

Thirty-year survey on airborne pollen concentrations in Genoa, Italy: Relationship with sensitizations, meteorological data, and air pollution

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ABSTRACT

Background: Pollen allergy represents a relevant health issue. Betulaceae sensitization significantly increased in Genoa, Italy, in the last decades. This study investigated possible relationships among pollen count, meteorological changes, air pollution, and sensitizations in this city during a 30-year period.

Methods: Betulaceae, Urticaceae, Gramineae, and Oleaceae pollen counts were measured from 1981 to 2010 in Genoa. Sensitization to these pollens was also considered in large populations of allergic patients. Meteorological parameters and pollutants were also measured in the same area.

Results: Betulaceae sensitization increased over time. All pollen species significantly increased over this time. Pollen season advanced for Betulaceae and Urticaceae. Only Urticaceae season significantly increased. Temperature increased while rainfall decreased over the time. Pollutants significantly decreased. There were some relationships between pollen changes and climatic and air pollution parameters.

Conclusion: This 30-year study conducted in an urbanized area provided evidence that Betulaceae sensitization significantly increased, pollen load significantly augmented, and climate and air pollution changed with a possible influence on pollen release.

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A recent review pointed out that pollen allergy has a remarkable clinical impact all over Europe.¹ Moreover, there is evidence that the prevalence of pollen-induced allergies has increased in the past decades.^{2,3} At present, the prevalence of pollen allergy is up 40% as recently reported.¹ Therefore, there is growing interest in this area of research.

The increased prevalence of allergic disorders has created the new term, "Allergy Epidemic." Several factors may have contributed to this phenomenon such as changes in climate after direct or indirect human actions, modifying plant characteristics and their distribution.^{4,5} In addition, weather changes have modified the pollen distribution and the duration of the pollen season. Another interfering factor may be air pollution because it has been outlined that it might affect the allergenic content of plants by increasing pollen production and modifying allergenic proteins of pollen grains.⁶ Furthermore, the antigenic characteristics of pollens may be modified by the interaction between fine particulate matter (PM₁₀) and pollen.⁷ Many taxa exist, but allergenic pollens are relatively few and their clinical relevance depends on several factors, including allergenic specificity, physical characteristics, quantity, air pollution, and climatic conditions of a specific geographic area. The Mediterranean area is characterized by temperate climate with mild winters, cool summers, and moderate rain. Several epidemiological surveys evidenced some allergenic plants that are more frequent causes of respiratory allergies: *Parietaria*, grasses, cypress, olive tree, birch and other trees (including other Betulaceae and Corylaceae), and Compositae.⁸ In this regard, a conspicuous increase in the percentage of patients sensitized to Betulaceae has been observed in Genoa in the last decades. Indeed, the first survey performed about 50 years ago, showed that only 7.5% of patients with pollen allergy was sensitized to Betulaceae.⁹ On the contrary, most recent surveys, conducted in Genoa, Italy, provided evidence that the percentage of patients sensitized to Betulaceae

progressively overgrew up to 50% of pollenosis patients.^{10–12} From a clinical point of view, it is relevant the association between increased sensitizations frequency and increased prevalence of both asthma and rhinitis. For these reasons, this study aimed at evaluating the trend of pollen count, addressing our attention on the four most relevant families (*i.e.*, Urticaceae, Gramineae, Oleaceae, and Betulaceae), during the 30-year period of 1981–2010 in Genoa. The purpose was to detect possible relationships among the clinical phenomena, such as sensitization, pollen count, meteorological data, and air pollution in this area.

MATERIALS AND METHODS

Clinical Data

Five periods were considered: 1981–1983, 1987–1990, 1997–2000, 2003–2005, and 2006. For this purpose, the case study reported in previous published studies has been used.^{12–16} The inclusion criteria, for all studies, were a new diagnosis of respiratory allergy (*e.g.*, rhinitis and/or asthma) and pollen sensitization. Sensitization was evaluated by skin-prick test. Skin-prick test was performed as stated by the European Academy of Allergy and Clinical Immunology.¹⁷ The panel consisted of grasses mix (*Phleum p*, *Dactylis g*, *Poa a*, *Lolium s*, and *Festuca e*), *Parietaria* mix (*Parietaria officinalis* and *Parietaria judaica*), birch, hazel, and olive tree (using extracts provided by Lofarma, Milan, Italy, or Stallergenes Italia, Milan, Italy). The San Martino

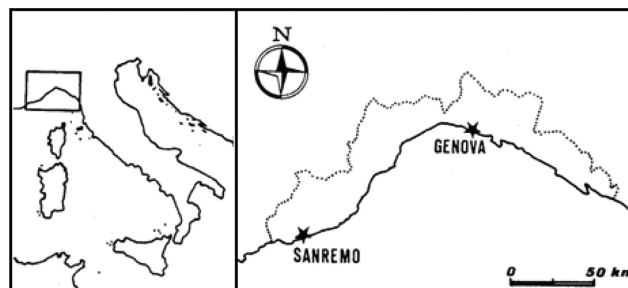


Figure 1. Localization of Genoa in northwestern Italy.

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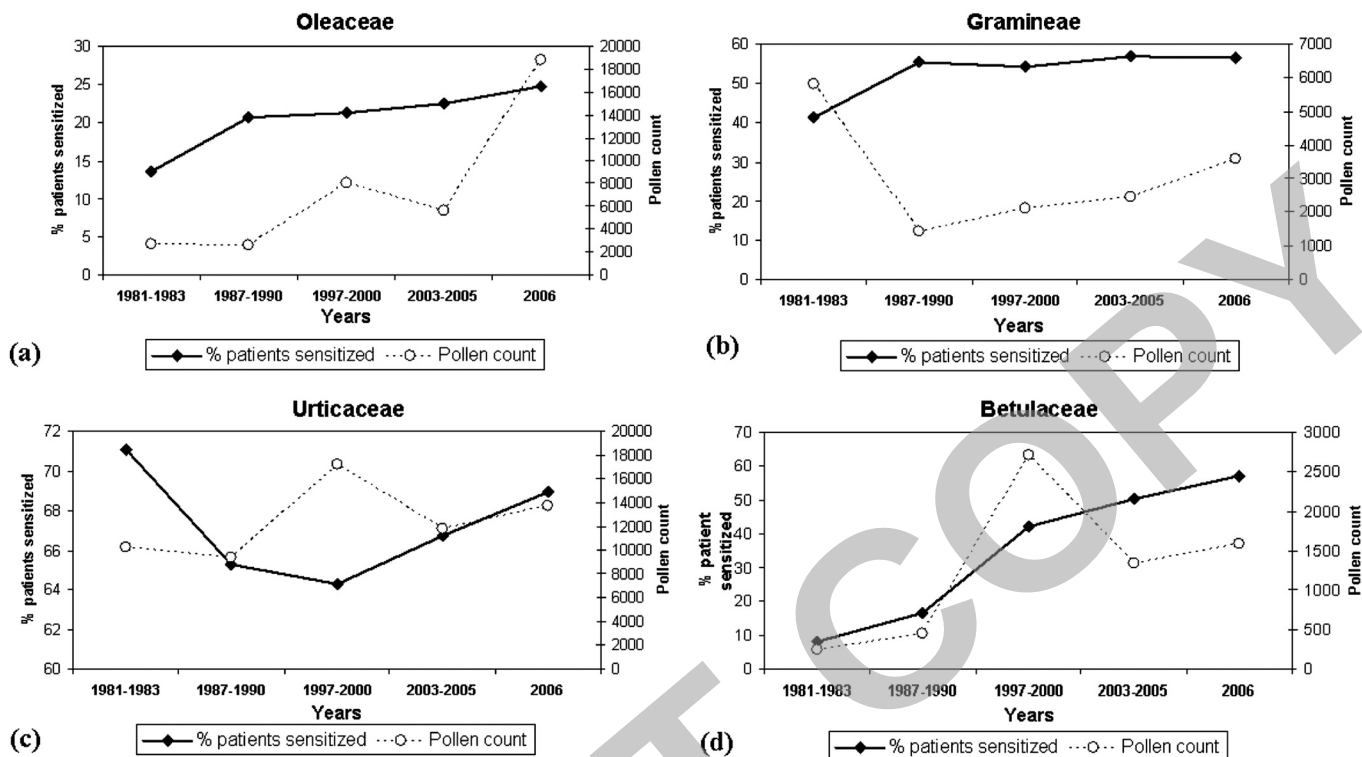


Figure 2. Trends, across the years, of percentages of sensitized patients and pollen count, evaluating separately (a) Oleaceae, (b) Gramineae, (c) Urticaceae, and (d) Betulaceae. Percentages of sensitized patients were available only for the year aggregations shown in the picture. Thus, even if pollen count was available throughout the years, in this picture only the counts for those year aggregations are reported.

hospital Review Board approved the study and an informed consent was obtained by each subject.

Aerobiological Data

Aerobiological data pertained to pollination period and pollen concentrations, recorded in the same period. The monitoring of pollens was performed at the survey center of San Martino Hospital concerning the first 20 years, and then further data were collected by Agenzia Regionale per la Protezione dell'Ambiente Ligure, according to standard methods of the Italian Aerobiology Association, now also recognized as UNI 11108. Both places are near and the collecting and reading procedures were the same. Airborne pollens were collected using a Burkard 7-day recording volumetric spore trap, standard equipment used for aerobiological sampling worldwide. This spore trap was placed 20 m above ground level and away from sources of air pollution.

The city of Genoa, with 612,872 inhabitants, lies near the Ligurian Sea (20 m above sea level; latitude, 44°25' N and longitude 8°56' E) and is in northwestern Italy (Fig. 1).

The choice of pollen species was based on two criteria: (i) they had to be the most important pollen types causing sensitizations and allergic symptoms in the people living in this geographic area and (ii) they had to be present in the atmosphere, every year, in relatively high or significant concentrations. For these reasons, four pollen families were measured: Gramineae, Betulaceae, Urticaceae, and Oleaceae.

Gramineae included *Phleum p*, *Dactylis g*, *Poa a*, *Lolium s*, and *Festuca e*. Betulaceae included *Betula alba*, *Alnus*, and *Corylaceae* (such as *Corylus a* and *Carpinus a*).

Urticaceae included *P. judaica* and *P. officinalis*.

Oleaceae included the olive tree.

For each taxon, 52 mean weekly pollen concentration values and peaks (grains/m³ of air) were recorded and the sum of 52 weekly

pollen concentration values gave an estimate of the annual amount of each pollen species. These four major allergenic families (Betulaceae, Gramineae, Oleaceae, and Urticaceae) were regularly recorded between January 1, 1981, and December 31, 2010.

Start, end, and duration of each season pollen season were also calculated for each species. The beginning of pollen season was established as the first 3 consecutive days with at least 10 grains/m³. Equally, the end of each pollen season was defined when there were 3 consecutive days with <10 grains/m³. Duration of pollen season was calculated from start to end as stated previously.

Meteorological Data

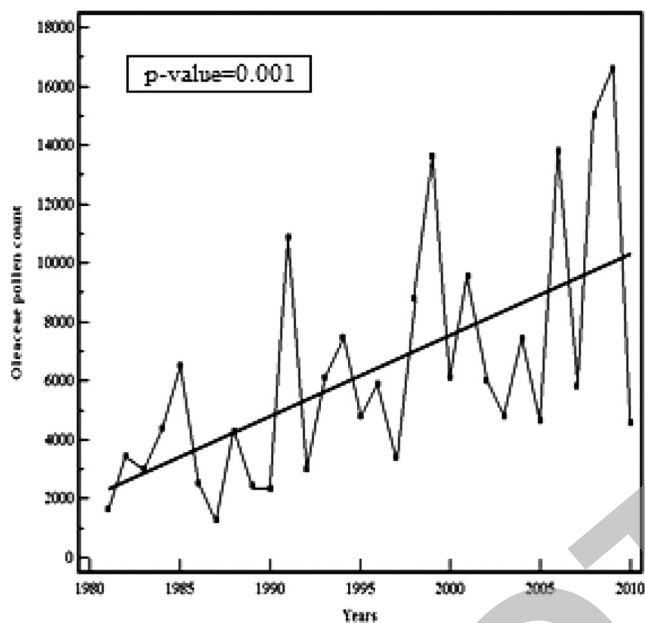
Meteorological data included the yearly average values for temperature (°C), rainfall (mm), relative humidity (%), dew point (°C), visibility distance (km), and wind speed (km/h). All of the data were collected and managed by "Il Meteo srl" (provided by Air Force Observatory of Genova Sestri Ponente). In particular, "visibility distance" and dew point correspond well with the humidity of the atmosphere.

Air Pollution Data

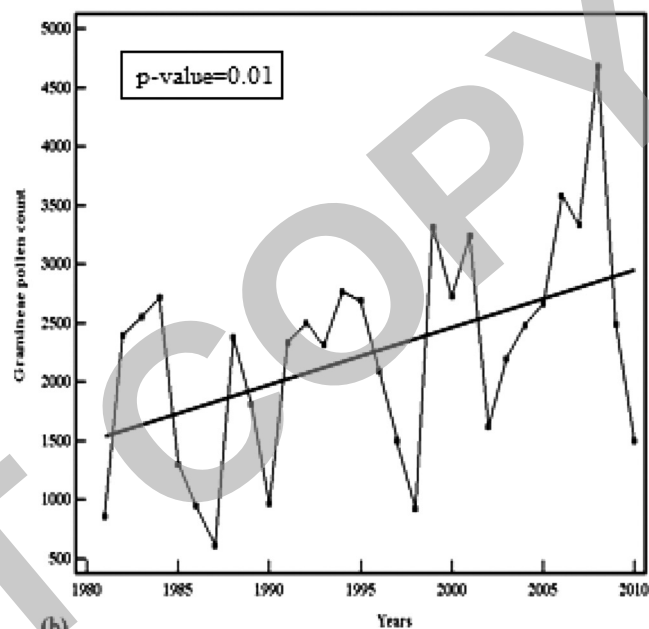
Air pollution data included the yearly (from 1993 to 2010) average values for carbon monoxide (CO; µg/m³), sulfur dioxide (SO₂; µg/m³), nitrogen oxide (NO; µg/m³), nitrogen dioxide (NO₂; µg/m³), ozone (O₃; µg/m³), and PM₁₀ (µg/m³). All of the data were collected and managed by the Environment and Territory Department of the "Provincia di Genova" (Observatory of Genova Quarto).

Statistical Analysis

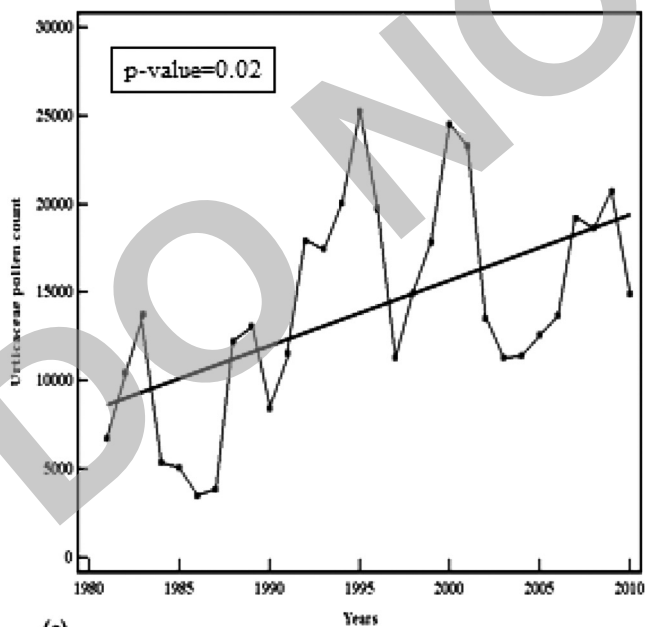
Correlation between quantitative variables was evaluated by means of the Spearman's correlation coefficient (ρ), because data



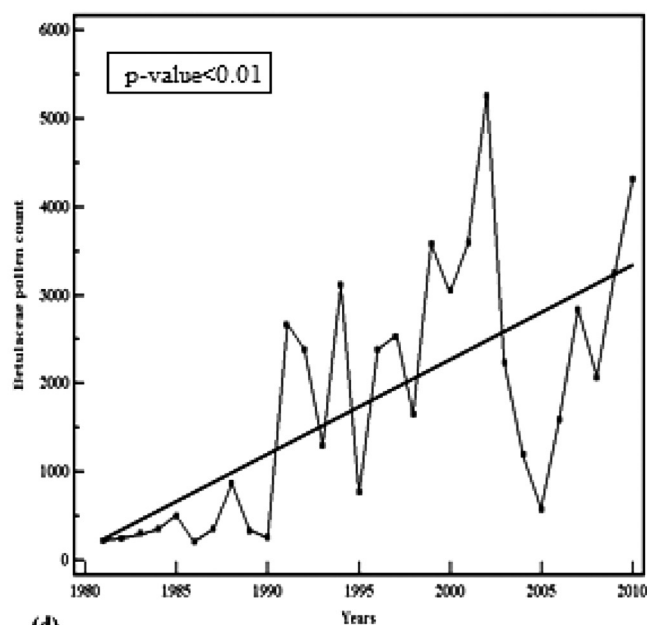
(a)



(b)

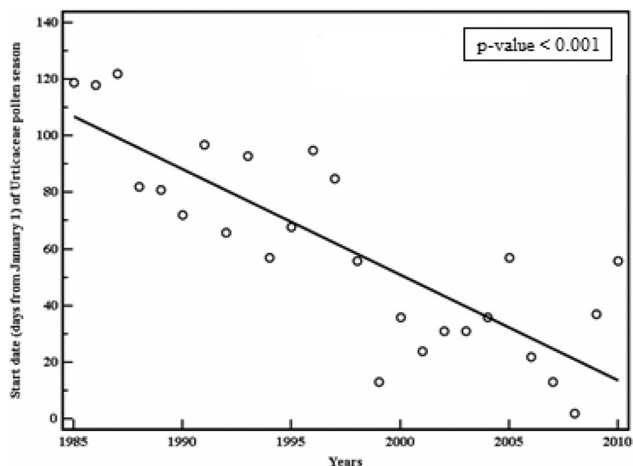


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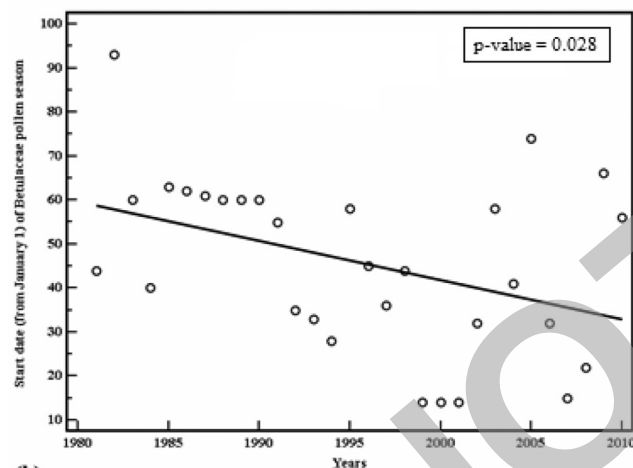


(d)

Figure 3. Trends, throughout the years, of pollen counts for (a) Oleaceae, (b) Gramineae, (c) Urticaceae, and (d) Betulaceae, expressed with p value.



(a)



(b)

Figure 4. Trends, throughout the years, of the pollen season start date for (a) Urticaceae and (c) Betulaceae (c).

were not normal distributed. Regression was used to describe temporal trends. Moreover, the association between total pollen count and pollen season and meteorological and air pollution data was estimated by multiple linear regression analysis to find independent predictors of the count. All tests were two sided and a value of $p < 0.05$ was considered statistically significant. The software Medcalc 9 (MedCalc Software, Mariakerke, Belgium) was used for all the analyses.

RESULTS

Patients and Sensitizations

During the considered periods, the data concerning 10,466 allergic patients (aged 5–64 years; 56% male patients) were included in the database. The percentage of patients sensitized to the considered species significantly increased only for Betulaceae allergens; however, an increasing trend was observed also for Gramineae and Oleaceae. Figure 2 shows the trends, across the years, of the percentages of sensitized patients and the relative pollen counts, evaluating separately each allergenic species.

Pollen Count and Pollen Season

As shown in Fig. 3, pollen counts significantly increased throughout the years for Oleaceae ($p = 0.001$), Gramineae ($p = 0.01$), Urticaceae ($p = 0.002$), and Betulaceae pollens ($p < 0.01$).

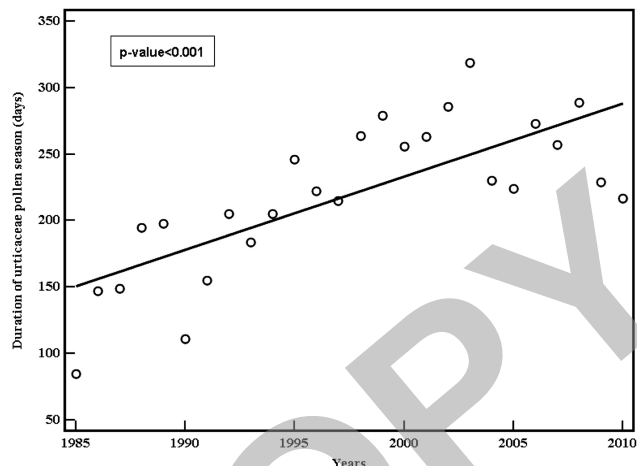


Figure 5. Trend, throughout the years, of duration of the pollen season for Urticaceae, expressed with p value.

The pollen season start date, such as the first 3 consecutive days with at least 10 grains/ m^3 since New Year's Day, tended to be in advance (such as to begin earlier) during the years for Urticaceae ($p < 0.001$; Fig. 4 a) and Betulaceae ($p = 0.028$; Fig. 4 b), but not for Oleaceae and Gramineae.

The duration of the pollen seasons for Urticaceae progressively increased ($p < 0.001$; Fig. 5) over time. The duration of the pollen seasons was approximately unchanged for Gramineae, Oleaceae, and Betulaceae.

Meteorological Data

Temperature ($p = 0.003$; Fig. 6 a), dew point ($p = 0.029$; Fig. 6 b), visibility distance ($p = 0.003$; Fig. 6 c), and wind speed ($p = 0.003$; Fig. 6 d) significantly increased, whereas rainfall significantly decreased ($p = 0.015$) throughout the years, as shown in Fig. 6 e.

There were significant correlations between temperature and total pollen count for Urticaceae ($p = 0.015$; $\rho = 0.449$; Fig. 7 a), Betulaceae ($p = 0.011$; $\rho = 0.472$; Fig. 7 b), and Oleaceae ($p = 0.05$; $\rho = 0.36$; Fig. 7 c).

There were significant correlations between wind speed and total pollen count for Urticaceae ($p = 0.0347$; $\rho = 0.392$; Fig. 8 a) and for Betulaceae ($p = 0.0383$; $\rho = 0.385$; Fig. 8 b).

There were significant correlations between visibility distance and total pollen count for Urticaceae ($p = 0.0001$; $\rho = 0.705$; Fig. 9 a), Betulaceae ($p = 0.0021$; $\rho = 0.572$; Fig. 9 b), and Oleaceae ($p = 0.0017$; $\rho = 0.583$; Fig. 9 c).

There were significant correlations for Urticaceae between duration of pollen season and temperature ($p = 0.0073$; $\rho = 0.537$; Fig. 10 a), dew point ($p = 0.0113$; $\rho = 0.507$; Fig. 10 b), wind speed ($p = 0.0012$; $\rho = 0.649$; Fig. 10 c), and visibility distance ($p = 0.0123$; $\rho = 0.501$; Fig. 10 d).

Air Pollution Data

Across the years, SO_2 ($p = 0.012$; Fig. 11 a), CO ($p = 0.003$; Fig. 11 b) and PM_{10} ($p = 0.004$; Fig. 11 c) significantly decreased.

There was a significant negative correlation for Gramineae between total pollen count and SO_2 ($p = 0.0498$; $\rho = -0.490$), as shown in Fig. 12.

There was a significant positive correlation for Urticaceae between total pollen count and NO_2 ($p = 0.0173$; $\rho = 0.577$), as shown in Fig. 13.

No correlations were found between air pollution data and pollen season duration data.

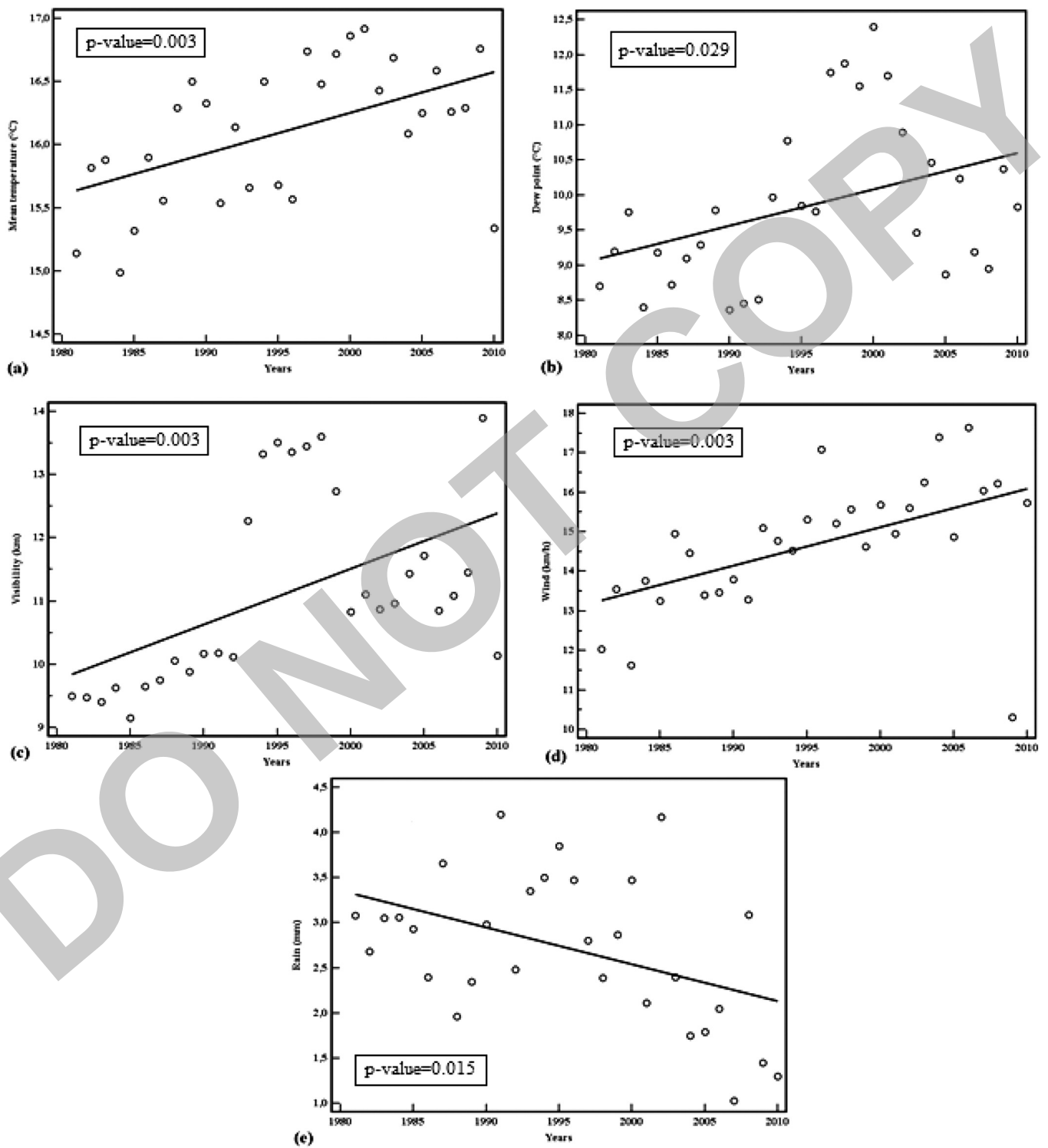
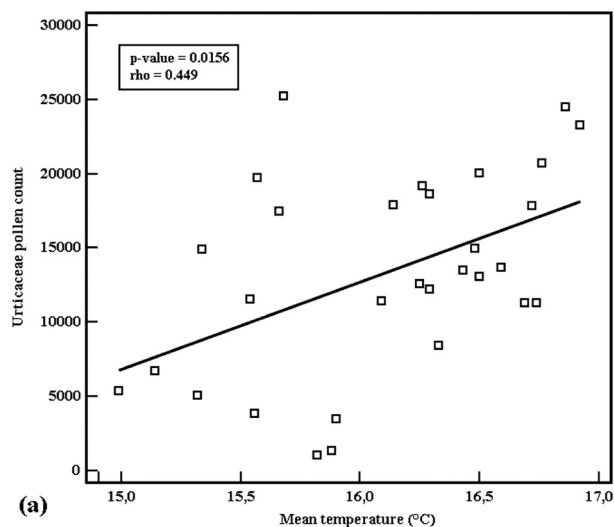
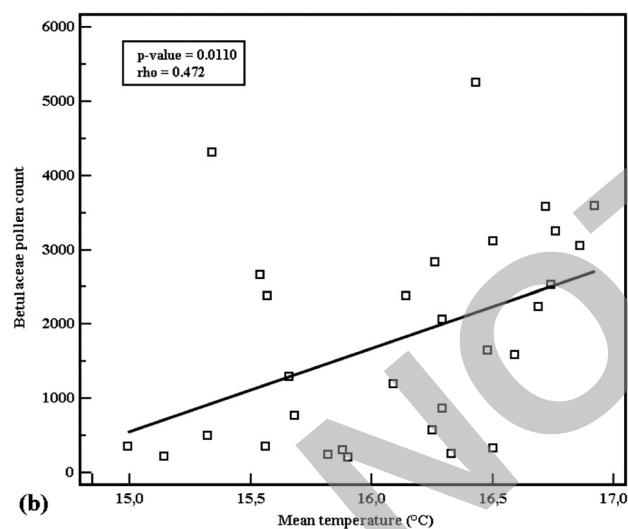


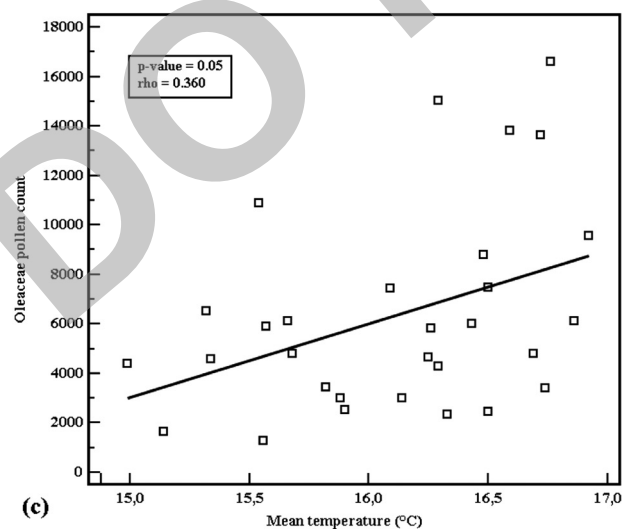
Figure 6. Trends, throughout the years, of (a) temperature, (b) dew point, (c) visibility distance, (d) wind speed, and (e) rainfall, expressed with p value.



(a)

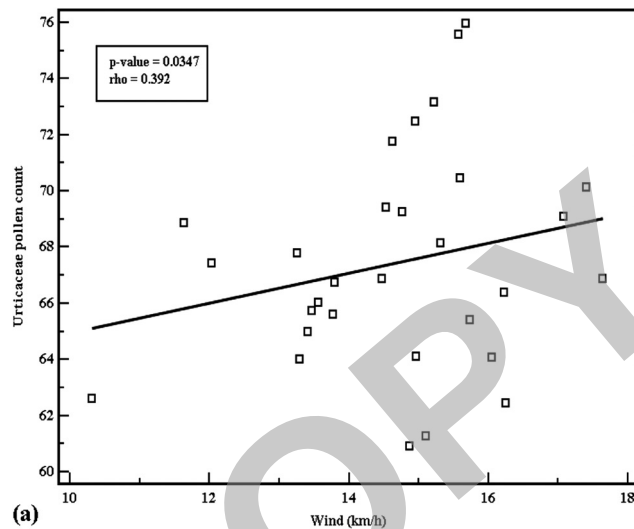


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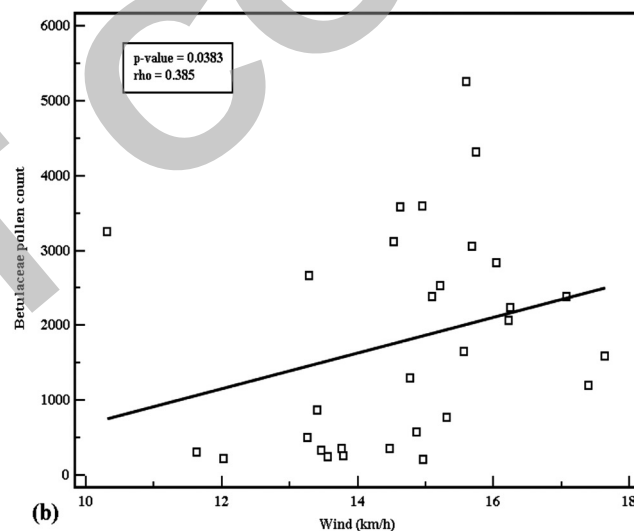


(c)

Figure 7. Correlations, expressed with Spearman's correlation coefficient (ρ) and p value, between temperature ($^{\circ}\text{C}$) and total pollen count (grains/ m^3) evaluated separately for (a) Urticaceae, (b) Betulaceae, and (c) Oleaceae.



(a)



(b)

Figure 8. Correlations, expressed with Spearman's correlation coefficient (ρ) and p value, between wind speed (km/h) and total pollen count (grains/ m^3) evaluated separately for (a) Urticaceae and (b) Betulaceae.

Multiple Regression Analysis

Total pollen count for Oleaceae was significantly correlated with CO , SO_2 , and temperature, after correction for rainfall, and the best ($p = 0.042$) multivariate model combined these variables (see Table 1). Total pollen count for Gramineae was significantly correlated with CO , PM_{10} , SO_2 , duration of the pollen season, dew point, temperature, and humidity and the best ($p < 0.001$) multivariate model combined these variables (Table 2). Moreover, total pollen count for Betulaceae was significantly ($p = 0.034$) associated with duration of the pollen season, NO , CO , and SO_2 , after correction for humidity (Table 3).

DISCUSSION

Theoretically, any plant can cause an allergic reaction. In reality, only about 30 major botanical families worldwide are recognized as causing clinical symptoms, such as pollenosis, in the majority of patients.¹⁸ Leaving aside the question of possible cross-reactivity between different plants, the individual allergenic species have been identified (e.g., *Plantago lanceolata*), or the genus (e.g., *Parietaria* and *Ambrosia*), or the family (Gramineae). The main aller-

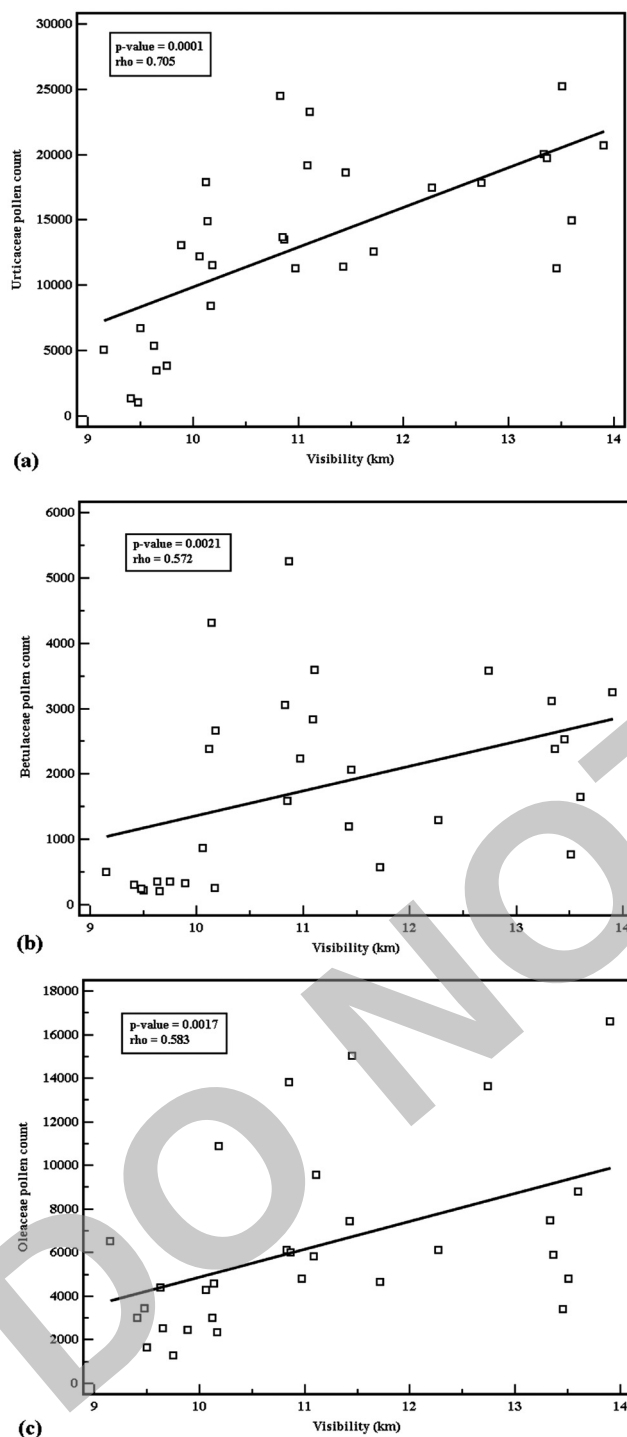


Figure 9. Correlations, expressed with Spearman's correlation coefficient (ρ) and p value, between visibility distance (km) and total pollen count (grains/m³) evaluated separately for (a) Urticaceae, (b) Betulaceae, and (c) Oleaceae.

genic agents causing pollenosis vary from region to region according to the environment, the vegetation, and the climate of each specific geographic area. Nonetheless, Gramineae are the most important cause of pollenosis worldwide, whereas *Parietaria* and *Olea* are predominant in the Mediterranean area.^{19,20}

Pollen calendars are very familiar and are consulted by clinicians as well as by patients for comprehending and defining temporal rela-

tionship (*i.e.*, the cause-effect link) between pollen exposure and symptom occurrence.

Previously, a 5-year aerobiological study (1984–1988) concerning the principal allergenic pollens in Italy has been reported.¹⁸ This survey provided evidence that pollenosis in Italy differed from pollenosis in other parts of Europe. In addition, a relevant increase of sensitization to Betulaceae and Corylaceae has been previously reported in the last decades in the Genoa area.^{10,11,21,22} Therefore, this 30-year study has been performed for better defining this phenomenon and for establishing possible relationships among pollen count, meteorological data, air pollution, and sensitization.

This study provided several interesting findings. First, all four families significantly increased the pollen production during the observation period, despite that only the percentage of birch-sensitized patients significantly increased over time. Some possible explanations might be proposed: (i) Betulaceae trees were intensively planted around Genoa to promote the reforestation in the previous decades, (ii) birch allergen exerts intense proinflammatory allergic response,²³ (iii) birch-allergic patients show more severe symptoms than other pollen-sensitized subjects,²⁴ and (iv) air pollution might have increased the expression of allergenic proteins (*i.e.*, thaumatin-like) that could determine an increase of sensitizations.

Second, the pollen seasons of Urticaceae, Gramineae, and Betulaceae significantly came early, and the Urticaceae season also was significantly prolonged. These findings could have a relevant impact on duration of symptoms over time.

Third, in the Genoa area the temperature, dew point, and visibility distance (expressions of air humidity), and wind speed significantly increased over time, whereas rainfall significantly diminished. Warm temperature associated with high humidity allowing plant growth as well as sustained wind may favor pollen dispersion. All of these findings also confirm the well-known worldwide weather changes. More interestingly, these weather changes significantly affected pollen counts for all families but Gramineae. However, it is noteworthy that grass pollens are relatively scarce in Genoa, probably because of the intense urbanization of the city and the geographic localization, such as near the sea.

Fourth, air pollution parameters significantly diminished over time; it was the result of rigorous restrictions of urban traffic and local antipollution laws. In addition, NO₂ levels were positively related to Urticaceae pollen counts, this fact might partially confirm a previous experimental study that evidenced that *Phleum pratense* pollen releases more granules when exposed to increasing concentrations of such pollutants.²⁵ On the other hand, sulfur dioxide was negatively related to Gramineae pollen counts; it may be that this pollutant might exert inhibiting effects on biological activities of some vegetables. However, these considerations basically derive from pure statistical relationships without a real indication of a cause-effect link between air pollution changes and pollen counts. Thus, further experimental studies should confirm these possible effects exerted by pollutants on pollens. However, the multivariate analysis allows hypothesizing that several combined factors (both climatic and pollutants) can affect pollen counts, mainly for Gramineae ($R^2 = 0.96$), but also fairly for Betulaceae ($R^2 = 0.66$) and less for Oleaceae ($R^2 = 0.38$).

Therefore, the present study provided relevant information about a possible link between environmental factors and humans. Particularly, sensitization prevalence may depend on allergen load as well as pollen counts may depend on both climate and air pollution.

The present study, conducted on a highly urbanized area, furthermore confirms two previous recent Italian studies. The first study was conducted in a relatively small town, Parma, surrounded by wide rural zone and some industry; it reported a significant decrease of grasses.²⁶

The most recent study was performed in Bordighera—a tourist coast village, characterized by very few industry and intensive

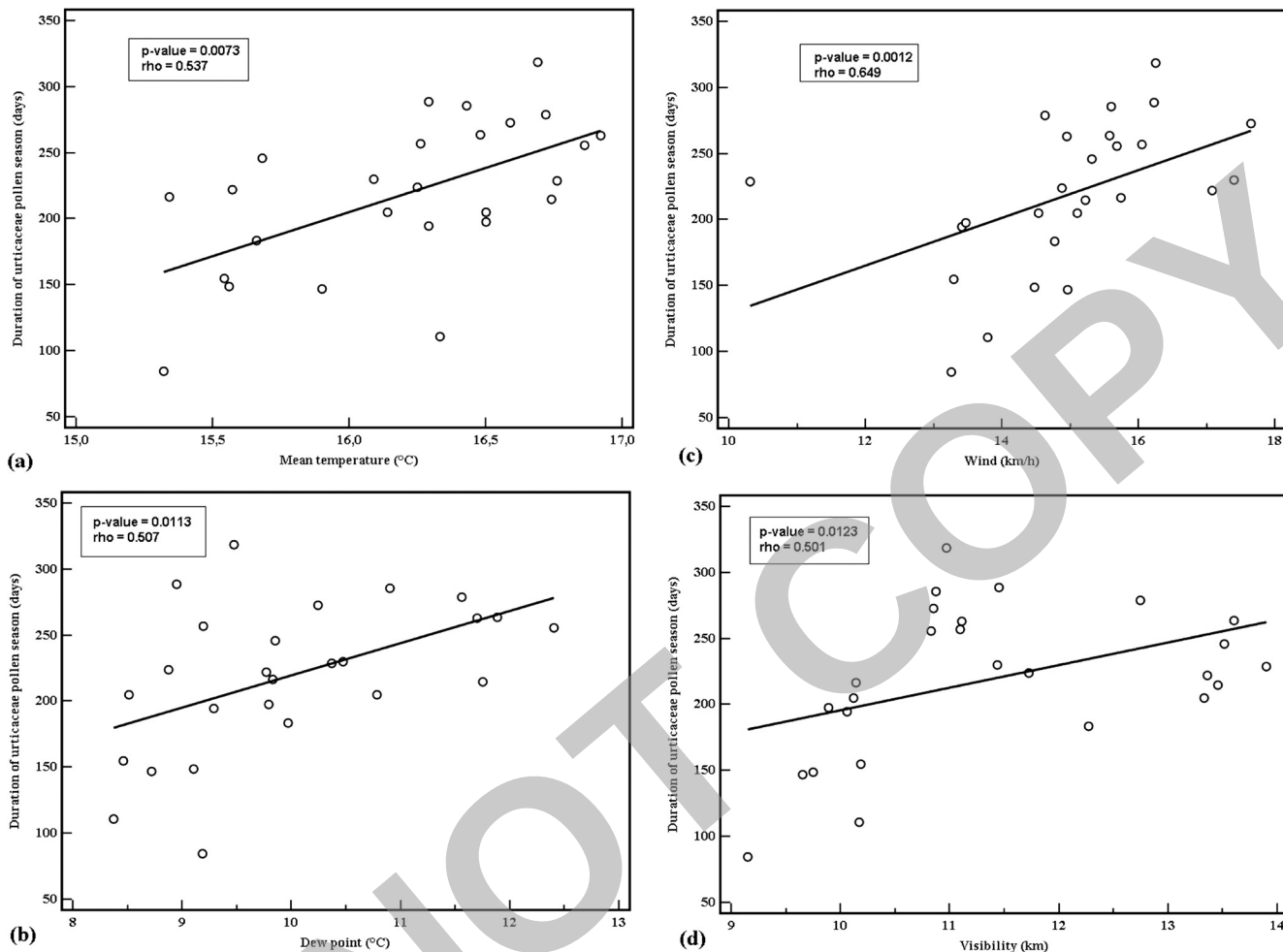


Figure 10. Correlations, expressed with Spearman's correlation coefficient (ρ) and p value, between duration of pollen season for (a) Urticaceae (days) and temperature, (b) dew point, (c) wind speed, and (d) visibility distance.

flower growing and olive growing, overlooking the Ligurian sea and ~150 km from Genoa.²⁷ That study reported that there was a progressive increase in the duration of *Parietaria*, olive, and cypress pollen seasons; also, the total pollen load progressively increased for the considered species except for grasses; sensitized patient percentages increased over time; these changes paralleled the constant increase in direct radiation, temperature, and number of days with >30°C temperature.²⁷ The data concerning duration, beginning, and end of pollen seasons of both studies are substantially consistent, even though *Parietaria* season doubled in Genoa area. However, pollen counts and sensitization data, reported by the

Bordighera study,²⁷ are partially different in comparison with the present study. Probably, there are substantial geographical, climatic, and environmental (mainly concerning the air pollution) differences between the two evaluated areas. Nevertheless, the present study provided further data on pollution and gave new findings on the possible effects of some pollutants on some pollen species.

Table 1 Multivariate analyses of factors associated with total pollen count for Oleaceae

Variable	Coefficient	Standard Error	t	p Value
Constant	-92068.01			
Carbon monoxide	-6637.27	2965.26	3.19	0.0077
Temperature	6470.85	2026.1	-2.24	0.0449
Sulfur dioxide	-486.09	213.15	-2.3	0.0416
Rainfall	1924.98	1233.21	1.561	0.1445

Model adjustment: $R^2 = 0.3827$.

Overall, $p = 0.042$.

Table 2 Multivariate analyses of factors associated with total pollen count for Gramineae

Variable	Coefficient	Standard Error	t	p Value
Constant	-32387.76			
Carbon monoxide	-610.86	184.78	-3.31	0.013
Sulfur dioxide	-141.32	17.95	-7.87	0.0001
Temperature	2012.16	189.1	10.64	<0.0001
Humidity	237.41	28.93	8.21	0.0001
Dew point	-1305.17	125.76	10.38	<0.0001
Duration of the pollen season	7.9	2.84	2.78	0.0272
PM ₁₀	35.85	7.04	5.09	0.0014

Model adjustment: $R^2 = 0.9635$.

Overall, $p < 0.001$.

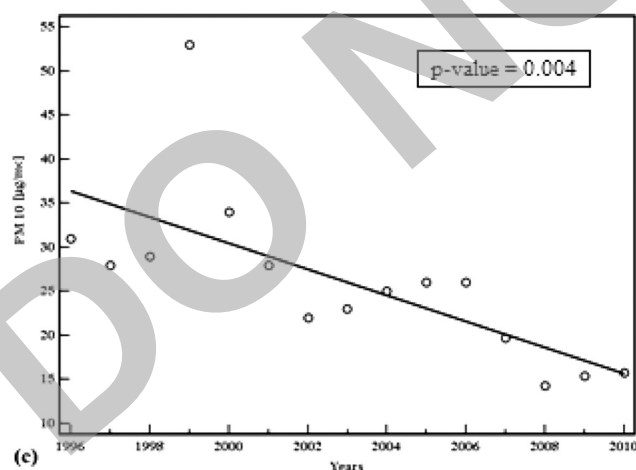
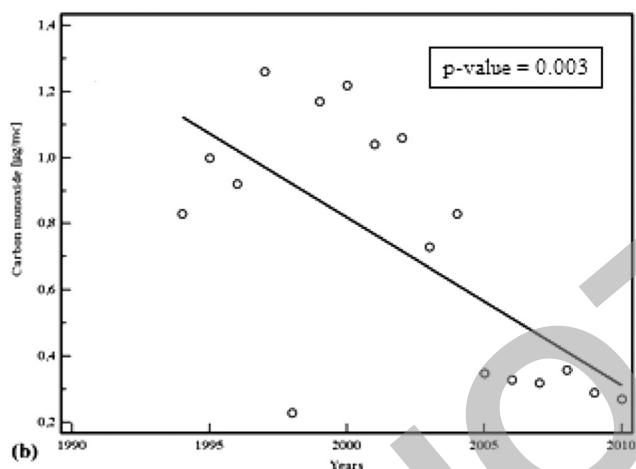
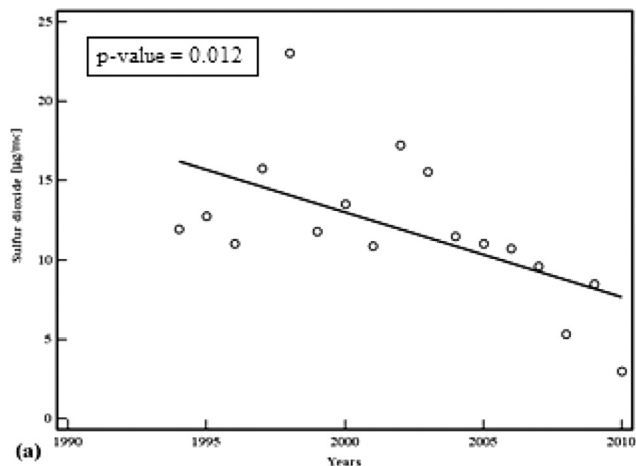


Figure 11. Trends, during the years, of (a) sulfur dioxide, (b) carbon monoxide, and (c) PM₁₀, expressed with *p* value.

Therefore, this study might support the concept that pollens are increasing in some areas and this fact might justify the increase of sensitizations for some species, also climate and air pollution might exert additional effects. In this regard, some recent studies investigated this issue. A study by Frei and Gassner, conducted in Switzerland, found a possible causal relationship between the decrease of hay fever prevalence (after 1992–1993) and a reduced pollen exposure caused by decreasing pollen counts.²⁸ A further airborne Poaceae pollen count study of four Galician cities in Spain

Table 3 Multivariate analyses of factors associated with total pollen count for Betulaceae

Variable	Coefficient	Standard Error	<i>t</i>	<i>p</i> Value
Constant	4324.29			
Carbon monoxide	17.02.78	780.84	2.18	0.0542
Duration of the pollen season	35.93	10.15	3.54	0.0053
Nitrogen oxide	284.37	98.23	2.90	0.016
Sulfur dioxide	193.41	84.57	2.29	0.045
Humidity	−152.45	84.99	−1.79	0.1031

Model adjustment: $R^2 = 0.6561$.

Overall, $p = 0.034$.

by Jato *et al.* reported a trend toward lower annual total Poaceae pollen counts, lower peak values, and a smaller number of days in which counts exceeded 30, 50, and 100 pollen grains/m³.²⁹ Another study reviewed five trials evaluating the relationship between aeroallergens and climate changes; the findings confirmed the impact of these parameters on health outcomes in children.³⁰ Finally, a more recent study reflected a strong relationship to climate change and pollination of ragweed in North America.³¹ Of course, further studies should be performed to confirm these outcomes.

In addition, a further step should investigate the effect of these changes on clinical symptoms. In this regard, a recent review article discussed these issues and provided a series of recommendations about new studies.³² The present research added new information about some requested aspects.

In conclusion, this survey conducted in an urbanized area provided evidence that some sensitization significantly increased, pollen load significantly augmented, and climate and air pollution changed with a possible influence on pollen release.

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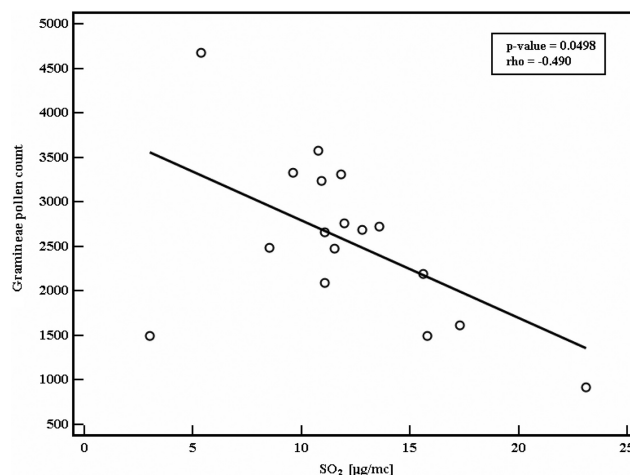


Figure 12. Correlations, expressed with Spearman's correlation coefficient (ρ) and *p* value, between total pollen count for Gramineae (grains/m³) and sulfur dioxide (µg/m³).

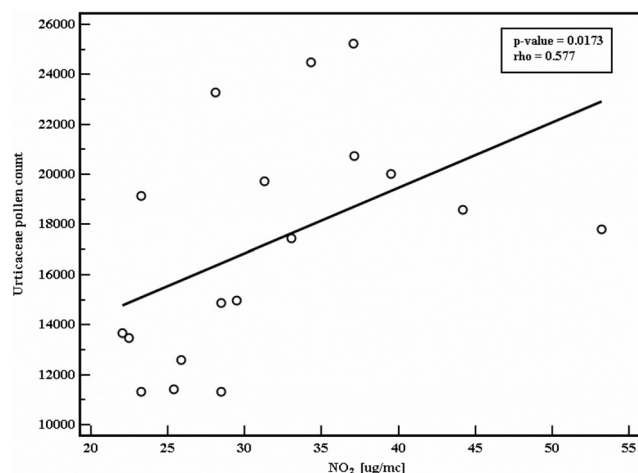


Figure 13. Correlations, expressed with Spearman's correlation coefficient (ρ) and p value, between total pollen count for Urticaceae (grains/ m^3) and nitrogen dioxide ($\mu\text{g}/\text{m}^3$).

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