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METHODS OF BIOMONITORING IN URBAN ENVIRONMENT: ALLERGENIC POLLEN IN WESTERN ROMANIA AND RELATIONSHIPS WITH METEOROLOGICAL VARIABLES

Nicoleta IANOVICI*, Dorina TUDORICĂ, Florentina ŞTEFLEA
[1]Department of Biology and Chemistry, University of Timisoara, Romania
**Corresponding author e-mail: nicole_ianovici@yahoo.com*
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ABSTRACT
Biomonitoring results showed that a total of 23 pollen types were located in the air of the study area. The analysis of the airborne pollen content concerned 20 taxa, whose pollen has allergenic properties and occurs in large quantities in the air of Timisoara: Alnus, Taxaceae/Cupressaceae, Salix, Populus, Ulmus, Fraxinus, Juglans, Quercus, Tilia, Pinaceae, Corylus, Carpinus, Betula, Poaceae, Rumex, Plantago, Urticaceae, Chenopodiaceae/Amaranthaceae, Ambrosia and Artemisia. Analysis of the pollen count in Timisoara was performed on the basis of the data collected in the year 2009. Ambrosia artemisiifolia, Urticaceae and Poaceae reached the highest atmospheric pollen concentrations in Timisoara. Multiple regression analysis was found to be a very valuable tool for identifying the weather variables most closely associated with atmospheric pollen seasons.
KEY WORDS: *phenology, biomonitoring, volumetric method, multiple regression analysis, atmospheric pollen concentrations*

INTRODUCTION

The environmental sensitivity of many plant life cycles reflects different life-history strategies in natural and agricultural areas (Wilczek *et al*, 2010). Plant phenology models are important tools in a wide range of issues such as agricultural practices, forestry and aerobiology. Phenology is a major bio-indicator of climate change impact on ecosystems and helpful in prediction of the impact of global warming (Schleip *et al*, 2006). Plant phenology include reproductive events such as flowering, fruiting, seed dispersal and germination. Flowering phenology is a critical stage of development that strongly influences reproductive success of plants (Faur *et al*, 2003a). The timing, duration and intensity of flowering may be affected by some biotic factors. Several climatic parameters such as temperature, precipitation, irradiance, soil nutrient concentration and other variables can trigger flowering in different taxa (Bustamante & Búrquez, 2008; Forrest & Miller-Rushing, 2010). As many authors have observed, the aerobiological processes (emission, dispersion, transport, deposition of pollen grains) are affected by the meteorological variables (Cecchi *et al*, 2010).

One of modern methods of knowing the impact of environment factors, extremely valuable in the medical practice, is the volumetric method of sampling, identifying and quantifying the airborne pollen, which belongs to the category of inhaling allergens (Faur *et al,* 2001). In Romania, at the Department of Biology of the West University of Timisoara, continuous pollen biomonitoring has been carried out since 1999. This type of work was started by Ianovici & Faur (2001). The results of the studies were published in numerous papers (Juhasz *et al*, 2001; Radišič *et al*, 2003; Faur *et al*, 2003b; Juhasz *et al*, 2004; Radisič *et al*, 2004; Juhasz *et al*, 2005; Ianovici, 2008a; Ianovici, 2008b; Ianovici *et al*, 2013a; Ianovici, 2014). The pollen spectrum show the distribution of frequently occurring taxa, particularly those having allergenic importance (Ianovici & Sîrbu, 2007; Ianovici, 2007c; Ianovici, 2008c). Airborne pollen content at Timisoara led us to identify the flowering plants that correspond to the three seasons corresponding to spring (12–23 weeks), summer (24–37weeks) and autumn (37–49weeks) (Ianovici, 2007b). The population with pollen allergy increases every year. An extremely high increase of patients allergic to *Ambrosia* was observed in the western part of Romania (Ianovici *et al,* 2009; Panaitescu *et al*, 2013; Ianovici *et al*, 2013b; Panaitescu *et al*, 2014; Leru *et al*, 2015). The symptoms of pollen allergy confirm a good correlation with the airborne pollen count (Obtułowicz *et al*, 1991; Florido *et al*, 1999; Ianovici, 2007a; Rodríguez *et al,* 2011; Ščevková *et al*, 2015].

The aim of the study was to analyse the pollen counts of selected allergenic taxa (20) in the air of Timisoara City (western Romania). Using data from 2009, the effect of climatic variables on pollen concentrations was examined with multiple regression analysis. The analysis was useful to detect which of the climatic variables had the strongest impact on onset and duration.

MATERIALS AND METHODS

Timisoara and its surroundings is located at an altitude of 86 m above sea level and characterized by arable lands and mixed forests. Many woody taxa are distributed in parks, alongside roads and cemeteries. The urban flora includes several plants used for ornamental purposes: Taxaceae/Cupressaceae and Pinaceae (Ianovici, 2007d; Ianovici, 2009). Herbaceous taxa occurs spontaneously in open areas, or it is cultivated in parks and private gardens (Ianovici, 2007e). *Ambrosia artemisifolia* and *Artemisia vulgaris* are cited as the more important species of the Timisoara City and are relatively common, especially bordering avenues and roads and could be considered the most relevant pollen sources for these types (Ianovici *et al,* 2013b; Ianovici, 2015).

Airborne pollen was monitored throughout the year. Airborne pollen were monitored using Lanzoni pollen trap. The sampling airflow rate was 10 l/min. Pollen was caught on a 24 mm wide transparent tape coated by a thin film of silicon oil. The tape was mounted on a cylinder rotating at a speed of 2 mm per hour. A complete rotation of the cylinder took seven days. Weekly tape strips were cut into 7 pieces, each 48 mm in length. Each piece corresponded to one day sampling. They were then mounted and stained in glycerine jelly mixed with basic fuchsine. Identification of the pollen was done by light microscopy, at a magnification of x 400. Pollen concentration was expressed as the daily average of pollen grains per cubic meter of air. A small fraction, of airborne pollen flora remained unidentified. Due to the difficulty on distinguishing some pollen of species belonging to the same family, because of its similar morphology, it is common to aggregate them by the family names (Sousa *et al,* 2010). Qualitative and quantitative analyses carried out using standard protocol in association with the comparison of reference slides (fig 1-20) and consulting the published literatures. To obtain the APS (atmospheric pollen season), 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).

All statistical tests were performed using the software package SPPS. The multiple regression analysis was performed in order to determine how much of total variance in pollen counts can be explained by meteorological variables. A total of nine meteorological factors were selected for this investigation (table 1).

TABLE 1. 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)	1008.4	-2.4	80	-1.6	4	7	2.1	4	2
March	1001.1	6.7	67	7.2	4.4	7.3	2.3	4.4	3.2
April	1001.8	14.71	56	17.9	9.1	4.1	1.6	4	3.2
May	1006.6	18.1	61	23.3	7.4	5.2	1.9	4	4.1
June	1002.3	20.3	72	23.9	7.3	6	1.8	4	8.8
July	1003.7	23.1	67	27.8	9.1	3.6	1.7	3	4.5
August	1005.9	22.7	68	27.2	8.2	4.1	1.6	3	3.2
September	1007.6	19	67	22	7	4.4	1.5	3	0.6
October (1-10)	1005.8	16	77	18	6.3	4.7	1.6	3	4.1

RESULTS AND DISCUSSIONS

A total of 23 pollen taxa, of these 16 taxa arboreal and 7 non-arboreal, were identified during 2009. The number of pollens in the atmosphere from herbaceous taxa was highest during summer and early fall (June to October) whereas pollens from woody taxa dominated during spring (February to May). Types representing arboreal pollen were reached the highest values during April. A maximum concentration of non-arboreal taxa was recorded in August. The richness of the pollen types varied throughout the year. The maximum number of pollen types was registered in April (18 types).

The twenty pollen taxa were then subjected to analyses. The pollen of these plants cause the majority of pollinosis in Europe. We noticed the abundance of the pollen coming from *Ambrosia artemisiifolia* which is adventives in our country's flora. Multiple regression analysis was performed in order to identify the major variables likely to influence the dynamic of the airpollen. Multiple linear regression is a commonly used method in environmental sciences. The aim was not to develop predictive model, but rather to give information on types of environmental factors that might be controlling dispersion of each pollen species. Table 2 summarizes the descriptive statistics and analysis results. Only the highest values of the statistically significant regression coefficients were selected. Among the meteorological parameters analysed, daily average relative humidity was the one that most influenced the pollen airborne concentrations. Mean daily average temperature and near-surface soil temperature also showed to have some influence on the pollen dispersion. For Urticaceae, meteorological parameters explained 43.25% of the total variance. The most important agents influencing the Urticaceae pollen count (in multiple regression) are atmospheric pressure + daily average relative humidity + near-surface soil temperature + sunshine hours + daily average wind speed. Pollen concentrations of species examined showed significant associations with mean daily average temperature *(Populus, Juglans, Pinaceae, Poaceae, Ambrosia, Plantago),* daily average relative humidity *(Corylus, Taxaceae/Cupressaceae, Betula, Carpinus, Salix, Ulmus, Fraxinus, Populus, Juglans, Quercus, Pinaceae, Poaceae, Urticaceae, Plantago),* near-surface soil temperature *(Alnus, Corylus, Salix, Ulmus, Fraxinus, Populus, Poaceae, Urticaceae, Plantago),* sunshine hours *(Tilia, Urticaceae),* nebulosity *(Carpinus, Pinaceae),* atmospheric pressure *(Taxaceae/Cupressaceae, Ulmus, Urticaceae),* daily average wind speed *(Betula, Carpinus, Ulmus, Fraxinus, Urticaceae),* daily maximum wind speed *(Alnus, Carpinus, Ulmus, Fraxinus)* and quantities of precipitations *(Tilia).* Average wind speeds were found to be more important than maximum wind speeds. Amounts of rain were not found to be significant factor. *Artemisia, Rumex* and Chenopodiaceae/Amaranthaceae were found not to be associated with weather variables.

In multiple regression analysis, significant associations between the pollen counts and daily average relative humidity, mean daily average temperature and near-surface soil temperature were noted. The multiple regression showed strong associations with daily average relative humidity for 14 taxa, while for 9 taxa a association was noted with the near-surface soil temperature. The positive association between pollen counts and the maximum wind speed indicates that several pollen types present in the air above Timisoara can be the result of long-distance transport.The other values here are the regression coefficients. More interesting for our understanding and interpretation are the unstandardized coefficients (B). Unstandardized coefficients indicate how much the dependent variable (pollen concentration) varies with an independent variable when all other independent variables are held constant. These indicates that for every unit increase in explanatory variables (apart from daily max. wind speed), the model predicts a decrease in pollen concentrations for *Alnus, Corylus,* Taxaceae/Cupressaceae, *Betula, Carpinus, Salix,* *Ulmus, Fraxinus, Populus, Juglans, Quercus*. For the pollen types who appear in the summer season-early fall season (Pinaceae, *Tilia,* Poaceae, *Ambrosia*, Urticaceae, *Plantago*), significant effects of the variables are mostly positive (increasing concentrations). Looking at the p-value of the t-test for each predictor, we can see that each weather variables contributes to the model, but not equally and not on the same pollen types. The most important factors statistically significantly different to 0 were not the same for each pollen type. The results indicate that mean daily average temperature is a powerful predictor of *Ambrosia* pollen. Daily average wind speed make a significant contribution to the explanation of variance in Urticaceae pollen concentrations. Taking into account regression results, the fluctuations of concentrations pollen types were better explained in some cases (Urticaceae*, Fraxinus, Ulmus*, Poaceae).

First we analysed the effect of meteorological factors on pollen concentrations, taking the season as a whole, on the days of measurement in the air. When we restricted the analysis only during the atmospheric pollen season, we find the changes of proportions that explain the variance. The spread of some pollen types is it seems more dependent on meteorological factors (*Alnus,* Taxaceae/Cupressaceae, *Salix, Populus, Ulmus, Fraxinus, Juglans, Quercus,* Pinaceae). In general, the present in the atmosphere (during atmospheric pollen season) for nonarboreal pollen is less associated with meteorological factors. The variance of concentrations of some arboreal pollen types also seems less explained by the weather during atmospheric pollen season (*Corylus, Betula, Carpinus, Tilia*). The resulting regression analysis is only an approximate indication of which variables are useful for predicting pollen counts (Makra *et al,* 2004). Wind pollination is much less precise than biotic pollination (Gomez-Casero *et al,* 2004).

The seasonal cycle of plants is influenced to the greatest extent by temperature, photoperiod and water availability (Wolkovich *et al,* 2014). Timing developmental stages to coincide with favourable seasonal conditions is critical for plant growth, reproduction and survival. Responses of plant assume cellular, metabolic, morphological or developmental changes that require time to complete (Wilczek *et al,* 2010). Flowering is the best characterized of seasonal responses. The release and dispersion of pollen are very important stages in the reproduction of most anemophilous plants (Ianovici et al, 2013a). Weather parameters are known to affect the dispersion dynamics of pollen. Plant uses of several indicators of season and thus resource availability, of which light and temperature are usually most important in temperate plant species (Wilczek *et al,* 2010). Different plants have different temperature requirements. Growth and development rates typically increase with ambient temperatures up to some optimum or maximum, and then decline as warming continues. In temperate environments, optimum ambient temperatures for growth are rarely exceeded (Schaber & Badeck, 2002). Ambient temperature is a seasonal indicator in temperate climates, following patterns of day length and insolation (Wilczek *et al,* 2010). In particular spring development in the mid latitudes depends especially on temperature in winter and spring (Cook *et al*, 2012). Regression analysis showed that rainfall and maximum temperature were the most important variables in Mediterranean areas, while both maximum and minimum temperatures were the most weighty meteorological parameters in Eurosiberian areas (García-Mozo *et al,* 2006).

Light quantity contributes to plant growth and development. The day length (also called photoperiod) can also serve as an important developmental indicator. Photoperiod is defined as the period of sunshine hours necessary for flowering. Thus, sunshine hours are correlated directly with the photoperiod (Vazquez *et al*, 2003). Increasing day duration indicates the arrival of spring. Declining photoperiods are a indicator of the end of the growing season (Bohlenius *et al*, 2006; Savolainen *et al,* 2007). Day length can also serve as an powerful indication for the appropriate timing of flowering and fruiting with respect to seasonal patterns of temperature and precipitation (Wilczek *et al*, 2010).

Plant water requirements differ according to taxa and soil types. Water availability may determine the duration of the growing season and thus can have important effects on the flowering phenology (Franks *et al,* 2007). In our study, while in some cases, daily pollen counts show significant negative associations with average relative humidity and precipitation, in other cases no associations were found.

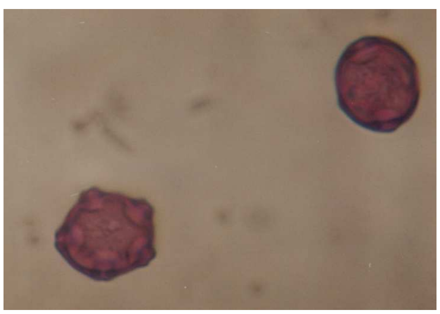

FIG.1. ***Alnus*** **type**

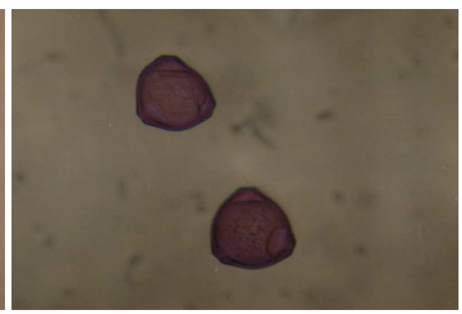

FIG.2. ***Corylus*** **type**

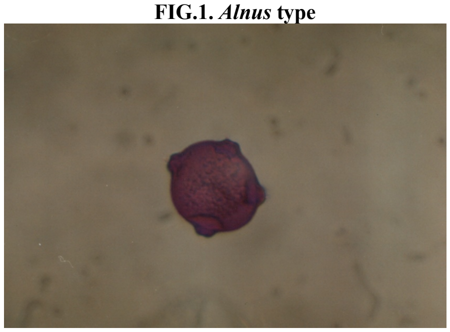

FIG.3. ***Carpinus*** **type**

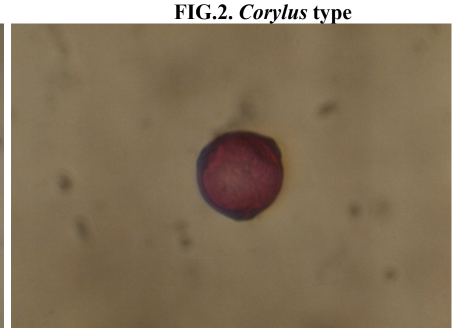

FIG.4. ***Betula*** **type**

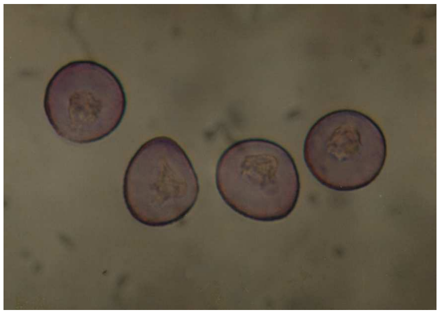

FIG.5. Taxaceae/Cupressaceae type

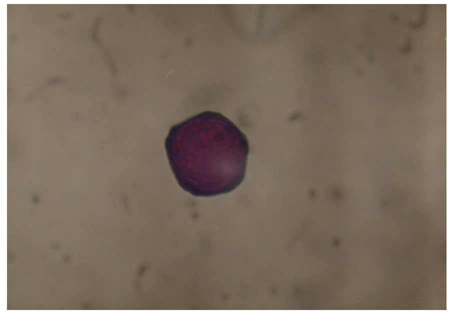

FIG.6. *Ulmus* type

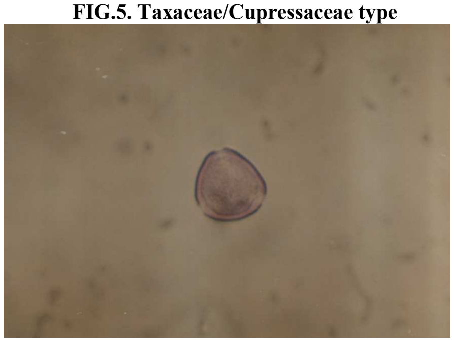

FIG.7. *Fraxinus* type

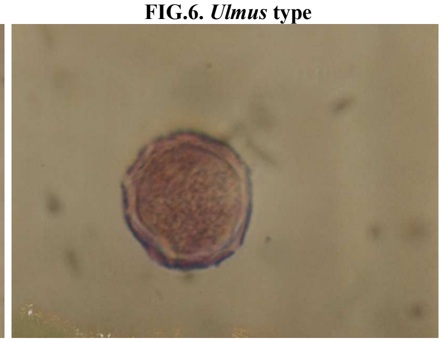

FIG.8. *Populus* type

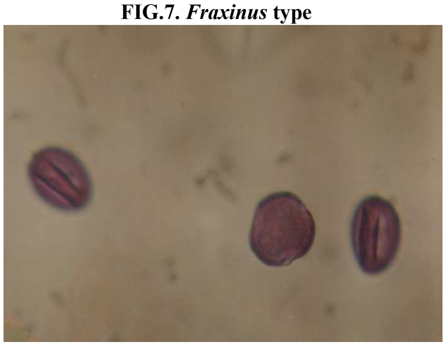

FIG.9. *Salix* type

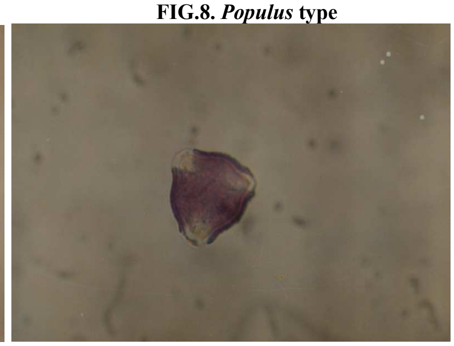

FIG.10. *Quercus* type

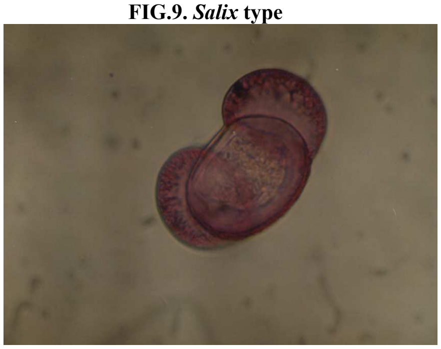

FIG.11. Pinaceae type

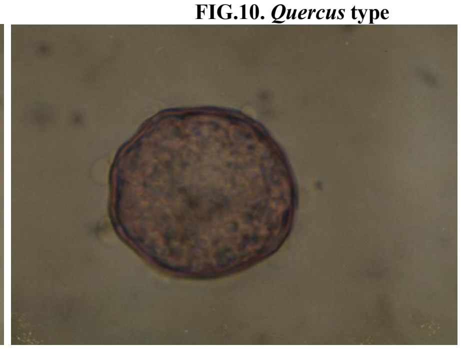

FIG.12. *Juglans* type

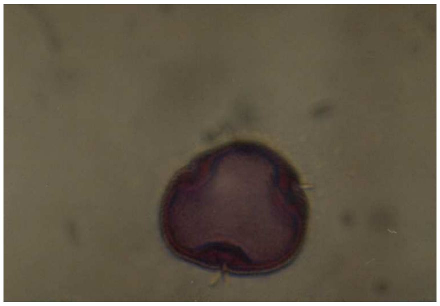

FIG.13. ***Tilia*** **type**

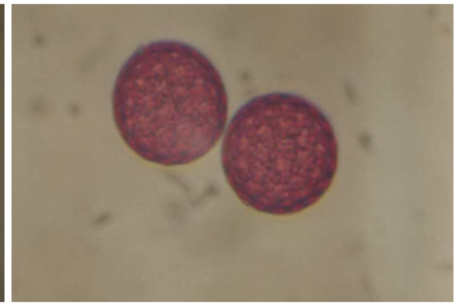

FIG.14. Chenopodiaceae/Amaranthaceae type

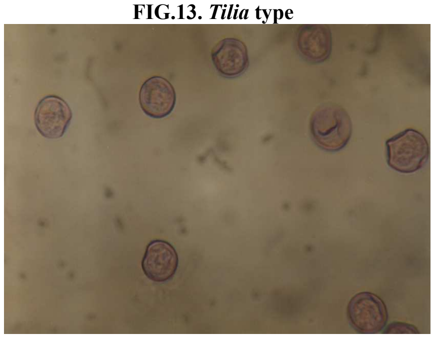

FIG.15. Urticaceae type

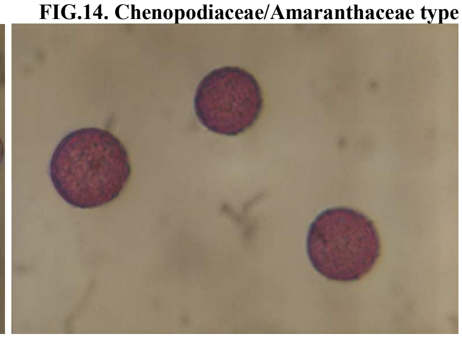

FIG.16. ***Plantago*** **type**

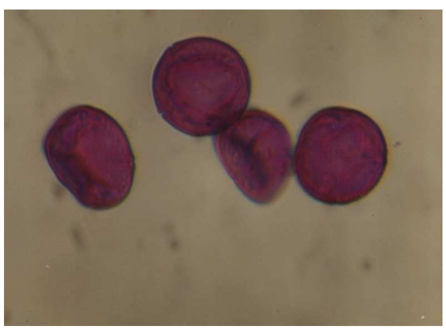

FIG.17. ***Rumex*** **type**

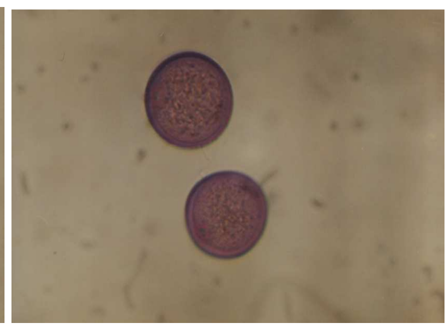

FIG.18. Poaceae type

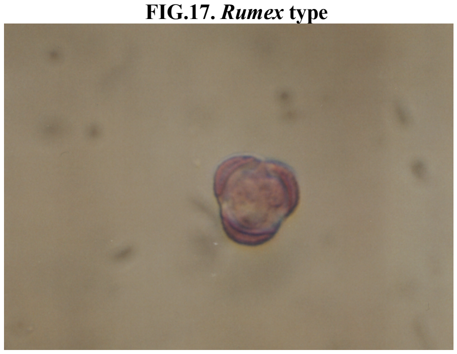

FIG.19. ***Artemisia*** **type**

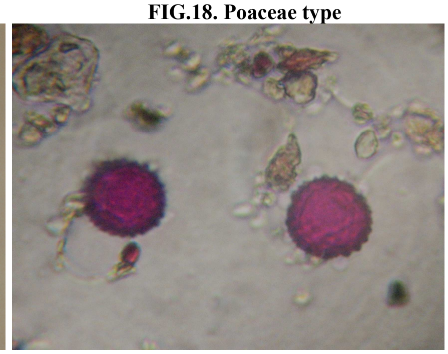

FIG.20. ***Ambrosia*** **type**

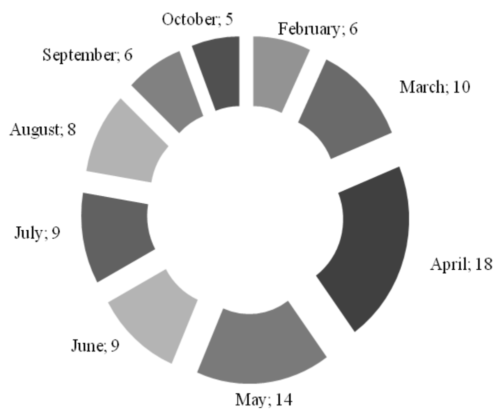

FIG. 21. Pollen richness (R) as number of pollen types for month

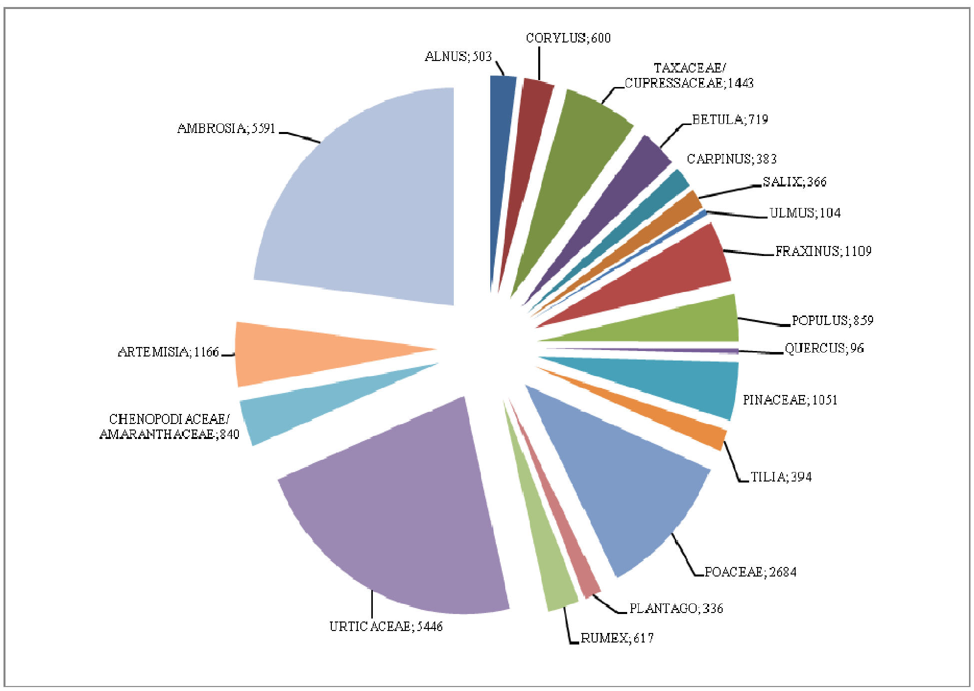

FIG .22. Annual pollen count values during 2009

TABLE 2. Summary statistics and results from the regression analysis

Taxa	proportion of variance explained	explanatory variables	value of regression coefficient (B)	t-ratio	prob(t)
Alnus	22.88%	near-surface soil temperature	-0.4975	-2.43761	0.01555
		daily max. wind speed	-0.71276	-1.99176	0.0476
Corylus	16.63%	daily average relative humidity	-0.18429	-3.16951	0.00174
		near-surface soil temperature	-0.83835	-2.98059	0.00319
Taxaceae/Cupressaceae	28.05%	atmospheric pressure	-0.77126	-3.40121	0.00079
		daily average relative humidity	-0.37049	-3.12436	0.00201
Betula	16.47%	daily average relative humidity	-0.36792	-5.4005	0
		daily average wind speed	-2.85886	-2.80399	0.00548
Carpinus	19.41%	daily average relative humidity	-0.17068	-4.22065	0.00004
		nebulosity	-0.69661	-3.1448	0.00188
		daily average wind speed	-1.59926	-2.64252	0.0088
		daily max. wind speed	1.039688	3.03119	0.00272
Salix	10.26%	daily average relative humidity	-0.15065	-3.21021	0.00152
		near-surface soil temperature	-0.50227	-2.21263	0.02792
Ulmus	30.61%	atmospheric pressure	-0.13189	-6.31817	0
		daily average relative humidity	-3.90E-02	-3.57441	0.00043
		near-surface soil temperature	-0.11422	-2.16302	0.03158
		daily average wind speed	-0.49077	-3.00405	0.00296
		daily max. wind speed	0.1935	2.089882	0.03774
Fraxinus	33.71%	daily average relative humidity	-0.50108	-6.81458	0
		near-surface soil temperature	-0.9157	-2.57443	0.01068
		daily average wind speed	-3.00148	-2.72754	0.00688
		daily max. wind speed	1.231935	1.975308	0.04944
Populus	13.00%	daily average relative humidity	-0.24116	-2.6231	0.0093
		near-surface soil temperature	-1.1564	-2.60019	0.00993
Juglans	10.86%	mean daily average temperature	-0.37948	-2.13803	0.03358
		daily average relative humidity	-0.12315	-3.9251	0.00011
Quercus	8.93%	daily average relative humidity	-0.04494	-3.44555	0.00068
Pinaceae	21.20%	mean daily average temperature	-1.2063	-2.73218	0.00679
		daily average relative humidity	-0.44573	-5.71087	0
		near-surface soil temperature	1.152749	3.053199	0.00253
		nebulosity	1.294771	3.028513	0.00274
Tilia	16.79%	sunshine hours	0.525194	2.379101	0.01818
		quantities of precipitations	0.476893	4.463034	0.00001
Poaceae	28.56%	mean daily average temperature	-1.30763	-2.2035	0.02856
		daily average relative humidity	0.311549	2.969821	0.0033
		near-surface soil temperature	2.029127	3.998548	0.00009
Ambrosia	14.75%	mean daily average temperature	6.235735	2.817827	0.00526
Urticaceae	43.25%	atmospheric pressure	-0.9533	-2.59309	0.01013
		daily average relative humidity	1.110957	5.778806	0
		near-surface soil temperature	1.941829	2.088051	0.03791
		sunshine hours	3.255665	3.388248	0.00083
		daily average wind speed	6.113398	2.124824	0.03468
Plantago	17.96%	mean daily average temperature	-0.23643	-2.0174	0.04483
		daily average relative humidity	4.54E-02	2.192906	0.02933
		near-surface soil temperature	0.300639	2.999907	0.003
Rumex	7.81%	-	-	-	-
Artemisia	15.35%	-	-	-	-
Chenopodiaceae/Amaranthaceae	15.00%	-	-	-	-

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TABLE 3. Summary statistics and results from the regression analysis (during APS)

Taxa	proportion of variance explained (during APS)	explanatory variables	value of regression coefficient (B)	t-ratio	prob(t)
Alnus	67.17%	quantities of precipitations	1,616	2,604	0,035
Salix	31.46%	atmospheric pressure	2,711	2,183	0,043
Ulmus	71.10%	mean daily average temperature	-1,658	-2,494	0,019
		near-surface soil temperature	1,635	2,495	0,019
		daily average wind speed	-1,598	-2,643	0,013
		daily max. wind speed	1,234	5,281	0,00001
		quantities of precipitations	0,556	3,231	0,00314
Fraxinus	48.55%	mean daily average temperature	6,966	2,089	0,0453
		daily average relative humidity	-1,132	-3,264	0,00274
		near-surface soil temperature	-7,086	-2,240	0,03265
Rumex	23.52%	mean daily average temperature	-1,977	-2,624	0,011
		daily max. wind speed	2,787	2,256	0,027
Poaceae	21.57%	atmospheric pressure	-1,001	-2,217	0,028
		quantities of precipitations	1,074	3,179	0,002
Ambrosia	20.15%	daily average relative humidity	-2,907	-2,060	0,044
Artemisia	13.40%	atmospheric pressure	1,020	2,339	0,021
Urticaceae	20.89%	mean daily average temperature	4,681	2,142	0,035
		daily average relative humidity	1,074	2,175	0,032
		sunshine hours	3,920	2,097	0,039
Corylus	16.97%	-	-	-	-
Taxaceae/Cupressaceae	47.06%	-	-	-	-
Betula	18.67%	-	-	-	-
Carpinus	26.61%	-	-	-	-
Populus	20.86%	-	-	-	-
Quercus	75.47%	-	-	-	-
Juglans	54.01%	-	-	-	-
Pinaceae	61.31%	-	-	-	-
Plantago	3.70%	-	-	-	-
Tilia	17.46%	-	-	-	-
Chenopodiaceae/Amaranthaceae	10.35%	-	-	-	-

According to the literature and aerobiological and ecological data, temperature and average relative humidity are the environmental factors which most strongly affects the generative and vegetative development and the occurrence of pollen in air (Kasprzyk, 2008). In our opinion, multiple regression analysis reveals a more nuanced effects of meteorological factors. It has been observed that there is not a clear relationship between the amount of pollen collected in the air and the meteorological variables. In fact, meteorological factors plays an ambivalent role, partly having a positive impact by increasing pollen shed from the anthers, partly a negative association by diluting pollen from the air. On the other hand, the plants species behave differently: in some cases the concentrations of pollen in the air are dependent on weather parameters, and in other cases less dependent.

Allergy to the pollen of anemophilous plants has significantly impacts on the health of people in many parts of the world. Determining the amount of each pollen type in the air is a mean for evaluating a risk of exposure. In our study, not all 20 pollen types are equally allergenic. For example, the relative new allergenic pollen (*Ambrosia artemisiifolia*) demand some time for organization of tests and therapeutic solutions. Identifying, quantifying and biomonitoring of allergenic pollen may contribute to: targeting specific preventive measures, assessing the role played by the allergens in sensitization, directing immediate and future therapeutic plans, directing production of allergenic extracts and vaccines according to the presence of allergophytes in a certain area, producing plant pollen calendars which show the dynamics of pollen and anemophilous plants, the management of parks and green areas (Ianovici, 2012).

CONCLUSIONS

The objective of this study was to examine the relationships between environmental factors and pollen concentrations. The relationships between pollen concentration and meteorological variables were assessed using multiple linear regressions. Some pollen types recorded during this study could be involved in pollinosis. The best associations were found between daily values of concentrations and two meteorological variables (temperature and relative humidity). When making comparisons, it is important to specify exactly which data are analyzed. The relevance of certain meteorological factors may increase or decrease by comparing pollen concentrations recorded during an entire year or during atmospheric pollen season.This is a preliminary study and effects of meteorological variables on pollen counts could not be clearly identified with the evaluation of one-year data yet. On the other hand, the contribution of this paper lies in its potential informations to public health surveillance and clinical guidance in our area.

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