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Research article

Bioaerosols in urban environments: Trends and interactions with pollutants and meteorological variables based on quasi-climatological series

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ABSTRACT

Pollen grains emitted by urban vegetation are the main primary biological airborne particles (PBAPs) which alter the biological quality of urban air and have a significant impact on human health. This work analyses the interactions which exist between pollen-type PBAPs, meteorological variables, and air pollutants in the urban atmosphere so that the complex relationships and trends in future scenarios of changing environmental conditions can be assessed. For this study, the 1992–2018 pollen data series from the city of Granada (southeast Spain) was used, in which the dynamics of the total pollen as well as the 8 main pollen types (Cupressaceae, Olea, Pinus, Platanus, Poaceae, Populus, Quercus and Urticaceae) were analysed. The trend analysis showed that all except Urticaceae trended upward throughout the series. Spearman's correlations with meteorological variables showed that, in general, the most influential variables on the pollen concentrations were the daily maximum temperature, relative humidity, water vapor pressure, global radiation, and insolation, with different effects on different pollen types. Parallel analysis by neural networks (ANN) confirmed these variables as the predominant ones, especially global radiation. The correlation with atmospheric pollutants revealed that ozone was the pollutant with the highest influence, although some pollen types also showed correlation with NO_2, SO_2, CO and PM_{10}. The Generalized Linear Models (GLM) between pollen and pollutants also indicated O_3 as the most prominent variable. These results highlight the active role that pollen-type PBAPs have on urban air quality by establishing their interactions with meteorological variables and pollutants, thereby providing information on the behaviour of pollen emissions under changing environmental conditions.

1. Introduction

1.1. Bioaerosols in the urban environment

Pollen grains transported through the atmosphere are considered the most numerous primary biological airborne particles (PBAPs) among the different categories of material that comprise atmospheric bioaerosol (Després et al., 2012; Fröhlich-Nowoisky et al., 2016). The necessary role that PBAPs have in plant reproduction processes means that they are present in all terrestrial ecosystems and that once released they can be incorporated into the dispersive dynamics of the atmosphere. The aerodynamic shape and size of PBAPs (between 10 and 100 μm in diameter) (Faegri et al., 2000) make it easier for them to remain in the proximity of the emission source or to be transported at different distances, from just a few meters to hundreds of thousands of kilometres (Griffin, 2007; Prospero et al., 2015). Although traditionally the concentration of pollen in the atmosphere has been considered insignificant compared to that of other types of non-biological aerosol (Penner et al., 2011), some authors estimate that their concentrations are quite high, since their measurements and contributions have not been correctly interpreted (Elbert et al., 2007; Núñez et al., 2016). Thus, it has been suggested that up to 1000 Tg/year of bioaerosols are emitted (compared to 3300 Tg/year for sea salt and 2000 Tg/year for mineral dust) [Jaenicke, 2005; Després et al., 2012]. Pollen contributes between 47 and 84

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Tg/year to these global emissions, with its numerical concentration around 10 #/m^3 (up to 103 #/m^3) and its mass concentration of the order of 1 μg/m^3 (Manninen et al., 2014). What is known is that their concentrations are the highest in the atmospheric boundary layer (ABL) and decrease with altitude (Damialis et al., 2017), although this phenomenon can be modified by thermal inversion layers (Cariñanos et al., 2000; Williams, 2020) and other processes of vertical dynamics (Sicard et al., 2016; Núñez and Moreno, 2020).

One of the most frequent impacts associated with the presence of pollen in the atmosphere is its direct participation in the spread of diseases in plants, animals, and humans. In the case of plants, the transmission of viruses and pollen-mediated fungal infections after pollination can cause significant problems because the infection may persist for years to come (Atsumi et al., 2015; Antonovics, 2004). In the case of animals and humans, the presence of pollen is closely related to the development and aggravation of some respiratory diseases, constituting a modern global health burden (Traidl-Hoffman et al., 2003; Kim et al., 2018). Pollen emissions during the flowering period, particularly those derived from species with a wind-pollinated strategy, are recognized as the main causative agents of allergic rhinitis in 30% of the world population (Pawankar, 2014) and up to 15% of asthma episodes are caused by the presence of allergens in the pollen cover (Kim et al., 2018).

1.2. *Bioaerosols interactions with pollutants and meteorological variables*

In urban environments, the diseases associated with the presence of pollen in the atmosphere can be aggravated, since in addition to there being diverse sources of allergen emissions (Cariñanos and Casares-Porcel, 2011; Cariñanos et al., 2016), interactions with other atmospheric pollutants can be established (Sénéchal et al., 2015; Sedghy et al., 2018), and be subject to urban microclimate conditions that interfere with their dispersion dynamics (Cariñanos et al., 2002; Hjort et al., 2016). In the presence of atmospheric pollutants, pollen grains, in addition to having their fertility and viability altered (Cuinica et al., 2014), experience alterations in the protein composition in their exines, modifying their allergenic potency (Chakra et al., 2010; Sénéchal et al., 2015) and even function as transporters of other polluting particles on its surface (Okuyama et al., 2007). The physico-chemical reactions that occur in the atmosphere as a result of anthropogenic activities can in turn facilitate an availability of allergens under certain conditions of pH and temperature (Behrendt and Becker, 2001; Beggs, 2016). Moreover, in the face of high concentrations of CO_2, some species increase the production of pollen per flower by more than 50% (Albertine et al., 2014; Zisca and Caulfield, 2000).

Urban microclimate conditions have a direct effect on the phenology and reproductive biology of different plant species (Neil and Wu, 2006), and also cause environmental stress to pollen emissions which effects their morphology and functionality (Bosh Cano et al., 2011; Azzazy, 2016). Thus, the sudden changes in temperature and relative humidity in the urban environment can facilitate the release of sub-pollen particles and sub-micrometer fragments that keep their allergenic potency intact (Asam et al., 2015). The interception of ultraviolet B radiation by urban vegetation also has direct effects on the growth rate and pollen maturity due to the absorption of more than 80% of this radiation by the flavonoid compounds in the outer wall (Torabinejad et al., 1998; Zhang et al., 2014). The atmospheric dynamics itself, which makes possible the long-range transport of particulate material of remote origin (e.g. Veriankaitė et al., 2010; Szczepanek et al., 2017) and rapid movements of vertical ascent and descent (Jones and Harrison, 2004), can also promote physical contacts among the different components, causing degradation and breakdown of pollen grains by mechanical processes (Cariñanos et al., 2004; Manninen et al., 2014).

This close relationship between meteorological conditions and the presence of pollen in the atmosphere becomes more evident when long-term series are considered, where it is possible to analyse in detail the effect of changing environmental factors on different species and different ecosystems over a series of years (Hájkova et al., 2020; Cariñanos et al., 2014). In the current climate change scenario, the atmospheric pollen data series has been indicated as an efficient indicator to know the intensity of this impact and the response to it at different scales. A study of 17 locations across three continents has shown that most locations had significant increases in seasonal cumulative pollen or annual pollen load associated with an annual cumulative increase in temperature (Zisca et al., 2019). In Europe, the changes detected have been more pronounced in urban than in semi-rural/rural areas (Ziello et al., 2012). Several studies in Mediterranean countries highlight that the expected changes in the rainfall distribution regime, water availability, and the rise of temperatures – mainly during winter season – are the factors that most affect the intensity of the flowering of the species (Algarra et al., 2019), and therefore, the production and pollen emission (Damialis et al., 2007; Galán et al., 2016). However, few studies consider the effect that a set of long-term meteorological variables can have on pollen emissions from different plant species. When the correlations among pollen concentrations from selected taxa and the atmospheric pollutants in urban environments have been analysed, it is observed that pollutants can also influence the pollination periods of some species (Honour et al., 2009; Puc and Bosiacka, 2011). Nevertheless, the interactions that can be established between pollen, pollutants and meteorological variables in urban environments are still far from clear, since very different correlation results have been obtained according to the pollen type (Grundström et al., 2017; Sauliene et al., 2019), as well as on the same pollen type under different environmental conditions (Cuinica et al., 2014; Ørby et al., 2015). Sometimes the interactions with several pollen types have been analysed, but then the data series has been very short (Puc and Bosiacka, 2011; Rahman et al., 2019), or the number of pollen taxa considered has been very low (Honor et al., 2009; Oduber et al., 2019). Therefore, there is still an important knowledge gap when it comes to knowing the response of some main plants to changing environmental conditions, which can affect the emission of pollen-type bioaerosols into the urban atmosphere. This is even more relevant in the face of significant growth prospects for both diseases related to urban air quality (Goodman et al., 2017; Khaltaev and Axelrod, 2019), and the percentage of the population that will continue to move from rural areas to cities in the coming decades (UN-Habitat, 2019).

Given the great impact that PBAPs have on air quality and health, this work aims to learn more about the interactions that may exist between atmospheric pollutants and meteorological variables with pollen-type PBAP concentrations in an urban atmosphere, so that trends throughout a long-term series can be evaluated and the possible response to the effects of changing weather conditions can be estimated. The local situation recorded in a medium-sized city in the southeast of the Iberian Peninsula, and strongly impacted by urban pollution, will be shown as a case study, representative of a frequent situation in many cities of the Mediterranean region. The results enhance the understanding of the complex relationships that may be established between PBAPs, pollutants, and meteorological variables and help in the implementation of measures to mitigate the health effects associated with their presence.

2. Material and methods

2.1. *Study area*

The city of Granada is located in the southeast of the Iberian Peninsula (37.18°N, 3.60°W), at the foot of one of the highest mountain ranges in Europe, the Sierra Nevada (Fig. 1). Its geographical location favours the prevalence of a marked Mediterranean-continental climate (annual average temperature 15.6 °C, annual average rainfall 359 mm for the period 1981–2010, AEMET, 2018). It is strongly conditioned by the altitude at which the city is located (680 m a.s.l.) and influenced by

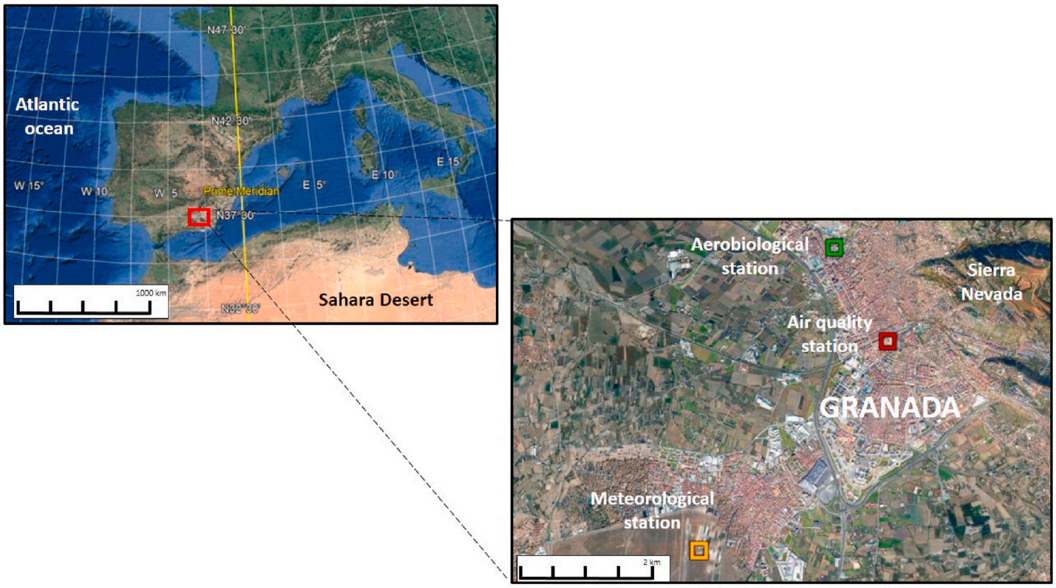

Fig. 1. Location of study area.

the mountainous massif, which actively participates in the wind regime of the city (Viedma-Muñoz, 1998). The main sources of pollen-type PBAPs emissions come from the different types of vegetation in the area. This includes natural vegetation in the surroundings of the city of which the Sierra Nevada is the main contributor due to the presence of various ecosystems dominated by anemophilous species. These species include grasslands, holm oaks (*Quercus* spp.), junipers (*Juniperus* spp.), and pine forests (*Pinus sylvestris, P. nigra*), among others (Cariñanos et al., 2019a,b). Another source of pollen-type PBAPs emissions come from cultivated vegetation, predominately olive groves and the vegetable and cereal crops of La Vega, as well as ornamental vegetation which make up urban forests and can be considered one of the main sources of pollen emissions in the urban environment due to the presence of several wind-pollinated species such as those of genus *Cupressus, Platanus, Populus, Acer, Ulmus* and *Fraxinus* (Díaz de la Guardia et al., 2006; Cariñanos et al., 2016).

The main anthropogenic emission sources come from traffic, diesel heating systems, and biomass burning (agricultural waste burning) (Titos et al., 2014, 2017). Mineral material from African dust events should also be included as they are increasingly frequent during the spring and summer (e.gLyamani et al., 2005; Guerrero-Rascado et al., 2009), but some of these dust events might also occur in winter (Cazorla et al., 2017). Due to the diversity and intensity of the emission sources, the concentrations of some pollutants sometimes exceed the limit values established by the European Union Air Quality Standards (Casquero-Vera et al., 2016, 2019). There are continuous episodes of air pollution in the city. This ranks Granada among the most polluted cities in Spain in terms of nitrogen oxides, together with Madrid and Barcelona, which have a significantly higher populations and significantly higher industrial activity (Report of the Evaluation of Air Quality in Spain, 2019; Casquero-Vera et al., 2019).

2.2. Bioaerosol sampling

Aerobiological records were obtained from a volumetric suction Hirst-type sampler (Hirst, 1952) Lanzoni VPPS 2000(Lanzoni, s.r.l., Bologna, Italy), installed in the Faculty of Sciences, Fuentenueva Campus of the University of Granada (37.18°N, 3.61°W, Fig. 1). This sampler actively collects PBAPs by sucking a constant flow of air of 10 l/min, uninterrupted 24 h per day, 365 days per year. The qualitative and quantitative analysis by optical microscopy of the samples allows for the identification of the different pollen types and the expression of the results in pollen grains/m^3 on daily basis (Galán et al., 2007). From these results, the values of Annual Pollen Integral (APIn) of each year, obtained by summing the daily concentrations of all pollen types (Galán et al., 2017) and the Seasonal Pollen Integral (SPIn) of the eight most prominent pollen types in the city's atmospheric spectrum (Cupressaceae, *Olea*, *Pinus*, *Platanus*, Poaceae, *Populus*, *Quercus* and Urticaceae) were calculated for the period between 1992 and 2018. In the case of the SPIn, the daily pollen concentrations recorded during the Main Pollen Season (MPS) have been considered (García-Mozo et al., 2009).

2.3. Meteorological and air pollution data

The daily average of relative humidity (RH), total daily precipitation (Pr), daily global radiation (G) and diffuse radiation (D), daily maximum and minimum temperature (T_M, T_m), wind gust speed (W_{GS}) and wind gust direction (W_{GD}), Hours of Insolation (I), and water vapor pressure (e), measured at the station located at Armilla Air Base (37.81° N, 3.38° W), 7 km southwest of the location of the aerobiological sampler (Fig. 1), were supplied by the State Meteorological Agency (AEMET) for the same data period as the pollen dataset, from 1992 to 2018.

Data on the main gaseous atmospheric pollutants (CO, NO, NO_x, NO_2, O_3, SO_2) and particles (PM_{10}) measured in the Air Quality Surveillance and Control Network Cabinet located in the Palacio de Congresos of the city of Granada (37.16°N, 3.69°W, Fig. 1), were supplied by the Environmental Information Network (REDIAM) of the Counselling of Environment and Territorial Planning of the Junta de Andalucía. The available series covers from 2009 to 2018.

2.4. Statistical analysis

The analysis of trends of the pollen-type PBAPs, the main air pollutants, and the meteorological variables throughout the series was carried out with the Mann Kendall Trend Test (Kendall, 1955). The potential relationship existing among the daily pollen variables and the daily mean values of meteorological factors and atmospheric pollutants was analysed with Spearman's non-parametric correlation tests in order to highlight the strength and direction of the association between pairs of variables. Artificial Neural Networks (ANN), specifically a multilayer perceptron (MLP) (Fausset, 1994; Haykin, 1994), was also used to analyse the relative importance of a set of meteorological variables on the APIn and SPIn of the eight pollen taxa considered. In a simplified way, ANN is a computational model that mimics somewhat the learning processes of a human brain, generating relationships between interrelated input variables, which cross a neural network where operations are combined and performed, producing output values organized according to the value of Normalized Relative Importance (NRI) that they have on the pollen type considered (Suppl. Mat. Fig. 1). In the selection of the input variables, the results previously obtained by the Spearman correlation were taken into account. More details of this tool can be obtained in Alados et al. (2004, 2007). The statistical analysis was carried out using the SPSS 25 software.

In the case of pollutants, as it was not possible to run ANN because of the smaller and insufficient number of valid cases to train the network, the independent variables (pollutants) were adjusted to Generalized Linear Models (GLMs) with Poisson distribution and logarithmic link function (Dalgaard, 2008). For each dependent variable (total Pollen and each of the 8 pollen types concentrations), several models were created, selecting those that best fit the following criteria: 1) the model did not include variables which showed multicollinearity, verified by the Variance Inflaction Factor (VIF), understanding multicollinearity as VIF> 5; 2) the best model that performed according to the Akaike Information Criterion (AIC); and 3) the model that presented the highest Deviance (D^2), understood as how much of the variability of the variable is explained by the model. All the GLM models were carried out using R Software (R Core Team, 2019).

3. Results

The results are presented in Fig. 2 with the descriptive statistics for APIn (mean, minimum, and maximum values along with the variability coefficient, defined as the ratio between the difference of the maximum and minimum values divided by the mean value). Fig. 3 shows the boxcharts by year for the total pollen concentrations. During the 1992–2018 series, the APIn obtained an average value of 58316 pollen*day/m^3 (Suppl. Mat. Table 1), with values that have ranged from 31259 pollen*day/m^3 in 1999–101767 pollen*day/m^3 in 2018. The year 2018 is also the one with the highest number of cases between the 25th-75th interquartile range (Fig. 3), followed by 2007 and 2009. The years 1995, 1999, 2003, 2009, 2011 and 2015 are the ones with the highest number of outliers in the entire series. The 8 pollen types considered in this study, namely Cupressaceae, *Olea, Pinus, Platanus*, Poaceae, *Populus*, *Quercus* and Urticaceae, represent 92% of the total pollen that was recorded in the atmosphere of Granada in the period analysed. Particularly, *Olea* comprised 36% of total atmospheric pollen and Cupressaceae comprised 30%. Other taxa, such as *Populus* and *Pinus*, comprised just 2% to the total pollen spectrum. The variability coefficient ranged from less than 130% for Urticaceae to almost 300% for *Pinus*.

Detailed analysis of the SPIn for each taxon and for every year (Supplementary Material Table 1) revealed that 2018 was the year in which the records were the highest for *Olea, Populus,* and *Pinus*. Poaceae and Urticaceae registered the maximum SPIn in 2004, *Platanus* in 2008, and Cupressaceae and *Quercus* in 2009. On the contrary, pollen records were the lowest in 1994–1995 for most taxa, except for *Populus* and *Platanus* (both in 2004), Urticaceae in 2012, and *Quercus* in 2013.

The statistical Mann-Kendall test applied to the quasi-climatological pollen series indicated that, with the exception of Urticaceae, the trends were positive for almost all pollen types, although not significant trends ($p > 0.05$) were obtained in the case of *Platanus*, Poaceae, and *Populus* (Supplementary Material Fig. 2). In the case of total pollen, the increase throughout the series is 1461 pollen*day/(m^3·year) (2.5% annual increase). In the case of Cupressaceae, the increase is 616 pollen*day/(m^3·year) (3.4%), in the case of *Olea* 780 pollen*day/(m^3·year) (3.7%), and in the case of *Pinus*, 62 pollen*day/(m^3·year) (5%). Urticaceae lost 2.3% of pollen*day/(m^3·year) throughout the analysed period.

The meteorological variables (Fig. 4) showed important interannual variations, in particular precipitation (Pr) with a variability coefficient of 134%, resulting from a maximum value of 665.1 mm in 1996 and a minimum of 181 mm in 2005. The meteorological factor with the lowest variability was wind gust speed (W_{GS}), 14%, with speeds ranging between 7.9 and 9.2 m/s. Maximum temperatures (T_M) and insolation (I) are other variables with low variability coefficients, 15% for both.

The Spearman correlation analysis between the meteorological variables and the APIn of the total pollen and the SPIn of each pollen type (Fig. 5) revealed that the most correlated variables were the daily RH, the Pr, e and T_m,. Precipitation showed negative sign with all pollen types, and RH also negative with all except with Cupressaceae and Urticaceae.T_M, G, D and Iwere the variables that showed the most positive correlation with a greater number of pollen types and with the total. .

Cupressaceae was the one that showed the highest correlation, with all the coefficients above 0.5 for the variables T_m, T_M and G. *Quercus*, with values above 0.3 for e and RH (both with negative correlations) showed the second highest correlation.

The parallel analysis of neural networks, which jointly used all the variables with which the Spearman's analysis had previously shown significant correlation, was utilized as an additional technique to corroborate the results in order to provide importance value of Normalized Relative Importance (NRI), in percentages, to the variables analysed. This complementary technique indicated that G and D radiation, e, and, T_M were the variables with the highest NRI (100%) for all pollen types and total pollen (Table 1). Specifically, e was the variable with the greatest influence for Cupressaceae, *Olea*, *Pinus,* and *Populus*. G

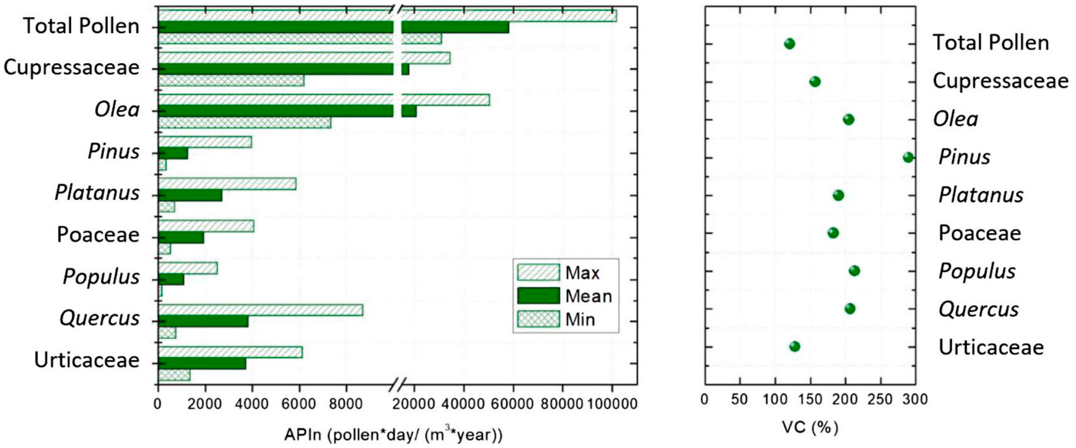

Fig. 2. Mean, extremes values and variability coefficient (VC) (difference between maximum and minimum divided by the mean) of total pollen and of the eight most abundant taxa in the atmosphere of Granada during the period 1992–2018.

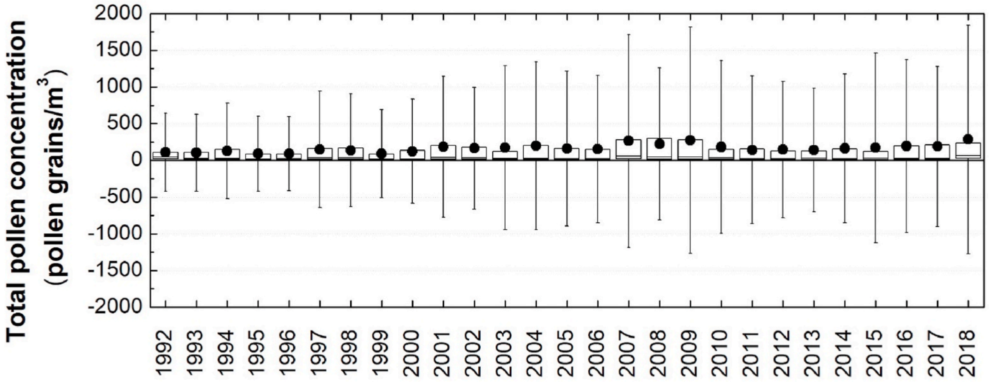

Fig. 3. Box chart of total pollen for each year. The bottom and the top of the box mark the 25th percentile and 75th percentile, respectively. Dot in the box marks the mean and the vertical line marks the standard deviation (SD).

Table 1
Value of the normalized relative importance (NRI) obtained when applying a model of Artificial Neural Networks (ANN) to determine the total pollen and for the eight types of pollen where the set of meteorological variables with level of significance $p < 0.05$ for each type of pollen have been included.

	Total Pollen	Cupressac.	*Olea*	*Pinus*	*Platanus*	Poaceae	*Populus*	*Quercus*	Urticaceae
NRI (%)	G (100.0) D (99.1) T_m (90.2) T_M (85.9) e (85.8) RH (84.1) I (82.1) Pr (82.1) W_{GS} (67.0)	e (100.0) RH (97.8) D (94.9) G (93.2) T_M (90.9) Pr (85.3) I (82.9) T_m (82.5) W_{GS} (70.1) W_{GD} (63.1)	e (100.0) G (91.7) D (89.1) RH (85.2) T_M (81.7) T_m (81.6) I (75.6) W_{GS} (74.2) W_{GD} (59.8)	e (100.0) Pr(86.9) RH (81.4) T_m (76.5) W_{GD} (69.7)	D (100.0) e (92.9) T_M (91.6) RH (85.6) Pr (71.4)	G (100.0) Pr (94.8) e (94.3) T_m (90.6) RH (82.5) I (78.1) W_{GS}(74.6)	e (100.0) RH (98.4) I (93.4) T_M (89.1) T_m (83.8) Pr (69.2)	G (100.0) e (92.8) D (91.8) RH (91.1) T_M (86.5) T_m (85.3) Pr (85.2) I (83.6) W_{GD} (79.5)	G (100.0) e (100.0) T_M (95.4) T_m (95.0) D (93.1) RH (86.7) Pr (85.2) I (83.0) W_{GS} (76.6) W_{GD} (72.3)

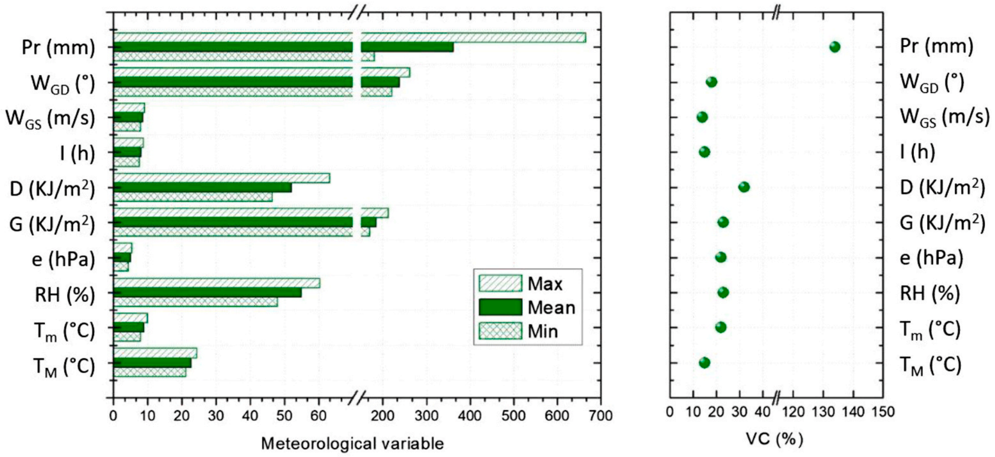

Fig. 4. Descriptive statistics of the meteorological variables (Annual Mean Values) during the period 1992–2018: mean value (Mean), minimum value (Min), maximum value (Max) and variability coefficient (VC). T_M: daily maximum temperature; T_m: daily minimum temperature; RH: daily average of relative humidity; e: water vapor pressure; G: daily global radiation; D: daily diffuse radiation; I: hours of insolation; W_{GS}: wind gust speed; W_{GD}: wind gust direction; Pr: total daily precipitation.

was an influence variable for all pollen types except for *Platanus* and *Pinus*, while D was also an influence variable for all except *Pinus*, *Populus,* and Poaceae. T_M and RH had an influence of over 90% in Cupressaceae, T_M in *Platanus*, Precipitation in Poaceae, RH and I in *Populus*, RH in *Quercus* and T_m and T_M in Urticaceae.

Spearman's correlation analysis between total pollen values and the different taxa with atmospheric pollutants revealed that O_3 was the pollutant with the highest correlation (Fig. 6). Specifically, O_3 was revealed as the pollutant specie with the highest correlation coefficient ($p < 0.01$), with a negative sign for Cupressaceae, *Platanus* and *Populus* and a positive sign for *Olea, Pinus,* Poaceae, *Quercus* and Urticaceae. PM_{10} also appeared to be the first most correlated variable for *Quercus*, *Olea* and Poaceae, all of them with a positive sign. CO was revealed as the most dependent pollutant specie with a positive sign for *Pinus*. Other dependent species were nitrogen dioxide (NO_2) for *Pinus*, Poaceae and *Quercus,* and SO_2 for *Populus* and Urticaceae. Again, Cupressaceae was the taxa with the highest correlation with atmospheric pollutants.

The importance of O_3 as a variable of influence in the APIn and SPIn of the different pollen types was also highlighted by the different GLMs, being present in all models. CO was also a prominent variable, except for *Platanus* and *Populus* (Suppl. Mat Tables 2–10). The multicollinearity existing between nitrogen oxides (NO, NO_x, NO_2) is the reason why each model selects the one that results in the best AIC, that is, the one with the best goodness of fit for the complexity of the model (Table 2). The D^2 of the models ranged from less than 10% in those of *Olea*, Poaceae, *Populus,* and Urticaceae, between 10 and 20% for Total, Cupressaceae, *Platanus*

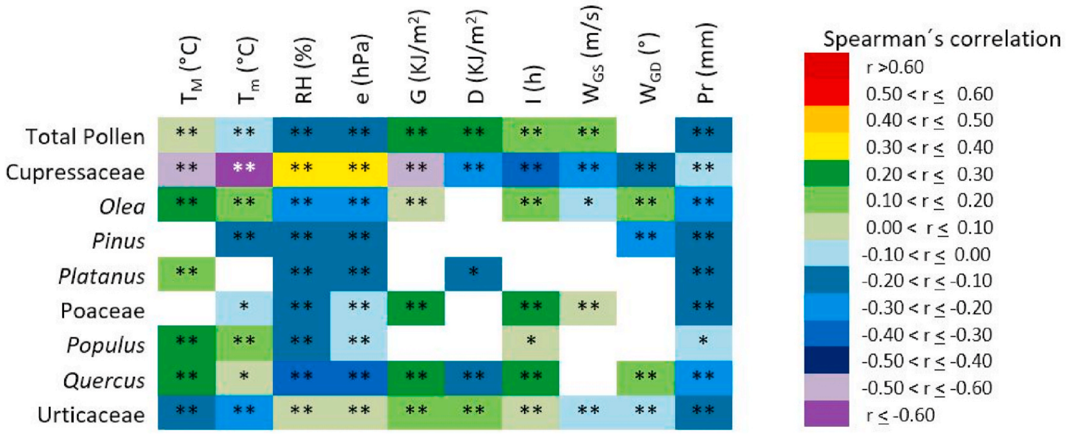

Fig. 5. Analysis of the Spearman's correlation for daily concentrations of total pollen and the eight most frequent pollen-types in Granada with respect to the daily mean values of different meteorological variables. Only the correlations that have obtained a significance value of $p < 0.05$ (*) and $p < 0.01$ (**) are presented.

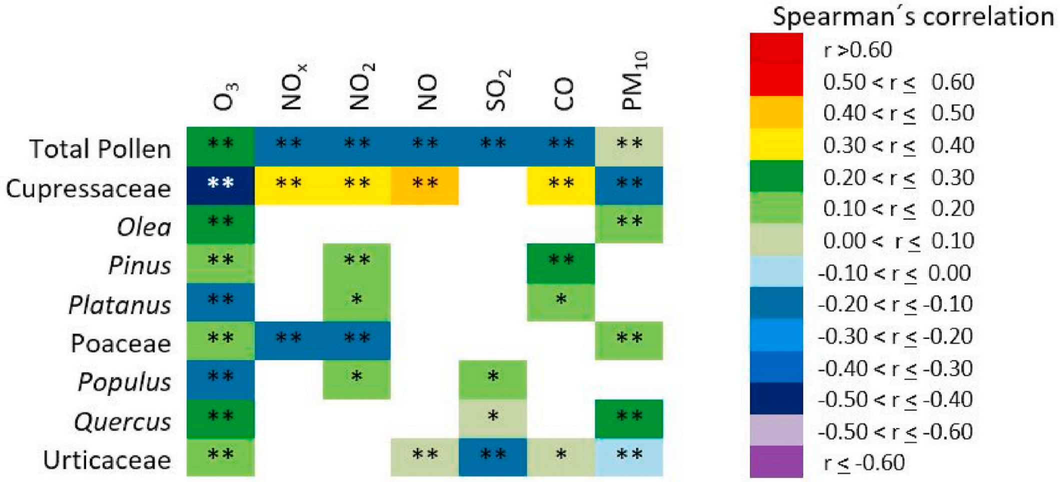

Fig. 6. Analysis of the Spearman's correlation for daily concentrations of total pollen and the eight most frequent pollen-types in Granada with respect to the daily mean values of atmospheric pollutants. Only the correlations that have obtained a significance value of $p < 0.05$ (*) and $p < 0.01$ (**) are presented.

Table 2
Pollutant variables included in the GLM models that best fit the total pollen and the eight pollen types. The coefficients and significance levels of each model are shown in Tables 2–10 Supplementary material. PM_{10} data has been excluded because the series is incomplete.

	Variables in GLMs	AIC	D^2(%)
Total pollen	CO, NO, NO_2, O_3, SO_2	1087628	11.77
Cupresaceae	CO, NO_2, O_3	412660	16.38
Olea	O_3	172586	4.27
Pinus	CO, NO_2, O_3	23680	22.16
Platanus	NO_2, O_3	22394	13.54
Poaceae	CO, NO_x, O_3	18166	3.24
Populus	NO_2, O_3, SO_2	14363	2.64
Quercus	O_3, SO_2	101532	17.07
Urticaceae	CO, NO_2, O_3, SO_2	26742	8.92

and *Quercus*, and more than 20% *Pinus*.

4. Discussion

4.1. PBAPs: trends and variability

The presence of PBAPs in the atmosphere is directly related to an impact on public health, in particular with infectious and respiratory diseases and allergies (Pawankar, 2014; Kim et al., 2018). The quasi-climatological analysis of pollen-type PBAPs in the atmosphere of Granada revealed an average feature of 58316 pollen*day/m^3, so that the concentrations of pollen grains in the city air at certain periods of the year widely exceeded the threshold for triggering symptoms in almost 30% of the population affected by allergic rhinitis, set at 100 pollen grains/m^3 day^{-1} (Waisel et al., 2004; Toro et al., 2015). The pollen spectrum comprised emissions made by urban ornamental vegetation (Cupressaceae, *Platanus*, Urticaceae), natural vegetation (*Pinus, Quercus*, Poaceae) and woody crops from the peri-urban (*Olea, Populus*). The dynamics of these taxa throughout the series was very different, since not only a different response to environmental conditions was found between woody and herbaceous species (Moreno-Gutiérrez et al., 2015; Fei et al., 2017), but also in terms of whether they came from the natural, urban, or peri-urban environment (Nicolau et al., 2005; Bosch-Cano et al., 2011). In other words, in addition to having a similar reaction to general meteorological variables, such as rainfall pattern and temperature, plant species were also subject to environmental conditions more or less modified by human activities, such as the urban microclimate and the presence of atmospheric pollutants (D'Amato, 2011.). This was reflected in the fact that the variability coefficient in the pollen concentrations of the species typical of urban vegetation was lower than that of non-urban species, which withstood greater oscillation in meteorological variables such as temperature and precipitation

(Parmesan and Harley, 2015; Zipper et al., 2016). In this sense, the emissions derived from the pine forests (*Pinus* spp.) and holm oak (*Quercus* spp.) in Sierra Nevada, and those of *Populus* from the peri-urban plantations were the ones with the greatest variability in the SPIn throughout the series (Velasco-Jiménez et al., 2017; Recio et al., 2018).

The analysis of the trends along the quasi-climatological series showed that the amount of total pollen in the atmosphere of the city of Granada increased significantly every year by a ratio of 1500 pollen*-day/(m^3·year), approximately. The two pollen types contributing most to this increase were *Olea* and Cupressaceae. In the case of the olive tree, the increase in the area dedicated to its cultivation in the region, the selection of varieties best adapted to local climatic conditions, and above all the incorporation of irrigation systems on a significant number of hectares has led to its expansion and confirmation as the main woody crop in the area (Sánchez-Martínez and Paniza-Cabrera, 2015; Rodriguez-Sousa et al., 2019). Moreover, it was also as the main agent causing pollen allergies in the Mediterranean population (Gaussorgues, 2009; Ovidiu, 2014). In the case of Cupressaceae, its emission sources can be considered totally urban. The historical use that links the cypresses to the city of Granada, of which they are a symbol of urban and historical identity (Casares-Porcel, 2010), has made it one of the most abundant tree species of urban vegetation with almost 10000 individuals in the elements of urban green infrastructure (Cariñanos 2016, Cariñanos et al., 2019a). This fact positions the city in one of the first Mediterranean cities in terms of percentage of population sensitized to its pollen (Charpin et al., 2013). Its ability to adapt to climatic stress conditions (Brodribb et al., 2014) suggests that in the coming years it will continue to be one of the largest pollen emitters affecting urban air quality.

The rest of the pollen types contribute less to the total spectrum, but they become relevant in seasonal periods. *Quercus* and Urticaceae contribute more than 6% to total atmospheric pollen. Oak pollen is concentrated during the spring (García-Mozo et al., 2008) while Urticaceae (*Parietaria*) is detected in the atmosphere throughout the year, with sufficient levels to rank Granada among the main Mediterranean cities in degree of incidence of this pollen type (Díaz de la Guardia et al., 2006). Less contributory is *Platanus* (4.64%), which nevertheless dominates the pollen spectrum between late winter and early spring and its degree of incidence on the local population has increased by 30 points in recent decades due to its frequent use as an element of Green Infrastructure and its positive response to environmental changes (Cariñanos et al., 2020).

4.2. *Effect of meteorological variables: trends and interactions with PBAPs*

The variability coefficient of the meteorological variables in Granada over the last 30 years has shown that precipitation is the variable that presented the greatest interannual variation, with a difference of more than 130% between the driest and the wettest years. This variability is much higher than the rest of the variables that are below 30%. These changes detected in the rainfall pattern, both in quantity and in the period in which they occur, have already been pointed out as an indicator of the effects of climate change for the Mediterranean region (Giorgi and Lionello, 2008). It is expected that the impact of climate change will be greater for species less adapted to summer drought conditions and for those that are not native to the territory (Gordo and Sanz, 2009). However, in our case it was observed that years of severe drought throughout the series (1993, 1994, 1995, 1998, 2005 and 2015) also had an effect on the pollen levels of native and naturalized species (Cupressaceae, *Olea, Pinus*, Poaceae, *Populus* and *Quercus*). In those years, these species all recorded the lowest SPIn of the entire series (Tabla 1 Suppl. Material). Spearman's correlations between the total APIn and the SPIn of the eight pollen types and the meteorological variables revealed that precipitation is one of the meteorological factors that has the greatest effect on pollen levels, especially in the case of *Olea*, and always with a negative value for the effect of atmospheric washing that rainfall produces (Kluska et al., 2020). Environmental humidity is another factor with a significant, negative correlation for most pollen types, results that coincide with those obtained in other European areas with a temperate continental climate such as Lithuania (Sauliene et al., 2019) or Poland (Puc and Bosiacka, 2011). The sudden changes in environmental humidity are related to the breakdown of the pollen grains, generating sub-pollen particles in the respirable fraction (Behrendt and Becker, 2001; Wang et al., 2011). The pollen from grasses and Cupressaceae are the ones that release the highest amount of submicron allergens (Abou-Chakra et al., 2012; Ruggiero and Bedini, 2018), so during the flowering period they will worsen the air quality together with the intact pollen grains. For other pollen types such as *Olea*, *Platanus,* and *Parietaria*, allergenic activity associated with sub-pollen particles was recorded both before and after the flowering period (Suárez-Cervera et al., 2005; Moreno-Grau et al., 2006).

Temperature was another variable that showed the highest correlation, being the daily T_M in *Platanus* and *Populus*, and the daily T_m in Cupressaceae and Urticaceae. The daily T_M was one of the variables that showed the lowest variability coefficient throughout the series, and its direct relationship with a significant increase in seasonal pollen load has already been highlighted in previous works (Zisca et al., 2019). But it is the variations in the T_m that can have the greatest effect on the reproductive biology of the species, altering the threshold temperature necessary to break the dormancy period of deciduous species (Pletser et al., 2015; Kubik-Komar et al., 2018). The 22% variation registered by the T_m throughout the series represents more than a 1 °C increase in the nearly 30 years considered, in line with the T_m projections for the Mediterranean region (Giorgi and Lionello, 2008). This impact may be more important for winter flowering species, such as Cupressaceae, or early spring species such as *Platanus* and *Populus* (Cariñanos et al., 2020).

The neural network analysis indicated that e and G were the two variables that had the most influence on pollen concentrations. Water vapor pressure (e) was the first factor of the set of variables for *Olea*, *Pinus,* and *Populus*, all of them tree species from the peri-urban natural environment of the city of Granada, in agricultural or forest formations. This factor highlights the relationship between meteorological variables, since water vapor pressure patterns are related to temperature and atmospheric moisture transport (Ellison et al., 2017). This is of great importance in the Mediterranean climate where the water regime can be compensated with a supplementary contribution of orographic precipitations (favoured by the proximity of Sierra Nevada) (Costa et al., 2010). Global radiation was another variable of influence, especially for Poaceae, *Quercus,* and Urticaceae. The greater the amount of solar radiation that plants receive, the greater stimulation in biological activity. This includes higher amounts of diffuse radiation as well, since plants are able to take advantage of the diffuse component in the visible range, the so-called photosynthetic radiation (Foyo-Moreno et al., 2018), which translates into a higher potential rate of productive growth (Amthor, 2010) and bioaerosol emission (Jones and Harrison, 2004). In fact, the partitioning of global radiation in its components and the relationship between them is of great interest in many disciplines, e.g. for predicting canopy photosynthetic productivities (Foyo-Moreno et al., 2014). In a study that analysed the effects of different meteorological variables on pollen emission and dispersion in central Spain, it was found that solar radiation and hours of insolation have a positive influence on pollen counts even during the post-peak period (Rojo et al., 2015), especially for *Quercus* and Poaceae.

4.3. *Effect of pollutants: trends and interaction with PBAPs*

Understanding the relationship between pollen-type PBAPs and atmospheric pollutants is particularly interesting in the city of Granada, since the strong impact of urban pollution over the city (Casquero-Vera et al., 2016; Report of the Evaluation of Air Quality in Spain, 2019). Although since 2010 air quality policies have been implemented to control and reduce pollution (National Air Quality Plan, 2017-2019, 2017), O_3 levels continued to show a slight upward trend throughout the series (Ceballos, 2018), followed by NO_x (Casquero-Vera et al., 2019). Hence, one of the most novel results of our study is the fact that O_3 showed the highest level of correlation with the different pollen types and total pollen. This was also supported by the results of the GLMs. Various studies have already highlighted the relationships that may exist between ozone levels and pollen concentrations Adhikari et al. (2006); Rahman et al. (2019), frequently with a negative sign due to the possible negative effect that O_3 can have on plant growth (Ziello et al., 2012), and on the intrinsic allergenicity of pollen grains (Albertine et al., 2014; Frank and Ernst, 2016). A positive relationship has resulted for *Olea, Quercus*, and *Pinus*. Some studies highlight the O_3 tolerance of the Mediterranean evergreen broadleaves forests (*Quercus*) due to their sclerophilic leaves, low exchange rates, and their ability to tolerate oxidative stress (Paoletti and Grulke, 2005; Paoletti, 2006), as well as the emission of BVOCs of these species which protect them from the effects of atmospheric O_3 (Kesselmeier and Standt, 1999). Moreover, *Pinus sylvestris*, a species present in Sierra Nevada, has been indicated as one of the most tolerant to concentrations of environmental O_3 (Paoletti, 2006). Some mechanisms of tolerance to O_3 have been also observed in olive trees, in which an adaptative mechanism is developed according to atmospheric O_3 concentrations for the opening and closing of stomata (Sebastiani et al., 2002). The relationship between *Olea* pollen and O_3 is confirmed by the GLM model, in which only O_3 has participated as a variable.

Nitrogen oxides, NO_2 in particular, is a pollutant that has been shown to have a direct adverse effect on human health (Boningari and Smirniotis, 2016), but also on plants and their pollen (Frank and Ernst, 2016). Some studies with pollen from three species of the Betulaceae family have shown that germination and soluble proteins in the exine of pollen grains are affected by NO_2 exposure in vitro even below the current atmospheric hour-limit value acceptable for human health protection (Cuinica et al., 2014). These modifications can increase pollen allergenicity, both due to the nitration experienced by some of the main allergenic proteins (Shiraiwa et al., 2012), and due to the increased expression of some of them by degrading the pollen structure (Zhao et al., 2015). A similar effect occurs when various pollen grains are subjected to low CO concentrations, where after exposure a change is observed in the water-soluble proteins and the antigenicity of the pollen grains (Ruffin et al., 1986). In Granada, NO_2 atmospheric concentrations still often exceed the European Union air quality standard limit of 40 $\mu g/m^3$ (Casquero-Vera et al., 2019). Paradoxically, some of the species included in this study are recommended for their good performance in urban air pollution abatement (Barwise and Kumar, 2020), but in this consideration the trade-offs associated with their implementation are not valued (Grote et al., 2016). For example, *Populus* and *Quercus* are intense emitters of isoprene, which in the presence of sufficient levels of NO_2 can contribute to the formation of ozone (Churkina et al., 2015). The monoterpenes and sesquiterpenes emitted by *Pinus* are in turn related to the formation of secondary organic aerosols (Préndez et al., 2014).

SO_2 is another pollutant whose primary emission source is the burning of fossil fuel and the production of energy in thermoelectric plants (Titos et al., 2014; Casquero-Vera et al., 2016) and whose presence in the atmosphere in values that exceed 20 $\mu g/m^3$ 24-hr are already considered harmful to animal and plant life (WHO, 2010). In the city of Granada, during the study period, the levels of this pollutant were very low due to the low industrial activity in the city (Casquero-Vera et al., 2016). However, several of the pollen types considered presented a correlation with this pollutant although very low. In the case of *Populus*, the relationship was positive which would confirm that species of this genus have greater tolerance to moderate concentrations of SO_2, decreasing the conductance in the leaves by closing the stomata (Linzon, 1972; Kimmerer and Kozlowski, 1981). The results for Urticaceae differ from those obtained in Serbia, where no significant relationship was found between the pollen levels of Urticaceae and those of SO_2 (Sabo et al., 2016). This would confirm the good adaptability of this plant species to the urban environmental conditions of Granada, where it stands out both for its continuous presence on altered walls and terrains (Yalcinalp and Meral, 2017), and for the presence in the aerobiological records throughout the year (Alba et al., 2003).

Some pollen types, such as *Olea*, Poaceae, and *Quercus,* presented a significant positive correlation with PM_{10} levels. The total pollen count showed this positive correlation as well. Some previous studies had not found a significant relationship between pollen and PM (Adhikari et al., 2006; Sousa et al., 2008), nor found a correlation depending on the pollen type and the levels of environmental contamination (Rahman et al., 2019); while other studies did find a strong correlation between PM and the variation in pollen concentrations (Puc and Bosiacka, 2011; Manninen et al., 2014). The increase in PM_{10} during the pollen season can be explained both by the abundance of biological material in certain periods, in which mechanical breakdown can occur due to the interaction between this material (Laakso et al., 2003; Cariñanos et al., 2004), and by the release of micron-sized allergens not associated with pollen grains. In some pollen types, such as Poaceae, these sub-micron particles can cause symptoms in the sensitized population up to 15 days after flowering ends (Cariñanos et al., 2020), and be present in different-sized particles (De Linares et al., 2009; Plaza et al., 2016). *Quercus* subpollen particles, derived from carbohydrate fragments that have intact pollen grains, have shown to have the ability to activate as cloud condensation nuclei (CCN) due to the nanometric size they can have (Steiner et al., 2015), which would also have an impact on the meteorology.

5. Conclusions

The results achieved in this work highlight the significant, but complex and ambiguous, influence that meteorological conditions and atmospheric pollutants have on atmospheric pollen of different plant species present in urban environments. In relation to meteorological variables, rainfall stands out as one of the most influential variables. This is very relevant in the Mediterranean region in which this study is located due to the expected effects of climate change. The influence of other meteorological factors, such as radiation and water vapor pressure, are also relevant which can provide more information on the behaviour of pollen emissions under changing environmental conditions. The positive response of most taxa to the meteorological variations that have occurred in the last 30 years suggest a positive response to the impacts of climate change expected for the area, mainly in temperature and precipitation. As for atmospheric pollutants, O_3 and NO_2 have turned out to be the pollutants with the highest correlation with pollen concentration, reinforced by the upward trend that both have experienced in the area in recent years. This information reveals not only the health impact that interactions between atmospheric pollutants and PBAPs can cause, but also the tolerance of some plant species to the moderate presence of these elements in the atmosphere. Also important are the contributions to the PM content that the presence of high amounts of pollen can make, derived from its degradation and breakdown during atmospheric transport.

It should be noted that of all the pollen types considered in this work, *Cupressus* showed the greatest sensitivity, highlighted by a significant correlation of a negative sign with most of the meteorological variables, and also with O_3. Given its relevant presence in the green infrastructure of many Mediterranean cities and landscapes, and its high capacity to emit allergenic pollen, it is to be expected that its role as an allergen will continue to be very relevant in the future.

Faced with a climate future in which important effects on the reproductive biology of plants in urban environments are expected, the results of this work can help to understand the impact that pollen emissions can have on urban air quality, and on public health, particularly in those areas that have been declared the most vulnerable to the effects of climate change.

Credit author statement

PC: Conceptualization; Data Curation; Formal analysis; Investigation; Writing-original draft; Writing-review&editing. IFM: Data Curation; Formal analysis; Writing-original draft. IA: Data Curation; Formal analysis; Writing-original draft. JLGR: Data Curation; Formal analysis; Writing-original draft. SRP: Data Curation; Formal analysis. GT: Validation; Writing-review&editing. AC: Validation, Writing-review&editing. LAA: Writing-review&editing. CDG: Investigation; Methodology.

Declaration of competing interest

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.

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Appendix A. Supplementary data

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List of acronyms

PBAPs: Primary Biological Airborne Particles

ANN: Artificial Neural Networks

GLM: Generalized Linear Models

AEMET: State Meteorological Agency

REDIAM: Environmental Information Network

MLP: multilayer perceptron

APIn: Annual Pollen Integral

SPIn: seasonal Pollen Integral

NRI: Normalized Relative Importance

VIF: Variance Inflaction Factor

AIC: Akaike Information Criterion

D: Deviance

TM: daily maximum temperature

Tm: daily minimum temperature

RH: daily average of relative humidity

E: water vapor pressure

G: daily global radiation

D: daily diffuse radiation

I: hours of insolation

WGS: wind gust speed

WGD: wind gust direction

Pr: total daily precipitation