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ORIGINAL PAPER

The effect of geographical and climatic properties on grass pollen and Phl p 5 allergen release

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Abstract

The Poaceae family, including grasses, comprises several cosmopolitan and allergenic species. The aim of this study was to determine the correlations between Poaceae pollen and Phl p 5 allergen concentrations in two cities with different geographical and climatic properties in Turkey. Pollen were collected from Burkard traps in Ankara and Zonguldak. Phl p 5 sampling was carried out between March and October in both 2015 and 2016 using a BGI900 Cascade High Volume Air Sampler (900 L/min.). The concentrations of Phl p 5 were measured by the enzyme-linked immunosorbent assay (ELISA) technique. The annual sum of Poaceae pollen (pollen index) during 2015–2016 was 5454 in Ankara and 4142 in Zonguldak. The total Phl p 5 concentration was 1309 pg/m^3 in Zonguldak, whereas it was 8181 pg/m^3 in Ankara over 2 years. About 90% of the allergen was found in the fraction with particulate matter (PM) > 10 μm in both cities. It was found that the main meteorological parameter which affected pollen and Phl p 5 was temperature in both stations. Rainfall was also found to be important for Zonguldak, due to its climatic and geographic properties. Lastly, we suggest that the primary wind direction, which is from the south of Zonguldak, could have a 'drift effect' for allergens because of the airborne pollen concentrations and the dates on which the allergen is released into the atmosphere. The wind direction may be an important factor in the distribution of allergen and pollen grains in stations, especially those with a hilly topography.

Keywords Aeroallergen · Ankara · Zonguldak · Turkey · Phl p 5 · Monitoring

Introduction

The grass family (Poaceae) includes more than 12,000 known species belonging to 771 genera with a worldwide distribution (Soreng et al. 2015). The majority of these species produce highly allergenic pollen, which is the primary cause of pollinosis in several countries (Jaggi et al. 1989; Fischer et al. 1996; García-Mozo 2017). Poaceae pollen allergens have been classified into 10 different groups (Pablos et al. 2016). Phl p 5 belongs to Group 5 allergens and shares sequence identity with other pollen allergens such as Lol p 1 from *Lolium perenne*. Almost 90% of grass pollen-allergic patients are sensitised against Group 5 grass pollen allergens (Flicker et al. 2000). Phl p 5 consists of 287 amino acid residues. Group 5 allergens have been shown to demonstrate ribonuclease activity (Laaidi et al. 2003). Several isoforms of grass pollen allergen are also known to exist, which are primarily caused by the multiple gene products or by their combinations. Modification of Poaceae allergens, such as by glycosylation, is very important due to cross-reactions with other allergens such as food allergens (Andersson and Lidholm 2003).

There are approximately 602 taxa belonging to the Poaceae family distributed in Turkey (Bıçakçı et al. 2009). In Central Anatolia, in particular, the steppe vegetation primarily consists of the members of the Poaceae family. The pollen season of the Poaceae family in Turkey varies according to the geographical region from March to August. In Ankara, the Poaceae family comprises 8% of the total pollen load, whereas 2.7% in Zonguldak (Kaplan 2004). Long-term studies in Ankara have shown that annual Poaceae pollen concentrations have tended to be lower over the years (Stach et al. 2008; Acar et al. 2017) because of urbanisation.

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The relationship between allergic diseases and atmospheric pollen concentrations has been a well-documented phenomenon. It has been supposed that airborne pollen concentrations represent allergen exposure. However, some studies conducted in the last two decades about respirable particles containing pollen allergens, that are generally smaller than 10 μm and released from pollen, have demonstrated that these particles are responsible for causing thunderstorm asthma (Suphioglu 1998; D'Amato et al. 2007). Therefore, recent studies have been focusing on the allergen content of the atmosphere. There is some evidence showing that pollen loads do not adequately represent the allergen exposure of sensitised patients. It has been suggested that the allergen amount (Bet v 1/Phl p 5) has a strong but regionally dependent impact on the symptom load of pollen allergy sufferers (Bastl et al. 2016).

On the other hand, several studies have emphasised that aeroallergen monitoring also allows the detection of non-pollen-bound allergens. It has been reported that more than 89% ± 11% of the allergen collected across Europe was contained in particulate matter (PM) > 10 μm. However, on some days, the allergen proportions with 10 μm > PM > 2.5 μm reached 20% of the total airborne allergen (Buters et al. 2015). Plaza et al. (2016) reported another study on non-pollen-containing fractions of ambient air in Córdoba, in which they recorded the allergen levels when there was no pollen in the atmosphere.

The geographical and climatic properties of stations could also be important for expressing the allergen exposure to sensitised patients. Atmospheric pollen and allergen concentrations tend to be lower at higher altitudes (Jochner et al. 2015). Moreover, it has been reported that the release amount of Group 5 allergens from each pollen grain varied naturally across Europe (Buters et al. 2015).

In this study, our aim was to determine the correlations between Poaceae pollen and Phl p 5 allergen concentrations in two cities with different geographical and climatic properties.

Material and methods

Study area

The study was carried out during 2015–2016 in Ankara (41° 27′ 13.3" N, 31° 45′ 42.4″ E) and Zonguldak (39° 56′ 12.8" N, 32° 49′ 50.8″ E) cities (Fig. 1). Ankara is the capital city of Turkey with 5.5 million inhabitants and located in Central Anatolia which has a steppe vegetation. Average annual rainfall is 387.2 mm and the annual average temperature is 11.9 °C (Table 1).

Zonguldak is a small city with about 110,000 inhabitants. It is also situated in the North West part of Turkey. The settlement is mostly located on the coastline due to the hilly topography (Fig. 2). Fifty-six percent of the city is made up of mountains, and 52% of Zonguldak is covered with forests mainly consisting of leafy trees such as *Fagus*, *Carpinus*, *Ostrya*, *Corylus* and *Quercus* (Güner et al. 2012). Average annual rainfall is 1040.5 mm and annual average temperature is 13.6 °C from data obtained from the Turkish State Meteorological Service (Table 1). The changes of some meteorological parameters during 2015 and 2016 and seasonal normals of the both cities were given in Fig. 3.

Since Ankara is located in the Irano-Turanian phytogeographic region and has an arid climate, many Poaceae species are distributed naturally. One among them is endemic (*Festuca longipanicula* Markgr.-Dann.), 81 taxa are found in Ankara, whereas about 10 taxa are identified in Zonguldak (Davis 1965; Akaydin and Erik 2002). Only species *Hordeum murinum* L. subsp. *glaucum* (Steud.) Tzvelev and *Bromus japonicus* Thunb. subsp. *japonicus* are common.

Sampling and measuring of airborne pollen and aeroallergens

Airborne Poaceae pollen and Phl p 5 submicronic particles were sampled during 2 years in Ankara and Zonguldak. Samplers were placed on the flat roof of the building at about a height of 30 m above ground level. Airborne Poaceae pollen were collected using a 7-day Hirst-type volumetric spore trap (Hirst 1952), and counted on the full area of the slide. Pollen are expressed in pollen/cubic metre. For defining the pollen season, 98% of total pollen index were taken (Emberlin et al. 1993).

Air was sampled by using BGI900 (MesaLabs, USA) high volume cascade impactor for Phl p 5 sampling. The suction rate of the sampler is 900 L/min. Two different cascades were used for sampling. While the first cascade was collecting particles which PM > 10 μm, the second cascade was collecting particles in diameters between 10 and 2.5 μm onto polyurethane filters. Samplings were made simultaneously in both Ankara and Zonguldak. The filters changed daily in the pollen season at 12:00 AM. Then, the filters were cut into three identical parts. Two filter parts were used for Phl p 5 analysis, the rest was stored at − 20 °C as a backup.

Extraction and quantification of airborne allergens

At least two different filter parts for each cascade were extracted separately in 0.1 M NH4HCO3, pH 8.1 with 0.1% bovine serum albumin (BSA) in overhead rotator for 4 h in the dark (Buters et al. 2010, 2012). The extracts were clarified by centrifugation and lyophilized. Phl p 5 levels were determined by sandwich ELISA with the monoclonal antibodies (Indoor Biotechnologies) in redissolved samples in 1/10 of original volume. Allergens are expressed in picogramme/cubic metre. For each day, at least two filter parts were independently analysed.

Fig. 1 Map of Turkey showing location of Ankara and Zonguldak cities

Table 1 Yearly average, minimum, maximum temperature, relative humidity and total rainfall in Ankara and Zonguldak (2015–2016)

	Ankara		Zonguldak	
	2015	2016	2015	2016
Total rainfall (mm)	447.8	883.8	983.2	1329.4
Average temperature (°C)	12.48	13.06	13.69	14.27
Average relative humidity (%)	60.55	57.32	78.36	77.66
Average air pressure (hPa)	914.85	914.08	1001.85	1000.74

Meteorological Data and Back-Trajectory Analysis

Meteorological data (average, maximum and minimum daily temperature, average relative humidity, average wind speed, rainfall and air pressure) were obtained from Ankara and Zonguldak stations of the national weather services.

The Hybrid Single-Particle Lagrangian Integrated Trajectory (HYSPLIT) modelling system (Stein et al. 2015) was used to calculate the backward trajectories. The backward trajectories were prepared for 9 July 2015 and 13 and 26 June of Episode III in 2016 for Zonguldak.

Meteorological data which are used for preparing back trajectories were taken from the operational system of the National Weather Service's National Centers for Environmental Prediction (NCEP), Global Data Assimilation System (GDAS, 0.5 degree, global, 09/2007-present, the number of trajectories = 12). We used 72 h and 12 h as the backward trajectories with 6-h intervals and 70 m altitudes for Zonguldak (41.45 N, 31.76 E).

Statistical analysis

Spearman correlation analysis was performed to identify potential correlations between mean daily Poaceae pollen concentrations, airborne Phl p 5 concentrations and weather variables. Also, principal component analysis (PCA) was taken into account for showing the most favourable meteorological factors during both pollen and allergen seasons. Daily pollen and allergen levels during

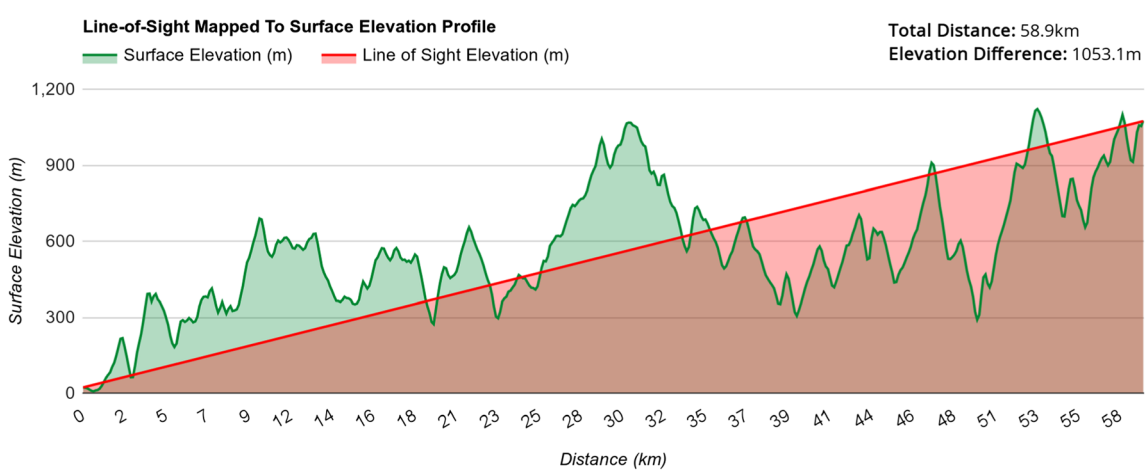

Fig. 2 Elevation chart of Zonguldak starting from the sampling area to the south

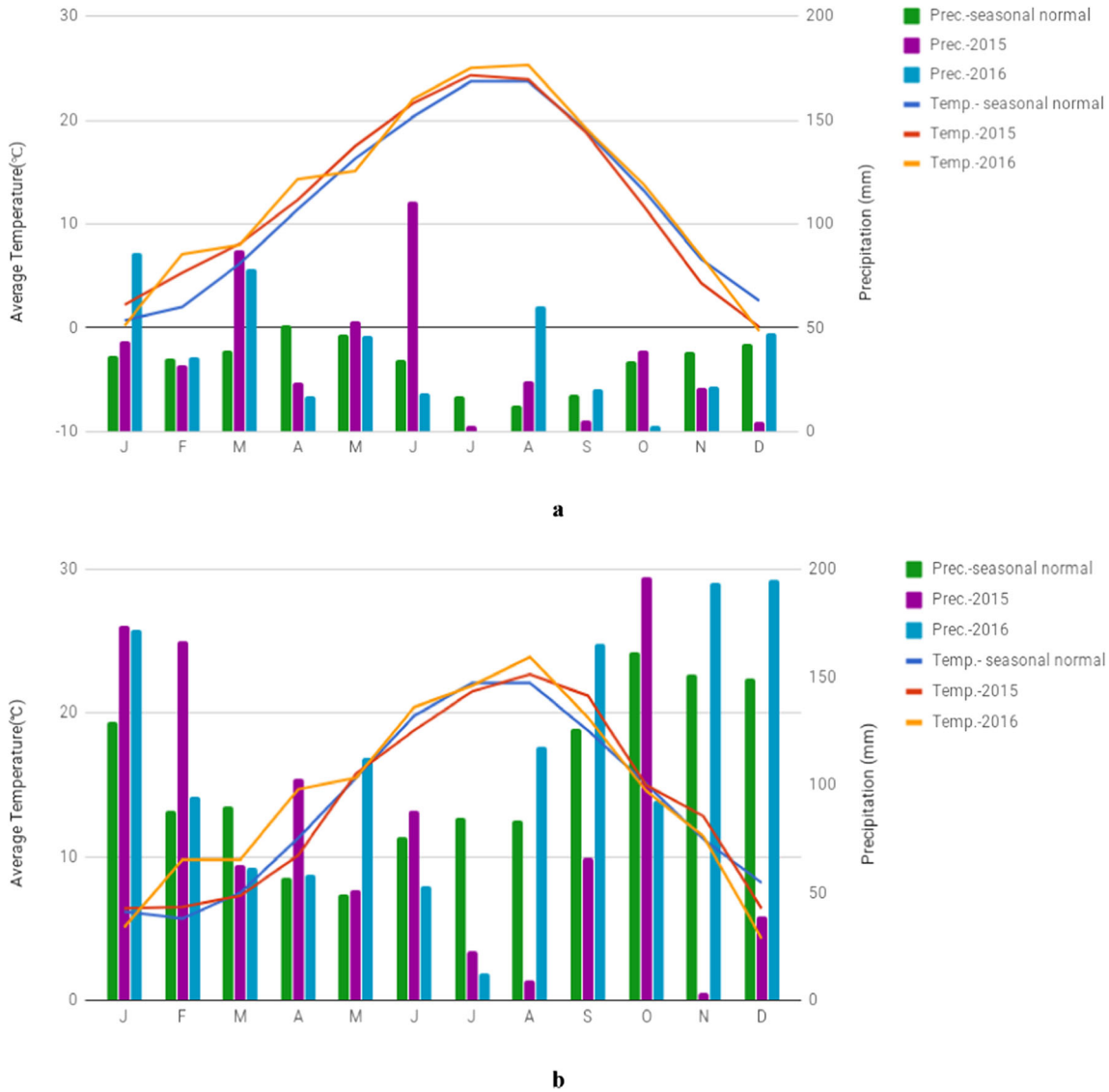

Fig. 3 Intraseasonal variations of rainfall and temperature in Ankara (**a**) and Zonguldak (**b**)

the common season, average temperature (AT), average relative humidity (AH), average pressure (AP), wind speed (WS) and total rainfall (RF) were studied variables, influences of each on pollen and allergen features were investigated. The results of factor loadings showing correlations between weather parameters and the obtained factors (PC1 and PC2) as well as in the form of factor score scatterplots with the PC1-PC2 coordinate systems are presented in Online Resource 1 (Parrado et al. 2009). The results are shown in a two-dimensional graph with the first and second principal components. Unrotated, data were used to create a covariance matrix, and three eigenvectors were extracted. The values of the eigenvectors and the total cumulative variance are represented in Online Resource 1. All statistical analyses were performed using the R programming language (R Core Team 2013).

Results

Pollen counts

The annual sum of the Poaceae pollen index (PI) during 2015 was 4006 for the Ankara station and 2101 for the Zonguldak station. The pollen season length of the stations for 2015 was 143 days for Ankara and 133 days for Zonguldak. The major peak was recorded on 8 June with 60.6 pollen/m^3 for Ankara and on 27 May with 15.3 pollen/m^3 for Zonguldak (Fig. 4.).

In 2016, the PI was 1448 for Ankara and 2041 for Zonguldak. It was observed that while the pollen seasons were longer in both stations, the pollen concentrations in both stations were lower than in 2015. The pollen season was calculated as 166 days for Ankara and 176 days for Zonguldak. The major pollen peak was recorded on 17 September with 4.9 pollen/m^3 for Ankara and on 30 May with 12.4 pollen/m^3

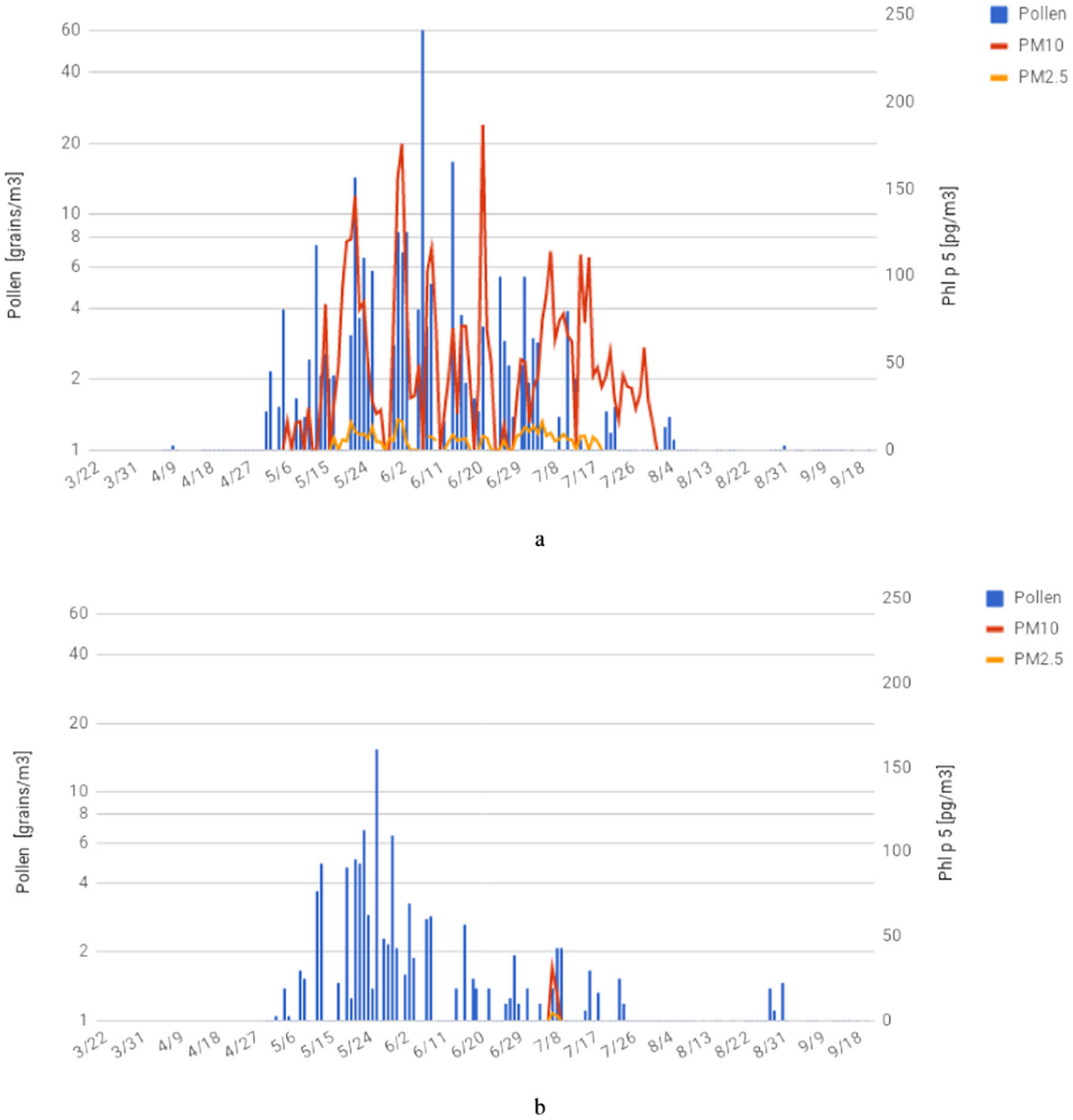

Fig. 4 Seasonal variation of Poaceae pollen and Phl p 5 in PM > 10 μm and 10 μm > PM > 2.5 μm in 2015 and 2016 from Ankara and Zonguldak stations. **a** 2015, Ankara. **b** 2015, Zonguldak. **c** 2016, Ankara. **d** 2016, Zonguldak

for Zonguldak. Some characteristics of the pollen and the allergen during the study period in both cities are shown in Table 2.

Only the values that were higher than the detection limit of the ELISA kit (4 ng/ml) were taken for evaluation. The annual total allergen index (AI) for 2015 was 4685.5 pg/m^3 in Ankara. The majority of Phl p 5 allergen were recorded in PM10 > 10 μm filters (Table 2). The major allergen peak was recorded on 21 June with 194.9 pg/m^3. One of the most interesting results of this study is that the allergen concentrations were recorded on only 2 days in 2015 in Zonguldak. The allergen concentrations in both cascades were obtained on 8 and 9 July, approximately 1 month later than when the major pollen peak was observed, with a total of 56.72 pg/m^3.

In 2016, the AI was lower than that in 2015 for Ankara, with a total of 3495.5 pg/m^3, whereas the AI recorded was much higher than that in 2015, with a total of 1252.04 pg/m^3 for Zonguldak. In 2016, the correlation coefficient between airborne grass pollen concentration and Phl p 5 concentration was higher than that in 2015 for Ankara. On the other hand, a significant proportion of Phl p 5 was recorded on days on which the airborne grass pollen concentrations were lower or absent for 2015. The graph of the seasonal variation of pollen and allergens depicted in Fig. 4d showed that there were three distinct episodes for pollen only in Zonguldak in 2016 (Table 3). Correlation analyses were also carried out for the episodes to determine whether the allergen levels that were obtained in these episodes originated from the pollen recorded

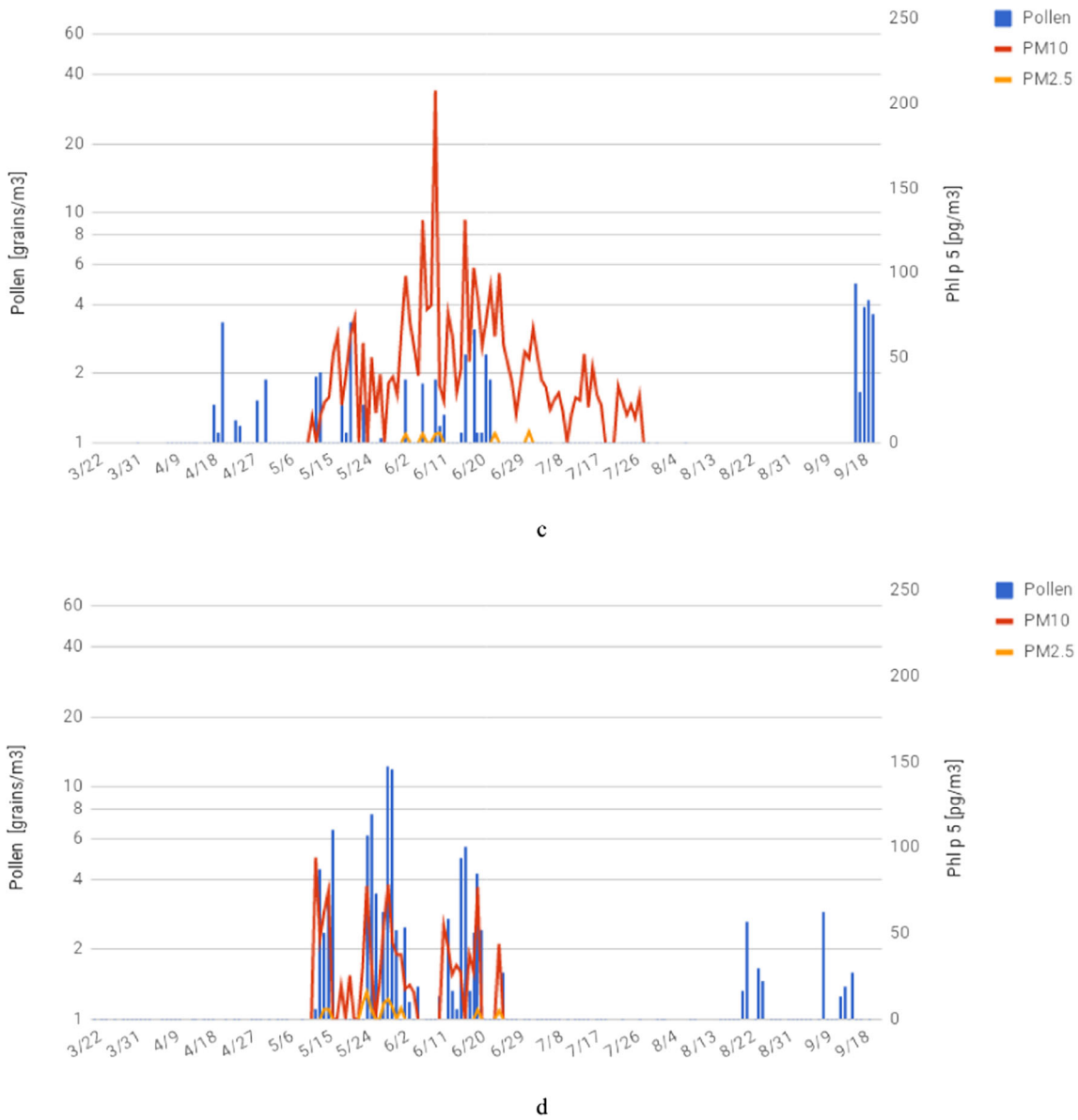

Fig. 4 (continued)

during the episodes. The results of these analyses are shown in Table 3. In episodes I and II, there was a strong positive correlation between the pollen and the allergen. Nevertheless, in episode III, this tendency did not fit for Zonguldak.

Correlation with meteorological parameters

The mean values of temperature, air pressure, relative humidity and total rainfall in Ankara and Zonguldak showed few differences between 2015 and 2016 (Table 1). Regarding the average temperatures across the years, it appeared to be about 1 °C higher in 2016 than that in 2015 in both cities. The increase in the total rainfall was especially striking in both Ankara and Zonguldak.

Only the days on which pollen and Phl p 5 allergens were recorded for correlation analysis were selected. In both stations, a positive correlation was observed between airborne Poaceae pollen and Phl p 5 concentrations. We did not conduct a correlation analysis for allergen data in Zonguldak because we obtained the Phl p 5 concentration for only 2 days during 2015. For the other days, the allergen levels were measured under the threshold values.

A strong negative correlation was found between temperature and pollen in both stations in 2015. On the other hand, a positive correlation was detected between relative humidity and pollen in both stations. However, in Ankara, a positive correlation was observed between Phl p 5 and maximum temperature as well as average temperature in 2015. The correlations observed in 2016 between pollen and temperature

Table 2 Characteristics of the 2015–2016 pollen and allergen seasons of Ankara and Zonguldak

	Ankara		Zonguldak	
	2015	2016	2015	2016
Pollen				
Pollen concentration (pollen/m^3)	272.6	98.5	142.9	138.9
Pollen index (whole year)	4006	1448	2101	2041
Pollen season start	9/4	8/4	2/5	22/3
Pollen season end	30/8	21/9	12/9	14/9
Season length (days)	143	166	133	176
Peak day	8/6	17/9	27/5	30/5
Peak value (pollen/m^3)	60.6	4.9	15.3	12.4
Phl p 5				
Total allergen (pg/m^3)	4685.5	3495.5	56.72	1252.04
PM > 10 μm (pg/m^3)	4310.2 (92%)	3462.5 (99.1%)	49.3 (86.9%)	1164.9 (93%)
10 μm > PM > 2.5 μm (pg/m^3)	375.3 (8%)	33.0 (0.9%)	7.4 (13.1%)	87.1 (7%)
Peak value	194.9	212.7	35.9	94.2
Peak day (month/day)	6/21	6/9	7/8	5/13

appeared to be the opposite to those observed in 2015 in both stations. The Phl p 5 concentrations also showed a positive correlation with temperature in 2015 for Ankara and in 2016 for Zonguldak. Air pressure was found to have a positive effect in Ankara in 2015, whereas it exerted a negative effect on both the pollen and the allergen in Zonguldak in 2016 (Table 3).

The primary wind directions during the episodes, for the entire year of 2015 and 2016 and the days in 2015 on which the allergen concentrations were recorded in Zonguldak, are depicted in Fig. 5. In episode III, the primary wind direction was NNE (Fig 5b). The pattern that was observed in 2015 was also observed in episode III of 2016 (Fig. 5a). The wind blowing in the NNE direction was responsible for the observation of allergen in 2015; however, it also caused the correlation between pollen and Phl p 5 in episode III in 2016 not to be observed, in contrast to episodes I and II (Table 3).

Table 3 Results of Spearman correlations between Poaceae pollen concentrations, airborne allergen Phl p 5 concentrations and weather variables

			N	Phl p 5	Maximum temperature (°C)	Minimum temperature (°C)	Average temperature (°C)	Rainfall (mm)	Relative humidity (%)	Wind speed	Air pressure (hPa)
Ankara	2015	Phl p 5	91		0.306**	0.241*	0.308**	−0.09	−0.100	0.063	0.257*
		Pollen	91	0.226*	−0.265*	−0.263*	−0.367**	0.025	0.367**	−0.198	0.230
	2016	Phl p 5	78		0.052	−0.052	0.020	0.026	0.013	0.104	0.196
		Pollen	78	0.365**	−0.226*	−0.370**	−0.251*	0.132	0.120	−0.190	0.165
Zonguldak	2015	Phl p 5	2	–	–	–	–	–	–	–	–
		Pollen	134		−0.529**	−0.498**	−0.548**	0.100	0.185*	−0.030	0.134
	2016	Phl p 5	43		0.333*	0.439**	0.402**	0.078	−0.424**	0.335*	−0.564**
		Pollen	176	0.822**	0.294	0.458**	0.383*	0.063	−0.280	0.350*	−0.460**
	Episode I (14/05–22/-05)	Phl p 5	9		0.525	0.813**	0.644	0.120	−0.118	0.512	−0.772*
		Pollen		0.877**	0.445	0.870**	0.644	0.076	−0.008	0.451	−0.745*
	Episode II (24/05–06/-06)	Phl p 5	14		0.202	0.604*	0.421	0.058	−0.621*	0.305	−0.433
		Pollen		0.797**	0.008	0.42	0.262	0.148	−0.535*	0.586*	−0.433
	Episode III (13/06–26/-06)	Phl p 5	9		0.225	0.533	0.766*	−0.410	−0.250	0.118	−0.166
		Pollen		0.175	0.211	0.680*	0.420	−0.552	−0.075	−0.063	0.479

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

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

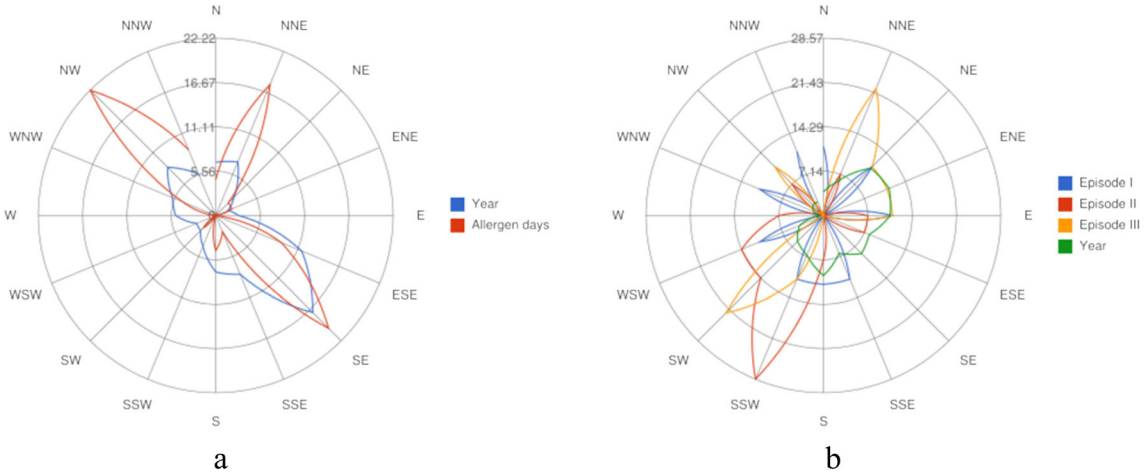

Fig. 5 Wind rose plot of Zonguldak station for episodes **a** 8–9 July 2015, **b** 2016

Principal component analysis was also performed to compare the weather conditions during the pollen and allergen seasons for both provinces (2015–2016). The PCA for Ankara (2015) explained 69% of the variation with the first three axes (Fig. 6a; Online Resource 1). The first principal component explained 32% of the total variation. Average relative humidity and average temperature were the most significant variables, because they have the highest relative variation rate. Average pressure and wind speed had the strongest influence on the seasons in the second principal component; this explained 21% of the total variation. Similarly, the PCA for Ankara (2016) explained 74% of the variation with the first three axes (Fig. 6b; Online Resource 1). The first principal component explained 35% of the total variation and the average temperature and the average relative humidity had the highest rate. In Zonguldak (2015), the PCA was performed only with the pollen season due the lack of days which are recorded allergen. The first three components explained 74% of the variation. Two primary valuable variables were total rainfall and average pressure in the first component with 29%. Average relative humidity and the wind speed were the secondary important variables with 26% of the variation (Fig. 6c; Online Resource 1). However, it was observed in Zonguldak (2016), the PCA explained 74% of the variation with the first three axes. While the average humidity and the average pressure were the most significant variables with 38% variation, the total rainfall and the wind speed were the secondary important variables (Fig. 6d; Online Resource 1).

Back-trajectory analysis

The back-trajectory analysis is a useful tool for showing the impact of air mass patterns on weather conditions. The movement of air masses may indicate the sources of pollen and allergens. Therefore, the path taken by air masses were analysed using the HYSPLIT model (Stein et al. 2015) to examine the possible sources of Phl p 5 allergen concentrations in 2015 and 2016.

We conducted 72-h and 12-h back-trajectory analyses to identify the possible sources of the allergen Phl p 5 in 2015. The results showed that there are two major possible ways for the transportation of the Phl p 5 allergen. The first possibility is a long-distance transport from Ukraine and Crimea according to 72-h back trajectories, and the other one is a short-distance transport from the south of Zonguldak (Fig. 7).

Furthermore, the back-trajectory analyses of 2016 (72-h) indicated that the primary routes for the movement of air masses in episode III were from both the northwest and the northeast coast of the Black Sea (Fig. 8). On the other hand, the 12-h trajectories clarified that the majority of air masses did not directly reach Zonguldak. These air masses reached Zonguldak after moving around the hills to the south of Zonguldak. This pattern was also observed in 2015 (Fig. 7).

Discussion

Airborne grass pollen is one of the most important aeroallergens across the world (Mari 2003; Andersson and Lidholm 2003; Weber 2004; García-Mozo et al. 2009), which is responsible for causing several allergic symptoms (Mohapatra et al. 2005). Turkey is also one of two gene centers of the Poaceae family (Dogan et al. 2016). Several species belonging to Poaceae are distributed naturally in Turkey. Members of the Poaceae family grow naturally, and landscaping is quite common in Ankara and Zonguldak. Previous investigations have reported that Poaceae pollen is a major cause of seasonal allergic rhinitis in Turkey, and affects 1.3–6.4% of the population. There is also evidence showing that respiratory allergy episodes have increased in Ankara (Bostanci et al. 1999; Sin et al. 2001; Çelik et al. 2004). Based on skin prick test results in Eskişehir, Turkey, 58.5% of 130 patients with allergic rhinitis were severely sensitised to grasses-cereals

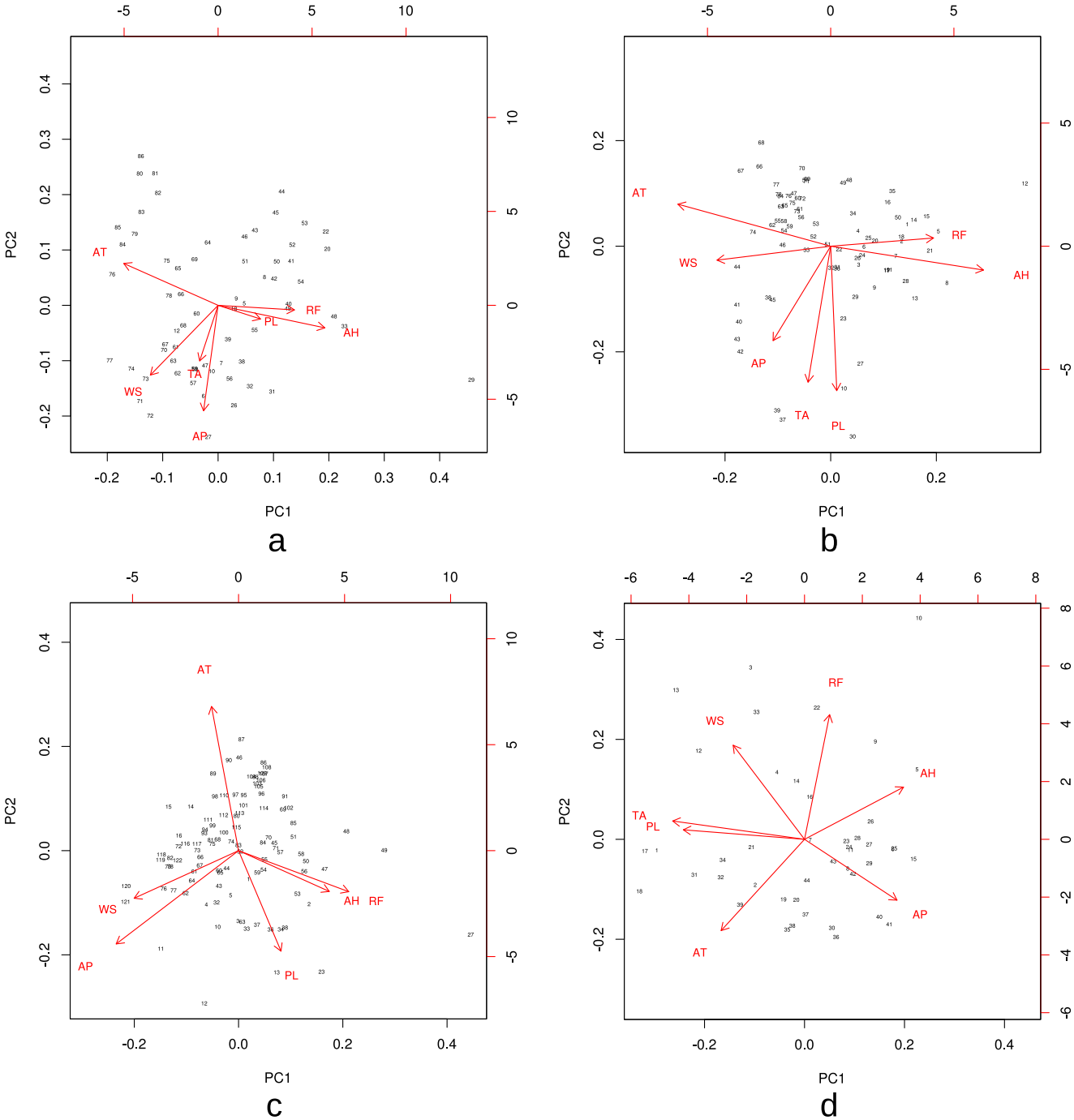

Fig. 6 Biplots of a principal component analysis were performed on the interaction between the pollen, allergen concentration and meteorological parameters. **a** Ankara (2015). **b** Ankara (2016). **c** Zonguldak (2015). **d** Zonguldak (2016)

(Erkara et al. 2009). On the other hand, there is a lack of aeroallergen monitoring studies in Turkey, with only one or two studies being conducted till date (Buters et al. 2015). This is the first study on aeroallergens conducted in Ankara and Zonguldak.

It was shown that the temperature and rainfall significantly affect the change of pollen concentration between years with the PCA analysis (Makra et al. 2004). The result of the PCA analysis represented the effect of the climatic properties on pollen and allergen distribution. Generally, in terms of temperature, the pollen seasons in Ankara during 2015–2016 represented an extreme fluctuation. In addition, it can be seen that the average temperature is secondly important after the rainfall for Zonguldak (2015). According to the PCA graphs, most observations are above the PC1 axis, which means that these seasons (Ankara-2015-2016) can be considered to be relatively warm, contrary to the season 2016 in Zonguldak.

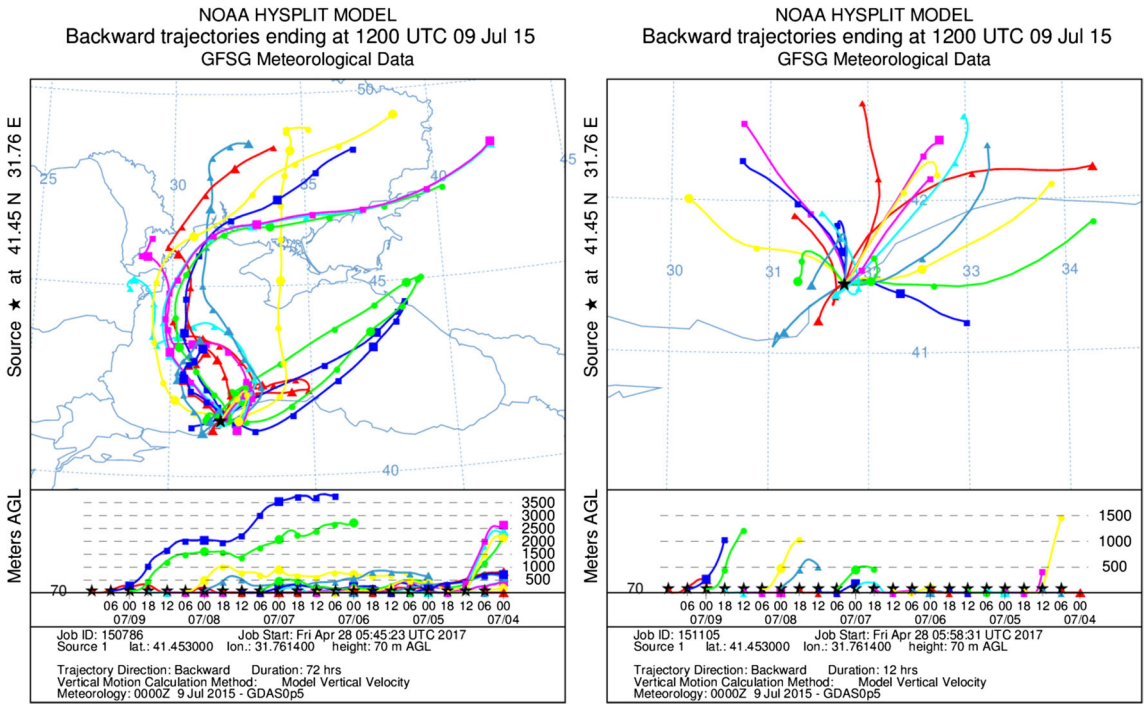

Fig. 7 72-h and 12-h backward trajectories showing long- and short-distance transportation of Phl p 5 recorded on 8–9 July 2015

Results of the pollen concentrations of 2016 did not reveal vegetation differences between the stations. Although more Poaceae members are distributed in Ankara (Akman and Ketenoğlu 1986), we recorded a lower pollen concentration than that in Zonguldak in 2016. The declining trend associated with urbanisation, reported earlier in Ankara, also continued in 2015 and 2016 (Acar et al. 2017). The decrease in grass pollen concentrations is believed to be caused due to the destruction and reduction of large grassland areas of large cities such as Ankara. Furthermore, it was reported that grass pollen was more abundant in rural areas than urban areas, lacking of grassy areas, and city patients were exposed to grass pollen less often than country patients (Bosch-Cano et al. 2011).

It was also found that rainfall and air pressure of which the most different meteorological parameters between stations were a remarkable effect on pollen and allergen distribution in Zonguldak during 2015–2016. The average temperature and average relative humidity were found to influence airborne pollen concentrations in other studies conducted using PCA analysis (Makra et al. 2004; Piotrowska and Kubik-Komar 2012). It is also reported that higher relative humidity in the Black Sea and the coastal parallel mountain chains cause occurrences of orographic and cyclonic rainfall (Şen 2018). This is a feature of the Black Sea region and the North Atlantic has air masses that descend southwesterly over Europe and then the Black Sea. Therefore, it could be said that orographic rainfall, which is related with the hilly topography of Zonguldak, is a significant meteorological factor which affect distribution of pollen and Phl p 5 allergen.

A generally strong positive correlation between Poaceae pollen and temperature has been found in several studies. In a study conducted on airborne Poaceae pollen in the city of Timisoara in Romania during 2000–2010, a strong positive correlation was found between pollen concentrations and temperature (average, minimum and maximum) for the years studied (Ianovici 2015). A similar interaction was observed for Poaceae pollen in the atmosphere of Zagreb in 2002–2005 (Peternel et al. 2006). Although we obtained a similar result for Zonguldak in 2016, a negative correlation was observed for both cities in 2015 and for Ankara in 2016. An explanation for this finding, also based on the PCA results, is the observation of pollen peaks in the months when the temperatures are relatively low. This is why there was a decrease in the pollen concentrations with an increase in temperature.

The reason for the positive correlation with temperature observed in 2016 for Zonguldak was that the starting date of the pollen season for Zonguldak in 2016 was earlier than that in others. An anomalous monthly average temperature was found during the spring of 2016 in Zonguldak (Seasonal normal, 11.3 °C (1981–2010); 2015, 8 °C; 2016, 13.3 °C), which was higher than both the seasonal normal temperatures and those of 2015. This increase could so have led to early pollination and the positive correlation between temperature and pollen levels. There was another anomaly in monthly rainfall in Ankara for 2015 and 2016 in comparison with seasonal normal levels (Fig. 3), which could therefore be the other reason for the decrease in pollen and Phl p 5 levels in 2016 in Ankara.

A positive correlation was observed between pollen counts and allergen levels in both 2015 and 2016 in Ankara. Meanwhile, a strong correlation was found between airborne grass pollen concentrations and allergen levels during 2016,

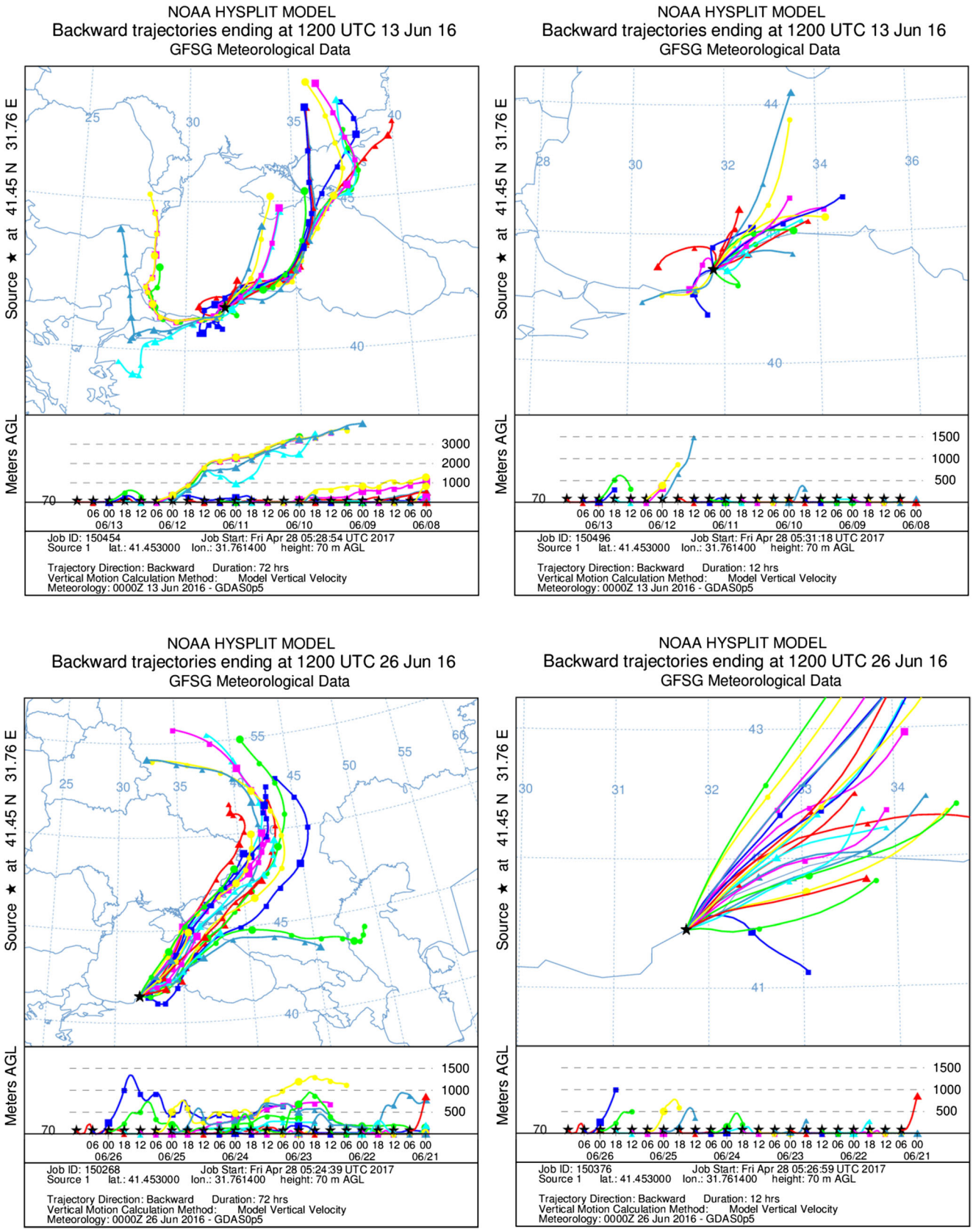

Fig. 8 72-h and 12-h backward trajectories showing movement of air mass for 13 and 26 June in episode III for 2016 in Zonguldak

except in episode III, in Zonguldak. Similar results have been reported by several studies (De Linares et al. 2010; Rodríguez-Rajo et al. 2011; González Parrado et al. 2014). Studies that have analysed pollen and allergens together have demonstrated a correlation between pollen and meteorological factors rather than allergens. Our results showed that the pollen distribution and the allergen release are affected by meteorological factors and vary from year to year, independent of stations. In both stations, there were some external pollen peaks observed in late August and early September, with no corresponding allergen. These peaks could have been originated due to the distribution of different Poaceae species. This situation could be a contributing factor to the apparent contradiction observed between the years. Considering that species belonging to the Poaceae family are primarily distributed in Ankara (Davis 1965; Akaydin and Erik 2002), there is an understandable difference between the Phl p 5 allergen concentrations of the two cities. However, it is difficult to explain the differences in Phl p 5 allergen concentrations between the cities solely based on plant distribution. Lower airborne allergen concentrations in relation to pollen were observed in Zonguldak in 2015 and 2016, when compared to those of Ankara. Because both the pollen and allergens have different aerobiological dynamics, correlation could not be observed between them. This supported the phenomenon that there is a difference between dispersion dynamics of pollen and allergen. Some studies have reported the presence of allergen levels when pollen grains were fewer in number or absent in the atmosphere (De Linares et al. 2007; Plaza et al. 2016).

High magnitude airborne allergen concentrations recorded in Zonguldak in both 2015 and 2016 were observed after the major pollen season in Zonguldak. Air mass analysis suggests that these allergens may have originated from the sources to the south of Zonguldak. The hills that are situated just behind the city of Zonguldak could have blocked airborne allergens from reaching the sampler. Airborne allergens originating from a higher altitude than Zonguldak could only have reached to the sampler on the days when the wind direction was NNE. The study by Plaza et al. (2016) also reported that aeroallergen concentrations could be recorded based on a specific wind speed and direction. The wind direction, which is from the mainland to the Black Sea in the Zonguldak region, could have a drift effect on allergens rather than on pollen. This could explain why airborne allergen concentration was only recorded on 2 days in 2015 and a lower total allergen level was recorded in Zonguldak compared to Ankara in 2016.

Conclusion

This study considers the airborne grass pollen and Phl p 5 allergens could be more of a risk for patients who live in Ankara than Zonguldak, due to higher total pollen and Phl p 5 allergen levels. Urbanisation has a decreasing effect on some pollen concentrations, but increases in air pollution associated with the built environment can increase allergen expression and so the continuity of the aeroallergen monitoring studies will provide more accurate data. Members of the Poaceae family are found more frequently in Ankara, and the results of back trajectory analysis indicate that the hills behind Zonguldak have a protective feature against the allergens carried from the mainland. The peak date of pollen concentration and the starting date of pollination related to the temperatures in spring are important to determining the direction of correlation between temperature and pollen concentration. Not only the climatic features of sampling site but also the geographic properties that change them are quite effective in the dispersion of pollen and allergens. Our results suggest that changes in wind direction are particularly important for the transportation of allergens that originate from nearby sources in areas with a hilly topography such as Zonguldak.

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The Effect of Geographical and Climatic Properties on Grass Pollen and Phl p 5 Allergen Release

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Online Resource 1. PCA variable loadings for the first 3 components.

	Variables	**PC 1**	**PC 2**	**PC 3**		**Variables**	**PC 1**	**PC 2**	**PC 3**
ANKARA 2015	Pollen level (PL)	0.233	-0.093	0.505	**ANKARA 2016**	Pollen level (PL)	0.022	-0.64	0.191
	Total allergen (TA)	-0.101	-0.378	-0.652		Total allergen (TA)	-0.083	-0.603	0.256
	Average temperature (AT)	-0.518	0.285	-0.188		Average temperature (AT)	-0.561	0.186	0.021
	Average pressure (AP)	-0.079	-0.718	0.185		Average pressure (AP)	-0.211	-0.418	-0.587
	Average humidity (AH)	0.587	-0.153	-0.054		Average humidity (AH)	0.561	-0.105	-0.16
	Wind speed (WS)	-0.372	-0.475	0.077		Wind speed (WS)	-0.417	-0.061	-0.48
	Total rainfall (RF)	0.419	-0.031	-0.49		Total rainfall (RF)	0.377	-0.036	-0.543
	Standard deviation	1.499	1.211	1.052		**Standard deviation**	1.563	1.296	1.013
	Proportion of Variance	0.321	0.209	0.158		**Proportion of Variance**	0.349	0.24	0.147
	Cumulative Proportion %	0.321	0.531	0.689		**Cumulative Proportion %**	0.349	0.589	0.736
	Variables	**PC 1**	**PC 2**	**PC 3**		**Variables**	**PC 1**	**PC 2**	**PC 3**
ZONGULDAK 2015	Pollen level (PL)	0.192	-0.472	0.481	**ZONGULDAK 2016**	Pollen level (PL)	-0.482	0.047	-0.415
	Total allergen (TA)	0	0	0		Total allergen (TA)	-0.524	0.092	-0.373
	Average temperature (AT)	-0.122	0.677	-0.158		Average temperature (AT)	-0.332	-0.458	-0.134
	Average pressure (AP)	-0.554	-0.438	-0.077		Average pressure (AP)	0.368	-0.305	-0.104
	Average humidity (AH)	0.410	-0.191	-0.526		Average humidity (AH)	0.394	0.262	-0.619
	Wind speed (WS)	-0.472	-0.224	-0.549		Wind speed (WS)	-0.284	0.473	0.494
	Total rainfall (RF)	0.498	-0.193	-0.397		Total rainfall (RF)	0.099	0.626	-0.176
	Standard deviation	1.308	1.260	1.062		**Standard deviation**	1.637	1.297	0.894
	Proportion of Variance	0.285	0.264	0.188		**Proportion of Variance**	0.383	0.24	0.114
	Cumulative Proportion %	0.285	0.550	0.738		**Cumulative Proportion %**	0.383	0.623	0.738