Turk J Med Sci
36 (2006) 31-36

CLINICAL INVESTIGATION

Identification and Seasonal Distribution of Airborne Fungi in Urban Outdoor Air in an Eastern Black Sea Turkish Town

Murat TOPBAŞ, İlknur TOSUN, Gamze ÇAN, Neşe KAKLIKKAYA, Faruk AYDIN

[1]Department of Public Health, Faculty of Medicine, Karadeniz Technical University, Trabzon - Turkey

[2]Department of Microbiology and Clinical Microbiology, Faculty of Medicine, Karadeniz Technical University, Trabzon - Turkey

Received: February 08, 2005

Abstract: The aim of the present report was to evaluate the prevalent species of airborne fungi in the outdoor environment in the city of Trabzon, Turkey. The area is surrounded by high mountains running parallel to the Black Sea, and has a mostly rainy and temperate climate together with a most varied and widespread flora. Air samples were collected in four seasons by means of the gravitational settling method using petri dishes with SDA culture media. Those fungi colonies that formed after an incubation period of 7-10 days at 25 ºC were determined on the basis of micro and macromorphological features. With respect to seasons, it was found that in Summer *Penicillium*, *Alternaria* and *Fusarium*, in Autumn and Winter *Penicillium* and in Spring and Summer *Alternaria* were the most prevalent fungal genera.

Key Words: Airborne fungi, fungal flora, allergen, seasonal distribution

Introduction

Fungi are widespread all over the world, and high environmental burdens have been shown to be affected by various factors such as wind, moisture, and temperature and air pollution leading to variations with respect to species and quantities from one season to another. Fungi thrive better in moist and warm places. Airborne fungi originate from different environments such as soil, plants and water. Fungal spores in aquatic environments may be transferred to the air by wave action. The concentration of airborne fungal spores has been linked to wind, humidity, temperature, rainfall, altitude, vegetation and various specific reservoirs of contamination. In addition, fungal propagative units may be dispersed in the air by insects (1).

Based on the microbiological analysis of air samples from inhabited areas, it has been reported that airborne fungi are among the most common organisms correlated with air pollution that have adverse effects on human health. Fungi are known to be one of the major causative allergens of allergenic diseases such as bronchial asthma, allergic rhinitis and atopic dermatitis (2-4). In a study conducted in our region, it was reported that 11.2% of the adult population wheezed, 3.3% wheezed even in the absence of the common cold, 11.1% suffered from shortness of breath, 2.2% were receiving treatment for asthma, and 16.7% suffered from rhinitis (5). In another study it was shown that 12.8% of all allergic cases among children living in Trabzon were due to airborne fungi spores (6). Similar studies from different geographies also indicate that bronchial asthma and allergic rhinitis reacted positively to fungal extracts and that higher symptom scores in asthmatic children correlate well with higher fungal exposures in indoor dwellings (7,8). For instance, a recent study carried out in England showed that exceptional rates of admission for asthma tended to occur on days with high total mould spore counts (9). Moreover, with radiotherapy, corticosteroid and immunosuppressive treatments, there is a tendency towards opportunistic systematic fungus infections such as aspergillosis, mucormycosis, penicillosis, brain abscess, pneumonitis and endocarditis in diabetes mellitus, bronchiectasia, emphysema,

tuberculosis, transplantation and AIDS cases. In addition to opportunistic fungus infections most of the fungus species responsible for gastrointestinal and central nervous system infection can exist in the air. The roles of various airborne fungus species in causing opportunistic infections are currently the subject of debate, and it has been reported that these fungi have a low virulence potential (10-12). Even if the probability is not high, it is thought that airborne fungi may be related to such infections.

The present study was conducted in the center of Trabzon, a large city in the Eastern Black Sea region in Turkey, surrounded by high mountains running parallel to the Black Sea, with relatively warm temperatures and uniform rainfall all year round. The flora is very rich and widespread compared with the other regions of Turkey. Our purpose was to determine the genus, quantity and seasonal distributions of airborne fungi that may be important causative allergens. To the best of our knowledge, despite a few clinical studies on allergic status, no study of this kind has previously been conducted in this region.

Materials and Methods

The city of Trabzon with a population of 980,000 (2004 estimate), is the largest in the Eastern Black Sea region of Turkey. The vicinity is backed by the Zigana Mountains. It lies on latitude 40º58.8'N and longitude 39º46.2'E. The study was carried out in the town over *four seasons*: *Autumn* (September, 2001) *Winter,* (January) *Spring* (April) and *Summer* (July, 2002). Sampling locations are shown in Figure 1. Fungal flora of the air was determined from 5 different parts of the town for 10 days a month, which is regarded as a normal meteorological status (Table 1) (Personal communication with Trabzon Meteorology Directorate)

Study areas and their features are as follows:

1. Region I: 0 m above the sea level and coastline.
2. Region II: 50 m above sea level in the center of the city
3. Region III: Woodland 20-30 m above sea level
4. Region IV: Woodland 200 m above sea level
5. Region V: 10 m above sea level; a new and orderly urbanized settlement.

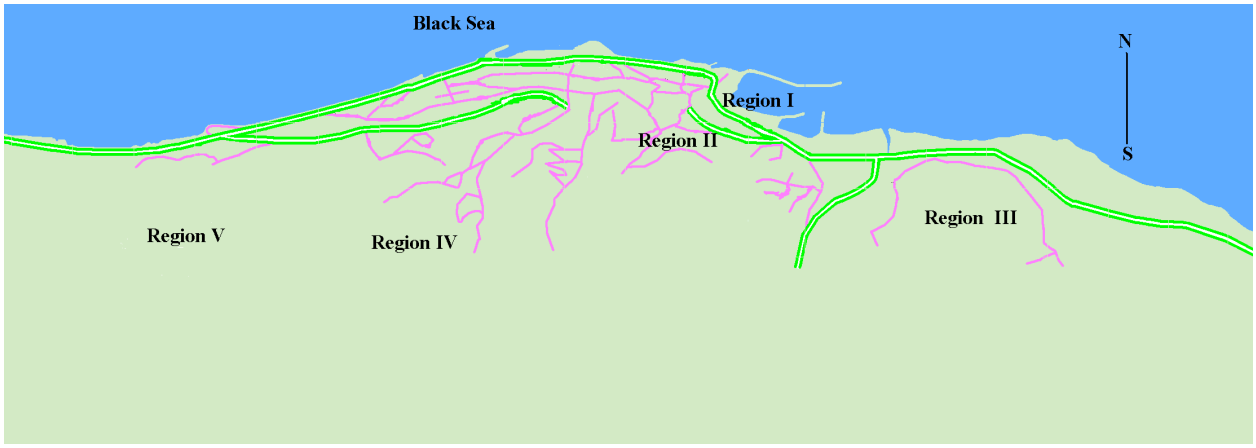

Figure 1. The map of Trabzon City and Sampling Locations.

Table 1. Average meteorological measurements in Trabzon.

Months	Temperature (ºC)	Moisture (%)	Wind speed (m/s)
January	7.3	67	2.0
April	11.6	74	1.7
July	22.6	74	1.6
October	16.3	72	1.9

A total of 200 samples were taken in the morning (09.00-11.00) using the gravitational settling method with petri plates, as described in the literature (13-15). Beriefly, SDA containing plates were exposed to the air at a height of 1.5 m above the ground for 15 minutes by opening the plate cover. Those fungi colonies formed after an incubation period of 7–10 days at 25 °C were determined on the basis of micro- and macromorphological features, and were cultivated in Malt Extract Agar (MEA), Czapek Dox Agar (CDA) and Potato Dextrose Agar (PDA) (16). Extra-thin lamellae were used in the preparation and identification of fungi as described elsewhere (17,18).

Results

Two hundred culture plates from various locations in Trabzon as described in the materials and methods produced a total of 433 colonies belonging to various genus of fungi, and an overall 192 (91.0 %) culture media reproduction was observed.

A total of 11 genus were determined. The most reproductive of these were *Penicillium* with 116 (26.8%) colonies, *Alternaria* with 114 (26.3%) colonies and *Fusarium* with 58 (13.2%) colonies, followed by *Aspergillus* with 43 (9.9%) colonies and *Cladosporium* with 35 (8.1%) colonies.

As shown in Table 2, the highest level of reproduction in terms of the number of colonies was observed in region III (116 colonies, 26.8%) and in region I (98 colonies, 22.6%). When the results were analyzed with respect to the number of fungi found, region III ranked first with 10 genus, region V second with 9 genus, regions I and IV third with 8 genus, and region II with 7 genus.

The seasonal distribution of colonies of airborne fungi is summarized in Table 3, and shows that the highest reproduction level was observed in Summer (129 colonies, 29.8%). The most reproductive fungus species were *Penicillium* in Summer and Winter with 30 and 34 colonies, respectively, and *Alternaria* in Spring and in Summer with 38 and with 42 colonies, respectively. In the Autumn, all species reproduced.

Table 2. Total count of airborne fungi associated with reproduction sites.

Fungi types		Region 1	Region II	Region III	Region IV	Region V	TOTAL	
							count	%*
Penicillium		32	18	25	28	13	116	26.8
Alternaria		24	18	26	23	23	114	26.3
Fusarium		19	6	14	10	9	58	13.4
Aspergillus		8	6	13	10	6	43	9.9
Cladosporium		3	7	19	4	2	35	8.1
Rhizopus		-	-	-	1	7	8	1.8
Aureobasidium		2	2	-	-	1	5	1.2
Epicoccum		1	-	2	-	1	4	0.9
Mucor		-	-	1	1	-	2	0.5
Scopulariopsis		-	-	1	-	-	1	0.2
Verticillium		-	-	1	-	-	1	0.2
Other (Sterile)		9	6	14	12	5	46	10.6
TOTAL	n	98	63	116	89	67	433	100.0
	%**	22.6	14.5	26.8	20.6	15.5		

*Column percent
**Line percent

Table 3. Genera and total count of occurrence of airborne fungi by seasons.

Fungi types		Fall	Winter	Spring	Summer	TOTAL	
						count	%*
Penicillium		26	34	26	30	116	26.8
Alternaria		22	12	38	42	114	26.3
Fusarium		16	5	17	20	58	13.4
Aspergillus		24	6	9	4	43	9.9
Cladosporium		10	12	5	8	35	8.1
Rhizopus		4	-	-	4	8	1.8
Aureobasidium		5	-	-	-	5	1.2
Epicoccum		4	-	-	-	4	0.9
Mucor		1	-	-	1	2	0.5
Scopulariopsis		1	-	-	-	1	0.2
Verticillium		1	-	-	-	1	0.2
Other (sterile)		6	20	-	20	46	10.6
TOTAL	n	120	89	95	129	433	100.0
	%**	27.7	20.6	21.6	29.8		

* Column percent
** Line percent

Discussion

Knowledge of species and density of outdoor airborne fungi in a given environment can be especially important in the diagnosis and treatment of various allergic diseases. This study was therefore conducted in Trabzon which, compared to other parts of Turkey, has different features in terms of climate, geography and flora. Twelve genus of fungi including *Penicillium*, *Aspergillus*, *Alternaria*, *Fusarium* and *Cladosporium* were identified in outdoor. *Penicillium* and *Alternaria* were the most prevalent and appeared to be the most common genera in almost all of the zones. Soil is an important source for airborne fungi. A study on soil fungi in Trabzon by Soylu (19) showed that the most abundant genera were *Penicillium, Aspergillus, Cladosporidium* and *Fusarium*.

The *Penicillium* species have been identified as important causative agents of extrinsic bronchial asthma (20). Furthermore, it has been reported that the most common genera namely *Aspergillus, Penicillium*, *Cladosporium* and *Alternaria* should always be considered as a cause of fungal allergy (21).

Alternaria, which also exhibited a high level of reproduction in Trabzon, is known to be allergenic and is one of the most common fungi worldwide. In a study conducted with 399 school children in Australia, Downs *et al.* (22) reported that *Alternaria* allergens contributed to severe asthma in regions where exposure to the fungus was high. It is of interest that *Alternaria* species were detected mostly along the coastline (Region I) in our study.

In a study performed in Ankara, Turkey (23), it was reported that the most prevalent fungal genera were of *Rhizopus* (54%), *Cladosporium* (14.3%), *Penicillium* (12.4%) and *Alternaria* (4.7%). The most abundant genera isolated in the Belgrad Forest near the Marmara Sea in Turkey by Çolakoğlu (24) were *Aspergillus* (33%), *Penicillium* (19%), *Cladosporum* (17%) and *Rhizopus* (9%). Elsewhere, in a study performed in outdoor air in Riyadh (25) it was reported that the genera of *Alternaria*, *Aspergillus*, *Cladosporium*, *Penicillium* and *Ulocladium* were the most common, whereas *Drechslera*, *Fusarium*, *Rhizopus* and *Stachybotrytis* species were minor components or else sporadic.

In the present study, although some degrees of seasonal variations of the major genera were detected, the most notable ones were Autumun the *Aspergillus* and *Alternaria* genus in that they were in higher numbers in the Autumun and in Summer, respectively.

Rainfall and relative humidity almost always have profound effects on the level of fungi spores. It has been stated that *Alternaria* levels may decrease in Winter as opposed to *Penicillium* and *Aspergillus* levels which may be high in Autumun and Spring, despite the fact that they may be found in the atmosphere all year round (25,26). Our results also showed that overall the total number of fungi colonies decrease in winter, but in contrast to the results of other studies, *Penicillium* reproduces more in Winter and Summer.

There are several methods for measurement of fungi and in one, recently airborne spores are sampled by either filtration or impaction using volumetric air samplers. This method is very useful quantitative correlation of airborne organisms, a person may be exposed to (27).

In conclusion, the present study suggests that the city of Trabzon, as in any of the costal dwellings in the region, harbors various species of fungi due to its warm and rainy climate and very rich flora. It is of significance that our findings may be of use with regard to the diagnosis and prophylaxis of allergic diseases thought to be resulting from airborne fungi, and this should be born in mind when using allergic tests the spectrum of the fungal genera examined in this region. This study may thus be of considerable assistance to scientists and clinicians working in this field in adopting preventive measures and/or selecting an appropriate antigen for diagnostic purposes.

Acknowledgement

We gratefully appreciate Professor Murat Erturk's help in writing up.

Corresponding author:

İlknur TOSUN

Karadeniz Technical University, Faculty of Medicine,

Department of Microbiology and Clinical Microbiology,

61080 Trabzon TURKEY

E-mail: ilknurt@meds.ktu.edu.tr

References

1. Kerssies A. Horizontal and vertical distribution of airborne conidia of Botrytis cinerea in a gerbera crop grown under glass. Neth J Plant Pathol 99: 303–311, 1993.
2. Burge HA, Rogers CA. Outdoor allergens. Environ Health Perspect 108: 653-9, 2000.
3. Terui T, Makino Y, Hashimoto A et al. Learning from fungus allergy in atopic dermatitis patients. Nippon Ishinkin Gakkai Zasshi, 41:157-60, 2000.
4. Akiyama K. Fungal allergy-clinical aspect. Nippon Ishinkin Gakkai Zasshi 42: 109-111, 2001.
5. Ozlu T, Can G, Torun P. TEPAP Study Group. Self-reported asthma and respiratory symptoms prevalence of adult population of Trabzon from the region of Eastern Black-Sea in Turkey. J Tuberculosis and Thorax 47:65-7, 1999.
6. Ayvaz A. Doğu Karadeniz Bölgesi'ndeki çocuklarda allerji testi (skin prick test) sonuçları. Karadeniz Teknik Üniversitesi Tıp Fakültesi (unpublished thesis study in Turkish), 2002.
7. Al-Suwaine AS, Bahkali AH, Hasnain SM. Airborne viable fungi in Riyadh and allergenic response of their extracts. Mycoses, 44: 401-4, 2001.
8. Su HJ, Wu PC, Lin CY. Fungal exposure of children at homes and schools: a health perspective. Arch Environ Health, 56: 144-9, 2001.
9. Newson, R, Strachan, D, Corden, J et al. 2000. Fungal and other spore counts as predictors of admissions for asthma in the Trent region. Occup. Environ. Med. 57, 786-92.
10. John PU. Sistemik Mantar İnfeksiyonları. In: The Merck Manual Teşhis / Tedavi El Kitabı (R. Berkow,Editor), Merck and Co. Inc (in Turkish). 1985, pp:115-121.
11. Bennett JE. Aspergillus species. In: Principles and Practice of Infectious Diseases (Eds. GL MANDELL, JE BENNETT, R DOLIN).Churchill Livingstone Inc, New York. 1995, pp: 2306-11.
12. Sugar AM. Agents of mucormycosis and related species. In: Principles and Practice of Infectious Diseases (Eds. GL MANDELL, JE BENNETT, R DOLIN) Churchill Livingstone Inc, New York 1995, pp:2311-21.
13. Asan A, Sen B, Sarıca S. Airborne fungi in urban air of Edirne city (Turkey). Biologia, 57: 59–68, 2002.
14. Savino E, Caretta G. Airborne fungi in an Italian rice mill. Aerobiologia, 8: 267–274, 1992.

15. Rosas I, Calderon C, Ulloa M et al. Abundance of Penicillium CFU in relation to urbanization in Mexico city. Appl Environ Microbiol, 59: 2648–265, 1993.

16. Smith G. An Introduction to Industrial Mycology. Edward Arnold Ltd., London. 1971, pp.117-134.

17. Larone DH. Medically Important Fungi. ASM Press, Washington DC. 1993, pp:86-169.

18. Fisher F and Cook NB. Fundamentals of Diagnostic Mycology. W.B. Saunders Company, Philadelphia. 1998, pp:36-100.

19. Soylu N. Trabzon Merkez ilçede kültüre alınmış topraklarla kültüre alınmamış toprakların mikrofungus florası. KTÜ Fen Bilimleri Ens Yük Lis tezi, Trabzon, (in Turkish) 1997, pp:64-66.

20. Shen HD, Han SH. Characterization of allergens of Penicillium and Aspergillus species. J Microbiol Immunol Infect 31: 141-5, 1998.

21. Peat JK, Tovey E, Mellis C M et al. Importance of house dust mite and Alternaria allergens in childhood asthma-an epidemiologic-study in two climatic regions of Australia. Clinical and Exper Allergy 23: 812–820, 1993.

22. Downs SH, Mitakakis TZ, Marks GB et al. Clinical importance of Alternaria exposure in children. Am J Respir Crit Care Med, 164: 455-459, 2001.

23. Mete E, Özkaragöz F, Cerrahoğlu K et al. Ankara'nın dört semtinde havanın 6 aylık fungal florası. J The New Medicine 18: 197-201 (in Turkish), 2001.

24. Çolakoğlu G. Airborne fungal spores in the Belgrad forest near the city of İstanbul (Turkey) in the year 2001 and their relation to allergic diseases. J Basic Microbiol 5: 376-384, 2003.

25. Al-suwaine AS, Bahkali AH, Hasnain SM. Seasonal incidence of airborne fungal allergens in Riyadh, Saudi Arabia. Mycopathol 145: 15-22, 1999.

26. Al-Doory Y. Air-borne fungi. In: Mouldy Allergy (Eds: Y Al-Doory, JF Domson,) Lea and Febiger, Philadelphia, 1984, pp. 27.

27. Tovey ER, Green BJ. Measurement of environmental fungal exposure. Med Mycol 43 suppl 1: 76-70, 2005.