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Indoor Aeromycological Study in Manisa, Turkey

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Abstract: In this study, a systematical survey on the indoor airborne fungi was carried out for one year in Manisa. Fungal samples were collected in each month with Merck MAS100 air sampler from four sampling sites. Fungal concentrations were reported as colony forming units per cubic meter of air using the MAS100 conversion unit table. Results showed that the average fungal concentration is 320 colony forming units cfu m⁻³. Twenty one species belonging to 7 genera were identified according to their microscopic, macromorphological properties and through references. *Penicillium*, with the most abundant genera, which comprised more than 38 of the total isolated fungal species. *Cladosporium* were the most dominant fungal group, followed by *Penicillium*, *Aspergillus* and *Alternaria*.

Key words: Airborne fungi, indoor air, bioaerosol

INTRODUCTION

Airborne fungi are among the most common organisms in nature. They were considered to be correlated with air pollution and were proposed as a cause of adverse health effects on humans, animals and plants (Bush and Portnoy, 2001; Shelton *et al.*, 2002). Elevated levels of particle air pollution were associated with a decreased lung function, increased respiratory symptoms such as coughing, shortness of breath, wheezing and asthma attacks, as well as chronic obstructive pulmonary disease, cardiovascular disease and lung cancer (Hargreaves *et al.*, 2003; Fang *et al.*, 2005). More than 80 genera of fungi were reported to be associated with symptoms of respiratory tract allergies (Horner *et al.*, 1995; Colakoglu, 2004) and over 100 species of fungi were involved with serious human and animal infections, while many other species caused serious plant diseases (Cvetnic and Pepelnjak, 1997). *Alternaria*, *Cladosporium*, *Aspergillus*, *Penicillium* and *Fusarium* were amongst the most common allergenic genera. For example, elevated concentrations of *Cladosporium* were usually associated with respiratory symptoms (Su *et al.*, 2002). Similarly, higher concentrations of *Cladosporium* and *Penicillium* could cause allergenic diseases (Gelincik *et al.*, 2005).

For determination of their adverse effects on the human health, many studies were carried out about the fungal community both in outdoor and indoor environments. It was reported that the dominant fungal genera were *Penicillium*, *Aspergillus*, *Alternaria* and *Cladosporium* in the atmosphere (Singh, 2001; Huang *et al.*, 2002; Shelton *et al.*, 2002; Hargreaves *et al.*, 2003; Adhikari *et al.*, 2004; Asan *et al.*, 2004) and their concentrations differed from place to place because of local environmental conditions such as temperature and humidity, fungal substrates and human activities (Shelton *et al.*, 2002). However, little is known about the species, number and distribution of indoor airborne fungi in Manisa. Therefore, it is indispensable to survey on concentration, distribution and species of airborne fungi systematically and extensively across different research sites in Manisa. Four sampling sites in different functional areas were selected for the research on community structure and dynamic change of indoor fungi in Manisa city. The objectives of this study were to describe the groups and concentrations of indoor airborne fungi in Manisa.

MATERIALS AND METHODS

Sampling Sites

The city of Manisa, (250,000 inhabitants) in the Ege Region of Turkey's west. The average altitude of city is 74 m. The climate is continental and annual mean temperature is 17.28°C and annual mean relative humidity is 67.63% and annual mean rainfall is 569.5 mm. The coldest month is January but the average temperature does not fall below 6.2°C. The hottest months are July and August when the average temperature is 28.3°C. The most precipitation takes place in the winter and the least in summer. The average number of the snowy days is less than 5 days in a year. The direction of the dominant wind is mostly S or SE. Climatic data was obtained from the Department of Meteorology, Manisa.

Four buildings were selected for the study in Manisa (Table 1). This buildings vary according to age, construction, size and number of persons who using for various purpose. Hygiene conditions at these sites were average. All buildings were constructed of concrete. However, floor coverings in each site varied; carpet covered the floors in station one and four, mosaic tiles were used in station two and three. All research stations were situated on different floors in each building. All the buildings were equipped with a central heating system. In all buildings, windows provided the primary source of building ventilation.

Sampling, Isolation and Identification of Fungi

Indoor air samples were collected from the four research stations monthly between January and December 2005. Fungi were sampled by the using Merck MAS 100 air sampler and this sampler operated at 50 L air min⁻¹ (Gelincik *et al.*, 2005). The samples were taken in the morning (08:00-10:00) at 1.50 m above ground level when the weather was stable and dry (Asan *et al.*, 2004; Gelincik *et al.*, 2005). The sampling time was always at the same time of the day. Rosebengal Chloramphenicol Agar was used to collect fungal airborne propagules (Asan *et al.*, 2004; Colakoglu, 2004). The sampling period was 1 min with an airflow rate of 50 L min⁻¹ and three Petri dishes used each station. In total, 144 samples taken from the indoor air in research period. After exposure, the sampled agar plates were incubated at 27°C and examined after 7-10 days after which the resulting colonies were counted (Colakoglu, 2004; Okten *et al.*, 2005).

Each colony of fungi was inoculated onto Malt Ekstrakt Agar (MEA), Potato Dextrose Agar (PDA), Czapek Dox Agar (CZ), Czapek Yeast Agar (CYA) and Gliserol Nitrate Agar (G25N) media for identification and incubated at 27°C for a period of seven days after which colony diameters were measured. Petri plates were first examined under the dissecting microscope (stereomicroscope) and then under a high resolution light microscope to determine the colonial features and the morphological structures of the fungi. The determination of the morphological structures was carried out on material mounted in lactophenol (Asan *et al.*, 2004; Colakoglu, 2004; Gelincik *et al.*, 2005).

Fungi were identified to genus level using Barnett (1960). Cultures were identified to species level according to various mycological references (Raper and Thorn, 1949; Raper and Fennel, 1965; Pitt, 1979, 2000; Domsch *et al.*, 1980; Samson and Pitt, 2000; Klich, 2002; Samson *et al.*, 2004).

Table 1: Some features of the selected research stations

No.	Name	Locality coordinates	Average used persons (monthly)
1	An apartment flat	38°36'51.41"N/27°25'35.27"E	10
2	Large railway station waiting hall	38°37'13.23"N/27°26'2.42"E	10000
3	Faculty of medicine dining hall	38°36'30.52"N/27°22'31.22"E	6000
4	Faculty of science lecture room	38°40'35.28"N/27°18'42.42"E	2000

Statistical Analysis

All the experimental data were analyzed using SPSS Version 10.0 (SPSS, Standard Version) for one-way analysis of variance (ANOVA).

RESULTS AND DISCUSSION

Classified according to the four different districts of Manisa where the sampling was done. During the one year period of the study, 144 samples were collected using Merck MAS 100 air sampler and fungi were isolated from these samples (Table 2). These were cultured to a total of 2304 colonies. Identification of the fungi isolated showed 7 genera, 21 species from the indoor air had been collected. Also the average culturable fungal concentration is 320 cfu m⁻³ in Manisa air.

The results showed that the most widespread genus was *Cladosporium* followed by *Penicillium*, *Aspergillus* and *Alternaria*. The species *Cladosporium herbarum* that was generally found as the predominant fungus. *Penicillium* species more than 38% of the total isolated fungal species (Table 2). Other genera isolated (*Rhizopus*, *Mucor* and *Geotrichum*) (Table 2) were found in accordance with reported literature (Adhikari *et al.*, 2004; Asan *et al.*, 2004; Colakoglu, 2004; Fang *et al.*, 2005; Gelincik *et al.*, 2005; Okten *et al.*, 2005).

High fungal spore densities were observed in November, October and September (490, 465 and 455 cfu m⁻³, respectively) and the months in which least fungi were observed were January, March and April (105, 140 and 190 cfu m⁻³, respectively).

Statistical analysis of the data showed that, there was a positive correlation between the some research stations by fungal spore density and fungal species (Table 3).

Table 2: Fungal species which identified in this research and the sampling sites

Fungal species	Sampling sites			
	1	2	3	4
<i>Alternaria alternata</i> (Fr.) Keissl.*	+	+	+	+
<i>Alternaria tenuissima</i> (Kunze) Wiltshire	-	+	+	-
<i>Aspergillus flavus</i> Link.*	+	+	+	-
<i>Aspergillus foetidus</i> Thom and raper	-	+	+	-
<i>Aspergillus fumigatus</i> Fresen.*	+	+	+	-
<i>Aspergillus niger</i> var. <i>niger</i> Tiegh.*	+	+	+	+
<i>Aspergillus terreus</i> Thom	+	+	+	+
<i>Cladosporium herbarum</i> (Pers.) Link*	+	+	+	+
<i>Cladosporium oxysporum</i> Berk. and curtis*	+	+	+	+
<i>Geotrichum candidum</i> Link*	-	+	+	+
<i>Mucor racemosus</i> Fresen.*	-	+	+	-
<i>Penicillium chrysogenum</i> Thom*	+	+	+	+
<i>Penicillium citrinum</i> Thom*	+	+	+	+
<i>Penicillium funiculosum</i> Thom	+	+	+	-
<i>Penicillium glabrum</i> (Wehmer) Westling	+	+	+	+
<i>Penicillium italicum</i> Wehmer	+	+	+	+
<i>Penicillium purpurogenum</i> Stoll	+	+	+	+
<i>Penicillium verrucosum</i> Dierckx	+	+	+	+
<i>Penicillium verruculosum</i> Peyronel	+	+	-	+
<i>Rhizopus oryzae</i> Went and Prins. Geerl.	+	+	-	+
<i>Rhizopus stolonifer</i> var. <i>stolonifer</i> (Ehrenb.) Vuill.	+	-	-	-

*Fungi which are known allergens. +: Positive, -: Negative

Table 3: Correlations between sampling sites by fungal species in relation to their spore density

Sampling sites		1	2	3
2	r	0.686		
	p	0.014		
3	r	0.834*	0.645	
	p	0.001	0.024	
4	r	0.720	0.597	0.678
	p	0.008	0.040	0.015

*Correlation is statistically significant at the 0.01 level

CONCLUSION

Many of the species of fungi found in indoor air in this study are known to cause allergy. Examples are *Alternaria alternata*, *Cladosporium herbarum*, *Aspergillus flavus*, *A. fumigatus*, *A. niger*, *Geotrichum candidum*, *Mucor racemosus*, *P. chrysogenum* and *P. citrinum* (Colakoglu, 2004; Gelincik *et al.*, 2005) (Table 2). Also, some fungal species are known to producing some mycotoxins. These are *Alternaria alternata*, *A. tenuissima*, *Aspergillus flavus*, *A. fumigatus*, *A. niger*, *A. terreus*, *P. chrysogenum*, *P. citrinum* and *P. verrucosum* (Picco and Rodolfi, 2000; Sen and Asan, 2001; Su *et al.*, 2002; Singh, 2005) (Table 2). This study has shown that fungi which may cause diseases of the respiratory system and producing mycotoxins were encountered frequently in the research areas.

Manisa with its climatic conditions presents a convenient habitat for the reproduction of fungi. As a result, there must be a high percentage of airborne fungal spores which can cause allergy (Colakoglu, 2004) and asthma (Gelincik *et al.*, 2005). Isolating and identifying the type and density of fungal spores and where they are most commonly found will be a useful contribution to the community health services. Details of the distribution of them in the different districts could be of assistance for the treatment of pertinent diseases.

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