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Research Article

Airborne Spore Analysis of Karabük Atmosphere

Karabük Atmosferinin Spor Analizi

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Abstract

In order to identify types and amounts of airborne allergenic spore dispersal in the atmosphere of Karabük by gravimetric method in 2006 and 2007, two Durham samplers were situated on roof and garden of Technical Education Faculty of Karabük University between the dates January 1, 2006 and December 31, 2007. As a result of the analysis a total of 2822.2±625.01 spore/cm2 spore quantity belonging to 21 types was identified. Of this total, 1106±250.33 spore/cm² was observed in 2006 and 1716±374.68 spore/cm² was observed in 2007. Spore concentrations revealed no statistically significant differences between two samplers (t=0.1527-1.1355, p>0.05). The relationship between spore concentrations and meteorological factors was displayed by Spearman Correlation analysis. The highest quantity of fungal spores and Myxomycetes were determined in June and July. *Cladosporium, Alternaria, Ustilago*, Myxomycetes and unidentified Ascomycetes spores were recorded as dominant. In the end of this study, a two-year spore calendar was prepared.

Keywords: Karabük, Fungal spores, Myxomycetes, Meteorological factors

1. Introduction

Airborne fungal spores and myxomycetes are allergenic biological particulates found in high quantities in the atmosphere (Salvi et al. 2001, Recer 2004, Corden et al. 2003). There are several studies on the determination of the quantity of airborne fungal spores in various countries (Juozaitis et al. 1997, Waisel et al. 1997, Subai 2002, Pepeljnjak and Segviã 2002, Bianchi and Olabuenaga 2006, Herrero et al. 2006).

In Turkey, indoor and outdoor fungi studies have been made by many researches (Özkaragöz 1967, Ayata et al. 1991, Atik and Tamer 1994, Çolakoğlu 1996a, b, c, Şimsekli et al. 1999, Bıcakcı et al. 2001, Tatlıdil et al. 2001, Şen and Asan 2001, Asan et al. 2002, 2003, 2004, Sarıca et al. 2002, Şakıyan and İnceoğlu 2003, Çolakoğlu 2003, Ökten et al. 2005, Boyacıoğlu et al. 2007, Ataygul et al. 2007, Kalyoncu 2008, Menteşe et al. 2009, Bülbül et al. 2011, Docampo et al. 2011).

There are not any former studies made for Karabük Province. Therefore, the purpose of this study is to make an analysis of the allergenic fungal spores in Karabük atmosphere for the years 2006 and 2007. At the end of the study, a two-year spore calendar was produced for the atmosphere of Karabük.

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2. Materials and Methods

2.1. Site Information

Karabük Province, which has a surface area of 4,145 km² is situated in Western Black Sea Region and between the North latitudes of 40° 57′ and 41° 34′ and between the East longitudes of 32° 04′ and 33° 06′. The province has an undulating geographical structure with moderate elevations. There are some arable lands in the valley floor though not being extensive. The majority of the population is clustered in the vicinities of the valley floor (Karabük Governorship 2008).

2.2. Sampling Method

For the investigation of the allergenic fungal spores in Karabük atmosphere in the years 2006 and 2007, two Durham gravimetric samplers were used. Ground sampler was located on garden, roof sampler was mounted on the roof of a 10 m high of Technical Education Faculty Building (Figure 1). Weekly slides were inserted on the slide fixers of the Durham samplers and mounted in safranin-glycerine jelly (Woodehouse 1965). For the identification, count and photography of the spores was used Nikon Eclipse 200 trinocular investigation microscope. Counting were made on a 24x50 mm (12 cm²) area of the slide, which was calculated on 1 cm² bases (Charpin and Surinyach 1974, Bıçakcı et al. 2001). Some spore morphology book (Smith 2002) and reference preparations of the local herbaceous plants infected by fungi were utilized during the identification of the spores.

2.3. Site Climate

Since Karabük in the zone of transition from Black Sea climate to Terrestrial climate, it exhibits the characteristics of a transition type climate. The characteristics of Black Sea Climate are partially seen. But in the interior part of the city can not benefit from the humid weather of the Black Sea and is rather influenced by terrestrial climate. The amounts of total precipitation, average temperature, wind speed and relative humidity in 2006 and 2007 were given in Table 1. The prevailing wind direction was SouthWest in 2006 and West for 2007. Meteorological data of Karabük city was obtained from Goverment Meteorological Institute.

2.4. Statistical Analyses

According to spore counts, unpaired t test was made between two samplers using Graphpad on line calculator (graphpad.com) and no statistically significant differences were found (t=0,1527-1,1355, $p>0.05$). Therefore only monthly mean spore quantities and standart deviations were calculated.

For relationship between spore concentrations and meteorological parameters Spearman Rank Correlation analysis were made with two durham samplers by using SPSS 19.0 program.

3. Results

In this study, total 2822,2±625,01 spore/cm^2 belonging to 21 taxa were detected in Karabük atmosphere. Of these, 1106±250,33 spore/cm^2 were found in 2006, 1716±374,68 spore/cm^2 in 2007 (Table 2).

The percentage of two years total fungal spores was 94,04% and percents of the most frequent taxa as follows; *Cladosporium* 37.03%, *Alternaria* 30.74%, *Ustilago* 17.52%, Myxomycetes 4.50% and Ascospores 4.25% in two years (Table 2).

Monthly distributions of six dominant taxa were given below;

Cladosporium: The highest amount was observed 188,5±14,85 spore/cm^2 in July 2006, 192±14,14 spore/cm^2 in May 2007. The two years total was found as 1045±109,58 spore/cm^2 (Tables 2-4, Figures 2-4).

Alternaria: The highest concentrations were 193,5±13,44 spore/cm^2 in July 2006, 245±16,97 spore/cm^2 in 2007. Total amount was 867,5±160,51 spore/cm^2 in two years (Tables 2-4, Figures 2-4).

Ustilago: Two years total was recorded 494,5±168,98 spore/cm^2. The highest counts were 161±33,94 spore/cm^2 in June 2006, 138,5±33,23 spore/cm^2 in July 2007.

Mxomycetes: Mountly highest quantity was achieved in July both in 2006 and 2007 (37,5±3,54 spore/cm^2 in 2006, 36±28,28 spore/cm^2 in 2007, respectively) (Tables 2, 3, 4 and Figures 2-3).

Ascomycetes spores is observed starting from March. While the highest quantities were observed in March and April 2006 (10,5±2,81 spore/cm^2), highest abundance was recorded in May 2007 (36,5±6,36 spore/cm^2) (Tables 3, 4, Figure 4).

4. Discussion

Cladosporium and *Alternaria* spores were determined to be the most frequent in Karabük atmosphere, as also found to be the most frequent of all by numerous former studies in Turkey and the other countries (Şakıyan and

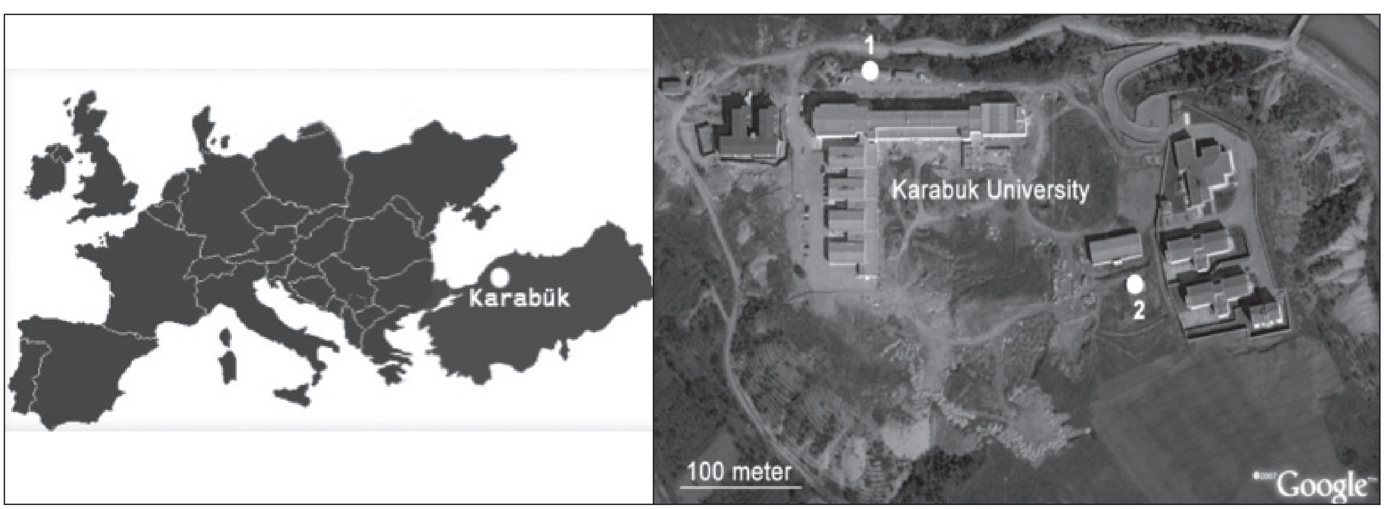

Figure 1. Karabük city and sampling stations (1: roof sampler, 2: ground sampler).

Table 1. Monthly meteorological data of Karabük atmosphere in 2006 and 2007

Meteorological factors	January		February		March		April		May		June		July		August		September		October		November		December	
	2006	2007	2006	2007	2006	2007	2006	2007	2006	2007	2006	2007	2006	2007	2006	2007	2006	2007	2006	2007	2006	2007	2006	2007
Relative humidity (%)	76	83	68	72	62	70	56	63	59	64	61	59	53	46	52	56	61	56	71	71	77	79	75	82
Mean wind speed (m/sn)	0,37	1,5	0,34	1,6	0,74	1,9	0,68	2,1	0,45	1,8	0,39	2,0	0,48	2,1	0,53	2,2	0,27	2,0	0,20	1,5	0,25	0,85	0,24	0,77
Mean temperature (°C)	1,10	3,1	3,17	4,4	9,13	8,2	13,26	9,9	17,15	19,4	20,91	22,5	23,40	25,3	27,05	25,3	19,43	20,6	14,89	15,3	5,73	7,42	2,27	8,07
Precipitation (mm)	24,8	103,8	48,4	3,4	25	48,8	1,2	33,6	37,9	23,0	66,3	24,6	7,8	0	0,2	3,8	61	1,0	28,8	0	43,6	31,6	23,6	59,4

Table 2. Monthly Total Spore Quantities and Standart Deviations of two samplers in 2006 and 2007

Taxa/Months	1	2	3	4	5	6	7	8	9	10	11	12	Total	SD	%
Cladosporium	1	2	13	26	210	224,5	256	202,5	86,5	17,5	5	1	1045	109,58	37,03
Alternaria	0	0	1,5	3	99	277	247,5	127	87	21,5	4	0	867,5	160,51	30,74
Ustilago	1,5	1	0	0	70	176	174,5	45	25,5	1	0	0	494,5	168,98	17,52
Myxomycetes	1	1	1	1	6,5	26,5	84,5	5	0	0,5	0	0	127	51,60	4,50
Ascospore	0,5	0,5	10,5	14,5	44	13	18	15	3	1	0	0	120	36,03	4,25
Uromyces	0	0	0	0	54,5	7	2	1,5	1	0	0	0	66	25,46	2,34
Stemphylium	0	0	0	0	5,5	1	6	5	2,5	0,5	1,5	0	22	14,85	0,78
Aspergillus/ Penicillum	2	0	0	0,5	7	0	0	1	0	0	0	0	10,5	4,95	0,37
Epicocum	0	0	0	0	2	1	2	1,5	1,5	2	0	0	10	4,24	0,35
Bipolaris	0	0	0	0	2	1,5	2,5	2	0,5	0	0	0	8,5	4,24	0,30
Eryisiphe	0	0	0	0	0	0	0	2	5	0,5	0	0	7,5	7,08	0,27
Rust	0	0	0	0,5	0	0	0	1	1	3,5	1,5	0	7,5	4,96	0,27
Periconia	0	0	0	0	0	0	1,5	2	2	0,5	1	0	7	5,66	0,25
Pleospora	0	0	1	3	2,5	0	0	0	0	0	0	0	6,5	7,79	0,23
Coprinus	0	0	3	0,5	2	0	0	0	0,5	0	0	0	6	2,82	0,21
Teliospore	6	0	0	0	0	0	0	0	0	0	0	0	6	8,49	0,21
Pithomyces	0	0	0	0	0	0	0	3	1,5	0	0	0	4,5	2,12	0,16
Pteridophyta	0	0	0	0	0	0	0	0,5	2	0	0	0	2,5	2,12	0,09
Monodictys	0,5	0	0	1,5	0	0	0	0	0	0	0	0	2	1,41	0,07
Torula	0	0	0,5	0	0,5	0	0	0	0	0	0	0	1	1,41	0,04
Leptosphaeria	0	0	0	0	0,5	0	0	0	0	0	0	0	0,5	2,13	0,02
Total	12,5	4,5	30,5	50,5	506	727,5	794,5	414	219,5	48,7	13	1	2822,2	625,01	100,00

Table 3. Monthly Spore Quantities and Standart Deviations of two samplers in 2006

Taxa/Months	1	2	3	4	5	6	7	8	9	10	11	12	Total
Cladosporium	0	0	9±1,41	17±1,41	18±4,24	83,5±3,54	188,5±14,85	52,5±23,33	15,5±3,54	13,7±0,71	2,5±3,54	0,5±0,71	400,7±79,91
Alternaria	0	0	1,5±0,71	3±1,41	16±2,83	32±22,63	193,5±13,44	62±33,94	40±4,24	15,5±0,71	3±0	0	366,5±57,28
Ustilago	1±1,41	0	0	0	1±1,41	15±16,97	138,5±33,23	25±2,83	8,5±2,12	0,5±0,71	0	0	189,5±58,68
Ascospore	0	0,5±0,71	10,5±2,12	10,5±0,71	7,5±0,71	10±0	9±1,41	5±1,41	2±1,41	1±1,41	0	0	56±9,89
Myxomycetes	0	0	0	0	0	3±1,41	37,5±3,54	2,5±2,12	0	0	0	0	43±7,07
Stemphylium	0	0	0	0	0	0	6±1,41	1±0	2±1,41	0,5±0,71	1,5±0,71	0	11±3,23
Aspergillus/ Penicillium	2±2,83	0	0	0	6±0	0	0	0	0	0	0	0	8±2,83
Uromyces	0	0	0	0	0	2,5±3,54	1±1,41	0	1,5±0,71	1±0	0	0	6±5,65
Periconia	0	0	0	0	0	0	0,5±0,71	0,5±0,71	2±1,41	0,5±0,71	1±1,41	0	4,5±4,95
Epicocum	0	0	0	0	0	0	0	0	1,5±0,71	2±1,41	0	0	3,5±2,12
Rust	0	0	0	0	0	0	0	1±0	0,5±0,71	0,5±0,71	1,5±0,71	0	3,5±2,13
Bipolaris	0	0	0	0	0	0	2,5±0,71	1±0	0	0	0	0	3,5±0,71
Eryisiphe	0	0	0	0	0	0	0	2±0,71	0,5±0,71	0,5±0,71	0	0	3±2,13
Coprinus	0	0	3±0	0	0	0	0	0	0	0	0	0	3±0
Pleospora	0	0	0,5±0,71	1	0	0	0	0	0	0	0	0	1,5±0,71
Pithomyces	0	0	0	0	0	0	0	1±0	0,5±0,71	0	0	0	1,5±0,71
Pteridophyta	0	0	0	0	0	0	0	0,5±0,71	0,5±0,71	0	0	0	1±1,42
Leptosphaeria	0	0	0	0	0,5±0,71	0	0	0	0	0	0	0	0,5±0,71
Total	3	0,5	24,5	31,5	51,5	144,5	574,5	154,5	74,5	36,7	7,5	0,5	1106,2±250,33

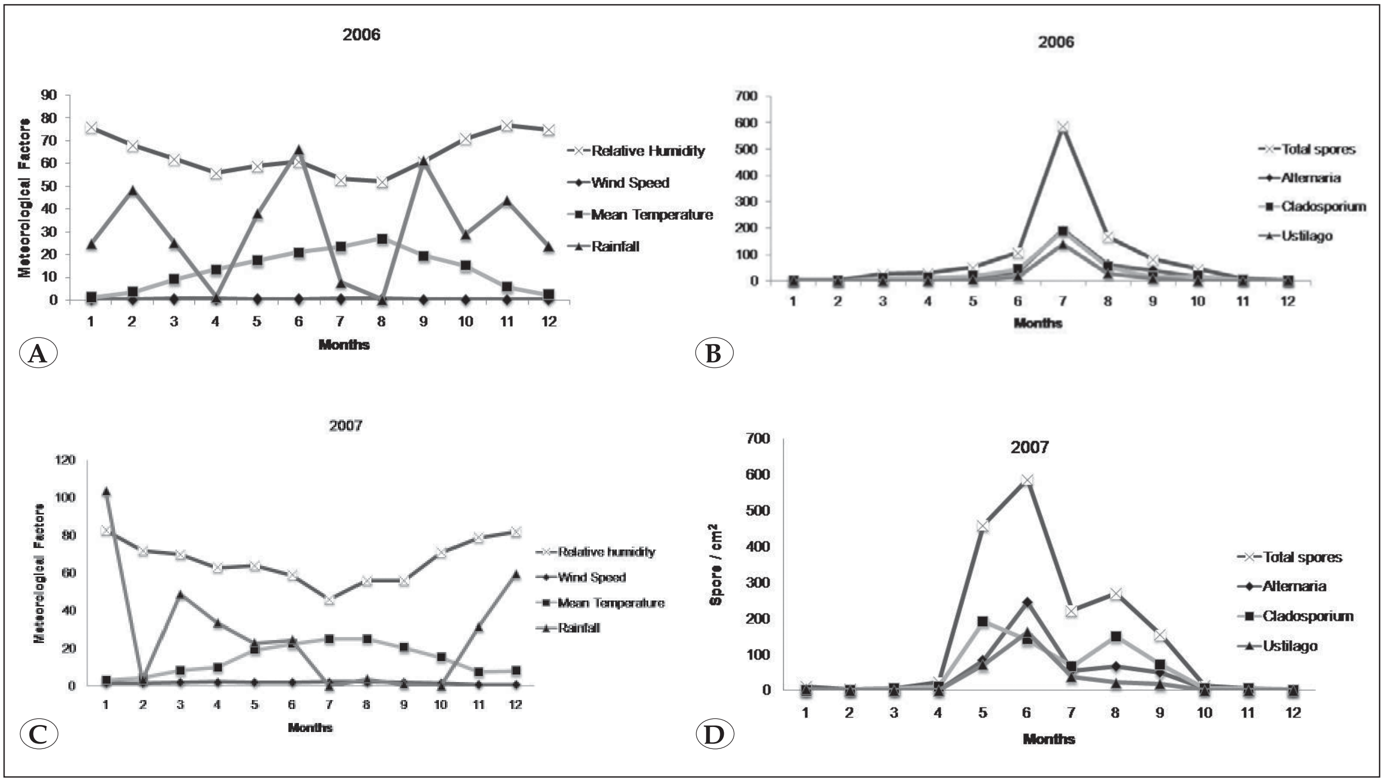

Figure 2. Variations in monthly concentrations of total and dominant spores with meteorological factors in 2006 and 2007. **A-B:** 2006, **C-D:** 2007.

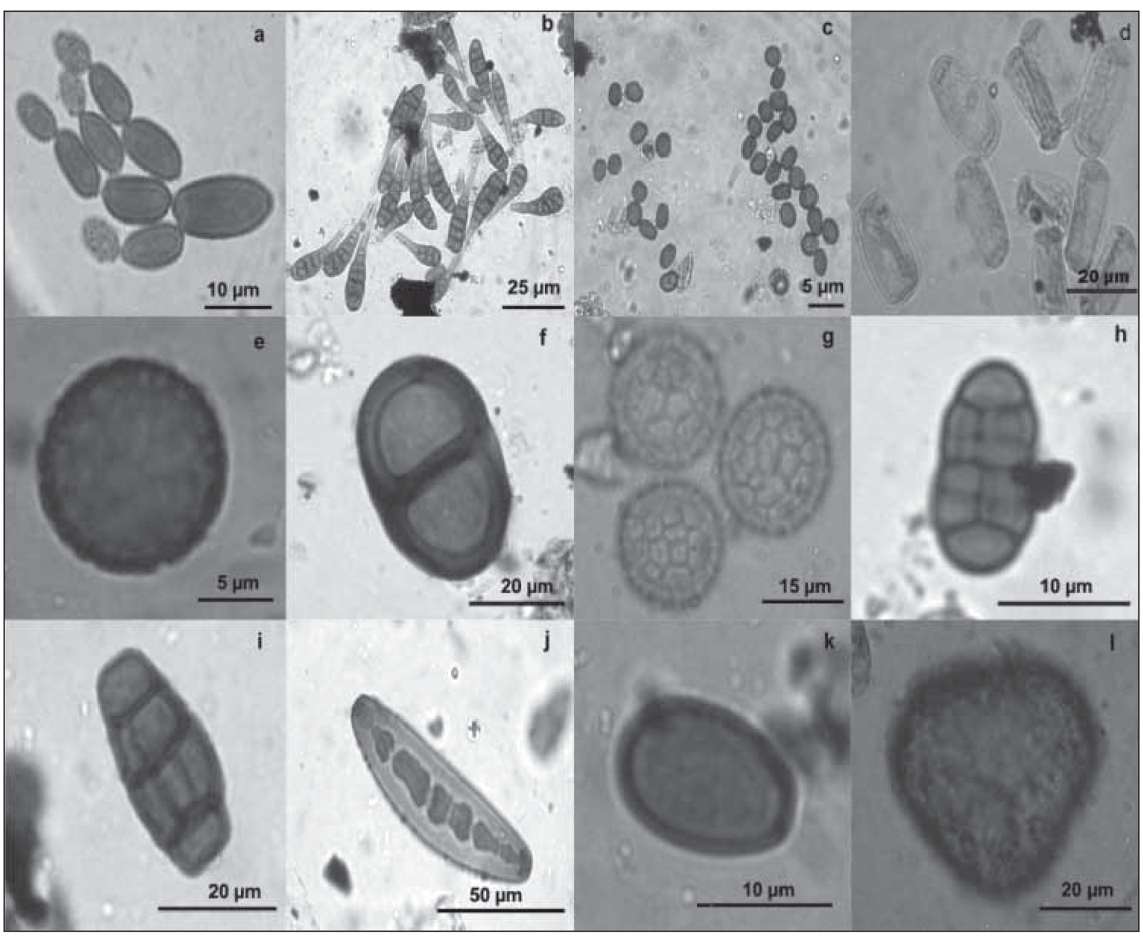

Figure 3. Some spores found in Karabük Atmosphere. a- *Cladosporium*, b- *Alternaria*, c- *Ustilago*, d- *Erysiphe*, e- *Periconia*, f- *Puccinia*, g- Myxomycetes, h- *Pleospora*, i- *Pithomyces*, j- *Bipolaris* type, k- *Coprinus*, l- Pteridophyta.

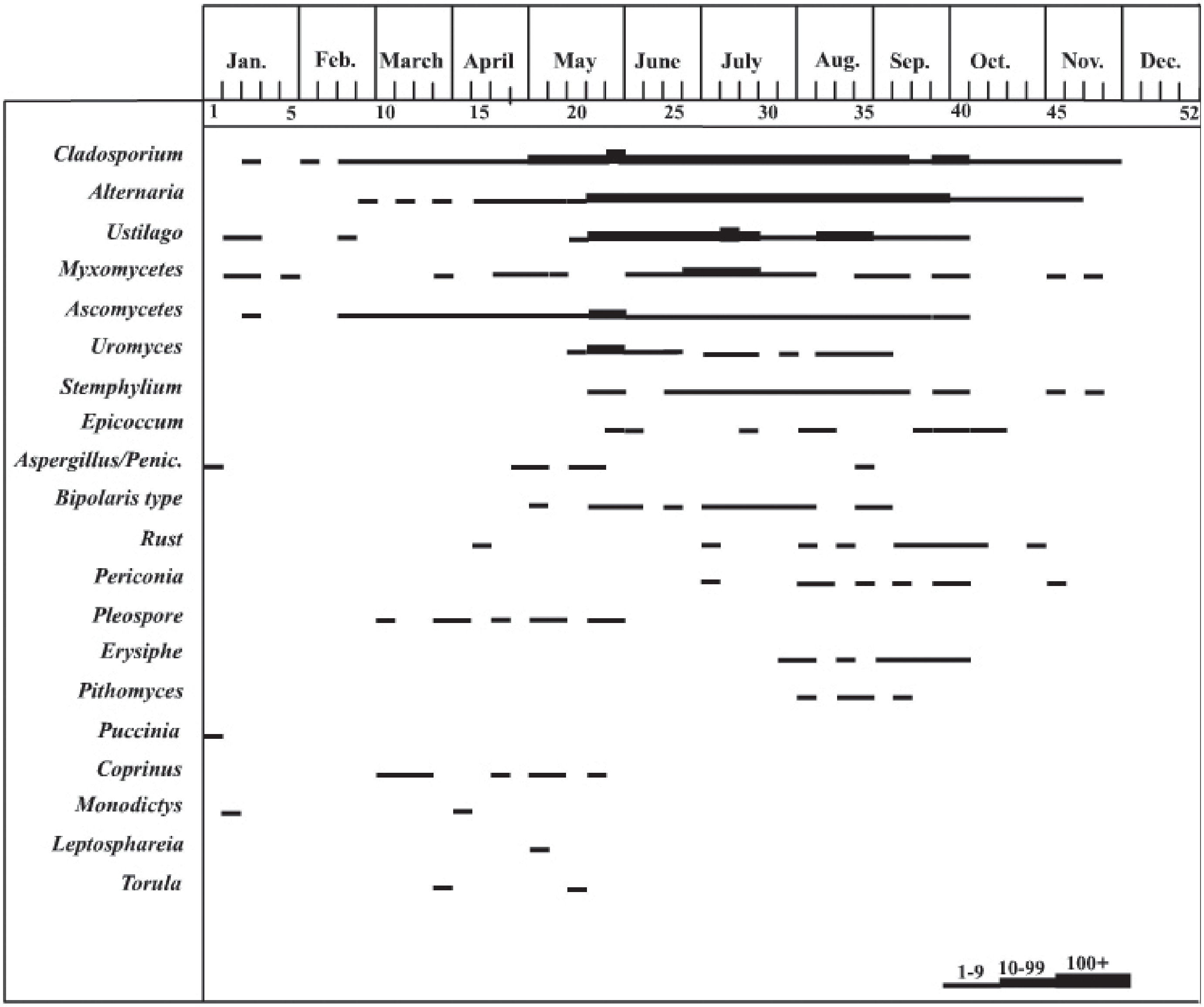

Figure 4. The two-years atmospheric spore calendar for Karabük Province.

İnceoğlu 2003, Stepelska et al. 2005, Herrera et al. 2006, Boyacıoğlu et al. 2007, Patoğlu Erkara et al. 2008, Bülbül et al. 2011, Mallo et al. 2010).

Ustilago spores were the third frequently observed in Karabük atmosphere following *Cladosporium* and *Alternaria*. In Spain, Morales et al. (2006) indicated that *Ustilago* and *Coprinus* are and the most frequently seen basidiospores among the other genera of Basidiomycota division.

The distribution of fungal spores in the years 2006 and 2007 indicate an increase in their quantities in the months of May, June and July (Tables 3, 4). In other studies, they have revealed that the existence of fungal spores varies according to seasons due to their sensitivity towards meteorological factors (Corden and Millington 2001, Bülbül et al. 2011). Stepalska and Wolek (2005) examined the variations in the concentrations of fungal spore species with respect to seasonal meteorological parameters and concluded that there is a positive correlation between spore concentrations and minimum, maximum temperatures as well as sunlight.

There are a number of significant correlations ($p < 0.01$, $p < 0.05$) between weekly spore counts from the most frequently recorded taxa and meteorological data (Tables 5, 6). Although there is no diffrences between the two samplers in terms of the spore quantities, the effect of meteorological parameters on spore concentration is different between the samplers (Tables 5, 6).

There is a strong negative correlation between total spores and average humidity rate in 2006 (Table 5). Increase in humidity rate was accompanied by a decrease in the number of spores. *Cladosporium* and total spore

Table 4. Monthly Spore Quantities and Standart Deviations of two samplers in 2007

Taxa/Months	1	2	3	4	5	6	7	8	9	10	11	12	Total
Cladosporium	1±0	2±0,71	4±0	9±4,24	192±14,14	141±12,73	67,5±2,12	150±7,07	71±12,07	4±1,41	2,5±3,54	0,5±0,71	644,5±58,67
Alternaria	0	0	0	0	83±53,74	245±16,97	54±1,41	65±1,41	47±7,07	6±0,00	1±0,00	0	501±80,60
Ustilago	0,5±0,71	1±1,41	0	0	69±38,18	161±33,94	36±28,28	20±4,24	17±2,83	0,5±0,71	0	0	305±110,30
Myxomycetes	1±0	1±1,41	1±0	1±1,41	6,5±3,53	23,5±17,68	47±18,38	2,5±1,41	0	0,5±0,71	0	0	84±44,53
Ascospore	0,5±0,71	0	0	4±4,24	36,5±6,36	3±4,24	9±9,89	10±7,07	1±0	0	0	0	64±32,51
Uromyces	0	0	0	0	52±14,14	6±2,83	2±2,83	0	0	0	0	0	60±19,80
Stemphylium	0	0	0	0	5,5±2,12	1±0	0	4±1,41	0,5±0,71	0	0	0	11±4,24
Epicocum	0	0	0	0	2±1,41	1±0	2±0	1,5±0,71	0	0	0	0	6,5±2,12
Teliospore	6±8,49	0	0	0	0	0	0	0	0	0	0	0	6±8,49
Pleospora	0	0	0,5±0,71	2±1,41	2,5±3,54	0	0	0	0	0	0	0	5±5,67
Bipolaris	0	0	0	0	2±1,41	1,5±1,41	0	1±0	0,5±0,71	0	0	0	5±3,53
Erysiphe	0	0	0	0	0	0	0	0	4,5±4,95	0	0	0	4,5±4,95
Rust	0	0	0	0,5±0,71	0	0	0	0,5±0,71	3	0	0	0	4±1,41
Pithomyces	0	0	0	0	0	0	0	2±1,41	1	0	0	0	3±1,41
Coprinus	0	0	0	0,5±0,71	2±1,41	0	0	0	0,5±0,71	0	0	0	3±2,82
Aspergillus/ Penicillum	0	0	0	0,5±0,71	1	0	0	1±1,41	0	0	0	0	2,5±2,12
Periconia	0	0	0	0	0	0	1±0	1,5±0,71	0	0	0	0	2,5±0,71
Monodictys	0,5±0,71	0	0	1,5±0,71	0	0	0	0	0	0	0	0	2±1,41
Pterodophyta	0	0	0	0	0	0	0	0	1,5±0,71	0	0	0	1,5±0,71
Torula	0	0	0,5±0,71	0	0,5 ±0,71	0	0	0	0	0	0	0	1±1,41
Total	9,5±10,61	4±3,53	6±1,41	19±14,14	454,5±140,69	583±89,80	218,5±628,9	259±13,42	147,5±31,11	11±2,83	3,5±3,54	0,5±0,71	1716±374,68

quantity and average wind speed are strongly positively correlated (Table 5). The increase in wind speed lead to an increase in spore quantity. There is a strong positive correlation between all spores and average temperature (Table 5). Increase in temperature was accompanied by an increase in spore quantities (Tables 5, 6).

There is a strong negative correlation between average humidity rate and quantities of Alternaria and *Cladosporium* in 2007 (Table 6). Increase in average humidity rate was accompanied by a decrease in the quantities of *Alternaria* and *Cladosporium*. Average humidity rate is weakly negatively correlated to remaining spores. The remainder of the spores were not influenced by average humidity rate. No significant relationship was identified between total precipitation amount and the spores. There is a strong positive correlation between quantitites of all spores and maximum wind speed (Table 6).

Total spores have a strong positive correlation with average temperature values. Increase in temperature was accompanied by an increase in spore quantities (Table 6). There has been an increase in the number and type of fungal spores in 2007 relative to 2006. This increase can be attributed to the increased wind speed in 2007 (1.78 m/sec) and the prevailing wind direction (West) in 2007. Since the wind roses are available on yearly basis, no correlation analysis could be performed between wind directions and fungal spores.

5. Conclusion

As a conclusion, the most frequent and the most detrimental are *Cladosporium, Alternaria* and *Ustilago* spores. These spores are abundant in June, July, August and September. The treatment of the patients allergic to fungal spores need to be sustained in these months as

Table 5. Monthly Correlations between spore counts and meteorological factors in 2006.

Taxa	2006									
	Roof Sampler					Ground Sampler				
	n	%	m/sec	°C	mm	n	%	m/sec	°C	mm
Alternaria	9	-0,765	0,268	0,949	0,116	9	-0,765**	0,268	0,949**	-0,116
Cladosporium	10	-0,680	0,643*	0,653*	-0,311	8	-0,845**	0,413	0,968**	-0,135
Ustilago	5	-0,492	0,234	0,655*	-0,047	6	-0,703*	0,160	0,922**	-0,022
Ascospore	8	-0,719**	0,882**	0,482	-0,154	8	-0,739**	0,750**	0,643*	-0,146
Myxomycetes	3	-0,639*	0,376	0,753**	-0,275	3	-0,598*	0,349	0,734**	-0,174
Stemphylium	5	-0,222	-0,191	0,550	-0,136	4	-0,290	-0,008	0,508	-0,067
Uromyces	4	-0,538	0,212	0,645*	0,216	3	-0,444	0,097	0,532	-0,118
Total spore	12	-0,898**	0,754**	0,951**	-0,582*	11	-0,801**	0,345	0,979**	-0,092

* Correlation is significant at the 0.05 level (2-tailed). ** Correlation is significant at the 0.01 level (2-tailed).
n: monthly spore season, **%:** relative humidity, **m/sec:** wind speed, **°C:** mean temperature, **mm:** rainfall.

Table 6. Monthly Correlations between spore counts and meteorological factors in 2007

Taxa	2007									
	Roof Sampler					Ground Sampler				
	n	%	m/sec	°C	mm	n	%	m/sec	°C	mm
Cladosporium	10	-0,784**	0,714*	0,815**	-0,278	11	-0,732*	0,570	0,847**	-0,300
Alternaria	7	-0,616*	0,365	0,825**	-0,438	7	-0,695*	0,464	0,891**	-0,490
Ustilago	5	-0,671*	0,512	0,780**	-0,271	8	-0,211	0,169	0,215	-0,191
Myxomycetes	8	-0,529	0,566	0,577	-0,226	6	-0,612*	0,501	0,687*	-0,092
Ascospore	7	-0,619*	0,613*	0,594	-0,068	5	-0,680*	0,643*	0,653*	-0,311
Uromyces	3	-0,310	0,282	0,501	0,012	3	-0,438	0,273	0,528	-0,261
Stemphyllium	4	-0,549	0,486	0,646*	-0,247	3	-0,368	0,364	0,533	-0,092
Pleospora	3	-0,092	0,212	0,000	0,267	1	-	-	-	-
Epicoccum	4	-0,720**	0,588*	0,712**	-0,524	4	-0,646*	0,520	0,679*	-0,468
Total spore	11	-0,793**	0,691*	0,861**	-0,342	11	-0,592	0,444	0,752**	-0,214

* Correlation is significant at the 0.05 level (2-tailed). ** Correlation is significant at the 0.01 level (2-tailed).
n: mounthly spore season, **%:** relative humidity, **m/sec:** wind speed, **°C:** mean temperature, **mm:** rainfall.

well. The further studies are planned to be conducted by volumetric method. The influence of wind directions on the distribution of fungal spores will also be investigated in the forthcoming studies.

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7. References

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