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Comparative Analysis of some Vernal Pollen Concentrations in Timisoara (Romania), Szeged (Hungary), Novi Sad (Serbia) and Ljubljana (Slovenia)

Nicoleta IANOVICI[1)], Miklos JUHASZ[2)], Andrea KOFOL-SELIGER[3)], Branko SIKOPARIJA[4)]

[1)] *West University of Timisoara, Faculty of Chemistry-Biology-Geography, Romania; nicoleta.ianovici@gmail.com*

[2)] *University of Szeged, Department of Botany, Hungary; juhaszm@bio.u-szeged.hu*

[3)] *Institute of Public Health of the Republic of Slovenia; andreja.seliger@ivz-rs*

[4)] *University of Novi Sad, Faculty of Sciences, Laboratory of Palynology, Insitute of Biology; sikoparijabranko@yahoo.co.uk*

Abstract

The aim of the study was to compare the airborne concentrations of pollen produced by vernal flowering trees taxa (*Alnus, Betula, Carpinus, Corylus, Fraxinus, Platanus, Populus, Quercus, Taxaceae/Cupressaceae*) in the cities of Timisoara (Romania), Szeged (Hungary), Novi Sad (Serbia) and Ljubljana (Slovenia) during the years 2006–2008. Annual variations in the concentration of pollen in the atmosphere were analysed by the volumetric method. In these cities, the period with the greatest diversity of pollen types is spring. These trees are found in mixed forests and are used in urban landscaping and home gardens. Inter-annual differences can be seen in the seasonal behaviour of the pollen in Novi Sad, 2008 being the year in which the highest levels of airborne pollen were reached. During the 3-year period, pollen of the representatives of the family *Betulaceae* accounted for a significant proportion of total pollen, predominated by *Betula* pollen and a considerably lower proportion of *Alnus*, *Carpinus* and *Corylus* airpollen. *Taxaceae/Cupressaceae* pollen appears in the atmospheric pollen spectra of all localities in high concentrations. These pollen grains are the main source of allergens in springtime. Results of the study reveal important differences between the cities.

Keywords: airpollen grains (PG), allergenic potential, anemophilous trees

Introduction

Phytoallergens include taxa of various botanical families. According to their migration they are classified as anemophilous or entomophilous allergens. Anemophilous species often causing allergic diseases produce a huge quantity of readily airborne pollen grains. They gently dispersed in the air and are carried a long distances (Bartkova-Ščevkova, 2003). Monitoring of the pollen counts in the aeroplankton of cities is of relevant medical importance. The number of people allergic to plant aeroallergens has substantially increased in big cities and industrial areas (Nilsson and Persson, 1981). In each geographical region, there is a succession of different flowering species throughout the year, following a rhythm mainly established by meteorological conditions. Such conditions influence both the ontogeny of pollen grains and their dispersal. The relief and height of relatively close areas can produce local microclimates, which explain the differences observed regarding the onset of flowering within the same species (Rodriguez-Rajo *et al.,* 2003). The aim of the study was to determine the periods of occurrence of pollen grains of nine selected taxa in the atmosphere of four European cities.

Materials and methods

In all the stations (Timisoara, Szeged, Novi Sad and Ljubljana), Hirst volumetric spore traps were used. They are located on buildings approximately 15 m above ground level. These traps have an autonomy of 7 days and collect pollen continuously with a given absorption flux, enabling daily and even hourly pollen concentration data to be obtained. The tape was divided in 7 segments, each one of 48 mm in length, corresponding to each sampling day, and adhered to standard glass slides covered with gelatin-glycerin and stained with basic fuchsin. In each glass slide, 4 longitudinal scans were made under the optical microscope using a 400× objective lens. 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. For taxa identification the works of Faegri and Iversen (1989) and Smith (1990) were used. Daily mean data are expressed in terms of pollen grains per cubic metre of air (PG/m^3) (Mandrioli *et al.*, 1998). To calculate trends, a linear regression has been performed: the annual cumulative pollen count of 3 years of records for each taxa was correlated with the increase of time (Tab. 1.).

Szeged is situated 79 m meters above sea level (46° 15′ 18″ N, 20° 8′42″E). The town is located in the south-east of Hungary, in the center of the Carpathian Basin, along the banks of Tisza River. The study area has continental climate with a long warm season and has a certain Mediterranean influence. The main average meteorological parameters of the Szeged region are as follows: mean annual temperature 11.2°C, mean January and July temperatures -1.2°C and 22.4°C respectively, mean annual precipitation 573 mm.

Novi Sad is situated 80 meters above sea level (45°15′N 19° 51″ E). Novi Sad is located on the border of the Bačka and Srem regions, on the banks of the Danube river and Danube-Tisa-Danube Canal, while facing the northern slopes of Fruška Gora mountain. Novi Sad is capital administrative center of the Province Vojvodina, Republic of Serbia. Climate in the area of Novi Sad is moderate-continental. In January the average is -1°C, while in July it is 21.6°C. The annual rainfall is 686 mm.

Timisoara is situated in the western region of Romania at 88 meters above sea level (45°45′35″ N 21°13′ 48″ E). River Bega passes through Timisoara. The climate, which defines Timisoara city, is temperate continental moderate, which characterizes the Southern-Eastern part of the Panonic Field. The average annual temperature is of 10.6°C while the hottest month of the year is July (22.42°C). Thus the average thermic amplitude of 22.7°C is lower than that of the Romanian Plain which testifies the beneficial influence of oceanic air masses. The annual rainfall is 592 mm.

Ljubljana, the capital of Slovenia, is situated 299 m above see level (46° 3′ 5″ N, 14° 30′ 20″ E), on a natural passage called the Ljubljana Gate, connecting Central Europe and Mediterranean, on the Ljubljanica river. The city's climate is the continental type. Ljubljana has a climate influenced by its situation in a large basin, surrounded by sub-Alpine and Karst regions. Summers are warmed by the balmy breezes from the Mediterranean, making the weather usually sunny and hot. During winter temperature inversions cause the foggy air to hang heavy over the city, bringing cold, moist conditions. The mean annual air temperature is 10.2°C, the mean temperature for July is 20.4 and for January it is -0.2°C. The annual rainfall is 1368 mm.

Results and discussion

Flora in our study areas is relatively diverse. New taxa have been introduced which could have allergenic potential. The pollen seasons show three main parts: vernal season (January–April), early summer season (May–July), late summer - autumn season (July–October). Pollen grains of trees were determined during the vernal pollen season in the atmosphere of our cities: *Alnus*, *Betula*, *Carpinus*, *Corylus, Fraxinus*, *Platanus*, *Populus*, *Quercus*, *Taxaceae/ Cupressaceae, Salix, Acer, Ulmus, Morus, Juglans.* Spring allergies are a result of pollen from trees, which can start pollinating from January to May. *Alnus* (alder), *Betula* (birch), *Carpinus* (hornbeam), *Corylus (hazelnut), Fraxinus* (ash), *Platanus* (plane tree), *Populus* (poplar), *Quercus* (oak), *Taxaceae/Cupressaceae* (yew and cypress family) are very abundant anemophilous plants in the metropolitan area of Timisoara, Szeged, Novi Sad and Ljubljana, frequently used as ornate plants in gardens and public parks, streets, avenues, sidewalks and private gardens. At all the sites during the three years of investigation, these taxa were recognised. The analysis of pollen grain concentrations was conducted in these cities based on data from the years 2006-2008. According to previous studies it has been recognized that the species of these genera are etiological agents of pollinosis in diverse places all over the world (Hemmer *et al.*, 2000; Laurent *et al.*, 1998; Peeters, 2000; Rocha-Estrada *et al.*, 2008). The urban landscape and its surroundings consist of ruderal vegetation, forests, semi-natural community of grasses and antropomorphic habitats. Natural conditions, especially the composition of the tree flora, are mirrored in the quantitative and qualitative composition of pollen fall (Kasprzyk, 1999). Trees belong to the main pollen producers because of their common incidence and large pollen production per anther, inflorescence or individual tree. This pollen can be transported with air-masses over large areas (Molina *et al.*, 1996). Quantitative differences in annual totals of airborne vernal trees concentrations in Ljubljana between the years are relatively small. The maximum value of these trees was recorded in Ljubljana in 2006 with 18832 PG compared to 17270 PG and 16109 PG in 2007 and 2008 respectively. The results of pollen count for all years are presented in Fig. 1.

In Novi Sad the annual values of pollen vernal trees are the highest than other three areas during this study (Fig. 2.). The maximum value of these trees was recorded in Novi Sad in 2008 with 41756 PG compared to 17822 PG and 33279 PG in 2007 and 2006 respectively. The best-represented pollen type through the entire period was *Taxaceae/Cupressaceae* in 2006 with 18146 PG.

Since 2006, pollen concentrations in Szeged have been much higher (Fig. 3.), probably due to the increase in the number of trees planted in the city, although some differences between years could also be due to meteorological factors. The maximum value of this trees was recorded in Szeged in 2008 with 12947 PG compared to 9016 PG and 4320 PG in 2007 and 2006 respectively. The best-represented pollen type through the entire period was *Populus* in 2008 with 6192 PG.

In Timisoara the annual values of pollen vernal trees are a lot lower than those of cities such as Ljubljana and Novi Sad (Fig. 4.). These differences are evidently related to the greater abundance of trees in the parks and streets. The maximum value of this trees was recorded in Timisoara in 2007 with 7679 PG compared to 7286 PG and 4285 PG in 2008 and 2006 respectively. The characteristic vegetation of Timisoara is that of anthropogene forest steppe which also defines the whole Panonic Field.

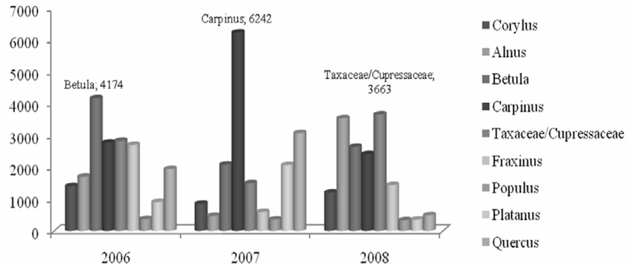

Fig. 1. Concentrations of vernal airpollen grains (PG) measured in Ljubljana, 2006-2008

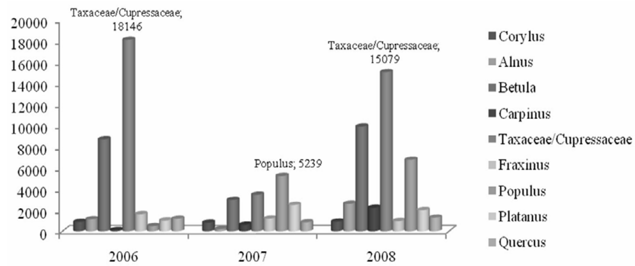

Fig. 2. Concentrations of vernal airpollen grains (PG) measured in Novi Sad, 2006-2008

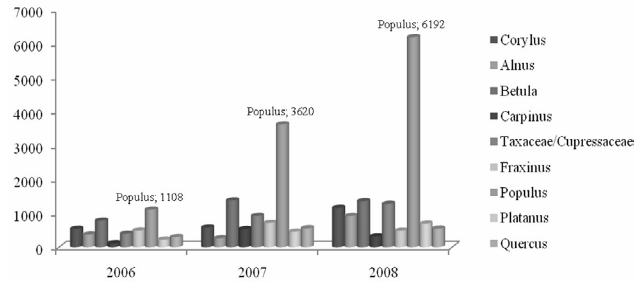

Fig. 3. Concentrations of vernal airpollen grains (PG) measured in Szeged, 2006-2008

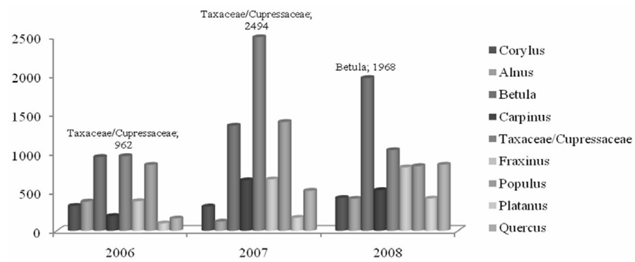

Fig. 3. Concentrations of vernal airpollen grains (PG) measured in Timisoara, 2006-2008

In the atmosphere of the city of Ljubljana the annual sums of Corylus were rather variable in the studied period. The mean value was 1157 PG with the highest total of 1410 in 2006 and the lowest total of 854 in 2007. The comparison of mean annual totals of *Corylus* grain pollen demonstrates that in Ljubljana were three as high than in Timisoara. We observed an alternation in the total annual pollen of *Corylus* in Novi Sad, Timisoara and Ljubljana. In Timisoara, the hazel pollen counts were very low. Increasing trend in the annual totals is observed at Szeged station. The *Corylus* pollen grains (20-25 x 26-28 μm) gives allergic reaction even at very low concentrations of 20–30 PG/m3/24 h (Weryszko-Chmielewska *et al.*, 2001).

In all sites the concentration of Alnus pollen in 2007 was low. The comparison of annual totals of Alnus grain pollen demonstrates that in Ljubljana they were twice as high. The mean annual totals of the concentrations of airborne alder pollen are 1907 PG. In Ljubljana, the lowest amount of Alnus pollen recorded in a season was 473 grains in 2007, and the highest was 3543 grains in 2008. In Timisoara and Szeged, the alder pollen counts were very low compared to those in Novi Sad and Ljubljana. These differences may be due to a different composition of local flora and the influence of weather. A greater number of pollen grains were collected in Novi Sad, especially in 2008 (2604 PG). The pollen count of Alnus was higher in 2008 than in 2006 and 2007. In 2008, the pollen count of Alnus in all the cities was from 3.5–12 times higher than in 2007. The behaviour of Alnus was irregular, with pollen concentrations fluctuating considerably between the years 2006-2008. Despite the limited number of years included in this study, we observed an alternation in the total annual pollen of *Alnus*. This behaviour applies to different tree species and is explained by alternation in the mobilisation of nutrient reserves towards the vegetative growth of the tree or towards the reproductive structures, according to the year (Rodriguez-Rajo *et al.,* 2003). The *Alnus* pollen grains (19-21 x 23-30 μm) gives allergic reaction even at low concentrations of 50 PG/m^3/24 h.

Birch is planted particularly frequently on account of its fast growth and its ability to utilize a broad range of habitats, which create possibilities for restoration of degraded soils. The highest annual pollen count was measured in 2008 for Novi Sad site (9921PG).The mean annual totals of the concentrations of airborne birch pollen are higher at Novi Sad and Ljubljana than at Szeged and Timisoara. The lowest number of *Betula* pollen grains recorded in Szeged was similar to that of Timisoara. This difference is due to the fact that there are many more birch trees in the direct environments of the pollen traps at Novi Sad and Ljubljana than at the other stations. We observed an alternation in the total annual pollen of *Betula* in Novi Sad and Ljubljana. Increasing trend in the annual totals is observed at Timisoara station. In central and northern Europe, where *Betula* pollen (18-23 x 21-30 μm in size) is rated one of the most important allergenic pollen types, an estimated 10-20% of the population are allergic to birch pollen (Smith *et al.*, 2007). Clinical symptoms can appear at the pollen grain concentration of about 80 PG/m^3/24 h. Rapiejko *et al.* (2004) reported a threshold value for birch pollen of 20 PG/m^3 in Poland. The number of pollen grains per inflorescence was estimated by Erdtman (1954) as 6×10^6. *Betula* pollen is one of the main European aeroallergens, triggering symptoms of asthma and seasonal rhinitis. It has been also shown that there is a cross reactivity of *Betula* pollen with *Corylus* and *Alnus*

Tab. 1. Trend in vernal pollen concentrations (2006-2008)

Pollen type	Szeged	Novi Sad	Timisoara	Ljubljana
Corylus	y = 310x + 136,6 R^2 = 0,797	y = 13x + 836 R^2 = 0,071	y = 52x + 248 R^2 = 0,692	y = -101,5x + 1360 R^2 = 0,130
Alnus	y = 273x - 28,33 R^2 = 0,601	y = 736,5x - 158 R^2 = 0,372	y = 18,5x + 265,3 R^2 = 0,013	y = 919x + 69 R^2 = 0,353
Betula	y = 285,5x + 601,3 R^2 = 0,719	y = 606,5x + 5984 R^2 = 0,026	y = 508,5x + 407,3 R^2 = 0,985	y = -765x + 4498 R^2 = 0,500
Carpinus	y = 102x + 116,3 R^2 = 0,238	y = 1052,x - 1118 R^2 = 0,916	y = 167x + 121 R^2 = 0,491	y = -179x + 4172 R^2 = 0,007
Taxaceae/Cupressaceae	y = 440x - 15,33 R^2 = 0,988	y = -1533,x + 15294 R^2 = 0,039	y = 38,5x + 1421 R^2 = 0,002	y = 414,5x + 1837 R^2 = 0,144
Fraxinus	y = -1,5x + 568 R^2 = 0,000	y = -325x + 1909 R^2 = 0,97	y = 217,5x + 185 R^2 = 0,971	y = -629x + 2841 R^2 = 0,352
Populus	y = 2542x - 1444 R^2 = 1	y = 3143,x - 2120 R^2 = 0,919	y = -8x + 1044 R^2 = 0,000	y = -18x + 392 R^2 = 0,964
Platanus	y = 237,5x - 17,66 R^2 = 1	y = 493,5x + 850 R^2 = 0,430	y = 160x - 93 R^2 = 0,910	y = -276x + 1664 R^2 = 0,099
Quercus	y = 125x + 217 R^2 = 0,72	y = 51,5x + 998,6 R^2 = 0,049	y = 346,5x - 184 R^2 = 0,999	y = -726,5x + 3292 R^2 = 0,313

pollen, as well as with apple, hazelnut and peach (Radišić and Sikoparija, 2005).

Carpinus pollen grains (26-31 x 28- 41.5 μm) presented the shortest season and lowest concentrations in Szeged, Novi Sad and Timisoara. The highest values for *Carpinus* occurred in 2007 in Ljubljana (6242 PG). The 2006-2008 mean at Ljubljana (3814 PG) was twelve times higher than in Szeged and eight times higher than in Timisoara. The pollen count of *Carpinus* was higher in 2007 than in 2006 and 2008. The differences in the pollen counts of *Carpinus* taxa in the three seasons were also significant. In Timisoara, the *Carpinus* pollen counts were very low. Increasing trend in the annual totals is observed at Novi Sad station. In Geneva, 71.7% of the patients allergic to *Betulaceae* pollen were found to be sensitive to hornbeam, and 12.5% of the same group of patients were sensitive to hornbeam pollen only (Gumowski *et al.* , 2000).

In Novi Sad, *Taxaceae/Cupressaceae* shows a mean value of 12227.33, ranging from 18146 PG in 2006 and 3457 PG in 2007. In Szeged the mean values of the annual sums were 864.7, with the highest total of 1277 PG in 2008. The lowest annual total of 397 was reached in 2006. Increasing trends in the annual totals is observed at Szeged station. *Taxaceae/Cupressaceae* pollen accounts for a large proportion of the total pollen spectrum recorded in the atmosphere of Novi Sad. *Taxaceae/Cupressaceae* pollen has been cited by different authors as an important allergen in the Mediterranean region (Caiaffa *et al.*, 1988; Bousquet *et al.*, 1993; D'Amato and Liccardi, 1994; Nardi *et al.*, 1996). However, different aerobiological studies show that its presence is also very considerable outside that area (Hirotaka *et al.*, 1996; Galán *et al.*, 1998; Levetin, 1998) since different species of this family are frequently used for ornamental purposes in parks and gardens in many cities. Airborne pollen counts do not usually separate *Cupressaceae* pollen from that of *Taxaceae* and *Taxodiaceae*, because of their similar morphology (Galan *et al.*, 1998). After the experience of the Catalan research group, the pollen clearly causes allergic responses in people when concentrations reach level 4 (>100 PG/m^3/24 h) (Belmonte *et al.*, 1999). In last decades, *Cupressaceae* pollen has been identified as source of increasing pollinosis in Mediterranean countries such as France, Israel, Spain and Italy (D'Amato *et al.*, 2007). The grouping of *Taxus* and *Cupressaceae* in one type in the records has artificially augmented the importance of this group.

The minimum annual totals of *Fraxinus* were registered in Timisoara and Szeged. A greater number of pollen grains were collected in Ljubljana, especially in 2006 (2704 PG). Increasing trend in the annual totals is observed at Timisoara station. Quantitative differences in annual totals of airborne *Fraxinus* concentrations between the four monitoring stations are relatively small. Horak *et al.* (1980, cited by Peeters, 2000) consider for *Fraxinus* pollen the daily average of 167 PG/m^3 of air are a critical concentration concerning the appearance of allergic symptoms in humans. Hofman and Michalik (1998) reported 50 PG/m^3 for ash in Poland. Anemophily of the *Fraxinus* genus has been observed in France, Hungary, Argentine, Poland, Spain, Switzerland, Mexico and Austria (Jarai-Komlodi, 1991; Laurent *et al.*, 1998; Hemmer *et al.*, 2000; Peeters, 2000; Nitiu andMallo, 2002; Gattuso *et al.*, 2003; Rocha-Estrada *et al.*, 2008). Schmid-Grendelmeier *et al.* (1994) have suggested that in France and Switzerland the ash tree is a strongly allergenic species. The results obtained by Wahl *et al.* indicate the occurrence of the cross-

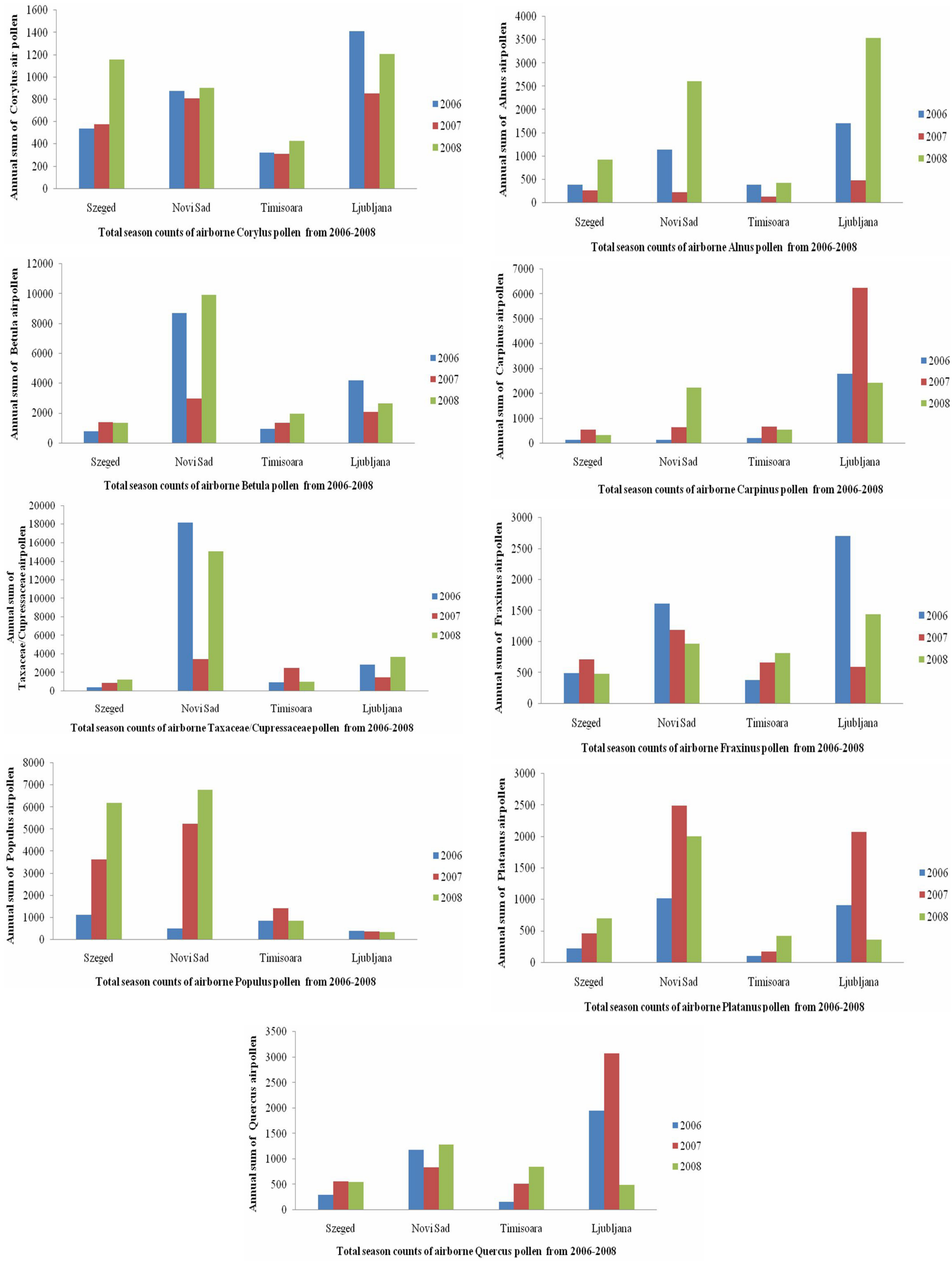

Fig.4. Annual values of airpollen types during the years studied

reactivity between birch and ash pollen allergens (Wahl *et al.*, 1996). The period of the presence of airborne ash tree pollen (April, May) coincides with the pollen season of birch trees. In spite of locally high concentrations, ash pollen rarely causes allergy symptoms in our regions.

Populus belongs to anemophilous trees and produces large amounts of airborne pollen. The poplar tree releases most of its pollen grains (30 × 36 µm) in the morning making use of the ascending convectional currents. Although the poplar tree pollen reaches high concentrations in the air it rarely produces allergic response. The cities of maximum pollen concentration were Szeged (2006 - 1108 PG) and Novi Sad (2007 – 5239 PG; 2008 – 6774 PG). The highest annual pollen count of *Populus* measured in 2008 in Novi Sad was fourteen times higher than in 2006. In Szeged the annual pollen counts of this taxon were also higher in 2008. Increasing trends in the annual totals are observed at Szeged and Novi Sad stations. The 2006-2008 mean at Ljubljana was 356 PG. These differences may be due to a different composition of local flora and the influence of weather.

Platanus is common in the ornamental flora of our cities and its pollen is present in the pollen spectra. This pollen type appears abruptly in high concentrations in the air (Alcázar *et al.*, 2004). In Novi Sad the mean value of the annual sums was 1837, with the highest total of 2492 in 2007 and the lowest of 1016 in 2006. The minimum annual totals of *Platanus* were registered in Timisoara and Szeged. The pollen count of *Platanus* was higher in 2007 than in 2006 and 2008. Increasing trends in the annual totals are observed at Szeged and Timisoara stations. Concentrations of airborne *Platanus* pollen never reach high values in Timisoara site. Most of the patients reported that symptoms usually began a few days after *Platanus* pollen concentrations had reached 50 PG/m^3 (Alcázar *et al.*, 2004). Many authors have considered that *Platanus* pollen (18 – 25 µm) has a moderate allergenic power. This pollen type has been detected as an important cause of pollinosis in cities such as Madrid (Varela *et al.,* 1997), Ourense (Arenas *et al.,* 1996) or Santiago de Compostela (Dopazo, 2001), where sensitisation rates of around 8–9% were reached. The majority of patients with *Platanus* pollen allergy symptoms were also sensitive to Poaceae and *Olea* (Alcázar *et al.*, 2004).

The *Quercus* species are anemophilous trees producing stenopalynus pollen grains (24-38 µm) in high quantities that are well dispersed through the air (Tormo *et al.*, 1996; Gómez-Casero *et al.*, 2004). In spring, tree pollen grains of the Fagales order which includes *Quercus* genus, are a main allergen source in the northern hemisphere, with cross-reactivity occurring between pollen within the same order (Matthiesen *et al.*, 1991; Mothes and Valenta, 2004). The mean annual pollen count obtained during the period studied in Ljubljana was 1839.7, the lowest value being recorded in 2008 (493 PG). The highest annual pollen count of *Quercus* was measured in 2007 (3080 PG/m^3). The mean annual pollen count obtained during the period studied in Novi Sad was 1101.7 PG. The minimum annual totals of *Quercus* were registered in Timisoara and Szeged. Increasing trends in the annual totals is observed at Timisoara station. Rodriguez-Rajo *et al.* (2003) reported a threshold value for oak pollen of 50 PG/m^3 in Spain. Studies conducted in Hungary showed that among patients with pollen allergies 10.5% react positively to oak pollen allergens (Kadocsa and Juhász, 2002). Several studies consider *Quercus* pollen type responsible for allergy in areas with abundant *Quercus* vegetation (Butland *et al.*, 1997; Luscri *et al.*, 1996; Negrini and Arobba, 1992; Ross *et al.*, 1996; Subiza *et al.*, 1995). The high abundance of these species in natural and semi-natural vegetation areas increases allergy risk for agriculture and forestry-related workers. The specific aerobiological behaviour of *Quercus* pollen has been studied in different parts of Europe and the USA (Leventin, 1998; Recio *et al.*, 1999; Corden and Millington, 1999; Jato *et al.*, 2002; García-Mozo *et al.*, 2006). *Quercus* genus, in spite of the great pollen production (Tormo Molina *et al.,* 1996), could only be responsible for allergenic symptoms when airborne pollen grains are abundant, playing only, therefore, a supporting role in inducing pollinosis in Europe (Ickovic and Thibaudon 1991; Holmquist *et al.,* 2005; Rodrıguez de la Cruz et al, 2008).

In our opinion, some pollen types recorded during this study could be involved in allergenic pollinosis (*Betula, Carpinus, Taxaceae/Cupressaceae*). Two of these pollens (*Betula, Taxaceae/Cupressaceae*) are in the list of the most important aeroallergens in Europe. Other pollen types recorded in this study and cited as allergenic do not account for high concentrations and are less allergenic significance (*Alnus, Corylus, Fraxinus, Populus, Platanus, Quercus*).

Airpalynologic values recorded during the years 2006-2008 show huge differences for our regions concerning the incidence of the pollen of some trees with airborne allergen pollen.

Conclusions

The analysis of the airborne pollen content concerned nine taxa, whose pollen has allergenic properties and occurs in large quantities in the atmosphere of Timisoara (Romania), Szeged (Hungary), Novi Sad (Serbia) and Liubliana (Slovenia) during the years 2006–2008. Some pollen types (B*etulaceae, Taxaceae/Cupressaceae*) recorded during this study could be involved in allergenic pollinosis. Airborne pollen concentrations of the *Betula* are relatively high at all four stations, especially in Novi Sad.

Obviously, the study of the trend of any given atmospheric pollen has to be carried out over a period of several consecutive years (Arobba *et al.*, 1992). This study was based on 3-year investigations; additional studies over a longer period are needed to provide a more profound insight into the relationship between pollen content in the air and seasonal allergic manifestations affecting patients during plant pollen seasons.

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