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# **Spatiotemporal assessment of airborne pollen in the urban environment: the pollenscape of Thessaloniki as a case study**

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# URBAN POLLENSCAPE EXPLORATION

### **Spatiotemporal assessment of airborne pollen in the urban environment: the pollenscape of**

### **Thessaloniki as a case study**

4 Athanasios Charalampopoulos<sup>1\*</sup>, Athanasios Damialis<sup>1,2</sup>, Maria Lazarina<sup>1</sup>, John M. Halley<sup>3</sup>, Despoina Vokou<sup>1</sup> 

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### **Abstract**

Pollen is indispensable for life. But, as it may trigger allergic reactions, it can become a biological pollutant, thus requiring monitoring. In urban ecosystems, this is usually done with sampling at rooftop level; exposure to allergenic pollen at ground level is largely unknown. Using the Hirst-type methodology, we explore here how the qualitative, quantitative and phenological features of airborne pollen change horizontally, in different sites of the urban environment, and vertically, when pollen sources are primarily local. We sampled for two years in Thessaloniki, Greece, at six near-ground stations (at 1.5 m) and one at rooftop-level (30 m high). There was a large variability in quantitative pollen features among stations, but Urticaceae, Cupressaceae, *Platanus,* Pinaceae, and *Quercus* were the five most abundantly represented taxa in the air, both near the ground and at rooftop level, exceeding there an 34 annual pollen integral of 1,000 grains  $m<sup>-3</sup>$ . We found height to have a clear effect on pollen concentration: near the ground, it was three times higher for the entire pollen spectrum and up to 11 times higher for individual taxa. Assuming an exponential decay of pollen with distance from the ground, we calculated pollen concentration for the entire spectrum to decline to half the near-ground value every 19 m and at higher rates for individual taxa. Pollen season also varied largely among stations; a semi-natural station, next to a peri-urban forest, differed from the purely urban stations in having higher pollen concentration and shorter pollen season. For only two taxa, Urticaceae and Cupressaceae, pollen concentrations exceeded thresholds associated with high risk for more than 5% of the year. We conclude that pollen is far from homogeneously distributed within the urban environment, and that height has a strong effect on the low-altitude vertical profile of pollen. At an applied level, this study provides necessary tures of airborne pollen change horizontally, in different<br>d vertically, when pollen sources are primarily local. We sai<br>sirecec, at six near-ground stations (at 1.5 m) and one at<br>s a large variability in quantitative poll



### **1. Introduction**

Pollen grains of anemophilous species are essential for plant reproduction. However, once they become airborne, they can enter the airways of the human respiratory system and induce allergic reactions in sensitized individuals. Because of the health-related implications, the indispensable-for-life pollen has been characterized as 'bio-pollutant'. Allergy was 73 considered a rare disease in the early  $20<sup>th</sup>$  century, but it is the most prevalent chronic disease 74 in the  $21^{st}$  century. It is estimated that 100 million EU citizens suffer from allergic rhinitis and 70 million from asthma, with the prediction being that by 2025 more than half of the EU population will be affected (EAACI, 2015). At the global scale, it is estimated that half a billion people suffer from rhinitis and more than 300 million from asthma (GBD, 2017). As pollen grains in the form of bioaerosols are main causes of allergic diseases, Cecchi et al. (2010) urge for continuous monitoring and readiness to take measures when pollen atmospheric concentrations get high. e disease in the early 20<sup>th</sup> century, but it is the most preva<br>y. It is estimated that 100 million EU citizens suffer from all<br>hma, with the prediction being that by 2025 more the<br>affected (EAACI, 2015). At the global sca

Given that the diversity and abundance of airborne pollen, and the phenology of the pollen season have been changing dramatically over the last few decades as a result of climate change (Damialis et al., 2019b; Ziska et al., 2019) and other factors, forecasting the phenological, qualitative and quantitative features of the pollen season and assessing the impacts of exposure to allergenic pollen have become important research issues. To monitor airborne pollen, aerobiological stations have been established, primarily in urban areas, where the majority of people spend most of their time (Velasco-Jiménez et al., 2013); also, it is documented that people living in urban environments are more susceptible to allergenic pollen than people living in rural ones (D'Amato and Cecchi, 2008).

Airborne-pollen counting has a history of more than 50 years allowing for method standardization and specifications for sampling to be developed (Galán et al., 2014) and validated (Rojo et al., 2019) so that results from different places become comparable. The recommended method includes use of a volumetric sampler (Hirst, 1952), positioned at the roof of a high building, where neighboring buildings or other infrastructure do not impede airflow towards it. It is a common practice to use only one sampler for a whole city. However, concerns have been risen about the suitability of such a sampling to provide accurate information, as the actual exposure to pollen in the places where people spend their time may be considerably different to the one estimated at the sampler's location (Charalampopoulos et al., 2018; Katz and Batterman, 2020).

The current study aims at investigating the pollenscape in an urban environment. More specifically, it explores the extent to which the pollen season and the qualitative and quantitative features of airborne pollen differ horizontally, at small scale, at different sites within the city, and vertically, between near-ground level and approximately 30-m high. Apart from hosting most people and human activities, urban environments are, in general, far more complex than rural ones regarding movement of the air and airborne particles, with urban landscape features possibly affecting markedly pollen transport; for instance, the canyoning effect of buildings (Peel et al., 2014) may limit pollen dispersion and prolong exposure to allergenic pollen at street level. t. It is a common practice to use only one sampler for a w<br>been risen about the suitability of such a sampling to<br>he actual exposure to pollen in the places where people sp<br>different to the one estimated at the sampler's l

Various studies have attempted to provide insight on the spatial distribution of pollen in the urban environment and the variation of pollen season patterns within cities of Australia (Katelaris et al., 2004), USA (Barnes et al., 2001), Germany (Mücke et al., 2014; Simoleit et al.,

2017; Werchan et al., 2017), Denmark (Skjøth et al., 2013), Italy (Arroba et al., 2000; Fornaciari et al., 1996), Poland (Puc and Puc, 2004; Rodríguez-Rajo et al., 2010), Spain (Alcázar et al., 2004; Cariñanos et al., 2002; Fernández-Rodríguez et al., 2014; Gonzalo-Garijo et al., 2006; Velasco-Jiménez et al., 2013), Turkey (Celenk et al., 2010), and elsewhere. Some studies used several samplers (e.g. Emberlin and Noris-Hill, 1991; Hjort et al., 2016; Ishibashi et al., 2008; Katz and Batterman, 2020; Katz and Carey, 2014; Werchan et al., 2017), but mainly gravimetric or rotorod-type. In the few cases that samplers were volumetric (Hirst-type), the study lasted for a few days and/or was conducted in a few sites (e.g. Antón et al., 2020; Bilińska et al., 2019; de Weger et al., 2020). Some studies explored also the vertical distribution of pollen in the urban environment either for individual taxa like *Ambrosia* (Alcázar and Comtois, 2010), *Alnus* or *Betula* (Borycka and Kasprzyk, 2018), or for the entire pollen spectrum (Bryant et al., 1989; Gálan Soldevilla et al., 1995; Fernández-Rodríguez et al., 2014; Xiao et al., 2013) leading, nevertheless, to rather inconclusive results. Recently, Rojo et al. (2019) used data from 25 cities around the world pairing near-ground and higher stations, located up to 10 km apart and at a height of 1.5 m up to 50 m, and found a clear but limited effect of height on pollen concentration, with larger values recorded at the lower stations. Our study contributes to the exploration of airborne pollen spatial patterns and variability in the urban environment using the same Hirst-type methodology for sampling at rooftop level and in six locations near the ground during a two-year period, thus allowing direct comparisons of measurements at 131 different heights. There are not many studies monitoring pollen volumetrically in so many locations and for so long (Weinberger et al., 2015) and, therefore, the collected dataset and results taken make a worthwhile contribution to literature. The study also attempts a i; Katz and Carey, 2014; Werchan et al., 2017), but maintained few cases that samplers were volumetric (Hirst-type), the set was conducted in a few sites (e.g. Antón et al., 2020; Bilir (0). Some studies explored also the

quantification of pollen decay with distance from the pollen source. We chose the major area of Thessaloniki, Greece, as a case study. Detailed information is available on the woody vegetation of the city (Charalampopoulos et al., 2018), which can be used to interpret airborne pollen data. Based on pollen findings and thresholds for symptom manifestation, this study also aims at assessing the risk associated with exposure to pollen for sites within the city differing in various features, among which in the degree of urbanization, therefore, contributing necessary information for medical praxis and background knowledge for decision making in urban planning.

### **2. Materials and methods**

### *2.1 The study area, pollen sampling and analysis*

The study took place in Thessaloniki, Greece. The city is bordered by Thermaikos gulf to the south and southwest and by the Seih Sou forest to the northeast. Land uses and vegetation types in and around Thessaloniki are shown in the CORINE Land Cover (CLC) map (European Union, Copernicus Land Monitoring Service, 2012) (Figure 1). Some CLC categories of similar type were combined and/or renamed as follows: 'Continuous urban fabric' and 'Discontinuous urban fabric' are combined under 'Urban fabric'; 'Industrial or commercial units' and 'Port areas' are combined under 'Industrial, port & commercial areas'; 'Mineral extraction sites' and 'Construction sites' are together under 'Mineral extraction & construction sites'; 'Complex cultivation patterns', 'Permanently irrigated land' and 'Non-irrigated arable land' are combined under 'Cultivations'; 'Land principally occupied by agriculture with significant areas of natural which in the degree of urbanization, therefore, con<br>medical praxis and background knowledge for decision<br>methods<br>a, pollen sampling and analysis<br>took place in Thessaloniki, Greece. The city is bordered by<br>uthwest and by th

vegetation' is under 'Agricultural land mixed with natural vegetation'; and 'Water bodies' and 'Wetlands' are combined into 'Water bodies and Wetlands'. For a number of woody species that are present in the different vegetation types and contribute pollen in the air of Thessaloniki, flowering phenology and pollen productivity are also known (Damialis et al., 2011, 2020).

Pollen sampling was conducted in 2012 and 2013, starting at the end of January and ending in early November for each of the two sampling years. Meteorological data for the two years were provided by the National Oceanic and Atmospheric Administration - National Centers for Environmental Information (NOOA - NCEI) (2013).

Six stations (sites) of high visitation and representing different environmental conditions were selected for sampling in the major area of Thessaloniki (Figure 1). These are: Aristotelous street (Ari), in the downtown area; KTEL Makedonia (Kte), the main intercity bus station, away from the city centre; the Zoological Park (Zoo), at the foot of a hill and at the edge of the peri-urban forest of Seih Sou; Ethnikis Amynis street (EAm), near the Aristotle University campus; Aretsou street (Are), at the eastern coastal zone of the city; and Platia Chimiou (Uni), inside the Aristotle University campus. For all these stations, there are detailed data of the local woody vegetation (Charalampopoulos et al., 2018). Sampling at them was conducted with a portable volumetric sampler (Burkard Manufacturing Co. Ltd., UK; http://burkard.co.uk/product/personal-174 volumetric-air-sampler) near the ground, at 1.5 m. In parallel, a 7-day volumetric Burkard sampler operated inside the Aristotle University campus, at 30 m, fixed at the roof of the Biology building, right above the near-ground Uni station. This was the rooftop University station (HUn) representing the classic method of pollen monitoring. Both samplers operate at a nominal flow mpling was conducted in 2012 and 2013, starting at the<br>ovember for each of the two sampling years. Meteorologi<br>ided by the National Oceanic and Atmospheric Admin<br>onmental Information (NOOA - NCEI) (2013).<br>Is (sites) of hig

178 of 10 L min<sup>-1</sup> through a 2x14 mm orifice.

Sampling at 'HUn' was continuous (24 h per day, 7 days per week). At the near-ground stations, sampling took place once every week, as described in Charalampopoulos et al. (2018). Throughout the sampling period, stations were visited from 9.00 to 15.00, in the order and time indicated in Figure 1. Sampling at near-ground stations was done always within a specific for each two-hour period, which corresponds to the minimum resolution of the continuous sampler.

To collect pollen, the tapes that were needed for the 7-day sampler and the slides for the portable sampler were coated with a gelvatol-glycerol-phenol mixture (Hirst, 1952) before being put in place. Pollen grains were stained with a mixture of glycerol-distilled water-gelatine-phenol (25:21:4:1) and safranin (1%), and were stored under cover slips, 24x50 mm, for the 7- day sampler, and 22x22 mm, for the portable sampler. Pollen grains were counted and identified at x400 magnification (Damialis et al., 2007) using an optical microscope (Nikon Eclipse E200, Nikon Instruments Inc., NΥ, USA). Asteroideae taxa other than *Ambrosia* and *Artemisia* were included under o. Asteroideae (other Asteroideae), and Oleaceae taxa other than *Fraxinus, Ligustrum* and *Olea* were included under o. Oleaceae (other Oleaceae). Chenopodiaceae family is now considered a synonym of Amaranthaceae (POWO, 2019), so their representatives are under Amaranthaceae. beriod, which corresponds to the minimum resolution<br>pollen, the tapes that were needed for the 7-day sample<br>pler were coated with a gelvatol-glycerol-phenol mixture<br>e. Pollen grains were stained with a mixture of glycerol-

To compare data collected by use of a fixed volumetric sampler of continuous operation at rooftop level and of a portable volumetric sampler of intermittent operation, near the ground, we assume (a) that spatial differences within the 6-hr sampling time are not due to intra-diurnal variations of pollen concentrations, and (b) that fluctuation in the flow input of the

two sampler types (fixed and portable) is of the same magnitude, so their operational behaviour is the same. Regarding intra-diurnal variations, previous research (Damialis, 2010) showed no differences of considerable magnitude in total pollen concentration within the specific 6-hr period, from 9.00 to 15.00; among the taxa reported as the most abundant in the city (Damialis et al., 2007), only for *Platanus* there was a notable variation during this time of the day (Figure A1). Regarding flow input, we checked it regularly at the rooftop sampler, every week, and corrected if necessary. For the portable sampler, we accepted the input flow given 207 by the manufacturer. In addition, we checked its performance by letting it operate next to the continuous sampler for a number of days. Comparison of their performance showed that pollen concentration of the entire spectrum was 1.5 times higher with the fixed sampler. 1). Regarding flow input, we checked it regularly at the rocted if necessary. For the portable sampler, we accepted turer. In addition, we checked its performance by letting it ler for a number of days. Comparison of thei

### *2.2 Data analysis*

From the pollen counts that we recorded, we estimated pollen concentrations per unit 213 of air volume (pollen grains  $m^{-3}$  of air) corresponding to different time scales, depending on the issue addressed each time.

To examine whether there were differences in pollen concentrations between near-ground and rooftop measurements, we used the *Wilcoxon Matched-Pairs Test*. To compare as precisely as possible pollen concentrations between each of the stations near the ground and the rooftop station, we used for the latter the data recorded during specific two-hour periods corresponding to the different times of the overall sampling, from 9.00 to 15.00, that sampling took place in the compared near-ground stations. In consequence, data for the rooftop station were not the same in the different comparisons. We name the new datasets thus produced for

the rooftop station 'HUn-sd2'. Given the rather large yearly fluctuations of pollen concentrations, to increase the robustness of the dataset, we used for this analysis pollen integrals for the entire study period, i.e. we summed for all sampling days of the two years the 225 concentration values (pollen grains  $m^{-3}$  of air) of the taxa for the specific to each comparison two-hour period. Before comparison, pollen values were transformed according to the formula *C* = log(x+1) (Damialis et al., 2015; Yadav et al., 2004), where *x* = a taxon's biannual pollen sum.

To get near-ground values that would represent pollen concentrations for the entire city and further compare them with the rooftop ones, we estimated at each near-ground station 230 pollen concentrations (pollen grains  $m^{-3}$  of air) corresponding to an hour. These values for the six stations were then summed to provide pollen concentrations near the ground for the six-hour period, from 9.00 to 15.00. We produced in a straightforward way a comparable dataset from the recordings of the continuous rooftop sampler (HUn-sd6) by estimating pollen concentrations corresponding to the same (9.00 to 15.00) six-hour period, and we further calculated the yearly values for both datasets. ialis et al., 2015; Yadav et al., 2004), where  $x = a \tan \alpha$ 's bian-ground values that would represent pollen concentration<br>pare them with the rooftop ones, we estimated at each lions (pollen grains  $m^3$  of air) correspondin

We checked if pollen concentrations for the 'Zoo' station, located next to the peri-urban forest of Seih Sou that is dominated by anemophilous species (Charalampopoulos et al., 2018), are significantly higher than in the other strictly urban stations. Given the lack of normality of the distribution and the large number of zeros in the dataset, we applied a randomisation test*.*  For this, we selected the seven most abundant taxa at the near-ground stations and used yearly 241 and maximum concentrations from each station. Data were randomized by shuffling the values at random among stations, species by species. Using a macro in Visual Basic, we repeated this procedure 1,000 times and used this set of simulations to estimate the significance of

244 differences among stations. For this, we calculated the mean rank over all species at each 245 station. If the observed mean rank for 'Zoo' was higher for more than 97.5% of the randomized 246 datasets, then we judged the difference to be significant.

We used values from the two stations having the same coordinates but differing in height by 30 m (Uni and HUn) for the specific time when measurements were taken at the near-ground station and we calculated the ratio of pollen concentration at near-ground level divided 250 by that at rooftop level. We describe the pollen concentration change in the atmosphere by a negative exponential using the 'Uni' and 'HUn-sd2(Uni)' datasets in Table A2. Negative exponential formulas are common in the atmospheric sciences. Examples include the barometric formula for air pressure (Lente and Ősz, 2020) or the Beer-Lambert law for the attenuation of light (Mayerhöfer and Popp, 2019); exponential decrease vertically is also reported for pollen (Huang et al., 2015). In our case, the negative exponential formula is as follows: nd we calculated the ratio of pollen concentration at near-<br>
p level. We describe the pollen concentration change in the intial using the 'Uni' and 'HUn-sd2(Uni)' datasets in<br>
mulas are common in the atmospheric sciences.

257 
$$
y(h) = y_0 \exp[-h/H]
$$
 ...(1)

258 In this model, where *H* is reference height, pollen concentration has a value of  $y_0$  at *h*=0; it 259 decreases with increasing height, *h*, falling to half ground-level values (y<sub>0</sub>/2), when the height is 260 exactly:

261 
$$
L_{50} = \ln(2) \cdot H
$$
 ... (2)

262 If we know the concentration at height  $h_1$  to be  $y_1$ , we can rearrange Eq.(1) to find the 263 parameter *H* and then insert Eq.(2) to get:

264 
$$
L_{50} = \frac{0.693h}{\ln(y_0/y_1)} \qquad \dots (2)
$$

Large values of *L*50 characterize slow rates of decline of pollen concentration and *vice versa*. This negative exponential form is the simplest model with the required characteristics of asymptotic 267 decline to 0 starting at a value of  $y_0$  at ground level. Other forms of decay, such as linear, Weibull or various types of power law could also have been used, but, over the examined range, the exponential model, which is a simple and commonly used distance-decay model, will 270 not differ appreciably from most other forms. We assume that this model holds true for the 271 vertical profile of pollen close to the ground and for pollen rising into the air from local sources; 272 it may not hold true at much larger altitudes and when long-range transport dominates the patterns of pollen in the air (Raynor et al., 1973).

For each station, we also estimated the start, end, and duration of pollen seasons for 275 the entire pollen spectrum and for the seven most abundant taxa in the air of the city. Start and end were defined as the day when the cumulative pollen concentration reached 2.5% and 97.5%, respectively, of the annual concentration (Andersen, 1991). iably from most other forms. We assume that this mode<br>pollen close to the ground and for pollen rising into the air<br>true at much larger altitudes and when long-range trans<br>in the air (Raynor et al., 1973).<br>station, we also

R software (version 3.3.2, R Core Team 2016) was used for the analyses.

### *2.3 Assessment of exposure levels*

There are no published results on the relationships of allergic symptoms of human cohorts with levels of exposure to airborne pollen for the Greek population, hence, there are no allergenic pollen thresholds established for this population. Given that the study area of Thessaloniki has a typically Mediterranean climate and vegetation, to have some estimation of the potential allergy risk from exposure to airborne pollen, we used thresholds (Galán et al., 2007) established for the population of Spain (Table 1), another Mediterranean country sharing

similar vegetation and climate. For the rooftop station, we calculated the number of days over the entire year when pollen was present in the air. We then calculated the number of days with pollen concentrations above the upper threshold values (Galán et al., 2007) corresponding to high exposure levels. We applied accordingly the same analysis to data from the stations near the ground, but now the period of reference was the days of the year when sampling took place. We expressed results for the rooftop station as percentages over the entire year, allowing comparisons with other cities of the world, and for the near-ground stations as 294 percentages of the sum of days when sampling took place there, allowing comparisons among them. ssed results for the rooftop station as percentages ov<br>
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reproof and the sampling were quite similar in aver

### **3 Results**

### *3.1 Airborne pollen patterns*

The two years of sampling were quite similar in average temperature, but 2012 was a little wetter than 2013 and with a different rain distribution. In 2012, there were only few rain events in late winter to mid-spring (from mid-February to April) and several from mid-spring to early summer (April to July); in 2013, it was the opposite (Figure A2). Pollen from 42 taxa was recorded at the rooftop station during the two years of study. Cupressaceae, Urticaceae, *Quercus, Platanus* and Pinaceae had the highest representation in the air of the city, each 305 contributing yearly more than 1,000 pollen grains  $m^{-3}$  (Table 2). These five taxa were responsible for more than 85% of the annual airborne pollen concentration. There was no marked difference between the two years of study for the entire pollen spectrum, but there was for individual taxa like *Fagus* and *Olea* with at least five times more pollen in 2013.

In the air near the ground, Cupressaceae, Urticaceae, *Quercus, Platanus* and Pinaceae were again first in rank, followed by *Olea* and Poaceae, whereas at the rooftop station *Carpinus*  preceded Poaceae (Table 2). Pollen concentrations of individual taxa also differed near the ground between the years of study resulting into different yearly ranks (Table 2). Of the taxa represented in the pollen spectrum after the rooftop sampler, Cichorioideae and *Ligustrum* were not recorded near the ground, whereas *Betula*, Cyperaceae, Juncaceae and Myrtaceae were represented only in one year; all six taxa were of very low annual pollen concentration (≤6 316 pollen grains  $m<sup>3</sup>$ ). To have fully comparable sets of data from sampling at rooftop and near-ground levels, apart from the annual pollen concentration calculated for the entire year at the rooftop station (HUn), we also estimated the rooftop concentration corresponding to the near-ground sampling period (Table 2). In total, 29 taxa were represented in the new rooftop dataset (HUn-sd6). The number of taxa identified at each of the near-ground stations was close to this number, from 30 to 34. All missing taxa in the new dataset were minor taxa contributing pollen to the air of 0.1% or less. ed near the ground, whereas *Betula*, Cyperaceae, Juncac<br>
Jonly in one year; all six taxa were of very low annual polle<br>
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art from the annual pollen concentration ca

There was a large variability in the amount of pollen recorded at the different sites of the city. Figure 2 shows pollen concentrations of the seven most abundant taxa and of the entire spectrum (Total) at each station. As a result of this variability, the rank of pollen taxa (Table A1) changed from station to station not only for minor but also for well represented taxa as is *Platanus* that moved from position 1 at 'Uni' down to position 10 at 'Ari'. Nevertheless, Cupressaceae was the first in rank taxon at all near-ground stations, except at 'Uni' (Figure 2, Table A1). The highest amount of pollen was recorded at the semi-natural Zoo station; as the randomization test showed (Figure A3), for both yearly and maximum pollen concentrations,

values were significantly higher there. This was mainly due to Cupressaceae and Pinaceae: compared to the other stations, pollen concentrations of these two taxa at the Zoo station were more than ten times higher (Figure 2).

Comparisons of pollen concentrations for the entire pollen spectrum (Figure 3) showed that they were higher at all sites examined with the near-ground sampler. Airborne pollen concentration of the entire spectrum was 3.0 times higher at the near-ground station compared to the station right above [Uni and HUn-sd2(Uni) datasets in Table A2]. The ratio of pollen at ground level divided by that at the same location but elevated 30 m is given in Figure 4. For all but three taxa (*Olea Salix*, Apiaceae) for which measurements exist, the pollen ratio is greater than unity and may be as high as 11; on average it is 4.55. If we take into account the 341 1.5 times underestimation of pollen concentration with the portable sampler, this average ratio would be even higher. Since these values represent the total pollen over two years of study, these measure long-term patterns rather than reflecting short-term stochasticity. Using Eq.(3), we calculated the total pollen concentration to be halved every 19 m. For the individual taxa, 345 LD<sub>50</sub> ranged from 9 m to well above 30 m, corresponding to taxa with pollen ratio 10 to 11 and lower than 2, respectively (Figure 4). F the entire spectrum was 3.0 times higher at the r<br>station right above [Uni and HUn-sd2(Uni) datasets in Tal<br>level divided by that at the same location but elevated 30<br>ee taxa (*Olea Salix*, Apiaceae) for which measureme

The Seih Sou peri-urban forest is a major source of airborne pollen. Comparing pollen concentrations at the Zoo station, at the edge of this forest, with those at the rooftop station, 1.1 km away and about 150 m lower in altitude [Zoo and HUn-sd2(Zoo) datasets in Table A2], we find them much higher at the 'Zoo' for all taxa contributing at least 0.3% of pollen to the air and most of the remaining taxa. Pollen concentration for the entire spectrum was 9.1 times

higher at the 'Zoo' and at least 20 times higher for individual taxa like *Fagus,* Pinaceae, *Plantago* or *Populus*.

The pollen season features for the seven most abundantly represented taxa for each year of study, as derived with the continuous sampler for all days of the year and with the portable sampler with intermittent sampling, are shown in Figures 5 and A4. Exploration of the pollen season start, end, and peak, at the different stations, shows that these phenological features differed among sampling stations and did not remain the same in the two years of study. The duration of a taxon's pollen season varied considerably among sampling stations, from a few days [e.g. *Carpinus* (2013), *Quercus* (2012), *Olea* (2012, 2013)] to more than three months [e.g. Amaranthaceae (2013), Pinaceae (2013), *Plantago* (2013)]. Peaks of the pollen season also had the tendency to vary largely from station to station; they remained rather fixed only for Urticaceae. The pollen season had the lowest duration values at the 'Zoo' station for the entire pollen spectrum and for the major taxa Cupressaceae and Pinaceae; it was shortest for Urticaceae at rooftop level. art, end, and peak, at the different stations, shows that<br>among sampling stations and did not remain the same<br>ion of a taxon's pollen season varied considerably among<br>[e.g. *Carpinus* (2013), *Quercus* (2012), *Olea* (2012

### *3.2 Exposure levels*

Results of the assessment of exposure levels to the seven most abundantly represented pollen taxa in the air, in the six sites of the city examined, are shown in Figure 6. The sampling period for the rooftop station 'HUn' corresponded to the entire year, whereas for the other near-ground stations to the days when sampling took place. Assessment of data for HUn representing the standard pollen monitoring method, showed that there was pollen in the air of the city from all seven taxa for a considerable part of the year, from 11.2% in the case of

*Quercus* (2012) up to 38.6% in the case of Cupressaceae (2012). Pollen concentrations of these taxa exceeded the upper thresholds, as defined by Galán et al. (2007), for at least one day during the two-year sampling period. Nevertheless, high concentrations corresponded always to less than 2.5% of the year, except for Urticaceae, for which they accounted for a little more than 5%.

Near the ground, there was a lot of variability among stations and between years. In several cases, days with pollen were either far more (e.g. Poaceae at several stations in 2012) or far less (e.g. *Quercus* at all near-ground stations in both years) than at rooftop level. Occurrence of high pollen concentrations, exceeding 5% of the yearly sampling period, were detected for Urticaceae and Cupressaceae; for the latter, this was the case only at the Zoo station, whereas for Urticaceae at several stations (Kte, Eam, Uni and Zoo). In fact, days of high concentrations of Urticaceae pollen were detected at all near-ground stations, at each corresponding to longer parts of the year than for any other pollen taxon. ground, there was a lot of variability among stations and<br>ys with pollen were either far more (e.g. Poaceae at sevel<br>Quercus at all near-ground stations in both years) tha<br>gh pollen concentrations, exceeding 5% of the year

### **4. Discussion**

Annual concentration of the entire pollen spectrum, as estimated after the classic rooftop method, was similar in the two years of study, but there were some marked differences for individual taxa. One herbaceous and four woody taxa, the same as in the past (1996-2005; Damialis et al., 2007), viz. Cupressaceae, Urticaceae, *Quercus, Platanus* and Pinaceae, were the five most abundantly represented in the air of Thessaloniki. The same pollen taxa were the most abundantly represented also near the ground.

The amount of airborne pollen present, of the entire spectrum and of individual taxa, varied largely among stations indicating a non-uniform pollen distribution within the city. Pollen transportation and dispersal are affected by pollen features and prevailing meteorological conditions, but also very important are the composition and local presence of pollen-producing vegetation and the features of the urban environment (Charalampopoulos et al., 2018; Silva Palacios et al., 2007). Micro-environmental conditions can play key roles in the movement and circulation of pollen grains in the air resulting into noteworthy differences in pollen concentration within a city (Peel et al., 2014). Our finding regarding the non-homogeneous pollen distribution in the city of Thessaloniki agrees with several other studies that examined airborne pollen at ground level, among which in other Mediterranean cities like in Spain (Fernández-Rodríguez et al., 2014; Gonzalo-Garijo et al., 2006). These findings also indicate that, while pollen monitoring with a single sampler in an urban environment provides background information on the pollen seasons' features (Šikoparija et al., 2018), other approaches, alternative or complementary, may be needed when less regional and more local information is required (Charalampopoulos et al., 2018; Katz and Batterman, 2020), as in the case of personalized preventive-allergy risk alerts (de Weger et al., 2020). 007). Micro-environmental conditions can play key roles in<br>ollen grains in the air resulting into noteworthy dif<br>thin a city (Peel et al., 2014). Our finding regarding the<br>n in the city of Thessaloniki agrees with several

The Seih Sou peri-urban forest, next to the Zoo station, is a major source of airborne pollen. Comparing pollen concentration of the entire spectrum at the 'Zoo' with that at the rooftop station we found it 9.1 times higher at the 'Zoo'; this is the highest difference in pollen abundance between any of the near-ground stations and the rooftop one. Results from the rooftop station and the near-ground one, of same coordinates but 30 m lower, enabled us to arrive at quantitative descriptions of the vertical changes of pollen concentration. Assuming an exponential decay of pollen with distance from the source on the ground and using the values at 418 1.5 m and 30 m, we calculated pollen concentration of the entire spectrum to be halved vertically every 19 m, suggesting a rapid decay; for individual species like *Platanus*, for which the Uni station is an important source (Charalampopoulos et al., 2018), the corresponding value was much smaller (9 m).

What is the appropriate *a priori* assumption for the near-ground vertical profile of pollen remains unanswered. Different studies have found increasing, decreasing or non-differing near-ground profiles (Alcázar et al., 1999; Fernández-Rodríguez et al., 2014; Spieksma et al., 2000; Raynor et al., 1973). Our results are in line with the findings of a large-scale study by Rojo et al. (2019), namely that pollen shows a decrease in concentration with height. However, while these authors found pollen ratios between 0.5 and 2, our results show variation from 0.43 to 11.0, on average 4.55, suggesting a much faster decrease; we note also the 1.5 times underestimation of pollen abundance with the portable sampler suggesting a higher average ratio than the one calculated. This difference can be attributed to the fact that Rojo et al. (2019) are considering stations at a more regional perspective, with pairs up to 10 km apart, something which will subdue differences arising from local geographical features and sources. By contrast, our pair of stations occur at the same geographical location. Tenfold concentration differences like those we found for a number of taxa between emission (near-ground) and higher levels were also reported by Šikoparija et al. (2018) for *Ambrosia,* in a detailed field study. the appropriate *a priori* assumption for the near-ground<br>unanswered. Different studies have found increasing,<br>bund profiles (Alcázar et al., 1999; Fernández-Rodríguez et<br>nor et al., 1973). Our results are in line with the

Studies on pollen vertical profiles deal with complex systems and differ in many aspects; hence, they also differ in their results. Apart from differences in the position of samplers, the

height and density of surrounding buildings, blowing winds and other factors, presence (or absence) of pollen producing individuals in the proximity of the pollen-sampler site seems to determine to a large extent the recorded vertical pollen profile. In an earlier study in Thessaloniki, Charalampopoulos et al. (2018) showed that distant sources may influence considerably the diversity of pollen recorded locally but not its abundance, whereas, in the early '70s, Raynor et al., (1973), having concluded that ragweed pollen concentrations do not change appreciably up to 108 m above ground, stated that these conclusions would not apply when pollen concentrations are estimated close to local pollen sources; near-ground concentrations there would be much greater than at higher elevations. Therefore, studies on pollen vertical profiles should be better distinguished in two fundamentally different groups after the location of pollen sources, and then assessed separately. Our study, showing concentrations decreasing rapidly with height, falls within the group of studies where pollen is mainly produced locally. r et al., (1973), having concluded that ragweed pollen co<br>bly up to 108 m above ground, stated that these conclusion<br>incentrations are estimated close to local pollen so<br>nere would be much greater than at higher elevations

The phenological aspects of the pollen season varied largely among stations and between years, with variation being expressed in all features, i.e. start, end, duration, and peak of the season. In some cases, the season ended later at the near-ground stations as other researchers observed (Rojo et al., 2020), but this was not a consistent pattern. Rantio-Lehtimäki et al. (1991) and Bastl et al. (2019) found an earlier and/or longer pollen season for grasses near the ground than at rooftop level. Such a pattern for grasses was not evident in our results. In our case, another herbaceous taxon, the very abundant Urticaceae, presented the shortest pollen season at the rooftop station compared to all near-ground ones. The only recognizable pattern in this variability was the shorter duration of the pollen season at the 'Zoo'. This station

is a major pollen source for woody taxa. Charalampopoulos et al. (2018) found that individuals of pollen-contributing woody species at the 'Zoo' accounted for 60% of those recorded at all six near-ground stations with Pinaceae individuals making there 82% of the individuals recorded in all stations. The season was shortest there in both years of study for the entire pollen spectrum and for the two major taxa, Pinaceae and Cupressaceae.

Compared to areas of varying levels of urbanization, higher pollen concentrations in semi-natural areas are reported for part or the whole pollen spectrum from Poland (Kasprzyk, 2006; Rodríguez-Rajo et al., 2010), Serbia and Montenegro (Šikoparija et al., 2006), Spain (Cariñanos et al., 2002; Rodríguez-Rajo et al., 2010) and France (Bosch-Cano et al., 2011). Apart from pollen circulation, urbanization influences several other plant parameters, such as plant growth and biomass (Ziska et al., 2003; 2004), and pollen productivity (Cariñanos et al., 2002). 472 The thermal island phenomenon, which is associated with increased thermal radiation (e.g. from the asphalt or structures of concrete) and decreased levels of wind speed and relative humidity, may lead to longer periods of plant growth (Ziska et al., 2004). The shorter pollen seasons at the semi-natural Zoo station may be related to its being a major pollen source. The other purely urban stations, with less importance as pollen sources, seem to be influenced to a 477 larger degree by factors, such as blowing winds and features of the urban environment like the city landscape and air flow through it. This results into a larger heterogeneity in the urban stations than in the 'Zoo' leading to pollen seasons of variable length, start, end, and peak and random changes from station to station. The results of our small-scale study of airborne pollen phenology not showing any pattern of change of the pollen season, particularly among the semi-natural and the urban stations, is in agreement with a large-scale study investigating d to areas of varying levels of urbanization, higher polle<br>as are reported for part or the whole pollen spectrum fro<br>Rajo et al., 2010), Serbia and Montenegro (Šikoparija<br>2002; Rodríguez-Rajo et al., 2010) and France (Bosc

phenological changes, among which in flowering, in many European sites for the period 1981- 2010 (Wohlfahrt et al., 2019). Authors of this study concluded that temporal trends in plant phenology (with the exception of leaf senescence) are unaffected by the degree of urbanization and that plant phenology is a poor quantitative predictor of the urban heat island.

With respect to the risk associated with exposure to pollen, using data derived from the continuous sampler, we estimated that pollen of the seven most abundantly represented taxa was present in the air of the city for a considerable amount of time. Nevertheless, pollen concentrations exceeded the upper thresholds for a very limited period, less than 2.5% of the year; only for Urticaceae such concentrations were recorded for longer, up to 5.5% of the year. At the near-ground stations, apart from Urticaceae, Cupressaceae pollen was also found to exceed the upper threshold for more than 5% of the year, but only in the semi-natural Zoo station. Concentrations of the typical Mediterranean plant *Olea* did not exceed the upper threshold at any of the near-ground stations in any of the sampling years. Also, the risk associated with the highly allergenic pollen of *Ambrosia* (ragweed), which has created many problems in other European countries (Šikoparija et al., 2016), is low in Thessaloniki. We note that allergy risk in Thessaloniki was estimated after threshold values pertaining to the population of Spain (Galán et al., 2007); it is an open question how much applicable to the Greek population these thresholds are. ler, we estimated that pollen of the seven most abundant<br>the air of the city for a considerable amount of time. Note<br>acceded the upper thresholds for a very limited period, le<br>icaceae such concentrations were recorded for

Comparing the exposure levels in Thessaloniki to those in other cities, we find high-risk days to account for more than 5% for Cupressaceae, Poaceae, *Quercus* and Urticaceae for most years of study in the city of Toledo (Perez-Badia et al., 2010), whereas less than 5% for all studied taxa in the city of Guadalajara (Rojo et al., 2016) and Funchan (Camacho et al., 2015). In

mid-Europe, Piotrowska-Weryszko and Weryszko-Chmielewska (2014) estimated days of high risk to account for more than 5% for eight of the 18 pollen taxa recorded in Lublin (Poland) including Cupressaceae, *Quercus* and Poaceae; Jochner et al. (2015) estimated a similar frequency for Poaceae in the German Alps, whereas Nowak et al. (2012) found for the single studied taxon *Platanus* a period of high risk less than 5% for Poland. RNSA in France (https://www.pollens.fr/en/) estimated the days of high risk to be more than 5% of the year for Cupressaceae and Urticaceae in Nice and for Cupressaceae, Poaceae and *Quercus* in Montpelier (for 2012-2013).

Among the near-ground stations in Thessaloniki, we can identify comparably safe or risk areas for the people suffering from pollen allergies. To the safer end, the stations Ari, Kte and Are can be grouped together; to the other end is 'Zoo', the most pollen loaded. This means that downtown Thessaloniki and both the western and eastern parts of the city are areas where sensitized individuals are not exposed to high pollen concentrations for long periods. Such individuals should avoid the area neighboring the Zoo station for outdoor activities during the pollen season and, if possible, as their residential area. However, individuals in Thessaloniki and other cities of the world should not be left alone to make their best-judgement decisions to minimize their allergy-related problems. Urban green is almost entirely human made and, therefore, it can have any desirable quality to satisfy the citizens' needs. As the population of sensitized individuals keeps increasing (EAACI, 2015), municipal authorities and responsible agencies should take into consideration pollen-triggered allergies when designing and managing the urban green spaces. Towards this end, existing information on airborne pollen abundance of the different taxa and trends of its change with time is very valuable. We note Ilens.fr/en/) estimated the days of high risk to be more tha<br>d Urticaceae in Nice and for Cupressaceae, Poaceae and  $Q$ <br>e near-ground stations in Thessaloniki, we can identify con<br>pple suffering from pollen allergies. To t

that, in Thessaloniki, the two taxa with pollen concentration exceeding the upper thresholds for more than 5% of the year (Urticaceae and Cupressaceae) were among the taxa with the highest rate of long-term trend of increase in atmospheric pollen concentration (Damialis et al., 2007).

There have been several attempts to estimate allergy risk from airborne pollen. But this is not an easy task. Pollen threshold levels for sensitization are not known, whereas pollen concentrations that induce symptom manifestation vary largely among populations (de Weger et al., 2013). For instance, the lower thresholds for *Ambrosia* cover a range from 1(-3) pollen 534 grains  $m<sup>-3</sup>$  of air in Canada (Comptois and Gagnon, 1988) to 20(-25) in Russia (Ostroumov, 535 1989). Similarly, for Poaceae, they cover a range from 4 pollen grains  $m^{-3}$  of air in Israel (Weisel et al., 2004) to 20 in Poland (Rapiejko et al., 2007). Additionally, there is great variability in the severity of symptoms among individual patients for a given type of allergy (Blume et al., 2013; Bryborn et al., 2010). Recently, Damialis et al. (2019a) found that thresholds can become higher or lower because of environmental co-factors like relative humidity: higher humidity values reduce thresholds even to half the initial levels for the same patient cohort who thus exhibit more intense symptoms of allergic rhinitis, allergic conjunctivitis and, especially, allergic asthma. Sensitization to a certain pollen allergen depends greatly on the genetic characteristics of the population and, hence, it varies geographically, with vegetation present, prevailing meteorological conditions, and level of urbanization having important contribution in determining exposure histories (Peden and Reed, 2010). Differences among populations but also among individuals of the same population are highly preventive factors for the establishment of global thresholds for airborne pollen like the ones that have been established for chemical pollutants (Cecchi, 2013). Given the lack of such thresholds, there is always the risk nat induce symptom manifestation vary largely among pop<br>instance, the lower thresholds for *Ambrosia* cover a rang<br>in Canada (Comptois and Gagnon, 1988) to 20(-25) in<br>for Poaceae, they cover a range from 4 pollen grains m<sup></sup>

of misinforming interested people when communicating pollen monitoring information to the public, as those who will possibly use it will be both locals and visitors, with potentially largely different responses to similar pollen concentrations. Harmonizing outputs that target the wider public is an important issue that should be addressed by the relevant scientific community.

### **5. Conclusions**

This spatial study of airborne pollen in an urban environment showed clearly that (i) pollen is not homogenously distributed within the mosaically patterned urban ecosystems, and (ii) that height has a strong effect on the low-altitude vertical profile of pollen, with concentrations rapidly decreasing with distance from local sources, on the ground. While showing that Thessaloniki is a city of rather limited pollen allergy risk, the study raises important issues concerning urban environments and public health, highlighting the need for alternative or complementary monitoring approaches, at a spatial scale finer than that of the standard method with only one rooftop station, and also for harmonizing communication of monitoring results to the wider public and for making use of these results to decision making in urban planning. At a time when climate change increases the demand for urban green, the allergy aspect is an important factor to be addressed. Exploration of a city's pollenscape can help in improving the quality of life in the urban environment, indicating specific areas where the allergy related issues are more acute and, hence, the need for measures to be taken and the plants and management practices that are better to be avoided. al study of airborne pollen in an urban environment show<br>nogenously distributed within the mosaically patterned urb<br>has a strong effect on the low-altitude vertical prof<br>apidly decreasing with distance from local sources,

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**BEERINGS** 

### **Figure captions**

**Figure 1:** CORINE Land Cover (CLC) map of Thessaloniki and its surroundings (European Union, Copernicus Land Monitoring Service, 2012), and location of sampling stations (in parenthesis, the time of the day when they were visited). 1. **Ari**: Aristotelous street (09.00-10.00), 2. **Κte**: Ktel Makedonia (10.00-11.00), intercity bus station, 3. **Ζoo**: Zoological Park (11.00-12.00), 4. **EAm**: Ethnikis Aminis street (12.00-13.00), 5. **Are**: Aretsou street (13.00-14.00), 6. **Uni**: Aristotle University campus (14.00-15.00), **HUn**: rooftop station, above the Uni one. Numbers before the names of stations indicate the order in which they were visited. Some initial CLC categories of similar use have been combined and/or renamed (see 2.1 in Materials and methods for details). (10.00-11.00), intercity bus station, 3. **200**: Zoological Pa<br>
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sindicate the order in which t

**Figure 2:** Pollen concentrations for the seven most abundant taxa and the entire pollen spectrum (total) in the atmosphere of Thessaloniki for each of the near-ground sampling stations and the rooftop one (red circles) for the entire sampling period. The sizes of circles indicating pollen abundance are taxon-specific (note the different abundance scales per taxon). Abbreviations of stations are as in Figure 1.

**Figure 3:** Box and whisker plots of pollen concentrations (in log scale) at each near-ground station and the rooftop one, and results of the Wilcoxon Matched Pairs Signed Rank Test; for each comparison, measurements for the rooftop sampler correspond to the specific two-hour period, when sampling took place at the near-ground station HUn-sd2). In each boxplot, the horizontal line separating the two boxes represents the median, whereas the 'x' symbol represents the mean. Top and bottom sides of the box correspond to the Q3 and Q1 quartiles,

whereas the whiskers the minimum and maximum values. Given is the probability *p* for all 851 compared pairs. Abbreviations of stations are as described in Figure 1. Axis y corresponds to pollen concentration in log values.

**Figure 4**: Ratio of airborne pollen concentration over the duration of observations at near-ground level (1.5 m) divided by that at the same location but elevated 30 m for 26 taxa. For the calculation of ratios, the Uni and HUn-sd2(Uni) datasets in Table A2 were used.

**Figure 5:** Pollen seasons (PS) and dates of peak concentrations for the seven most abundantly 857 represented pollen taxa in the air near to the ground as well as for the entire pollen spectrum (Total) at each sampling station, for the two years of sampling (2012, 2013). Abbreviations of stations are as in Figure 1. Start and end of the pollen season were defined as the day when the cumulative pollen concentration reached 2.5% and 97.5%, respectively, of the annual concentration. ios, the Uni and HUn-sd2(Uni) datasets in Table A2 were us<br>seasons (PS) and dates of peak concentrations for the seven<br>n taxa in the air near to the ground as well as for the ent<br>ampling station, for the two years of sampl

**Figure 6:** Percentage of days when pollen was present in the air and of days when pollen concentrations were above the higher threshold value (after Galán et al., 2007) for the seven most abundantly represented taxa in the air of Thessaloniki, in each year of sampling and station. For the rooftop station (Hun), percentages are after the entire year; for the near-ground stations, they are after the days of sampling. Abbreviations of stations are as in Figure 1.

Table 1: Pollen concentrations corresponding to threshold values for symptom manifestation (Galán et al. 2007) that were used to assess the level of exposure to allergenic pollen. Low threshold levels denote no symptom manifestation, while medium-level and high-level thresholds provoke the manifestation of mild and severe symptoms, respectively.



Table 2: Pollen concentration (pollen grains m<sup>-3</sup> of air) [average pollen concentration (APC) and yearly values] and relative abundance (RA) of each taxon's airborne pollen, and taxa ranks after RA. 'HUn' corresponds to the rooftop station, with the entire year as sampling period. 'Near-ground' corresponds to the days when sampling took place near the ground during the 6-hr period, 9.00-15.00; values for this are calculated with summing up concentrations, on an hourly basis, at each of the six near-ground stations. 'HUn-sd6' refers again to the rooftop station but corresponds only to the days when sampling took place at the near-ground stations during the 6-hr period, 9.00-15.00; it is the equivalent at rooftop level of 'Nearground'. Taxa are consecutively ranked after average concentration at the rooftop station.





Table A1: Representation and rank of pollen taxa at the sampling stations in Thessaloniki after their average yearly pollen concentration. Rank for the rooftop station (HUn-sd6) is after the 6-hr period, 9.00- 15.00, when sampling took place at all the near-ground stations. Taxa are presented according to their rank at HUn (Table 2); the dash symbol means that pollen of a taxon was not detected.



Table A2: Average yearly concentrations of airborne pollen ± sd for the rooftop, 'Zoo' and 'Uni' stations. To be comparable, values for the rooftop station were estimated for the 2-hr period, within which sampling took place at the corresponding near-ground station; the corresponding datasets are HUn-sd2 (Zoo) and HUn-sd2 (Uni). Taxa are presented according to their rank at HUn (Table 2).





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Sampling stations



Figure A1: Intra-diurnal (in a 24-hour scale) patterns of average pollen concentration for the five most abundant taxa and the total pollen spectrum in the air of Thessaloniki, during the years 2003-2005



Figure A2: Mean, maximum and minimum daily temperatures (Y axis on the left) and daily precipitation (Y axis on the right) for the years 2012-2013. Given are also average values ± standard deviation, total precipitation, and days with precipitation as percent of the year.



**Figure A3:** Randomization results for comparison of ranks for the annual and maximum pollen concentrations of the seven most abundant taxa at the Zoo station with the corresponding concentrations of the same taxa at each of the other near-ground stations. For each of the attributes examined, significance values appear as labels; **Α:** maximum values of 2013, **B:** annual values of 2012, **C:** maximum values of 2012, **D:** annual values of 2013. Given the 2.5% significance level adopted, only A is marginally no t significant.

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Figure A4: Daily pollen concentrations during the pollen season for the seven most abundantly represented pollen taxa as well as for the entire pollen spectrum (Total) in the air of Thessaloniki, at the rooftop station (HUn) (over the entire year) and near the ground (over the sampling days), as estimated after measurements at the six near-ground stations (see Table 2), for the two years of sampling (2012, 2013). Abbreviations of stations are as in Figure 1. For each taxon, given is also the total number of pollen grains per year.

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### **Highlights**

- Pollen is not homogenously distributed in the urban environment
- Pollen concentrations near the ground are much higher than at roof-top level
- Pollen concentrations are lower and seasons longer in urban compared to semi-natural sites

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### **Declaration of interests**

 $\boxtimes$  The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

 $J_{\alpha}$  ,  $J_{\alpha}$  ,  $J_{\alpha}$