EVALUATING FUNGI INDOOR PRESENCE IN HOMES THROUGH VIABLE AND NON-VIABLE SAMPLING

(Evaluación de la presencia de hongos en el interior de domicilios a través de muestreo viable o no viable)

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ABSTRACT

Moulds are common and important allergens. They are more abundant outdoors but patients affected by mould allergy stay indoors much longer than outdoors. So, indoor sampling could help to assess the influence of the concentration of allergens in allergic symptoms. The aim of this study was to assess the relative efficiencies of two air sampling methods, viable and non viable, for the quantification of airborne indoor fungi in the homes of patients sensitized to Alternaria. Furthermore, outdoor sampling was carried out to compare results. Samples were taken over six months in Badajoz (SW Spain). Two houses were selected according to the presence of allergic patients to Alternaria. They were sampled once a month using both viable and non viable personal samplers at solar noon. A Burkard personal sampler was used to record spores and a Sampl'air AES Chemunex sampler was used for colonies. Three rooms were selected in each home: living room, kitchen and bathroom. Temperature and relative humidity were registered at each sample. Outdoor sampling was performed one day per week at the Faculty of Science, using a seven day Burkard sampler for spores and the same personal sampler for colonies. On average, 200-300 CFU/m^3 were found from more than 40 taxa identified. The highest number of colonies was recorded in the kitchen, then in the bathroom and finally in the living room.
Nevertheless, there were minor differences between rooms. The houses studied showed a similar temporal pattern, with maximum values in December and minimum in January. Cladosporium colonies showed statistical differences between homes, but these differences were not found with Alternaria, Aspergillus or Penicillium colonies. Differences between rooms appeared for Alternaria colonies and Cladosporium herbarium spores. Temperature was positively correlated in most cases and relative humidity negatively with Alternaria spores. The number of spores collected was as twice as that of colonies, with an average concentration between 600-700 colonies/m^3, and nearly 1400 spores/m^3 as maximum concentration. The temporal pattern of spores was similar to that found for colonies. Comparing colonies to spores, Alternaria and Aspergillus-Penicillium showed similar values. Notwithstanding, Cladosporium spores were nearly five times more abundant than colonies. The advantage of viable methods is the identification to species level, but they have the disadvantage that spores from some ubiquitous species, as Cladosporium, do not always grow in those media, which raises the interest to use additional non-viable methods.

RESUMEN

Los hongos son alérgenos comunes e importantes. Son más abundantes en exteriores pero los pacientes afectados por alergia a los hongos permanecen en interiores mucho más tiempo que en exteriores. Por esto, el muestreo en interiores puede ayudar a evaluar la influencia de la concentración de alérgenos en los síntomas de la alergia. El objetivo de este trabajo ha sido valorar la eficiencia relativa de dos métodos de muestreo del aire, viable y no viable, para la cuantificación de hongos aerovagantes de interiores en hogares de pacientes sensibilizados a Alternaria. Adicionalmente, se ha realizado un muestreo en exteriores para comparar los resultados. Las muestras se tomaron durante seis meses en Badajoz (SO de España). Dos casas fueron seleccionadas de acuerdo a la presencia de pacientes alérgicos a Alternaria spp. Fueron muestreadas hacia el mediodía de forma mensual utilizando simultáneamente captadores personales con métodos viables y no viables. Un captador

personal Burkard se utilizó para el registro de las esporas y un captador Sampl'air AES Chemunex para las colonias de hongos. Se seleccionaron tres habitaciones en cada casa, el salón, la cocina y el cuarto de baño. La temperatura y la humedad relativa fueron registradas en cada muestreo. El muestreo en el exterior se llevó cabo un día a la semana en la Facultad de Ciencias utilizando un captador Burkard 7-day para las esporas y el mismo captador personal para las colonias. En promedio se encontraron 200-300 CFU/ m^3 pertenecientes a más de 40 taxones identificados. El mayor número de colonias fue registrado en la cocina, luego en el cuarto de baño y finalmente en el salón. Sin embargo las diferencias entre las habitaciones fueron mínimas. Las casas estudiadas mostraron un patrón temporal similar, con valores máximos en Diciembre y mínimos en Enero. Las colonias de Cladosporium spp. mostraron diferencias estadísticamente significativas entre las casas, sin embargo, estas diferencias no aparecieron en las colonias de Alternaria, Aspergillus o Penicillium spp. Aparecieron diferencias entre las habitaciones para las colonias de Alternaria spp. y las esporas de Cladosporium spp. Las esporas de Alternaria spp. mostraron en la mayoría de los casos correlación estadísticamente significativa y positiva con la temperatura y negativa con la humedad relativa. El número de esporas registrado fue doble respecto al de colonias, con una concentración promedio de 600-700 esporas/m3, y casi 1400 esporas/m3 de concentración máxima. El modelo temporal encontrado fue similar al de colonias. La comparación entre esporas y colonias mostró valores similares para Alternaria spp y Aspergillus-Penicillium spp. Sin embargo, las esporas de Cladosporium spp. fueron casi cinco veces más abundantes que las colonias. La ventaja de los métodos viables es la capacidad de identificación a nivel específico, pero tiene la desventaja que las esporas de algunas especies ubicuistas, como Cladosporium spp, no siempre crecen en los medios usados, lo que enfatiza el interés de utilizar adicionalmente métodos no viables.

INTRODUCTION

It is widely accepted that moulds are common and important allergens. Although they are more abundant outdoors, patients affected by mould allergy stay indoors much longer. Some authors suggested that proper indoor sampling is the best way to study their possible influence on allergic symptoms (Ammann et al., 2008). Moulds are not only important allergens, moreover, they are an important source of mycotoxins (Zain, 2011) and they are even considered responsible, to some extent, for the Sick Building Syndrome (Crook & Burton, 2010).

Although indoor airborne fungi may be found as a consequence of their outdoor presence, and accordingly the seasonal pattern may be followed indoors, in many cases this seasonal pattern disappears (Herbarth et al., 2003) or even changes. For instance, indoor concentration does not decrease during winter, as it frequently occurs outdoors; on the contrary, it may increase (Tuñón de Lara et al., 1990). In any case, indoor sources should be taken into account to explain fungus indoor presence.

Most research works agree that some fungi genus are the most frequent indoors: *Cladosporium*, *Penicillium* and *Aspergillus* (Beguien & Nolard, 1994; Escamilla et al., 1995; Emberlin et al., 1995; Cosentino & Palmas, 1996; Palmas et al., 1999; Lee et al., 2006; Gómez de Ana et al., 2006; Aríngoli et al., 2008; Basilico et al., 2007). Notwithstanding, *Penicillium* has been recorded as the most frequent, at least in some season or conditions (Palmas et al., 1999; Solomon, 1976; Docampo et al., 2011; Cabral, 2010; Codina et al., 2008; Barnes et al., 2007; Hass et al., 2007; Pasanen et al., 1992). Furthermore, most fungal genera were found in higher concentrations outdoors compared to indoors, but *Penicillium* was more commonly recorded indoors in some cases (Garret et al., 1997).

A wide range of samplers were used to record indoor moulds. For non-viable airborne propagule capture, Burkard personal sampler was widely used either for limited period sampling (Escamilla et al., 1995; Emberlin et al., 1995; Garret et al., 1997; Pei-Chih et al., 2000) or for a continuous sampling (Fairs et al., 2010). For viable capture, Malt Extract Agar (MEA) is considered a suitable medium for growing most airborne fungi (Ren et al.; 2001). If non-viable and viable methods are working simultaneously, a useful index is the culturability, which has been defined by Lee et al. (2006) as the rate between colonies and spores. As outdoor sampling is often performed in addition to indoor sampling, another useful index is the Indoor/Outdoor (I/O) ratio (Nevalainen et al., 1991; Lee et al., 2006). Furthermore, as the data provided by these techniques can show an important level of variability, it is useful to give general data as arithmetic average as well as geometric median (Lee et al., 2006; Pei-Chih et al., 2000; Nevalainen et al., 1991; Dassonville et al., 2008; Pasanen et al., 1992).

Identification of fungi from airborne samples may present some degree of difficulty, which is why some biochemical techniques have been performed, like PCR (Meklin et al., 2007). Nevertheless, sampling methods that involve viable and non-viable capture will continue to provide valuable information about organisms in indoor air (Flannigan, 1997).

The aim of this study was to assess the relative efficiencies of two air sampling methods, viable and non viable, for the quantification of airborne indoor fungi in the homes of patients sensitized to *Alternaria* and to compare this data with those from outdoor sampling, taking into account environmental factors to explain possible differences. Although the number of homes sampled is low, they were selected using medical criteria from a higher number

of cases and it is only a preliminary study to assess the efficiency of the methodology.

MATERIAL AND METHODS

Sampling was performed over six months in Badajoz (SW Spain), from October 2010 to April 2011. Two houses were selected according to the presence of allergic patients to *Alternaria*, one in the centre of the city (home A) and the other one in outskirts (home B). The former was a flat in the 7th floor of a nine floor building, and the latter was a two floor house with a small garden (home B). They were sampled once a month, between 11:00 to 13:00 h in the morning, using both viable and non-viable personal samplers at solar noon. A Burkard personal sampler was used to record spores for 5 minutes at 10 litres/minute flow rate, using white petrolatum as adhesive (CAS number 8009-03-8). A Sampl'air AES Chemunex sampler was used for viable colonies for 1 minute at 100 litres/minute, using MEA as culture media. Indoor samples were taken by placing the sampler at ground level in the center of the rooms, keeping doors and windows closed.

Three rooms were selected in each home: living room, kitchen and bathroom. Temperature and relative humidity were registered at each sample. Non-viable samples were analysed at x1000 magnification optical microscope, and data were given as spores per cubic meter (spores/m^3). Petri dishes were cultivated at 27ºC for 5-7 days to identify colonies and data were given as colonies forming units per cubic meter (CFU/m^3). Outdoor sampling was performed one day per week at the Faculty of Science (University of Extremadura), 2980 m and 3520 m away from the homes studied, respectively, using a seven day Burkard sampler for spores and the same personal AES Chemunex sampler for colonies following the same sampling procedures. Monthly data from this sampling was compared to indoor sampling data. Data were given as average concentrations per cubic meter and geometric mean (GM) in order to compare them with the literature. Two ratio values have been calculated, indoor/outdoor (I/O) ratio and colonies/spores (C/S) ratio, the former to express the level of fungi infiltration and the latter to express in some way the viability of propagules.

Non parametric statistics were used: Spearman correlation and Kruskall-Wallis test were performed in order to compare the data from outdoor-indoor homes and rooms. For the former, correlation coefficient (r) and p-value (p) were given and chi-square value (cs) and p-value (p)were calculated for the later.

RESULTS

Table 1 shows date and meteorological data from sampling. Indoor temperature was 6-7ºC higher than outdoors, and indoor relative humidity was 24-26% lower than outdoors. However, both indoor and outdoor temperature and relative humidity had a strong negative correlation (r 0.533, $p < 0.001$)

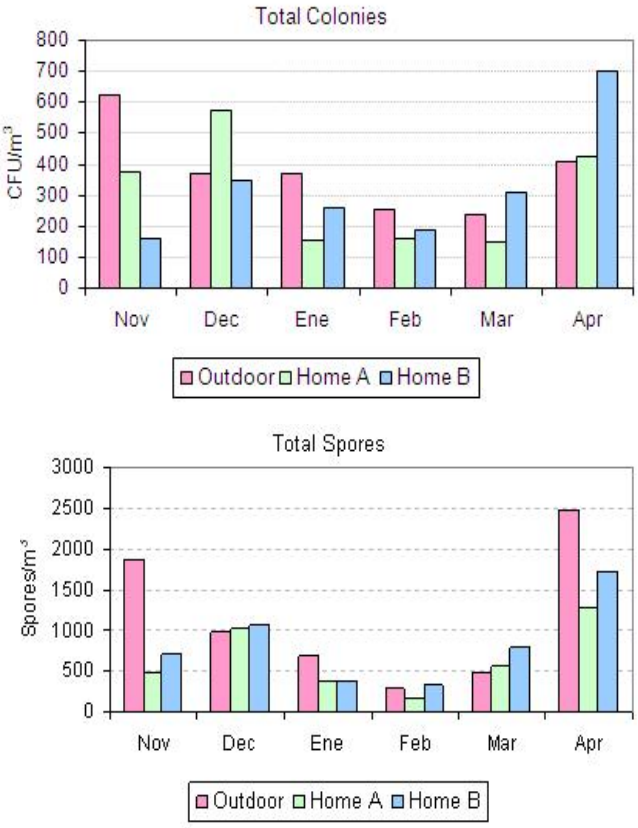

Fig. 1. Outdoor and indoor concentrations for total spores and colonies.

On average, for the whole samples, we recorded 741 spores/m^3 and 317 CFU/m^3 (GM: 267 spores/m^3 and 536 CFU/m^3) indoors, whereas 1890 spores/m^3 and 487 CFU/m^3 (GM: 1032 spores/m^3 and 402 CFU/m^3) were recorded outdoors (Table 2). Monthly total values are shown in Fig. 1. Minimum values were found usually in February and maximum values recorded depended on sampling type and home. Maximum concentrations of spores were recorded in April, both indoors and outdoors. For indoor colonies, maximum concentrations were recorded in December (home A) and April (home B), and maximum outdoor colonies were recorded in November.

Figs. 2-4 show monthly values for indoor and outdoor *Cladosporium*, *Alternaria*, *Aspergillus* and *Penicillium* spores and colonies. Outdoor *Cladosporium* spores were not always more abundant than indoor, even with lower values in some cases, as in December for *Cladosporium cladosporioides*. Colonies of *Cladosporium* showed a great variation, home A showed lower values than home B outdoors, and home B showed some higher values than outdoors in two occasions (Fig. 2). *Alternaria* showed on average higher values outdoors than indoors, mainly in colonies, except in one case, which is April in home A (Fig. 3). *Aspergillus*-

	Outdoor		Home A			Outdoor		Home B	
Date	Temp	HR	Temp	HR	Date	Temp	HR	Temp	HR
05-11-10	14.6	85.7	21.4	59.3	10-11-10	13.3	80.0	19.5	54.7
14-12-10	7.9	100.0	17.1	63.7	13-12-10	9.7	100.0	17.8	64.0
17-01-11	7.6	100.0	15.6	58.0	18-01-11	9.3	100.0	14.8	71.3
17-02-11	7.8	69.7	15.6	53.0	21-02-11	12.9	87.9	17.5	61.3
17-03-11	12.0	76.9	16.4	57.3	21-03-11	14.0	74.6	19.9	56.3
08-04-11	16.6	51.7	23.1	45.3	11-04-11	15.2	64.2	22.9	44.7

Table1. Outdoor and indoor temperature in degrees Celsius and relative humidity in percentage for sampling days.

	CFU/m3					Spores/m3					CFU/Spores	
	Outdoor	Home A	Home B	Indoor	I/O	Outdoor	Home A	Home B	Indoor	I/O	Indoor	Outdoor
Total	487	307	327	317	0,7	1890	653	829	741	0,4	0,43	0,26
Cladosporium spp.	206	35	129	80	0,4	356	274	305	290	0,8	0,28	0,58
C. cladosporioides						1010	236	252	244	0,2		
C. herbarum						110	38	53	46	0,4		
Alternaria spp.	31	10	6	8	0,3	37	4	9	7	0,2	1,14	0,84
Aspergillus-Penicillium spp.	91	58	41	50	0,5	59	106	79	92	1,6	0,54	1,54
Aspergillus spp.	63	28	14	21	0,3							
Penicillium spp.	18	30	27	29	1,6							

Table 2. Average concentration of colonies and spores for outdoor and home sampled.

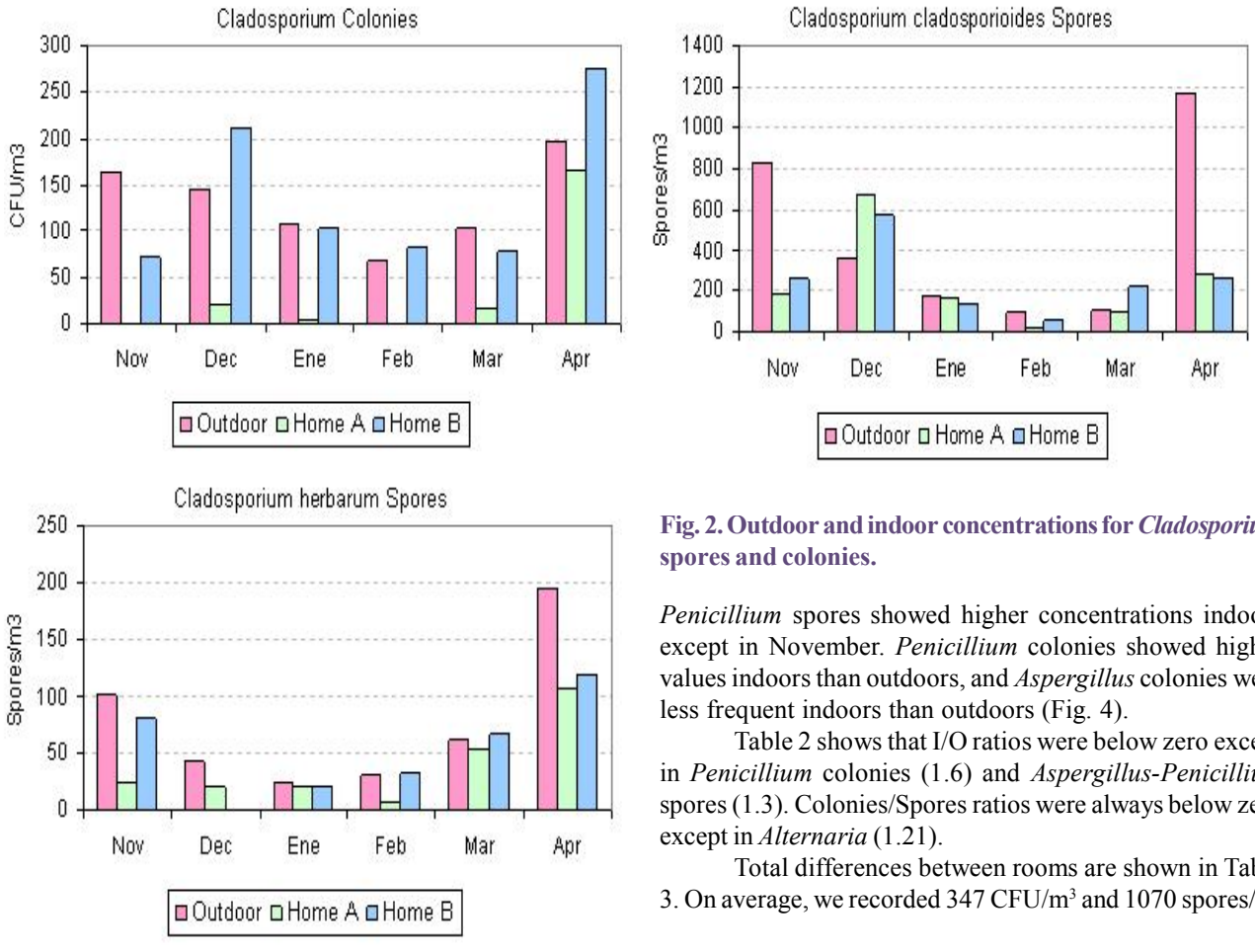

Fig. 2. Outdoor and indoor concentrations for *Cladosporium* spores and colonies.

Penicillium spores showed higher concentrations indoors except in November. *Penicillium* colonies showed higher values indoors than outdoors, and *Aspergillus* colonies were less frequent indoors than outdoors (Fig. 4).

Table 2 shows that I/O ratios were below zero except in *Penicillium* colonies (1.6) and *Aspergillus-Penicillium* spores (1.3). Colonies/Spores ratios were always below zero except in *Alternaria* (1.21).

Total differences between rooms are shown in Table 3. On average, we recorded 347 CFU/m^3 and 1070 spores/m^3

in the kitchen, 306 CFU/m^3 and 635 spores/m^3 in the bathroom, and finally 296 CFU/m^3 and 518 spores/m^3 in the living room.

The differences between the rooms of the two homes studied are showed in Figs. 5-8. There were no statistically significant differences between the two homes, except for *Cladosporium* colonies (cs 15.658, p<0.001) with higher values in the house B (Fig. 6). Comparisons between rooms showed that *Alternaria* colonies had statistically significant

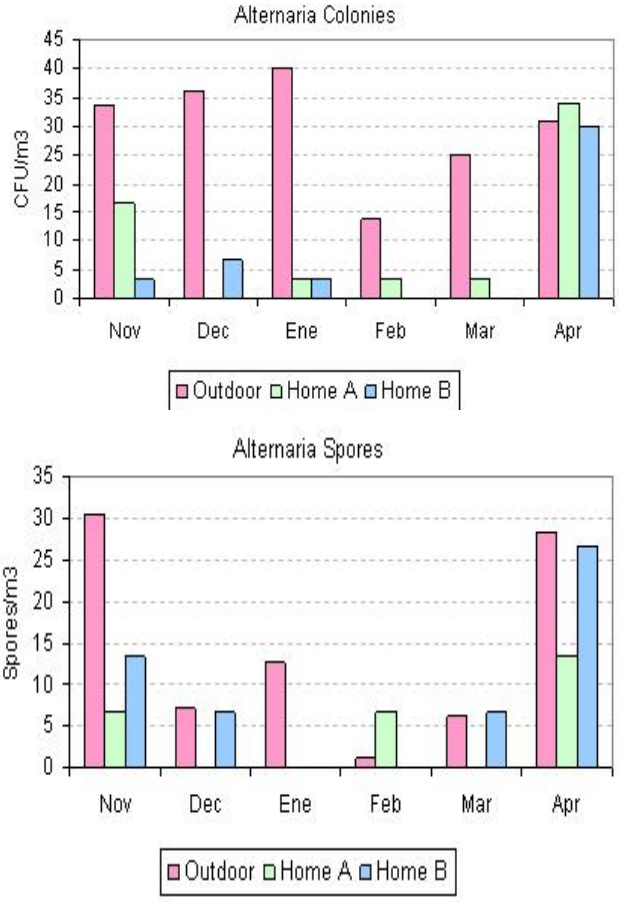

Fig. 3. Outdoor and indoor concentrations for *Alternaria* spores and colonies.

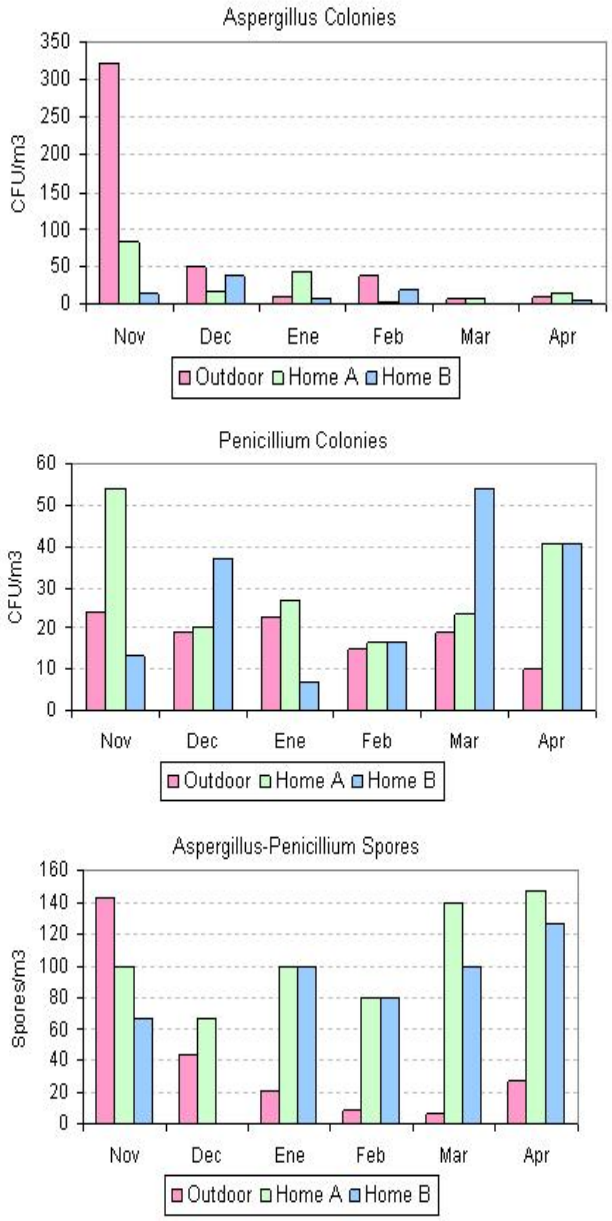

Fig. 4. Outdoor and indoor concentrations for *Aspergillus* and *Penicillium* spores and colonies.

	CFU/m^3				Spores/m^3			
	Total	Kitchen	Bathroom	Living r.	Total	Kitchen	Bathroom	Living r.
Total	317	349	306	296	741	1070	635	518
Cladosporium	80	101	89	47	290	383	287	199
C. cladosporioides					244	298	269	165
C. herbarum					46	86	18	34
Alternaria spp.	8	12	3	10	7	8	5	7
Aspergillus-Penicillium	50	52	60	37	92	135	71	71
Aspergillus spp.	21	26	20	17				
Penicillium spp.	29	25	40	20				

Table 3. Average concentration of colonies and spores for the room sampled.

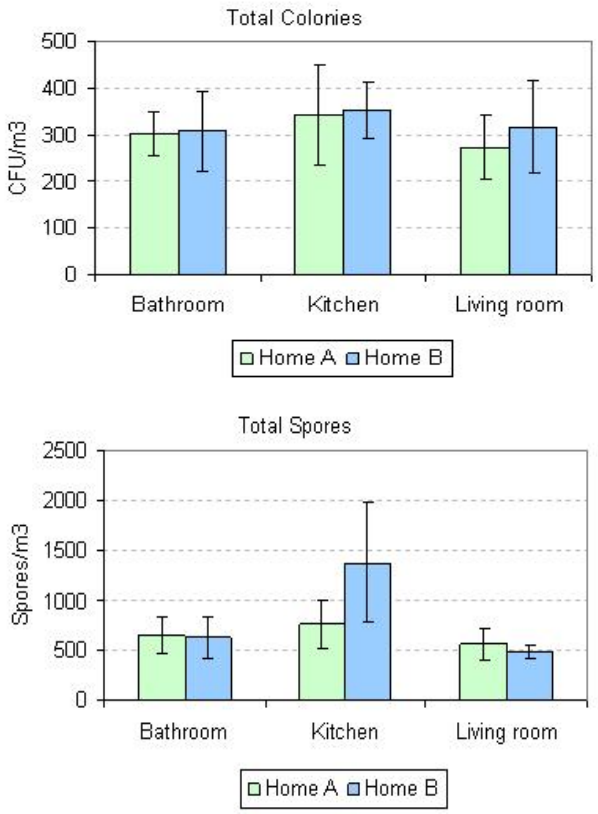

Fig. 5. Average total concentration indoors for spores and CFU depending on the room, bars represent errors.

differences (cs 9.998, p 0.007), see Fig. 7, and *Cladosporium herbarium* (cs 6.366, p 0.041), see Fig. 6. Both of them with the lowest concentrations in the bathroom.

DISCUSSION

Values of spores and colonies recorded in our study are in the range of comparable works using similar methodologies (Pasanen et al., 1992; Beguin & Nolard, 1994; Escamilla et al., 1995; Pei-Chih et al., 2000; Ren et al.; 2001; Lee et al., 2006; Gómez de Ana et al., 2006; Basilico et al., 2007; Meklin et al., 2007; Haas et al., 2007; Dassonville et al., 2008; O'Gorman & Fuller, 2008).

The indoor/outdoor ratio is always below 1 except for *Penicillium* colonies and *Aspergillus-Penicillium* spores. This could mean that, at least for *Penicillium*, indoor environment would be more suitable for growth and that there could be other sources inside that would increase their concentrations. This data agree with those found by other authors (Medrela-Kuder, 2003; Lee et al., 2006; Gómez de Ana et al., 2006; Docampo et al., 2011). These fungi seem to be more abundant in the bathroom and in the kitchen than in the living room (Fig. 8), probably because there are more suitable media or environmental conditions for proliferation.

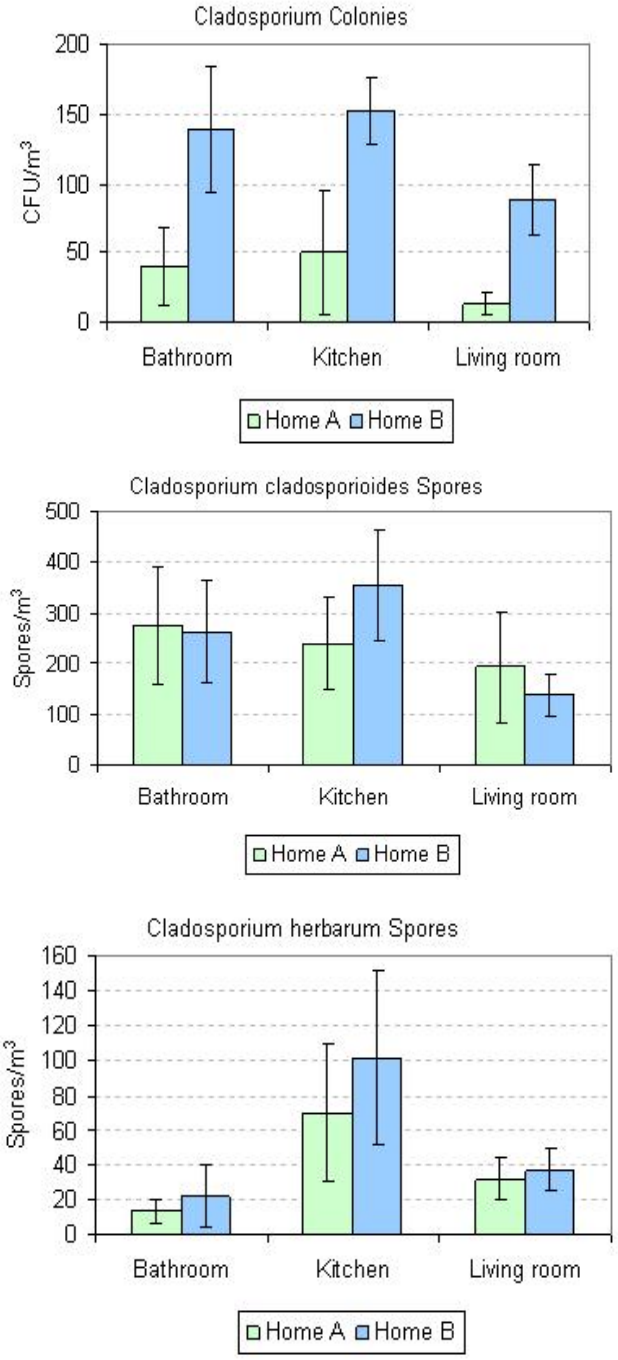

Fig. 6. Average *Cladosporium* concentration indoors for spores and CFU depending on the room, bars represent errors.

Regarding the ratio between colonies and spores, about half of the spores were able to grow in the culture media provided. Nevertheless, there were differences between fungi type. *Cladosporium* spores seem to be the least able to grow, while *Alternaria* colonies outnumber spores. This could be possible because some colonies of *Alternaria* grew from hyphal fragments, or because some conidia grow in chains with the apical conidia so different and smaller than the basal ones that they could not be counted under light microscope

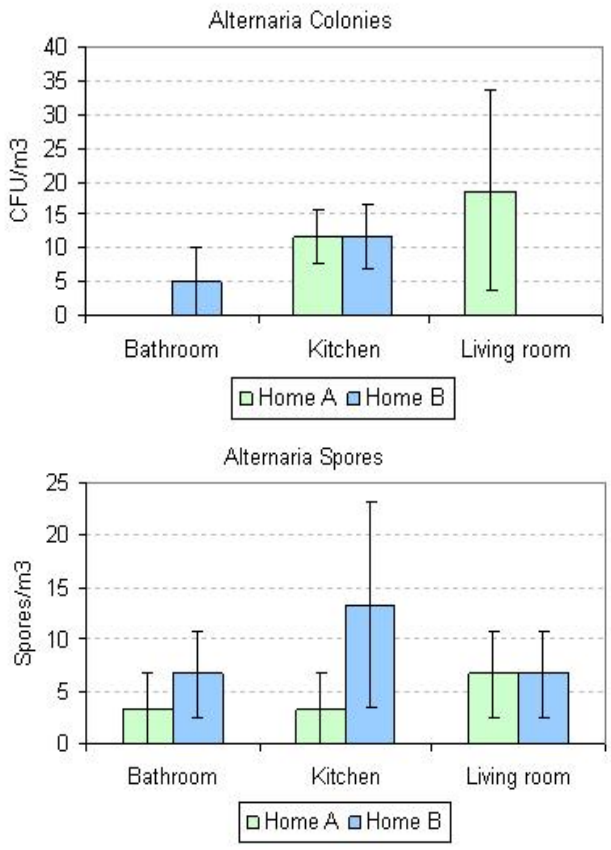

Fig. 7. Average *Alternaria* concentration indoors for spores and CFU depending on the room, bars represent errors.

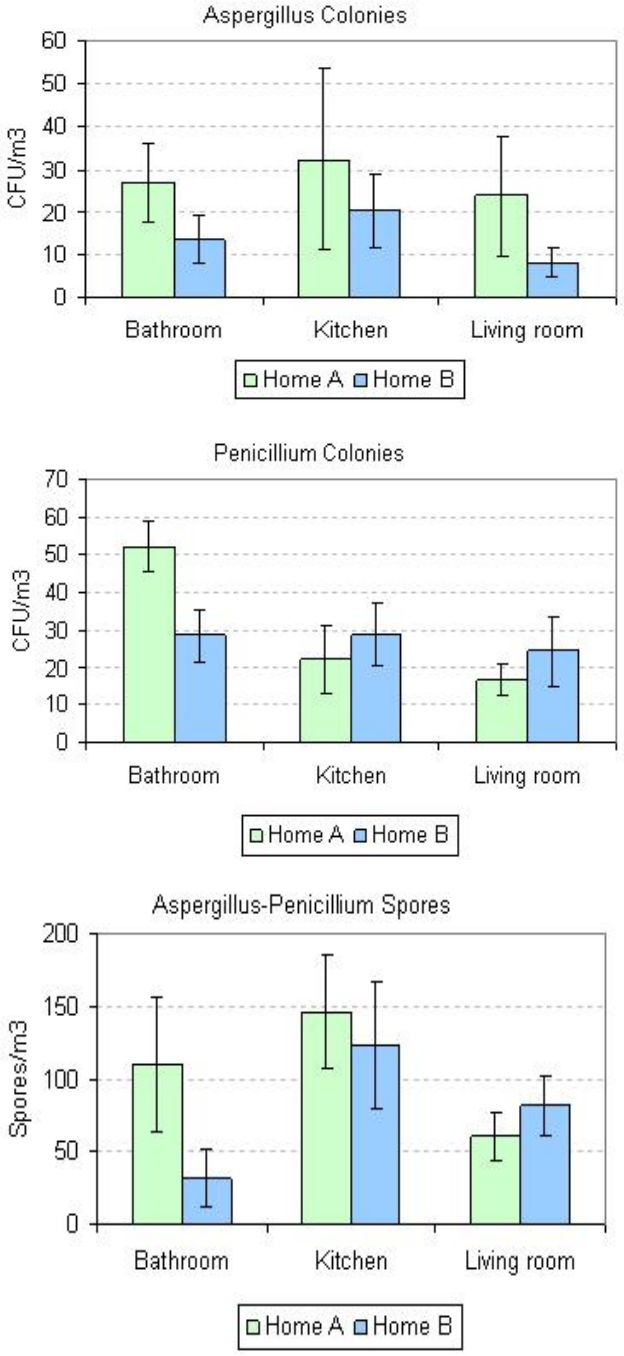

Fig. 8. Average *Aspergillus* and *Penicillium* concentration indoors for spores and CFU depending on the room, bars represent errors.

as *Alternaria* type. Other possible explanation could be that multicellular conidia of *Alternaria* are more efficient than unicellular conidia of *Cladosporium* to develop colonies.

There are no clear differences between both houses analysed. They follow a similar monthly pattern with similar concentrations (Figs. 1-4), even the pattern for the three rooms shows similarities. However, home B showed a statistically significant concentration of *Cladosporium* colonies higher than that of home A. This difference could be attributed to a closer contact with rural environment (Pasanen, 1992; Mitakakis & McGee, 2000, Rodríguez-Rajo et al., 2005). Nevertheless, the number of *Cladosporium* spores is only slightly higher in home B with no statistically significant difference. Perhaps home B could provide better conditions for the growth of *Cladosporium* colonies, but temperature and relative humidity are quite similar in both places. One difference that could explain these differences is that home A is higher than home B, nevertheless this should affect only *Cladosporium* colonies. Other possible explanations could be that fungi may be introduced into buildings through the dust and dirt swept away by the shoes and clothing or that the environmental conditions of house B may influence the growth of fungi, including heating and ventilation systems, furniture etc.

The indoor and outdoor viabilities of fungi, ratio between colonies and spores (C/S), were on average 0.43 and 0.26, respectively, which indicates that indoor environment provides more favourable survival conditions for airborne fungi. These values are quite similar to those found by Lee et al. (2006). Nevertheless, this ratio was higher for *Alternaria* indoors and *Aspergillus-Penicillium* outdoors. In the former case we can assume better conditions indoors for *Alternaria* and in the later better condition for *Aspergillus-Penicillium*. Nevertheless, as low

values of concentrations have been recorded in some cases, this should be taken with care when calculating the ratio, as in the case for both fungi types, since small figures could lead to great differences in ratio calculation.

CONCLUSIONS

As the differences between outdoor and indoor colonies and spores, on average, are not quite relevant, and the monthly pattern is similar too, we can conclude that most indoor fungi comes from outdoors, and, only a small proportion depends on indoor sources, as in *Penicillium*. After using simultaneously viable and non-viable sampling we consider that the only advantage of viable methods is the identification to species level, but they have the disadvantage that spores from some ubiquitous species, as *Cladosporium*, do not always grow in those media, so their presence is often underestimated and the interest to use additionally non-viable methods is proved.

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REFERENCES

Ammann, H.; Hodgson, M.; Nevalaine, A. & Presant, B. (2008). Basic for health concerns. In B. Prezant, D. M. Weekes, J. D. Miller (eds) Recognition, Evaluation, and Control of Indoor Mold. AIHA, Fairfax. 1:3-20

Aríngoli, E.E.; Basílico, M.L.Z.; Altahus, R.L. & Basílico, J. C. (2008). Multivariate analysis of fungal associations in the indoor air of Argentinean houses. Internat. Biodet. Biodegrad. 62: 281-286

Barnes C.S.: Dowling, P.; Van Osdol, T. & Portnoy, J. (2007). Comparison of indoor fungal spore levels before and after professional home remediation. Ann. Allergy Asthma Immunol. 98:262-268

Basilico, M.L.Z.; Chiericatti, C.; Aringoli, E.E.; Althaus, R.L. & Basilico, J.C. (2007). Influence of environmental factors on airborne fungi in houses of Santa Fe City, Argentina. Sci. Total Environ. 376:143-150

Beguin, H. & Nolard, H. (1994). Mould biodiversity in homes I. Air and surface analysis of 130 dwellings. Aerobiologia 10:157-166

Cabral, J.P.S. (2010). Can we use indoor fungi as bioindicators of indoor air quality? Historical perspectives and open questions. Sci. Total Environ. 408:4285-4295

Codina, R.; Fox, R.W.; Lockey, R.F.; DeMarco, P. & Bagg, A. (2008). Typical levels of airborne fungal spores in houses without obvious moisture problems during a rainy season in Florida, USA. J. Investig. Allergol. Clin. Immunol. 18:156-162

Cosentino, S. & Palmas, F. (1996). Occurrence of fungal spores in the respiratory tract and homes of patients with positive skin test to fungi. Aerobiologia 12:155-160

Crook, B. & Burton, N.C. (2010). Indoor moulds, Sick Building Syndrome and building related illness. Fungal Biol. Rev. 24:106-113

Dassonville, C.; Demattei, C.; Detaint, B.; Barral, S.; Bex-Capelle, V. & Momas, I. (2008). Assessment and predictors determination of indoor airborne fungal concentrations in Paris newborn babies' homes. Environ. Res. 108: 80-85

Docampo, S.; Trigo, M.M.; Recio, M.; Melgar, M.; García-Sánchez, J. & Cabezudo, B. (2011). Fungal spore content of the atmosphere of the Cave of Nerja (southern Spain): Diversity and origin. Sci. Total Environ. 409: 835-843

Garcia, E.; Comtois; P. & Borrego, P.C. (1995). Fungal content of air samples from some asthmatic childrens' homes in Mexico City Beatriz. Aerobiologia 11:95-100

Emberlin, J.; Newman, T. & Bryant, R.. . (1995). The incidence of fungal spores in the ambient air and inside homes: Evidence from London. Aerobiologia 11:253-258.

Escamilla, G.B.; Comtois, P. & Cortés, B.P. (1995) Fungal content of air samples from some asthmatic childrens' homes in Mexico City. Aerobiologia 11:95-100

Fairs, A.; Wardlaw, A.J.; Thompson, Jr. & Pashley, C.H. (2010). Guidelines on ambient intramural airborne fungal spores. J. Investig. Allergol. Clin. Immunol. 20:490-498

Flannigan, B. (1997) Air sampling for fungi in indoor environments. J. Aerosol Sci. (1997) 28(3): 381-392.

Garrett, M.H.; Hooper, B.M.; Cole, F:M. & Hooper, M.A. (1997). Airborne fungal spores in 80 homes in the Latrobe Valley, Australia: levels, seasonality and indoor-outdoor relationship. Aerobiologia 13:121-126

Gómez de Ana, S.; Torres-Rodríguez, J.-M.; Alvarado-Ramírez, E.; Mojal-García, S. & Belmonte-Soler, J. (2006). Seasonal Distribution of Alternaria, Aspergillus, Cladosporium and Penicillium Species Isolated in Homes of Fungal Allergic Patients. J. Invest. Allergol. Clin. Immunol. 16:357-363

Haas, D., Habib, J.; Galler, H.; Buzina, W.; Schlacher, R.; Marth, E. & Reinthaler, F.F. (2007). Assessment of indoor air in Austrian apartments with and without visible mold growth. Atmos. Environ. 41: 5192-5201

Herbarth, O., Schlink, U.; Müller, A. & Richter, M. (2003). Spatiotemporal distribution of airborne mould spores in apartments. Mycol. Res. 107:1361-1371

Lee, T.; Grinshpun, S.A.; Martuzevicius, D.; Adhikari, A.; Crawford, C.M. & Reponen, T. (2006). Culturability and concentration of indoor and outdoor airborne fungi in six single-family homes. Atmos. Environ. 40:2902-2910

Medrela-Kuder, E. (2003). Seasonal variations in the occurrence of culturable airborne fungi in outdoor and indoor air in Craców. Biodet. & Biodegrad. 52:203-205

Meklin, T., Reponen, T.; McKinstry, C.; Cho, S.-H.; Grinshpun, S.A.; Nevalainen, A.; Vepsäläinen, A.; Haugland, R.A.; LeMasters, G. & Vesper, S.J. (2007). Comparison of mold

concentrations quantified by MSQPCR in indoor and outdoor air sampled simultaneously, Sci. Total Environ. 382:130-134

Mitakakis, T.Z. & McGee, P.A. (2000). Reliability of measures of spores of Alternaria and pollen concentrations in air over two towns in rural Australia. Grana 39: 141–145

Nevalainen, A.; Pasanen, A.-L.; Niininen, M.; Reponen, T.; Kalliokoski, P. & Jantunenm, M.J. (1991). The indoor air quality in Finnish homes with mold problems. Environ. Internat. 17(4):299-302

O'Gorman, C.M. & Fuller, H.T. (2008). Prevalence of culturable airborne spores of selected allergenic and pathogenic fungi in outdoor air. Atmos. Environ. 42(18): 4355-4368

Pei-Chih, W.; Huey-Jen, S. & Chia-Yin, L. (2000). Characteristics of indoor and outdoor airborne fungi at suburban and urban homes in two seasons. Sci. Total Environ. 253:111-118

Palmas, F.; Cosentino, S.; Meloni, V. & Fadda, M.E. (1999). Occurrence of mites and fungi in the homes of patients with allergic manifestations. Aerobiologia 15:109-114

Pasanen, A.L. (1992). Airborne mesophilic fungal spores in various residential environments. Atmos. Environ. 26:2861-2868

Pasanen, A.-L.; Niininen, M.; Kalliokoski, P.; Nevalainen, A. & Jantunen. M.J. (1992). Airborne Cladosporium and other fungi in damp versus reference residences. Atmos. Environ. 26: 121-124

Ren, P.; Jankun, T.M.; Belanger, K.; Bracken, M.B. & Leaderer, B.P. (2001). The relation between fungal propagules in indoor air and home characteristics. Allergy 56:419-424

Rodríguez-Rajo, F.J.; Iglesias, I. & Jato, V. (2005). Variation assessment of airborne *Alternaria* and *Cladosporium* spores at different bioclimatical conditions. Mycol. Res. 109:497-507

Solomon, W.R. (1976). A volumetric study of winter fungus prevalence in the air of midwestern homes. J. Allergy Clin. Immunol. 57:46-55

Tuñon De Lara, J.M.; Tessier, J.F.; Lafond-Grellety, J.; Domblides, J.M.; Faugère, J.-G. & Taytard, A. (1990). Indoor moulds in asthmatic patients homes. Aerobiologia 6:98-101

Zain, M.E. (2011). Impact of mycotoxins on humans and animals. J. Saudi Chem. Soc. 15(2):129-144