

Preliminary survey of indoor and outdoor airborne microfungi at coastal buildings in Egypt

EL-Sayed M. EL-Morsy

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Abstract Forty six species and two sterile fungi and yeast species were isolated from samples collected both indoors and outdoors of coastal buildings located in an Egyptian coastal city. Twenty flats from ten buildings were investigated; children living in these buildings have been reported to suffer from respiratory illnesses. Samples were taken using a New Brunswick sampler (model STA-101) operating for 3.0 min at a flow rate of 6.0 l/min. Most of the species isolated have been associated with symptoms of respiratory allergies. Indoors the total culturable fungal count was 1548 CFU/m³; outdoors, it was 1452 CFU/m³. Indoor values of culturable fungal count, total spores count and ergosterol content ranged from 52 to 124 CFU/m³, 100 to 400 spore/m³ and 5 to 27.7 mg/m³, respectively, whereas outdoor levels typically varied between 25 and 222 CFU/m³, 110 and 900 spore/m³ and 3.3 and 67.2 mg/m³, respectively. The maxima for these parameters were detected indoors in house no. 6 and outdoors, outside of house no. 7. The most abundant species were primarily mitosporic (2832 CFU/m³). The most frequent species in both the indoor and outdoor samples were *Cladosporium cladosporioides* followed by *Alternaria alternata* and

Penicillium chrysogenum, with inside:outside ratios of 1.4, 1.8 and 1.9, respectively. The patterns of fungal abundance were influenced to some extent by changes in the relative humidity and temperature. Other factors, such as type of culture media, rate of sedimentation, size, survival rates of spore and species competition, also affected fungal counts and should be taken into consideration during any analysis of bioaerosol data.

Keywords Airborne viable fungi · Egypt · Ergosterol content · New Damietta · Total spore count

1 Introduction

Fungi are omnipresent, appearing in the atmosphere, hydrosphere, lithosphere and ontosphere (on biota). Airborne fungi have been found to be associated with certain respiratory illness and allergy (Kowalski & Bahnfleth, 1998), while exposure to fungi and other microbes, their fragments and metabolites may constitute a health risk: for example, increases in asthma attacks and bronchial hyperactivity and other respiratory symptoms such as lung cancer have been correlated to increased microbial and particulate levels in the aerosphere (Ross et al., 2000; World Health Organization, 2000). In addition, an association

E.-S. M. EL-Morsy (✉)
Botany Department, Damietta Faculty of Science,
New Damietta, Egypt
e-mail: el_morsy@mum.mans.edu.eg

has been found between damp environments, microbial exposure and higher prevalences of respiratory symptoms and allergic diseases (Nielsen, Holm, Uttrup, & Nielsen, 2004). More than 80 genera of fungi have been linked with symptoms of respiratory tract allergies (Horner, Helbling, Salvaggio, & Lehrer, 1995), with the most common allergenic genera being *Cladosporium*, *Alternaria*, *Aspergillus* and *Fusarium*. Exposure to large concentrations of the spores of these four genera cause aspergillosis (Anderson, Morris, & Kennedy, 1996), asthma and pneumonitis (Cuijpers, Swaen, Wesseling, & Sturmans, 1995; Hu, Persky, Flay, & Richardson, 1997), allergic alveolitis and toxicosis (Flannigan, McCabe, & McGarry, 1991). Concurrently, fungal metabolites are also believed to irritate the respiratory system. The indoor/outdoor fungal ratio of sick syndrome homes has been found to be higher (2.16%) than that (0.56%) of healthy homes (Gniadek, Macura, Oksieczuk, Krajewska-Kulak, & Lukaszuk, 2005).

The presence of airborne fungi in buildings has been highlighted by Hunter, Grant, Flannigan, and Bravery (1988) and Flannigan, McCabe, Jupe, and Jeffrey (1993), and subsequent studies determined that *Aspergillus*, *Cladosporium* and *Penicillium* were the major building species (Bogacka, 1997; Cooley, Wong, Jumper, & Straus, 1998; Gniadek et al., 2005; Macura & Gniadek, 2000; Nilsson et al., 2004; Pei-Chih, Huey-Jenu, & Chia-Yin, 2000). High indoor viable counts were invariably associated with damp buildings (DeKoster & Thorne, 1995; Nielsen et al., 2004). Nielsen et al. (2004) reported that almost exclusively only *Penicillium*, *Aspergillus* and *Eurotium* species grew on building materials and that the production of secondary metabolites and mycotoxins decreased with humidity. Conversely, mycotoxin-producing moulds were more prominent in poorly aerated buildings (Garrison, Robertson, Koehn, & Wynn, 1993). *Cladosporium* spp., *Phoma* and *Ulocladium* spp. commonly grow on damp indoor surfaces whereas in very damp conditions *Stachybotrys chartarum* may be prominent (Grant, Hunter, Flannigan, & Bravery, 1989).

In general, the incidence and prevalences of fungal distributions in the aerosol vary among geographic areas and are greatly influenced by

environmental factors (Burge, 1990; Cosentino, Pisano, Fadda, & Palmas, 1990; Halwagy, 1989; Lara, Tessier, & Lafont-Grellety, 1990). Differences in the size and sedimentation rate of spores also affect what is detected in the air samples (Hunter et al., 1988). The distribution of airspora also differs with regional locations and different survival rates of species, both in the environment and during cultivation (Flannigan, McCabe, & Jupe, 1996). Spores of certain species may survive for long periods, while others may decline very rapidly (Flannigan & Miller, 1994). Fungal spores floating in the open air are exposed to air currents and can travel very long distances, with the result that the numbers and species of airborne fungi vary with the season of the year (Medrela-Kuder, 2003; Picco & Rodol, 2000; Ren, Jankun, Belanger, Bracken, & Leaderer, 2001). Consequently, the greatest numbers of fungi are observed during the summer and autumn than the spring and winter (Gniadek et al., 2005; Medrela-Kuder, 2003; Ren et al., 2001). The most frequent airborne genera outdoors are *Cladosporium*, *Penicillium*, *Aspergillus* and *Acremonium* (Gniadek et al., 2005; Pei-Chih et al., 2000), while *Cladosporium* sp., has been found to be the mould most frequently isolated from indoor air during the summer (Bogacka, 1997; Ren et al., 2001). Bogacka (1997) noted that in both summer and autumn there are likely to be increased numbers of *Alternaria* spores (Bogacka, 1997).

The membrane lipid ergosterol is an almost exclusive property of fungi and is frequently used as an indicator of living fungal biomass based on the assumption that ergosterol is labile and, therefore, rapidly degraded after the death of fungal hyphae. However, Mille-Lindblom, von Wachenfeldt, and Tranvik (2004) reported that ergosterol persists after fungal death and, therefore, caution must be taken when using ergosterol as a biomarker of living fungi. Ergosterol content has been found to be closely correlated with the amount of mycelium (Gutarowska, 1999; Matcham, Jordan, & Wood, 1985). Other investigators have reported a great dependence between ergosterol content and the number of spores (Schnpurer, 1991) as well as the colony-forming units (CFU) of mould spores on rye (Magan, 1993), in house dust (Saraf,

Larsson, Burge, & Milton, 1997), building materials (Gutarowska & Zakowska, 2002) and wood samples (Pasanen, Yli-Pietila, Pasanen, Kalliokoski, & Tarhanen, 1999). The level of ergosterol in the fungal cell has been found to depend on the physiological forms of the fungus, the species, the age, amount of the nutrient medium, moisture content and environmental factors (Bjurman, 1994; Gutarowska, 1999; Pasanen et al., 1999).

The aim of this study was to determine the indoor and outdoor airborne fungal concentrations in coastal buildings occupied by children suffering from respiratory diseases.

2 Materials and methods

2.1 Study area

New Damietta is one of the newer coastal cities, located north of the delta region of the Nile, in the northwestern part of Damietta province, Egypt. It has a surface area of 18 km² and extends for 9.5 km along the coast of the Mediterranean Sea. It is situated at a distance of 4.5 km from Damietta Harbor. The city is surrounded from its east, west and south with a green belt comprised predominantly of palm and fruit gardens. As the city is still under construction, halophytic vegetations are well represented, especially in undeveloped constructed zones and the coastal strip. The city can be broadly classified into six main areas (437 acres) in addition to the industrial zone.

The study area consisted of the first and second areas, whereas the third and fourth are construction areas. Each area was divided into six zones. These zones are not densely populated but the population density differs throughout the year. The population in the summer is higher than any other season of the year. Five zones were selected, and two buildings, represented by two apartment buildings, were investigated in each zone. Sampling was conducted indoors, in a naturally ventilated bedroom of one flat in each of the 20 buildings, and outdoors, outside of the window of the same bedroom, in all zones in May. The investigated areas have an incidence of

children suffering from respiratory illnesses. During sampling, the windows were kept closed.

2.2 Isolation of fungi

Fungi were sampled using a New Brunswick single-stage air sampler Model STA-101 (New Brunswick Scientific, Edison, N.J.). The samplers was operated for 3.0 min at a flow rate of 60.0 l/min and a total volume of 180 l. The sampler was loaded with Czapek Dox agar plates amended with Rose Bengal (0.035 g/ml) as isolation medium. Penicillin G and streptomycin were added to prevent bacterial growth. Between measurements the samplers were swabbed with 70% ethanol. Samples were taken at a height of 150 cm near the human breathing zone both indoors in the bedroom and outdoors just outside the windows. After sampling, the plates were incubated at 28°C for up to 14 days to enable identification. The fungi were identified morphologically and the aerosol concentrations (CFU/m³) were calculated. The number of CFU per cubic metre was calculated as: number of colonies $\times$ 1000/sampling time $\times$ velocity of air flow (Fanga et al., 2005). Based on the frequency of occurrence, the isolated species were classified as very frequent (>20%) frequent (10–20%) and infrequent (<10%) (Tan & Leong, 1989).

2.3 Classification of fungi

Isolates were identified using various agar media to promote sporulation. Non-sporulating strains were grouped within Class Agonomycetes (sterile fungi) of the anamorphic fungi according to similarities in colony morphology and production of chlamydospores or sclerotia (Lacap, Liew, & Hyde, 2003).

2.4 Direct microscopic observation

The spores were trapped in a New Brunswick single-stage air sampler (model and parameters as described in preceding section). A 9-cm petri dish coated with a film of glycerol (10 ml/plate) and fixative (lactophenol cotton blue) was used in each sampler to trap spores. The trapped spores were examined using a light microscope (Carl

Zeiss Jena, GDR). Total counts of spores/ml of mounting media was determined using a haemocytometer. The counts were then converted into the number of spores per cubic meter of air (spores/m³) as above.

2.5 Determination of the ergosterol content

Ergosterol content was determined using the Seitz method (Seitz, Sauer, Burroughs, Mohr, & Hubbard, 1979). Spores trapped on the petri plates coated with a very thin film of glycerol (5 ml) were transferred into 50 ml of methanol, placed in a 250-ml flask, processed as described earlier and analysed at $\lambda = 282$ nm on a Turner (Cole Parmer, Vernon Hills, Ill.) spectrophotometer with a far-UV source (model 390). A standard ergosterol calibration curve were prepared from the basic solution containing 1 mg of ergosterol in 1 ml methanol (standard solution of Sigma, St. Louis, Mo.). The ergosterol content was read from the calibration curve. The resulting ergosterol content was expressed in milligrams of ergosterol per cubic metre of air as follows: ergosterol content $\times$ 1000/sampling time $\times$ velocity of air flow.

2.6 Meteorological parameters

Relative humidity and temperature were recorded in each sampled apartment as well as just outside the apartment using a thermohygrometer (UK model no. 2030012).

2.7 Data analysis

Correlation and analysis of variance (ANOVA) were performed using SPSS.10 FOR WINDOWS (SPSS, Chicago, Ill.) . Averages and standard deviations were determined using MS EXCEL 6. Only species with a prevalence of 20% for both the indoor and outdoor measurements were used for analysis.

3 Results

3.1 Occurrence

Forty six species in addition to two sterile fungi and yeast species were isolated on Czapek's-Dox

agar from air samples collected both indoors (naturally ventilated bedroom) and outdoors (outside of bedroom window) of 20 apartment buildings in New Damietta, Egypt (Table 1). Most were anamorphic taxa, with the majority of anamorphic fungi being hyphomycetes (indoor: 1518 CFU/m³; outdoors: 1314 CFU/m³). The number of hyphomycetes species varied from 30 plus two sterile species and one yeast species (indoors) to 37 species plus one yeast sp. (outdoors), and the frequency of occurrence varied from 0 to 100% (indoors and outdoors) (Table 1). Zygosporic species were isolated frequently (40%) from outdoor samples (five species = 26 CFU/m³) and infrequently (15%) from indoor samples (three species = 18 CFU/m³). Ascomycota were represented only with a single species that was infrequently isolated (10%) in both indoor and outdoor samples (12 CFU/m³) (Table 1).

The most common genus was *Cladosporium* (100%) both indoors (615 CFU/m³) and outdoors (614 CFU/m³). *Alternaria* and *Penicillium* in addition to *Aspergillus* were also frequently isolated in both indoor (75, 70 and 60%, respectively) and outdoor (65, 70 and 50%, respectively) samples with viable counts of 242, 200 and 214 CFU/m³ in the indoor samples and 134, 100 and 104 CFU/m³ in outdoor samples. As shown in Table 1, the most common aerobiologic airborne species was *Cladosporium cladosporioides* (100%) in both indoor (615 CFU/m³) and outdoor (591 CFU/m³) samples. *Alternaria alternata* and *Penicillium chrysogenum* were also common in both indoor (70–50%) and outdoor (55 and 25%) samples with viable counts of 236 and 127 CFU/m³ indoors and 123 and 66 CFU/m³ outdoors (Table 1).

3.2 Relation between meteorological parameters and fungal parameters

Relative humidity (RH%) was 75.1% (SD: 4.1) indoors and 76.9% (SD: 5.05) outdoors. Ambient temperature was 21.6°C (SD: 2.21) indoors samples and 26.5°C (SD: 1.83) outdoors.

ANOVA indicated that there was no statistically significant differences in temperature and relative humidity between the buildings and the

Table 1 Record of airborne indoor (I) and outdoor (O) species isolated from 20 apartments in New Damietta city

Taxa	Indoor CFU/ m ³	Outdoor CFU/ m ³	Percentage of occurrence Indoor	Percentage of occurrence outdoor	I/O ratio
Hyphomycetes	1518	1314	100	100	
<i>Acremonium</i>	36	12	25	10	3
<i>Acremonium charicola</i> (Lindau) Gams	24	6	15	5	4
<i>Acremonium guillematii</i> Gams	12	6	10	5	2
<i>Alternaria</i>	242	135	75	65	1.7
<i>Alternaria alternata</i> (Fres.) Keissler	236	123	70	55	1.8
<i>Alternaria</i> sp	6	12	5	10	0.5
<i>Aspergillus</i>	0	104	60	50	2.06
<i>Aspergillus conjunctus</i> Kwon et Fennell	22	0	5	0	
<i>A. flavus</i> Link	58	12	20	10	4.8
<i>A. flavipes</i> Bainier et Sartory	6	12	5	10	0.5
<i>A. fumigatus</i> Fres.	12	12	10	10	1
<i>A. niger</i> Van Tiegh	72	28	20	10	2.6
<i>A. parasiticus</i> Speare	6	6	5	5	1
<i>A. sydowii</i> (Bainier et Sartory) Thom et Church	16	16	10	10	1
<i>A. versicolor</i> Tiraboschi	22	18	5	10	1.2
<i>Cladosporium</i>	632	608	100	100	1.06
<i>Cl. cladosporioides</i> (Fres.) de Varies	615	591	100	100	1.04
<i>Cladosporium</i> sp.	17	17	5	5	1
<i>Curvularia</i>	24	18	5	10	1.3
<i>C. elatum</i> (Harz) Nannf	18	6	15	5	3
<i>C. pallescens</i> Boedijn	6	12	5	10	0.5
<i>Drechslera</i>	0	18	0	15	
<i>D. coisis</i> (Nisikado) Subrum. et Jain	0	12	0	10	
<i>D. hawaiiensis</i> (Bungicourt) Subrum. et Jain ex Ellis	0	6	0	5	
<i>Epicoccum nigrum</i> Link	24	23	20	10	1.04
<i>Fusarium</i>	6	12	10	10	0.5
<i>Fusarium avenaceum</i> (Fres.) Sacc.	6	6	5	5	1
<i>F. oxysporum</i> Schlecht	0	6	0	5	
<i>Genticularisporium repens</i> Chesters et Greenhalg	26	22	10	5	1.18
<i>Gilmanella macrospora</i> Moustafa	6	0	5	0	
<i>Glicoladium viride</i> Mart.	12	6	10	5	2
<i>Scopulariopsis brevicaulis</i> (Sacc.) Bainier	0	6	0	5	
<i>Myrothecium</i> sp.	18	12	15	10	1.5
<i>Nigrospora sphaerica</i> (Sacc.) Mason	6	12	5	10	0.5
<i>Paecilomyces variotii</i> Bainier	12	100	10	1	1
<i>Penicillium</i>	200	100	70	70	2
<i>Penicillium chrysogenum</i> Thom	127	66	50	25	1.92
<i>P. citrinum</i> Thom	46	12	20	10	3.8
<i>P. digitatum</i> (Pers.) Sacc.	0	6	0	5	

Table 1 continued

Taxa	Indoor CFU/ m ³	Outdoor CFU/ m ³	Percentage of occurrence Indoor	Percentage of occurrence outdoor	I/O ratio
<i>P. piscarium</i> Westling	28	6	10	5	4.66
<i>Phoma leveillei</i> Boerema et Bollen	0	12	0	10	
<i>Stachybotrys chartarum</i> (Ehrenb.ex Link) Hughes	0	12	0	10	
<i>Trichoderma koningi</i> Oud.	12	6	10	5	2
<i>Trimmatostroma</i> sp.	0	18	0	15	
<i>Ulocladium</i>	0	42	0	25	
<i>Ulocladium alternariae</i> (Cooke) Simmons	0	24	0	15	
<i>U. tuberculatum</i> Simmons	0	18	0	10	
Unknown hyphomycetes	12	29	10	15	0.41
D. Sterile mycelium (brown)	12	0	10	0	
D. Sterile mycelium (Black)	12	0	10	0	
Yeast	12	17	10	5	0.7
Ascomycota	12	12	10	10	
<i>Chaetomium globosum</i> Kunze ex Steud.	12	12	10	10	1
Zygomycota	18	126	15	40	
<i>Absidia corymbifera</i> (Cohn) Sacc. et Trotter	6	6	5	5	1
<i>Circinella rigida</i> Smith	0	12	0	10	
<i>Cunninghamella echinulata</i> (Thaxt.) Thaxt.	6	6	5	5	1
<i>Mucor circinelloides</i> Van Tiegham	6	12	5	5	0.5
<i>Rhizopus oryzae</i> Went et Gerling	0	90	0	25	
Total count	1548	1452			1.06

$$\% \text{ of occurrence} = \frac{(\text{Number of times of isolation} \times 100)}{(\text{Number of examined houses} = 20)}$$

recorded fungal parameters in general. A significant effect ($P > 0.01$) was found only between indoor counts of *Al. alternata* and RH, and between ergosterol content and outdoor temperature. Conversely, *Cl. cladosporioides* and *P. chrysogenum* did not show any significance with temperature and RH.

The correlation of the culturable count, spore count and ergosterol content in both indoor and outdoor samples was conducted using the Pearson rank order correlation two-tailed data (SPSS 10.0). The Pearson rank order correlation coefficient for indoor culturable count and spore count was found to be 0.955 ($P > 0.01$), which indicates that there is a good positive correlation between the two variables (Table 2). Similarly, a high correlation of 0.974 ($P > 0.01$) was found between the outdoor culturable count and spore count. Conversely, a significant negative correlation was detected between outdoor ergosterol content and outdoor culturable count ($P > 0.01$), spore count ($P > 0.05$) and indoor ergosterol content ($P > 0.01$).

3.3 Indoor-outdoor records

The indoor:outdoor ratio (I/O) was calculated as a means to compare the indoor-outdoor fungal parameters with respect to the potential source of species (Table 1). Thirty three species in addition to two sterile species and one yeast species (count: 1548 CFU/m³) were isolated from the indoor samples, and 42 species in addition to one sterile species and one yeast species (count: 1452 CFU/m³) were isolated from out-

door samples. Consequently the total I/O ratio was 1.06 (Table 1).

The I/O for the very frequent genera was 1.06 for *Cladosporium*, 1.7 for *Alternaria*, 2.0 for *Penicillium* and 2.06 for *Aspergillus*. The I/O ratio for the three most frequent species of these genera was 1.04 for *C. cladosporioides*, 1.8 for *Al. alternata* and 1.92 for *Penicillium chrysogenum*. The highest I/O ratio was obtained for the infrequent species *A. flavus* (4.8) and *Penicillium piscarinum* (4.66). The highest I/O ratio indoors was 3.6 in house no. 17, while the lowest ones were 0.3, 0.4, 0.5 and 0.6 in houses 10, 9, 7 and 8, respectively (Table 3).

Table 1 also shows that a few species were isolated only from indoor samples—*A. conjunctus*, *Gilmaniella macrospora* and sterile species—while some others were isolated only from outdoor samples—*Drechslera coisis*, *D. hawaiiensis*, *Fusarium oxysporum*, *Scopulariopsis brevicaulis*, *Penicillium digitatum*, *Phoma leveillei*, *Stachybotrys chartarum*, *Trimmatostroma* sp., *Ulocladium alternariae*, *U. tuberculatum*, *Circinella rigida* and *Rhizopus oryzae*.

Indoors spore counts ranged from 100 to 400 spores/m³ on each sample, with a total spore count of 5000/m³ (Table 3). The culturable fungal count ranged from 30 to 124 CFU/m³, with a total of 1548 CFU/m³. The ergosterol content ranged from 5 to 37.2 mg/m³, with a total of 341.6 mg/m³. Comparatively, in the outdoor samples spore counts ranged from 110 to 9000 spore/m³, with a total spore count of 5233 spore/m³; culturable fungal count ranged from 25 to 222 CFU/m³, with a total of 1452 CFU/m³; ergosterol content

Table 2 Summary of Pearson correlations of culturable fungal and spore counts and ergosterol content in indoor and outdoor samples

Parameters ^a	IC	OC	IS	OS	IE	OE
IC	1.000	0.235	0.955**	0.339	0.309	0.390
OC	0.235	1.000	0.250	0.974**	0.986**	-0.593**
IS	0.955**	0.250	1.000	0.379	0.335	0.403
OS	0.339	0.974**	0.379	1.000	0.987**	-0.493*
IE	0.986**	0.309	0.335	0.987**	1.000	-0.538*
OE	0.390	-0.593**	0.403	-0.493*	-0.538*	1.000

* $P > 0.05$; ** $P > 0.01$

^a IC, Indoor culturable count/m³; OC, outdoor culturable count/m³; IS, indoor spore/m³; OS, outdoor spore/m³; IE, indoor ergosterol/m³; OE, outdoor ergosterol/m³

Table 3 Indoor/outdoor ratio (I/O) of culturable fungal count, spore count and ergosterol content recorded from twenty apartments

House no.	Indoor CFU/m ³	Outdoor CFU/m ³	I/O ratio	Indoor spore/m ³	Outdoor spore/m ³	I/O ratio	ergosterol mg/m ³	Outdoor ergosterol mg/m ³	I/O ratio
1	109	84	1.3	300	260	1.2	25	20.5	1.2
2	101	42	2.4	280	170	1.6	24.4	7.8	3.1
3	85	52	1.6	250	185	1.4	21.1	6.7	3.15
4	114	61	1.9	350	195	1.8	24.4	11.7	2
5	58	97	0.6	200	300	0.66	12.2	21.7	0.6
6	124	145	0.9	400	550	0.7	37.2	43.8	0.85
7	118	222	0.5	390	900	0.4	25	67.2	0.4
8	58	103	0.6	150	320	0.5	11.1	23.9	0.5
9	30	80	0.4	130	250	0.5	8.3	18.9	0.4
10	28	112	0.3	100	310	0.3	5	23.3	0.2
11	58	30	1.9	210	130	1.6	7.7	4.4	1.8
12	79	69	1.1	280	250	1.1	17.7	13.9	1.3
13	119	34	3.5	350	145	2.4	27.7	5	5.5
14	62	46	1.3	190	178	1.1	16.6	11.1	1.5
15	57	30	1.9	185	125	1.5	8.3	5	1.7
16	79	30	2.6	290	135	2.1	17.7	5	3.5
17	79	25	3.6	295	110	2.7	17.7	3.3	5.4
18	86	72	1.2	260	280	0.9	21.1	16.6	1.3
19	52	63	.8	190	230	0.8	6.7	13.3	0.5
20	52	54	1.0	200	210	0.95	6.7	7.2	0.9
Total	1548	1452	1.07	5000	5233	0.96	341.6	330.3	1.03
Average	77.4	72.6	1.5	250.0	261.7	1.2	16.5	16.5	1.8
SD	29.3	47.4	0.9	84.3	180.1	0.7	15.4	1.6	1.5

ranged from 3.3 to 67.2 mg/m³, with a total of 330.3 mg/m³. Indoors, the highest culturable count, spore count and ergosterol content (124 CFU/m³, 400 spore/m³ and 37.2 mg/m³, respectively) were detected in flat no. 6, while outdoors the highest counts for all three parameters (222 CFU/m³, 900 spore/m³ and 67.2 mg/m³ were detected outside flat no. 7. A significant correlation was found between indoor ergosterol, indoor culturable and spore counts. Outdoor levels of ergosterol were correlated significantly with outdoor viable and spore counts and indoor ergosterol (Table 3).

4 Discussion

4.1 Occurrence

Forty six species in addition to two sterile species and a yeast species were isolated and identified to have a total colony concentration of 3000 CFU/m³ in both indoor and outdoor samples. Mitosporic species constitute the main bulk of these species (2832 CFU/m³), while the remainder consist of zygomycota and ascomycota, although at very low counts (144 CFU/m³ and 24 CFU/m³, respectively). These fungi have previously been detected in a wide variety of geographical areas (Al-Suwaine, Hasnain, & Bahkali, 1999; Al-Subai, 2002; Burhan & Asan, 2001; Fanga et al., 2005; Gniadek et al., 2005; Halwagy, 1989; Hargreaves et al., 2003; Henríquez, Villegas, & Nolla, 2001; Macura & Gniadek, 2000; Medrela-Kuder, 2003; Stepalska, Harmata, Kasprzyk, Myszkowska, & Stach, 1999; Picco & Rodol, 2000; Wu Pei-Chih et al., 2000).

The most common genera were *Cladosporium*, *Alternaria* and *Penicillium* in addition to *Aspergillus*, and their species, in both the indoor and outdoor samples. *Aspergillus*, *Cladosporium* and *Penicillium* have also been reported as major building molds (Bogacka, 1997; Cooley et al., 1998; Gniadek et al., 2005; Macura & Gniadek, 2000; Nilsson et al., 2004; Pei-Chih et al., 2000). The most frequent species was *C. cladosporioides* in both indoor and outdoor samples, but with a higher count indoors than outdoors. Other frequent species were *Al. alternata* and *P. chrysog-*

enum. These three species have been reported to be the major airspora all over the world (Al-Suwaine et al., 1999; Al-Subai, 2002; Burhan & Asan 2001; Fanga et al., 2005; Gniadek et al., 2005; Hargreaves et al., 2003; Henríquez et al., 2001; Medrela-Kuder 2003; Palska St et al., 1999; Wu Pei-Chih et al., 2000). The prevalence of these melanized fungi is likely attributable to the dark pigmentation which protects them against damage from UV light. A few authors have suggested that fungi have different spatio-temporal distributions, with *Penicillium* species being representative of indoor moulds and *Cladosporium* being representative of the outdoor air (Herbarth, Schlink, Muller, & Richter, 2003); however, the converse was found in Taiwan (Pei-Chih et al., 2000). In the present study, both species were detected from the indoor and outdoor samples of the New Damietta buildings at almost equal concentrations.

4.2 Factors affecting the distribution of airborne fungi

The distributions of airborne fungi have been found to be influenced by seasonal and environmental factors (Burge, 1990; Cosentino et al., 1990; Halwagy, 1989; Lara et al., 1990). Humidity (DeKoster & Thorne, 1995; Nielsen et al., 2004), with temperature (Gniadek et al., 2005; Medrela-Kuder, 2003; Ren et al., 2001) being the major factor affecting the concentration of airborne fungi. However, in the present study, of the species isolated, a significant correlation was only established between indoor *Al. alternata*, spore count, ergosterol content and RH, and also between ergosterol content and outdoor temperature.

The type of culture media may also affect the isolation of certain species. In most of the investigations reported to date, the isolation media have been nutritionally rich—for example, malt extract agar or Sabouraud's agar—which may have introduced a bias in the count of fungi by enhancing the growth of rapidly growing species (Flannigan, 1992; Flannigan and Miller, 1994). Samson et al. (1994) recommended that non-selective media be used for the general detection of fungi indoors and that rich media should not be

used. Consequently, the medium of choice in the present experiment for isolation of the fungi was a potentially non-rich medium amended with Rose Bengal (0.035 g/ml) to suppress the growth of fast-growing species. A further confounding factor may be that of competition, where some species do not compete well with others on isolation plates, even if the medium is non-rich and suitable for their growth, and are consequently not recorded as frequently as they originally present; this may be true of *Alternaria* species and those of *Ulocladium*, *Drechslera*, *Nigrospora*, among others.

The rate of sedimentation, size (Hunter et al., 1988) and survival rates of the spores (Flannigan & Miller, 1994; Flannigan et al., 1996) can also affect fungal counts. Stokes' law states that particles with diameters between 1.6 and 70 μm remain suspended in the aerosol for long periods of time due to the action of wind and turbulence (Chatigny, Dimmick, & Harrington, 1979). Thus, in addition to the action of UV, in which dark-pigmented fungi, such as *Cl. cladosporioides* and *Al. alternata*, have a survival advantage, the fact that these species also have spore sizes between 1.6 and 70 μm is relevant. Large *Ulocladium* spores, for example, settle relatively rapidly in the sediment of damp houses (Flannigan & Miller, 1994). It has been stated that up to 90% of airborne spores may not be viable (Nikulin, Pasanen, Berg, & Hintikka, 1994). *Aspergillus* and *Penicillium* spores may survive longer than those of *Stachybotrys chartarum* (Flannigan & Miller, 1994). These differences in survival rate increase the complexity of the data analysis. Thus, the occurrence of rare species such as *S. chartarum* and *Chaetomium globosum*, for example, should be interpreted differently than that of *Cladosporia* and *Penicillia* isolated from the same plates. The isolation from the former from the aerosol is difficult since they are normally present in cracks and cavities, and their spores, either in slime heads or ascoma, are of a form that makes it difficult for them to get air-borne (Billups, Tilton, & Warden, 1999; Fung, Clark, & Williams, 1998; von Arx, Guarro, & Figuera, 1986). Thus, because the spores of *S. chartarum* and *Ch. globosum* do not readily become air-borne a contact plates should be used instead of air sampler for their

isolation (Andersen & Nissen, 2000). Moreover, *S. chartarum* is extremely hazardous at even very low doses, causing infections of the skin and several potentially fatal infant diseases (Woods, Grimsrud, & Boschi, 1997).

Owing to these problems, including an insufficient culture methodology for assessing the bio-aerosol and the fact that culturable species encompass a small portion of the total bioaerosol entities, there is a strong argument for using non-cultural rapid methods to determine the aerosol fungal biomass over extended periods rather than total counts. Thus, ergosterol has also been used as the best indicator of exposure and to help in assessing of the airborne fungal biomass (Flannigan, 1997). In the present investigation a strong correlation was found between the ergosterol content and the culturable and spore count in indoor samples. Various researchers have found a high reliance between ergosterol content and spore count and CFU in various samples (Gutarowska & Zakowska, 2002; Magan, 1993; Pasanen et al., 1999; Saraf et al., 1997; Schnpurer, 1991). To convert ergosterol values to biomass equivalents a general multiplicative factor of 182 has been proposed but only for aquatic hyphomycetes (Gessner & Chauvet, 1993). Thus, for an accurate estimation of biomass, the ergosterol content of the most common airspora must be determined.

4.3 Indoor-outdoor ratios

An examination of the averaged ratios of indoor to outdoor concentrations for the identified genera indicated that the most predominant fungal genera were *Cladosporium*, *Alternaria*, *Penicillium* and *Aspergillus*, with low I/O ratios of 1.06, 107, 2.0 and 2.06 respectively. The presence of these fungi at a certain concentration is potentially hazardous. Bioaerosols have been reported to play a significant role in indoor air pollution as they can be pathogenic or cause an allergic reaction following inhalation (Kalogerakisa et al., 2005). In particular, the presence of *Aspergillus* above 50 CFU/m³ is associated with coughs, colds, and eye and skin irritation (Anderson et al., 1996; Dales, Burnett, & Zwanenburg, 1991). Moreover, *Aspergillus* is well known as the

causal agent of aspergillosis and to be a mycotoxin producer (Howard & Howard, 1983; Samson, 1994). *Penicillium* and *Cladosporium* have also been reported to be associated with respiratory illnesses and allergy (Cuijpers et al., 1995; Flannigan et al., 1991; Hu et al., 1997; Pope, Patterson, & Burge, 1993).

In this investigation, higher I/O ratios were found for the more infrequent species: *A. flavus* (I/O = 4.8), *Penicillium piscarinum* (I/O = 4.7), *Acremonium charticola* (I/O = 4.0), *P. citrinum* (I/O = 3.8) and *Curvularia elatum* (I/O = 3.0). Conversely, very frequent species—*Cl. cladosporioides*, *Al. alternata* and *P. chrysogenum*—showed low I/O ratios of 1.4, 1.8 and 1.9, respectively. Very few species were isolated from only the indoor samples and, similarly, very few other species were isolated from only the outdoor samples. Recent studies have indicated that while the outdoor population of airspora influence the presence of fungi in indoor environments, the indoor airspora are not a simple reflection of the presence of fungi in the outdoor air. The present data support the previous results that outdoor air is a major source of indoor fungi and can interfere and mask those from indoor sources (Reponen, Nevalainen, Jantunen, Pellika, & Kalliokoski, 1992). However, indoor sources of spores may also be present, and this is decisive factor when carrying out comparative analyses (Fradkin, Tobin, Tarlo, Tucic-Poretta, & Mallock, 1987; Koch, Heilemann, Bischof, Heinrich, & Wichmann, 2000; Verhoeff, 1992). In general, with respect to more frequent spores (CFU/m³), the indoor/outdoor data of the present study were very similar. This observation may be due to the competition of the cultured spores on the culture media and not a real reflection of the relative abundances.

In discussing the potential source of indoor airspora it is worth noting that the accumulation of spores may occur as the airborne spores settle on various surfaces. These settled spores may be re-suspended in the indoor air by air movements caused by human activities or by changes in air humidity (Flannigan & Hunter, 1988; Hirsh & Josman, 1976; Hunter et al., 1988; Kozak, Gallup, Cummins, & Gillman, 1979; Pasanen, Pasanen, Jantunen, & Kalliokoski, 1991; Pasanen,

Heinonen-Tanski, Kalliokoski, & Jantunen, 1992). They may develop as invisible fungal micro-colonies on any temporarily wet surface (Pasanen et al., 1992). Organic materials, such as plant debris, are sources of *Aspergillus* spores (Muilins, Harvey, & Seaton, 1976). *Aspergillus* spores have also been isolated from the soil of house plants (Botzenhart, Altenhoff, & Leithold, 1984). Botzenhart et al. (1984) showed that green plants may increase the concentrations of airborne fungal spores. Fungal spores may also be carried into the home on people's clothes (Lundqvist, Aalykke, & Bonde, 1990; Pasanen, Kalliokoski, Pasanen, Salmi, & Tossavainen, 1989). Unwashed root vegetables have also been shown capable to being sources of airborne *Cladosporium* and *Penicillium* spores (Lehtonen, Reponen, & Nevalainen, 1993).

In general, the outdoor levels of culturable count and total spore typically varied between 25 and 222 CFU/m³ and 110 and 900 spore/m³, respectively. This is in agreement with levels reported in the literature in which outdoor levels have been found to typically vary between 100 and 1000 CFU/m³, mainly built up by the external environment. Likewise, indoor levels reported in the literature vary between 100 and 400 spore/m³, and 52 and 124 CFU/m³. Maximum indoor limits have been set by various agencies: 1000 CFU/m³ for the total number of bioaerosol particles [The National Institute of Occupational Safety and Health (NIOSH)]; 1000 CFU/m³ [American Conference of Governmental Industrial Hygienists (ACGIH) and American Industrial Hygiene Association (AIHA)] with the culturable count for total bacteria not to exceed 500 CFU/m³. Furthermore, the Commission of the European Communities (CEC) defines 2000 CFU/m³ as a "very high" level (Cox & Wathes, 1995; Godish 1995; Jensen & Schafer, 1998). Reponen et al. (1992) reported that the upper limit of the normal range in suburban homes was 500 CFU/m³.

In conclusion, the indoor and outdoor counts determined in this study are not above the level assessed to be hazardous, with a few exceptions. However, indoor environments, can become a serious risks to their inhabitants if their contamination exceeds the recommended maxima. The highest indoor values (124 CFU/m³ and

400 spore/m³) of flat 6 and other few apartments could be considered to be at the hazardous level for indoor environments if we consider that these counts may represent only a part of the total biota of the bioaerosol.

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References

- Al-Subai, A. A. T. (2002). Air-borne fungi at Doha, Qatar. *Aerobiologia*, 18, 175–183.
- Al-Suwayne, A. S., Hasnain, S. M., & Bahkali, A. H. (1999). Viable airborne fungi in Riyadh, Saudi Arabia. *Aerobiologia*, 15, 121–130.
- Andersen, B., & Nissen, A. T. (2000). Evaluation of media for detection of *Stachybotrys* and *Chaetomium* species associated with water-damaged buildings. *International Biodeterioration and Biodegradation*, 46, 111–116.
- Anderson, K., Morris, G., & Kennedy, H. (1996). Aspergillosis in immunocompromised paediatric patients: Associations with building hygiene, design, and indoor air. *Thorax*, 51, 256–261.
- Billups, R. A., Tilton, K. S., & Warden, P. S. (1999). Identification of *Stachybotrys chartarum* utilizing various media and two temperature settings. In: *First NSF International Conference on Indoor Air Health* (pp. 166–174), Denver, Colo.
- Björman, J. (1994). Ergosterol as an indicator of mould growth on wood in relation to culture age, humidity stress and nutrient level. *International Biodeterioration and Biodegradation*, 33, 355–368.
- Bogacka, E. (1997). Sick building syndrome. *Mikologia Lekarska*, 4, 233–237.
- Botzenhart, K., Altenhoff, K., & Leithold, T. H. (1984). Molds in the air of greenhouse homes. In B. Seiferrt, H. Esdorn, M. Fischer, H. Ruden, & J. Wegner (Eds.), *Indoor air sensitivity and hyperactivity, reactions to sick buildings* (Vol. 84, pp. 277–82). Stockholm, Sweden: Swedish Council for Building Research.
- Burge, H. (1990). Bioaerosols: prevalence and health effects in the indoor environment. *The Journal of Allergy and Clinical Immunology*, 86, 687–701.
- Burhan, E. N., & Asan, A. (2001). Airborne fungi in vegetable growing areas of Edirne, Turkey. *Aerobiologia*, 17, 69–75.
- Chatigny, M. A., Dimmick, R. L., & Harrington, J. B. (1979). Aerobiology: The ecological systems approach. Dowden, Hutchinson and Ross, Stroudsburg, Pa. *US/IBP Synthesis Series*, 10, 111–150.
- Cooley, J. D., Wong, W. C., Jumper, C. A., & Straus, D. C. (1998). Correlation between the prevalence of certain fungi and sick building syndrome. *Occupational and Environmental Medicine*, 55, 579–584.
- Cosentino S., Pisano P. L., Fadda M. E., & Palmas, F. (1990). Pollen and mold allergy: Aerobiologic survey in the atmosphere of Cagliari, Italy 1986–1988. *Annals of Allergy*, 65, 393.
- Cox, C. S., & Wathes C. M. (1995). *Bioaerosols handbook*. New York: Lewis Publishers.
- Cuijpers, C. E. J., Swaen, G. M. H., Wesseling, G., Sturmans, F., & Wouters, E. F. M. (1995). Adverse effects of the indoor environment on respiratory health in primary school children. *Environmental Research*, 68, 11–23.
- Dales, R. E., Burnett, R., & Zwanenburg, H. (1991). Adverse health effects among adults exposed to home dampness and molds. *The American Review of Respiratory Disease*, 143, 505–509.
- DeKoster, J. A., & Thorne, P. S. (1995). Bioaerosol concentrations in noncomplaint, complaint, and intervention homes in the midwest. *American Industrial Hygiene Association Journal*, 56, 573–580.
- Fanga, Z., Ouyanga, Z., Hub, L., Wang, X., Zhenga, H., & Lina, X. (2005). Culturable airborne fungi in outdoor environments in Beijing, China. *Science of the Total Environment*, 350, 47–58.
- Fung, F., Clark, R., & Williams, S. (1998). *Stachybotrys*, a mycotoxinproducing fungus of increasing toxicologic importance. *Clinical Toxicology*, 36, 79–86.
- Flannigan, B. (1992). Indoor microbiological pollutants—sources, species, characterisation and evaluation. In H. Knoppel, & P. Wolkoff (Eds.), *Chemical, microbiological, health and comfort aspects of indoor air quality* (pp. 733–798). Dordrecht: Kluwer.
- Flannigan, B. (1997). Air sampling for fungi in indoor environments. *Journal of Aerosol Science*, 28, 381–392.
- Flannigan, B., & Hunter, C. A. (1988). Factors affecting airborne moulds in domestic dwellings. In R. Perry, & P. W. Kirk (Eds.), *Indoor and ambient air quality* (pp. 461–468). London.
- Flannigan, B., & Miller, J. D. (1994). Health implications of fungi in indoor environments—an overview. In R. A. Samson, B. Flannigan, M. E. Flannigan, A. P. Verhoeff, O. C. G. Adan, & E. S. Hoekstra (Eds.), *Health implications of fungi in indoor environments* (pp. 1–28). Amsterdam: Elsevier.
- Flannigan, B., McCabe, E. M., & McGarry, F. (1991). Allergic and toxigenic micro-organisms in houses. *Journal of Applied Bacteriology Symposium Supplement*, 70, 61S–73S.
- Flannigan, B., McCabe, E. M., Jupe, S. V., & Jeffrey, I. G. (1993). Mycological and acarological investigation of complaint and non-complaint houses in Scotland. In *Indoor Air '93, Proc 6th Int. Conf on Indoor Air Quality and Climate Indoor Air '93* (pp. 143–148). Helsinki.
- Flannigan, B., McCabe, E. M., & Jupe, S. V. (1996). Quantification of air- and dust-borne detriogenic microorganisms in homes. In W. Sard (Ed.), *Proc. 10th Biodeterioration and Biodegradation Symp* (pp. 377–384). Dechema, Frankfurt am Main.
- Fradkin, A., Tobin, R. S., Tarlo, S. M., Tucic-Poretta, M., & Mallock, D. (1987). Species identification of air-

- borne molds and its significance for the detection of indoor pollution. *Japca*, 35, 51–53.
- Garrison, R. A., Robertson, L. D., Koehn, R. D., & Wynn, S. R. (1993). Effect of heating-ventilation-air conditioning system sanitation on airborne fungal populations in residential environments. *Annals of Allergy*, 71, 548–556.
- Gessner, M. O., & Chauvet, E. (1993). Ergosterol-to-biomass conversion factors for aquatic hyphomycetes. *Applied Environmental Microbiology*, 59, 502–507.
- Gniadek, A., Macura, A. B., Oksiejczuk, E., Krajewska-Kulak, E., & Lukaszuk, C. (2005). Fungi in the air of selected social welfare homes in the Malopolskie and Podlaskie provinces—a comparative study. *International Biodeterioration and Biodegradation*, 55, 85–91.
- Godish, T. (1995). Sick buildings: Definition, diagnosis and mitigation. Boca Raton: Lewis Publishers.
- Grant, C., Hunter, C. A., Flannigan, B., & Bravery, A. F. (1989). Water activity requirements of moulds isolated from domestic dwellings. *International Biodeterioration and Biodegradation*, 25, 259–284.
- Gutarowska, B. (1999). Ergosterol as an indicator of degree of mould's contamination of plant materials. PhD thesis, Technical University, Irodz, Poland.
- Gutarowska, B., & Zakowska, Z. (2002). Elaboration and application of mathematical model for estimation of mould contamination of some building materials based on ergosterol content determination. *International Biodeterioration and Biodegradation*, 49, 299–305.
- Halwagy, M. (1989). Seasonal airspora at three sites in Kuwait 1977–1982. *Mycological Research*, 93, 208.
- Hargreaves, M., Parappukkaran, S., Morawska, L., Hitchins, J., He, C., & Gilbert, D. (2003). A pilot investigation into associations between indoor airborne fungal and non-biological particle concentrations in residential houses in Brisbane, Australia. *The Science of the Total Environment*, 312, 89–101.
- Henríquez, V. I., Villegas, G. R., & Nolla, J. M. R. (2001). Airborne fungi monitoring in Santiago. *Chile Aerobiologia*, 17, 137–142.
- Herbarth, O., Schlink, U., Muller, A., & Richter, M. (2003). Spatiotemporal distribution of airborne mould spores in apartments. *Mycology Research*, 107, 1361–1371.
- Hirsh, S. R., & Josman, J. A. (1976). A one year survey of mold growth inside twelve homes. *Annals of Allergy*, 36, 30–38.
- Horner, W. E., Helbling, A., Salvaggio, J. E., & Lehrer, S. B. (1995). Fungal allergens. *Clinical Microbiology Reviews*, 8, 161–179.
- Howard, D. H., & Howard, L. F. (1983). *Fungi pathogenic for humans and animals*. New York: Marcel Dekker.
- Hu, F. B., Persky, V., Flay, B. R., & Richardson, J. (1997). An epidemiological study of asthma prevalence and related factors among young adult. *British Medical Journal*, 34, 67–76.
- Hunter, C. A., Grant, C., Flannigan, B., & Bravery, A. F. (1988). Mould in buildings: The air spora of domestic dwellings. *International Biodeterioration*, 24, 81–101.
- Jensen, P. A., & Schafer, M. P. (1998). Sampling and characterization of bioaerosols. In NIOSH manual of analytical methods. Method 0800, Issue 1. pp. 82–112.
- Kalogerakisa, N., Paschalia, D., Lekaditisa, V., Pantidoua, A., Eleftheriadis, K., & Lazaridis, M. (2005). Indoor air quality—bioaerosol measurements in domestic and office premises. *Journal of Aerosol Science*, 36, 751–761.
- Koch, A., Heilemann, K. J., Bischof, W., Heinrich, J., & Wichmann, H. E. (2000). Indoor viable mold spores—a comparison between two cities, Erfurt (eastern Germany) and Hamburg (western Germany). *Allergy*, 55, 176–180.
- Kowalski, W., & Bahnfleth, W. P. (1998). Airborne respiratory diseases and technologies for control of microbes. HPAC 70.
- Kozak, P. P., Gallup, J., Cummins, L. H., & Gillman, S. A. (1979). Factors of importance in determining the prevalence of indoor molds. *Annals of Allergy*, 43, 88–94.
- Lacap, D. C., Liew, E. C. Y., & Hyde, K. D. (2003). An evaluation of the fungal 'morphotype' concept based on ribosomal DNA sequences. *Fungal Diversity*, 12, 53–66.
- Lara, T. J. M., Tessier, J. F., & Lafont-Grellety, J. (1990). Indoor moulds in asthmatic patients homes. *Aerobiologia*, 6, 98.
- Lehtonen, M., Reponen, T., & Nevalainen, A. (1993). Everyday activities and variation of fungal spore concentrations in indoor air. *International Biodeterioration and Biodegradation*, 31, 25–39.
- Lundqvist, G. R., Aalykke, C., & Bonde G. J. (1990). Evaluation of children as sources of bioaerosols in a climate chamber study. *Environment International*, 16, 213–218.
- Macura, A. B., & Gniadek, A. (2000). Fungi present in the indoor environment of a social welfare home. Preliminary study. *Mikologia Lekarska*, 7, 13–17.
- Magan, N. (1993). Early detection of mould growth in stored grain. *Aspects of Applied Biology*, 36, 417–426.
- Matcham, E., Jordan, B. R., & Wood, D. A. (1985). Estimation of fungal biomass in a solid substrate by three independent methods. *Applied Microbiology and Biotechnology*, 21, 108–112.
- Medrela-Kuder, E. (2003). Seasonal variations in the occurrence of culturable airborne fungi in outdoor and indoor air in Cracow. *International Biodeterioration and Biodegradation*, 52, 203–205.
- Mille-Lindblom, C., von Wachenfeldt, E., & Tranvik, L. J. (2004). Ergosterol as a measure of living fungal biomass: persistence in environmental samples after fungal death. *Journal of Microbiological Methods*, 59, 253–262.
- Muilins, J., Harvey, R., & Seaton, A. (1976). Sources and incidence of airborne *Aspergillus fumigatus* (Fres). *Clinical Allergy*, 6, 209–217.
- Nielsen, K. F., Holm, G., Uttrup, L. P., & Nielsen, P. A. (2004). Mould growth on building materials under low water activities. Influence of humidity and temperature on fungal growth and secondary metabolism.

- International Biodeterioration and Biodegradation, 54, 325–336.
- Nilsson, A., Kihlstrom, E., Lagesson, V., Wessen, B., Szponar, B., Larsson, L., & Tagesson, C. (2004). Microorganisms and volatile organic compounds in airborne dust from damp residences. *Indoor Air*, 14, 74–82.
- Nikulin, M., Pasanen, A. L., Berg, S., & Hintikka, E. L. (1994). *Stachybotrys atra* growth and toxin production in some building materials and fodder under different relative humidity. *Applied and Environmental Microbiology*, 60, 3421–3424.
- Palska, St D., Harmata, K., Kasprzyk, I., Myszkowska, D., & Stach, A. (1999). Occurrence of airborne *Cladosporium* and *Alternaria* spores in Southern and Central Poland in 1995–1996. *Aerobiologia*, 15, 39–47.
- Pasanen, A.-L., Kalliokoski, P., Pasanen, P., Salmi, T., & Tossavainen, A. (1989). Fungi carried from farmer's work into farm homes. *American Industrial Hygiene Association Journal*, 50, 631–633.
- Pasanen, A.-L., Pasanen, P., Jantunen, M. J., & Kalliokoski, P. (1991). Significance of air humidity and air velocity for fungal spore release into the air. *Atmospheric Environment*, 25A, 459–462.
- Pasanen, A.-L., Heinonen-Tanski, H., Kalliokoski, P., & Jantunen, M. J. (1992). Fungal micro-colonies on indoor surfaces—an explanation for the base level fungal spore counts in indoor air. *Atmospheric Environment*, 26B, 117–120.
- Pasanen, A., Yli-Pietila, K., Pasanen, P., Kalliokoski, P., & Tarhanen, J. (1999). Ergosterol content in various fungal species and biocontaminated building materials. *Applied and Environmental Microbiology*, 65, 138–142.
- Pei-Chih, W., Huey-Jenu, S., & Chia-Yin, L. (2000). Characteristics of indoor and outdoor airborne fungi at suburban and urban homes in two seasons. *The Science of the Total Environment*, 253, 111–118.
- Picco, A. M., & Rodol, M. (2000). Airborne fungi as biocontaminants at two Milan underground station. *International Biodeterioration and Biodegradation*, 45, 43–47.
- Pope, A. M., Patterson, R., & Burge, H. (1993). *Indoor Allergens*. Washington D.C.: National Academy Press.
- 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.
- Reponen, T., Nevalainen, A., Jantunen, M., Pellikka, M., & Kalliokoski, P. (1992). Normal range criteria for indoor air bacteria and fungal spores in a subarctic climate. *Indoor Air*, 2, 26–31.
- Ross, M. A., Curtis, L., Scheff, P. A., Hryhorczuk, D. O., Ramakrishnan, V., Wadden, R. A., & Persky, V. W. (2000). Association of asthma symptoms and severity with indoor bioaerosols. *Allergy*, 55, 705–711.
- Samson, R. A. (1994). *Health implications of fungi in indoor environments*. Amsterdam: Elsevier.
- Samson, R. A., Flannigan, B., Flannigan, M. E., Verhoe, A. P., Adan, O. C. G., & Hoekstra, E. S. (1994). Recommendations. In R. A. Samson, B. Flannigan, M. E. Flannigan, A. P. Verhoe, O. C. G. Adan, & E. S. Hoekstra (Eds.), *Health implications of fungi in indoor environments* (pp. 531–538). Amsterdam: Elsevier.
- Saraf, A., Larsson, L., Burge, H., & Milton, D. (1997). Quantification of ergosterol and 3-hydroxy fatty acids in settled house dust by gas chromatography-mass spectrometry: comparison with fungal culture and determination of endotoxin by *Limulus* amebocyte lysate assay. *Applied and Environmental Microbiology*, 63, 2554–2559.
- Schnpurer, J. (1991). Distribution of fungal biomass among fine bran, coarse bran, and gour from wheat stored at four deferent moisture levels. *Cereal Chemistry*, 68, 434–437.
- Seitz, L. M., Sauer, D. B., Burroughs, R., Mohr, H. E., & Hubbard, J. D. (1979). Ergosterol as a measure of fungal growth. *Phytopathology*, 69, 1202–1203.
- Tan, T. K., & Leong, W. F. (1989). Succession of fungi on wood of *Avicennia alba* and *A. lanata* in Singapore. *Canadian Journal of Botany*, 67, 2686–2691.
- Verhoeff, A. P., Van Wijnen, J. H., Brunekreef, B., Fischer, P., Van Reenenhoekstra, E. S., & Samson, R. A. (1992). The presence of viable mold propagules in indoor air in relation to home dampness and outdoor air. *Allergy*, 47, 83–91.
- von Arx, J. A., Guarro, J., & Figuera, M. J. (1986). *The ascomycete genus Chaetomium*. Berlin: J Cramer.
- Woods, J. E., Grimsrud, D. T., & Boschi, N. (1997). *Healthy Buildings/IAQ '97*. Washington, D.C.: ASHRAE.
- World Health Organization (2000). *Guidelines for concentration and exposure-response measurements of fine and ultra fine particulate matter for use in epidemiological studies*. Geneva: World Health Organization.