http://www.trakya.edu.tr/Enstituler/FenBilimleri/fenbilder/index.php
Trakya Univ J Sci, 6(1): 97-106, 2005
ISSN 1302 647X
DIC: 159SSOET65057705

Research Article/Araştırma Makalesi

AIRBORNE FUNGAL CONCENTRATIONS IN EAST PATCH OF EDIRNE CITY (TURKEY) IN AUTUMN USING TWO SAMPLING METHODS

Suzan SARICA OKTEN[1*], Ahmet ASAN[1], Yasemin TUNGAN[1], Mevlut TURE[2]
[1]Trakya University Faculty of Arts and Sciences Department of Biology TR-22030 Edirne – Turkey.
[2]Trakya University Medical Faculty Department of Biostatistics TR-22030 Edirne – Turkey.
*Corresponding Author: Tel.: +90 284 2352825-1145, Fax: +90 284 2354010, e-mail: suzanokten@hotmail.com

Received : 10.06.2004
Accepted : 07.10.2004

Abstract: Fungal spore concentrations vary during day and night. Dry spore types are released during the day, whereas wet spores that have more humidity ratio are released into the air at night. Studies on airborne fungi in Turkey are very rare, and they are daily and weekly monitored only in some cities. The aim of our study was to determine the daily fungal concentrations in Edirne, Turkey. All samples were taken from a level of 11 m. above ground. The sampling time was carried out bihourly in the day, from 8 am to 20 pm, once a week from October 11 to November 15, 2002. Two sampling methods were used for the isolation of fungi: A gravitational and a volumetric method. Statistical analysis was performed in order to determine the relationship between some environmental factors and airborne counts and some correlation were found between the fungal numbers, relative humidity, sampling hours and temperature. A total of 800 fungal colonies in 42 petri dishes were isolated, quantified to determine the frequency of occurrence and then identified as genus level. 9337 cfu/m^3 microfungi were determined by volumetric sampling. Seven fungal genera could be determined, among them *Cladosporium* and *Alternaria* were found as the most predominant fungi, followed by *Penicillium* and *Trichoderma*.

Key words: Airborne fungi, airspora, biomass, diurnal differences, gravitational sampling volumetric sampling

Sonbahar Mevsiminde İki Örnekleme Metodu Kullanarak Edirne İlinin Doğusunda Havayla Taşınan Fungal Konsantrasyonların Belirlenmesi

Özet: Gece ve gündüz saatlerinde fungal konsantrasyonlar değişiklik gösterebilir. Kuru sporlar havaya gündüz salınırken nem oranı daha yüksek olan sporlar gece salınmaktadırlar. Türkiye'de havayla taşınan funguslar ile ilgili çalışmalar çok azdır ve yalnızca bazı şehirlerde günlük ve haftalık incelemeler yapılmıştır. Bu çalışmada Edirne şehri günlük fungal konsantrasyonlarının belirlenmesi amaçlanmıştır. Örnekler yerden 11 m yükseklikten alınmıştır. Örneklemeler 11 Ekim 2002 – 15 Kasım 2002 tarihleri arasında, hergün aynı saatlerde olmak üzere birer haftalık zaman dilimlerinde sabah 08.00 ve akşam 20.00'de yapılmıştır. Fungus izolasyonu için yerçekimine dayalı petri plak metodu ve volumetrik örnekleme metodu kullanılmıştır. Sıcaklık ve nısbi nem iklimsel faktörlerin fungal sporları etkileyip etkilemedikleri istatistiki olarak incelenmiş ve fungus sayıları, nısbi nem ve sıcaklık arasında korelasyon bulunmuştur. 42 petri plağına izole edilen toplam 800 fungus kolonisi bulunma sıklıkları belirlenerek cins düzeyinde tanımlamaları yapılmıştır. Volumetrik metot ile m^3 de 9337 cfu mikrofungus belirlenmiştir. En baskınları *Alternaria, Penicillium* ve *Trichoderma* olan 7 fungus cinsi teşhis edilebilmiştir.

Anahtar kelimeler: Havayla taşınan funguslar, fungal konsantrasyon değişiklikleri, volumetrik metod

Introduction

Concentration and types of the fungal spores change in 24-h period in the atmosphere. Many factors such as wind, temperature, humidity, season, precipitation influence fungal spores in air. In addition, method for collection and analysis, type of culture medium, time of day, type of vegetation and soil may influence presence

and distributions of airborne fungi (Nussbaum, 1991; Wang et al, 2001, Pepeljnjak and Segvic, 2003). Common allergenic molds have dry spores, so they release their spores to the atmosphere in dry and windy weather. But, some fungi need more humidity or rain to release their spores into the air. Fungal spore concentrations may vary during day and night. While dry spore types are released into the air during the day, spores that have more humidity ratio are released to the air at night (source: www.healthieryou.com/air.html). Dry and wet spore types are well known. Dry air-spora occur in genera such as *Cladosporium*, *Alternaria*, *Epicoccum*, *Drechslera*, *Pithomyces*, and *Curvularia* (Troutt and Levetin, 2001).

Studies on airborne fungi in Turkey are very rare. These studies were performed only in some cities such as Istanbul (Colakoglu 1996a, b, c and 2003; Asan et al., 2003a), Edirne (Sen and Asan, 2001; Asan et al., 2002; Sarica et al., 2002), Bursa (Simsekli et al., 1999), Ankara (Sakiyan and Inceoglu, 2003; Okuyan et al., 1976), Eskisehir (Asan et al., 2004, Atik and Tamer, 1994) and Izmir (Ayata et al., 1991). Of these, concerning Edirne, the monthly fungal biotas of different localities of the city were revealed. However, the daily and intradiurnal concentrations of Edirne city have not been studied up to now since the above mentioned studies didn't include this criterion. So, objective of our study was determination of intradiurnal fungal concentrations in Edirne city, Turkey. The relationships among fungal spore numbers and some meteorological factors such as temperature and relative humidity were examined using statistical analysis.

Materials and Methods

Sampling Site

The city of Edirne (115,000 inhabitants) is situated in the northwest region of Turkey and has borders with Bulgaria and Greece. The climate is continental and annual mean temperature is 13.5 °C, and annual mean relative humidity is 69.7 %, and annual mean rainfall is 584.3 mm. Research station is Trakya University Campus, rare natural forest and has different trees (*Cerasus vulgaris*, *Ficus carica*, *Morus alba*, *Populus alba*, *P. canadensis*, *Pyrus communis*, *Rosa canina*, and shrubs) and agricultural area (*Triticum vulgaris* and *Helianthus annus fields are common*), vegetation is rich. Site is exposed to wind, there is University Arboretum in the east. Also there are different trees in mentioned arboretum such as *Ulmus minor*, *Salix alba, Robinia pseudoacacia,* and *Populus nigra.* Residential area is far away 4 km from the sampling site.

Sampling and Isolation Methods

Sampling time was performed bi-hourly always at the same time of the day between 08.00 am and 20.00 pm, at a one week interval during 6 weeks (from October 11 to November 15, 2002). The amount of samples is thus 42 per sampling method.

The fungal culture method (= Petri plate gravitational or Settle plate method) (PPM) (Ismail et al., 1999; Martinez Ordaz et al., 2002) and sampler based volumetric sampling (Made in Turkey) (*Volumetric sampler was used first time by Andersen, 1958*) were used for the isolation of fungi. Rose Bengal streptomycin agar medium (Sigma Chemical Co., USA) was used for isolation of fungi from air. Two petri dishes were used for one sampling for PPM and the other for volumetric method (VM). After incubation at 27°C±1, concentrations of airborne fungi were calculated as CFU (Colony forming units) (CFU/plate/15 min.) and CFU/m^3.

Each colony of fungi was inoculated to malt extract agar (MEA) (Merck, Germany), czapek-dox agar (CZ) (Merck, Germany) and potato dextrose agar (PDA) (Difco, USA) media for identification at genus level and incubated at room temperature (27°C±1) for a period of 7 days. Petri plates were first examined under a dissecting microscope (= stereomicroscope). Then we used a high resolution microscope (= light microscope) to determine the colonial features and morphological structures of the fungi. The determination of the morphological structures of fungi was carried out on material mounted in a modified mounting medium, Lacto-Cotton Blue, proposed by Sime et al. (2002).

Meteorological Data

We measured temperature and relative humidity of air using a thermometer and hygrometer device (*TFA-Dostmann GmbH, Germany*) in the study site (Table 1).

Table 1. Sampling hours and meteorlogical data.

	Relative humidity (%) / Temperature (°C)						Relative humidity (%) / Temperature (°C)					
Sampling hour	**OCT I**	**OCT II**	**OCT III**	**OCT I**	**OCT II**	**OCT III**	**NOV I**	**NOV II**	**NOV III**	**NOV I**	**NOV II**	**NOV III**
8 00	87	92	75	18	14	10	70	70	80	19	07	13
10 00	83	85	71	20	16	13	69	65	78	11	08	17
12 00	77	84	59	22	18	15	67	60	78	14	11	17
14 00	78	83	54	24	20	17	67	53	77	14	14	19
16 00	81	78	56	23	21	17	67	53	80	14	14	19
18 00	84	83	65	21	20	16	71	58	89	13	12	15
20 00	85	85	74	20	18	15	75	60	89	11	09	14

OCT I : October 11, 2002, OCT II: October 18, 2002, OCT III: October 25, 2002, NOV I : November 01, 2002, NOV II : November 12, 2002. NOV III: November 15, 2002

Statistical Analysis

We used a Kolmogorov-Smirnov test for the fitness of the variables to normal distribution. Following the results of this statistical analysis, a Pearson Correlation analysis was performed to reveal the relationships between temperature, humidity, used sampling methods and sampling times.

Identification

Many different culture media were used for identification of microfungi. Fungal genera were identified based on micro- and macro-morphology, reverse and surface coloration of colonies grown on CZ, MEA and PDA media. Fungi were identified to genus level using Barnett and Hunter's work (1999) and some mycological references (Pitt, 1979; Raper and Fennell, 1965; Klich, 2002; Ellis, 1971; Ellis and Ellis, 1997; Nelson et al., 1983 and Samson et al., 2002)

Results

A total of 800 fungal colonies in 42 petri dishes were isolated, quantified to determine the frequency of occurrence and then identified as genus level by PPM. Also 9337 cfu/m^3 microfungi were determined by VM. Seven fungal genera could be determined, among them *Cladosporium* and *Alternaria* genera generally found the most predominant fungi (for *Cladosporium*: in VM: 40.7 %, in PPM: 32.9 % and for *Alternaria*: 21.4 % and 32.4 %, respectively), followed by *Penicillium* and *Trichoderma* (in VM: 8.6 %, in PPM: 1.9 % and 1.4 % and 0.1 %, respectively) (Table 2, Figure 1). We sampled fungi from air totally 42 times during the 35 days. *Alternaria* species determined 15 times in VM (15 of 42) and 37 times in PPM (37 of 42)*;* these numbers are 14 and 35 for *Cladosporium* species.

Table 2. Total Colony numbers and percentages of genera determined between October 11 - November 15, 2002.

	C		Al		P		X		As		F		T		R		U	
	VM	PPM	VM	PPM	VM	PPM	VM	PPM	VM	PPM	VM	PPM	VM	PPM	VM	PPM	VM	PPM
Total number	3801	263	2001	267	800	15	601	111	67	16	-	5	134	1	-	8	1933	110
Total Percentages	40.7	32.9	21.4	32.4	8.6	1.9	6.4	13.9	0.7	2	-	0.6	1.4	0.1	-	1	20.7	13.4

All number of colonies: 9337-800

Letters indicate: **C**: *Cladosporium*, **Al**: *Alternaria*, **P**: *Penicillium*, **X**: Refers to the unidentified specimens which were found to belong Dematiaceae, **As**: *Aspergillus*, **F***: Fusarium*, **T**: *Trichoderma*, **R**: *Rhizopus*, **U**: Unidentified; **V**: VM (cfu/m^3), **PPM**: Petri plate gravitational settling method (cfu/plate/15 min.)

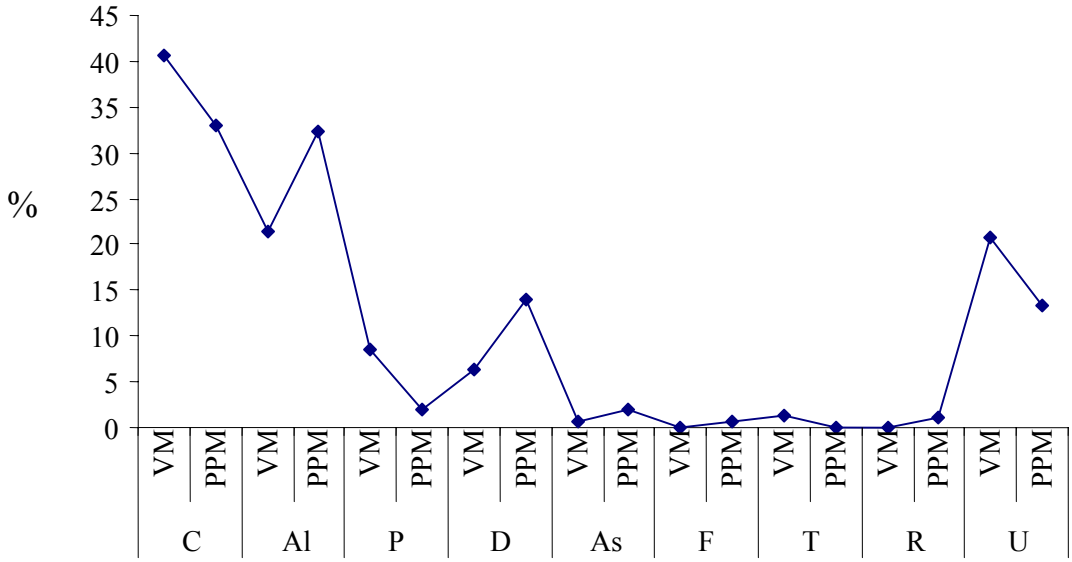

Figure 1. Total percentages of genera determined between the October 11 - November 15, 2002.

Letters indicate: **C**: *Cladosporium*, **Al**: *Alternaria*, **P**: *Penicillium*, **X**: Refers to the unidentified specimens which were found to belong Dematiaceae, **As**: *Aspergillus*, **F**: *Fusarium*, **T**: *Trichoderma*, **R**: *Rhizopus*, **U**: Unidentified; **V**: VM (cfu/m^3), **PPM**: Petri plate gravitational settling method (cfu/plate/15 min.)

During the 35 days of sampling time, the maximum concentration of airborne fungi was found in the first week (in VM: 56.4 %, in PPM: 32.8 %), followed by second week (in VM: 20.1 %, in PPM: 29.4 %). The minor peak week for microfungi were observed in 5th week (in VM: 3.6 %, in PPM: 3.3 %). The number of fungal colonies on culture media plates was between 0 to maximum count 25 cfu/plate/15 min; these numbers are 0 and 867 cfu/m^3 for VM. The daily concentrations ranged from 333 cfu/m^3 to 5266 cfu/m^3 for VM (highest peak is in the evening hours) 26 to 276 cfu/plate/15 min for PPM (highest peak is in the evening hours). In addition to the identified fungi, 20.7 % (in VM) and 13.8 % (in PPM) of the fungi determined in our study could not be identified as genus level by the literature used, because some genera have the same morphological aspect, no distinctive characteristics, and bacterial contamination.

Results of the Pearson correlation analysis were presented in Table 3.

Table 3. Pearson Correlation Analysis of the data.

		VM		PPM	
	Sampling Hours	Humidity	Temperature	Humidity	Temperature
Colonial Counts	08.00	r=0.417	r=0.328	r=0.983**	r=0.367
	10.00	r=0.41	r=0.314	r=0.862*	r=0.791
	12.00	r=0.514	r=0.877*	r=0.359	r=0.947**
	14.00	r=0.423	r=0.828*	r=0.945**	r=0.718
	16.00	r=0.653	r=0.842*	r=0.733	r=0.877*
	18.00	r=0.503	r=0.907*	r=0.648	r=0.856
	20.00	r=0.464	r=0.719	r=0.524	r=0.840*

** Correlation is significant at the 0.01 level (2-tailed), * Correlation is significant at the 0,05 level (2 failed) are not statistically significant.

Discussion

The previous studies on airborne fungi in Edirne city (Sen and Asan, 2001; Asan et al., 2002) focused only to determine the diversity of these particles, while the present study deals with the distribution of airborne fungi at different times of the day.

Our present study made an important contribution for determination of levels and types of airborne fungi in Edirne (Turkey). Although there are similar studies [Sen and Asan, 2001; Asan et al., 2002], both studies focus on determination of airborne fungi in Edirne; whereas our study aims to study the distribution of airborne fungi in different times of the day; consequently we determined microfungal concentrations only at genus level. In addition, further studies are needed to be carried out in Edirne because of rapid urbanization, increasing air pollution and industrialization. We determined some microfungal genera in our study, but it was difficult to determine all airborne fungal concentrations; so we used two different isolation methods for this purpose. Average numbers obtained in 19 studies reflected that the five fungal genera (*Cladosporium* 29 %, *Alternaria* 14 %, *Penicillium* 9 %, *Aspergillus* 6 % and *Aureobasidium* 5 %) were predominant in the atmospheric air (Scholte et al., 2002), they accounted for almost 70 % of the whole mould concentrations.

It is well known that microfungi can live under extreme conditions in almost all regions and all climates. They usually live in soil and can be dispersed into the atmosphere owing to various factors. In recent years, aerobiologists have shown a great interest in airborne fungi due to both their constant existence in the air and the increase of allergies caused by them (Pasanen, 1992; Larsen and Gravesen, 1991). Monitoring airborne fungi in outdoor and indoor environment would provide valuable data for evaluating fungal spore counts. *Aspergillus* and *Penicillium* spores are the most widespread aeroallergens in the world. According to qualitative and quantitative reports, the former is the dominant species in tropical regions while the latter is dominant all over the world (Rosas et al., 1992). Everything may expose to molds, but fungal density in air and exposed time are very important for effect of molds.

Cladosporium was the most frequent and predominant genus detected in our study, followed by *Alternaria*, *Penicillium*, and *Trichoderma* (Table 4-9). *Cladosporium* species are very common in air and found predominant in many studies (Hargreaves et al. 2003; Pepeljnjak and Segvic, 2003). Also Sakiyan and Inceoglu (2003) found *Cladosporium* species to be predominant in air followed by *Alternaria* in Ankara (Turkey). According to the Gambale et al. [1985], the genus *Alternaria* with strong allergenic power has been isolated with 16 % frequency. Downs et al. [2001] noted that *Alternaria* is known to be allergic and is one of the most common fungi worldwide and they suggested *Alternaria* allergens contribute to severe asthma. Myszkowska et al. [2002] noted that 4-7 % of the European population show sensitivity to *Alternaria* and *Cladosporium* spores. *Alternaria* and *Cladosporium* were also the predominant genera in our study. Therefore, it is necessary to reveal the concentration percentages of these fungi in order to take some health measure for allergen sensitive people.

Table 4. Colony numbers and percentages of genera determined the October 11 , 2002.

Sampling Day and Hour	C		Al		P		X		As		F		T		R		U		Total		%	
	VM	PPM	VM	PPM	VM	PPM	VM	PPM	VM	PPM	VM	PPM	VM	PPM	VM	PPM	VM	PPM	VM	PPM	VM	PPM
8	267	14	67	3	200	2	133	4	-	-	-	-	-	-	-	-	400	8	1067	31	11.4	3.9
10	133	20	67	13	133	-	-	-	-	-	-	-	-	-	-	-	-	8	333	41	3.6	5.1
12	400	16	-	19	-	-	-	-	-	6	-	-	-	-	-	-	133	7	533	32	5.7	4.0
14	867	6	333	14	-	-	-	5	-	-	-	-	-	-	-	-	-	3	1200	28	12.9	3.5
16	200	12	400	25	-	-	-	-	-	-	-	-	-	-	-	-	-	4	600	41	6.4	5.1
18	867	14	133	22	-	-	-	-	-	-	-	-	-	-	-	4	200	-	1200	40	12.9	5.0
20	67	14	133	22	-	-		-	-	-	-	-	-	-	-	4	133	7	333	47	3.6	5.9
Average	400	12	162	17	48	0.3	19	1.3	-	0.9	-	-	-	-	-	1.1	124	53				
Total	2801	82	1133	118	333	2	133	9	-	6	-	-	-	-	-	8	866	37				
%	30	10.3	12.1	14.8	3.6	0.3	1.4	1.1	-	0.8	-	-	-	-	-	1	9.3	4.6				
	General total				5266	262																
	%				56.4	32.8																

Table 5. Colony numbers and percentages of genera determined the October 18, 2002.

Sampling Day and Hour	C		Al		P		X		As		F		T		R		U		Total		%	
	VM	PPM	VM	PPM	VM	PPM	VM	PPM	VM	PPM	VM	PPM	VM	PPM	VM	PPM	VM	PPM	VM	PPM	VM	PPM
8	-	12	67	4	67	-	-	6	-	-	-	-	-	-	-	-	-	9	134	31	1.4	3.9
10	-	30	-	2	-	1	-	-	-	-	-	-	-	-	-	-	67	2	67	35	0.7	4.4
12	133	6	-	2	-	-	-	4	-	1	-	-	-	-	-	-	67	-	200	13	2.1	1.6
14	133	5	67	6	-	3	-	20	-	5	-	-	-	-	-	-	-	-	200	39	2.1	4.9
16	67	12	-	25	-	-	67	8	-	-	-	-	-	-	-	-	67	6	201	51	2.2	6.4
18	333	5	67	18	-	-	-	3	-	-	-	-	67	-	-	-	200	3	667	29	7.1	3.6
20	67	17	200	14	-	-	-	3	-	-	-	-	-	-	-	-	133	3	400	37	4.3	4.6
Average	105	13.4	57	10.1	9.6	0.6	9.6	6.3	-	0.9	-	-	9.6	-	-	-	76	3.3				
Total	733	87	401	71	67	4	67	44	-	6	-	-	67	-	-	-	534	23				
%	7.9	10.9	4.3	8.9	0.7	5.5	0.7	5.5	-	0.8	-	-	0.7	-	-	-	5.7	2.9				
General total:					1869	235																
%					20.1	29.4																

Autumn appears to be the best season for fungal colonisation. Excessive decaying leaf material, high humidity and the suitable temperature for optimal fungal growth during this season might account for this (Simsekli, 1997). Therefore we performed our study during autumn months.

Our sampling was performed between the hours 08.00 am and 20.00 pm at two hour intervals. So, we sampled 7 times in a day. Nussbaum (1991) sampled air-spora four times a day (morning, midday, afternoon and evening) at weekly intervals (totaly five weeks). Some results of Nussbaum (1991) resemble our results. *Cladosporium* was the predominant genus in both studies. Colakoglu (1996a) studied variability of fungal flora in the air during morning and evening in 1994. She sampled fungi from air between the 07.15 – 07.30 am and 19.15 – 19.30 pm using PPM in six locations in Istanbul (Turkey). She found the most common fungi to be *Aspergillus* genus. She also determined the concentrations of fungi were different in morning and evening hours.

Table 6. Colony numbers and percentages of genera determined the October24, 2002.

Sampling Day and Hour	C		Al		P		X		As		F		T		R		U		Total		%	
	VM	PPM	VM	PPM	VM	PPM	VM	PPM	VM	PPM	VM	PPM	VM	PPM	VM	PPM	VM	PPM	VM	PPM	VM	PPM
8	-	5	-	2	-	1	-	-	-	-	-	-	-	-	-	-	-	5	-	13	-	6
10	133	3	-	3	-	-	67	5	-	-	-	-	-	-	-	-	67	5	267	16	2.9	2
12	-	3	-	3	-	-	67	5	-	-	-	-	-	-	-	-	-	3	67	14	0.7	1.8
14	-	1	67	6	-	-	-	5	-	-	-	-	-	-	-	-	67	-	134	12	1.4	1.5
16	-	2	-	4	-	-	-	3	-	-	-	-	-	-	-	-	-	-	-	9	-	1
18	-	1	-	3	67	-	-	2	-	-	-	-	-	-	-	-	-	3	67	9	0.7	1.1
20	-	2	-	7	-	1	67	-	-	-	-	-	-	-	-	-	-	3	67	13	0.7	1.6
Average	19	2.4	9.6	4	9.6	0.3	29	2.9	-	-	-	-	-	-	-	-	19.1	2.7				
Total	133	17	67	28	67	2	201	20	-	-	-	-	-	-	-	-	134	19				
%	1.4	2.1	0.7	3.5	0.7	0.3	2.2	2.5	-	-	-	-	-	-	-	-	1.4	2.4				
General total:					602	86																
%					6.5	10.8																

Table 7. Colony numbers and percentages of genera determined the November 01, 2002.

Sampling Day and Hour	C		Al		P		X		As		F		T		R		U		Total		%	
	VM	PPM	VM	PPM	VM	PPM	VM	PPM	VM	PPM	VM	PPM	VM	PPM	VM	PPM	VM	PPM	VM	PPM	VM	PPM
8	-	2	-	-	-	-	-	-	-	3	-	-	-	-	-	-	-	-	-	5	-	0.6
10	-	-	200	4	67	2	-	2	-	1	-	-	-	-	-	-	133	7	400	16	4.3	2
12	-	3	-	2	-	1	-	-	-	-	-	-	-	-	-	-	133	2	133	8	1.4	1
14	67	10	-	5	-	-	-	-	-	1	-	-	-	-	-	-	-	2	67	18	0.7	2.2
16	-	2	-	3	-	-	-	-	-	-	-	-	-	-	-	-	-	2	-	7	-	0.9
18	-	4	-	6	-	-	-	-	-	2	-	-	-	-	-	-	-	7	-	19	-	23
20	-	4	-	8	-	1	67	3	-	-	-	-	-	-	-	-	133	4	200	20	2.1	2.5
Average	9.6	3.6	28.6	4	9.6	0.6	9.6	0.7	-	1	-	-	-	-	-	-	57	3.4				
Total	67	25	200	28	67	4	67	5	-	7	-	-	-	-	-	-	399	24				
%	0.7	3.1	2.1	3.5	0.7	0.5	0.7	0.6	-	0.9	-	-	-	-	-	-	4.3	3				
General total:				800		93																
%				8.6		11.6																

Although there are different methods for sampling fungi from air, we used PPM and VM for the isolation of airborne fungi. PPM is useful for the enumeration of fungal spores, but gives only a rough approximation of the types and numbers of airborne fungi (Pelczar et al., 1993). This method may be recommended to obtain preliminary or qualitative information (Hoekstra et al., 2002). Some researchers choose PPM; for instance, Nussbaum (1991) explained that the PPM is a traditional method, because it allowed the investigator to move quickly among the study sites insuring that subsequent data comparisons were based on a nearly simultaneous collection time. According to Chen et al. (1998), there is no official and universally accepted bioaerosol sampling method. Nussbaum (1991) also indicated that no ideal method for collecting and evaluating airspora has been developed.

Table 8. Colony numbers and percentages of genera determined in November 12, 2002.

Sampling Day and Hour	C		Al		P		X		As		F		T		R		U		Total		%	
	VM	PPM	VM	PPM	VM	PPM	VM	PPM	VM	PPM	VM	PPM	VM	PPM	VM	PPM	VM	PPM	VM	PPM	VM	PPM
8	-	4	133	-	133	-	-	-	-	-	-	-	-	-	-	-	-	-	266	4	2.9	0.5
10	-	1	-	-	-	-	-	1	-	-	-	1	-	-	-	-	-	-	-	3	-	0.5
12	-	-	-	-	-	1	-	-	-	1	-	-	-	1	-	-	-	-	-	3	-	0.5
14	-	1	67	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	67	2	0.7	0.2
16	-	-	-	1	-	-	-	2	-	-	-	2	-	-	-	-	-	-	-	5	-	0.6
18	-	-	-	3	-	2	-	-	-	-	-	-	-	-	-	-	-	-	-	5	-	0.6
20	-	-	-	2	-	-	-	2	-	-	-	-	-	-	-	-	-	-	-	4	-	0.5
Average	-	0.9	2.9	0.9	19	0.4	-	0.7	-	0.1	-	0.4	-	0.1	-	-	-	0.1				
Total	-	6	200	6	133	3	-	5	-	1	-	3	-	1	-	-	-	1				
%	-	0.8	2.1	0.8	1.4	0.4	-	0.6	-	0.1	-	0.4	-	0.1	-	-	-	0.1				
General total:				333		26																
%				3.6		3.3																

Table 9. Colony numbers and percentages of genera determined the November 15, 2002.

Sampling Day and Hour	C		Al		P		X		As		F		T		R		U		Total		%	
	VM	PPM	VM	PPM	VM	PPM	VM	PPM	VM	PPM	VM	PPM	VM	PPM	VM	PPM	VM	PPM	VM	PPM	VM	PPM
8	-	14	-	-	-	-	-	-	-	-	-	2	-	-	-	-	-	3	-	19	-	2.4
10	67	6	-	1	-	-	133	5	-	-	-	-	-	-	-	-	-	-	200	12	2.1	1.5
12	-	7	-	-	-	-	-	-	-	-	-	-	67	-	-	-	-	-	67	7	0.7	0.9
14	-	7	-	5	133	-	-	12	-	-	-	-	-	-	-	-	-	-	133	24	1.4	3
16	-	5	-	5	-	-	-	6	67	-	-	-	-	-	-	-	-	-	67	16	0.7	2
18	-	6	-	4	-	-	-	3	-	-	-	-	-	-	-	-	-	-	-	13	-	1.6
20	-	1	-	1	-	-	-	2	-	-	-	-	-	-	-	-	-	3	-	7	-	0.9
Average	9.6	6.6	-	2.6	19	-	19	4	9.6	-	-	0.3	9.6	-	-	-	-	0.9				
Total	67	46	-	16	133	-	133	28	67	-	-	2	67	-	-	-	-	6				
%	0.7	5.8	-	2	1.4	-	1.4	3.5	0.7	-	-	0.3	0.7	-	-	-	-	0.8				
General total:					467	98																
%					5.0	12.3																

In addition, many types of culture media are used for sampling of microorganisms from the air; we used Rose-Bengal streptomycin agar medium for sampling. According to Madan et al. (1982), this medium is the most-suitable to sample fungi from air. Also according to the Morring et al. [1983], Rose Bengal streptomycin agar can be used for aeromycological sampling. Streptomycin antibiotic was used to control reproduction of bacteria and Rose-Bengal stain was used to limit the growth of fast-growing molds (e.g., *Rhizopus* and *Trichoderma* spp.).

Depending upon our statistical analysis results, it can be said that in early morning samples, there is an increase in colonial counts obtained with PPM with increased humidity in morning. This increase in number may depend on the effect of humidity on fungal spores since humidity may make it easy for spores to fall onto petri plates. But for certainty of this conclusion, further studies must be performed.

There is also an increase in colonial counts of afternoon samples obtained with both methods with increasing temperature. This migth be an expected result since temperature facilitates the release of fungal spores into the air from soil, water and plant surfaces.

One of the conspicuous results of our study is the dominating fungal taxa; *Alternaria* is one of them with relatively high numbers. Humilton (1959) reported diurnal periodicity of this genus as 10% for both morning and evening, respectively (Srivastava & Wadhwani 1992). Contrary, morning and evening colonial counts of *Alternaria* are not the same for both methods in our study. *Alternaria* colonies were obtained more in the evening.

Our study also revealed the existence of a rich airborne fungi in Edirne. But culture methods reflect only a portion of airborne fungi because some of them were not able to grow in all culture media: So, fungal concentrations in Edirne's air might be richer than our results.

References

1 ANDERSEN AA. New sampler for the collection sizing and enumeration of viable airborne particles.J *Bacteriol*. 76: 471-484, 1958.

2 ASAN A, SEN B, SARICA S. Airborne Fungi in Urban Air of Edirne city (Turkey). *Biologia* 57:59-68, 2002.

3 ASAN A, KIRGIZ T, SEN B, CAMUR-ELIPEK B, GUNER U, GUHER H. Isolation, identification and seasonal distribution of airborne and waterborne fungi in Terkos Lake (Istanbul-Turkey). J Basic *Microbiol*. 43: 83-95a, 2003a.

4 ASAN A, ILHAN S, SEN, POTOGLU-ERKARA I, FILIK C, CABUK A, DEMIREL R, TURE M, SARICA-OKTEN S, TOKUR S. Airborne fungal and *Actinomycetes* concentrations in urban air of Eskisehir City (Turkey). *Indoor Built Environ*. 13: 63-75, 2004.

5 ATIK S, TAMER AS. Eskişehir (merkez ilçe)'de mikrofungal hava kirliliği. *Ege Univ Fen Fak Derg Seri* B 16: 227-238,1994

6 AYATA C, COSKUN S, OKYAY T. 1989 yılında aylara göre Izmir ilinin çeşitli semtlerinde havanın fungal florası ve bunun allerjik hastalıklar yönünden önemi. *Türk Mikrobiyol Cem Derg*. 21:219-226, 1991. (Fungal flora of the air in several parts of Izmir according to months in 1989 and its importance regarding in the allergic diseases) (Turkish, with English abstract).

7 BARNETT HL, HUNTER BB. Illustrated genera of imperfect fungi. Fourth (ed.) 218 pp. APS Press, St. Paul, Minnesota, USA. 1999.

8 CHEN CC, YU TS, CHANG JY, CHANG CW, SHIH TS, HWANG JS. A computer simulation study on bioaerosol colony counting error due to masking effect. *Ann Occup Hyg* 42:501-510, 1998.

9 COLAKOGLU G. Fungal spore concentrations in the atmosphere at the Anatolia Quarter of Istanbul, Turkey. *J Basic Microbiol* 36: 155-162, 1996a.

10 COLAKOGLU G. Mould counts in the atmosphere at the Europe Quarter of Istanbul, Turkey. *J Basic Microbiol* 36: 389-392.,1996b.

11 COLAKOGLU G. The variability of fungal flora in the air during morning and evening in 1994. *J Basic Microbiol* 36 393-398, 1996c.

12 COLAKOGLU G. Airborne fungal spores at the Belgrad forest near the city of Istanbul (Turkey) in the year 2001 and their relation to allergic diseases. *J Basic Microbiol*. 43: 376-384, 2003.

13 DOWNS SH, MITAKAKIS TZ, MARKS GB, CAR NG, BELOUSOVA EG, LEUPPI JD, XUAN W, DOWNIE SR, TOBIAS A, PEAT JK. Clinical importance of *Alternaria* exposure in children. *Amer J Resp Critical Care Med* 164:455-459, 2001.

14 ELLIS MB. Dematiaceous Hyphomycetes. 608 pp. London and Reading. Commonwealth Mycological Institute. The Eastern Press Ltd. Kew, Surrey, UK, 1971

15 ELLIS MB, ELLIS JP. Microfungi on land plants. An identification handbook. Enlarged Ed. 868 pp. The Richmond Publishing Co. Ltd. UK, 1997.

16 GAMBALE, W, PAULA, RC, BUCK, N, GAMBALE V. Airborne fungi of presidente prudente SP, Brasil. *Rev. Microbiol*.16:9-14, 1985.

17 HARGREAVES M, PARAPPUKKARAN S, MORAWSKA L, HITCHINS J, HE C AND GILBERT D. 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 (1-3): 89-101, 2003.

18 HOEKSTRA ES, SAMSON RA, SUMMERBELL RC. Methods for the detection and isolation of fungi in the indoor environments. [In: RA Samson, ES Hoekstra, JC Frisvad, O Filtenborg (Eds). Introduction to food- and airborne fungi. Sixth Ed. 389 pp. Centraalbureau Voor Schimmelcultures, Utrecht-The Netherlands], 2002.

19 http://www.healthieryou.com/air.html

20 ISMAIL MA, CHEBON SK. Nakamya R: Preliminary surveys of outdoor and indoor aeromycobiota in Uganda. *Mycopathol*; 148:41-51, 1999

21 KLICH MA. Identification of common Aspergillus species. First Ed. 122 pp. Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands, 2002.

22 LARSEN L, GRAVESEN S. Seasonal variation of outdoor airborne viable microfungi in Copenhagen, Denmark. *Grana* 30:467-471, 1991.

23 MADAN P, LAMBA LC, ANEJA KR. The most-suitable medium for trapping fungi from air. *Sci Cult* 48:77-78, 1982.

24 MARTINEZ ORDAZ VA, RINCON-CASTANEDA CB, ESQUIVEL LOPEZ G, LAZO-SAENZ JG, LLORENZ MERAZ MT, VELASCO RODRIGUEZ VM. Fungal spores in the environment of the asthmatic patient in a semi-desert area of Mexico. *Rev Alerg Mex* 49: 2-7, 2002.

25 MORRING, KL, SORENSON WG, ATTFIELD MD. Sampling for airborne fungi: A statistical comparison of media. *Am Ind Hyg Assoc* J. 44:662-664, 1983.

26 MYSZKOWSKA D, STĘPALSKA D, KRYSTYNA K, PORĘBSKI G. The relationship between airborne pollen and fungal spore concentrations and seasonal pollen allergy symptoms in Cracow in 1997–1999. *Aerobiologia* **18**:153-161, 2002.

27 NELSON PE, TOUSSOUN TA, MARASAS WFO. *Fusarium* species- An illustrated manual for identification. 193 pp. The Pennsylvania State University Press, University Park and London, USA, 1983.

28 NUSSBAUM, F. Variation in the airborne fungal spore population of the Tuscarawas Valley II. *Mycopathologia* 116:181-198,1991.

29 OKUYAN M, AKSOZ N, VARAN A. 1972 ve 1974 Ocak aylarında Ankara'nın çeşitli semtlerinde havanın küf ve maya florasındaki değişiklik ve bunun allerjik hastalıklar yönünden önemi. *Mikrobiol Bült*. 10: 351-358, 1976.

30 PASANEN, AL. Airborne mesophilic fungal spores in various residental environments. *Atmos Environ* 26A: 2861-2868, 1992.

31 PELCZAR MJ, CHAN ECS, KRIEG NR. Microbiology: Concepts and applications. 966 pp. International Ed. P. 796. McGraw- Hill, Inc. New York, : 1993.

32 PEPELJNJAK S, ŠEGVIĆ M. Occurrence of fungi in air and on plants in vegetation of different climatic regions in Croatia. *Aerobiologia* **19**: 11-19, 2003.

33 PITT JI. The genus *Penicillium* and its teleomorphic states *Eupenicillium* and *Talaromyces*. 634 pp. Academic Press. Inc. London,1979.

34 RAPER KB, FENNELL DI. The genus *Aspergillus*. 686 pp. The Williams & Wilkins Comp. Baltimore, USA, 1965.

35 ROSAS I, CALDERON C, ESCAMILLA B, ULLOA M. Seasonal distribution of *Aspergillus* in the air of an urban area: Mexico City. *Grana* 31:315-319, 1992.

36 SAKIYAN N, INCEOGLU O. Atmospheric concentration of *Cladosporium* and *Alternaria* spores in Ankara and the effect of meteorological factors. *Turk J Bot* 27:77-81, 2003.

37 SAMSON RA, HOEKSTRA ES, FRISVAD JC, FILTENBORG O. Introduction to food- and airborne fungi. Sixth Ed. 389 pp. Centraalbureau Voor Schimmelcultures – Utrecht–The Netherlands, 2002.

38 SARICA S, ASAN A, TATMAN-OTKUN M, TURE M. Monitoring indoor airborne fungi and bacteria in the different areas of Trakya University Hospital (Edirne-Turkey). *Indoor Built Environ* 11:285-292, 2002.

39 SCHOLTE RPM, SAMSON RA, DIJKSTERHUIS J. Spoilage fungi in the industrial processing of food. [In: RA Samson, ES Hoekstra, JC Frisvad, O Filtenborg (Eds). Introduction to food- and airborne fungi. Sixth Ed. 389 pp., Centraalbureau Voor Schimmelcultures, Utrecht-The Netherlands], 2002.

40 SEN B, ASAN A.: 2001. Airborne fungi in vegetable growing areas of Edirne, Turkey. *Aerobiologia* 17:69-75.

41 SIME AD, ABBOTT LL, ABBOTT SP. Mounting medium for use in Indoor Air Quality spore-trap analyses. *Mycologia* 94: 1087-1088, 2002.

42 SIMSEKLI Y. Bursa ev dışı havasında bulunan fungal sporlar. *Turk J Biol*. 21: 359-365, 1997.

43 SIMSEKLI Y, GUCIN F, ASAN A. Isolation and identification of indoor airborne fungal contaminants of food production facilities and warehouses in Bursa, Turkey. *Aerobiologia* 15:225-231, 1999.

44 SRIVASTAVA AK, WADHWANI K. Dispersion and allergenic manifestations of *Alternaria airspora*. *Grana* 31: 61-66, 1992.

45 TROUTT C, LEVETIN E. Correlation of spring spore concentrations and meteorological conditions in Tulsa, Oklahoma. *Int J Biometeorol* 45: 64-74, 2001.

46 WANG Z, REPONEN T, GRINSHPUN SA, GÓRNY RL AND WILLEKE K. Effect of sampling time and air humidity on the bioefficiency of filter samplers for bioaerosol collection. *J Aerosol Sci*, 32: 661-674, 2001.