Airborne Fungi in the Atmosphere in Beyazıt Square, Istanbul, Turkey

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Abstract

This study aimed at determining the airborne fungal concentration and genera/species diversity (especially potential allergens and opportunist pathogenic species) in Beyazit Square, Istanbul, where people congregate heavily, between September 2012 and August 2013. Air samples were collected each month using a volumetric air sampling device. Dichloran glycerol 18 agar (DG-18) and malt extract agar (MEA) were used for isolation, and the traditional morphologic diagnosis method enabled naming the fungal isolates at genera and species levels. The average minimum and maximum fungal concentrations in the DG-18 were found to be 10–705 CFU/m^3 and in the MEA, 20–710 CFU/m^3. The highest fungal concentrations were recorded in the fall season. The most populated airborne fungi were *Cladosporium*, *Penicillium*, and *Alternaria*, but the most isolated fungal species were, in descending order, *Cladosporium sphaerospermum*, *Alternaria alternata*, and *Penicillium brevicompactum*. Many fungal species that can cause asthma and allergic respiratory tract infections were also isolated. For people sensitive to fungal spores, the determination of fungal flora in their locality is very important. This study's results might play a leading role in providing support to existing knowledge, as well as in human protection and treatment.

Keywords—Airborne fungi, *Alternaria*, *Cladosporium*, Istanbul, volumetric air sampling.

1 Introduction

Fungi are eukaryotic and (mostly) saprophyte micro-organisms. Fungal spores and hyphae are constantly present in the air all over the world and grow in a wide range of habitats such as terrestrial, aquatic, and atmospheric environments [1]. Meteorological factors (such as wind, humidity, temperature, and rainfall), geographical location, air pollution, vegetation, and human activities (such as agriculture) can affect the concentration and composition of fungal spores in the atmosphere [2].

Alternaria, *Aspergillus*, *Cladosporium*, and *Penicillium* are the most dominant fungal genera in outdoor environments. Some of these genera are recognized outdoor allergens, importantly causing allergic diseases and asthma. It has also been reported that more than 80 genera are associated with allergic respiratory symptoms [3]. About 30% of the world's population is affected by different allergy problems, about 30% of which are caused by fungal spores [4]. Mapping fungal spores in the local geographic region will help clinicians to diagnose and treat allergic

illnesses caused by fungi and will help people with suppressed immune systems take necessary precautions [4,5]. However, there is limited knowledge about the species and number of airborne fungi in the atmosphere of Istanbul [6–8].

The main campus of Istanbul University is located at Beyazıt Square in Fatih, in the center of both the historical peninsula of Istanbul and the city's main transportation hub. This area was selected as the sampling area due to its high human population density. The aims of this study were (1) to determine the concentration of culturable airborne fungi in the atmosphere of Beyazıt Square, Istanbul, (2) to identify the mycobiota, especially the potential allergic and opportunistic fungal pathogens, and (3) to investigate the relationship between physical meteorological parameters and airborne fungi.

2 Material and Methods

2.1 Sampling area

The city of Istanbul, the most populated city in the country, is located in the northwest of Turkey, north of

the Marmara Sea. Beyazıt Square at the center of the historical peninsula is located in the city's Fatih district. The geographic location coordinates for Beyazit Square are latitude: 41° 0′' 26.01° N and longitude: 28° 6′ 51.75° E (Figure 1).

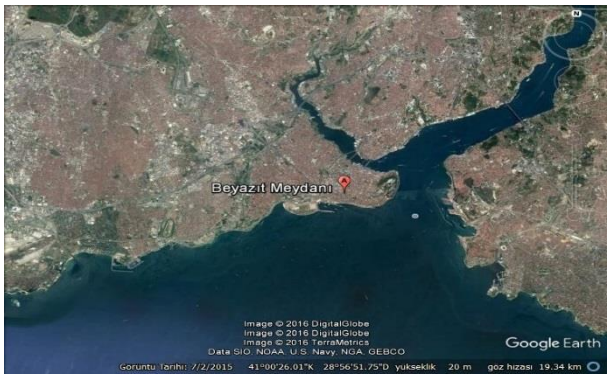

Figure 1. Map of Beyazıt square in the Fatih district, Istanbul

The trees found in the square's surroundings include *Pinus pinea* L., *P. pinaster* Ait., *P. nigra* Arnold, *P. brutia* Ten., *Cedrus deodara* (Roxburg) G. Don, *Cedrus libani* A. Rich., and *Platanus orientalis* L. During the sampling date period, the annual mean temperature was 15.9 °C, the annual mean relative humidity was 70.27 %, and the annual mean rainfall was 4.41 mm. Climatic data was obtained from the Republic of Turkey Ministry of Forestry and Water Affairs and the Turkish State Meteorological Service.

2.2 Sampling methods

Air samples were collected over a one-year period at one-month intervals (performed 12.00–13.00) between September 2012 and August 2013 using a volumetric air sampler (HiAirflow, HiMedia). The air sampler was used to collect 100 L of air for one min. at 1.5 m above ground level [9]. It was operated three times per two isolation media (dichloran glycerol 18 agar [DG-18] and malt extract agar [MEA] with streptomycin) in the sampling area. Samples were then transferred to the laboratory within 30 min. During the sampling, temperature and relative humidity values were recorded using a digital recorder (TM Instruments).

2.3 Mycological analysis

DG-18 and MEA with streptomycin were used for the isolation and enumeration of airborne fungi (AF), and the plates were incubated for up to 10 days at 25 °C [2]. After incubation, the colonies were counted and calculated using CFU/m^3 for the air samples. The fungal isolates were subcultured on MEA slants.

2.4 Identification

Identification of the fungi was based on their macro and microscopic characteristics. Fungal isolates belonging to the *Dematiaceous Hyphomycetes* group were inoculated into the MEA, and potato dextrose agar (PDA) plates were incubated for 7–14 days at 25 °C [10,11]. Fungal isolates belonging to the genus *Penicillium* and teleomorphic state *Talaromyces* were inoculated into Czapek yeast autolysate agar (CYA), 25% glycerol nitrate agar, and the MEA plates were incubated for 7–14 days at 25 °C. Two CYA plates were also incubated for 5 days, one at 5 °C and one at 37 °C [12]. Fungal isolates belonging to the genus *Aspergillus* were inoculated into CYA, Czapek-Dox agar, and CYA with 20% sucrose. The MEA plates were incubated for 7 days at 25 °C and one CYA plate at 37 °C [13]. At the end of the incubation period, the macroscopical colony size (mm), the colony shape, colony color (top and bottom), exudation, and pigmentation of the co-

lonies in the plates and their microscopic characteristics were examined by a stereomicroscope and a light microscope (Table 1). Lacto-cotton blue was used as the mounting media for the determination of micromorphological structures of the fungi [14]. The fungal isolates were then identified to genus and species level according to internationally accepted manuals [10–13]. Fungal names were standardized according to the Index Fungorum website and Hawsksworth et al. [15,16].

Table 1. The general macroscopic and microscopic identifying characteristics of the fungi

Macroscopic & Microscophic characteristcs	**Media x/ x°C**
Colony (diameter/color)	
Mycelium	
Exudate	
Reverse	
Soluble pigment	
Cleistothecia/Sclerotia/Gymnothecia	
Stipe (length/width/surface texture)	
Conidia (length/shape/color/ surface texture)	
Vesicle (diameter/shape)	
Penicilli type:	
Seriation: Uniseriate/Biseriate	
Cleistothecia/Sclerotia/Gymnothecia (diameter/shape/color)	

2.5 Statistical analysis

The mean and standard deviations of AF concentrations in the two media were calculated. The Wilcoxon signed-rank test was employed to detect statistically significant changes in the mean AF in the two different media. Significant differences are reported at $P<0.05$. Statistical analyses were carried out using Spearman's correlation coefficient test (IBM SPSS, version 21, USA). The test was used to examine the relationship between AF concentrations and selected parameters.

3 Results

3.1 Outdoor airborne fungal concentrations

The concentration of airborne fungi in the outdoor samples ranged from 10 to 705 CFU/m^3 on DG-18 and from 20 to 710 CFU/m^3 on MEA. The mean concentrations of outdoor airborne fungi were 213.33 and 155.41 CFU/m^3 on DG-18 and MEA, respectively (Table 2).

Table 2. The monthly distributions of airborne fungal concentrations

MONTH	**CFU/m^3**	
	DG-18	**MEA**
January	80 ± 4.2	30 ± 1.4
February	10 ± 0	25 ± 0.7
March	50 ± 4.2	25 ± 2.1
April	110 ± 2.8	100 ± 5.6
May	135 ± 2.1	155 ± 7.7
June	295 ± 4.2	160 ± 1.4
July	215 ± 0.7	115 ± 4.9
August	205 ± 2.1	150 ± 0
September	360 ± 4.2	95 ± 4.9
October	705 ± 73.5	710 ± 22.6
November	375 ± 6.3	280 ± 1.4
December	20 ± 0	20 ± 1.4
TOTAL (n = 12)	2560	1865
MEAN (n = 12)	213.33	155.41

DG-18: Dichloran glycerol 18 agar, MEA: Malt extract agar, CFU/m^3: Colony forming unit per cubic meter, ±: Standard deviation

The Wilcoxon signed-rank test was used to compare the differences between the DG-18 and the MEA airborne fungal concentrations. The total airborne fungal concentrations grown on DG-18 were significantly higher than those on MEA (P=0.000). On DG-18, the lowest fungal concentration was detected in February (10 CFU/m^3) and the highest in October (705 CFU/m^3). On MEA, the lowest fungal concentration was detected in December (20 CFU/m^3) and the highest in October (700 CFU/m^3).

3.2 Measurements of temperature and relative humidity

The lowest and the highest temperatures were 6.4 ˚C (December) and 38.5 ˚C (September). The lowest and the highest relative humidity levels were recorded at 13% (November) and 57% (June) (Table 3).

Table 3. The monthly records of the physical parameters of the outdoor air

MONTH	**˚C**	**%**
January	10.5	49.9
February	10.8	41.2
March	14	28.9

April	17.8	41.7
May	14.1	49
June	22.7	57
July	29.3	51.6
August	36.5	45.7
September	33.9	43.9
October	38.5	36.8
November	17.9	13
December	6.4	27

°C: Celsius-temperature, %: Relative humidity

While the statistical analyses indicated no correlation between airborne fungal concentration and relative humidity (P=0.601, r=0.168 for MEA) (P=0.779, r=0.091 for DG-18), there was a positive correlation between airborne fungal concentration and temperature (P=0.014, r=0.683 for MEA) (P=0.02, r=0.804 for DG-18).

3.3 Identification of airborne fungal isolates

In total, 221 MF strains were isolated (120 from DG-18 and 101 from MEA), and a total of 18 genera and 40 species were identified during the one-year sampling period (Table 4).

Table 4. Identified airborne fungi and the distribution in different media and months

Fungal identification	Isolation media	Isolation month	Total CFU
**Acremonium zonatum*	D	5	10
Alternaria sp.	D, M	2, 6–8	40
Alternaria alternata	D, M	1, 3–5, 7–11	620
**Alternaria atra*	D, M	6, 7	10
**Alternaria citri*	D, M	2, 6–8	50
Aspergillus sp.	D, M	10	5
**Aspergillus chevalieri*	M	4	10
Aspergillus flavus	M	10	5
Aspergillus niger	D, M	5, 9–11	110
Aspergillus ochraceus	D	10	10
**Aspergillus sydowii*	D	1	5
**Aspergillus versicolor*	D	12	5
**Chaetomium globosum*	D	3, 7, 8	70
Cladosporium sp.	D, M	6, 8	45
Cladosporium cladosporioides	D, M	3, 6, 9, 10	225
Cladosporium herbarum	D, M	1, 8	20
Cladosporium sphaerospermum	D, M	1, 2, 4–12	900
**Cladosporium spongiosum*	D, M	10	375
**Cladosporium variabile*	D, M	6, 7	40
**Curvularia affinis*	M	7	5
**Curvularia hawaiiensis*	D, M	1, 5–7, 11	185
**Didymella pomorum*	D	1, 10	10
**Dreschlera sp.*	D	9	20
Fusarium sp.	M	11	10
**Fusarium concolor*	M	11	20
**Fusarium culmorum*	D, M	7, 10	30
Fusarium oxysporium	M	8	10
Penicillium sp.	D, M	5, 11	215
**Penicillium aethiopicum*	M	4	5
Penicillium brevicompactum	D	10	410
Penicillium chrysogenum	D, M	2, 3, 7, 12	25
**Penicillium citrinum*	D	12	10
**Penicillium corylophilum*	M	4, 9	10
**Penicillium griseofulvum*	D	9	10
**Penicillium simplicissimum*	M	7	5
**Penicillium solitum*	D	10	35
Phoma sp.	D, M	5–7, 10, 12	85
**Phoma glomerata*	D, M	4–7	70
**Rhizopus stolonifer*	M	5, 9	30
**Scytalidium lignicola*	D	6	5
**Stemphylium globuliferum*	D, M	1, 4	15
**Talaromyces flavus*	D	4	10
**Torulomyces indicus*	D, M	5–7	20
**Trichoderma longibrachiatum*	M	7	15
**Trichoderma pseudokoningii*	M	7	5
Ulocladium sp.	D, M	8, 9	155
**Ulocladium chartarum*	M	10	15
**Ulocladium consortiale*	D, M	10	45
Non-sporulating	D, M	1, 6, 7, 11	65

fungi			

D: Dichloran glycerol 18 agar, M: Malt extract agar, CFU: Colony forming unit, *: first record in Istanbul

The highest numbers of species by genus were *Penicillium* (8 species), *Aspergillus* (6 species), *Cladosporium* (5 species), *Fusarium* (3 species), *Alternaria* (3 species), *Curvularia* (2 species), *Trichoderma* (2 species), and *Ulocladium* (2 species). The lowest numbers of species by genus were *Acremonium*, *Chaetomium*, *Phoma*, *Rhizopus*, *Scytalidium*, *Stemphylium*, *Talaromyces*, and *Torulomyces*. The most commonly isolated microfungal genera were *Cladosporium*, *Penicillium*, and *Alternaria* (1605 CFU, 725 CFU, and 720 CFU, respectively). The most commonly identified AF were *Cladosporium sphaerospermum* (900 CFU), *Alternaria alternata* (620 CFU), and *Penicillium brevicompactum* (410 CFU). Nonsporulating fungi (NSF) were detected in the outdoor air at 65 CFU.

More fungal genus/species diversity was detected in the months of July (15 species and 9 genera), October (10 species and 6 genera), and May (8 species and 9 genera) than the rest of the months. In July, the most isolated fungal genera were identified as being *Alternaria* and *Cladosporium*. *Pleosporaceae*, *Trichocomaceae*, *Hypocreaceae*, *Nectriaceae*, *Cladosporiaceae*, *Gnomoniaceae*, and *Chaetomiaceae* were also detected. In October, the most prevalent fungal genera were identified as being *Cladosporium* and *Penicillium*, while members of *Pleosporaceae*, *Trichocomaceae*, *Nectriaceae*, *Cladosporiaceae*, and *Gnomoniaceae* were also detected. In May, the most dominant fungal genus was identified as being *Alternaria*, with members of *Pleosporaceae*, *Trichocomaceae*, and *Hypocreales* also detected.

4 Discussion

The present study will contribute to the knowledge of the volumetric levels of culturable airborne fungi in the urban air of Istanbul. Although a similar study was conducted in Istanbul, it sampled different districts and used the gravimetric sampling method [7,8]. It was carried out over six months in five different districts of Istanbul where the factories and human population are high and reported that the most widespread genera were *Cladosporium* and *Alternaria* [7]. Another gravimetric sampling study was carried out in Belgrad Forest, outside the city center, over the course of a year, finding that *Aspergillus* and *Penicillium* were the most populated genera [8]. Çolakoğlu took outdoor air samples from the Marmara University campus area and a crowded street over a yearlong period using a volumetric spore trap. The most widespread fungal spores were identified as those of the genus *Cladosporium*, and *Penicillium* [6]. These previous studies' results, especially the volumetric study by Çolakoğlu, are similar to the presented study [6]. Outdoor studies carried out in Turkey using the gravimetric method [1,5,7,8,17–29] have been more common than those featuring the volumetric method [4,6,30–37]. Researchers have mostly preferred Petri dishes filled with PDA, rose bengal streptomycin agar, Sabouraud dextrose agar, MEA, and rose bengal chloramphenicol agar in the gravimetric method [17–20,35,38].

Previous studies have more commonly isolated *Cladosporium*, *Penicillium*, and *Alternaria* than other fungal genera, similar to this study. *Cladosporium* was the most prevalent fungal genera identified in the outdoor air of the Turkish cities of Ankara, Çanakkale, Gemlik-Bursa, Edirne, Eskişehir, Istanbul, Karabük, Kırşehir, Konya, Manisa, and Mersin [7,22–24,26,28,29,31,34,36,37]. *Penicillium* was predominant in the atmosphere of the cities of Afyon and Trabzon [17,21], while *Alternaria* was the most isolated in the cities of Kırklareli and Tekirdağ [1,25].

Istanbul's climate is changeable, somewhere between the Black Sea climate and the Mediterranean climate, and is therefore generally mild in character. Summers in Istanbul are hot and humid, winters cold and rainy but rarely snowy. The present study determined that the highest fungal concentration was in October, while the highest temperature was also measured in October. In fact, in the fall season (i.e., September, October, and November) the temperature is particularly suitable for fungal development when vegetation is dense; therefore, with precipitations, it is not surprising to find fungal spore concentrations being high due to seasonal degradations. In contrast, in winter, (i.e., December through February), the temperature is too low for fungal development and, since there is less food available, fungal concentration is at its minimum. Statistical comparisons showed that temperature and fungal counts correlated (P=0.014, r=0.683 for

MEA) (P=0.02, r=0.804 for DG-18). Menteşe et al., Kalyoncu & Ekmekçi, and Asan et al. also reported the same results in their studies [1,34,38]. Although the distribution of fungi is related to the temperature, human activity in the study area may also cause an increase in the distribution.

To our knowledge, different from the other volumetric outdoor air-sampling studies in Turkey [4,34,35,37], DG-18, which is suitable for isolation of xerophilics, was used for the first time. MEA was also chosen as it identifies a wide fungal spectrum, including hydrophilic and fast-growth fungi. Consequently, the total genera/species diversity could be determined in a more realistic fashion. In fact, fast-growing hydrophilic *Trichoderma* spp. was isolated only from MEA, whereas xerophilics helped in the isolation of some *Aspergillus* and *Penicillium* members from DG-18. In addition, this study is different from those conducted in several districts of Istanbul in that, for the first time, we have reported the *Dreschlera*, *Scytalidum*, and *Talaromyces* genera and 28 species (Table 4) [6,7].

Many of the fungal species (such as *Aspergillus niger*, *Cladosporium cladosporioides*, *C. herbarum*, *Chaetomium globosum*, *Fusarium oxysporum*, *Penicillium brevicompactum*, *P. chrysogenum*, *Phoma glomerata*, and *Trichoderma longibrachiatum*) listed in Table 4 have been reported as antigen sources of pulmonary and bronchial infections (such as fungal sinusitis and hypersensitivity pneumonitis) and cutaneous infections [39–42]. The highest fungal count was observed to be *Cladosporium sphaerospermum* and *Alternaria alternata*, which were isolated almost every month. *C. sphaerospermum* and *A. alternata* are known as being the most widely distributed fungi in outdoor air. While *A. alternata* is mainly associated with asthma, allergic rhinosinusitis, and allergic bronchopulmonary mycosis, *Cladosporium* sp. is associated with respiratory allergies [43–45]. There is a case report on *C. sphaerospermum*, detecting it in human intrabronchial lesions. Apart from this, it is also known as an agent of cutaneous infections [46]. Although people are generally exposed to fungal allergens in indoor environments, outdoor exposure generally has more of a relationship with mold sensitization and respiratory disease [47]. European Community respiratory health research has found that *A. alternate* is associated with a greater severity of asthma than other environmental aeroallergens and also that *Alternaria* allergenic proteins are commonly associated with the development of *Cladosporium*, *Penicillium*, and *Aspergillus* allergies [45]. A few epidemiologic studies demonstrated the prevalence of mold sensitization in Turkey [31,33,37,48,49]. Inal et al. reported a correlation between the symptoms of children with asthma and/or rhinitis (monosensitized) and outdoor airborne fungi [33]. They reported that, although outdoor *Cladosporium* spores were higher in number than *Alternaria* spores, the prevalence of *Alternaria* sensitization was higher than was *Cladosporium*. A high number of spores may not be directly related to mold sensitivity. Differences in environmental conditions and genetic predisposition, and differences in the number of respiratory mold spores may affect individual mold sensitivities. As a result, researchers have advised that long-term studies should be conducted to help to explain the relationship between the concentration of outdoor molds and sensitization [31,37].

5 Conclusion

Using two different media, this study has revealed the culturable airborne fungal concentration and diversity of Beyazit Square, Istanbul. The highest fungal counts were obtained in the fall season, covering September, October, and November. After a sampling period of one year, the most isolated species from the air were *Cladosporium*, *Alternaria*, and *Penicillium*. It is important to control fungal concentrations in order to protect people with allergic complaints. Those with allergies to fungal spores are recommended not to leave home unless they need to, since organic plant waste levels are high and thus fungal development is higher. Physicians are advised to note the range of months described above when considering their treatments.

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