

The statistical investigation on airborne fungi and pollen grains of atmosphere in Izmir-Turkey

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Abstract This study aims to investigate the differences in the concentrations of airborne fungi and pollens between the towns located in the province of Izmir and to determine the factors contributing to these differences. Five stations in each of four towns (Buca, Konak, Bornova, and Karsiyaka) were randomly selected as the research areas. Fungus (cfu/m³) and pollen counts (cm²/pollen count) in the air samples collected from each station between June 2003 and May 2004 were measured. The results revealed that whereas Karsiyaka had the highest fungus concentration (521.33±777.1), Buca and Bornova had the lowest concentration (482.67±308.44). The mean fungus concentration in the province of İzmir was 501.5±486.7. Pollen concentration was the

highest in Konak (486.67±839.06) and the lowest in Bornova (369.83±551.13). Fungus and pollen concentrations revealed no difference between the towns ($p>0.05$). The relationship between pollen-fungus concentrations and temperature-dust-humidity-sulphur-dioxide was investigated but it was found statistically insignificant ($p>0.05$). As a result of regression analysis, it was determined that correlation of atmospheric parameters had no effects on pollen and fungus concentrations ($p>0.05$).

Keywords Agar medium · Airborne fungi · Gravimetric method · Pollen grains

Introduction

The atmosphere is characterized by high light intensities, extreme temperature variations, low concentrations of organic matter and a scarcity of available water, making the atmosphere a hostile environment for microorganism and a generally unsuitable habitat for microbial growth. Nevertheless, substantial numbers of microorganisms are found in the lower regions of the atmosphere (Mahdy and El-Sehravi 1997). These microorganisms do not grow within the atmosphere but transported into the atmosphere from aquatic and terrestrial habitats. Many species, representing the various microbial groups, have been isolated from the atmosphere and have an important

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role in determining of out-door air microbial concentrations (Mezzari et al. 2002; Pepeljnjak and Segvic 2003).

Atmospheric surveys of airborne fungi and pollen grains are being carried out all over the world in order to deduce the number and frequency of various aeroallergens. Fungi live everywhere and they originate from different environments such as soil, plants and water. Fungal contamination of indoor and outdoor air environments comes from a variety of sources. Airborne fungal spore numbers and types vary with the time of the day, geographical region, air pollution, meteorological parameters, presence of local source and vegetation types (Al-Subai 2002; Asan et al. 2002; Lin and Li 2000; Pepeljnjak and Segvic 2003). The determination of airborne fungi and pollen concentration in a region is very important. Pollens and fungi cause allergic reactions and asthma on especially atopic people (Burge 2002; Burge and Rogers 2000; Flückiger et al. 2000; Myszkowska et al. 2002; Sterling 1998).

Aeropalinological studies in Turkey have been conducted in Marmara, Mediterranean, Aegean, Black Sea Regions and Central Anatolia (Bıçakçı et al. 2000, 2002, 2003, 2004; Çelik et al. 2005; Gemici et al. 1989; Güvensen and Öztürk 2002; İnce 1994; İnceoğlu et al. 1994; Kaplan 2004; Kaya and Aras 2004; Pehlivan 1995; Pınar et al. 1999). This is the first research conducted on determining airborne fungal flora concentration in the province of İzmir. Airborne fungi researches performed in Turkey are very limited and were conducted in certain provinces; Edirne (Asan et al. 2002; Sarica et al. 2002; Sarica-

Ökten et al. 2005; and Şen and Asan 2001), Eskişehir (Asan et al. 2004; Atik and Tamer 1994), İstanbul (Asan et al. 2003; Çolakoğlu 1996a, b, c and 2003), Bursa (Şimsekli et al. 1999) and Ankara (Okuyan et al. 1976; Şakıyan and İnceoğlu 2003; Yuluğ and Kuştımur 1977). In İzmir, the only other research was accomplished in 1991, but it was conducted only with the gravimetric method. Therefore, we aimed to determine the concentrations of airborne fungi and pollens in four towns (Buca, Konak, Bornova, and Karşıyaka) in the province of İzmir, the third biggest city in Turkey. The determination of airborne fungal concentration in an area is important because some of them can be aeroallergens. Thus, this study will help to take necessary precautions against allergies seen in people who are allergic to pollens and fungi in accordance with the recommendation by medical authorities.

Materials and methods

Sampling sites

İzmir metropolitan area lies between 37° 49'–28° 29' N; 26° 19'–28° 39' E in the western part of Turkey. İzmir, which is located on the Aegean coast of Turkey, has Mediterranean climatic conditions, *Pinus nigra* Arn. ssp. *pallasiana* (Lamb) Holmboe are dominant over the elevated parts of the mountainous regions extending from the east to the west of the city. *Pinus brutia* Ten. are present between the elevations of 0–600 m, maquis elements such as *Quercus*

Table 1 Pollen counts (pollen grains/cm²) obtained at the stations in the towns in the province of İzmir

Pollen counts (pollen grains/cm ²)	A Buca	B Konak	C Bornova	D Karşıyaka	Total pollen
06-07/2003	257	395	331	315	1,298
08-09/2003	350	257	330	277	1,214
10-11/2003	79	74	81	88	322
12-01/2003	14	18	12	13	57
02-03/2004	9	5	11	9	34
04-05/2004	1,540	2,171	1,454	2,022	7,187
TOTAL	2,249	2,920	2,219	2,724	
Mean	374.83	486.67	369.83	454	
Standard Deviation	587.14	839.06	551.13	779.20	

A, B, C, D: administrative districts (A: Buca, B: Konak, C: Bornova, D: Karşıyaka).

coccifera L., *Q. cerris* var. *cerris* L., *Q. infectoria* Oliver. ssp. *boissieri* (Reuter) O. Schwarz., *Q. pubescens* Willd., *Pistacia lentiscus* L., *P. terebinthus* L., *Olea europaea* L. var. *sylvestris* (Miller) Lehr., *Phillyrea latifolia* L., *Calicotome villosa* (Poiret) Link., *Spartium junceum* L., *Cercis siliquastrum* L., *Ceratonia siliqua* L., *Crataegus monogyna* Jacq., *Myrtus communis* L., *Laurus nobilis* L., *Arbutus unedo* L., *A. andrachne* L., *Erica arborea* L., *Vitex agnus-castus* L., *Rosa canina* L., *Jasminum fruticans* L. and *Paliurus spina-christii* Miller are present between the elevations of 0–750 m. Phrygana elements such as *Sarcopoterium spinosum* (L.) Spach,

Asphodelus aestivus Brot., *Coridothymus capitatus* (L.) Reichb., *Lavandula stoechas* L., *Cistus creticus* L. and *Origanum onites* L. are the plant taxa widely present over the lower elevations of the mountainous regions.

Pollen sampling and isolation methods

In order to place airborne pollen counts of plant taxa for each month in an annual calendar, gravimetric method was used. In this method which was conducted with Durham Sampler and which is used to determine the amount of pollens per cm² falling due

Table 2 Fungus counts (cfu/m³ fungus) obtained at the stations in the towns in the province of Izmir

Descriptive statistics				
Dependent variable: fungus				
Town	Station	Mean	Standard Deviation	Number
A	Station 1	523,3333	392,00340	6
	Station 2	433,3333	209,25264	6
	Station 3	390,0000	342,63683	6
	Station 4	610,0000	336,98665	6
	Station 5	456,6667	296,49058	6
	Total	482,6667	308,44363	30
B	Station 1	503,3333	464,31311	6
	Station 2	513,3333	319,16558	6
	Station 3	586,6667	311,04126	6
	Station 4	433,3333	240,55491	6
	Station 5	560,0000	248,55491	6
	Total	519,3333	308,02970	30
C	Station 1	463,3333	372,75551	6
	Station 2	550,0000	597,96321	6
	Station 3	410,0000	283,61946	6
	Station 4	380,0000	226,62745	6
	Station 5	610,0000	592,99241	6
	Total	482,6667	420,23748	30
D	Station 1	523,3333	301,57365	6
	Station 2	226,6667	208,86998	6
	Station 3	443,3333	599,78885	6
	Station 4	1043,3333	1572,43336	6
	Station 5	370,0000	263,43880	6
	Total	521,3333	777,06669	30
Total	Station 1	503,3333	361,76700	24
	Station 2	430,8333	367,74184	24
	Station 3	457,5000	385,26388	24
	Station 4	616,6667	810,44629	24
	Station 5	449,1667	364,72642	24
	Total	501,5000	486,67738	120

A, B, C, D: administrative districts (A: Buca, B: Konak, C: Bornova, D: Karşıyaka).

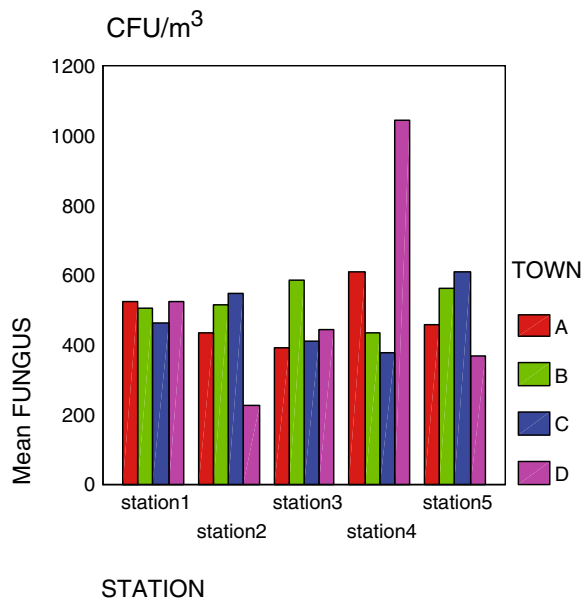


Fig. 1 Histogram on the fungus counts(CFU/m³) of the province of İzmir

to gravity, slides were replaced with the new once every month and data on pollen were evaluated as monthly mean values. For this purpose a Durham samplers was mounted on the roof of a 20 m high building in each district. The slides smeared with glycerin-jell stained with safranin were changed monthly. For identification B-3000 I binocular “Prior” laboratory microscope was used and counting was done on a 20×20 mm (4 cm²) area of the slide. The data was then calculated on 1 cm² bases (Charpin et al. 1974; Louveaux 1970; Moore et al. 1991).

Fungi sampling and isolation methods

In this study, five stations in each of four towns (Buca, Konak, Bornova, Karşıyaka) in the province of İzmir were randomly selected as the research centers. Samples from 20 stations were collected by using volumetric impactor Merck’s MAS 100 air sampler, a volumetric sampling method (Andersen 1958; Moring et al. 1983), since these months represent similar climatic characteristics. Mean fungus concentrations in the air samples collected from each station during 06-07/2003, 08-09/2003, 10-11/2003, 12-1/2003, 02-03/2004, and 04-05/2004 all of which have similar seasonal characteristics were shown as cfu/m³. The samples were taken in the afternoon (12⁰⁰–13⁰⁰)

at the height of 1.40 m above the ground (Asan et al. 2002, 2003, 2004). The sampling time was always at the same time of the day. During the 12 months research period, every third week of each month, 100 l of air was sucked into each petri plates.

The Rose-Bengal Chloramphenicol Agar Base (Oxoid CM549) and Chloramphenicol Selective Supplement (Oxoid SR78) was used for isolation of the fungi. The agar medium was made from: mycological peptone 5.0 g, glucose 10.0 g, di-potassium phosphate 1.0 g, magnesium sulphate 0.5 g, Rose- Bengal, 0.05 g, agar 15.5 g, sterile pure water 1,000 ml. Chloramphenicol Selective Supplement contains 0.05 g chloramphenicol, equivalent 0.1 g/lof medium. After incubation at 27±1°C in standard petri plates (Ø 90 mm), the concentration of fungi was calculated as CFU (colony forming units)/m³.

Statistical analyses

Firstly, statistics based on fungus-pollen counts were measured and then the graphics were prepared. Secondly, it was determined whether the counts showed any differences between the towns by using analysis of variance (ANOVA). After that, the relationship between fungus-pollen concentrations and temperature-dust-humidity-sulphurdioxide was

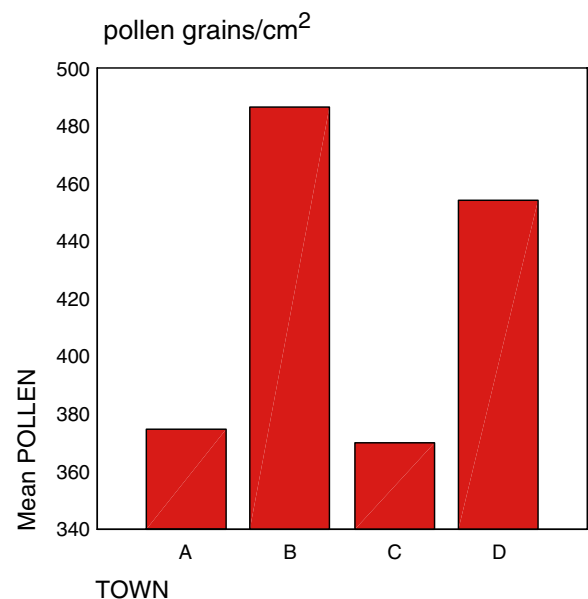


Fig. 2 Histogram on the pollen counts(pollen grains/cm²) of the province of İzmir

Table 3 Variance analysis table of fungus and pollen counts in the province of Izmir

Source	sd(Fungus)	Mean squares (Fungus)	sd(Pollen)	Mean squares (Pollen)	P(Fungus)	P(Pollen)
Town	3	14,207.78	3	20,300.11	0.981	0.988
Error	116	242,612.99	20	489,910.95		

investigated with Pearson correlation analysis. Later, the effects of atmospheric parameters on pollen and fungus concentrations were investigated with regression analysis. In the evaluation of the analyses, “ α ” was taken into account as “0.05” (Daniel 1983; Dawson and Trapp 2001; Dunn and Everitt 1995; Motulsky 1995; Steel et al. 1997).

Results and discussion

The present study will contribute to our knowledge of the levels of airborne fungi in the urban air of İzmir for the first time. Although a similar study (Ayata et al. 1991) was undertaken in this city, the method was different and the Petri Plate Gravitational Method for sampling fungi from air was used. In our study, we used the volumetric sampling method, which is official and universally accepted bioaerosols sampling method according to Andersen (1958), Morring et al. (1983) and Chen et al. (1998).

Sampling time was between 12⁰⁰ and 13⁰⁰ in our study. Levetin and Horner (2002) pointed out that the spores of many asexual fungi peaked in the air in early and mid-afternoon (Asan et al. 2004). The samples were taken at the height of 1.40 m above the

ground at which human beings start to have difficulty breathing (Gorry and Dutkiewicz 2002).

Total pollen counts of the towns are shown in Table 1. The highest pollen concentration was in Konak (486.67 ± 839.06), whereas it was the lowest in Bornova (369.83 ± 551.13) (Fig. 2).

At all stations, total pollen concentrations were 7,187 per cm² during April–May/2004, 1,298 per cm² during June–July/2003, 1,214 per cm² during August–September/2003, 322 per cm² during October–November/2003, 57 per cm² during December–January/2003 and 34 per cm² during February–March/2004 (Table 1). Since, April–May/2004 is the period for the pollination of arboreal and non-arboreal and June–July/2003 and August–September/2003 for the pollination of only non-arboreal species, airborne pollen concentrations vary. October–November/2003, December–January/2003 and February–March/2004 are the periods during which pollination is very low; thus, airborne pollen concentrations are low too. Aeropalynological studies conducted in İzmir and in the cities around İzmir, also report that airborne pollen concentrations are the highest during April–May/2004 (Bıçakçı et al. 2000, 2003; Çelik et al. 2005; Gemici et al. 1989; Güvensen and Öztürk 2003). It was found that Karşıyaka had the highest fungus concentration

Table 4 Atmospheric parameters of the province of İzmir

Date	Temperature	Dust	Humidity	(SO ₂)	Izmir (Fungus)	Izmir (Pollen)
06-07/2003	27,5	33	53,5	23	27.35	1796.75
08-09/2003	29	41	53,2	14	32.45	303.5
10-11/2003	20,4	35	63,6	12	14	80.5
12-01/2003	10,3	63	74,3	55	17.45	14.25
02-03/2004	9,4	51	64,9	58	36.65	8.5
04-05/2004	16,6	23	60,6	18	22.55	324.5
Pearson Correlation	0.840	0.889	0.359	0.631		
(p) (Fungus)	$r = 0,107$	$r = 0,067$	$r = 0,459$	$r = 0,251$		
Pearson Correlation	0.190	0.413	0.176	0.552		
(p) (Pollen)	$r = 0,619$	$r = -0,416$	$r = -0,634$	$r = -0,309$		

Table 5 Regression analysis table of fungus and pollen concentrations

	B	Standard Error	P
Fungus			
Temperature	-37.575	6.897	0.116
Dust	13.265	2.878	0.136
Humidity	-56.829	5.866	0.065
SO ₂	-2.319	2.387	0.509
Pollen			
Temperature	284.397	33.231	0.074
Dust	-111.239	13.867	0.079
Humidity	156.957	28.264	0.113
SO ₂	101.538	11.503	0.072

(521.33±777.1) whereas Buca and Bornova had the lowest concentrations (482.67±308.44). The mean fungus concentration in the province of İzmir was 501.5±486.7 (Table 2) (Fig. 1). The mean pollen concentration was the highest in Konak (486.67±839.06) and the lowest in Bornova (369.83±551.13) (Table 1) (Fig. 2). Whether the fungus and pollen counts showed any difference was determined with one-way ANOVA ($p>0.05$) (Table 3). DiGiorgio et al. (1996) reported that various meteorological factors affected the types and concentrations of airborne fungi. Among these wind velocity, relative humidity, and temperature were particularly important. Paya et al. (1984) reported that the level of sulphurdioxide seemed to have a negative influence on the spore concentration and in addition, high temperature may induce fungal concentration. The relationship between pollen-fungus concentrations and temperature-dust-humidity-sulphurdioxide was investigated with Pearson correlation analysis, but it was found statistically not significant ($P>0.05$) (Table 4). Thus, it can be assumed that changes in fungus and pollen concentrations are not related with these factors. As a result of multi-linear regression analysis, it can be said that atmospheric parameters do not to affect fungus and pollen concentrations ($p>0.05$) (Table 5).

As a result, since the number of the researches on this topic is very limited, and since the region has intensive and varying atmospheric conditions, there aren't enough established counts. However, according to American Academy of Allergy, Asthma and Immunology Records (<http://www.aaaai.org/>), on the NAB scale, daily outdoor fungus concentration of

1–6499 cfu/m³ is considered low; 6500–12.999 cfu/m³ is normal, 13.000–49.999 cfu/m³ is high and > 50.000 cfu/m³ is very high. The counts from certified sites in each state are daily released. When these fungal spore concentrations are taken into consideration, the concentrations measured throughout the year in the province of İzmir can be regarded in the lower limits of the low counts.

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