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The Annual Variation of Allergenic *Cladosporium* and *Alternaria* Spores in the Atmosphere of Büyükorhan (Bursa), Turkey; Effects of Meteorological Factors

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Abstract: Fungal spores are important bio-allergens frequently encountered in the atmosphere. *Cladosporium* Link and *Alternaria* Nees spores are generally recorded as dominant in many atmospheric fungal spore studies and have high allergic effects on susceptible individuals. The species belonging to these two genera may live as pathogens on the plants and cause low yield. This study was carried out between January 2012 to December 2013. Durham sampler, which is the device of the gravimetric method, was used in the research. A total of 10817 spores were recorded in the Büyükorhan (Bursa) atmosphere during the consecutive years. 4119 spores belonged to the first year and 6698 to the second year. The total numbers of *Cladosporium* spores were observed much higher than the total numbers of *Alternaria* spores in both years. This study aims to determine the variation of atmospheric spores in these two genera during the two years by comparing them with meteorological factors. For both spore types, the maximum spore numbers were found between the June-August term. Summer months revealed a risky period in terms of atmospheric fungal spore allergy for sensitive individuals.

Key words: Airborne fungal spores, Aeromycology, Allergy, Meteorological factors, Northwest Turkey**.**

Büyükorhan (Bursa), Türkiye Atmosferindeki Allerjik *Cladosporium* ve *Alternaria* Sporlarının Yıllık Değişimi; Meteorolojik Faktörlerin Etkileri

Öz: Mantar sporları, atmosferde sıkça karşılaşılan önemli biyo-allerjenlerdendir. Çoğu atmosferik mantar sporu çalışmasında, *Cladosporium* Link (Havaküfü) ve *Alternaria* Nees (Arıküfü) sporları genellikle dominant olarak kaydedilmiş olup duyarlı bireyler üzerinde yüksek allerjik etkilere sahiptirler. Bu iki cinse ait türler bitkiler üzerinde patojen olarak yaşayabilir ve bitkilerde verim düşüklüğüne sebep olabilirler. Bu çalışma, Ocak 2012 ile Aralık 2013 tarihleri arasında gerçekleştirilmiştir. Çalışma, gravimetrik yönteme dayalı Durham cihazı kullanılarak yapılmıştır. İki yıl süresince toplamda 10817 adet spor kaydedilmiş olup, 4119 adet spor birinci yıla, 6698 adet spor ise ikinci yıla aittir. Her iki yılda da toplam *Cladosporium* sayısının toplam *Alternaria* sayısından çok daha fazla olduğu gözlemlenmiştir. Bu çalışma, bu iki cinse ait atmosferik sporların iki yıl içindeki değişimini meteorolojik faktörlerle karşılaştırarak belirlemeyi amaçlamaktadır. Her iki spor tipi için, maksimum spor sayıları Haziran-Ağustos ayları arasında bulunmuş ve özellikle yaz aylarının atmosferik mantar sporu allerjisi açısından, duyarlı bireyler için riskli dönemler olduğunu ortaya çıkarmıştır.

Anahtar kelimeler: Atmosferik mantar sporları, Aeromikoloji, Allerji, Meteorolojik faktörler, Kuzeybatı Türkiye.

Introduction

Fungi are known as cosmopolitan organisms that can live in various habitats worldwide. They reproduce through spores, and these spores are mostly dispersed into the atmosphere generally with the help of wind (Gregory, 1961; Money, 2015; Kireçci and Alagöz, 2019).

Cladosporium (Havaküfü) and *Alternaria* (Arıküfü) spores have been among the most common fungal spore types in the atmosphere (D'Amato, 1981; Hjelmroos, 1993; Sesli, et al., 2020). These spores cause many allergic reactions on susceptible individuals and also damage plants and animals. They can cause respiratory diseases such as asthma and allergic rhinitis in people and may cause skin diseases in animals and yield losses in plants (D'Amato et al., 1984; Buck and Levetin, 1985; Vjay et al., 1991; Hjelmroos 1993; Angulo-Romero et al., 1999).

Since the sporulation times are different for each fungus, the periods and the number of fungi spores in the atmosphere also differ. The types and densities of atmospheric spores may vary according to geographic, ecological, meteorological factors, and the region's floristic structure. Therefore, atmospheric spore calendars should be prepared by comparing atmospheric spores and their distribution during the year with meteorological factors in the different areas. For this purpose, many aeropalynological studies were prepared around the world (Hjelmroos, 1993; Gioulekas et al., 2004; Damialis and Gioulekas, 2006; Oliveira et al., 2010; Mallo et al., 2011; Grinn-Gofron et al., 2016; Kasprzyk et al., 2016; Ding et al., 2016; Olsen et al., 2020). The first aeromycological study of Turkey was conducted by Özkaragöz in 1968 with the settle plate method using open Petri-dishes and aimed at determining atmospheric fungal spores in Ankara (Özkaragöz, 1969). Atmospheric fungal spore studies have been continuing in Turkey in the different cities by gravimetric and volumetric atmospheric particle sampling methods since 1968 (Bıçakçı et al., 2001; Ataygul et al., 2007; Potoglu Erkara et al., 2009; Akgül et al., 2016; Saatcioglu et al, 2016; Yılmazkaya et al., 2019; Kilic et al., 2020).

The purpose of atmospheric fungal spore studies is that they can benefit the people of the region and allergologists in the treatment process and in taking precautions to prevent allergies in risky periods. At the same time, it can contribute to taking the necessary measures to prevent yield losses in the economically important plants growing in the region.

This study investigated the annual variation of atmospheric *Cladosporium* and *Alternaria* spores and the effects of meteorological factors (Mean temperature, relative humidity, total rainfall, and wind speed) on them in the Buyukorhan (Bursa) atmosphere.

Material and Metod

Study area, Flora, and Climate

Büyükorhan district (Bursa) is located on the southern part of the city center and slope of Uludag Mountain (39°46'.25.0"N, 28°53'.20.0′′E) at an altitude of nearly 850 m. Büyükorhan is surrounded by Harmancık district in the east, Mustafakemalpaşa district in the west, Orhaneli district in the north, and Dursunbey district (Balıkesir) in the south.

Mediterranean climate is seen in the region; summers are hot and dry, winters are cold and rainy. According to long-term meteorological data, the highest temperature in summer is 30-35°C, while in winter, the temperature drops to 4-6°C on average. According to meteorological data provided by the Turkish State Meteorological Service, for two years (2012-2013), the annual average temperature is 10.6°C. The yearly average minimum temperature is 0.12°C, observed in January, and the annual average maximum temperature is 20.6°C, in July. The annual average total rainfall is 61.5 mm; the annual average relative humidity is 69.8%, and the annual average maximum wind speed 7.39 m/s. (Figure 1).

The southern and southeastern part of the study area consists of natural forests and mostly *Pinus nigra* Arn. arid forests and *Pinus brutia* Ten. communities are dominant, but in places where these forests are destroyed, maquis vegetation is settled with the members; *Juniperus oxycedrus* L. subsp. *oxycedrus, Quercus infectoria* Oliv. subsp. *infectoria, Phyllirea latifolia* L., *Paliurus spina-christii* Mill., *Cistus creticus* L., *Arbutus andrachne* L., *Rhus coriaria* L., *Pistacia palaestina* Boiss. (Bağçıvan and Daşkın, 2019).

Cultivated plants and crop plants have taken an essential place in the region. The following species are also frequently observed in the parks, gardens, and streets of the district: *Cupressus sempervirens* L., *Cupressus arizonica* Green, *Alnus glutinosa* (L.) Gaertn., *Carpinus betulus* L., *Castanea sativa* Mill., *Cedrus libani* A. Rich., *Cercis siliquastrum* L., *Cornus mas* L., *Elaeagnus angustifolia* L., *Juglans regia* L., *Malus domestica* Borkh., *Morus* sp. L., *Olea europaea* L., *Platanus orientalis* L., *Salix babylonica* L., and *Tilia tomentosa* Moench. The field crops grown in the town area are *Triticum* L. species, *Zea mays* L., *Helianthus* L., *Solanum tuberosum* L., and *Fragaria* L.

Aeropalynological study

This study was conducted between 01 January 2012 to 31 December 2013. Durham sampler was used in the research; the pollen trap was placed at the top roof of a building, 3 meters above the ground level. Before being placed on the device, slides were covered with basic fuchsine added glycerine-jelly (Charpin et al., 1974) and were changed weekly. Counting was conducted on a 24 x 24 mm area of the slide and calculated to 1 cm^2 area later; all spore numbers were given per cm^2.

Results

In the two consecutive years of the study, a total of 10817 spores were recorded in the Büyükorhan (Bursa) atmosphere. In the first year, 4119 spores were identified; 3280 (79.63%) spores belonged to the *Cladosporium* and 839 (20.37%) spores to the *Alternaria*. In the second year, 6698 spores were observed; 5615 (83.83%) spores belong to the *Cladosporium* and 1083 (16.07%) to the *Alternaria*. Annually, a total of 5409 spores were determined; 4448 (81.73%) spores belong to the *Cladosporium*, and 961 (18.27%) spores to the *Alternaria*. It was observed that the total number of *Cladosporium* spores was much higher than the total amount of *Alternaria* spores (Table 1).

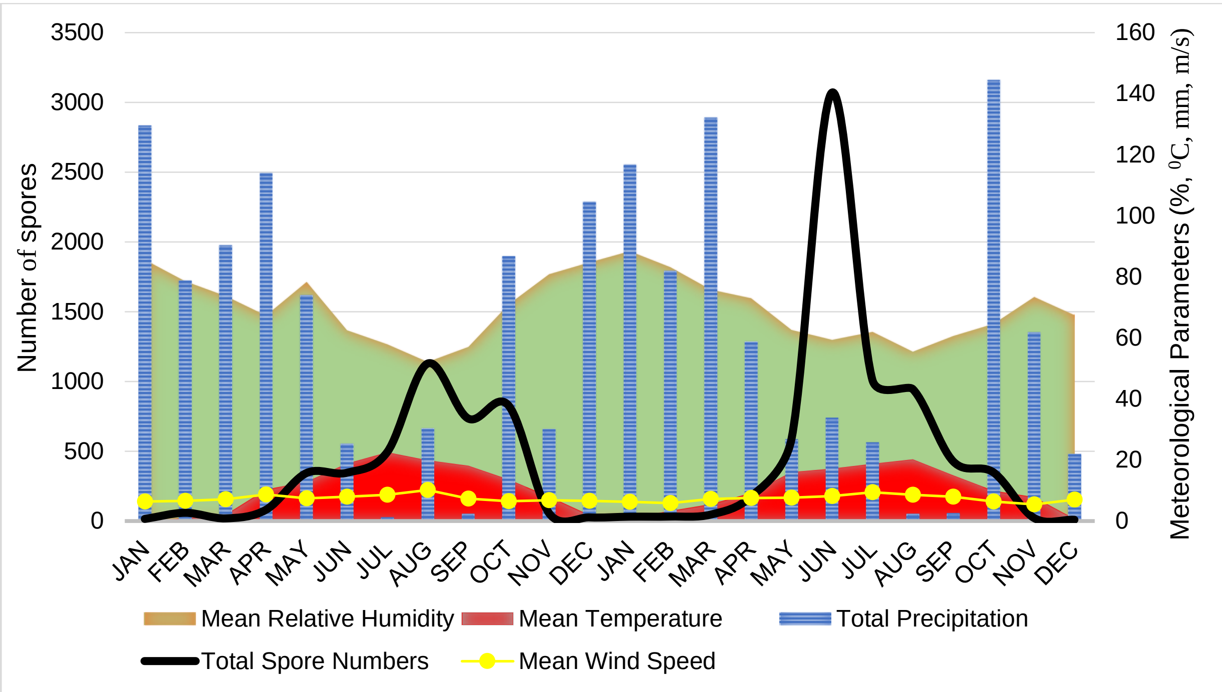

Figure 1**.** Monthly variation of meteorological parameters and total spore numbers in two consecutive years in Büyükorhan atmosphere.

Table 1. Total amounts of *Cladosporium* and *Alternaria* spores in Büyükorhan atmosphere in years 2012-2013 (mean and percentage values).

	2012	**%**	**2013**	**%**	**MEAN**	**%**	**TOTAL**
Cladosporium	3280	*79.63*	5615	*83.83*	4448	*81.73*	**8895**
Alternaria	839	*20.37*	1083	*16.17*	961	*18.27*	**1922**
TOTAL	4119	*100*	6698	*100*	5409	*100*	**10817**

In the atmosphere of Büyükorhan, *Cladosporium* and *Alternaria* spores were observed every month of the two years (Table 2). In both years, the total number of spores was found in low levels in January, February, and March. While the temperature was at low levels in these three months, precipitation and humidity levels were relatively high. In the year 2012, the number of spores was the lowest for the *Cladosporium* in January. However, the lowest amount of spores for *Alternaria* was recorded in March. The following year, For *Cladosporium* and *Alternaria* spores, the lowest values were recorded in December. Low temperature and heavy rain were kept the total number of spores at low levels for both (Figure 1, 2, and Table 2).

There has been a regular increase in the total number of spores from April in both years due to gradually increasing temperature. With favourable climatic conditions, the number of spores began to increase since this month in both sampling years. (Figure 1, Table 2). The total amount of spores were reached the highest level in August 2012 (1228 spores) and in July 2013 (3070 spores). For both spore types, the months in which the total number of spores reached the highest value were different in years, and at the same time, the total number of spores was more than doubled from 2013 to 2012 (Figure 1,2 and, Table 2). In both 2012 and 2013, the temperature was high, and humidity was low in the months (August 2012 and June 2013) when the total number of spores reached the highest level, and rainfall and wind-speed values were sufficient fungal spores to spread into the atmosphere. (Figure 1).

The total number of spores that peaked in August in the first year were started to decrease after this month, and the decline continued until September, were increased again, and showed the second peak in October. There was a sudden decrease in the total number of spores after October, and the total amount of spores in November and December was found very low (Figure 1,2 and, Table 2).

In the second year, after June, the total number of spores was decreased rapidly until August, but in August, the total number of spores was increased again slightly and were showed the second peak. August was the month with the highest temperature and the lowest rainfall in 2013. After August, the total number of spores was started to decrease gradually. A third small peak was observed in October. The total amount of spores in this month were less than the October peak of the previous year. As in 2012, the total number of spores belonging to both *Cladosporium* and *Alternaria* was low in November and December. The minimum spore concentration was recorded in December 2013 (8 spores) (Figure 1,2 and Table 2).

In the study period, meteorological parameters differed monthly between the two years due to their dynamic and variable nature. As a result, the total spore amount was variable during the months of peak spore concentrations. Considering the monthly average changes of *Cladosporium* and *Alternaria* spores, the highest level was recorded in June for both years. Also, August and October were the months in which an increase in the average number of spores was observed for both spore types (Figure 3, Table 2).

When the changes in the weekly amounts of spores *Cladosporium* and *Alternaria* taxa are examined during the two consecutive years;

During the two years, spores belonging to the genus *Cladosporium* encountered every week in the atmosphere of Büyükorhan. In the first year, the number of *Cladosporium* spores was started to increase after the 17^{th} week. The maximum level was recorded in the 35^{th} week (last week of August 588 spores) and decreased after the 44^{th} week. In the second year, the spore levels were beginning to increase after the 14^{th} week and reached the highest value in the 24^{th} week (second week of June 1244 spores) then, declined after the 41^{st} week. The average number of spores was observed in the 24^{th} week (649 spores) for *Cladosporium* taxa (Figure 4).

In the Büyükorhan atmosphere, *Alternaria* spores were found almost every week during the study period. In 2012, the number of *Alternaria* spores were started to increase after the 17^{th} week. The maximum spore levels were observed in the 35^{th} week (last week of August 59 spores) for *Alternaria*. Then decreased after the 42^{nd} week. In the following year, *Alternaria* spore levels were began to increase after the 14^{th} week. The maximum spore value was recorded in the 24^{th} week (second week of June 196 spores), for *Alternaria*, declined after the 43^{rd} week (Figure 4).

Considering the weekly average number of spore values for both taxa; While the week in which the spores belonging to the *Cladosporium* taxon were seen, the most was the 24^{th} (average 649 spores/cm^2) week. The week in which *Alternaria* spores were seen the most on average was again the same week (average 106 spores) (Figure 4).

Table 2. Monthly variation of *Cladosporium* and *Alternaria* spores in Büyükorhan atmosphere during the years 2012-2013 (Min. values shown in light, Max. values shown in dark grey font).

TAXA/MONTHS		JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP	OCT	NOV	DEC	TOTAL
Cladosporium	*2012*	9	52	14	67	273	270	360	931	569	683	37	15	**3280**
	%	*0.22*	*1.26*	*0.34*	*1.63*	*6.63*	*6.55*	*8.74*	*22.60*	*13.81*	*16.58*	*0.90*	*0.36*	***79.63***
	2013	25	27	43	153	521	2610	793	772	369	283	13	6	**5615**
	%	*0.37*	*0.40*	*0.64*	*2.28*	*7.78*	*38.97*	*11.84*	*11.53*	*5.51*	*4.23*	*0.19*	*0.09*	***83.83***
	Mean	17	40	29	110	397	1440	577	852	469	483	25	11	**4448**
	%	*0.30*	*0.83*	*0.49*	*1.96*	*7.20*	*22.76*	*10.29*	*17.06*	*9.66*	*10.40*	*0.55*	*0.23*	***81.73***
Alternaria	*2012*	5	6	4	13	67	75	134	197	165	144	19	10	**839**
	%	*0.12*	*0.15*	*0.10*	*0.32*	*1.63*	*1.82*	*3.25*	*4.78*	*4.01*	*3.50*	*0.46*	*0.24*	***20.37***
	2013	5	4	2	22	66	460	219	173	58	64	8	2	**1083**
	%	*0.07*	*0.06*	*0.03*	*0.33*	*0.99*	*6.87*	*3.27*	*2.58*	*0.87*	*0.96*	*0.12*	*0.03*	***16.17***
	Mean	5	5	3	18	67	268	177	185	112	104	14	6	**961**
	%	*0.10*	*0.10*	*0.06*	*0.32*	*1.31*	*4.34*	*3.26*	*3.68*	*2.44*	*2.23*	*0.29*	*0.14*	***18.27***
TOTAL	*2012*	14	58	18	80	340	345	494	1128	734	827	56	25	**4119**
	%	*0.34*	*1.41*	*0.44*	*1.94*	*8.25*	*8.38*	*11.99*	*27.39*	*17.82*	*20.08*	*1.36*	*0.61*	***100.00***
	2013	30	31	45	175	587	3070	1012	945	427	347	21	8	**6698**
	%	*0.45*	*0.46*	*0.67*	*2.61*	*8.76*	*45.83*	*15.11*	*14.11*	*6.38*	*5.18*	*0.31*	*0.12*	***100.00***
	Mean	22	45	32	128	464	1708	753	1037	581	587	39	17	**5409**
	%	*0.39*	*0.94*	*0.55*	*2.28*	*8.51*	*27.11*	*13.55*	*20.75*	*12.10*	*12.63*	*0.84*	*0.36*	***100.00***

Discussion

In the district of Büyükorhan (Bursa), study was performed between 1 January 2012 and 31 December 2013, aeropalynological research carried out by gravimetric method, a total of 10817 spores were detected. An average of 5409 spores were recorded in the consecutive years. They have belonged to *Cladosporium* with 81.73% and *Alternaria* with 18.27% (Table 1). The total number of *Cladosporium* spores were found much more than *Alternaria* spores. This situation has also been noted in many atmospheric fungal spore studies in Turkey and worldwide (Mitakasis and Guest, 2001; Gioulekas et al., 2004; Herrero at al., 2006; Ataygul et al., 2007; Pyrri and Kapsanki-Gotsi. 2007; Potoglu Erkara et al., 2008; Kizilpinar and Dogan, 2011; Akgül et al., 2016; Grinn-Gofron et al., 2016; Yılmazkaya et al., 2019). Both *Cladosporium* and *Alternaria* can survive on a wide variety of substrates and in different habitats. In some fungal spore studies, it was observed that *Cladosporium* formed much more colonies than *Alternaria.* Simultaneously, the number of species included in the *Cladosporium* genus was much higher than the *Alternaria* in the systematic studies (Şen and Asan, 2001; Dugan et al., 2004; Asan, 2015; Kireçci and Alagöz, 2019). These situations may explain why *Cladosporium* is seen more than *Alternaria* in atmospheric fungal studies.

In Turkey and worldwide, spores of these two genera are usually recorded as the dominant types in the atmosphere. It is essential to know the reproduction times of fungi belonging to these two genera and to understand the changes in the number of spores (sporulation periods) in the atmosphere during the year, as they cause many respiratory diseases on humans and also have pathogenic effects on many plants of economic importance.

Geography, vegetation, and climate (weather conditions) differences between the areas and the types of fungal spore, the total numbers, and sporulation periods of them may also differ in the atmosphere. In this context, a significant emphasis should be placed on weather conditions because the weather conditions have great importance for fungal growth and the spore concentration variation in the atmosphere. Many researchers have stated this situation and in many studies (Hjelmroos, 1993; Şakıyan and İnceoğlu, 2003; Potoglu Erkara et al., 2008; Ianovici, 2016; Sindt et al., 2016; Grinn-Gofron et al., 2016; Olsen et al., 2020).

The spores belonging to the *Cladosporium* and *Alternaria* taxa varied every month during the years. In the atmosphere of Büyükorhan, and have been encountered in all months of both years. At the beginning of April in 2012 and 2013, the number of spores in the atmosphere for both genera spores increased and reached the maximum level in August and June. Considering the monthly average number of spores for both taxa in two years, the month when the number of spores reached the maximum level was recorded as of June. Increasing temperature in summer months, suitable rainfall, sufficient humidity, and increasing wind kept the total number of spores in the atmosphere higher than in other months. On the contrary, in the cold period (late autumn, winter, and early spring), the total number of spores in the atmosphere was recorded at low levels due to the climatic conditions (particularly low temperature and heavy rain) that were not formed for. (Figure 1,2 and 3, Table 1).

Among the meteorological parameters, temperature is one of the most important factors affecting fungal spores density in the atmosphere (Hjelmroos, 1993; Ianovici, 2016). In this study, it was observed that the temperature was low in the months when the total amount of spores was at the lowest levels in both years (winter, early spring, late autumn). (Figure 1, Table 2). The low temperature negatively affected the growth of species belonging to the *Cladosporium* and *Alternaria*. Therefore, spores of these two genera were less common in the atmosphere during the cold months. Similar results have been obtained in other atmospheric fungal spore studies with similar climatic conditions (Bıçakçı et al., 2001; Şakıyan and İnceoğlu, 2003; Gioulekas et al., 2004; Ataygul et al., 2007; Potoglu Erkara et al., 2008 and 2009; Kizilpinar and Dogan, 2011). Other researchers have stated that; in addition to temperature, prolonged and frequent rainfall (generally fall of snow), high humidity, and low wind speed are important meteorological factors that suppress the increase in the number of fungal spores in the atmosphere (Kramer et al., 1959; Palmas et al., 1990; Hjelmroos, 1993). Early spring and winter periods, when the number of spores is observed at low levels in the atmosphere of Büyükorhan district, can be said to be the periods when the risk is the lowest for susceptible individuals with allergies to *Cladosporium* and *Alternaria* spores.

In parallel with the temperature increase since April, the number of spores of both taxa were increased in the Büyükorhan atmosphere in both years (Figure 1 and 2). In similar studies, the spore concentration in the atmosphere increased with rising temperature and sufficient precipitation (Kramer et al., 1959; Hjelmroos, 1993; Stepelska et al., 1999; Peternel et al., 2004; Potoglu Erkara et al., 2008; Serbes and Kaplan, 2014).

The amount of spores in the atmosphere of Büyükorhan is the highest value in the summer months in both years. The maximum spore level was observed in August for both taxa in the first year. In the second year, the top level of spores was recorded in June. In parallel with this study, in regions with similar climatic conditions, the maximum number of spores was generally reached in the summer months. (Figure 1,2,3, and Table 2). For example, in Bursa city center, maximum spore concentration was determined in June (Ataygul et al., 2007), in August in Ankara (Şakıyan and Inceoğlu, 2003), in July and August in Poland (Grinn-Gofron and Mika, 2008).

Rain is to be noted as another influential meteorological factor on atmospheric fungal spores (McCartney, 1991; Hjelmroos, 1993). In both years, when the total number of spores reached its peak, it was observed that rainfall was higher than in other summer months (August and June) (Figure 1). High temperature and sufficient rainfall can provide optimum sporulation conditions, and the concentration of spores may increase in the atmosphere after precipitation (Kramer et al., 1959; Hjelmroos, 1993). Different rainfall types are important for increasing or decreasing spore concentrations in the atmosphere. A storm with heavy rain (rainstorm, thunderstorm) may increase the spore concentration in the atmosphere. Because the spores of fungi such as *Cladosporium* and *Alternaria*, which can live on plants or in the soil, can be separated from the conidium by the storm's force and dispersed into the atmosphere. On the contrary, the light rains that fall continuously may decrease spores' concentration in the atmosphere (Rich and Waggoner, 1962). Besides, rainfall may cause a more accessible release of fungal spores to the atmosphere by splash and "tap-and-puff" mechanisms (Ho et al., 2005).

The total amount of spores after peaking in both years, but after the decreased, there were times when it increased for the second time and even for the third time. In the first year, the second peak was observed in October, while in the second year, it was in August. These peaks may be due to the relevant meteorological factors for sporulation and the sporulation time of different species belonging to both genera. Both *Cladosporium* and *Alternaria* genera are known to contain many species (Dugan et al., 2004; Asan, 2015). The reason for the low amount of total spores in October 2013 compared to 2012 was excessive rainfall. (Figure 1, Table 2).

Agricultural activities are carried out in areas where forest areas are destroyed in the region; this was another responsible for the high spore amounts in the atmosphere during the summer and early autumn months. Harvest time corresponds to between June and October in general, depending on the crop planted. Since species belonging to *Cladosporium* and *Alternaria* can live on plants as pathogens, spores of them can be separated from their conidia due to agricultural activities at harvest time. They can be released and increase the spore concentration in the atmosphere (Landecker, 1996).

In conclusion, the changes of spores belonging to the *Cladosporium* and *Alternaria* genera were observed in the Büyükorhan atmosphere during the two consecutive years. Simultaneously, the relationship between the number of spores' variation during the years and meteorological factors was investigated. Also, the effect of temperature and appropriate rainfall on the number of spores were found to be important, as well as humidity and wind speed. It has been determined that the riskiest times for susceptible individuals are summer months, especially June and August. Those who live in the region or who will visit the area have spore allergies. Besides, it has been predicted for the agriculturist in the region to take the necessary measures to prevent the loss of yield in the economically important plants growing in the area.

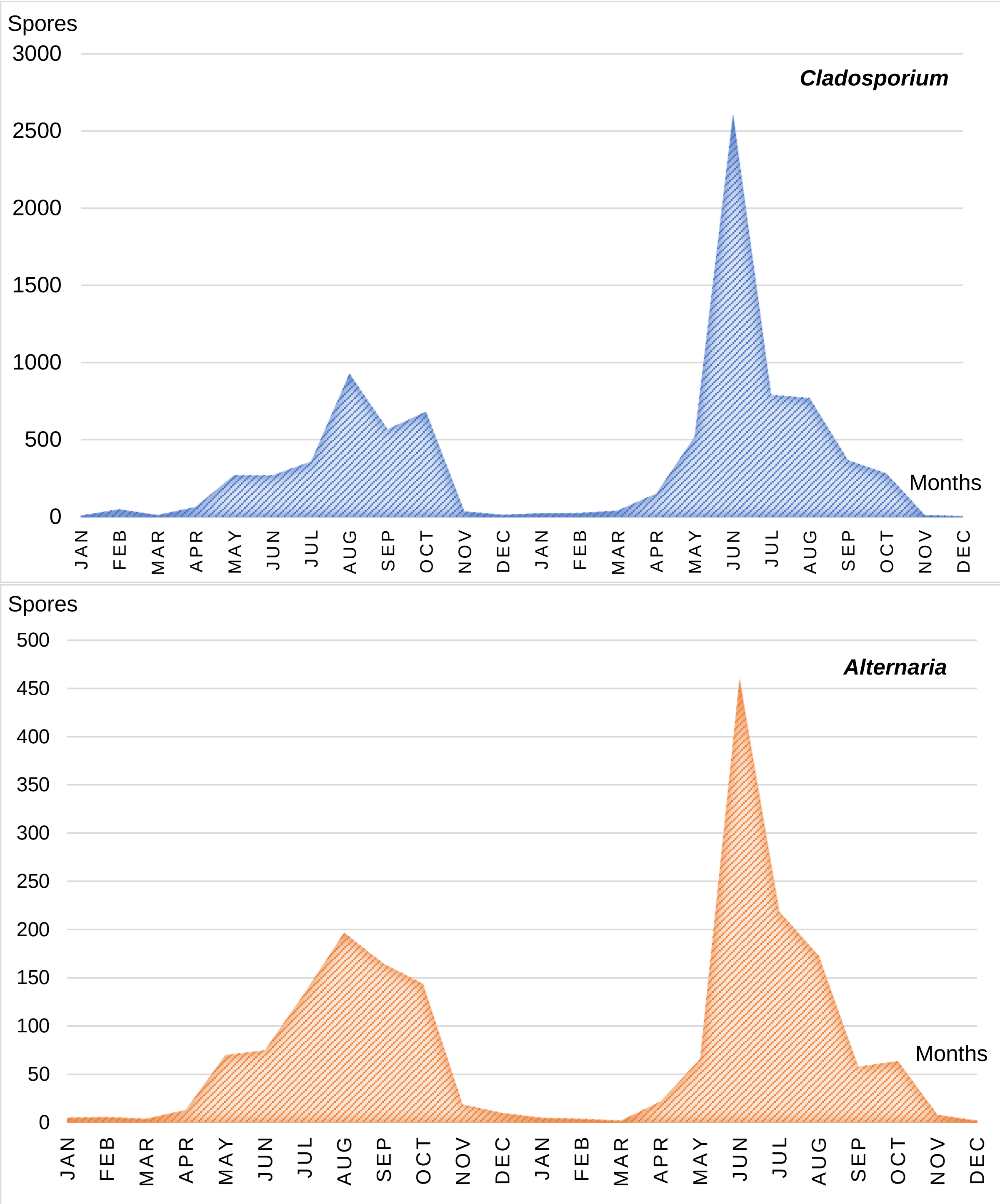

Figure 2. Monthly variation of atmospheric *Cladosporium* and *Alternaria* spores in the atmosphere of Büyükorhan during the years 2012-2013.

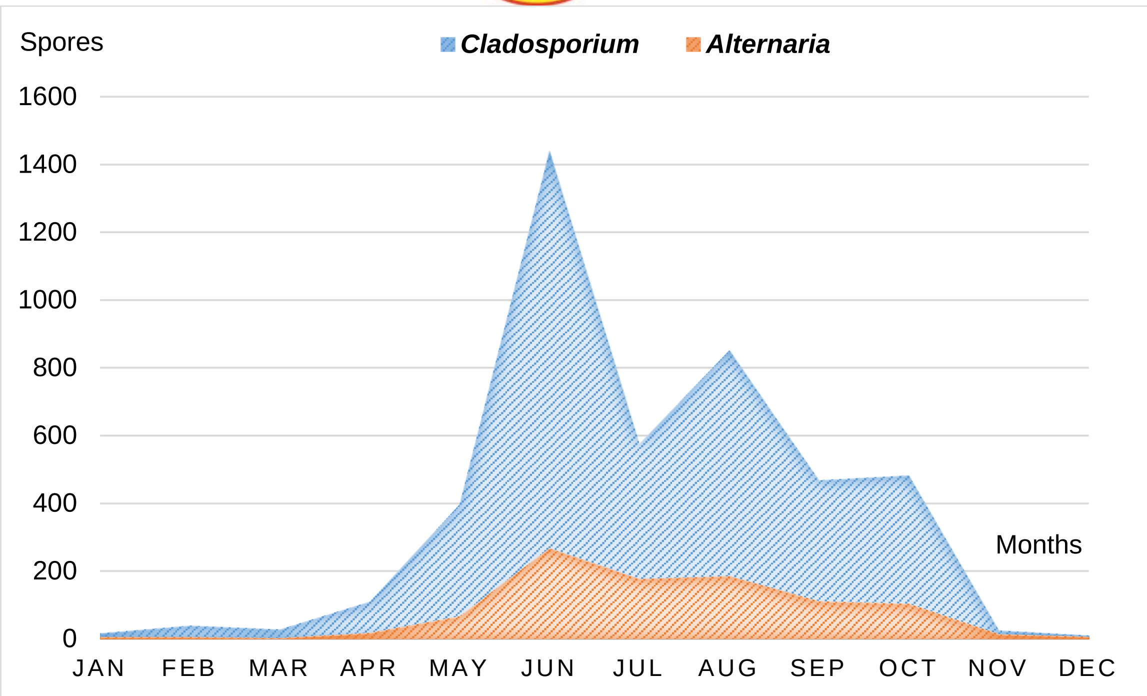

Figure 3. Monthly mean variation of atmospheric *Cladosporium* and *Alternaria* spores in the atmosphere of Büyükorhan during the years 2012-2013

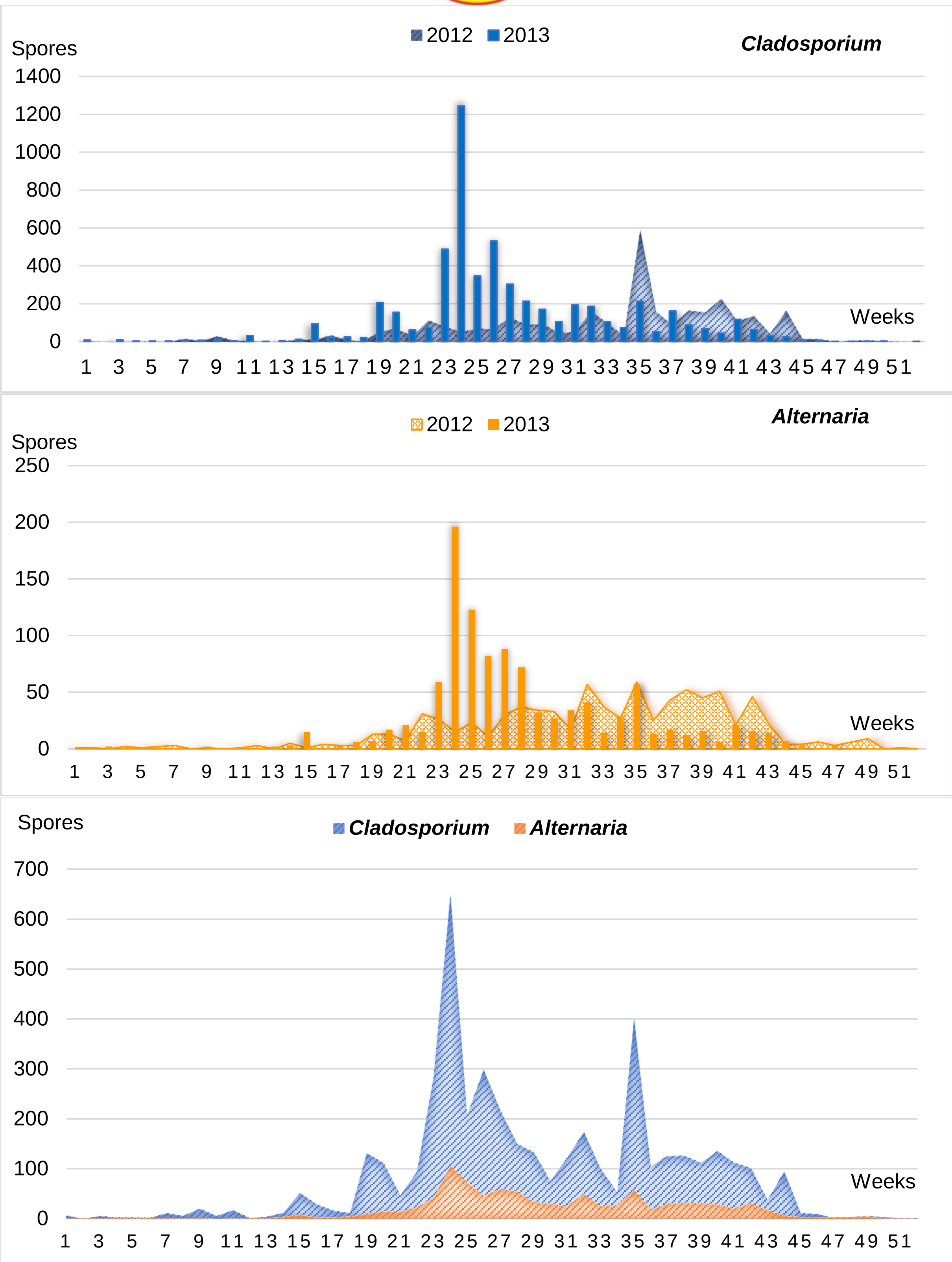

Figure 4. Weekly variation and weekly mean variation of *Cladosporium* and *Alternaria* spores in the atmosphere of Büyükorhan in the years 2012-2013.

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