Indoor air studies of fungi contamination in two kindergartens in Kavala, Greece

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A - Conception and study design, B - Data collection, C –Data analysis, D - Writing the paper,
E – Review article, F - Approval of the final version of the article

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ABSTRACT

Introduction: Fungi and moulds are biological hazards that are ubiquitous both in the communal and occupational environments. The aim of the study was to assess the presence of airborne fungi in two kindergartens in Kavala, Greece.

Materials and methods: Materials for the tests were: the air samples (in front of the buildings and the selected rooms) of the two kindergartens. The first Kavala kindergarten was located atop a hill and the second in the city center. The air pollution was determined using SAS SUPER 100.

Results: The mean number of fungal colonies isolated from air of the kindergarten rooms in the city center was 478.3±148.4 CFU/m^3. The mean number of fungal colonies isolated from air of the kindergarten on the hill was 343.6±188.8 CFU/m^3. *Aspergillus niger* was most frequently isolated in air samples from the kindergarten rooms in the city center, while *Penicillium species* predominated the kindergarten rooms on the hill. CFU values in the air samples outside the kindergartens were higher in the center than on the hill of Kavala.

CFU values of the examined air samples are varied. In the kindergarten rooms in the center of Kavala the most frequently isolated species was *Aspergillus niger*, and in the kindergarten on the hill it was *Penicillium species*.

Conclusions: The present study demonstrated considerable numbers of fungi in the air in two kindergartens in Kavala, Greece.

Key words: Indoor air fungi, kindergartens, SAS Super 100

DOI: 10.5604/01.3001.0010.1920

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Received: 08.03.2016
Accepted: 16.06.2016
Progress in Health Sciences
Vol. 6(1) 2016 pp 123-129

INTRODUCTION

Fungi and moulds are biological hazards that are ubiquitous both in the communal and occupational environments. Human exposure to airborne fungi in residential, occupational, and industrial settings has been shown to cause a variety of negative health effects. Numerous studies have demonstrated that onset of Sick Building Syndrome (SBS) could at least be partially owing to the exposure to the biological agents [1,2]. Common moulds such as *Alternaria, Aspergillus, Penicillium, Mucor, Drechslera, Cladosporium, Fusarium* and *Ulocladium,* which occur frequently in almost all the environments, are reported to cause various diseases like rhinitis, dermatitis and allergic asthma [3]. Moulds can pose a health hazard to adults and children.

In the literature [2,3], it is emphasized that all construction buildings create excellent conditions for the settlement, growth and reproduction of numerous and varied organisms.

It is estimated that several dozen species of bacteria can live in buildings (mainly Gram negative), more than 400 species of fungi (mainly *Aspergillus, Cladosporium, Penicillium, Fusarium* genus), several species of fungi causing decay processes of wood and wood-based materials, many species of algae, bryophytes, lichens, plant seeds, including decorative (e.g. *benjamin ficus, abutilon*), and over 30 species of mites (mainly in house dust), over 300 species of insects (posing parasitological and sanitation threats and destroying the structural wood of houses), several species of rodents, several species of birds (living on the roofs and external walls of buildings) and several species of bats [4,5].

There is now evidence that other consequences of exposure to spores of some fungi may be important. In particular, exposure to low molecular weight compounds retained in spores of some molds such as mycotoxins and β 1,3 glucans appears to contribute to some symptoms reported [6].

In many health effects research studies, children are considered as if they were small adults. This is not really true. There are many differences between children and adults in the ways that they respond to air pollution. For example, children take in more air per unit body weight at a given level of exertion than do adults. When a child is exercising at maximum levels, such as during a soccer game or other sports event, they may take in 20 percent to 50 percent more air - and more air pollution - than would an adult in comparable activity.

In the world, there are different fungi norms depending on the type of room.

According to Polish norm, (PN-89 Z-04111/03), in the atmospheric air number of detected fungi in 1 m³ can be: average clean, especially during spring, the total number of fungi in 1 m³ varies from 3000 to 5000; adverse effect for man with the detected number of fungi (5000 to 10, 000); threat for man, when the number of fungi is greater than 10,000. In Greece, there are no norms for fungal concentration indoor air. Studies of indoor bioaerosols conducted in Central and Eastern European countries, as a result of the scarcity of funding, mostly do not attain the level presented by similar studies in Northern America and Western Europe. The lack of reference limit values for bioaerosols seriously hinders interpretation of results obtained in various countries [7].

To our best knowledge, no articles have been published on the fungal air contamination of the kindergarten rooms in Greece.

The aim of the study was to assess the presence of airborne fungi in two kindergartens in Kavala, Greece.

MATERIALS AND METHODS

Air sampling was performed in two kindergardens in Kavala, Greece. The first kindergarten was located on the hill at an elevation of 129 meters above sea level. Seventy children attended kindergarten. This building was renovated in 2006-2007. The second building is located in the center of Kavala city at an elevation of 24 meters above sea level. Forty children attended kindergarten. Studies were performed in June.

Material into mycological studies was air sampled at the entrance of kindergarten buildings, and in the selected rooms. The monitoring of airborne fungi pollution was done using a SAS SUPER 100 (pbi international) with international measure standards (EN 50081-1, EN 500 50082-1). Sample has a flow rate of 100 liters air/min. At each site, a 100 liters sample was taken with the sampler placed at a height of 150 cm above floor level in the middle of the room, with all windows and doors closed. Plates from SAS SUPER 100 were incubated. After incubation number of fungal colonies and number of fungi in air volume was counted. In according to producer, at the first part of investigation number of fungal colonies at plates (real number of colonies - RNC) was corrected on statistical probability multiple passage of particle through the same hole (number of colonies corrected). In according to formula, it was estimated CFU (colony-forming unit - number of colonies at 1000 L of air): X = (P x 1000): V, where : V- volume of air sample, r – number of counted colonies at contact plate, P - corrected number of colonies (in according to producer instrument), X – number of colonies (CFU) at 1000 L (1 m^3) of air. Classification of isolated fungi was made with accordance to the current procedures. After incubation at 27°C for three days quantitative analysis and morphological evaluation of fungal colonies were carried out, and

depending on the nature of the fungi cultures the plates were incubated for up to 14 days to allow identification. The raw counts of colonies on the agar plates were adjusted by reference to statistical scaling tables applying to the particular sampler. The cultured fungi were identified from macroscopic and microscopic characteristics, and biochemical tests were appropriate. Yeast-like fungi were identified by means of original Candida ID (bioMerieux) medium, API 20C AUX (bioMerieux) identification sequence as well as CandiSelect (Bio-Rad) medium. For moulds, microscopical evaluation of morphological elements in the preparations was performed. Temperature and humidity were measured by thermo-hygrometer PWT-401 (Elmetron). Laboratory studies were performed in Poland.

The incidence of genus/species (indicator F) was calculated according to the formula:

$F = a / n \times 100\%$.

For which: F - frequency of species, a - the number of trials in which there was a strain, and n - number of attempts.

For moulds, a microscopical evaluation of the morphological elements used in preparations was performed. Temperature and humidity were measured using a thermo-hygrometer PWT-401 (Elmetron, Poland).

Wilocoxon's paired test and Speraman rank test were used. Significance was defined as a p value of 0.05.

RESULTS

Table 1 presents the fungal occurrence in the air of kindergarten rooms in the centre of Kavala. The mean number of fungal colonies isolated from air samples was 478.3±148.4, mean temperature: 27.3 ± 0.9°C, relative humidity: 55.2 ± 0.4, and air flow was 0.04 ± 0.02 m/s. No significant correlation (R=0.359; P=0.557) between CFU of fungi in the air and temperature was noted. Similarly, no relationship between CFU of fungi in the air and humidity (R=- 0.730; P=0.155), and as well as air flow (R=0.233; P=0.710) was found.

Table 1 shows the fungal occurrence in the air of kindergarten rooms on the hill of Kavala. The mean number of fungal colonies isolated from air samples was 343.6±188.8, mean temperature: 27.01 ± 1.3°C, relative humidity: 61.5. ± 3.2, and air flow was 0.02 ± 0.009 m/s.

No significant correlation (R=-0.023; p=0.955) between CFU of fungi in the air and temperature was noted. Similarly, no relationship between CFU of fungi in air and humidity (R= 0.012; P=0.967) and as well as air flow (R=- 0.577, P=0.133) was found. No significant differences were found in the CFU values, temperature, relative humidity, and air flow between kindergarten on the hill and in the city center (Table 1).

Table 1. Fungal occurrence, temperature, humidity and air flow in the air of the kindergartens in Kavala

Type of room	CFU/ 1 m^3 of air	Temperature	Humidity	Air flow
The city center of Kavala				
Cookhouse	430	27.2	55	0.07
Room I	300	27.2	55.5	0.02
Dinig room	290	27.7	55.7	0.02
Room II	670	28.2	55	0.02
Corridor I	610	27.6	55.5	0.06
Mean number	**478.3* ± 148.4**	**27.3* ± 0.9**	**55.2* ± 0.4**	**0.04* ± 0.02**
R=0.359 P=0.550 R-0.730, P=0.155, R=0,233; P=0.710				
The hill of of Kavala				
Corridor I	420	27.7	56.7	0.02
Room I	160	24.6	64	0.02
Bathroom	450	26.6	65.9	0.02
Cookhouse	380	26.9	61.4	0.02
Room II	110	29	57.4	0.03
Dinig room	320	26.6	61.4	0.02
Room III	560	27.1	61.2	0.01
Corridor II	350	27.6	63.9	0.01
Mean number	**343.6 ± 188.8**	**27.01 ± 1.3**	**61.5 ± 3.2**	**0.02 ± 0.009**
	R= - 0.023, P=0.955 R=-0.012, P=0.967 R=-0.577, P=0.133			

The following fungal pathogens isolated from air of the kindergarten rooms in the centre of Kavala were: *Acremonium strictum, Alternaria species, Aspergillus niger, Botrytis species, Candia albicans, Cladosporium species, Fusarium species and Penicillium species* (Table 2).

The following fungal pathogens isolated from air of the kindergarten rooms on the hill of

Kavala were: *A. strictum, A. species, A. niger, Botrytis species, C. albicans, Cladosporium species, Fusarium species and Penicillium species* (Table 2).

Table 2. Frequency of fungal occurrence, temperature, humidity and air flow in the kindergarten rooms of Kavala

Type of room/fungi	**The city center of Kavala**		**The hill of Kavala**	
	No	Type	No	Type
Bathroom	14	*Aspergillus niger*	16	*Penicillium sp.*
	10	*Penicillium sp.*	10	*Aspergillus niger*
	8	*Alternaria sp.*	5	*Alternaria sp.*
	7	*Candida albicans*	4	*Cladosporium sp.*
	6	*Cladosporium sp.*	3	*Acremonium strictum*
	6	*Botrytis sp.*	3	*Fusarium sp.*
	5	*Acremonium strictum*	2	*Botrytis sp.*
	1	*Fusarium sp.*	2	*Candida albicans*
Cookhouse	13	*Penicillium sp.*	11	*Penicillium sp.*
	12	*Aspergillus niger*	9	*Aspergillus niger*
	9	*Alternaria sp.*	8	*Cladosporium sp.*
	4	*Cladosporium sp.*	6	*Alternaria sp.*
	3	*Fusarium sp.*	4	*Botrytis sp.*
	2	*Acremonium strictum*		
Room I	10	*Penicillium sp.*	5	*Alternaria sp.*
	8	*Aspergillus niger*	4	*Cladosporium sp.*
	6	*Cladosporium sp.*	2	*Penicillium sp.*
	5	*Alternaria sp.*		
	1	*Botrytis sp.*		
Dinig room	16	*Penicillium sp.*	12	*Penicillium sp.*
	5	*Aspergillus niger*	11	*Aspergillus niger*
	4	*Cladosporium sp.*	5	*Alternaria sp.*
	3	*Alternaria sp.*	4	*Cladosporium sp.*
	3	*Fusarium sp.*		
	1	*Candida albicans*		
Room II	11	*Penicillium sp.*	18	*Aspergillus niger*
	10	*Cladosporium sp.*	11	*Penicillium sp.*
	8	*Alternaria sp.*	10	*Acremonium strictum*
	5	*Candida abicans*	6	*Alternaria sp.*
	3	*Botrytis sp.*	5	*Fusarium sp.*
	3	*Acremonium strictum*	4	*Cladosporium sp.*
			2	*Botrytis sp.*
Room III			4	*Penicillium sp.*
			4	*Cladosporium sp.*
			3	*Aspergillus niger*
			2	*Fusarium sp.*
			2	*Alternaria sp.*
			1	*Botrytis sp.*
Corridor I	18	*Penicillium sp.*	12	*Penicillium sp.*
	16	*Aspergillus niger*	10	*Aspergillus niger*
	10	*Cladosporium sp.*	6	*Cladosporium sp.*
	8	*Alternaria sp.*	5	*Alternaria sp.*
	4	*Acremonium strictum*	4	*Acremonium strictum*
	3	*Fusarium sp.*	3	*Fusarium sp.*
	2	*Botrytis sp.*	2	*Botrytis sp.*
Corridor II			14	*Penicillium sp.*
			12	*Aspergillus niger*
			6	*Alternaria sp.*
			5	*Cladosporium sp.*
			2	*Fusarium sp.*

In the kindergarten rooms in the center of Kavala, *A. niger* was most frequently isolated (20.9%), and from the kindergarten rooms on the hill of Kavala the most frequent was *P. species* (31.2%). (Table 3)

Table 3. Occurrence of genera/species of fungi types isolated from the kindergarten rooms of Kavala

	Fungi types							
	Aspergillus niger	***Penicilliu m sp.***	***Alternaria sp.***	***Candida albicans***	***Cladosporium sp.***	***Botrytis sp.***	***Acremoniu m strictum***	***Fusarium sp.***
Fungal occurrence	Kindergarten rooms in the centre of Kavala							
	20.9%	29.7%	15.6%	4.9%	15.2%	4.2%	5.3%	3.8%
	Kindergarten rooms on the hill of Kavala							
	27.8%	31.2%	15.2%	0.8%	14.0%	4.2%	6.5%	5.8%

In the air samples, from the kindergarten rooms in the center of city, *Fusarium species* was more rarely isolated (3.8%), and from the kindergarten rooms on the hill the most less was *Candida albicans* (0.8%). Table 4 presents the fungal incidence in the air of outside the kindergartens in Kavala, Greece. Higher values of CFU/m^3 were found outdoor kindergarten in the center of Kavala than on the hill of Kavala. Similarly, a higher temperature value was detected in the center of city than on the hill. Details are shown in Table 4.

Table 4. Fungal concentration, temperature, humidity and air flow in the air outside kindergartens of Kavala

Outside	**CFU/ 1 m^3 of air**	**Temperature**	**Humidity**	**Air flow**
Kindergarten in the centre of Kavala	570	29.1	56.1	0.1
Kindergarten on the hill of Kavala	330	27	65.5	0.04

A total of 5 genera/species were isolated in air samples collected outside kindergarten in the centre of Kavala. And, *Penicillium species* was more frequently isolated. A total of 4 genera/species were isolated in air samples collected outside kindergarten on the hill of Kavala. And, *Alternaria sp*. was more frequently isolated. Details are not shown.

DISCUSSION

In the present study, the authors demonstrated considerable numbers of fungi in the air in two kindergartnes in Kavala, Greece. The following fungal pathogens isolated from air were: *Asperegillus species, Penicillium species Candida albicans, Candida species, and Cladosporium sp.*. The study was performed in June. In the literature there is clear evidence of seasonal differences in the numbers of fungi in indoor air [8,9].

The typical fungal genera investigated are *Cladosporium***,** *Alternaria***,** *Aspergillus* and *Penicillium***,** probably because they are very often the most prevalent genera in ambient air [10-11]. In the present study, these fungi were also dominant genera in indoor and outdoor air.

The present findings are consistent with the previous report of Raisi et al. [12]. They examined the distribution of bacteria and fungi in air during April, May and June 2008 in a suburban, city of Chania (Crete, Greece). The mean number of fungal colonies isolated from air samples was 395 ± 338 CFU/m^3. In the present study, the fungal concentrations were comparable (478.3±148.4 CFU/m^3).

Climate and human activities are the main factors that influence the composition of outdoor atmosphere. In the temperate climates, these display, a typical pattern around the year. On the contrary, climate is not determinative in the mycoflora of the indoor atmosphere, but human activities and the quality and maintenance of the building do play a major role in these environments. For these reasons, dominant fungi indoors vary between buildings and can be used as monitors of indoor air quality [13]. In the present study, isolation of fungi for air was performed in summer. It would interesting to perform tests in other seasons.

The quality of the air in buildings is an important determinant of human health and well-being. The inadequate control of indoor air quality, therefore creates a considerable health burden [14].

Biological agents of relevance to health are widely heterogeneous, ranging from pollen and

spores of plants (mainly from outdoors), to bacteria, ungi, algae and some protozoa emitted outdoors or indoors [15].

Although the literature on biological contaminants in indoor air is vast, there is no universal agreement on the precise meaning of the terms used to describe the (micro-) environmental conditions determining their presence and proliferation.

There are few studies about indoor fungi in the kindergartens [16,17].

Karowska [16] performed research in a kindergarten room in classrooms of school in winter in Warsaw, Poland. The temperature of atmospheric air was about 1^0C and temperature indoors - 18-21.5^{o}C. Relative humidity of the air was 46-56%, depending on sampling place. The highest level of bacteriological contamination was detected in the corridor and in rooms during lessons. After the lessons a number of microorganisms were much lower.Among isolated from the air strains from genus *Aspergillus* (*A. ochraceus, A. flavipes, A. nidulans, A terreus and A. niger*) and *Penicillium* (*P. notatum, P. brevicompactum, P. implicatum*) predominated.

Vaattovaara and co-workers [17] evaluated risks associated with diaper changing in Finnish kindergartens. They determined enteric micro-organisms and ammonia in diaper-changing rooms in four kindergartens in autumn and winter in the ambient air. No coliphages were detected in the air. The numbers of faecal coliforms and enterococci in air were typically low regardless of whether the children used either paper or cloth diapers. Ammonia concentrations increased over the background level because of diaper changing.

Fungal growth can occur only in the presence of moisture, and many fungi grow readily on any surface that becomes wet or moistened; that is, virtually all fungi readily germinate and grow on substrates in equilibrium with a relative humidity below saturation (i.e. below 100%) [15]. In the present study, humidity ranged between 56% and 65%.

Many fungal species produce type I allergens, and immunoglobulin (Ig)E sensitization to the commonest outdoor and indoor fungal species, like *Alternaria, Penicillium, Aspergillus* and *Cladosporium spp*, is strongly associated with allergic respiratory disease, allergic rhinitis, sinusitis, and especially asthma. Fungi are also well-known sources of type III (or IgG-inducing) allergens. The species involved include many common genera such as *Penicillium* and *Aspergillus*, which can be found in most houses. At high concentrations, fungi may also be involved in combined type III and IV allergic reactions, including hypersensitivity pneumonitis [18]. Thus, fungi indoor air monitoring is very important in kindergartens.

Aerobiological sampling in Thesaoliniki, Greece was conducted over 1996–2002, using a Burkard trap [19]. Records of 18 meteorological parameters were used for the same period Significant variations in fungal spore atmospheric levels over time were found for both taxa. The time series of *Alternaria* spore counts was highly seasonal and slightly trended, whereas that of *Cladosporium* was seasonal but stationary. *Alternaria* seems to have a direct relationship with minimum air temperature, compared with *Cladosporium* which was affected by solar radiation

So, the quality of the air children breathe should be tested but in practice, it is performed very rarely.

Up to now in Poland and Greece have been no standard regulations concerning permitted levels of fungal contaminants in indoor air. It is suggested, the total number of fungi should be lower than 200 CFU/m^3. In the present research, the fungal concentrations in the kindergartens rooms were exceeded more than 100%. For example (Room I 300CFU/m^3, Room II 670 CFU/m^3).

Molds can be found almost anywhere; they can grow on virtually any organic substance, as long as moisture and oxygen are present. There are molds that can grow on wood, paper, carpet, foods, and insulation. When excessive moisture accumulates in buildings or on building materials, mold growth will often occur, particularly if the moisture problem remains undiscovered or unaddressed. Some building materials, such as dry wall with vinyl wallpaper over it or wood paneling, may act as vapor barriers, trapping moisture underneath their surfaces and thereby providing a moist environment where mold can grow [20].

In general, results of the current study demonstrated considerable numbers of fungi in the air in two kindergartens in Kavala, Greece. The following fungal pathogens isolated from air were: *Aspergillus species, Penicillium species Candida albicans, Candida sp, and Cladosporium sp.*. These fungi are involved in the etiology of fungal disease and allergies. Further studies are needed to determine the seasonal variations of the fungal air contamination in the kindergartens

Conflicts of interests

The authors declare that they have no competing interests.

CONCLUSIONS

1. CFU values of the examined air in the kindergarten rooms ranged between 100 and 670.
2. In the kindergarten rooms in the center of Kavala the most frequently isolated species was *Aspergillus niger*, and in the kindergarten on the hill it was *Penicillium species*.

3. CFU values in the air samples outside the kindergartens were higher in the center than on the hill of Kavala.
4. No significant correlations between CFU and temperature, humidity, and air flow were found.

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