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Airborne fungi in child day care centers in Edirne City, Turkey

Halide Aydogdu · Ahmet Asan

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Abstract The purpose of this study was to determine the concentration, in terms of monthly and seasonal distribution and in relation to meteorological factors, of indoor and outdoor microfungi at selected sites in several child day care centers in the city of Edirne, Turkey. Samples were collected at one month intervals over a period of 12 months between January–December 2004, by exposing petri plates containing Peptone Dextrose Agar with Rose-Bengal and Streptomycin medium to the air for 10–15 min. A total of 2,071 microfungal colonies were counted on 192 petri plates. Thirty microfungal genera (*Acremonium, Alternaria, Arthrinium, Aspergillus, Bahusakala, Beauveria, Ceuthospora, Chaetomium, Cladosporium, Curvularia, Drechslera, Epicoccum, Eurotium, Fusarium, Mycotypha, Myrotechium, Paecilomyces, Penicillium, Pestalotiopsis, Phoma, Ramichloridium, Rhizopus, Scopulariopsis, Stachybotrys, Stemphylium, Torula, Trichoderma, Trichothecium, Ulocladium, Verticillium*) and 75 microfungal species were isolated from the air indoor and outdoor of the day care centers. The dominant microfungal genera were *Cladosporium, Penicillium* and *Alternaria* (44.11%, 18.94%, 14.67% of the total respectively), while the genus with the most species richness was *Penicillium* (26 species). *Alternaria, Cladosporium, Penicillium* and non-sporulating microfungi were found every month. *Cladosporium* was the dominant genus in both indoor and outdoor air. Although the predominant genus was the same in both indoor and outdoor air, *Cladosporium* was followed by *Penicillium, Alternaria* and *Aspergillus* genera in indoor air and by *Alternaria, Penicillium* and *Aspergillus* genera in outdoor air. While a positive correlation was found between the concentration of monthly outdoor microfungi and monthly average temperature, a negative correlation was found between the concentration of monthly outdoor microfungi and monthly average wind velocity. Also, some relationships were found between the monthly concentrations of the most predominant microfungal genera (*Cladosporium, Penicillium* and *Alternaria*) and various meteorological factors.

Keywords Airborne fungi · Child day care centers · Meteorological parameters · Microfungi · Biomass

Abbreviations

CFU	colony-forming unit
CDCC	child day care center

H. Aydogdu
Arda Vocational College, Trakya University,
22030 Edirne, Turkey

A. Asan (✉)
Department of Biology, Trakya University,
Faculty of Arts and Sciences,
22030 Edirne, Turkey
e-mail: ahmetasan84@yahoo.com

Introduction

Microfungi are the most numerous and diverse particles both in indoor and outdoor environments. The concentrations and types of airborne microfungi in the atmosphere are affected by many biological and environmental factors (Stepalska and Wolek 2005). They vary greatly, by nature, with time, season, geographical, climatic and other physical factors (Abdel Hameed et al. 2007a). Moreover, meteorological parameters such as wind, humidity, temperature, rainfall, altitude and vegetation, affect the numbers and types of airborne microorganisms (Di Giorgio et al. 1996; Su et al. 2001; Asan et al. 2002; Topbas et al. 2006).

Airborne microfungi are one of the important indoor air biocontaminants. The main source of airborne fungi in indoor air is usually from outdoor air (Wu et al. 2000; Liao et al. 2004), but also from some indoor environmental factors such as dampness and high humidity levels that encourage fungal growth (Gelincik et al. 2005). Fungal growth in indoor environments releases infectious agents such as fungal spores and mycotoxins into the air inside buildings (Stetzenbach et al. 2004). Exposure to airborne mold contaminants or their metabolites can induce such responses as irritation and allergies, and may cause infections and acute reactions such as vomiting, diarrhea, hemorrhage, convulsions and, in some cases, death (Menetrez and Foarde 2004).

Studies of airborne microbial populations from indoor environments including houses, offices, hospitals, schools and day care centers are becoming increasingly important because of the adverse health effects associated with bioaerosols (Sivasubramani et al. 2004) and because many people are spending more time indoors. Children may be more likely to suffer from the adverse effects of airborne fungi than adults because of their age and susceptibility. Furthermore, the form of day care children receive is very important. Attendance at day care centers for children is unavoidable due to mothers involved in business life and is important to the children's socialization and education (Yalcin et al. 2004; Nafstad et al. 2004). However, according to Koskinen et al. (1997), children who attend day care centers are more prone to infectious agents than children who stay at home. Koskinen et al. (1995) has indicated that adverse infections with symptoms such as eye irritation, stomach pain, headache, nausea and fatigue were common among children in a day care center with mold problems.

The purposes of this study were: 1) to determine the concentrations of indoor and outdoor airborne microfungi, and sources of indoor microfungi in day care centers of Edirne, 2) to identify microfungi genera and species in indoor and outdoor air, 3) to evaluate the distributions of indoor and outdoor airborne microfungi at different times of the year, 4) to identify the microfungi species as potential allergens and pathogens and/or mycotoxin producers that pose a health risk for the children and teachers of the CDCCs studied. In addition, we evaluated relationships between the identified airborne fungi and some meteorological parameters such as average monthly and daily temperature, relative humidity, wind velocity, rainfall and amount of sunlight.

Children spend an average of 45 h a week in day care centers in Edirne. Identification of airborne fungi affecting health of children in day care centers is important for preventing diseases and many symptoms in the children. This investigation was also undertaken to help establish standards for future reference.

Materials and methods

Sampling of airborne fungi

Culturable airborne microfungi were collected using the Petri Plate Gravitational Settling Method (Abdalla 1988; Ismail et al. 1999; Colakoglu 2004) from four child day care centers in the city of Edirne. Samples were collected at one month intervals over a period of 12 months between January 2004–December 2004. Samplings were undertaken in three indoor sites (classroom, sleeping room and eating room) and one outside site at each day care center. Petri plates were exposed to the air 50–80 cm above ground level for each 10-minute indoor sampling session, while outdoor air samples were collected at a height of 150 cm above the surface of the ground for 15 min.

One petri plate was used over each sampling period for microfungi. Peptone Dextrose Agar with Rose-Bengal and Streptomycin was used to catch, enumerate, and isolate the fungi in the air. Rose-Bengal stain was added to limit the growth of fast growing moulds and streptomycin antibiotic was supplemented to

control the reproduction of bacteria in the medium. The plates were incubated at 25°C for 7–10 days; growing fungi were counted at the end of the incubation period (results reported as the number of colony forming units-CFU) and subcultured on slant Potato Dextrose Agar medium in tubes before identification.

Identification

Identification of the fungi was based on their macro- and microscopic features. The determination of micro morphological structures of fungi was carried out on material mounted in a modified mounting medium, Lacto-Cotton Blue, proposed by Sime et al. (2002). Barnett and Hunter's (1999) work provided the basis for the identification of fungi at the genus level. Pitt (1979 and 2000), Samson et al. (2002), Samson and Pitt (2000) were used for *Penicillium* Link: Fr. species. *Penicillium* cultures were inoculated for identification in triplicate on three different media (Czapek Yeast Autolysate Agar [CYA], Malt Extract Agar [MEA] and 25% Glycerol Nitrate Agar [$G_{25}N$]) instructed as detailed by Pitt (1979 and 2000) and incubated at three different temperatures (5, 25 and 37°C). *Aspergillus* species were identified according to the monographs by Raper and Fennell (1965), Klich (2002) and Samson et al. (2002). Czapek Solution Agar [CZ], CYA, MEA, Czapek Yeast Extract Agar with 20% sucrose [$CY_{20}S$] media were used for the identification of *Aspergillus* species. *Fusarium* species—An Illustrated Manual for Identification (Nelson et al. 1983) was used for the identification of *Fusarium* species inoculated PDA plates. Other genus and species inoculated PDA and MEA plates were identified according to Ellis (1971), Ellis and Ellis (1997), Samson et al. (2002) and Hasenekoglu (1991).

Fungal author names, as cited in this paper, are standardized in accordance with Authors of Fungal Names (Kirk and Ansell 1992) and the website, www.indexfungorum.org. List of accepted species and synonyms in the family *Trichocomonaceae* (Pitt et al. 2000) is followed for acceptability names of *Penicillium* and *Aspergillus* species.

Description of the study units in child day care centers

Edirne city is located in the North west region of Turkey and borders Bulgaria and Greece. The four child day care centers in which samplings were undertaken are in the eastern part of the city. Selected day care centers were used between 08.00–18.00 h and closed on weekends. The child day care centers varied according to building age, construction, size, and number of students and staff. Students, staff, protectors and other persons were not allowed to enter the CDCCs' rooms with their shoes on from the outside. All of the CDCCs were equipped with a central heating system and ventilation was provided naturally through windows. In all the CDCCs, floor were predominantly covered with carpet except for some parts (floor tile covered the floor in the eating room in Day care Center Number One; vinyl floor covering was used in the eating and sleeping rooms in Day care Center Number Two). Rooms in which sampling was carried out were situated on different floors in each building and were of different sizes. There were kitchens in all the CDCCs. The kitchen in Day care Center Number One was used both for preparation of meals and as an eating room.

Day care Center Number One was built in 1989, two in 1987, three in 1972 and four in 1984. When investigation began, Day care Center Number One had been used as day care center for 3 years, Number Two for 18 years, Three for 12 years and Four for 7 years. Although Day care Centers Number One and Three were located in more active places, Day care Centers number Two and Four were located in more inner parts.

Meteorological measurement

Monthly and sampling day average measurements of meteorological parameters such as temperature, relative humidity, wind velocity, rainfall and sunny time were supplied from the Directorate of Edirne Meteorological Office during the course of our study. Edirne Ministry of Environment and Forestry provided monthly and sampling days average concentrations of SO_2 and aerosol in urban air Edirne. Indoor air temperature and relative humidity were also monitored using a thermometer and hygrometer (TFA-Dostmann GmbH, Germany) at the study sites (Table 1).

Statistical analysis

Correlation analyses were performed to determine the relationships between meteorological factors and viable spore counts of microfungi genera isolated over

Table 1 Indoor environmental properties (temperature (°C) and relative humidity (%) values) that were measured in the CDCCs indoor air (with TFA-Dostmann GmbH, Germany)

Months 2004	CDCC 1			CDCC 2			CDCC 3			CDCC 4		
	1S	1E	1C	2S	2E	2C	3S	3E	3C	4S	4E	4C
January	22[a]	22	23	21	20	21	20	19	18	20	22	20
	70[b]	73	70	72.5	72	71	76	71.5	76	74	76	74
February	20	25	23	17	17	18	18	18	17	20	20	20
	60	65	60	65	65	65	66	66	65	65	68	65
March	23	22	24	20.6	21.8	22	19	21.5	19.7	19	22	18.5
	35	40	34	45	46	60	50	59	57	45	45	42
April	20.3	20.5	21.2	21	21.2	22.3	20	21	20.2	22	24	23.9
	51	52	62	50	54	55	49	59	58	46	38	41
May	19	19.5	19	19	19.3	20	21	21.6	20.9	19.7	19	19.7
	59	59	60	59	61	63	52	60	56	63	58	58
June	23.7	23.8	23.5	24	23.1	24.2	24.6	23.7	24.1	24.6	23.3	24.4
	60	65	63	58	61	63	59	65	58	66	67	63
July	26.8	27	28.2	28.4	27.4	27.2	28	27.1	27.7	27.1	26.8	26
	50	48	44	40	44	43	41	46	43	47	46	48
August	27.1	26.9	27.4	26	25.5	25.9	26.7	26	26.5	26.2	26	25.6
	47	52	50	46	42	44	50	50	52	62	55	48
September	23.5	24.6	24.4	23.1	22.7	22.6	23.6	24.4	23.7	23	24.3	23.9
	56	59	58	53	56	59	54	65	55	54	57	58
October	23	23.7	23.1	22.1	21.9	23.4	23.6	23	23.3	22.5	24.6	22.2
	60	61	60	59	61	55	50	58	51	58	53	60
November	21.8	20.8	22	18	18.6	17.5	17.9	19.4	17.5	16.3	17.9	18.4
	25	30	26	42	41	41	38	44	36	40	33	34
December	20.7	20.4	18.4	20.7	20.7	20.6	18.6	18.8	18.9	21.8	22	21.7
	65	70	68	56	56	60	59	62	63	59	59	61

S Sleeping room, *E* eating room, *C* classroom

[a] Temperature

[b] Relative humidity

the 12 months. Mann–Whitney U Test, ANOVA Test and Kruskal Wallis Analysis were used compare the differences and similarities among CDCCs and rooms. *P* values of <0.05 were considered as significant. Also, the ANOVA test and Kruskal Wallis Analysis was performed to compare indoor and outdoor air of CDCCs to microfungi genera found throughout the 12 month period.

Results

A total of 2,071 microfungi colonies (907 from indoors and 1,164 from outdoors) on 192 petri plates were isolated, enumerated and then identified. The highest viable spore counts in general total, indoor and outdoor air was found in June (451 CFU in general, 208 CFU in indoor air and 243 CFU in outdoor air). The lowest viable spore counts, 37 CFU, were observed in the months of January and February. For indoor air and outdoor air, the lowest viable spore counts were obtained in January (10 CFU) and February (26 CFU) respectively (Table 2).

The highest and lowest microfungi concentrations in the indoor air of CDCCs were determined in CDCC Number One (404 CFU) and Two (96 CFU), respectively. For the outdoor air, the highest microfungi concentration was found at CDCC Number Three (318 CFU), while CDCC number four total microfungi concentration was the lowest at 256 colonies (Table 1).

Samplings were made in three different indoor sampling areas (sleeping room, eating room and classroom) belonging to each CDCC. Indoor environment properties of the day care centers and total

Table 2 Monthly distributions and percentage (%) of fungal colony numbers counted in indoor and outdoor air of CDCCs

Months	CDCC 1		CDCC 2		CDCC 3		CDCC 4		Total		%		General Total	General %
	I	O	I	O	I	O	I	O	I	O	I	O		
January	2	4	1	3	1	10	6	10	10	27	27.03	72.97	37	1.78
February	0	2	2	19	8	2	1	3	11	26	29.73	70.27	37	1.78
March	51	2	0	4	22	1	3	22	76	29	72.38	27.62	105	5.07
April	7	3	2	54	5	6	5	5	19	68	21.84	78.16	87	4.20
May	18	32	3	29	17	59	18	50	56	170	24.78	75.22	226	10.91
June	18	58	19	56	132	99	39	30	208	243	46.12	53.88	451	21.78
July	25	29	15	38	27	42	25	26	92	135	40.53	59.47	227	10.96
August	18	36	23	27	18	19	13	18	72	100	41.86	58.14	172	8.31
September	6	9	4	14	6	12	4	11	20	46	30.30	69.70	66	3.19
October	53	58	19	30	24	40	12	31	108	159	40.45	59.55	267	12.89
November	19	21	5	32	7	17	7	30	38	100	27.54	72.46	138	6.67
December	187	20	3	10	2	11	5	20	197	61	76.36	23.64	258	12.46
Total	404	274	96	316	269	318	138	256						
Total %	44.55	23.54	10.58	27.15	29.66	27.32	15.21	21.99						
General Total (%)	678	(32.74)	412	(19.89)	587	(28.34)	394	(19.03)	907	1164	43.80	56.20	2071	100

I Indoor air, *O* outdoor air

microfungi spore counts isolated in sampling areas are shown in Table 3. During the investigation, we found that the indoor areas most isolated from microfungal spores were the eating rooms (362 CFU). This was followed by the sleeping rooms (287 CFU) and the classrooms (258 CFU). When the rooms of the CDCCs were compared with respect to total colony counts, the CDCC where the largest number of microfungi spores was isolated in each of the three rooms was CDCC Number One (121–162–121 CFU, respectively), while the lowest was CDCC Number Two (37–13–46 CFU, respectively) (Table 3).

Spores belonging to thirty fungal genera and 75 fungal species could be identified. *Cladosporium* (44.11%), *Penicillium* (18.94%), *Alternaria* (14.67%), *Aspergillus* (4.29%), *Epicoccum* (2.22%), and *Stemphylium* (1.30%) were the most abundant genera, forming over 85% of the total airborne fungi. The same genera were dominant in both indoor air and outdoor air with differences in sequence: *Cladosporium, Penicillium, Alternaria*, and *Aspergillus* (43.56%, 32.42%, 7.95% and 2.97%, respectively) in the indoor areas; *Cladosporium* (44.55%), *Alternaria* (19.91%), *Penicillium* (8.43%), and *Aspergillus* (5.34%) in the outdoor areas. *Cladosporium, Penicillium, Alternaria*, and non-sporulating microfungi were isolated every month. *Cladosporium, Penicillium,* and *Alternaria* genera were the most common in all indoor areas (sleeping rooms, eating rooms, classes) in CDCCs (Table 4).

Indoor and outdoor distributions of fungal genera for the CDCCs are shown in Table 4. Genera found in only indoor air but not outdoor air were *Bahusakala, Beauveria, Ramichloridium, Torula,* and *Trichothecium*. However, *Ceuthospora, Chaetomium, Curvularia, Mycotypha, Myrotecium, Paecilomyces, Pestalotiopsis*, and *Stachybotrys* were only encountered in the outdoor air of all four CDCCs.

Alternaria, Aspergillus, Cladosporium, Eurotium, Fusarium, Penicillium and non-sporulating microfungi were isolated in the indoor air of all the CDCCs, although *Alternaria, Aspergillus, Cladosporium, Epicoccum, Fusarium, Penicillium, Phoma, Stemphylium* genera and non-sporulating microfungi were found in the outdoor air of all the CDCCs (Table 4).

Seventy-five fungal species were identified. Among them, *Penicillium digitatum* was the most prevalent species in the indoor air and constituted 17.55%, the maximum percentage, of total indoor air microfungi; followed by *Cladosporium tenuissimum* (14.89% and 135 CFU) and *Cladosporium macrocarpum* (8.38% and 76 CFU). The most common

Table 3 Indoor environmental properties of day care centers and total microfungi spore counts isolated in sampling areas

	Part of the day-care center	No. of fungal colonies counted (CFU)	Environment properties	Number of children	Number of staff
Day-care center (1)	S	121	16 m^2, carpet covered, in underground floor	27	5
	E	162	12,5 m^2,floor tile covered, in underground floor		
	C	121	22 m^2, carpet cover, in underground floor		
	Total indoor	404			
	O	274			
	Total	678			
Day-care center (2)	S	37	62 m^2, vinyl floor covered, in ground floor	38	5
	E	13	43 m^2, vinyl floor covered, in ground floor		
	C	46	36 m^2, carpet covered, in ground floor		
	Total indoor	96			
	O	316			
	Total	412			
Day-care center (3)	S	78	30 m^2, carpet covered, in first floor	51	9
	E	130	30 m^2, carpet covered, in ground floor		
	C	61	30 m^2, carpet covered, in first floor		
	Total indoor	269			
	O	318			
	Total	587			
Day-care center (4)	S	51	30 m^2, carpet cover, in ground floor	64	11
	E	57	18 m^2, carpet cover, in ground floor		
	C	30	14 m^2, carpet cover, in ground floor		
	Total indoor	138			
	O	256			
	Total	394			
Total	S	287			
	E	362			
	C	258			
	Total indoor	907			
	O	1164			
	Total	2071			

S Sleeping room, *E* eating room, *C* classroom, *O* outdoor

species in outdoor air were *Cladosporium macrocarpum, Cladosporium variabile*, and *Alternaria alternata* (11.85% and 138 CFU, 11.59% and 135 CFU, 7.98% and 93 CFU) respectively. *Penicillium* genus had a maximum species number of 26; followed by *Cladosporium* and *Aspergillus* by nine species. Seven species were identified as belonging to the genus *Alternaria*. Spores belonging to eight microfungal genera could be identified only at the genus level (Table 5).

Table 4 Distributions of fungal isolates at the genera level in the indoor and outdoor air of day care centers

Isolates	CDCC 1		CDCC 2		CDCC 3		CDCC 4		Total					
	I	O	I	O	I	O	I	O	I	%	O	%	General	%
Acremonium spp.	–	–	1	–	3	–	1	–	5	0.55	0	0.00	5	0.25
Alternaria spp.	22	67	18	67	24	61	8	37	72	7.95	232	19.91	304	14.67
Arthrinium spp.	–	–	1	–	1	1	–	–	2	0.22	1	0.09	3	0.15
Aspergillus spp.	13	24	5	34	3	–	6	4	27	2.97	62	5.34	89	4.29
Bahusakala spp.	–	–	1	–	–	–	–	–	1	0.11	0	0.00	1	0.05
Beauveria spp.	–	–	1	–	1	–	–	–	2	0.22	0	0.00	2	0.10
Ceuthospora spp.	–	–	–	1	–	–	–	–	0	0.00	1	0.09	1	0.05
Chaetomium spp.	–	1	–	–	–	–	–	–	0	0.00	1	0.09	1	0.05
Cladosporium spp.	88	89	47	133	166	162	94	135	395	43.56	519	44.55	914	44.11
Curvularia spp.	–	1	–	–	–	–	–	–	0	0.00	1	0.09	1	0.05
Drechslera spp.	–	4	–	5	2	2	–	–	2	0.22	11	0.96	13	0.63
Epicoccum spp.	1	7	–	8	6	8	–	16	7	0.77	39	3.35	46	2.22
Eurotium spp.	1	–	1	–	6	2	3	1	11	1.21	3	0.25	14	0.67
Fusarium spp.	3	4	3	2	2	3	1	1	9	0.99	10	0.85	19	0.91
Mycotypha spp.	–	1	–	–	–	–	–	–	0	0.00	1	0.09	1	0.05
Myrotechium spp.	–	–	–	–	–	1	–	–	0	0.00	1	0.09	1	0.05
Paecilomyces spp.	–	–	–	–	–	–	–	2	0	0.00	2	0.18	2	0.10
Penicillium spp.	244	19	4	13	35	45	11	21	294	32.42	98	8.43	392	18.94
Pestalotiopsis spp.	–	–	–	–	–	1	–	–	0	0.00	1	0.09	1	0.05
Phoma spp.	1	3	–	8	–	3	–	4	1	0.11	18	1.54	19	0.91
Ramichloridium spp.	–	–	1	–	–	–	–	–	1	0.11	0	0.00	1	0.05
Rhizopus spp.	–	–	–	–	–	–	1	1	1	0.11	1	0.09	2	0.10
Scopulariopsis spp.	–	1	–	–	1	–	–	–	1	0.11	1	0.09	2	0.10
Stachybotrys spp.	–	1	–	–	–	–	–	–	0	0.00	1	0.09	1	0.05
Stemphylium spp.	–	13	3	4	2	2	–	3	5	0.55	22	1.89	27	1.30
Torula spp.	1	–	–	–	–	–	–	–	1	0.11	0	0.00	1	0.05
Trichoderma spp.	3	1	–	–	1	1	–	2	4	0.44	4	0.34	8	0.38
Trichothecium spp.	2	–	–	–	–	–	–	–	2	0.22	0	0.00	2	0.10
Ulocladium spp.	–	–	–	–	1	–	1	1	2	0.22	1	0.09	3	0.15
Verticillium spp.	1	–	–	1	–	–	–	–	1	0.11	1	0.09	2	0.10
Non-sporulating fungi	22	35	9	35	13	22	10	25	54	5.95	117	10.05	171	8.26
Unidentified	2	3	1	5	2	4	2	3	7	0.77	15	1.28	22	1.06
Total of indoor and outdoor	404	274	96	316	269	318	138	256						
Total	678		412		587		394		907	100	1164	100	2071	100
%	32.74		19.89		28.34		19.03							

Total viable spore counts and CFU numbers of dominant genera showed seasonal fluctuations. The maximum microfungal CFU number was found during the summer at 850 CFU (372 in indoor–478 in outdoor) and the minimum was found during the winter at 332 CFU (218 in indoor–114 in outdoor), respectively (Fig. 1). Seasonal concentrations of predominant indoor isolates were significantly lower in wintertime with the exception of *Penicillium* genus. Genus *Cladosporium* was isolated at the highest concentration during the summer and the lowest concentration during the winter indoors, outdoors and among all isolated microfungi. The lowest concentration for *Penicillium* was found in the summer. The genus *Alternaria* was found at highest concentration during the summer and lowest during the winter (Fig. 1). The indoor and outdoor *Cladosporium* concentration showed a peak in late spring (May and June) and again in autumn (October). The maximum concentration for *Cladosporium* was observed in June (Fig. 2). The genus *Penicillium* showed peak in March and December in the indoor air and in May in the outdoor air (Fig. 2). *Alternaria* genus concentration was at its peak in early summer

Table 5 Indoor and outdoor colony numbers, percentage (%), and sampling stations of fungal species found between January–December 2004

Genera and species	I		O		Total		Sampling months during which the samples were found and number of stations
	(CFU)	(%)	(CFU)	(%)	(CFU)	(%)	
Acremonium Link	5	0.55	0	0.00	5	0.25	Oc, N
Acremonium fusidioides (Nicot) W. Gams	1	0.11	0	0.00	1	0.05	N (3S1)
Acremonium spp.	4	0.44	0	0.00	4	0.20	Oc (2S1,3E2,4S1)
Alternaria Nees	72	7.95	232	19.91	304	14.67	J, F, Mr, Ap, My, Jn, Jl, Au, Sp, Oc, N
Alternaria alternata (Fr.) Keissl.	26	2.87	93	7.98	119	5.74	J (4O1); F (2O2,4O1); Mr (4E1,4O5); Ap (2O8,4O1); My (1C1,3C1,3O3,4O1); Jn (1E1,1O9,3S1,3E2,3O3, 4O2); Jl (1C1, 1O5,2C3,2O9,3S3,3O17,4S1,4E1,4O7); Au (1O1,2O1); Sp (2S1, 2O1,4S1,4O1); Oc (1E2,1C4,2O2,402); N (1C2,1O1,2O1,3O1,4O8)
Alternaria citri Ellis & N. Pierce	17	1.88	54	4.64	71	3.43	J (3S1); Ap (2O2); Jn (1O3,2O5,3S2, 3E1,3O4,4S3); Au (1E1,1C3,1O9,2S1,2O6,3E3,3O5); Sp (3O1); Oc (3O5); N (3O5, 2S1,2C1,2O8,3O1)
Alternaria petroselini (Neerg.) E.G. Simmons	2	0.22	0	0.00	2	0.10	O (2S2)
Alternaria radicina Meier, Drechsler & E.D. Eddy	0	0.00	1	0.09	1	0.05	Sp (1O1)
Alternaria raphani J.W. Groves & Skolko	1	0.11	0	0.00	1	0.05	Oc (2C1)
Alternaria tenuissima (Kunze) Wiltshire	11	1.21	51	4.38	62	2.99	Ap (1S1,3O1); Au (1O12,2S2,2O1,1E1,1C1,1O8,4E1, 4O5); Oc (1S1,1O10,2S1,2C1,2O7,3E1,3C1,3O3, 4O1); N (1O1, 3O2)
Alternaria triticicola Vasant Rao	1	0.11	6	0.51	7	0.33	Jl (1O6,2S1)
Alternaria spp	14	1.55	27	2.31	41	1.98	Ap (2O8); Jl (1E1, 1C1,1O2,2O2,3E4,3O1); Au (2S3,3E2,3O2); Sp (203,401); Oc (1S2,304); N (1E1,1O1,2O1,4O1); D (1O1)
Arthrinium Kunze	2	0.22	1	0.09	3	0.15	Au, Oc, D
Arthrinium phaeospermum (Corda) M.B. Ellis	2	0.22	1	0.09	3	0.15	Au (2C1); Oc (3C1); D (3O1)
Aspergillus Fr.:Fr.	27	2.97	62	5.34	89	4.29	J, F, Mr, Ap, Jn, Jl, Au, Sp, Oc, N
Aspergillus candidus Link	0	0.00	2	0.18	2	0.10	F (2O2)
Aspergillus flavus Link	6	0.66	22	1.89	28	1.35	Oc (1E2,1C2,1O20,2C2); N (4O2)
Aspergillus fumigatus Fresen.	3	0.33	3	0.25	6	0.28	F (2O2); Jn (1S2); Sp (2O1); Oc (1E1)
Aspergillus niger Tiegh.	6	0.66	32	2.75	38	1.83	F (2O1); Mr (1O2); Ap (2O27); Jn (1S1); Jl (1C1,4E1); Sp (2C1); Oc (1S1,1O1,4O1); N (1C1)
Aspergillus oryzae (Ahlb.) E. Cohn	1	0.11	0	0.00	1	0.05	N (1E1)
Aspergillus sclerotiorum G.A. Huber	1	0.11	0	0.00	1	0.05	Oc (4E1)
Aspergillus terreus Thom	1	0.11	0	0.00	1	0.05	Oc (1C1)
Aspergillus ustus (Bainier) Thom & Church	0	0.00	1	0.09	1	0.05	Sp (2O1)
Aspergillus versicolor (Vuill.) Tirab.	8	0.88	2	0.18	10	0.48	J (2S1,4E1); Mr (4C1); Ap (4O1); Jl (3E2); Sp (1O1); N (3E1,4E1,4C1)

Aspergillus sp.	1	0.11	0	0.00	1	0.05	Au (2C1)
Bahusakala Subramanian	1	0.11	0	0.09	1	0.05	Jn
Bahusakala olivaceonigra (Berk. & Broome) Subram.	1	0.11	0	0.00	1	0.05	Jn (2E1)
Beauveria Vuill.	2	0.22	0	0.00	2	0.10	My, Sp
Beauveria alba (Limber) Saccas	2	0.22	0	0.00	2	0.10	My (3S1); Sp (2E1)
Ceuthospora Grev.	1	0.11	0	0.00	1	0.05	Jl
Ceuthospora sp.	1	0.11	0	0.00	1	0.05	Jl (2O1)
Chaetomium Kunze	1	0.11	0	0.00	1	0.05	Sp
Chaetomium sp.	1	0.11	0	0.00	1	0.05	Sp (1O1)
Cladosporium Link	395	43.56	519	44.55	914	44.11	J, F, Mr, Ap, My, Jn, Jl, Au, Sp, Oc, N, D
Cladosporium chlorocephalum (Fresen.) E.W. Mason & M.B. Ellis	4	0.44	10	0.86	14	0.67	Ap (2O6,3C1); Jn (4O1); Au (3E2,3O2,4O1); Oc (3S1)
Cladosporium cladosporioides (Fresen.) G.A. de Vries	41	4.52	57	4.89	98	4.73	J (1O1,4E1); F (4S1); Ap (1C1,2C1,2O1,3O1,4S1, 4O1); My (3S1,3E1,3C2); Jn (1E5,1O13,2C4,2O1, 4E2,4C2,4O4); Jl (1O1,2S1,4E2); Au 2C2,2O1,4C1); Oc (1S2,1C3,1O6,2S1,2C1,2O2,3E3, 3O5,4S1,4O3); N (1O3,2E1,2O3,3O1,4S1,4O6); D (1O2,201,401)
Cladosporium cucumerinum Ellis & Arthur	15	1.65	7	0.60	22	1.06	Ap (4E1); My (1O1,2E1); Jn (1O1,3E4,4S7); Sp (3O1); Oc (1O2,3O2,4E1); N (1C1)
Cladosporium elatum (Harz) Nannf.	1	0.11	1	0.09	2	0.10	Mr (4O1); Ap (4C1)
Cladosporium herbarum (Pers.) Link	27	2.98	27	2.31	54	2.60	J (1E1,1O1,2O1,4O4); Mr (4O6); Ap (1E1); My (1E2,1C2,3E2); Jl(1E3,1C2,2C1,3E3,3C1,3O10,4E7); Oc (1S2,4O1); N (2O4)
Cladosporium macrocarpum Preuss	76	8.38	138	11.85	214	10.33	J (4O2); Ap (4O1); My (1E3,1O11,2S1,2O14,3E1,3C1,4S1,4C1); Jn (1S1,1E1,2S2,2C2,2O26,3S13,1E15,3C10,3O56, 4S7,4E3,4O12); Jl (1E3,1C2,2C1,2O6,3C1,4S1,4E2,4C1,4O6); Oc (1E2,2O1); D (2C1)
Cladosporium sphaerospermum Penz.	29	3.20	45	3.86	74	3.57	F (2O2,4O1);Ap (1E1); My (2O2,3O3,4O2); Jn (1S1,3C3,4C1,4O3); Jl (1E1,2C2,2O5,4S2,4E1); Au (1E2,1O1,2S1,2O3,4E1); Oc (1S2,2C1,2O2,3S1,3E4,3C1,3O6); N (1E1,1O1,2O6,3S1,3O2,4S1,4O3); D (3C1,4O3)
Cladosporium tenuissimum Cooke	135	14.89	76	6.53	211	10.19	Mr (2O1,4O2); Ap (1S1,1O1,3E1,3C1,4E1); My (1E1,1O1,2O1,3C2,4S2,4E1); Jn(1S4,1E1,2E1,2C3,3S24,3E18,3C12,4S3,4E2,4C4) Au (1S2,1C3,1O3,2S2,2E3,2O5,3S1,3E2,4E3); Sp (1C1,2S1,2O1,3E2,3O2,4O1),

Table 5 (continued)

Genera and species	I		O		Total		Sampling months during which the samples were found and number of stations
	(CFU)	(%)	(CFU)	(%)	(CFU)	(%)	
							Oc (1S4,1E6,1C3,1O1,2S5,2C1,2O5,3E1,3C1,3O4, 4S1,4O8); N (1C4,2S1,3O3,4O3); D(1S2,1E1,1O9,2O7,3O9,4E2,4C1,4O9)
Cladosporium variabile (Cooke) G.A. de Vries	53	5.84	135	11.59	188	9.08	My (1S1,1E1,1O14,2E1,2O11,3S1,3E1,3C1,3O20, 4S3, 4E7,4C1,4O29); Jn (1C1,1O10,2C2,2O14, 3S10,3E6,3C5,3O27,4C3,4O4); Jl (1C1,2C1,3S1); Au (1E2,3C1); Sp (3O3,4E1); D (2C1, 3O1, 4C1, 4O2)
Cladosporium spp.	14	1.55	23	1.97	37	1.78	Ap (3O1); My (1C1,1O1); Jn (4C2); Jl (3O2); Au (2S1,3E1,4E1,4C3,4O11); Sp (2O1,3C1,3O1,4O1); Oc (1S2,1O4); N (1C1,1O1,4S1)
Curvularia Boedijn	0	0.00	1	0.09	1	0.05	Ap
Curvularia inaequalis (Shear) Boedijn	0	0.00	1	0.09	1	0.05	Ap (1O1)
Drechslera Ito	2	0.22	11	0.96	13	0.63	Ap, Jl, Au, Sp, N
Cochliobolus sativus (Ito & Kuribayashi) Drechsler'un *Drechslera* devresi	1	0.11	1	0.09	2	0.10	Jl (3S1,3O1)
Cochliobolus spicifer (Nelson)' in *Drechslera* devresi	0	0.00	6	0.51	6	0.28	Ap (3O1); Au (1O3,2O2)
Drechslera australiensis M. B. Ellis	0	0.00	1	0.09	1	0.05	Sp (2O1)
Drechslera biseptata (Sacc. & Roum.) M.J. Richardson & E.M. Friser	0	0.00	1	0.09	1	0.05	Au (2O1)
Drechslera spp	1	0.11	2	0.18	3	0.15	Au (1O1); Sp (3E1); N (2O1)
Epicoccum Link	7	0.77	39	3.35	46	2.22	Mr, Jn, Jl, Au, Sp, Oc, N, D
Epicoccum spp.	7	0.77	39	3.35	46	2.22	Mr (4O2); Jn (2O2,3S2,304,4O4); Jl (4O1); Au (3S1,3E2,3O1); Sp (1O3); Oc (1C1,1O1,2O4,3E1,3O3,4O5); N (2O2,4O3); D (1O3,4O1)
Eurotium Link:Fr.	11	1.21	3	0.25	14	0.67	F, Au, Sp, Oc, N
Eurotium herbariorum (F.H. Wigg.) Link	11	1.21	3	0.25	14	0.67	F (4O1); Au (1C1,3E1); Sp (3E1,3O1); Oc (3S1,3E1,3C1,4S3); N (2C1,3E1,3O1)
Fusarium Link	9	0.99	10	0.85	19	0.91	My, Jl, Au, Sp, Oc, N
Fusarium moniliforme var. *subglutinans* Wollenw. & Reinking	5	0.55	4	0.34	9	0.43	Oc (1S2,1E1,2S1,2O1,3O2,4S1,4O1)
Fusarium oxysporum Schltdl.	1	0.11	0	0.00	1	0.05	My (3E1)
Fusarium trichothecioides Wollenw.	0	0.00	1	0.09	1	0.05	N (1O1)
Fusarium spp.	3	0.33	5	0.42	8	0.38	Jl (2S2); Au (1O3); Sp (2O1,3O1); N (3C1)
Mycotypha Fenner.	0	0.00	1	0.09	1	0.05	Jl

Mycotypha sp.	0	0.00	1	0.09	1	0.05	Jl (1O1)
Myrotechium Tode	0	0.00	1	0.09	1	0.05	Oc
Myrotechium verrucaria (Alb. & Schwein.) Ditmar	0	0.00	1	0.09	1	0.05	Oc (3O1)
Paecilomyces Bainier	0	0.00	2	0.18	2	0.10	Jl, D
Paecilomyces variotii Bainier	0	0.00	2	0.18	2	0.10	Jl (4O1); D (4O1)
Penicillium Link	294	32.42	98	8.43	392	18.94	J, F, Mr, Ap, My, Jn, Jl, Au, Sp, Oc, N, D
Penicillium atramentosum Thom	0	0.00	1	0.09	1	0.05	F (1O1)
Penicillium aurantiogriseum Dierckx	4	0.44	2	0.18	6	0.28	F (2O1,3S1); Mr (1E3); Au (2O1)
Penicillium brevicompactum Dierckx	10	1.10	5	0.42	15	0.72	J (3O1); F (2E1,2D1); Mr (1S1,1E5); Oc (3O1); N (1S1,1E1,1O1,3O1); D (2C1)
Penicillium chrysogenum Thom	38	4.19	4	0.34	42	2.03	F (2O1,3S3,3C1); Mr (3E21,3O1); Ap (1C1); My (3O1,4E1); Jl (1S1,4S1); Au (2S1); Sp (1S1,1O2); Oc (3E2,4S1,4E1); N (1C1); D (3E1)
Penicillium citreonigrum Dierckx	0	0.00	3	0.25	3	0.15	J (3O3)
Penicillium citrinum Thom	7	0.77	49	4.21	56	2.70	J (3O1,4E2,4O3); Mr (3C1); Ap (2C1,3E1); My (3O29,4O15); Oc 1C2,1O1)
Penicillium digitatum (Pers.: Fr.) Sacc.	159	17.55	0	0.00	159	7.68	D (1S57,1E48,1C54)
Penicillium echinulatum Fassat.	10		1	0.09	11	0.53	F (1O1); Mr (1E1O)
Penicillium expansum Link	0	0.00	1	0.09	1	0.05	Oc (1O1)
Penicillium fellutanum Biourge	1	0.11	1	0.09	2	0.10	F (2O1); D (4E1)
Penicillium glabrum (Wehmer) Westling	11		2	0.18	13	0.62	J (1O1); Mr (1S2,1E9); N (3O1)
Penicillium griseofulvum Dierckx	1	0.11	2	0.18	3	0.15	Au (3O1); N (1E1); D (1O1)
Penicillium hirsutum Dierckx	1	0.11	2	0.18	3	0.15	Ap (1S1,2O1); Jn (1O1)
Penicillium italicum Wehmer	21		0	0.00	21	1.01	D (1S6,1E10,1C5)
Penicillium janczewskii K. M. Zalessky	1	0.11	0	0.00	1	0.05	F (3S1)
Penicillium janthinellum Biourge	0	0.00	3	0.25	3	0.15	F (2O1); Ap (2O1); D (1O1)
Penicillium lividum Westling	1	0.11	0	0.00	1	0.05	D (1C1)
Penicillium miczynskii K. M. Zalessky	2	0.22	0	0.00	2	0.10	F (3S2)
Penicillium purpurescens (Soop) Biourge	0	0.00	2	0.18	2	0.10	N (1O2)
Penicillium purpurogenum Stool	0	0.00	4	0.34	4	0.20	Sp (2O1,4O3)
Penicillium restrictum J.C. Gilman & E.V. Abbott	1	0.11	1	0.09	2	0.10	My (2O1); N (4C1)
Penicillium roseopurpureum Dierckx	1	0.11	0	0.00	1	0.05	Mr (1S1)
Penicillium variabile Sopp	1	0.11	5	0.42	6	0.28	J (3O5); My (4E1)
Penicillium verruculosum Peyronel	3	0.33	0	0.00	3	0.15	Mr (1E3)
Penicillium viridicatum Westling	12		3	0.25	15	0.72	Mr (1S3,1E5,1C1); Oc (4C1); N (1S1,2O2); D (1C1,1O1)
Penicillium waksmanii K. M. Zalessky	0	0.00	1	0.09	1	0.05	Oc (1O1)
Penicillium spp.	9	0.99	6	0.51	15	0.72	J (4E1); F (3O1); Mr (1S1,1E6,2O1); My (1E1); Oc (1O4)
Pestalotiopsis Steyaert	1	0.11	0	0.00	1	0.05	Jl
Pestalotiopsis sp.	1	0.11	0	0.00	1	0.05	Jl (3O1)

Table 5 (continued)

Genera and species	I		O		Total		Sampling months during which the samples were found and number of stations
	(CFU)	(%)	(CFU)	(%)	(CFU)	(%)	
Phoma Sacc.	1	0.11	18	1.54	19	0.91	J, F, Mr, Ap, My, Jl, Oc, N, D
Phoma spp.	1	0.11	18	1.54	19	0.91	J (2O1); F (2O3); Mr (2O2,4O2); Ap (1O1,3O2); My (4O1); Jl (4O1); Oc (1C1,2O1); N (2O1,3O1); D (1O2)
Ramichloridium Stahel	1	0.11	0	0.00	1	0.05	Jn
Ramichloridium subulatum de Hoog	1	0.11	0	0.00	1	0.05	Jn (2C1)
Rhizopus Ehrenberger	1	0.11	0	0.00	1	0.05	Au
Rhizopus spp.	1	0.11	0	0.00	1	0.05	Au (4C1,4O1)
Scopulariopsis Bainier	1	0.11	1	0.09	2	0.10	My, Oc
Scopulariopsis chartarum (G. Sm.) F.J. Morton & G. Sm.	1	0.11	0	0.00	1	0.05	Oc (3S1)
Scopulariopsis fusca Zach	0	0.00	1	0.09	1	0.05	My (1O1)
Stachybotrys Corda	0	0.11	1	0.09	1	0.05	Jl
Stachybotrys chartarum (Ehrenb.) Z. Hughes	0	0.00	1	0.09	1	0.05	Jl (1O1)
Stemphylium Wallroth	5	0.55	22	1.89	27	1.30	Jn, Jl, Au, N, D
Stemphylium botryosum Sacc.	5	0.55	22	1.89	27	1.30	Jn (1O13,3E1,3C1,3O1); Jl (2O4); Au (2S1,2E1,2C1); N (3O1); D (4O3)
Torula Pers	1	0.11	0	0.00	1	0.05	Au
Torula herbarum (Pers.) Link	1	0.11	0	0.00	1	0.05	Au (1S1)
Trichoderma Pers	4	0.44	4	0.34	8	0.38	My, Oc, N
Trichoderma spp.	4	0.44	4	0.34	8	0.38	My (1O1); Oc 1C3); N (3E1,3O1,4O2)
Trichothecium Link	2	0.22	0	0.00	2	0.10	D
Trichothecium roseum (Pers.) Link	2	0.22	0	0.00	2	0.10	D (1S1,1E1)
Ulocladium Preuss	2	0.22	1	0.09	3	0.15	Mr, Jn
Ulocladium chartarum (Preuss) E.G. Simmons	2	0.22	1	0.09	3	0.15	Mr (4S1,4O1); Jn (3E1)
Verticillium Nees	1	0.11	1	0.09	2	0.10	Sp, N
Verticillium tenerum Nees	0	0.00	1	0.09	1	0.05	Sp (2O1)
Verticillium sp.	1	0.11	0	0.00	1	0.05	N (1E1)
Non sporulating fungi	54	5.95	117	10.05	171	8.26	J, F, Mr, Ap, My, Jn, Jl, Au, Sp, Oc, N, D
Unidentified	7	0.77	15	1.28	22	1.06	F, My, Jn, Jl, Au, Sp, Oc, N

The first number in parenthesis = number of day-care center (station number); the second number in parenthesis = fungal colony number

J January, *F* February, *Mr* March, *Ap* April, *My* May, *Jn* June, *Jl* July, *Au* August, *Sp* September, *Oc* October, *N* November, *D* December, *S* sleeping room, *E* eating room, *C* classroom, *O* outdoor

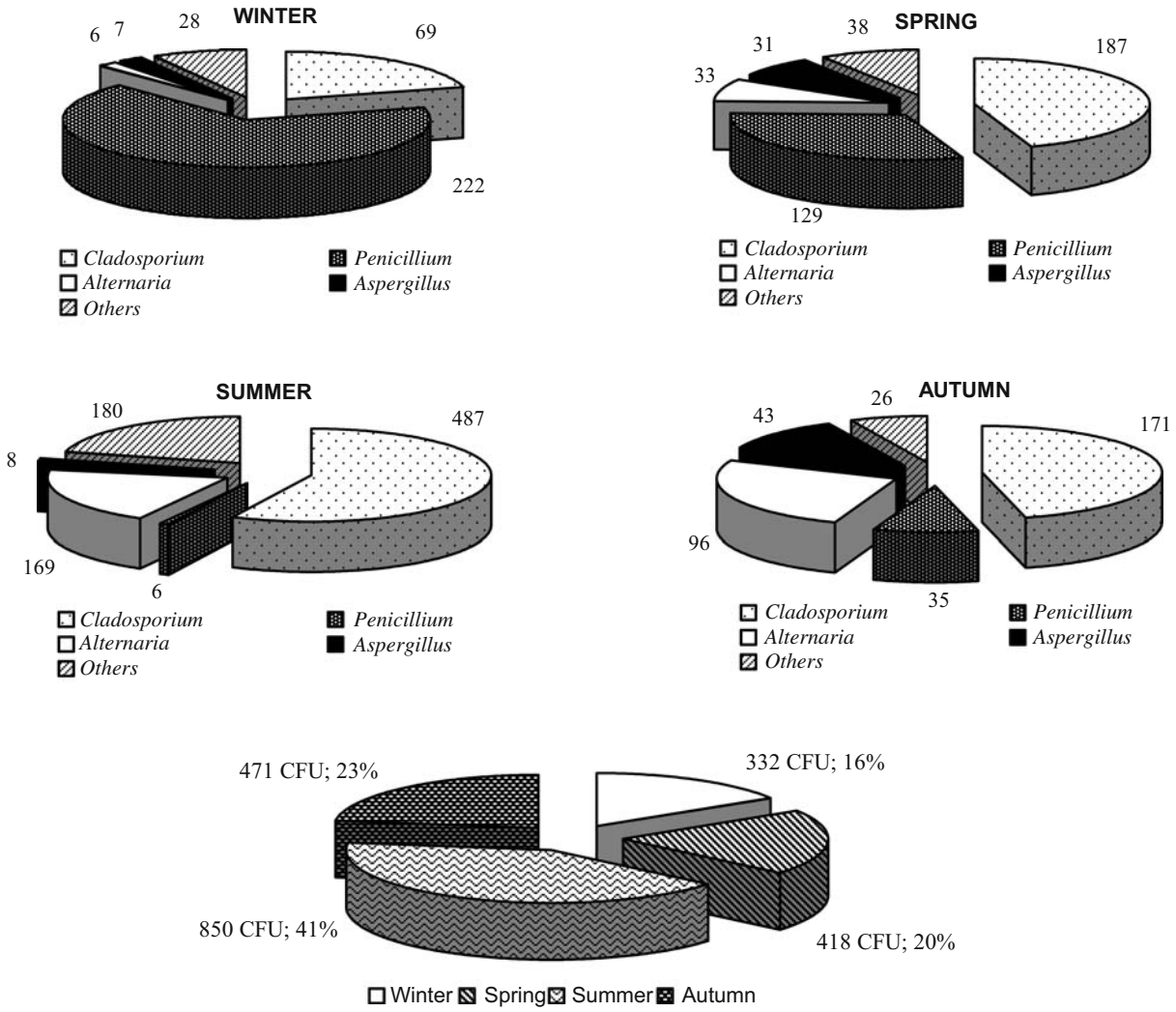

Fig. 1 Seasonal variations in CFU numbers of *Cladosporium, Penicillium, Alternaria* and *Aspergillus* spores

(June) and CFU numbers were higher in this season than other seasons. The maximum viable spore count for *Alternaria* was found in August (Fig. 2).

Distributions of total monthly outdoor CFU numbers and monthly outdoor CFU numbers of dominant genera according to monthly average values (MA) and sampling day's average values (SDA) for meteorological factors measured during the investigation are shown in Fig. 3, and significant findings from statistical analyses in Table 6.

Discussion

Indoor and outdoor airborne microfungal studies similar to this investigation have been carried out previously in Edirne city. Yazicioglu et al. (2004) examined indoor airborne fungi in the homes of asthmatic and nonatopic children. The other investigation of indoor airborne fungi and bacteria was performed on hospital air by Sarica et al. (2002). In addition, Aydogdu et al. (2005) studied fungi and bacteria in the indoor air of primary schools in Edirne city. Also, Asan et al. (2002) determined airborne fungal concentrations and compositions as well as their correlations with various meteorological factors. The prevalence of allergic diseases in primary schools in the city was evaluated by Selcuk et al. (1997). However, indoor and outdoor airborne microfungi in day care centers in Edirne city remain unknown.

In our study, the genera *Cladosporium, Penicillium, Alternaria* and *Aspergillus* were the most abundant

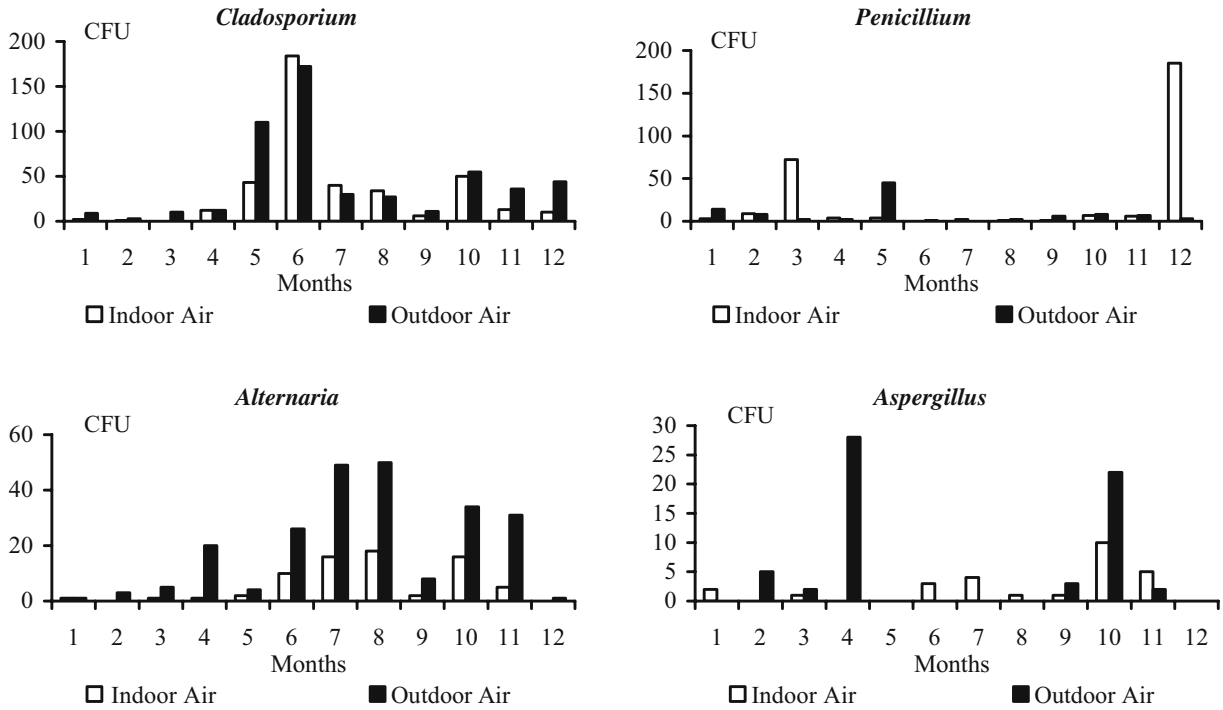

Fig. 2 Monthly variations in the indoor and outdoor CFU numbers of *Cladosporium, Penicillium, Alternaria* and *Aspergillus* spores

constituents of fungal aerosols in both indoor air and outdoor air. Some or all of these genera have been reported as the most common airborne fungi in different indoor and outdoor environments in other studies (Pyrri and Kapsanaki-Gotsi 2007; Abdel-Hameed 2007a, b; Basilico et al. 2007; Kim and Kim 2007; El-Morsy 2006; Fang et al. 2005; Adhikari et al. 2004; Hargreaves et al. 2003; Medrela Kuder 2003; Norbäck et al. 2000; Katz et al. 1999; Li et al. 1995). In addition to this, the predominant indoor microfungi reported in this study were consistent with previous indoor air studies carried out by Aydogdu et al. 2005; Sarica et al. 2002 and Yazicioglu et al. 2004 from Edirne, Turkey. Moreover, Asan et al. (2002) found *Alternaria, Penicillium, Cladosporium* and *Aspergillus* as the predominant genera in their study of airborne fungi in the urban air of Edirne.

Microfungal spores are ubiquitous worldwide and their numbers and species are known to vary with the season of the year to weather changes (Kasprzyk and Worek 2006; El-Morsy 2006; Stepalska and Wolek 2005; Bartlett et al. 2004; Ren et al. 2001). In our study, total indoor and outdoor fungi concentration was highest during the summer. While a positive correlation was found between monthly average temperature and monthly total outdoor CFU numbers, a strong negative correlation was found between monthly average wind speed and monthly total outdoor CFU counts. According to Fang et al. (2005) the vigorous growth of plants in summer can allow for the growth airborne microfungi and may induce more favourable growth conditions for fungi due to increased temperatures (Lee et al. 2006). Outdoor air is the main source of airborne fungi in indoor air. Therefore, seasonal variations in climatic conditions are also responsible for changes in the concentrations and types of microfungi in the air indoors. Fungal spore count in indoor air is usually high in the summer when outdoor fungal concentrations increase (Medrela-Kuder 2003). As ventilation is provided through windows in all CDCCs, higher indoor airborne fungal concentrations in the summer can be partly explained by higher outdoor concentrations. Airborne spore counts of microfungi were observed to be higher during the summer and autumn than the spring and winter. Increased temperature and suitable relative humidity might lead to higher microfungal counts. Ren et al. (2001) explained that

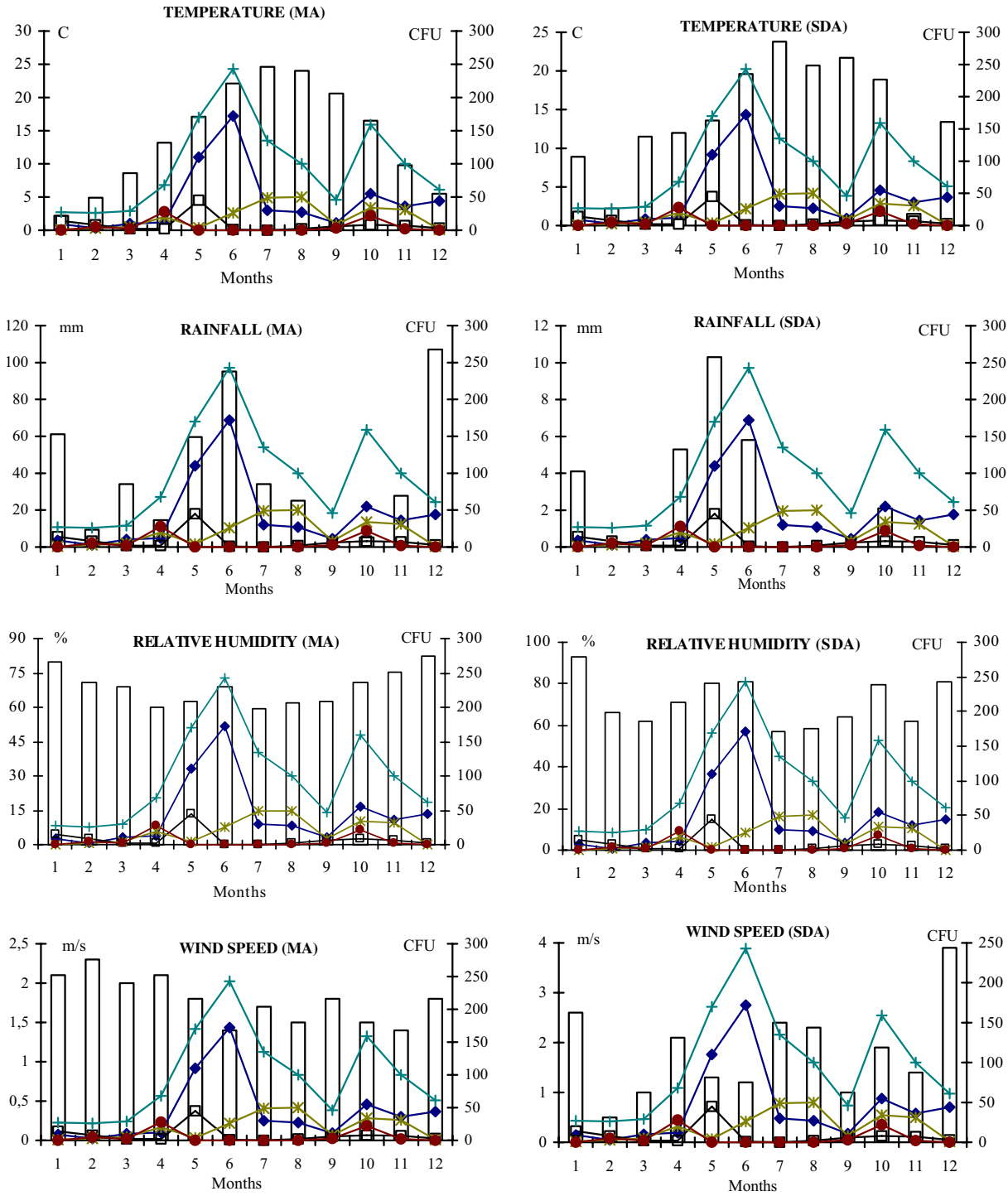

Fig. 3 Distributions of total monthly outdoor CFU numbers and monthly outdoor CFU numbers of dominant genera according to monthly values (*MA*) and sampling day's average values (*SDA*) for meteorological factors

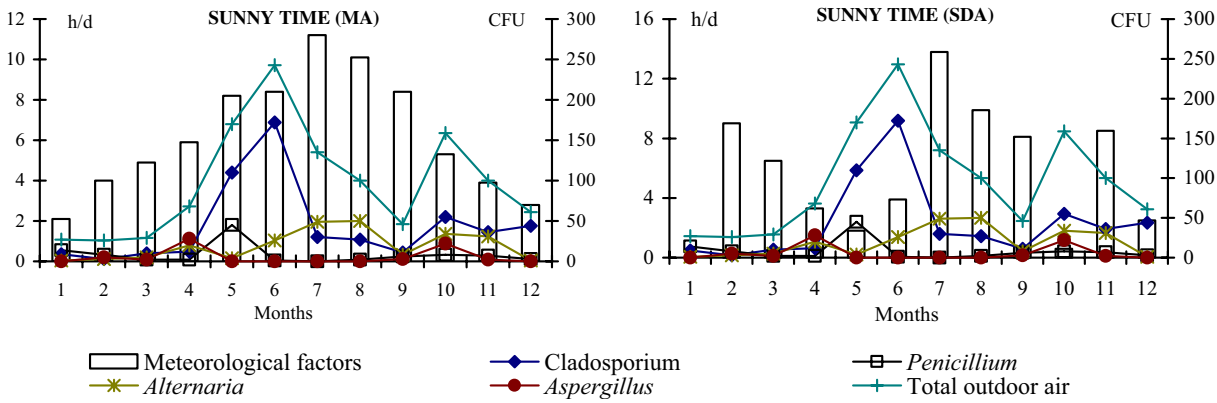

Fig. 3 (continued)

summer and autumn tend to have higher temperature and are more humid than spring and winter, conditions that favor fungal growth.

Cladosporium was the dominant and most important genus, constituting the majority in the total count. This genus was also predominant both indoors and outdoors, being the most common in the indoor air of all the CDCCs except CDCC Number One, and all indoor air sampling areas. Spores of *Cladosporium* species probably occur more abundantly worldwide than any other spore type and they are the dominant airborne spores in many areas, especially in temperate climates (Cooley et al. 1998; Kasprzyk and Worek 2006; Herrero et al. 2006). There was an important increase in indoor and outdoor *Cladosporium* concentrations in May, June and October. While temperature and rainfall continued to increase, the heaviest rainfall seemed to occur during these months except for December. Positive correlations were found between monthly average rainfall and sampling day average rainfall with monthly outdoor air *Cladosporium* concentrations. Sakiyan and Inceoglu (2003) pointed out that when high temperature is combined with a sufficient amount of precipitation, sporulation conditions for *Cladosporium* and *Alternaria* spores are optimal, hence a significant increase in CFU numbers after precipitation was determined. Furthermore, higher concentrations of fungal spores occur in seasons with higher precipitation and temperatures where there is abundant dead plant material for the fungi to grow on. The main sources of spores are considered to be the surrounding vegetation (Jones and Harrison 2004). In our study, indoor and outdoor *Cladosporium* concentrations were highest during the summer when there was increased vegetation. In other investigations as well, this genus was found most frequently both indoors and outdoors in the summer (Ren et al. 2001).

The genus *Penicillium* is among the most abundant mesophilic airborne fungi in nature and in the human environment. We found that the *Penicillium* genus was common indoor and outdoor of the CDCCs. The outdoor concentration of *Penicillium* was highest in May. The increase in relative humidity and the growth of vegetation with increasing the temperature and rainfall in this month contributed to increasing *Penicillium* spore counts in outdoor air. This finding was also supported by significant correlation found between monthly total outdoor air *Penicillium* concentration and sampling day average rainfall. The concentration of *Penicillium* decreased abruptly during the summer. Similarly, Pyrri and Kapsanaki-Gotsi (2007), in their study on the airborne fungi in Athens, also found that *Penicillium* concentrations decreased in summer. The highest concentration of *Penicillium* was found in CDCC Number One. Most of *Penicillium* concentration in this CDCC was isolated from its indoor air in December (185 CFU and 70.34%). Two species alone, *P. digitatum* and *P. italicum*, constituted 183 out of 185 colonies. Accordingly, air currents which occurred in the indoor environments of CDCC number one might have been responsible for the distribution of these two *Penicillium* species from their source to all indoor areas.

The spores of *Alternaria* genus are found in the atmosphere in many locations around the world (Stennett and Beggs 2004). *Alternaria* species live on most plants as saprophytes and parasites and mostly favor outdoor habitats. In present study, this

Table 6 The significant statistical findings from statistical analyses

	Monthly average temperature (C)	Monthly average wind speed (m/s)	Monthly average rainfall (mm)	Sampling days average rainfall (mm)	Monthly average sunny time (h/d)	Relative humidity (indoor sampling areas) (%)
Monthly total outdoor CFU numbers	r=0.647 p=0.023<0.05	r=−0.723 p=0.008<0.01				
Monthly CFU numbers of *Cladosporium* in outdoor air			r=0.589 p=0.044<0.05	r=0.620 p=0.032<0.05		
Monthly CFU numbers of *Penicillium* in outdoor air				r=0.721 p=0.008<0.01		
Monthly CFU numbers of *Alternaria* in outdoor air	r=0.716 p=0.009<0.01	r=−0.667 p=0.018<0.05			r=0.637 p=0.026<0.05	
Monthly CFU numbers of *Cladosporium* in indoor air of CDDC 2						r=0.590 p=0.043<0.05
Monthly total CFU numbers in indoor air						r=0.702 p=0.011<0.05
Monthly total CFU numbers in outdoor air of CDDC 1	r=0.611 p=0.035<0.05	r=−0.816 p=0.001<0.01				
Monthly total CFU numbers in outdoor air of CDDC 3	r=0.581 p=0.047<0.05	r=−0.599 p=0.04<0.05				
Monthly total CFU numbers in outdoor air of CDDC 4	r=0.665 p=0.018<0.05	r=−0.612 p=0.034<0.05				

genus was isolated more predominantly in the outdoor air than indoor air in all seasons. In the same way, some researchers showed that *Alternaria* was found in indoor air less often than in outdoor air (O'Connor et al. 2004; Andersson et al. 2003). Furthermore, *Alternaria* occurred in increasing numbers during the summer months when temperature and amount of sunlight increased markedly. However, its concentration was lowest in the winter. Researchers such as Sakiyan and Inceoglu (2003) and Pyrri and Kapsanaki-Gotsi (2007) have reported that the highest concentrations of airborne *Alternaria* spores were recorded during the summer. The positive relationships found between monthly average temperature and monthly average sunny time with outdoor monthly total *Alternaria* concentration supported this findings. A negative relationship was also determined between monthly outdoor *Alternaria* concentration and monthly average wind speed. Wind velocity is an important factor for the liberation and dispersion of airborne fungi. According to Sakiyan and Inceoglu (2003), changes in wind speed have affected the spore concentration significantly, especially when other climatic factors were optimal.

Airborne fungal spore concentration in enclosed environments depends on outdoor fungal concentration, the conditions of general hygiene, the microclimate, the occupancy and the use of the building (Medrela-Kuder 2003). In this study, airborne fungal concentration in CDCC Number One (404 CFU) was higher than in the other CDCCs. The sampling areas in CDCC number one were underground and smaller. Windows and ventilation were restricted, and the building was surrounded by vegetation, which is effective as a microfungal source. The lowest fungal concentration was in the CDCC Number Two. Although Number Two was situated at a very active place as an outdoor environment, the lowest number of microfungal colonies (96 CFU) was isolated from its indoor air. In this situation, we could say that the indoor environments of CDCC number two was not suitable for microfungal growth. Also, the positive correlations were found between indoor relative humidity measured during samplings with indoor air total fungi count and monthly total indoor air *Cladosporium* concentration in CDCC number two. There was no such correlation in the other CDCCs.

The number of microfungal colonies isolated from outdoor air in CDCC Number Three (318 CFU) and Number Four (316 CFU) was higher than the others. These stations were positioned at more active places compared to two other stations located at inner and closed regions. Hence, activity in environment, traffic, dust, and air currents might be responsible for the higher microfungal concentrations in these stations. Additionally, CDCC Number Three was surrounded by vast vegetation. While positive correlations were found between monthly average temperature and monthly total CFU counts in outdoor air of CDCC Numbers One, Three and Four, negative correlations were found between monthly wind speed and monthly total CFU counts in outdoors of the same CDCCs. This statistical data showed that an increase in temperature and a decrease in wind speed increased fungal concentrations in outdoor air of the CDCCs mentioned.

The highest microfungal concentration isolated from indoor air was in the eating room of CDCC Number One (162 CFU). In this CDCC, the eating room was used to prepare, cook, and serve up food in a different manner from the other CDCCs. Consequently, the increased temperature and relative humidity while the food was being cooked might provide more suitable conditions for fungal growth. Moreover, the dispersion of spores into the air from various substrates by air currents and human activities might be caused by processes carried out in this area. Spore release by air currents is likely the most prevalent mechanism for indoor fungi dispersal (Sivasubramani et al. 2004). The lowest microfungal concentration was in the eating room of CDCC Number Two. This room was used only for meal consumption for short periods and the ventilation was rather good. Therefore, this area was distant from contamination effects.

Comparing the findings of different studies on airborne microfungi is difficult. According to Adhikari et al. (2004), differences observed in the total and viable fungal spore concentrations in different studies depends on different climatic conditions, vegetations, and different fungal growth substrates. The sampling methods used by researchers are also of vital importance. In our study, The Petri Plate Exposure Method (also known as the gravity, open plate technique or settling plate method) was used to collect airborne fungi. The petri plates were held an average height of 50–80 cm between the ground and ceiling in indoor sampling areas while samplings were made at a height of 150 cm, which is the average breathing height for humans in the outdoor air. Petri plates were kept open

for a longer time outdoors than indoors to allow microfungi to make contact with the surface of the medium. This passive sampling technique is widely used due to its practical usage and low cost (Abdel Hameed et al. 2007a). Only viable fungi can be detected by this method; non-viable fungi cannot be counted. Additionally, factors such as spore size, shape, density and wind affect its reliability. As the sampling air volume cannot be known exactly, this method does not permit quantitative studies of the airborne fungal spore content. However, it does give a rough approximation and qualitative information about the types and concentrations of airborne fungi (Picco and Rodolfi 2000; Olonitola et al. 1994). In addition, the settling plate method was currently used for the above mentioned reasons but due to technological advances, has been replaced by better methods that permit us to determine exact counts of microorganisms present in a given amount of air.

Health effects caused by exposure to fungi and the role of fungal metabolites have been the focus of increased attention recently (Ren et al. 2001). *Aspergillus* and *Penicillium* genera are often associated with allergic symptoms in the respiratory system (Fischer and Dott 2003). In recent years, the role of indoor *Penicillium* as a cause of allergies in some people has been proven (Pitt 2000). Higher concentrations of *Cladosporium* and *Penicillium* indoor could cause allergic diseases (Fang et al. 2005). Su et al. (2001) and El-Morsy (2006) pointed out that microfungal genera that most commonly cause allergies are *Cladosporium, Alternaria, Aspergillus* and *Fusarium*. Exposure to *Alternaria* spores is an important agent for allergic rhinoconjunctivitis (Andersson et al. 2003). It was found that spores belonging to genus *Alternaria* were associated with severe asthma in the study carried out by Burch and Levetin (2002). Harmanci et al. (2000), indicated that *Cladosporium* and *Aspergillus* were the most common causes of allergy in a population study (adult patients with asthma and/or rhinitis) in Eskisehir, Turkey. According to Pastuszka et al. (2000), *Cladosporium, Alternaria* and *Aspergillus* are the main fungi to which children may be sensitive and by which allergic symptoms can be provoked. In our study, all the above mentioned microfungi genera were most prevalent and are likely to affect health of children in day care centers. The effects on human health of inhaled fungal spores depend not only on their concentration and composition, but also their size. While fungal spores larger than 10 μm, like *Alternaria* species, are deposited in the nose and pharynx, spores smaller than 5 μm in diameter are can penetrate the lungs and might lead to allergies and asthma (Fang et al. 2005). In particular, *Penicillium* and *Aspergillus* spores are smaller than 5 μm and can penetrate respiratory system barriers.

Mold contamination in buildings may cause "sick building syndrome" (SBS) including symptoms such as headaches; eye, nose, and throat irritation, a dry cough, dry or itchy skin, dizziness and nausea, difficulty in concentrating, fatigue, and sensitivity to odors (Handal et al. 2004; Cooley et al. 1998). Koskinen et al. (1995 and 1997) found that children in day care centers with mold problems have similar symptoms.

Some members of the genus *Aspergillus* may cause invasive aspergillosis when immunocompromised. *A. fumigatus* (most important agent for invasive aspergillosis), *A. flavus, A. niger, A. terreus* are among the most common species that cause invasive aspergillosis and were also picked up in our study (Kantarcioglu and Yucel 2003). *P. brevicompactum, P. chrysogenum, P. citrinum* isolated from human mycoses were among the *Penicillium* species found in our study. *P. chrysogenum* species may cause central nervous system infection, otomycoses, endophthalmitis, keratitis and endocarditis (Kantarcioglu et al. 2004).

In recent times, concerns have been raised about exposure to mycotoxin-producing fungi in indoor environments. Mycotoxins can easily become airborne and mobile in the adjacent air-stream. Mycotoxins contained in the spores are hazardous to humans and/or animals (Menetrez and Foarde 2004). Mycotoxin-producers common indoor fungi are some species of the *Aspergillus, Penicillium, Fusarium* genera and *Stachybotrys chartarum* species (Menetrez and Foarde 2004; Edmondson et al. 2005) as we found in our study. In addition to these genera and species, *A. alternata, A. flavus* and *A. versicolor* isolated in the present study are potential mycotoxin producers.

Conclusions

Indoor and outdoor viable fungal spore counts and compositions were determined at four child day care centers in our study. Many potential allergenic, pathogenic and mycotoxin-producers airborne microfungi were found in the indoor and outdoor air of the

child day care centers. *Cladosporium, Penicillium* and *Alternaria* as the most common aeroallergens were dominant in all the sampling sites. Filtration systems and appropriate air ventilation may ease this situation should this prove necessary. Humidity in the CDCCs must be controlled and filtration systems (especially high efficiency filters) should be used to prevent the ingress of mould spores, especially those coming from outdoor sources. Ventilation should also be adequate since poor ventilation together with water leaks cause increased humidity that result in increased fungal growth (Haverinen et al. 1999).

Monthly and seasonal differences were observed in the concentrations and distributions of indoor and outdoor airborne microfungi. Meteorological parameters showed positive and negative effects on airborne fungal counts.

Monitoring of airborne microfungi, of seasonal variations and the relationships with meteorological factors of their may provide a basis for future epidemiologic investigations of the role of fungal exposure as a risk factor for disease. In child day care centers, the investigations on fungi affecting children's health are important for prevention of fungal diseases and symptoms. Therefore, more clinical and epidemiological investigations must be undertaken.

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