dubuque, Iowa, 2000.
13. Kendrick B. Fungal allergens. In Smith EG. Sampling and identifying allergenic pollens and moulds. Blewstone Press, San Antonio Texas, USA. 1990; 41–49.
14. Palmas F, Cosentino S. Comparison between fungal airspore concentration at two different sites in the south of Sardinia. Grana 1990; 29: 87–95.
15. Abbott SP. Molds and other fungi in indoor environments: Summary of biology, known health effects and references. Natural Link MOLD LAB, 2004.

16. Caretta G. Epidemiology of allergic disease: the fungi. Aerobiologia 1992; 8: 439–445.
17. Garret MH, Hooper BM, Cole FM, Hooper MA. Airborne fungal spores in 80 homes in the Latrobe Valley, Australia: levels, seasonality and indoor-outdoor relationship. Aerobiologia 1997; 13: 121–126.
18. Nayar TS, Thripthi KM, Jotish PS. Status or airborne spores and pollen in a coir factory in Kerala, India. Aerobiologia 2007; 23: 131–143.
19. Escamilla B, Comtois P, Cortes P. Fungal content of air samples from some asthmatic childrens homes in Mexico City. Aerobiologia 1995; 11: 95–100.
20. Craig RL, Levetin E. Multi-year study of *Ganoderma* aerobiology. Aerobiologia 2000; 16: 75–81.
21. Mitakakis TZ, Guest DI. A fungal spore calendar for the atmosphere of Melbourne, Australia, for the year 1993. Aerobiologia 2001; 17: 171–176.
22. Beaumont F, Kauffman HF, Sluitter HJ, De Vries K. Sequential sampling of fungal air spores inside and outside the homes of mould-sensitive. Asthmatic patients: a search for a relationship to obstructive reactions. Ann Allergy. 1985; 55: 740–746
23. Cezar Fontana MB. Estudio epidemiológico de alergia a hongos y otros neumoalergénos en estudiantes de Medicina de la Universitat Autonoma de Barcelona, con relación a los niveles fúngicos ambientales. Tesis doctoral, Universidad Autónoma de Barcelona. 2009. p. 235 (in Spanish).
24. Chakraborty S, Kumar S, Bhattacharya K. Indoor and outdoor aeromycological survey in Burdwan, West Bengal, India. Aerobiologia 2000; 16: 211–219.
25. Aira MJ, Rodríguez-Rajo FJ, Jato V, Piontelli E. Análisis cuantitativo y cualitativo de la aeromicota aislada de la catedral de Santiago de Compostela (Galicia, España). Boletín Micológico 2006; 21: 27–34 (in Spanish).