

Seasonal and intradiurnal variation of airborne pollen concentrations in Bodrum, SW Turkey

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Abstract An aeropalynological study was performed in Bodrum, the famous tourism center in southwestern Turkey with a Hirst-type volumetric 7-day pollen and spore trap for 2 years (2007–2008). In Bodrum, 25,099 pollen grains as a mean value belonging to 41 taxa were recorded annually during the study period, and pollen grains from woody plant taxa had the largest atmospheric contribution of 86.99 % and 24 taxa. However, 17 herbaceous plant taxa constituted 12.82 % of the annual total pollen count, and 0.19 % were unidentified. An average annual pollen index of 22.66 % was recorded in March, despite differences from year to year. The highest pollen variability of 34 taxa was recorded in April and May. Predominant pollen types belonged to Cupressaceae/Taxaceae (42.73 %), *Quercus* (15.95 %), *Pinus* (9.78 %), *Olea europaea* (9.04 %), Poaceae (5.50 %), *Betula* (1.82 %), *Pistacia* (1.74 %), *Morus* (1.72 %), Urticaceae (1.46 %), and *Plantago* (1.28 %) and generated 91.03 % of the annual total. In total, 32.59 % of the mean annual total pollen index was recorded in the morning, and less pollen was recorded in the evening (18.71 %). Maximum pollen concentration was recorded between 11:00 and 12:00 a.m.

Keywords Airborne pollen · Pollen concentrations · Bodrum · Turkey

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Introduction

Pollen grains are common, natural aeroallergens that may cause mucus membrane irritation, chronic bronchitis, allergic rhinitis, asthma, extrinsic allergic alveolitis (hypersensitivity pneumonitis), inhalation fever, humidifier fever, organic dust toxic syndrome, and immunological effects (Lacey and Dutkiewicz 1994). Evidence suggests that the prevalence of pollen-induced respiratory allergic reactions has been increasing in Europe during the past several decades and is estimated to be approximately 40 % (D'Amato et al. 2007).

Determining type of atmospheric pollen grains and concentrations is useful for patients who are suffering from allergies. Therefore, many aeropalynological investigations have been performed worldwide to determine pollen dispersal and concentrations, especially in highly populated areas (Giner et al. 2002; Petermel et al. 2003; Gioulekas et al. 2004; Weryszko-Chmielewska and Piotrowska 2004).

In Turkey, palynological studies began in the 1960s, and Durham samplers were frequently used for aeropalynological samplings for many years (Ince 1994; Bicakci et al. 2000a, b; Bicakci et al. 2002; Guvensen and Ozturk 2003; Kaya and Aras 2004; Bicakci 2006). After the 2000s, volumetric pollen and spore traps were commonly used in many Turkish city centers (Inceoglu et al. 1994; Bicakci et al. 2003; Altunoglu et al. 2008; Celenk et al. 2010; Ceter et al. 2012; Tosunoglu et al. 2014). However, there are not many aeropalynological studies on some kind of cities, where multi-fold increase of population sighted just for

one season, like major tourism centers in Turkey, and also in the world.

The aims of this study are as follows: (1) to clarify what pollen types are present in the air, (2) to represent pollen seasons, (3) to prepare a pollen calendar for a world-known tourism center, Bodrum, and (4) to present the diurnal results from the airborne pollen sampling.

Materials and methods

Study area, flora, and climate

Bodrum is located in the Muğla province on the coast of the Aegean Sea (37° 02' N–27° 25' E) and is also known as the ancient city “Halicarnassus,” which is shaped like a peninsula and is surrounded by the Güllük and Gökova gulfs on the north and south (Fig. 1).

Mediterranean maquis elements are dominant in this region. A total of 346 plant species belonging to 72 families have been recorded in floristic studies (Tuzlaci 2000). The flora are dominated by *Pinus brutia* Ten. forests, and the forest openings are mostly covered by maquis elements such as *Quercus* spp., *Arbutus unedo* L., *Arbutus andrachne* L., *Pistacia lentiscus* L., *Cistus creticus* L., *Laurus nobilis* L., *Erica arborea* L., *Nerium oleander* L., and *Olea europaea* L. These species are widespread throughout the area; some of them are both in wild and in cultured forms.

Other represented plants, which are mostly found in parks and gardens, are *Juniperus drupacea* Labill., *Juniperus sabina* L., *Juniperus oxycedrus* L.,

Cupressus arizonica L., *Cupressus sempervirens* L. var. *pyramidalis*, *Thuja orientalis* L., *Abies cilicica* Ant. et Kotschy, *Acer platanoides* L., *Acer negundo* L., *Eucalyptus camaldulensis* Dehnh., *Alnus* spp., *Betula* spp., *Phoenix canariensis* Hort., *Phoenix dactylifera* L., *Phoenix theophrasti* Greuter, *Platanus orientalis* L., *Salix babylonica* L., *Schinus molle* L., *Mimosa* spp., *Melia azaderach* L., *Morus alba* L., and *Liquidambar orientalis* Mill.

Bodrum has a typical Mediterranean climate. Meteorological parameters analyzed in our study for sampling years, provided by Turkish State Meteorological Service, were mean humidity (%), wind speed (m/s), mean temperature (°C; hourly), and rainfall (mm; daily). The meteorological station is located in the city center and 0.80 km as the crow flies from the pollen trap. According to 25 years of meteorological data, July is the warmest month (28.3 °C), whereas January and February are the coldest months (11.4 °C). July was also the sunniest month with 10.9 h/day and was the least rainy month with a total of 1.0 mm of rain; December was the rainiest month with 13 rainy days with a total of 149.8 mm of rain. The most snow within the county was measured on January 4, 1979 at a depth of 3 cm.

Aerobiological method

An aeropalynological study was performed in Bodrum with a Hirst-type volumetric pollen trap during 2007 and 2008. The pollen trap was placed on a bank roof in the city center 13 m above the ground. The device operated continuously and was calibrated to aspirate 10 l/m³ air.

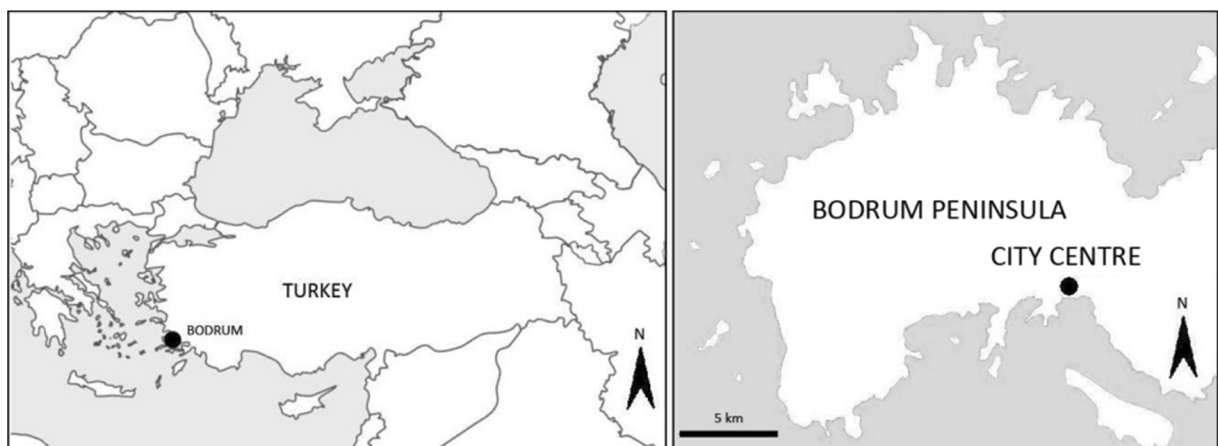


Fig. 1 Location of the study area (Bodrum)

Melinex® tape, which was first placed by applying silicone oil, was taken from the 7-day sampling drum together with the adherent atmospheric particles and was cut into daily fragments. The tape fragments were mounted to slides and covered with glycerin jelly mixed with basic fuchsin (Charpin et al. 1974) and were examined by light microscopy at $\times 400$ magnification. Atmospheric sampling and analysis were performed as described by the Spanish Aerobiology Network (REA), which ascertains intradiurnal variation by transversally dividing slides into 24 2-mm intervals (Galán et al. 2007). Pollen concentrations were stated as a number of pollen grains in one cubic meter of air. The pollen grains that could not be identified were considered to be unidentified types.

Pollen types that comprised more than 1 % of the annual total pollen concentration were considered to be dominant. The main pollen season (MPS) for dominant pollen types was calculated according to Andersen (1991), using the 95 % method; the start date of the MPS is defined as the day when the daily pollen count reached 2.5 % of the annual pollen index (API), starting from 1 January, and the end occurs when 97.5 % of the annual pollen count had been reached.

In general, four time zone periods (00:00–06:00 night, 06:00–12:00 morning, 12:00–18:00 afternoon, and 18:00–24:00 evening) were used to show the intradiurnal variations of pollen grains in Bodrum both for displaying total pollen and for different pollen types. For yearly classification, all days were taken into account, and for any taxa, all days with belonging pollen were taken into account.

The pollen calendar was constructed following the Spieksma model (Spieksma 1991); daily pollen counts from 10-day periods were summed and averaged for the years that were studied. These average sums were placed in 10-day mean pollen concentrations into a series of classes (Spieksma 1991) and were depicted by column growth heights in the calendar.

Statistical analysis

Statistical analysis were performed to correlate the mean daily pollen counts with the data of meteorological parameters (mean daily relative humidity, mean daily temperature, mean daily wind speed, and daily rainfall) of the same day and mean daily pollen counts of dominated pollen types (Cupressaceae/Taxaceae, *Quercus*, *Pinus*, *O. europaea*, Poaceae, *Betula*, *Pistacia*, *Morus*,

Urticaceae, and *Plantago*) with meteorological data using Spaerman's correlation analysis. Correlation analyses were performed between data of counts recorded during the MPS periods of frequented pollen types and same day meteorological factors. For correlating the daily concentrations of pollen grains in a year, whole data of daily counts have been used. The statistical tests were performed using the software package IBM SPSS version 20.0 (SPSS, Chicago, IL, USA).

Results

In Bodrum, a total of 25,099 pollen grains as a mean value belonging to 41 taxa were recorded annually during the study period (January 2007 to December 2008). In the first year, 28,591 pollen grains, and in the following year, 21,605 pollen grains were recorded, which was a remarkable decrease (Table 1).

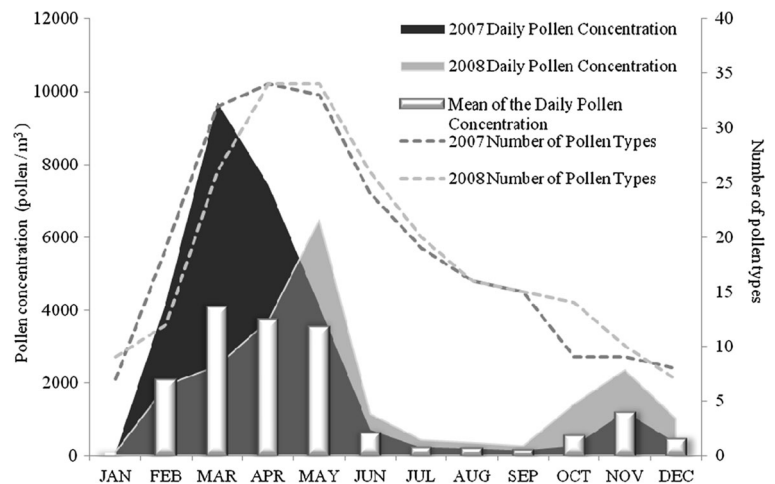
For both years, pollen grains from woody plant taxa (23 taxa) had the largest atmospheric contribution, with a mean value of 86.94 % (89.68 % in 2007 and 84.20 % in 2008) of the total pollen count. Moreover, 12.87 % (10.14 % in 2007 and 15.59 % in 2008) of the annual total pollen count was from herbaceous plants (18 taxa)—4.01 % is belonging to Poaceae in 2007 and 7.00 % in 2008, and 0.19 % (0.18 % in 2007 and 0.20 % in 2008) of the pollen was unidentifiable (Table 1).

In the first year of our study, there was an abrupt increase in number of pollen grains from January to March. Significant differences in pollen amounts were recorded between the studied years, especially during the main pollen season. In the first year of the study, the maximal pollen concentration was recorded in March with a total of 9702 pollen grains per cubic meter, or 33.93 % of the annual total, while May had the highest number of pollen grains in the following year, with 6472 pollen grains, or 29.96 % of the annual total (Fig. 2). We conclude that the spring peak in pollen grain number was a result of the woody plant taxa pollen season, which produces many pollen grains. In the summer, pollen grains from herbaceous plants were noted, and very low pollen concentrations were recorded in early autumn. In the late fall and early winter months of both years, there was an increase in pollen during November, which declined through the end of the year (Fig. 2). For both years, January had the lowest pollen concentrations (Fig. 2).

Table 1 Annual pollen counts and percentage of pollen taxa recorded in Bodrum atmosphere (2007–2008)

Year	2007		2008		Annual Mean	
Taxa	Pollen/m ³	Percentage	Pollen/m ³	Percentage	Pollen/m ³	Percentage
Cupress/Taxaceae	13,676	47.83	8131	37.64	10,904	42.73
<i>Quercus</i>	4006	14.01	3867	17.90	3936	15.95
<i>Pinus</i>	3894	13.62	1283	5.94	2589	9.78
<i>Olea europea</i>	1493	5.22	2779	12.86	2136	9.04
<i>Betula</i>	406	1.42	482	2.23	444	1.82
<i>Pistacia</i>	552	1.93	334	1.55	443	1.74
<i>Morus</i>	527	1.84	346	1.60	437	1.72
<i>Platanus</i>	241	0.84	204	0.94	222	0.89
<i>Fraxinus</i>	212	0.74	184	0.85	198	0.80
Rosaceae	139	0.48	155	0.72	147	0.60
<i>Eucalyptus</i>	161	0.56	135	0.62	148	0.59
<i>Salix</i>	50	0.18	46	0.21	48	0.20
Ericaceae	46	0.16	51	0.24	49	0.20
<i>Acer</i>	75	0.26	21	0.09	48	0.18
<i>Populus</i>	40	0.14	45	0.21	42	0.17
<i>Alnus</i>	46	0.16	32	0.15	39	0.15
<i>Ulmus</i>	24	0.08	49	0.23	36	0.15
<i>Juglans</i>	29	0.10	25	0.12	27	0.11
<i>Liquidambar orientalis</i>	10	0.03	9	0.04	10	0.04
Mimosaceae	7	0.02	5	0.03	6	0.02
<i>Ligustrum</i>	4	0.01	6	0.03	5	0.02
<i>Laurus nobilis</i>	3	0.01	3	0.01	3	0.01
<i>Abies</i>	2	0.01	2	0.01	2	0.01
Woody plants	25,642	89.68	18,193	84.20	21,917	86.94
Poaceae	1147	4.01	1512	7.00	1329	5.50
Urticaceae	391	1.37	334	1.54	362	1.46
<i>Plantago</i>	326	1.14	305	1.41	315	1.28
Cheno/Amaranthaceae	140	0.49	282	1.31	211	0.90
<i>Mercurialis</i>	221	0.77	159	0.74	190	0.75
Asteraceae	148	0.52	206	0.95	177	0.74
<i>Rumex</i>	113	0.40	101	0.47	107	0.43
<i>Xanthium</i>	67	0.23	113	0.52	90	0.38
Fabaceae	110	0.38	75	0.35	92	0.37
<i>Artemisia</i>	43	0.15	119	0.55	81	0.35
Boraginaceae	39	0.14	50	0.23	45	0.18
Brassicaceae	51	0.18	23	0.10	37	0.14
Apiaceae	32	0.11	34	0.16	33	0.13
Cyperaceae	31	0.11	26	0.12	29	0.11
<i>Humulus</i>	15	0.05	9	0.04	12	0.05
Lamiaceae	13	0.05	12	0.05	12	0.05
<i>Taraxacum</i>	8	0.03	7	0.03	8	0.03
<i>Papaver</i>	3	0.01	3	0.01	3	0.01
Herbaceous plants	2900	10.14	3368	15.59	3134	12.87
Unidentified	51	0.18	44	0.20	47	0.19
Total	28,591	100.00	21,605	100.00	25,098	100.00

Fig. 2 Monthly variation of pollen concentration and number of identified pollen types in years 2007–2008 in Bodrum atmosphere



The greatest pollen diversity was found during the spring. The greatest variation of pollen type was recorded in April with 34 taxa in the first year, whereas both April and May had the greatest variability and the same number of pollen types in the following year (Fig. 2).

A two-year period is too short to generalize and produce a pollen calendar; however, because the sampling was limited to 2 years, it was determined that pollen seasons, intensities, pollen grain variations and identified taxa would be put into a calendar, which was prepared using the average pollen concentration values from these 2 years (Fig. 3). Pollen calendar showed that the earliest pollen grains in the atmosphere of Bodrum were mostly belonging to winter and spring flowering arboreal plants like *Alnus*, *Betula*, Cupressaceae/Taxaceae, and Ericaceae. *Mercurialis* pollen grains are the unique non-arboreal pollen type, found present in the air from October to July. Most of identified taxa in the atmosphere were spring flowering plants, and pollen types were abruptly decreased with forthcoming summer in the study area. It may be characterized with low pollen concentrations in summer, and the most common types were Poaceae and Chenopodiaceae/Amaranthaceae pollen grains from non-arboreal plants. Also, *Artemisia* and *Xanthium* were the frequented pollen types found in the air of Bodrum in autumn (Fig. 3).

To show the intradiurnal variations of pollen grains in Bodrum, four time zone periods (00:00–06:00 night, 06:00–12:00 morning, 12:00–18:00 afternoon, and 18:00–24:00 evening) were used. Of the mean annual total pollen index, 32.59 % was recorded in the morning, and 31.10 % was recorded in the afternoon. Furthermore, less pollen (18.71 %) was recorded and

calculated in the evening (Fig. 4). Maximum pollen grain concentration was recorded between 10:00 and 11:00, together with gradual increase of temperature and wind speed therewithal graduated decrease of relative humidity and slightly lower pollen levels recorded between 22:00 and 23:00 hours together with gradual decrease of temperature and increase of relative humidity jointly with unstable wind speed (Fig. 5).

Comparing 2007 and 2008 about meteorological conditions, the most obvious differences were in rainfall and wind speed. The year 2007 was rainier and windier than 2008 (Fig. 6). There was no remarkable difference in mean temperature between the 2 years. The average relative humidity was also different for the studied years; however, monthly humidity was lower in April 2007 than in 2008 and in May 2008 than in 2007 (Fig. 6). The predominant winds in Bodrum during the study period usually blew from the north (north, north-northwest, and north-northeast) and the south (south, south-southwest, and southeast) (Fig. 7). The annual average wind speed in 2007 was 2.8 m/s, while the 2008 average was 2.3 m/s. Although this difference does not seem large, lower monthly and intradiurnal wind speed variation was noted in 2008 than in the previous year (Fig. 6).

Using the whole year data, some significant correlations ($P < 0.05$) were obtained between daily pollen counts and daily meteorological parameters in the studied years. Interestingly, a negative correlation was found between daily pollen count and mean daily temperature in the year 2007, but there were not any correlations between the same parameters for the next year. In addition, a significant positive correlation was also found

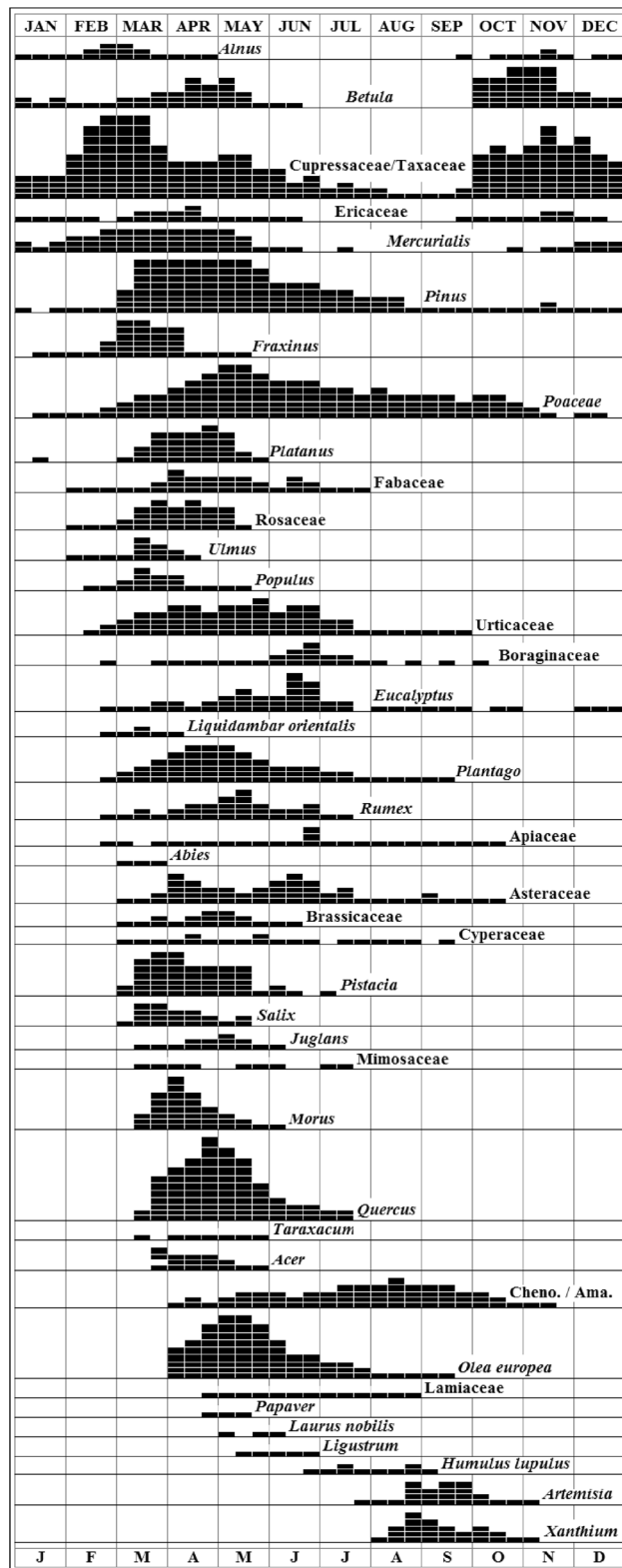


Fig. 3 Pollen calendar of Bodrum (average values of 2007–2008)

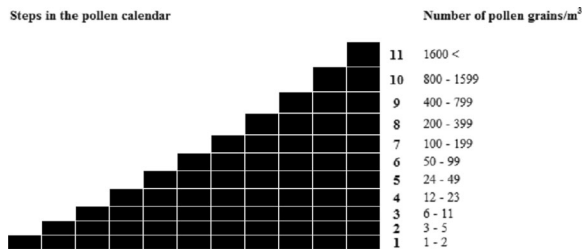


Fig. 3 (continued)

between daily pollen concentration and daily rainfall in the year 2008, and it was not in concordant with 2007 (Table 2). It is indicated by some authors that pollen levels were not significantly correlated with any single meteorological parameter, although significant correlations can be seen between certain pollen taxa and humidity and rainfall (Altintas et al. 2004; Abu-Dieyeh and Ratrouf 2012). In contrast, there were more calm periods in 2007 (28.98 %) than in 2008 (17.41 %).

Ten different plant taxa comprised more than 1 % of the total pollen content, taken as predominant pollen types with the greatest influence in Bodrum's atmosphere during the study period (Table 3). These pollen types belonged to Cupressaceae/Taxaceae (42.73 %), *Quercus* (15.95 %), *Pinus* (9.78 %), *O. europaea* (9.04 %), Poaceae (5.50 %), *Betula* (1.82 %), *Pistacia* (1.74 %), *Morus* (1.72 %), Urticaceae (1.46 %) and

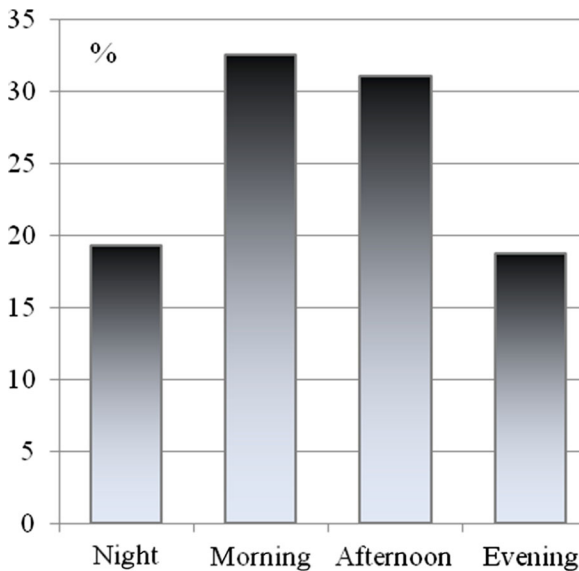


Fig. 4 Intradiurnal variation of pollen grains in Bodrum atmosphere (mean values of both years) (time zone periods: 00:00–06:00, night; 06:00–12:00, morning; 12:00–18:00, afternoon; and 18:00–24:00, evening)

Plantago (1.28 %), which accounted for 91.03 % of the annual total (Table 1).

Cupressaceae/Taxaceae was the most abundant pollen type in Bodrum's atmosphere, and visible differences in the annual pollen index were found between the 2 years especially about pollen number and annual percentages; 13,676 (47.83 %) pollen were accounted in 2007 and 8131 pollen (37.64 %) in 2008 (Fig. 8, Table 1). The MPS was calculated starting in January for the 2-year study duration and was recorded as 287–300 days in Bodrum. In our study, intradiurnal Cupressaceae/Taxaceae pollen concentration variation revealed that this type of pollen grains was common in the morning and reached their peak value between 09:00 and 10:00 am in Bodrum (Fig. 9).

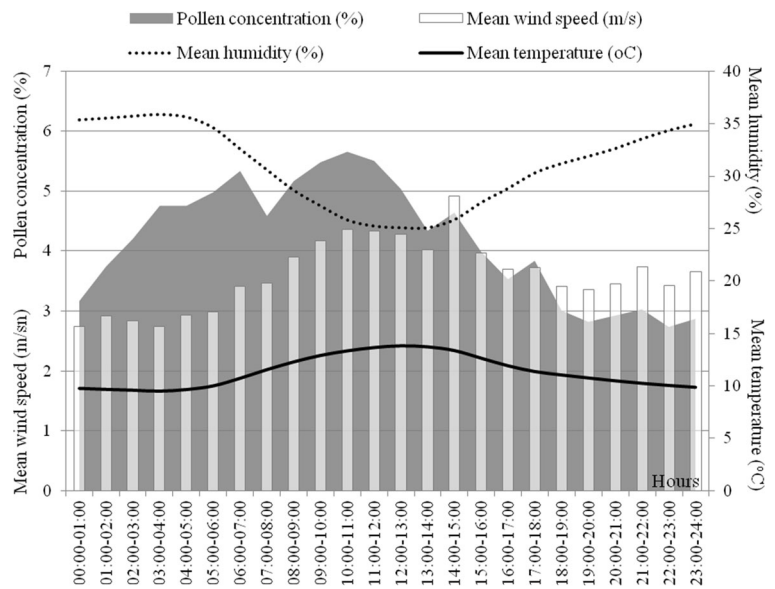
The second dominant pollen type in Bodrum was *Quercus*, with 15.95 % of the annual pollen index (Table 1). In Bodrum, *Quercus* pollen grains were detected from March to July (Fig. 3), with pollen concentration from this taxon reaching its peak in April (Table 3). The *Quercus* pollen grain MPS had one peak in both years, which was on April 24. The MPS was 33 days long in the first year and 21 days long with a 12-day delay in the second year (Fig. 8 and Table 3). Intradiurnal *Quercus* pollen variations were detected at high levels between 13:00 and 15:00 hours in the afternoon (Fig. 9).

The third most abundant pollen type was *Pinus*, which constituted 9.78 % of the annual pollen index (Table 1). *Pinus* pollen grains were detected in all months during the study period (Fig. 3); however, this taxon reached the highest concentration in April (Table 3). The MPS was 51–77 days long and they were detected at high levels (30.97 %) in the afternoon and early evening, between 11:00 and 16:00 (Fig. 9).

O. europaea pollen grains were recorded between April and September (Fig. 3), and the annual total was 9.04 % (Table 1). This pollen type reached its highest level in May (Table 3). In the second year, pollen concentration was two times as high as the first year. The MPS duration was 28–39 days long for *O. europaea* (Fig. 8), and intradiurnal variation was clustered in the morning, with slightly higher values recorded between 08:00 and 12:00 a.m. in Bodrum (Fig. 9).

Poaceae is the most abundant non-arboreal pollen type in Bodrum's atmosphere and represents 5.50 % of the annual pollen index (Table 1). Poaceae family pollen grains are obtained during the year but mostly in low

Fig. 5 Intradiurnal variation of pollen concentration, mean wind speed, humidity, and temperature in Bodrum for studied years



concentrations (Fig. 3) and were found in Bodrum in every month. This long pollen season is most likely due

to limited identification; Poaceae pollen may be identified at the family level and originates from many wild

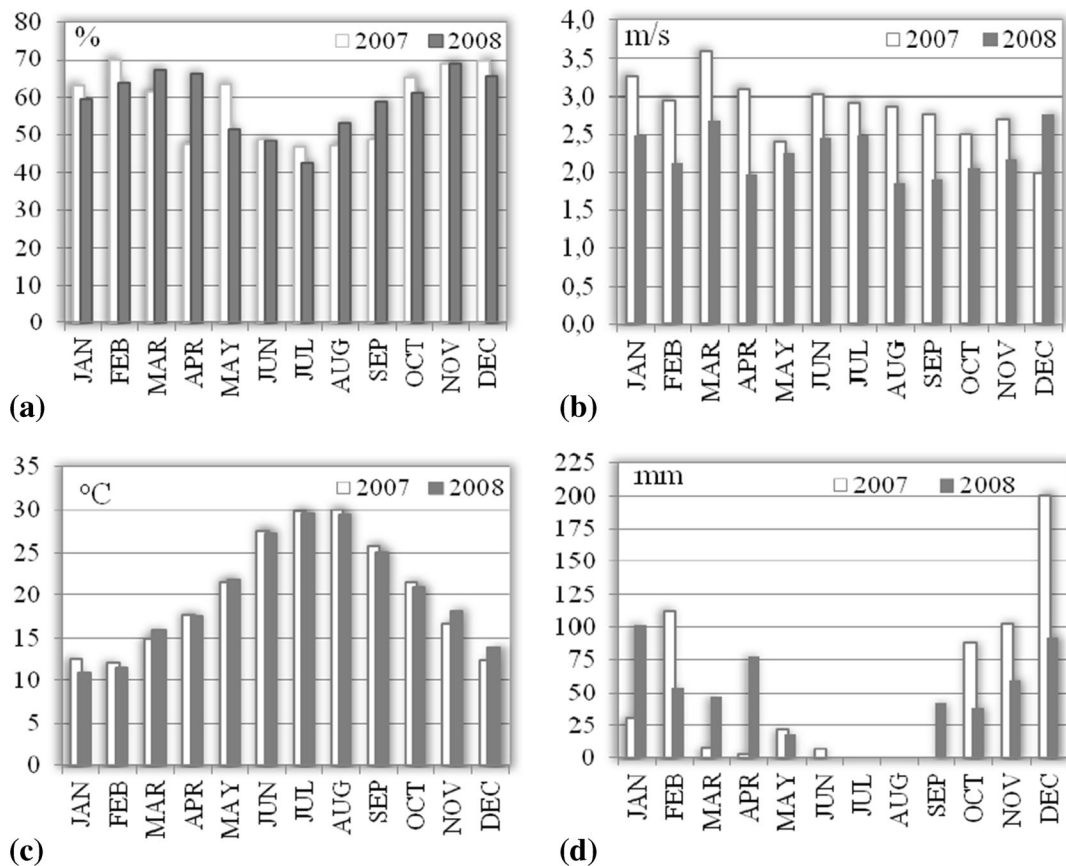


Fig. 6 Monthly variations of humidity (a), wind speed (b), mean temperature (c), and rainfall (d) in the study area between years 2007 and 2008

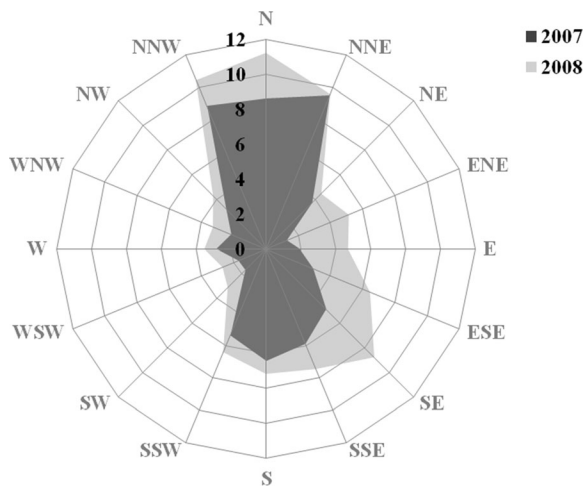


Fig. 7 Wind direction frequencies (%) in Bodrum during the study period

Table 2 The results of Spearman's correlation analysis between total daily pollen concentration or daily pollen concentrations of dominated taxa and meteorological parameters

		Mean daily relative humidity	Mean daily temperature	Mean daily wind speed	Daily rainfall
Daily pollen concentration	2007	0.003	−0.183**	0.015	0.052
	2008	−0.102	0.080	0.066	0.204**
Cupressaceae/Taxaceae	2007	0.336**	−0.800**	0.013	0.191**
	1st peak	−0.288	−0.119	−0.105	−0.180
	2nd peak	0.075	0.444	0.070	−0.451
	2008	0.233**	−0.586**	0.103	0.002
	1st peak	0.129	0.206	−0.165	−0.151
	2nd peak	−0.263*	0.005	0.230	−0.097
<i>Quercus</i>	2007	−0.338	0.045	0.036	−0.107
	2008	−0.229	−0.484*	0.196	0.433*
<i>Pinus</i>	2007	−0.429**	−0.143	0.118	−0.206
	2008	−0.461**	0.278*	0.414**	−0.224
<i>Olea europaea</i>	2007	0.075	0.759**	−0.199	0.087
	2008	−0.472**	0.079	0.276	−0.102
Poaceae	2007	−0.450**	−0.096	−0.349**	−0.199
	2008	−0.214	−0.214	0.476	−0.016
<i>Betula Pistacia Morus</i>	2007	0.219**	−0.699**	−0.099	0.274**
	1st peak	0.153	0.006	0.032	—
	2nd peak	−0.376	−0.484	−0.323	−0.030
	2008	−0.078	0.066	−0.062	0.214
	1st peak	—	—	—	—
	2nd peak	−0.089	0.125	−0.023	0.215
	2007	−0.170	0.084	0.161	−0.015
	2008	0.158	−0.402**	0.153	0.295
	2007	−0.284	0.270	−0.086	−0.549*
	2008	0.785**	−0.299	0.405	0.462
Urticaceae	2007	−0.406*	0.003	0.153	−0.213
	2008	−0.108	0.449*	0.294	0.024
<i>Plantago</i>	2007	−0.267	0.178	0.096	−0.298
	2008	0.139	−0.468**	0.199	0.259

*Correlation is significant at the 0.05 level (two-tailed); **correlation is significant at the 0.01 level (two-tailed)

grass species with different flowering times over several months. Pollen grains from this taxon reached their highest level in May (Table 3). The MPS was 8–22 days long and lasted throughout May (Fig. 8). Intradiurnal variation demonstrated that Poaceae pollen concentration was increased in the afternoon, especially between 10:00 and 17:00 (Fig. 9).

Betula pollen grains constituted 1.82 % of the annual pollen index (Table 1) and had two peaks during the year, similar to Cupressaceae/Taxaceae pollen grains (Figs. 3 and 8). In the first year, MPS in the spring peak was 20 days and the winter peak was 15 days long. But, in the second year, a clear winter peak was detected with a 41-day MPS, but there was no spring peak in this year (Fig. 8 and Table 3). Intradiurnal *Betula* pollen concentration variation demonstrated that pollen grains were

Table 3 Characteristics of the main pollen season for the most important taxa: start and ending dates, length, and maximum percentage of annual pollen index in the month

		2007	2008
Cupressaceae/Taxaceae	Main pollen season	15/02 to 27/11	12/02 to 07/12
	1st peak:	15/02 to 18/03	12/02 to 14/03)
	2nd peak:	10/11 to 27/11	12/10 to 07/12)
	Season length	287	300
	Max. percentage of pollen/month	25.04/Mar	9.97/Nov
	Max. daily pollen/m ³ – date	06/3 – 2029	17/11 – 768
<i>Quercus</i>	Main pollen season	10/04 to 12/05	23/04 to 13/05
	Season length	33	21
	Max. percentage of pollen/month	9.54/Apr	9.48/Apr
	Max. daily pollen/m ³ – date	24/04 – 635	24/04 – 861
<i>Pinus</i>	Main pollen season	27/03 to 16/05	14/03 to 29/05
	Season length	51	77
	Max. percentage of pollen/month	6.14/Apr	3.49/May
	Max. daily pollen/m ³ – date	24/04 – 213	08/05 – 121
<i>Olea europea</i>	Main pollen season	24/04 to 21/05	21/04 to 29/05
	Season length	28	39
	Max. percentage of pollen/month	3.69/May	9.75/May
	Max. daily pollen/m ³ – date	15/05 – 89	15/05 – 172
Poaceae	Main pollen season	24/04 to 16/06	08/05 to 15/05
	Season length	22	8
	Max. percentage of pollen/month	1.35/May	3.23/May
	Max. daily pollen/m ³ – date	24/04 – 36	09/05 – 69
<i>Betula</i>	Main pollen season	12/04 to 20/11	08/10 to 17/11
	1st peak:	12/04 to 01/05	–
	2nd peak:	06/11 to 20/11	08/10 – 17/11
	Season length	223	41
	Max. percentage of pollen/month	0.49/Apr	1.15/Oct
	Max. daily pollen/m ³ – date	07/11 – 77	10/10 – 33
<i>Pistacia</i>	Main pollen season	13/03 to 11/04	24/03 to 14/05
	Season length	30	52
	Max. percentage of pollen/month	1.02/Mar	0.62/Apr
	Max. daily pollen/m ³ – date	31/03 – 46	05/04 – 17
<i>Morus</i>	Main pollen season	25/03 to 13/04	31/03 to 13/04
	Season length	20	14
	Max. percentage of pollen/month	1.25/Apr	1.32/Apr
	Max. daily pollen/m ³ – date	07/04 – 61	05/04 – 40
Urticaceae	Main pollen season	14/03 to 12/04	08/05 to 29/05
	Season length	30	22
	Max. percentage of pollen/month	0.47/Apr	0.86/May
	Max. daily pollen/m ³ – date	09/04 – 24	28/05 – 18
<i>Plantago</i>	Main pollen season	09/04 to 06/05	20/04 to 05/06
	Season length	28	47
	Max. percentage of pollen/month	0.61/Apr	0.71/May
	Max. daily pollen/m ³ – date	10/04 – 14	09/05 – 19

common in the morning (33.24 %) and reached their peak value between 10:00 and 11:00 am in Bodrum (Fig. 9).

Members of the Mediterranean endemic genus *Pistacia* are an important element of forests, maquis, and orchards in the Mediterranean basin. Pollen grains of this genus constituted 1.74 % of the API and were recorded between March and July in Bodrum (Fig. 3), reaching their highest level in March (Table 3). The MPS began in mid-March and continued for 30 days until mid-April in the first year. In the second year, the MPS was 52 days and extended until mid-May (Fig. 8, Table 3). *Pistacia* pollen grain intradiurnal variations were prominent, and pollen concentrations were highest

in the afternoon (34.91 %), particularly between 12:00 and 13:00 (Fig. 9).

Morus pollen grains comprised 1.72 % of the annual pollen index, and their pollen season continued from March until June (Fig. 3). The highest pollen concentration was recorded in April (Table 3). The *Morus* species main pollen season in Bodrum began at the end of March, and the MPS lasted for 14–20 days (Fig. 8 and Table 3). Pollen concentration was intradiurnally centered in the afternoon (38.76 %), with the highest concentration recorded between 12:00 and 13:00 (Fig. 9).

Urticaceae is presented mostly by two genera (*Urtica* and *Parietaria*) in flora of Bodrum and because of

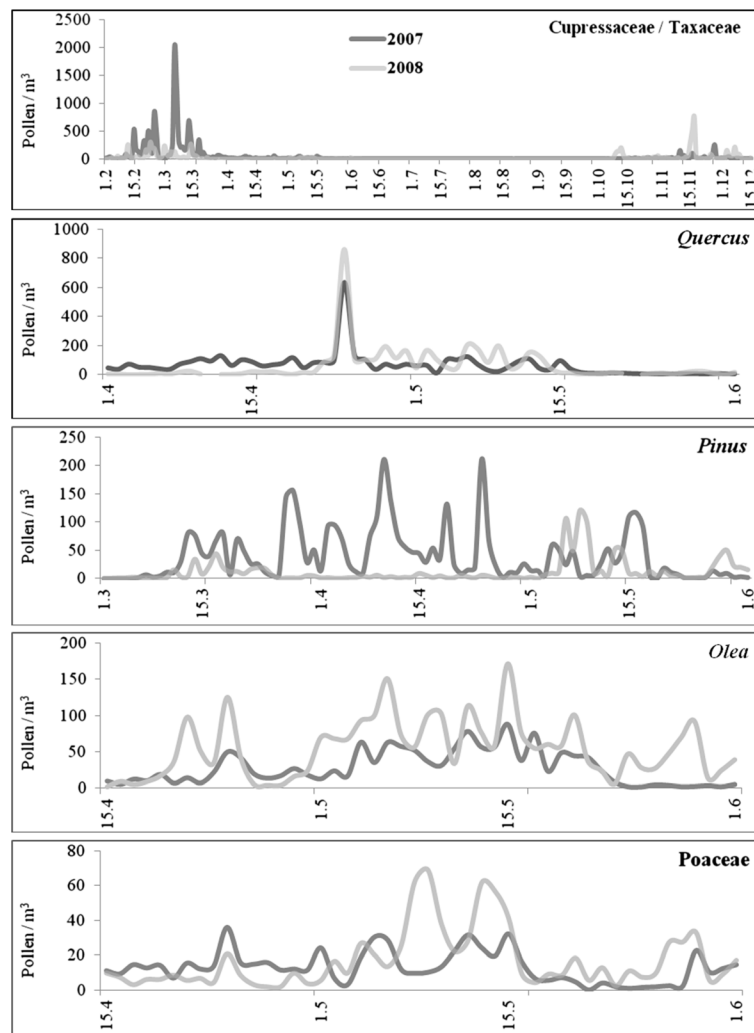


Fig. 8 Seasonal variation of dominated pollen types in Bodrum atmosphere

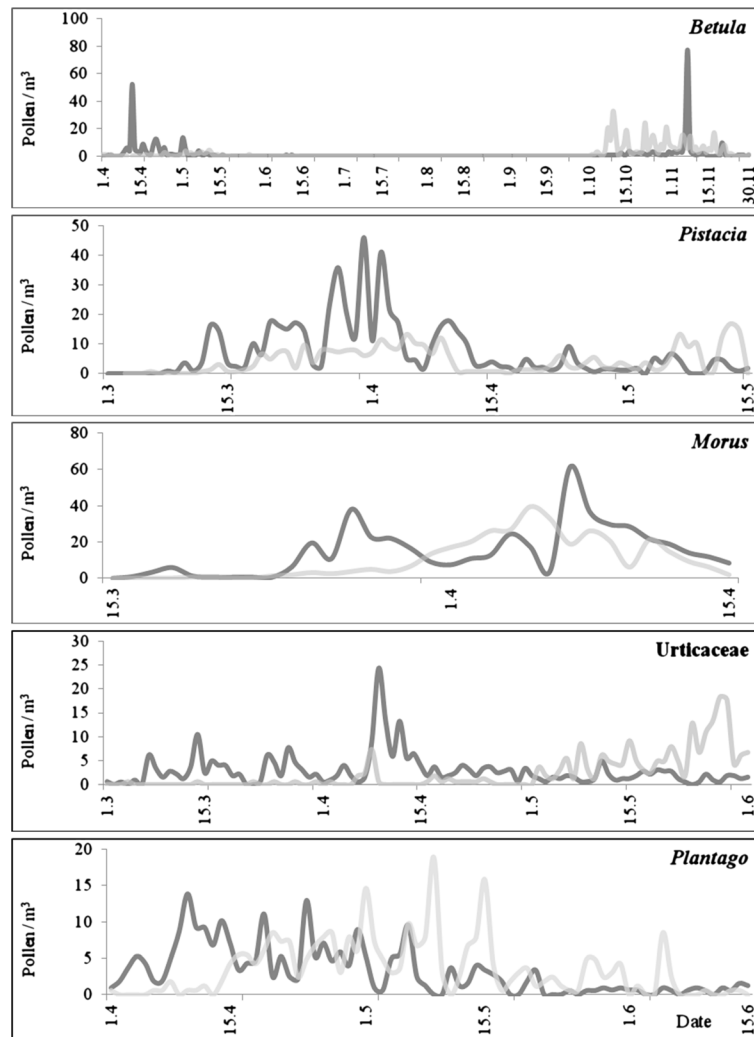


Fig. 8 (continued)

similarity of the pollen grains of these genera they identified in family level. Urticaceae was the second most abundant non-arboreal pollen type in Bodrum, and the annual rate was 1.46 % (Table 1). A long pollen season covered February–September (Fig. 3). Pollen grains from this taxon reached peak concentrations in May in Bodrum (Table 3). The MPS was 22–30 days long, with a nearly 2-month delay in the second year (Fig. 8). Pollen concentration was highest in the morning, with peak levels between 10:00 and 11:00 (Fig. 9).

Plantago was the last species in the list of dominated pollen types in Bodrum, which represented 1.28 % of the annual pollen index (Table 1). In our study, *Plantago* pollen grains were detected in the atmosphere between February and September

(Fig. 3). In our study, pollen concentrations reached peak levels in April at 0.55 % (Table 3). The MPS was 28–47 days long (Fig. 8). In Bodrum, high levels of *Plantago* pollen concentrations are observed in the afternoon with the highest quantities recorded between 14:00 and 15:00 (Fig. 9).

There were a number of significant correlations ($P < 0.05$) between daily mean pollen concentrations of ten dominated pollen types and meteorological data (Table 2). We found positive correlations between daily Cupressaceae/Taxaceae pollen concentrations and daily mean relative humidity in both years, but correlation was negative between daily *Pinus* pollen concentrations for the same parameter. Daily *Olea*, Poaceae, and Urticaceae pollen concentrations and mean daily

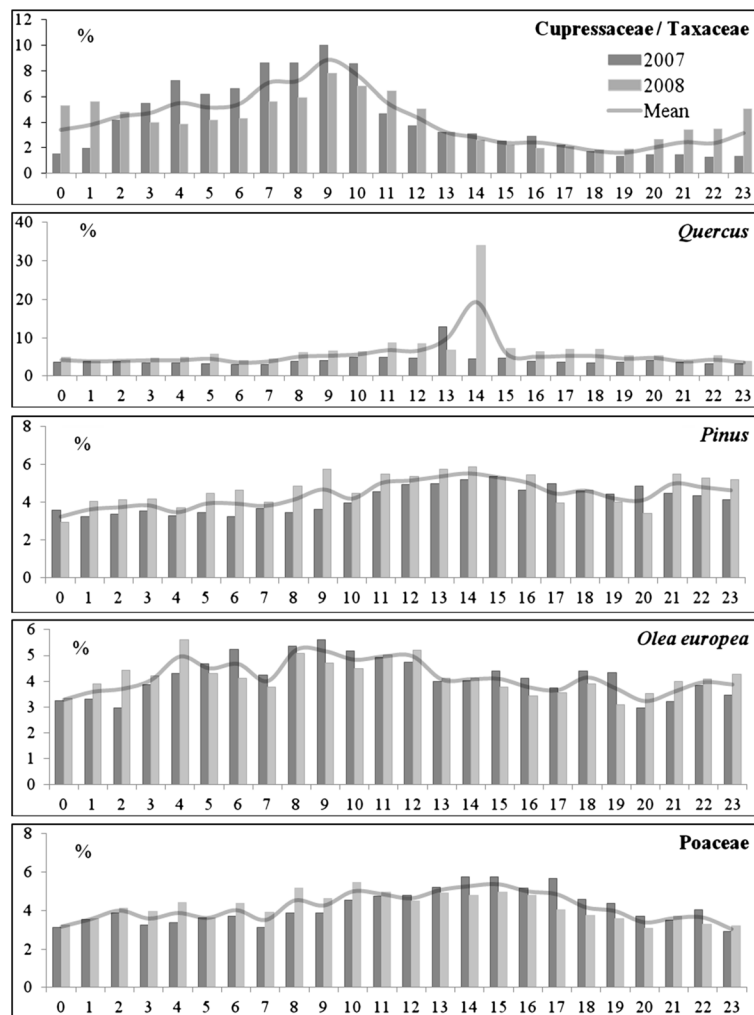


Fig. 9 Intradial variation of main pollen types in Bodrum atmosphere

relative humidity were negatively correlated just in one of the sampling years, but not in the other sampling year. On the other hand, there was a significant positive correlation found between daily *Betula* and *Morus* pollen concentrations and relative humidity in the one of the sampling years. There were no significant correlations found between *Quercus*, *Pistacia*, and *Plantago* pollen concentrations and relative humidity in both years (Table 2).

Using the mean daily temperature and pollen concentration data, we obtained a negative correlation with Cupressaceae/Taxaceae pollen concentration for both years. Also, we found a negative correlation between *Quercus*, *Betula*, *Pistacia*, and *Plantago* pollen concentrations and mean daily temperature for 1 year. In addition, a significant positive correlation was found

between *Pinus*, *O. europaea*, and *Urticaceae* daily pollen concentrations and mean daily temperature for one sampling year. On the contrary, there could not be found any correlation on daily Poaceae and *Morus* pollen concentrations and mean daily temperature (Table 2).

There were not any significant correlations for both years of any taxa for mean daily wind speed. While there were significant correlation between Poaceae pollen concentration and mean daily wind speed, a significant positive correlation was obtained for only one sampling year between daily *Pinus* pollen concentration and mean daily wind speed. There were not any significant correlations between wind speed and other pollen types (Table 2).

Looking up to the statistical data between pollen concentrations and daily rainfall, there were not any

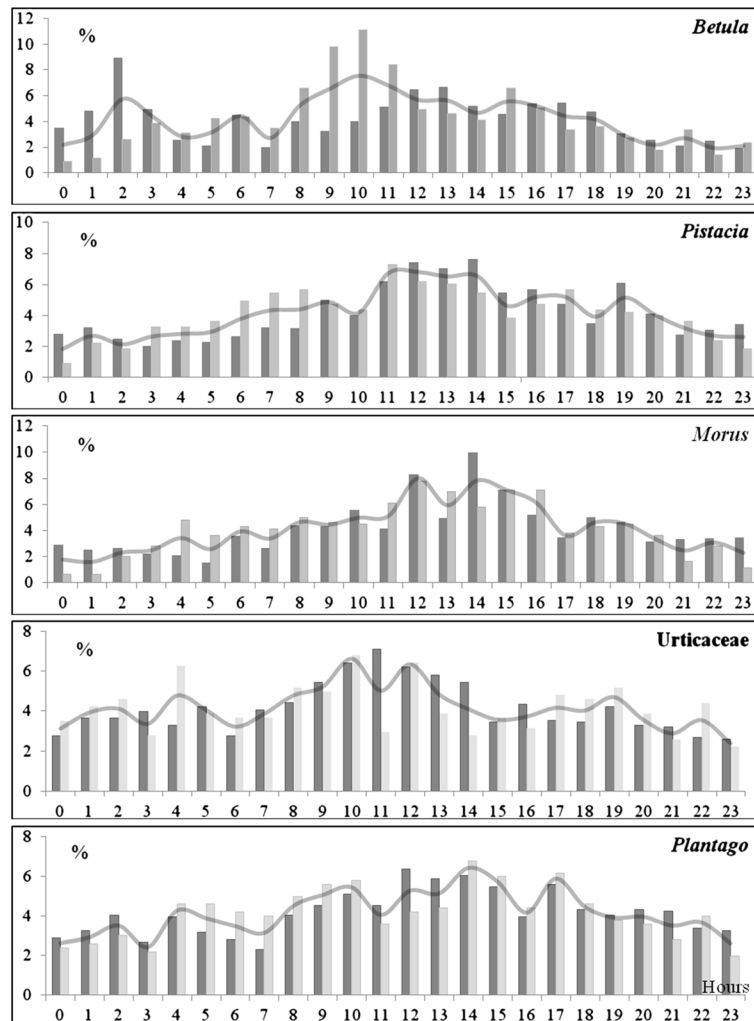


Fig. 9 (continued)

significant correlations obtained for both years of any taxa; a significant negative correlation was found between rainfall and *Morus* pollen concentrations for one of the studied years, and there was a significant positive correlation between daily Cupressaceae/Taxaceae, *Quercus*, and *Betula* pollen concentrations and daily rainfall. On the other hand, there could not be found any correlation between daily *Pinus*, *O. europaea*, Poaceae, *Pistacia*, Urticaceae, and *Plantago* pollen concentrations and daily rainfall (Table 2).

Discussion

In the present study, pollen grains of Bodrum were found to be belonging to 41 plant taxa, with 23 of them

from woody plants (23 taxa) with the largest atmospheric contribution of 86.94 % as a mean value. Cupressaceae/Taxaceae pollen was the most abundant pollen type in our study, which is not surprising because there are many cultivars in the city. Moreover, a predominance of this pollen type has also been recorded in Istanbul, Turkey (Celenk et al. 2010), in Cagliari, Italy (Ballero and Maxia 2003), in Porto, Portugal (Abreu et al. 2003), and in Thessaloniki, Greece (Gioulekas et al. 2004). However, in the other Turkish aeropalynological studies, the most abundant pollen type reported was *Pinus* (i.e., Bıçakci et al. 2000a, b; Bıçakci et al. 2004; Bilisik et al. 2008a, b; Tosunoglu et al. 2009; Gucl et al. 2012; Tosunoglu et al. 2015). The determinant winds that blew in the Bodrum atmosphere were usually coming from the north and south

(Fig. 7). The northern study area is mostly covered by pine forests; thus, northern winds are likely responsible for transporting pine pollen grains to the city center. The southern Bodrum peninsula is more arid than the northern part; thus, winds coming from the south are mostly blowing from the sea and are likely carrying pollen grains of maquis elements and cultivars from the city center and the immediate surroundings. The aeropalynological spectrum of Bodrum was found to be compatible with floral elements in this study. Unfortunately, the Bodrum area natural flora is rapidly changing because of urbanization and degradation, which occur in parallel to population growth in the area.

On the other hand, in Mediterranean countries, Cupressaceae/Taxaceae is reported as a main type of atmospheric pollen in winter and early spring (Papa et al. 2001; Docampo et al. 2007); also, these pollen types are known as an important cause of respiratory allergies in the region (Felice et al. 1994). The prevalence of allergic sensitization to Cupressaceae/Taxaceae pollen grains is increasing worldwide (D'Amato 1998), and this is probably due to the wide usage for gardening, reforestation, and high *Cupressus* plantation in graveyards especially in Turkey. Exposure to these type of pollen in sensitive individuals seems to be a strong risk factor for allergic rhino-conjunctivitis and, in some cases, for bronchial asthma (D'Amato 1998). In our study, pollen concentration from these taxa had two peaks, with the highest values in March, followed by February and November (Fig. 3). These two peaks of Cupressaceae/Taxaceae pollen in Bodrum are likely due to different species' flowering periods. For example, *Cupressus arizonica* and *Cupressus sempervirens* are common trees in the study area, and *Cupressus arizonica* was reported to flower 20–23 days before *C. sempervirens* (Hidalgo et al. 2003). Also, *Cupressus arizonica* trees are reported to produce second pool of flowers 2–4 weeks after the main flowering period, which is halted at the close of immature phase and remained at this stage during summer, autumn, and winter; so, these flowers were joined by the normal pool of the flowers set in early winter (Hidalgo et al. 2003). Furthermore, overlap of pollination seasons between *Cupressus sempervirens* and *Cupressus arizonica* causes another problem; pollen extracts are found highly cross-reactive at the IgE level (Barletta et al. 1996). The MPS was calculated as 287–300 days in Bodrum (Table 3), parallel to the MPS duration of Nerja, another Mediterranean city in southern Spain (Docampo et al.

2007). However, this period is too long to understand peak points and is therefore meaningless. If the MPS is calculated from the beginning of October, the MPS was 114–156 days long. Conversely, it may be more meaningful to divide the Cupressaceae/Taxaceae MPS into spring and winter seasons. In this case, the MPS is 32–33 days in early spring and 18–37 days in winter (Fig. 8, Table 3). Compared with the second year, the Cupressaceae/Taxaceae MPS was delayed by nearly 25 days, which was most likely because of greater precipitation and higher temperature in October in the first year (Fig. 6). On the other hand, there was a significant negative correlation found between daily mean Cupressaceae/Taxaceae pollen concentration and mean daily temperature in both 2007 and 2008; a significant positive correlation was found in both years with mean daily relative humidity (Table 2). In addition, if we divide the MPS of Cupressaceae/Taxaceae pollen grains in two seasons as spring-first peak and winter-second peak; there were not any significant correlations found between pollen concentration and meteorological parameters except the significant negative correlation of mean daily relative humidity in the winter period of 2008 (Table 2). In our study, Cupressaceae/Taxaceae pollen grains were common in the morning and reached their maximum value between 09:00 and 10:00 am, but in another study that was performed in Santiago de Compostela, another Mediterranean city on the NW Iberian Peninsula, the greatest pollen concentration was recorded in the afternoon between 11:00 and 13:00 (Aira et al. 2001) in contrast to our results.

Although Emberlin et al. (1990) and Spieksma et al. (2003) suggested a biennial or triennial alternating pattern in airborne oak pollen annual totals; it is difficult to support these data with our biennial sampling. The MPS was delayed for 12 days in the second year, and the reason for this delay may have been due to different meteorological factors in 2 years; in the second year, higher precipitation humidity in addition to lower wind speed in April may have caused this disparity (Fig. 6). Comparing the daily *Quercus* pollen concentrations with meteorological factors, there were significant negative correlations found with mean daily temperature and positive correlation found with daily rainfall (Table 2). Looking up to the intradiurnal variation of oak pollen grains, K  p  l   (1984) reported a peak of oak pollen at noon, and in the late afternoon, Corden and Wendy (1999) found a peak in the Derby area, UK, similar to our results, which occurred at approximately

15:00 hours; on the other hand, maximum concentrations were observed between 22:00 and 06:00 hours in Toledo (Spain) (Pérez-Badia et al. 2010). In general, *Quercus* pollen is not considered to be a potential aeroallergen, although there is positive skin reactions to oak pollen allergens reported from many regions (García-Mozo et al. 1999; Basak et al. 2011; Larenas-Linnemann et al. 2011; Grewling et al. 2014). *Quercus* pollen allergy may be related to the high level of immunological similarities and cross-reactions of pollen grains in Fagales order and especially with birch allergens (Egger et al. 2008; Wallner et al. 2009).

There has been controversy about the allergenicity of pine pollen; some authors previously suggested that pine pollen is not a significant allergen because of its low allergenicity and large size while there are some older studies supporting pine pollen as an allergen (Harris and German 1985). Therefore, it is thought that pine pollen allergy must be taken into account in patients living in areas with high *Pinus* pollen concentrations and with seasonal respiratory disease (Marcos et al. 2001). Inter-annual *Pinus* pollen variation in Bodrum created a bumpy chart for both years studied, and in the middle of the MPS, there was a long, stagnant, low-level period in the second year (Fig. 8). In the second year, *Pinus* pollen concentration greatly decreased; this decrease may be a result of meteorological factors, similar to *Quercus* pollen grains in April, such as large amount of rainfall and humidity (Fig. 6). Comparing with the meteorological factors statistically, there was a significant negative correlation found between daily *Pinus* pollen concentration and mean relative humidity in both years. On the other hand, we obtained a significant positive correlation between pollen concentration and temperature in the year 2008 (Table 2). Conversely, Atkinson and Larsson (1990) and Green et al. (2003) demonstrated an alternating tree-year pollination cycle pattern in *Pinus*. Similar MPS results were found with Perugia, Italy (Frenguelli et al. 2002), Brisbane, Australia (Green et al. 2003), and Nerja, southern Spain (Docampo et al. 2007) for pine pollen.

Difference between the pollen amounts of 2 years may be because the *O. europaea* pollination period length depends upon both temperature and rainfall; high temperature and low rainfall shorten the main pollination period (González Minero and Candau 1997a). Although the mean temperatures of the studied years were not significantly different, especially during the

MPS months, a significant disparity was noted in both rainfall and relative humidity at the beginning of the MPS in our study (Fig. 6). In the first year, there was a significant correlation found between daily pollen concentration of *O. europaea* and mean daily temperature, but in the second year, we obtained a significant negative correlation between the pollen concentration and mean daily relative humidity (Table 2). Other authors reportedly confirm that rainfall during the *O. europaea* vegetative period has a positive effect on pollen production (González Minero and Candau 1997a; González Minero et al. 1998; Galán et al. 2001). Conversely, *O. europaea* aeropalynology has a typical biannual pattern of alternate pollen production; high pollination will therefore result in both reduced flowering and pollination in the following year, as was previously observed (Domínguez-Vilches et al. 1993; González Minero and Candau 1997a). The MPS of *O. europaea* in Spain was reported as 26–62 days in Córdoba, 32–57 days in Jaén, 44–75 days in Málaga, 35–79 days in Granada, and 28–63 days in Seville (González Minero and Candau 1997a; Díaz de la Guardia et al. 2003) and found similar to our MPS durations. Inter-annual *O. europaea* pollen grain variation was also variable, and the MPS was mostly throughout May. Olive pollen is considered as one of the most important causes of respiratory allergic disease in the Mediterranean region, and also polysensitization to olive pollen is more frequent than monosensitization (Liccardi et al. 1996). The frequency of pollinosis to olive pollen grains is clinically characterized by rhinoconjunctival symptomatology than bronchial asthma (D'Amato et al. 2007).

While Poaceae family members are the most successful and important group of plants, both ecologically and economically, they are also the major cause of pollinosis in many parts of the world (D'Amato et al. 2007). They are responsible for the symptoms in the majority of allergic rhinitis patients and also can trigger asthma. Poaceae is the most abundant non-arboreal pollen type in the atmosphere of Bodrum. These types of pollen grains are obtained continuously in the atmosphere during the year (Fig. 3). Nevertheless, the short MPS of Poaceae pollen was found compatible with the other studies that were performed in southern Spain; the MPS was 21–76 days long in Córdoba, 11–52 days long in Priego, and 25–66 days long in Ciudad Real. The grass pollen season in Bodrum is also shorter than in England (Sánchez Mesa et al. 2003). Looking up to intradiurnal variation of Poaceae pollen; it can be seen

that concentration was increased in the afternoon, between 10:00 and 17:00, and some studies had similar findings (Norris-Hill 1999); however, other studies did not (Alcazar et al. 1999). Also, there was a significant negative correlation found between daily Poaceae pollen concentration and mean daily relative humidity and mean daily wind speed in year 2007 (Table 2).

In Turkey, species of *Betula* is mostly naturally growing in the north and east parts of the country, but especially *Betula pendula* Roth and *Betula nigra* L. are common plants which are frequently planted in parks, gardens, and roadsides of the study area. *Betula* pollen is a well-known significant aeroallergen especially in European countries (Eriksson and Holmen 1996). Pollen grains of birch, followed by alder and hazel, has the greatest allergenic potency in this group of allergenic trees (D'Amato et al. 2007). The cross-reactivity between the Betulaceae members and the other tree pollen allergens is also well documented, so clearly, this pollen type contributes to a prolonged season of symptoms for many patients (Florvaag et al. 1982). In our study, pollen grains of *Betula* had two peaks during the year, similar to Cupressaceae/Taxaceae pollen grains. The first period was between March and June, and the second period was from October to February. Two peaks of *Betula* is an unusual phenomenon, but winter and spring peaks of *Betula* pollen were also recorded before in Adana atmosphere, in south coast of Turkey (Altintas et al. 2004). That may be due to different pollen seasons of different species of the genus *Betula*, but there is not any evidence and any phenological observation to present any species as responsible of these peaks. Differences that appear in relation to the start of the pollen season for different species of winter–spring flowering trees in the temperate zone may be explained by the air temperature during the period preceding the pollen season (Rodríguez-Rajo et al. 2003). In addition, these flowering peaks and especially the winter peak may be a result of the light climate of the study area. Dormancy in autumn and early winter, driven mostly by accumulation of chilling temperatures, hinders the start of ontogenetic development. There is evidence that light climate plays a role in the start of ontogenetic development (Myking and Heide 1995; Häkkinen 1999; Linkosalo et al. 2000). The peak MPS in the spring was 20 days long between mid-April and the beginning of May, while the winter peak was 15 days long during November. In the following year, a clear winter peak was detected with a 41-day MPS

lasting from the beginning of October until mid-November; however, there was no spring peak, and the recorded pollen concentration in the spring was quite low (Fig. 8 and Table 3). *Betula* concentration may be affected by higher rain and humidity in April, contrary to *O. europaea* in the second year (Fig. 6). Looking up to the statistical data in the year 2007, there was a significant positive correlation found between daily pollen concentration with mean daily relative humidity and daily rainfall. Also, a significant negative correlation was obtained between mean daily temperature and pollen concentration in the same year. But, in the second year, there were not any correlation found between pollen concentration of *Betula* pollen grains and meteorological factors. Calculating the MPS of *Betula* pollen from 1 January results with included long and stagnant period to MPS between two peaks; because of this case, we divided the MPS period and statistically analyzed spring (first peak) and winter (second peak) peaks separately, but there were not any correlations found between daily pollen concentration and meteorological factors during these peaks for both years as in 2008 (Table 2). Additionally, conversely to our results of intradiurnal variation, *Betula* pollen concentrations were reported in lower levels in the early morning than in the afternoon, and the maximum levels were detected before midnight in Reykjavík (Iceland) (Halladóttir 1999); however, Jäger (1990) in Vienna, Austria, and Käpylä (1984) in Finland emphasized the variability of the daily peak time of *Betula* pollen concentration (Clot 2001).

Pollen grains of *Pistacia* in the atmosphere are unique for especially Mediterranean basin. Most of the species are spread around the Mediterranean basin, although a few of them grow in East Africa, some reach Central and Eastern Asia, and some in Mexico (Zohary 1972; Waisel and Alon 1980). The allergenicity of *Pistacia* pollen was discovered only a few years ago (Keynan et al. 1987; Keynan et al. 1997) and there are not many studies about it. *Pistacia* pollen was reported as a major cause of pollinosis, and the incidence of atopic patients to this pollen type was found very high (Keynan et al. 1997). Together with our study, *Pistacia* pollen was also detected as a predominant pollen type in the atmosphere of southern Croatia (Cvitanovic et al. 2004), in Didim, west Turkey (Bilisik et al. 2008b), and in both the Asian and European areas of Istanbul, north-west Turkey (Celenk et al. 2010). On the other hand, we found a significant negative correlation in the second year between daily *Pistacia* pollen concentrations and

mean daily temperature in Bodrum atmosphere (Table 2). In our study, *Pistacia* pollen grains were common in the afternoon and reached their maximum value between 12:00 and 13:00 am.

The mulberry tree is found throughout the world, including Asia, Eastern and Midwestern USA, and Southern Europe. Mulberry trees are the leading cause of pollen allergy (Targow 1971; Murioz et al. 1995). *Morus* pollen grains were reported as a predominant pollen type also from La Plata City, Argentina (Nitiu 2006), İzmir, west Turkey (Guvensen and Ozturk 2003), and Köyceğiz, southwest Turkey (Tosunoglu et al. 2009). There was a significant correlation between *Morus* pollen concentration and daily rainfall in 2007 and also a significant positive correlation between mean daily relative humidity in 2008 (Table 2). Intradiurnally, pollen concentration was centered in the afternoon, and the highest concentration was recorded between 12:00 and 13:00 in Bodrum. This finding was similar to a report from La Plata City, Argentina (Nitiu 2006); however, contradictory data were reported from Cordoba, Spain, where the highest *Morus* pollen concentration was reported at 20:00 in the afternoon (Garcia-Mozo et al. 2007).

In the Mediterranean region, pollen from Urticaceae family members is among the most allergenic in the air and is often implicated in respiratory allergies (Gioulekas et al. 2004; D'Amato et al. 2007). Urticaceae pollen season run along February to September in the study region, and the seasonal behavior of this pollen was similar to that recorded in other cities such as London (Emberlin and Norris-Hill 1991), Perugia (Fornaciari et al. 1992), Malaga (Trigo et al. 1996), Seville (González Minero et al. 1997b), Córdoba, Granada (Diaz de La Guardia et al. 1998), and Trieste (Rizzi Longo et al. 2004). The MPS duration was delayed for nearly 2-months in the second year. In the following year, Urticaceae family pollen concentrations remained very low, especially in March and April, which may be the result of a rainy spring in 2008. There was a significant negative correlation between pollen concentration and relative humidity in 2007 and significant positive correlation between daily temperatures in 2008 (Table 2). Pollen concentration recorded in high levels in the morning (between 10:00 and 11:00); however, maximum counts were recorded between 14:00 and 16:00 in another study (Diaz de La Guardia et al. 1998).

In the past years, several studies in different countries have shown the clinical importance of *Plantago* pollen grains. Although it is thought that *Plantago* pollen is a minor cause of pollinosis; the highest incidence of allergy to plantain has been described in Australia (Krilis et al. 1985) and in the Mediterranean area (D'Amato and Lobefalo 1989). On the other hand, most patients found positive to the skin prick test with plantain pollen extracts are hypersensitive to grass (Bousquet et al. 1984; Watson and Constable 1991), a fact which suggests that grass and plantain pollens cross-react (Asero et al. 2000). *Plantago* pollen was also present in the air from March to September in the southern Iberian Peninsula and from April to October in the northern Iberian Peninsula (Gutiérrez et al. 1999). The MPS was 28–47 days long, which was not as long as was recorded in Cordoba, Spain, and Ponzan, Poland (Alcázar et al. 2009), or Szczecin, Poland (Puc 2009). Looking up to the statistical data, there was a negative correlation between mean daily temperature and *Plantago* pollen concentration in the year 2008 (Table 2). In the other studies, different results were found from our study about intradiurnal variations of *Plantago* pollen; maximum levels were recorded between 10:00 and 12:00 in the SW Iberian Peninsula (Molina et al. 2001), between 12:00 and 16:00 in E-Central Iberian Peninsula (Pérez-Badia et al. 2011), and between 14:00 and 16:00 hours in Toledo (Spain) (Vaquero et al. 2012).

In conclusion, in the Bodrum atmosphere, pollen concentrations peaked in March and prominently decreased in the summer, in parallel with high temperatures. Thus, in this famous tourism center, allergenic pollen grains reportedly display minimum activity concurrent with summer dryness during the tourism season. Determination of pollen types, concentrations, and fluctuations through the year as well as intradiurnal variations will be useful for patients who suffer from pollen allergies, and the regional pollen calendar presented in this paper may be useful also for visitors of the city for timing and may be useful for allergologists to establish an exact diagnosis.

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval For this type of study, formal consent is not required. This article does not contain any studies with human participants or animals performed by any of the authors.

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