Aerobiologia (2013) 29:95–111
DOI 10.1007/s10453-012-9266-y

ORIGINAL PAPER

Analysis of airborne allergenic pollen spectrum for 2009 in Timişoara, Romania

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Received: 23 November 2010 / Accepted: 21 June 2012 / Published online: 1 July 2012

Abstract The objective of this investigation was to identify the overall pollen types and, more particularly, the allergenic pollen content in the investigated area and then to explore their seasonal variations. The measurement point was located in the Timişoara city, Romania. A Lanzoni volumetric trap was used for sample collection. Duration of the pollen season of allergenic plants and respective variation in airborne pollen concentration are presented in the pollen calendar for the year 2009. Among the identified pollen of 23 types, 20 were allergenic: Taxaceae/Cupressaceae, *Alnus* sp., *Fraxinus* sp., *Betula* sp., *Corylus* sp., *Carpinus* sp., *Salix* sp., *Populus* sp., *Ulmus* sp., *Juglans* sp., *Quercus* sp., Pinaceae, *Tilia,* Poaceae, Urticaceae, Chenopodiaceae/Amaranthaceae, *Rumex* sp., *Plantago* sp., *Artemisia* sp., *Ambrosia* sp. These species prevail throughout almost the entire pollen season, from February–October, accounting for 87.03 % of the total pollen count. The greatest diversity of pollen types is detected in the months of spring. The summer months were characterized mostly by non-arboreal pollen types. In late summer and early autumn, *Ambrosia* airpollen was the most abundant in the atmosphere. The relationships between pollen concentrations and nine meteorological parameters are presented too. To analyze the correlation between pollen data and variables, the Spearman rank correlation coefficient was used. The correlation analysis of daily pollen counts and meteorological parameters showed that arboreal pollen and non-arboreal pollen counts were significantly correlated with temperature. The prevalence of pollen sensitization resulted to be very high in our patients with respiratory symptoms.

Keywords Pollen spectrum · Allergenic pollen · *Ambrosia artemisiifolia*

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1 Introduction

Many airborne pollen grains and fungal spores are important biopollutants responsible for human respiratory allergy. Generally, the seasonal and diurnal periodicities of aeropollen and spores are monitored by volumetric traps (Chakraborty et al. 2001; Cosentino et al. 1995) to prepare pollen/spore calendars. Airborne pollen grains are inhalant allergens that are

Fig. 1 Location of Timişoara in Timiş County, Romania

natural-source related. When released by the sources in sufficient amounts, allergenic pollen may evoke allergic responses in sensitive patients, leading to pollinosis. These diseases appear especially during flowering periods of plants. For this reason, annual pollen calendars have been prepared in many countries (Nardi et al. 1986; Al-Eisavi and Dejani 1988; Sado and Takeshita 1990; D'Amato and Spieksma 1990; Spieksma et al. 1991; Galán et al. 1991; Jager et al. 1991; Romano et al. 1992; Latorre 1997; Waisel et al. 1997; Emberlin et al. 1999; Kobzar 1999; Mishra et al. 2002; Abreu et al. 2003; Guvensen and Ozturk 2003; Rodrıguez-Rajo et al. 2003; Peternel et al. 2003; Bicakci et al. 2003; Weryszko-Chmielewska and Piotrowska 2004; Kaplan 2004; Syed et al. 2005; Nitiu 2006; Garcıa-Mozo et al. 2006; Recio et al. 2006; Ianovici 2007a; Kosisky et al. 2010). A calendar of airborne and allergenic pollen grains compiled for each region of a country can be used in allergy clinics for symptoms correlation and subsequent selection of allergens for diagnosis and treatment. This also serves primary goal toward the prevention and control of environmental allergens. The city of Timişoara has been the subject of several aerobiological studies (Ianovici 2007b, 2008a, 2009b). The objective of this investigation was to identify the allergenic pollen content in the investigated area and then to explore their seasonal variations.

Timişoara (Timiş County) is situated in the western region of Romania at 86 meters above sea level (45°46′N, 21°15′E) (Fig. 1). River Bega passes through Timişoara. The climate, which defines Timişoara city, is temperate continental moderate. The average annual temperature is 10.6 °C, and the hottest month of the year is July (22.42 °C). Rainfall in Timişoara is concentrated mainly in the spring and autumn months. The average value in this urban area is 592 mm. The characteristic vegetation of Timişoara is that of anthropogene forest steppe, which defines the Banat Plain (Ianovici et al. 2009).

2 Materials and methods

2.1 Aerobiological methodology

A Lanzoni 7-day volumetric trap was placed on the roof of West University near the center of the Timişoara city, at a height of about 20 m. This is the only pollen allergen monitoring station that worked in Romania since 1999. No obstacles or sources of heat and pollution influenced the air circulation around the sampler. The area is completely urbanized with buildings several meters in height mixed with low buildings and abundant vegetation. The air was sucked in a flow rate of 10 L per minute. Pollen grains were impacted onto tapes that were coated with a thin film of liquid silicone. A tape was replaced each Monday morning and cut into segments corresponding to 24-hour periods. The slides were mounted and stained in glycerine jelly mixed with basic fuchsine and examined microscopically weekly. The pollen was counted at a magnification of 400× . Pollen grains were observed on the surface of 4 horizontal bands. Daily values were represented as the number of pollen grains per m^3 of air (Mandrioli et al. 1998). The obtained results of the count per glass slide were converted to values of density per air volume by multiplying the values by a correction factor. We must note that the large majority of the taxa examined are not monospecific; under the same pollen types, representatives of co-generic species or even representatives of a whole family are included (Damialis et al. 2007). The identification and counting of airborne pollen were accomplished at least up to family or genus levels, and those grains that could not be identified were considered as unidentified types. The received data were worked out using the Excel.

2.2 Statistical analysis

The Spearman's correlation coefficients between pollen concentration (expressed as PG/m^3) and some daily meteorological parameters (mean daily average temperature expressed in °C; near-surface soil temperature expressed in °C; relative humidity in %; mean wind speed expressed in m/s; daily maximum wind speed expressed in m/s, atmospheric pressure in millibars; sunshine hours in h; nebulosity in tenths, quantities of precipitations in L/m^2) were analyzed. The nebulosity (cloud amount) represents the fraction of the sky covered by clouds as seen by an observer at ground level. This quantity is defined in oktas (8 classes) or in tenths (10 classes). The nonparametric Spearman's coefficient was chosen because daily pollen concentration is not normally distributed. These correlations have been calculated for each taxon. Statistical analysis was carried out by SPSS software package. All the meteorological data were provided by the Meteorological Centre of Timişoara.

2.3 Clinical data

The final objective of this study was to evaluate the component of allergic respiratory pathology in Timişoara. The study group consisted of 1,036 patients who presented during 2009 to allergy specialist for advice, inside specialty Ambulatory Clinical Hospital Nr. 4 "Victor Babes" Timişoara. SPTs were performed using the airborne set of allergens, from DIAGNOSTIC TERAPIE HALCIS ALERGIE SRL, Romania. A positive SPT result was defined as an allergen wheal with mean diameter at least equal to the mean diameter of histamine wheal (Demoly et al. 2008). SPT was performed at a total of 18 outdoor and indoor allergens:

- early tree pollen mixture I (*Corylus avelana, Betula verucosa, Alnus glutinosa*)
- early tree pollen mixture II (*Fraxinus excelsior, Salix sp., Populus sp., Quercus robur, Fagus silvatica*);
- grass pollen mixture I (*Triticum aestivum, Avena sativa, Hordeum vulgare*);
- grass pollen mixture II (*Dactylis glomerata, Lolium perenne, Festuca rubra, Secale cereale, Phleum pratense, Holcus lanatus*);
- pollen of *Ambrosia artemisiifolia*;
- pollen of *Artemisia vulgaris;*
- *Dermatophagoides pteronyssinus;*
- *Dermatophagoides farinae*;
- dog hair;
- cat hair;
- mold mix (*Aspergillus fumigatus, Penicillium notatum, Alternaria alternata, Mucor mucedo, Cladosporium cladosporioides*);
- *A. fumigatus;*
- *P. notatum;*
- *A. alternata;*
- *C. cladosporioides;*
- *M. mucedo;*
- *Candida albicans;*
- *Saccharomyces mellis*.

The selection of pollen species was made according to the local vegetation and expected sensitization, as documented by past studies made in our country (Ianovici and Faur 2005; Ianovici 2008b, c). Western Romania is characterized by different geographical conditions associated with distinct patterns of the flora distribution and different botanical species. In Western Romania, the most common aeroallergen was grass pollen. Usually, testing was done with mixtures of allergens. Because recent studies have shown changes on airborne concentrations of pollen (in late summer and early autumn), in design of this study we introduced allergens of *Ambrosia* and *Artemisia*. The performance of SPT with a wide number of different allergens, according to pollen concentrations and geographical particularities, allows obtaining a better vision of the sensitization in specific populations (Bousquet et al. 2005; Kirmaz et al. 2005).

3 Results

The present report describes the airborne pollen spectrum for 2009 in Timişoara, Romania. The aims were to identify the airborne pollen content during a year and to recognize the atmospheric pollen seasons. In the first part of this paper, we discuss the results of aeropalynological observations based on airborne pollen concentrations. In the second part, the meteorological aspects will be discussed. Finally, data of prevalence of patients with skin prick test (SPT) positive toward pollens, coming from the analysis of about 1,036 outpatients skin-tested in our Allergy Unit in the year 2009, were compared with data of pollen amount.

3.1 The pollen data

Studies were carried out from February 16, 2009, till October 11, 2009. Pollen of 23 taxa was recorded in the atmosphere of the city. Out of these, 16 belong to the arboreal taxa and 7 belong to the non-arboreal ones. Airborne pollen of allergenic plants dominated in the air throughout the year. There are anemophilous species well known for the production of large amounts of airborne pollen in many parts of Europe (Puc and Wolski 2002; Radišić and Šikoparija 2005; Stefanic et al. 2007). Twenty of them (*Alnus, Corylus, Populus, Salix, Ulmus, Fraxinus, Quercus, Carpinus, Betula, Juglans, Tilia,* Pinaceae, Taxaceae/Cupressaceae, Poaceae, *Rumex*, *Plantago*, Urticaceae, *Artemisia, Ambrosia* and Chenopodiaceae/Amaranthaceae) formed 87.03 % of spectrum, and all of them are considered allergenic.

The number of pollen counted in the station was 28,190 PG/m^3. Plant taxa represented in the pollen spectrum in Timişoara are listed in Table 1 in decreasing abundance. A total of 16,680 pollen grains have been found as non-arboreal pollen (NAP, 59.17 %) and 7,854 pollen grains as arboreal pollen (AP, 27.86 %) (Fig. 2).

In this year, two marked pollen seasons were observed. One was characterized by arboreal pollen (AP). This season lasts from February to May, which coincides with the low temperatures that occur in late winter–early spring. This period showed the climatic seasonality of the area, which was mirrored in the vegetable phenology and reproduction. A maximum concentration of these taxa was recorded in April. The other season was characterized by herbaceous taxa, which prevail from June to October and coincide with the highest temperatures of the year. NAP season starts with a minor concentration in April and then dominated in the pollen spectrum in the air throughout the year. A maximum concentration of these taxa was recorded in August. The presence of two periods of pollen dominance, one of arboreal and shrub pollen (AP season) and other of herbaceous pollen (NAP season), is a characteristic of cities with temperate climates (Tejera and Beri 2005).

The highest monthly pollen concentration was observed in April (6,661 pollen grains) followed by August (5,277 pollen grains) and July (3,729 pollen grains). The lowest concentration of pollen was recorded during February (Fig. 3). The richness of the pollen types varied throughout the investigated period, and the maximum number of pollen types was registered in April (18 types), followed by May (14 types) and March (10 types), and the minimum was recorded in October (5 types). In April, 18 taxa were identified, out of which 16 of them belonged to AP. In May, 5 taxa belonged to AP.

The earliest pollen grains in the atmosphere of Timişoara were noted in February. In relation to the order in which the taxa appear in the atmosphere, the first pollen grains belong to *Corylus, Alnus* and Taxaceae/Cupressaceae. In Timişoara, the period with the greatest diversity of pollen types is spring (Ianovici 2009a, b). The percentage of the monthly pollen in relation to the total pollen counted increased in March, reaching its highest level in April (23,63 %). Pollen concentrations decrease in May since the spring species end their flowering. *Poaceae* (27.67 %) was identified as dominant pollen taxa in May. In May, the airborne samples are principally composed of pollen from the herbaceous taxa. Many of these types of pollen remain present in June, although their levels are lower (with the exception of Poaceae, which presents its peak values in June—871 pollen grains). The quantities of pollen captured into the atmosphere

Table 1 Annual incidence of daily pollen counts, atmospheric pollen season and peak day

Plant groups	Taxa	Annual incidence in %	Atmospheric pollen season (APS) in day	Peak day (PG/m^3 air)
Arboreal plants (AP) woody taxa	*Acer* sp.	6.79	28	187
	Morus sp.	5.28	26	233
	Taxaceae/Cupressaceae	5.12	74	107
	Fraxinus sp.	3.93	51	70
	Pinaceae	3.73	52	65
	Populus sp.	3.05	32	131
	Betula sp.	2.55	59	96
	Corylus sp.	2.13	52	60
	Alnus sp.	1.78	43	48
	Tilia sp.	1.4	78	43
	Carpinus sp.	1.36	44	53
	Salix sp.	1.3	31	63
	Juglans sp.	0.81	37	36
	Ulmus sp	0.37	39	14
	Quercus sp.	0.34	36	15
	Platanus sp.	0.08	18	9
Non-arboreal plants (NAP) herbaceous taxa	*Ambrosia* sp.	19.83	65	292
	Urticaceae	19.32	103	141
	Poaceae	9.52	133	111
	Artemisia sp.	4.14	106	73
	Chenopodiaceae/Amaranthaceae	2.98	108	68
	Rumex sp.	2.19	83	37
	Plantago sp.	1.19	124	20
Unidentified		0.81		

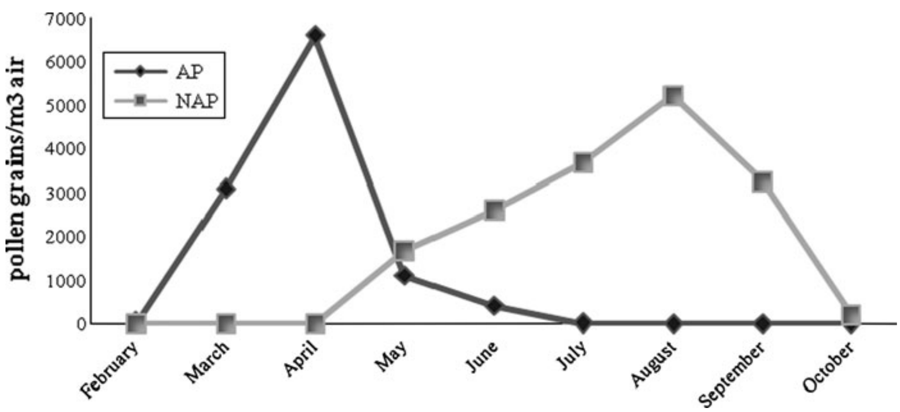
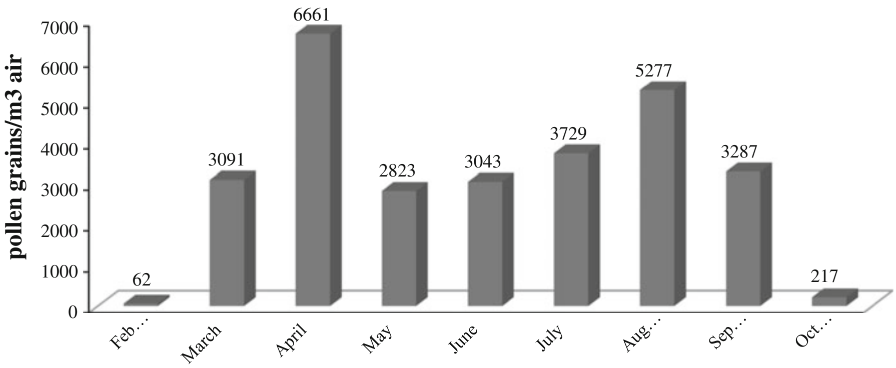


Fig. 2 Total monthly arboreal pollen (AP) and non-arboreal pollen (NAP) concentration

increase again during July. This increase is caused by the flowering of herbaceous species, among which the pollen types Poaceae and *Plantago* stand out, with *Tilia* and Pinaceae being the only trees species. From July, the pollen grains of weeds became dominant, but the amount of pollen was lower than in springtime. Urticaceae dominate the pollen spectrum of Timişoara in July (65.65 %). The summer peak was found to occur in August. This month accounts for an average of 18.72 % of the total annual pollen content.

Fig. 3 Monthly total variation in pollen grains in the atmosphere of Timişoara (2009)



In August, however, there is a high increase in overall levels, mainly due to the presence of pollen from *Ambrosia* (54.06 % monthly), whose pollen season continues until October, and *Artemisia*, which presents its peak concentrations in August. In autumn, pollen concentration starts to decrease due to the scarcity of flowering plants. In September, there is a gradual decline in pollen concentrations due to the decrease in the anemophilous pollen types. In September and October, the dominant pollen type is also *Ambrosia.* Very low values of airborne pollen were recorded in October. In Romania, the pollen season of these plants terminates toward the end of October (Ianovici 2007b, 2008b).

The annual incidence of each pollen type is expressed as a percentage of the total pollen registered in the studied period. The best-represented non-arboreal pollen type throughout the entire study period was *Ambrosia,* which attained an average of 19.83 % of the annual total. The best-represented arboreal pollen type through the entire period was *Acer* with 1,913 pollen grains (6.79 %). It is followed by pollen from *Morus* (5.28 %).

The dynamics of the different pollen types in the atmosphere in the course of the year provide very important information for completing the aerobiological description of a given locality. Fig. 4 presents the pollen calendar of Timişoara. The worked-out pollen calendar describes 20 allergenic pollen types. The pollen calendar was constructed following Spieksma's model (1991), which transforms 10-day mean pollen concentrations into a series of classes according to Stix and Ferretti (1974) and represented in pictogram form by columns of increasing height (Recio et al. 2006). In the calendar, each 10-day period corresponded approximately to a third of a month. To obtain the atmospheric pollen season (APS), we took into account the period covering 95 % of total annual pollen, discarding the initial period until 2.5 % is reached and the final period after reaching 97.5 % (Andersen 1991). The longest atmospheric pollen seasons were found for Poaceae (133 days) and the shortest for *Salix* and *Populus.* There are some taxa with long atmospheric pollen seasons, such as Urticaceae, Chenopodiaceae/Amaranthaceae, Poaceae, *Artemisia* and *Plantago* that are present in the atmosphere of the locality for more than 3 months.

In general, most taxa show a well-defined annual curve of pollen concentration. Some taxa, such as Taxaceae/Cupressaceae, Pinaceae, Chenopodiaceae/Amaranthaceae, *Rumex, Plantago,* Poaceae, *Alnus, Corylus, Ulmus, Quercus, Tilia, Artemisia* and Urticaceae, show a multi-peak curve, probably due to the number of species involved (Fig. 5b, c). Seven of the taxa analyzed, *Ambrosia*, *Betula, Carpinus, Salix, Fraxinus, Populus, Juglans*, show only one well-defined pollen peak during the season (Fig. 5a).

3.2 The meteorological data

In our region, the highest relative humidity values were recorded in the first month of the year, with minimum values being reached in the summer months. In Timişoara, the lowest temperatures were recorded in the first months of the year, with the maximum temperatures being reached in July (Table 2). The relation between airborne pollen and meteorological variability is given in Table 3. The twenty pollen taxa were then subjected to analyses. Spearman's correlation test was performed in order to identify the major variables likely to influence the dynamic of the airpollen. Wind direction was not considered when analysis was performed.

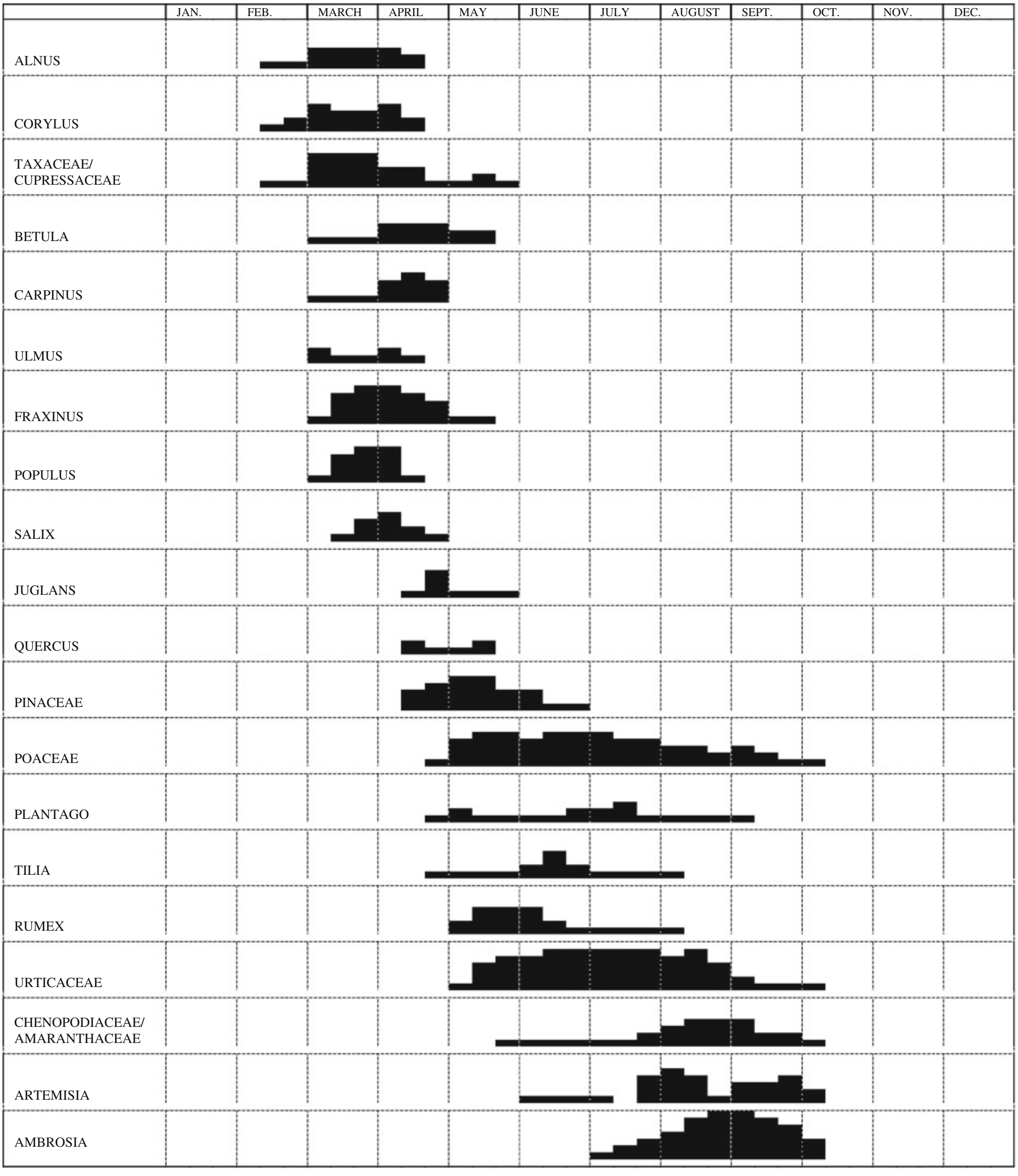


Fig. 4 Pollen calendar for 20 allergenic taxa, using data from February 2009 to October 2009 and following Spieksma's models

In the present work, *Alnus,* Taxaceae/Cupressaceae*, Betula, Ulmus* and Urticaceae pollen presented a significant negative correlation with pressure. The results have shown that level of Chenopodiaceae/Amaranthaceae, *Artemisia* and *Ambrosia* pollen is significantly positively affected by an atmospheric pressure.

Positive and statistically significant correlation was observed between the *Tilia, Rumex, Plantago*,

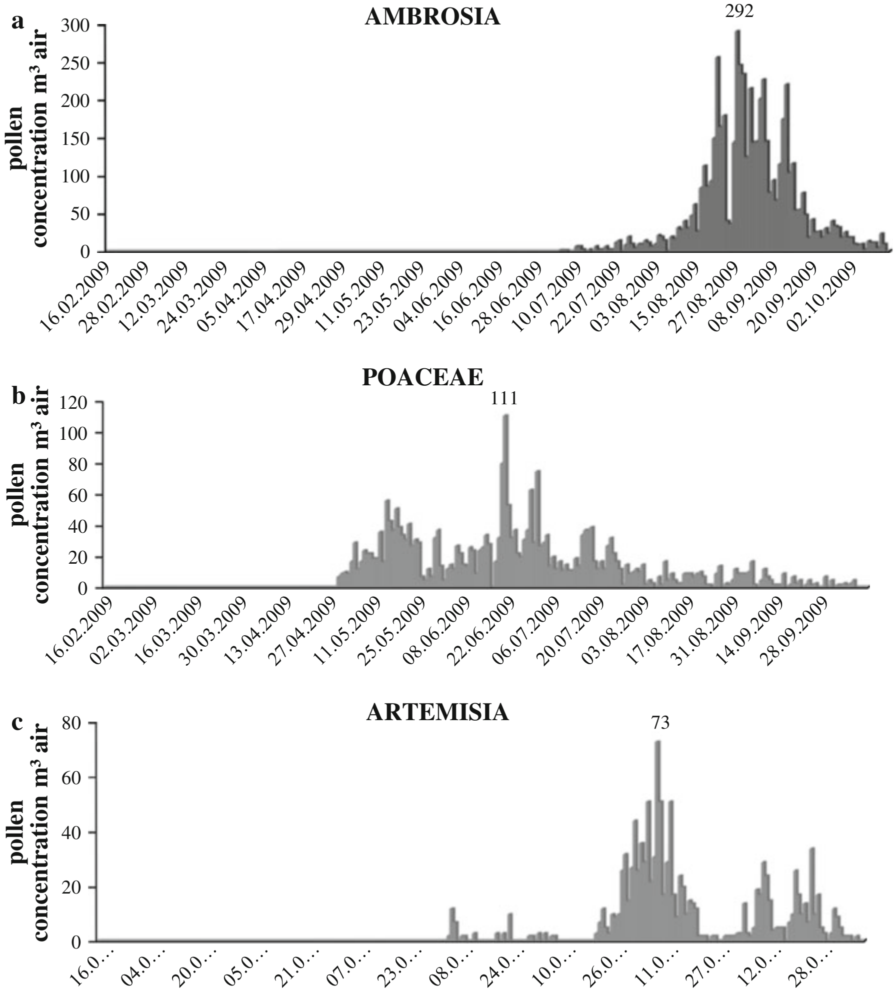


Fig. 5 **a-c** Daily pollen dynamics of the most abundant allergenic pollen types in the atmosphere above Timişoara, Romania

Urticaceae, Chenopodiaceae/Amaranthaceae, *Artemisia, Ambrosia* and Poaceae pollen and the mean daily average temperature. More notable is the significant negative relationship between mean daily average temperature and concentrations of airpollen *Alnus, Corylus,* Taxaceae/Cupressaceae*, Betula, Carpinus, Salix, Ulmus, Fraxinus, Populus, Acer, Juglans* and *Morus.* Similar results were observed for near-surface soil temperature.

In the case of *Alnus,* Taxaceae/Cupressaceae*, Betula, Carpinus, Salix, Ulmus, Fraxinus, Populus, Acer, Juglans, Quercus, Morus* and Pinaceae, negative significant correlation coefficients were only obtained with daily average relative humidity.

In the present study, we found a positive correlation between sunshine hours and *Acer, Juglans, Tilia, Plantago*, Urticaceae, Chenopodiaceae/Amaranthaceae, *Artemisia*, *Ambrosia* and Poaceae pollen counts. *Alnus, Corylus* and Taxaceae/Cupressaceae revealed significant negative correlations.

A positive and statistically significant correlation was found between the nebulosity and *Alnus, Corylus* and Taxaceae/Cupressaceae pollen concentrations. For *Plantago,* Urticaceae, Chenopodiaceae/Amaranthaceae, *Artemisia, Ambrosia* and Poaceae pollen grains were negative correlations detected between concentrations and nebulosity.

In relation to daily average wind speed, the correlation was significant and positive in the cases of Taxaceae/Cupressaceae. For this same factor, the correlation was significant and negative in the case of *Ambrosia* and *Artemisia.*

Table 2 The meteorological variables according to months (2009)

		Atmospheric pressure (millibars)	Mean daily average temperature (°C)	Daily average relative humidity (%)	Near-surface soil temperature (°C)	Sunshine hours (h)	Nebulosity in tenths	Daily average wind speed (m/s)	Daily max. wind speed (m/s)	Quantities of precipitations (l/m^2)
February (16–28)	Mean	1,008.4	−2.4	80	−1.6	4	7	2.1	4	2
	Maximum	1,015.5	2.8	89	2.4	8.7	10	5	7	3.7
	SD	4.72834	2.816348	7.158381	2.662307	3.956165	2.857248	1.220531	1.466804	1.824007
March	Mean	1,001.1	6.7	67	7.2	4.4	7.3	2.3	4.4	3.2
	Maximum	1,015.3	17.9	85	18.2	11.2	10	4.5	7	17.8
	SD	8.841873	3.738567	10.57241	3.464294	3.266063	2.177935	0.814389	1.350059	4.583802
April	Mean	1,001.8	14.7172	56	17.9	9.1	4.1	1.6	4	3.2
	Maximum	1,009.2	17.2	76	22	11.3	9	3.5	11	11
	SD	5.366796	1.364883	9.11665	1.960848	2.514206	2.45381	0.700686	1.822141	5.407314
May	Mean	1,006.6	18.1	61	23.3	7.4	5.2	1.9	4	4.1
	Maximum	1,010.3	22.9	90	30.1	11.4	8.5	3.3	6	16.9
	SD	2.280855	3.791127	10.49625	5.001178	2.747609	2.115322	0.56088	0.808717	6.022307
June	Mean	1,002.3	20.3	72	23.9	7.3	6	1.8	4	8.8
	Maximum	1,010.9	26.1	99	33.4	12.5	10	3.5	6	35.3
	SD	4.638312	3.307783	14.35184	4.878487	3.967158	2.255268	0.703807	1.023131	11.39581
July	Mean	1,003.7	23.1	67	27.8	9.1	3.6	1.7	3	4.5
	Maximum	1,009.6	27.5	85	39.7	11.7	8.8	3	6	14.1
	SD	3.965688	2.374358	10.48836	4.507511	2.645158	2.495476	0.52413	1.014833	4.283236
August	Mean	1,005.9	22.7	68	27.2	8.2	4.1	1.6	3	3.2
	Maximum	1,012.6	26.9	90	32.2	11.2	8	3.3	5	7.6
	SD	2.68	1.808502	9.443419	2.715433	2.866934	2.699936	0.612138	0.886683	3.222232
September	Mean	1,007.6	19	67	22	7	4.4	1.5	3	0.6
	Maximum	1,015.2	23.2	92	28.1	10.6	8.8	3.5	6	1.7
	SD	4	1.9	10	2.6	2.7	2.6	0.7	1.13606355	0.7
October (1–10)	Mean	1,005.8	16	77	18	6.3	4.7	1.6	3	4.1
	Maximum	1,009.6	18.8	89	20.7	9.1	9.8	2.8	4	5.7
	SD	2.69122	2.249667	7.527727	1.876433	2.609593	3.114482	0.567646	0.816497	2.262742

Table 3 Pollen concentrations for selected taxa versus meteorological parameters (*correlation is significant at the 0.05 level; **correlation is significant at the 0.01 level), in Timişoara (2009)

Taxa	Spearman correlation test									
	Spearman's coefficient	Atmospheric pressure (millibars)	Mean daily average temperature (°C)	Daily average relative humidity (%)	Near-surface soil temperature (°C)	Sunshine hours (h)	Nebulosity	Daily average wind speed (m/s)	Daily max. wind speed (m/s)	Quantities of precipitations (l/m^2)
Alnus	Spearman's rho	**−0.135***	**−0.529****	**−0.130***	**−0.544****	**−0.174****	**0.184****	0.119	**0.179****	−0.035
	Sig.(2 − tailed)	.037	.000	.044	.000	.007	.004	.066	.006	.593
Corylus	Spearman's rho	−0.118	**−0.590****	−0.056	**−0.608****	**−0.209****	**0.199****	0.073	**0.143***	0.037
	Sig. (2-tailed)	.069	.000	.393	.000	.001	.002	.259	.027	.567
Taxaceae/Cupressaceae	Spearman's rho	**−0.147***	**−0.558****	**−0.215****	**−0.541****	**−0.150***	**0.169****	**0.216****	**0.296****	0.025
	Sig. (2-tailed)	.023	.000	.001	.000	.020	.009	.001	.000	.701
Betula	Spearman's rho	**−0.132***	**−0.347****	**−0.370****	**−0.291****	0.096	−0.004	−0.015	0.076	−0.042
	Sig. (2-tailed)	.042	.000	.000	.000	.139	.948	.824	.242	.520
Carpinus	Spearman's rho	−0.054	**−0.356****	**−0.349****	**−0.344****	0.100	−0.066	−0.022	0.106	−0.112
	Sig. (2-tailed)	.410	.000	.000	.000	.123	.314	.732	.102	.085
Salix	Spearman's rho	−0.075	**−0.344****	**−0.264****	**−0.356****	−0.016	0.006	0.043	0.077	−0.061
	Sig. (2-tailed)	.248	.000	.000	.000	.810	.922	.511	.238	.345
Ulmus	Spearman's rho	**−0.185****	**−0.346****	**−0.138***	**−0.361****	−0.087	0.112	0.084	**0.173****	0.023
	Sig. (2-tailed)	.004	.000	.033	.000	.179	.083	.194	.007	.725
Fraxinus	Spearman's rho	−0.083	**−0.492****	**−0.349****	**−0.456****	0.023	0.009	0.097	**0.190****	−0.107
	Sig. (2-tailed)	.203	.000	.000	.000	.719	.886	.137	.003	.101
Populus	Spearman's rho	−0.027	**−0.424****	**−0.197****	**−0.442****	−0.071	0.049	0.113	**0.161***	−0.061
	Sig. (2-tailed)	.673	.000	.002	.000	.274	.453	.082	.013	.348
Acer	Spearman's rho	−0.027	**−0.289****	**−0.371****	**−0.239****	**0.195****	−0.109	−0.095	0.045	−0.070
	Sig. (2-tailed)	.681	.000	.000	.000	.002	.092	.143	.494	.284
Juglans	Spearman's rho	0.000	**−0.143***	**−0.336****	−0.041	**0.185****	−0.108	0.098	**0.129***	−0.067
	Sig. (2-tailed)	.999	.027	.000	.534	.004	.097	.132	.047	.301
Quercus	Spearman's rho	0.002	−0.098	**−0.299****	−0.014	0.105	−0.054	0.051	0.091	−0.061
	Sig. (2-tailed)	.978	.130	.000	.829	.107	.409	.437	.160	.350
Morus	Spearman's rho	−0.011	**−0.172****	**−0.327****	−0.071	0.112	0.029	0.003	0.012	0.012
	Sig. (2-tailed)	.860	.008	.000	.274	.084	.659	.967	.853	.854

Table 3 continued

Taxa	Spearman correlation test									
	Spearman's coefficient	Atmospheric pressure (millibars)	Mean daily average temperature (°C)	Daily average relative humidity (%)	Near-surface soil temperature (°C)	Sunshine hours (h)	Nebulosity	Daily average wind speed (m/s)	Daily max. wind speed (m/s)	Quantities of precipitations (l/m^2)
Platanus	Spearman's rho	−0.051	−0.108	−0.100	−0.091	0.006	0.013	−0.082	−0.035	0.009
	Sig. (2-tailed)	.437	.096	.123	.163	.923	.838	.209	.588	.887
Pinaceae	Spearman's rho	0.067	−0.072	**−0.284****	0.041	0.089	0.046	0.052	0.084	0.046
	Sig. (2-tailed)	.302	.272	.000	.526	.173	.485	.425	.197	.483
Tilia	Spearman's rho	−0.040	**0.228****	−0.091	**0.273****	**0.218****	−0.080	0.044	0.078	0.016
	Sig. (2-tailed)	.542	.000	.160	.000	.001	.217	.500	.228	.805
Rumex	Spearman's rho	−0.099	**0.292****	−0.084	**0.310****	0.117	−0.007	0.088	0.074	0.091
	Sig. (2-tailed)	.126	.000	.198	.000	.072	.909	.177	.258	.160
Plantago	Spearman's rho	−0.094	**0.464****	−0.047	**0.503****	**0.248****	**−0.167****	−0.017	−0.067	−0.050
	Sig. (2-tailed)	.148	.000	.475	.000	.000	.010	.788	.304	.443
Urtica	Spearman's rho	**−0.135***	**0.777****	0.068	**0.748****	**0.286****	**−0.194****	−0.047	**−0.130***	0.021
	Sig. (2-tailed)	.038	.000	.294	.000	.000	.003	.471	.045	.751
Chenopodiaceae/ Amaranthaceae	Spearman's rho	**0.165***	**0.531****	−0.057	**0.489****	**0.222****	**−0.306****	−0.076	**−0.153***	**−0.221****
	Sig. (2-tailed)	.011	.000	.384	.000	.001	.000	.240	.018	.001
Artemisia	Spearman's rho	**0.135***	**0.493****	0.025	**0.446****	**0.145***	**−0.229****	**−0.162***	**−0.190****	**−0.136***
	Sig. (2-tailed)	.037	.000	.707	.000	.026	.000	.012	.003	.036
Ambrosia	Spearman's rho	**0.195****	**0.494****	0.009	**0.436****	**0.168****	**−0.299****	**−0.149***	**−0.226****	**−0.169****
	Sig. (2-tailed)	.003	.000	.885	.000	.009	.000	.021	.000	.009
Poaceae	Spearman's rho	−0.078	**0.639****	0.012	**0.670****	**0.223****	**−0.150***	−0.029	−0.063	0.001
	Sig. (2-tailed)	.231	.000	.858	.000	.001	.021	.656	.333	.992

A positive, statistically significant correlation was noted between the *Alnus, Corylus*, Taxaceae/Cupressaceae, *Ulmus, Fraxinus, Populus* and *Juglans* pollen counts and the maximum wind speed. The results have shown that level of Urticaceae, Chenopodiaceae/Amaranthaceae, *Artemisia* and *Ambrosia* pollen is significantly negatively affected by a daily maximum wind speed.

Significant negative correlations were observed between the Chenopodiaceae/Amaranthaceae, *Artemisia* and *Ambrosia* pollen and precipitation. In Timişoara, no statistically significant correlation was observed between the precipitation and the rest of pollen concentrations.

3.3 The clinical data

The number of patients diagnosed with allergic pathology after skin prick test was 458, of which 221 were men and 237 were women.

Clinical and paraclinical assessment of patients has resulted in the following distributions on the diagnosis and outdoor and indoor allergens:

- Only 12 patients with allergic asthma—3 allergic to outdoor allergens (pollens), 6 allergic to indoor allergens (dust mites, animal scales, molds) and 3 allergic to both types of allergens.
- 276 patients with allergic rhinitis—139 allergic to outdoor allergens (pollens), 60 allergic to indoor allergens (dust mites, animal scales, molds) and 77 allergic to both types of allergens.
- 116 patients with both asthma and allergic rhinitis—33 allergic to outdoor allergens (pollens), 46 allergic to indoor allergens (dust mites, animal scales, molds) and 37 allergic to both types of allergens.
- 54 patients with other diagnoses (atopic dermatitis, contact dermatitis, subacute angioedema, chronic urticaria, allergic conjunctivitis, chronic bronchitis, etc.).

Of the 458 patients allergic to aeroallergens, 138 showed a single positive test (Fig. 6). Out of 138 study patients, 91 (65.94 %) patients showed pollen allergy, whereas allergy to other inhalation allergens (mite, animal hair, epithelium, and *Alternaria* and *Cladosporium* spores) was recorded in 34.06 % of study patients. The evaluation of sensitization patients shows that principal outdoor aeroallergen is the pollen of *Ambrosia* ($N = 71$). The less important allergens were *Artemisia* pollen ($N = 6$) and early tree pollen ($N = 5$).

The proportion of patients with polysensitization was considerably greater. The main allergen from outdoor environment in monosensitization and

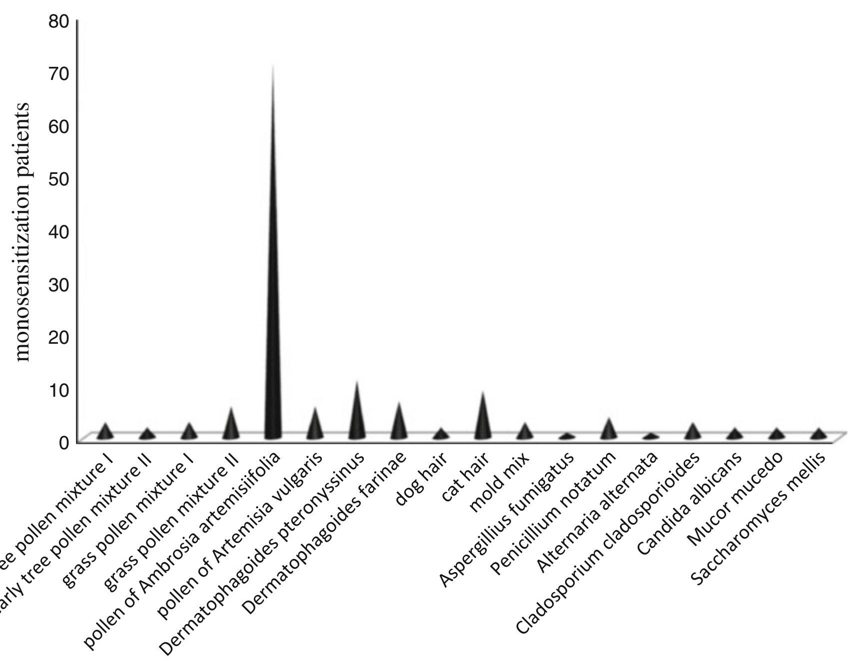


Fig. 6 Distribution of patients with positive SPT to one allergen

polysensitization patients (positive tests to two or more allergens) is the pollen of *Ambrosia*: 49.7 % ($N = 228$). In second place ranks grass pollen mixture (*D. glomerata, L. perenne, F. rubra, S. cereale, P. pratense, H. lanatus*)—33.6 % ($N = 154$), followed by in third place planted grass pollen (*T. aestivum, A. sativa, H. vulgare*)—25.3 % ($N = 116$).

4 Discussion

The allergy depends on the combined effects of several factors: the patient, the allergens, the timing, the duration of exposure and the qualities of the environment. Studying plants' pollen levels in relation to meteorological elements is of high practical importance because of its health concern (Makra et al. 2006).

Analyses of aeroallergens in Timişoara have been made since 1999 (Ianovici and Sirbu 2007; Ianovici 2007b).The pollen spectrum reflected the floristic diversity of the investigated region, and the majority of the sources of airborne pollen are present in local and regional flora. Some of them are used for ornamental purposes and do not represent the local flora such as Pinaceae and Taxaceae/Cupressaceae. The urban flora is mainly composed of exotic trees and shrub species that are cultivated for ornamental purposes in streets, avenues, parks and cemeteries over the entire city. Herbaceous vegetation occurs spontaneously in open areas, or it is cultivated in parks and private gardens. A positive correlation of pollen concentration and surrounding vegetation density has been described, indicating that changes in the floral composition of a given area have a direct influence on its airborne pollen spectrum (Abreu et al. 2003).

From the clinical point of view, pollen from herbs is much more of a problem than pollen from trees. Besides the natural or planted tree genera, herbs belonging to the taxa *Ambrosia,* Poaceae, Urticaceae, *Rumex,* Chenopodiaceae/Amaranthaceae, *Artemisia* and *Plantago* contribute significant amounts of airborne pollen to the atmosphere of Timişoara. Poaceae and *Artemisia* are very abundant weed species in the region and, moreover, are known as very potent aeroallergens. They are a common cause of pollinosis in the temperate zone (Puc and Puc 2004). Some species, for example, *Plantago* and *Rumex*, which induce pollinosis, might have a combined effect, and several types of pollutants may sum up and induce clinical responses, even at low airborne pollen concentrations (Waisel et al. 1997). Other most significant pollen types found in the air are Betulaceae pollen grains. However, the relatively low concentration of Betulaceae pollen (*Betula, Corylus, Carpinus, Alnus*) detected during our survey suggests to us that this pollen taxa cannot be a very important cause of pollinosis in the city.

Ambrosia sp. is the most allergenic plant of our climate (Makra et al. 2010). The dominance of *Ambrosia* pollen in the atmosphere of Timişoara has also been noted by other studies (Faur et al. 2000; Juhasz et al. 2004; Ianovici and Sirbu 2007; Ianovici 2007b; Skjøth et al. 2010). Because *A. artemisiifolia* (ragweed) is young adventive taxa of the Romanian flora, it does not have any natural competitors. Ragweed pollen load of Timişoara is most serious between 15 August and 15 September. Hence, this is the most dangerous period for pollinosis. We think that allergologists from western Romania should include *Ambrosia* pollen extracts in standard skin tests. Since 1910, having been spread all over the country, it has become the most common weed in Romania. There is an urgent need to organize interventions to stop ragweed expansion (Ianovici and Sirbu 2007; Ianovici 2009a).

A total of nine meteorological parameters were selected for this investigation. The weather conditions (independent variables) influence pollen concentrations (dependent variables). Coefficients for the same variable were positive in some cases and negative in others (atmospheric pressure, temperature, wind speed, sunshine and nebulosity). Temperature and humidity were found to be the most important influences on pollen concentrations in the spring. However, only the correlation with temperature was highly significant. The maximum counts of arboreal pollen were detected in April when the mean temperature was 14.7 °C. The maximum counts of non-arboreal pollen were detected in August when the mean temperature was 23.1 °C. *Ambrosia* is the most important pollen source at this moment.

According to the aerobiological and ecological data, temperature is the environmental factor that most strongly affects the generative and vegetative development and the occurrence of pollen in air (Kasprzyk 2008). The negative effect of increased humidity on pollen release is most marked in Timişoara. The heavy rain in spring may have delayed the stamens to open and may have depressed pollen dispersal (McDonalds 1980). The humidity decreased atmospheric pollen concentration by accelerating the process of deposition. On the contrary, the dry period facilitated pollen dispersal through the air. In several other studies, humidity and rainfall exerted a negative influence on pollen counts (Alwadie 2008). Trees that bloom in early spring are negatively influenced by the temperature at the soil surface. Dynamics of herbaceous plant pollen are positively correlated with this parameter. The temperature and moisture conditions near the soil surface change quite rapidly and are strongly influenced by small changes in weather patterns and soil types. Vegetation also strongly influences soil water and temperature conditions by controlling how much sunlight reaches the soil surface and how much heat is lost from the soil at night, when the air is cooler. Soil under a tree receives much less sunlight than bare soil or soil covered by a weed plant immediately adjacent to the tree. This causes a great deal of variation in how much heat is accumulated at different locations across a landscape (Pierson et al. 1996–2000). Photoperiod is defined as the period of sunshine hours necessary for flowering. Thus, sunshine hours are correlated directly with the photoperiod (Vázquez et al. 2003). The influence of sunshine accelerates pollen grain release, especially in long-day weed species. We found a statistically significant relationship between the non-arboreal pollen season and nebulosity in 2009 (negative correlation). The positive correlation between *Alnus, Corylus*, Taxaceae/Cupressaceae, *Ulmus, Fraxinus, Populus* and *Juglans* pollen counts and the maximum wind speed indicates that these pollen types present in the air above Timişoara can also be the result of long-distance transport. Wind speed and nebulosity for arboreal pollen, temperature and sunshine hours for non-arboreal pollen were significant risk factors for increased pollen counts. In other studies, pollen concentrations showed significant positive correlations with mean air temperature (Jato et al. 2002; Gioulekas et al. 2004; Stennett and Beggs 2004), minimum air temperature (Makra et al. 2004), maximum air temperature (Rodríguez-Rajo et al. 2004), dew point temperature (Stennett and Beggs 2004), sunshine duration (Gioulekas et al. 2004; Rodríguez-Rajo et al. 2004) and significant negative correlations with air pressure (Stennett and Beggs 2004). While in some cases, daily pollen counts show significant negative correlations with precipitation (Jato et al. 2002; Green et al. 2004), in other case no correlation was found between pollen and relative humidity and rainfall (Gioulekas et al. 2004). Pollen levels of species studied showed significant positive correlations with wind speed (Stennett and Beggs 2004; Gioulekas et al. 2004) and wind components, showing the importance of wind persistence in pollen transport (Damialis et al. 2005; Makra et al. 2006).

Our study showed that pollen was the major sensitizing aeroallergen in patients with symptoms of respiratory allergy. The rate of 63.76 % of patients with pollen allergy recorded in our study was only slightly lower than the rate of such patients reported in an epidemiological study carried out in Iran (Kashef et al. 2003) and Croatia (Peternel et al. 2007). Our clinical data show that patients react to these allergens. In most European and North American countries, the allergens with highest sensitization rates among patients with nasal symptoms are also pollen, followed by house dust mites and cat dander (Solomon and Platts-Mills 1998).

The most frequently implicated pollen in SPT sensitization of patients were *Ambrosia* (ragweed), Poaceae (grasses) and *Artemisia* (mugwort). The existence of genetic factors, influencing the differences in sensitization in various countries (Ruffilli and Bonini 1997), could partially explain these findings. These hypotheses need to be confirmed by genetic, epidemiological and aerobiological studies on a large scale and on a long period (Voltolini et al. 2000). The vast majority of patients appeared to have symptoms in August and September during the flowering of *Ambrosia*. They were followed by the group of patients developing symptoms during the pollinative period of grasses in May and June. Frequency of SPT sensitization did not necessarily present a positive relationship with pollen grain concentrations in the air of Timişoara.

Knowledge of the daily, monthly and annual pollen levels is important for clinicians and allergy sufferers alike. The possible causes of variations in atmospheric levels of pollen, their statistical analysis and the construction of prognostic models are also important (Docampo et al. 2007). An aeropalynological study of one year is not sufficient to analyze seasonal variations in airborne pollen. It would be necessary to extend these studies to more years and to other strategically placed areas to achieve a global pollen calendar for the city and to improve the knowledge of flowering

(Fernández-Illescas et al. 2010) and pollen dynamics and its relation to the meteorological parameters. The effect of any meteorological variable on concentration may differ from year to year because of extremes in other variables. Despite the seasonal effects, there remains a trend in the results that shows the correlations between concentration and temperature, and lower humidity, due to the release processes. To investigate the factors that might affect these different behaviors in this preliminary study, given that conclusions are to be drawn, a more prolonged study would be necessary. In the near future, we will analyze 10 years of monitoring data for a proper image on the dynamics of potentially allergenic pollen in Timişoara and surroundings.

5 Conclusion

The pollen spectrum includes several plants used for ornamental (Taxaceae/Cupressaceae and Pinaceae) and agricultural purposes, adventives taxa (*A. artemisiifolia*) and other typical anemophilous species of western Romania. Our results show that 20 allergenic taxa were observed in the atmosphere of the Timişoara surroundings. High counts of arboreal pollen were detected in spring; for non-arboreal pollen, late summer and fall were the periods with high and very high concentrations. The dominant pollen type during the investigation period belongs to the genus *Ambrosia.*

Our analysis showed strong correlations with mean daily average temperature for 20 taxa, while for 18 taxa, a correlation was noted with the near-surface soil temperature. The significant Spearman's correlation coefficients between the pollen counts and daily average relative humidity, sunshine hours, nebulosity and daily maximum wind speed were noted.

Pollen is the major aeroallergen provoking seasonal respiratory allergies in Timişoara, with a high proportion of patients being allergic to pollen of *A. artemisiifolia.*

This study permitted us to identify the airborne pollen types present in the Timişoara atmosphere and to give an indication of their atmospheric pollen seasons. It is hoped that the results obtained might allow allergy specialists to plan treatments and allergy sufferers to plan their activities. Determination of types and concentrations of airpollen grains will be helpful for patients suffering from these diseases.

Acknowledgments The study was financed by a Romanian national grant, Program 4 Partnership (PN2-41-011/2007-2013, PREVALERG)-Minister of Education, Research, Youth and Sports.

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