

AIRBORNE FUNGAL CONCENTRATIONS OF MORNING AND EVENING IN EAST PATCH OF EDİRNE CITY USING TWO SAMPLING METHODS

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Abstract: In order to determine the variations of fungal concentrations during morning and evening, fungal samples were taken three times a month between September- November 2002. Two sampling methods were employed. Samples were taken either by gravitation method for which Petri dishes containing Rose Bengal streptomycin agar were exposed to air for 15 min. or by using a volumetric sampler. A total of 9680 colonies belonging to *Alternaria* (Nees), *Aspergillus* (Fr.:Fr.), *Cladosporium* (Link), *Penicillium* (Link), *Rhizopus* (Ehrenberg), *Scopuloriopsis* (Bainier), *Trichoderma* Pers, *Ulocladium* Preus, and to unidentified genera from Dematiaceae were observed. Of these, *Cladosporium*, *Alternaria* and *Penicillium* were the dominating taxa with their colonial counts 3133, 2733 and 1134 for gravitational method and 120, 149 and 4 for volumetric sampling method, respectively. We used a Kolmogorov-Smirnov test for the fitness of the variables to normal distribution. For morning and evening counts of the sampler method, we found a significant relationship ($r = 0.489$, $P < 0.001$) as revealed by correlation of analysis. When colonial counts of morning and evening samples of the gravitational method were compared again a significant relationship was found ($r = 0.667$). Morning and evening counts of both methods were analysed statistically ($r = 0.486$ and $r = 0.761$ respectively).

Key Words: Airborne fungi, Diurnal fungal concentration variations, Volumetric Method, Edirne.

Edirne Şehrinin Doğusunda iki Örneklemeye Metodu Kullanılarak Havayla Taşınan Fungusların Sabah ve Akşam Konsantrasyonlarının Belirlenmesi

Özet: Eylül- Kasım 2002 tarihleri arasında sabah ve akşam saatlerinde havayla taşınan fungusların konsantrasyonlarının değişimlerini belirlemek amacı ile ayda üç kez olmak üzere fungal örneklemeye yapılmıştır. Bu amaç için iki metot uygulanmıştır. Örnekler, Yerçekimine Dayalı Petri Plak Metodu ile, Rose Bengal Streptomisin agar içeren petri plakları 15 dakika havayla temas ettirilerek ve Volumetrik bir örnekleyici kullanarak alınmıştır. *Alternaria*, *Aspergillus*, *Cladosporium*, *Penicillium*, *Rhizopus*, *Scopuloriopsis*, *Trichoderma*, *Ulocladium* cinslerine ve Dematiaceae familyasına ait 9680 koloni gözlenmiştir. Bunlardan *Cladosporium*, *Alternaria* ve *Penicillium* cinsleri Yerçekimine Dayalı Petri Plak Metodu için 120, 149 ve 4, Volumetrik örnekleme için de 3133, 2733 ve 1134 koloni sayıları ile baskın cinsleri oluşturmuşlardır. Değişkenlerin normal dağılıma uygun olup olmadıklarını belirlemek için Kolmogorov-Smirnov testi uygulanmıştır. Daha sonra yapılan Korelasyon analizi sonucunda Volumetrik metotla izole edilen cinslerin sabah ve akşamki koloni sayıları arasında anlamlı bir ilişki bulunmuştur ($r = 0.489$). Yerçekimine Dayalı Petri Plak Metoduna göre izole edilen cinslerin sabah ve akşamki koloni sayıları arasında da yine anlamlı bir ilişki bulunmuştur ($r = 0.667$). Her iki metoda göre izole edilen cinslerin sabah ve akşamki koloni sayıları istatistiki olarak analiz edilmiş ve aralarında bir ilişki olduğu saptanmıştır ($r = 0.486$ ve $r = 0.761$).

Anahtar Kelimeler: Havayla taşınan funguslar, günden fungal konsantrasyon değişiklikleri, volumetrik metod, Edirne.

Introduction

Besides water, insects, man and animals, atmospheric air is one of the dissemination source of fungal spores to the environment (Lacey & Dutkiewicz, 1994). Fungi whose spores are spread by the atmospheric air are termed as **anemophilous** (Mezzari et al., 2002). As these spores have the ability to cause some respiratory diseases such as asthma, rhinitis etc., when inhaled by humans it is important to reveal the atmospheric fungal composition of a given region. Some of these spores are also important in allergic cases and in plant pathology (Sakıyan & Inceoglu, 2003).

The medical profession began to associate fungi with allergy during the early 1900s, and in 1969. Bulmer (1969) summarized the factors that have affect on spore populations. These include diurnal periodicity, season, temperature, wind, precipitation, soil, and vegetation (Nussbaum, 1991).

Distribution of fungal spores in atmospheric air has a varying nature and is determined by a combination of many environmental and biological factors (De-Wei & Kendrick, 1996; Burch & Levetin, 2002). Winds, humidity, temperature, rainfall, altitude, geography, human activity, vegetation and fungal life cycles all affect the concentrations of airborne fungal spores at a given time at a given region. Therefore, it is normal for one to find not only seasonal and regional concentration differences but also daily differences can be found. These above mentioned environmental and biological factors affect different fungal spores in different manners so all these spores may exhibit varying diurnal concentration patterns, as shown by De-Wei & Kendrick (1995). Similarly, in one of their study about airborne fungi, Bandyopadhyay et al. (1991) revealed that diurnal periodicity of *Alternaria* and *Curvularia* had a similar pattern but this pattern differed from that of *Fusarium* (Michael et al., 1997).

Two methods, gravitational and volumetric sampling, are currently being used for airborne fungal sampling. By these two methods different spore counts are obtained. For example it is hard to efficiently sample some small-spored fungi such as *Penicillium* and *Aspergillus* by using the gravimetric method (Michael et al., 1997).

Studies concernig airborne fungal biota carried out in Turkey are generally resctricted to a few big cities, eg. İstanbul (Colakoglu, 1996 a,b,c, 2003; Ozyaral et al., 1988; Ozyaral & Johansson, 1990), Edirne (Sen & Asan, 2001; Asan et al., 2002; Sarıca et al., 2002), Bursa (Sapan et al., 1991, 1993; Simsekli et al., 1999), Ankara (Okuyan et al., 1976), Eskişehir (Atik, 1993) and İzmir (Ayata, 1990). Sen & Asan (2001) reported the first data about outdoor fungal biota in Edirne. Later, Asan et al., (2002) added to these data. However, these studies are not too much when compared to other countries. Therefore, the need of a further study to improve present data appeared to be important.

We aimed to identify and reveal the diurnal periodicity of airborne fungal spores in the atmosphere of an open field in Edirne city by using gravitational and volumetric sampling methods. It was also aimed to compare the counts of these two methods and to find a possible positive and/or a negative correlation between airborne fungal spores and meteorological data.

Sampling site

The study area is an open field exposed to different air currents. Güllapoğlu Arboretum, a 200 hectar woodland, is located around the study area with its nearest border only 10 meters away. Among the vegetation found in the Arboretum *Ulmus minor* M., *Salix alba* L., *Robinia pseudoacacia* L., *Populus nigra* L., *Populus alba* L., *Populus tremula* L., *Prunus spinosa* L., *Morus alba* L., *Rubus sanctus* Scereber, *Vitis vinifera* L. *Rosa canica* L., *Paliurus spinacristi* M. occur dominantly. There are also *Triticum* sp. and *Helianthus* sp. fields located at northern and western sides of the study area. The city of Edirne is 3 km. far away to the area.

Materials and Methods

Sampling, Isolation Methods and Identification

Concentrations of airborne fungi were monitored over a three months period between November and December 2002. Three samplings were performed for each month. Samples were taken twice a day, in morning at 09: ⁰⁰ a.m and in evening at 21: ⁰⁰ p.m.

Two methods were used for sampling; Petri Plate Gravitational or Settle Plate Method (Ismail et al., 1999) and Volumetric Spor Sampling Method (Martinez et al. 2002). Rose-Bengal streptomycin agar¹ (RBS agar) (Madan et. al., 1982) medium were used for both methods. The samples were incubated at 25-27 °C for 7 days. After incubation period, concentrations of airborne fungi were calculated as CFU (colony forming unit) (CFU/plate/15 min.) and CFU/m³. Morphological properties of the colonies such as colour, size and heigth were examined by placing petri plates under a stereo microscope. Then, a light microscope was used for determinations of morphological properties of the specimens. For the latter fungal fragments were mounted on Lacto – Cotton Blue (Sime et al., 2002).

¹ (Procedure: dextrose; 10 gr., peptone; 5gr., KH₂PO₄; 1 gr., MgSO₄.7H₂O; 0,5 gr., agar agar; 20 gr., distilled water; 1000 ml.)

Procedure of Rose-Begal stain and streptomycin antibiotic: 1 gr powdery streptomycin (Deva Inc., Turkey) dissolved in 33 mL. sterile - pure water; 2 mL. of this mixture was added to 1000 mL. of medium . 0,5 gr. Powdery Rose-Bengal stain (Fluka Chemika-BioChemika, Switzerland) dissolved in 150 mL. sterile – pure water; 10 mL. of mixture was added to 1000 mL. of medium)

Climatological Data

Temperature and relative humidity of the study site were also measured during whole research (Table 1).

Table 1. Average temperature and relative humidity values measured at sampling site.

Ss	Average Temperature (°C)		Relative Humidity (%)	
	Morning	Evening	Morning	Evening
05.09.2002	22	22	60	66
15.09.2002	16	19	55	58
25.09.2002	22	20	76	69
05.10.2002	14	17	68	72
15.10.2002	15	16	75	86
25.10.2002	10	14	75	75
05.11.2002	7	9	67	70
15.11.2002	9	11	70	68
25.11.2002	-6	-9	45	41

Results & Discussion

A total of 9680 colonies belonging to *Alternaria*, *Aspergillus*, *Cladosporium*, *Penicillium*, *Rhizopus*, *Scopuloriopsis*, *Tricoderma*, *Ulocladium*, *Dematiaceae* were observed. Of these, *Cladosporium*, *Alternaria* and *Penicillium* were the dominating taxa with their colonial counts 3133, 2933 and 1134 for gravitational method and 120, 149 and 4 for volumetric sampling method, respectively (Table 2 , Table 3).

Table 2. Fungal colonial numbers obtained with volumetric method (N; Number of sampling (1,2,3 correspond to September, 4,5,6 to October and 7,8 and 9 to November), t; time of sampling, ; S; Volumetric sampler counts as m³)

N	T	<i>Alternaria</i>	<i>Aspergillus</i>	<i>Cladosporium</i>	<i>Penicillium</i>	<i>Rhizopus</i>	<i>Scopuloriopsis</i>	<i>Tricoderma</i>	<i>Ulocladium</i>	Unidentified*
		S (m ³)	S (m ³)	S (m ³)	S (m ³)	S (m ³)	S (m ³)	S (m ³)	S (m ³)	S (m ³)
1	09.00	133	133	533	133	0	133	0	0	67
	20.00	133	0	200	67	0	0	0	67	0
2	09.00	67	67	333	333	0	0	0	0	200
	20.00	200	67	400	267	0	0	0	0	333
3	09.00	133	0	267	0	0	0	0	0	67
	20.00	1133	0	400	133	0	0	0	0	267
4	09.00	67	0	333	67	0	0	0	0	0
	20.00	467	67	267	67	0	0	0	0	267
5	09.00	133	67	133	0	0	0	0	0	67
	20.00	0	0	67	0	0	0	0	0	0
6	09.00	0	0	0	0	0	0	0	0	0
	20.00	267	0	133	0	0	0	67	0	200
7	09.00	0	0	0	0	0	0	0	0	0
	20.00	0	0	67	67	0	0	0	0	0
8	09.00	0	0	0	0	0	0	0	0	200
	20.00	0	0	0	0	0	0	0	0	0
9	09.00	0	0	0	0	0	0	0	0	0
	20.00	0	0	0	0	0	0	0	0	0
Total		2933	401	3133	1134	0	133	67	67	1668

*Unidentified genera from Dematiaceae.

Table 3. Fungal colonial numbers obtained with gravitational method (N; Number of sampling (1,2,3 correspond to September, 4,5,6 to October and 7,8 and 9 to November), t; time of sampling, ; G; Gravitational sampler counts as CFU).

N	T	<i>Alternaria</i>	<i>Aspergillus</i>	<i>Cladosporium</i>	<i>Penicillium</i>	<i>Rhizopus</i>	<i>Scopuloriotopsis</i>	<i>Tricoderma</i>	<i>Ullocladium</i>	Unidentified*
		G Cfu/plat e/15 /min.	G Cfu/plate/ 15/min.	G Cfu/plate/ 15/min.	G Cfu/plat e/15 /min.	G Cfu/pla te/15 /min.	G Cfu/plate /15 /min.	G Cfu/pla te/15 /min.	G Cfu/plat e/15 /min.	G Cfu/pl ate/15 /min.
1	09.00	29	0	2	0	0	0	0	0	0
	20.00	28	0	8	0	0	0	0	0	0
2	09.00	8	1	30	0	0	0	0	0	15
	20.00	5	0	0	0	0	0	0	0	3
3	09.00	10	1	30	0	0	0	0	0	28
	20.00	32	0	21	0	0	0	0	0	0
4	09.00	1	0	3	1	0	0	0	0	0
	20.00	9	0	7	1	0	0	0	0	0
5	09.00	5	0	2	0	0	0	0	0	1
	20.00	6	0	1	0	1	0	0	0	2
6	09.00	1	0	3	1	0	0	0	0	2
	20.00	6	0	2	0	0	0	0	0	7
7	09.00	1	0	0	0	0	0	0	0	3
	20.00	1	0	1	1	0	0	0	0	1
8	09.00	0	0	4	0	0	0	0	0	0
	20.00	1	0	3	0	0	0	1	0	2
9	09.00	5	0	3	0	0	0	0	0	2
	20.00	1	0	0	0	0	0	0	0	1
Total		149	2	120	4	1	0	1	0	67

*Unidentified genera from Dematiaceae.

It is a common problem in most countries that nasal or other respiratory allergy cases increase in number when the air temperature starts to decrease. One of the causes of these allergies could be fungal spores. Therefore it was essential to reveal the types and numbers of airborne fungal spores in Gullapoglu Campus in such a time period when the allergic cases accelerate. This study was carried on for this purpose with special respect to the comparison of two different sampling methods used; volumetric and gravitational methods. As diurnal periodicity of fungal spores are in relationship with respiratory allergies (De- Wei & Kenrick, 1995; Pepeljnjak & Segvic, 2003) samplings were performed twice a day, in morning and in evening.

When compared to volumetric sampling method, gravitational sampling method is low cost and more practical in usage. It is also useful for the enumeration of fungal spores but gives only a rough approximation of the kinds and numbers of airborne fungi (Pelczar et al., 1993). For this method Rose-Bengal streptomycin agar medium was used. According to the Madan et al., (1982) this medium is the most suitable one for sampling fungi from air. Streptomycin antibiotic contained in the medium controls the bacterial reproduction and Rose Bengal stain limits the growth of fast growing molds.

It is well known that airborne fungi have a global distribution (Soutworth, 1974). Fungal composition of the atmosphere of a given area depends on many biological and environmental factors. Of these, especially meteorological conditions have influences on the production, dispersal and deposition of airborne fungal spores. Rain, wind speed, humidity, temperature are among the major above mentioned conditions. Besides seasonal differences, there are also daily concentration differences among and within taxa.

We used a Kolmogorov-Smirnov test for the fitness of the variables to normal distribution. For morning and evening counts of the sampler method, we found a significant relationship ($r = 0.489$, $P < 0.001$) as revealed by correlation of analysis. When colonial counts of morning and evening samples of the gravitational method were

compared again a significant relationship was found ($r = 0.667$). Morning and evening counts of both methods were analysed statistically ($r = 0.486$ and $r = 0.761$ respectively).

One of the conspicuous results of our study is the dominating fungal taxa. *Alternaria* is the one of them with relatively high numbers. Humilton (1959) reported diurnal periodicity of this genus as 10% for both morning and evening, respectively (Srivastava & Wadhvani 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. The importance of this species mainly emerges in allergic cases (Downs et. al., 2001). Fungal airspora are known to incite respiratory allergic reaction in sensitized individuals. However, their exact means for eliciting allergic responses remains under investigation and their clinical impact on the development of asthma is not accurately known (Lopez & Salvaggio 1985). A standardization of allergens along the many fungal species may be needed. Progress toward standardization of allergens of *Alternaria* has recently been reported (Nussbaum, 1991).

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.

It is rather important to know about the fungi present in a city, from the point of view of ecological diagnosis and specific applications that could be used for the allergy cases caused by inhaled allergens. Several qualitative and quantitative techniques have been developed for determination of fungi dispersed through atmosphere via wind. In a former study (Mezzari et. al., 2002) samples were taken one day during every week for 24 hours. A total of 52 sampling was performed between April 2000 and March 2001 and the following ratio results were obtained; Ascospore forming fungi % 50.49, *Cladosporium* % 17.86, *Aspergillus / Penicillium* % 15.03, basidiospored fungi % 3.84, rasts % 3.82, *Helminthosporium* % 2.49, *Botrytis* % 1.22, *Alternaria* % 1.19, *Curvularia* % 0.87, *Nigrospora* % 0.61 and *Fusarium* % 0.08. Fungal spores were seen in summer frequently but their presence decreased in Autumn. This is why we chose autumn season for our study. Fungi disperse their spores to the environments such as atmospheric air, water, insects, human beings and animals. It is important to know the qualitative and quantitative features of the spores those carried by wind because these spores are causal agents, when inhaled, of respiratory diseases such as rhinitis and asthma. Therefore, the concentrations of airborne fungal spores in a given region, especially the diurnal patterns, are to be known to take better health measurements for people that are sensitive to fungal allergen spores. So, we believe that our report on Edirne's airborne fungal concentrations will be taken into consideration for above mentioned purposes.

Fungi are organisms that are able to survive extreme environmental conditions and that grow on varying substrates such as soil and plant animal waste materials. Fungal spores are generally airborne. Some of these airborne spores are known to cause chronic bronchitis, asthma, fungal allergens, high-sensitive pneumonia and Aspergillosis in humans. Tilak (1991) related the %2-30 of the respiratory allergies to fungal spores. He also reported *Alternaria* and *Cladosporium* species to be the most allergy causing fungi. Fungal allergens are primarily fungal spores. However, other structures such as mycellium are also dispersed to the environment. Airborne fungal concentrations depend on some outdoor factors; i.e. the time of the day, meteorological factors, seasonal climatic factors and type of vegetation (Pepeljnjak & Segvic, 2003).

Since fungal aerobiota of a region depends on many environmental and biological factors it is normal to find varying fungal spore concentrations in different regions, even if they are very close to each other, and even at different times of the day. This points out the importance of taxonomical studies on fungi supported with ecological ones. With these studies, one can make a conclusion about the fungal biota of the atmosphere in a region and help to the understanding of the relationship of these fungi with especially human health.

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