Annals of Agricultural and Environmental Medicine 2022, Vol 29, No 2, 232–237
www.aaem.pl

**ORIGINAL ARTICLE**



# Traffic-related $NO_2$ affects expression of *Cupressus sempervirens* L. pollen allergens

**Fabrizio Mattei[1,B,E], Gianni Della Rocca[2,B,E], Giovanna Schiavoni[1,B], Elena Paoletti[3,A,E], Claudia Afferni[1,A,C–F]**

[1] *Istituto Superiore di Sanità, Italy*
[2] *Institute for Sustainable Plant Protection, National Research Council (IPSP) – Consiglio Nazionale delle Ricerche (CNR), Italy*
[3] *Institute of Research on Terrestrial Ecosystems (IRET) – Consiglio Nazionale delle Ricerche (CNR), Italy*
*A – Research concept and design, B – Collection and/or assembly of data, C – Data analysis and interpretation,*
*D – Writing the article, E – Critical revision of the article, F – Final approval of the article*

Fabrizio Mattei, Gianni Della Rocca, Giovanna Schiavoni, Elena Paoletti, Claudia Afferni. Traffic-related $NO_2$ affects expression of *Cupressus sempervirens* L. pollen allergens. Ann Agric Environ Med. 2022; 29(2): 232–237. doi: 10.26444/aaem/146607

**Abstract**

**Introduction and Objective.** Traffic pollution has been recognized as directly worsening respiratory symptoms of allergic subjects, although whether urban air pollutants can also directly increase the allergenic potential of pollen has not yet been definitely proven. Therefore, the hypothesis that intra-urban air $NO_2$ variation influences allergens expression in *Cupressus sempervirens* (*Cs*) L. pollen was tested.

**Material and methods**. Mature microsporophylls were cut from *Cs* trees of similar age and height (14–17 m) present in three different sites of Florence (Italy) and processed in the laboratory. *Cs* pollen allergens amount was determined by a semi-quantitative analysis of electrophoretically separated pollen extracts fractions. $NO_2$ air concentrations were recorded by air monitoring stations located at a distance not exceeding 50 m from each pollen collection site, and the relative annual mean values were acquired by a publicly available database (Tuscan Regional Agency for Environment Protection).

**Results**. Expression of three major *Cs* pollen allergens was non-linearly correlated with mean annual $NO_2$ concentrations. Expression peak of all major allergens considered was reached at $NO_2$ air concentration (67µg/m$^3$), far below the value at risk for direct effect on the respiratory health (European Union Directive 2008/50/EC).

**Conclusions.** The findings suggest that intra-urban $NO_2$ variations do affect the expression of *Cs* pollen major allergens, and an apparent low risk $NO_2$ concentration should be regarded as indirectly harmful for increasing the allergenic potential of pollen.

**Key words**
urban air pollutants, $NO_2$, Pollinosis, pollen allergens, *Cupressus sempervirens* L.

## INTRODUCTION

Allergic rhinitis and asthma have a significant economic impact on patients, patients' families and society as a whole [1]. The growing prevalence of allergy also has major economic consequences for society due to absence from education and work or impaired performance, thereby placing a greater burden on healthcare resources and increasing medication costs [1]. For example, mean annual direct and indirect costs due to respiratory allergic diseases in Italy in 2013 were estimated to be 7.34 billion Euros [1]. Although recognized as the result of an interaction between multiple genetic and environmental factors [2], allergies are mainly considered as an environmental disease [3]. Indeed, the dramatic increase in allergy-related diseases observed in the past decades cannot be ascribed only to genetic factors [3]. Allergy to *Cs* pollen is widely diffused in the Mediterranean area [4, 5]. Since major *Cs* allergens are highly cross-reactive with allergens of both species of the same genus and species belonging to other genera (i.e. *Hesperocyparis*, *Platycladus*, *Cryptomeria*, *Chamaecyparis*, *Juniperus*), the prevalence of this allergy can depend on the geographic area and diffusion of allergenic genera [5]. In fact, the prevalence of *Cs* allergy in Italy is 18% of all allergic population, with a 28% prevalence peak in the regions of Tuscany and Umbria [6], and 43% in Lazio region [7] where the presence of *Cs* is significant in both rural and urban areas [8, 9], and it is increasing in metropolitan areas [10].

Several *in vitro* studies have shown that the bioavailability of allergens is directly influenced by air pollution [11]. In particular, recent data [12–16] indicate that exposure of allergenic plants to $NO_2$ gas can increase the expression of pollen allergens and/or the allergenicity of the pollens. Atmospheric $NO_2$ has long been known to be harmful both to plants [17] and humans [18]. To our knowledge, no data on $NO_2$ effect on *Cs* pollen allergenic potential are available so far.

## OBJECTIVES

We hypothesize that *Cupressus sempervirens* L.*(Cs)* plants exposure to traffic-related air pollution, particularly to the $NO_2$ gas component, could be associated with an increased allergenic potential of its pollen. Starting from these premises, this study focusses on assessing whether the *Cs* pollen major allergens were differently expressed in association to traffic-related $NO_2$ air pollutant, comparing pollen samples from differently polluted sites in the city of Florence (Italy).

Address for correspondence: Claudia Afferni, Istituto Superiore di Sanità, Viale Regina Elena n.299, 00161, Rome, Italy.
E-mail: claudia.afferni@iss.it

Received: 15.12.2021; accepted: 10.02.2022; *first published: 06.04.2022*

## MATERIALS AND METHOD

**Pollen collection and quantification.** Pollen of *Cs* was collected during the flowering season of 2011 in the city of Florence, Italy, where *Cupressus sempervirens* L. released pollen from 1 February – 5 April. The peak occurred on 7 March when 8,944 pollen granules/m$^3$ of air were sampled with a pollen catcher (volumetric sampler type Hirst, model VPPS 2000, Lanzoni, Bologna, Italy). The day of sampling, 16 February 2011, corresponded to the beginning of the pollen grains exponential release phase (1,958 granules/m$^3$), when most of the sporophylls were ripe but still sufficiently rich in pollen grains (http://sira.arpat.toscana.it/sira/). To exclude the influence of temporal and climatic variables, all pollen samples were collected on the same sunny day (16 February) at all the city sites. Three city sites characterized by medium-high traffic intensity were selected, in each of which were selected three *Cs* trees (less than 5 m apart from each other) of similar age (30–40 years) and height (14–17 m), planted by municipal services.

Air monitoring stations are located at a distance not exceeding 50 m from each pollen collection site (Fig. 1). The site names, corresponding to the nearest street name, were Bassi, Ponte alle Mosse (Mosse hereafter) and Gramsci. Mapped urban forests characterized by the presence of conifers are present in the indicated sites [19].

Eight twigs per *Cs* tree, bearing mature microsporophylls from the medium and the lower part of the crown were randomly cut from different orientations, placed in plastic bags and processed in the laboratory within two hours. The *Cs* twigs were arranged in vases placed on a sheet of wrapping paper and maintained at 25 °C to collect the shed pollen, as described in Barberini et al. [4]. The collected pollen was sieved with a 300 µm sieve to separate extraneous materials and then dehydrated in a laboratory dryer at room temperature (18–20 °C) using silica gel, until 30–35% of relative humidity (RH) was reached. The pollen was then stored at -20 °C in hermetically-sealed plastic tubes until the analyzes.

In two of the three trees per site, the total amount of microsporophylls and the total pollen produced per square meter of crown was estimated, as in [20] and [21]. Briefly, a flexible square (1 m$^2$) was randomly leaned to the lower part of each tree crown (three replicated squares per tree), and all the twigs bearing microsporophylls inside the square were collected and processed as previously described. The resulting pollen was weighted by a Mettler College 150 Digital Precision Laboratory Balance.

**Pollen proteins extraction.** Pollen grains of *Cs* from each tree and from each city site were separately incubated overnight at room temperature (r.t.) in a rotating stirrer in phosphate buffer saline (PBS) pH 7.4 (10%, w:v) with 1% (v:v) of protease inhibition mixture (Halt protease Inhibitor Cocktail 87785; Thermo Fisher Scientific, Waltham (MA), USA) and 0.001% (v:v) of ethylenediaminetetraacetic acid (EDTA).

After centrifugation (18,000 g, 4 °C, 20 minutes), the supernatant was collected and stored in aliquots at -20 °C until use. *Cs* pollen grains were purchased (Allergon AB; Valingevagen 309, SE-262 92 Angelholm, Sweden) and

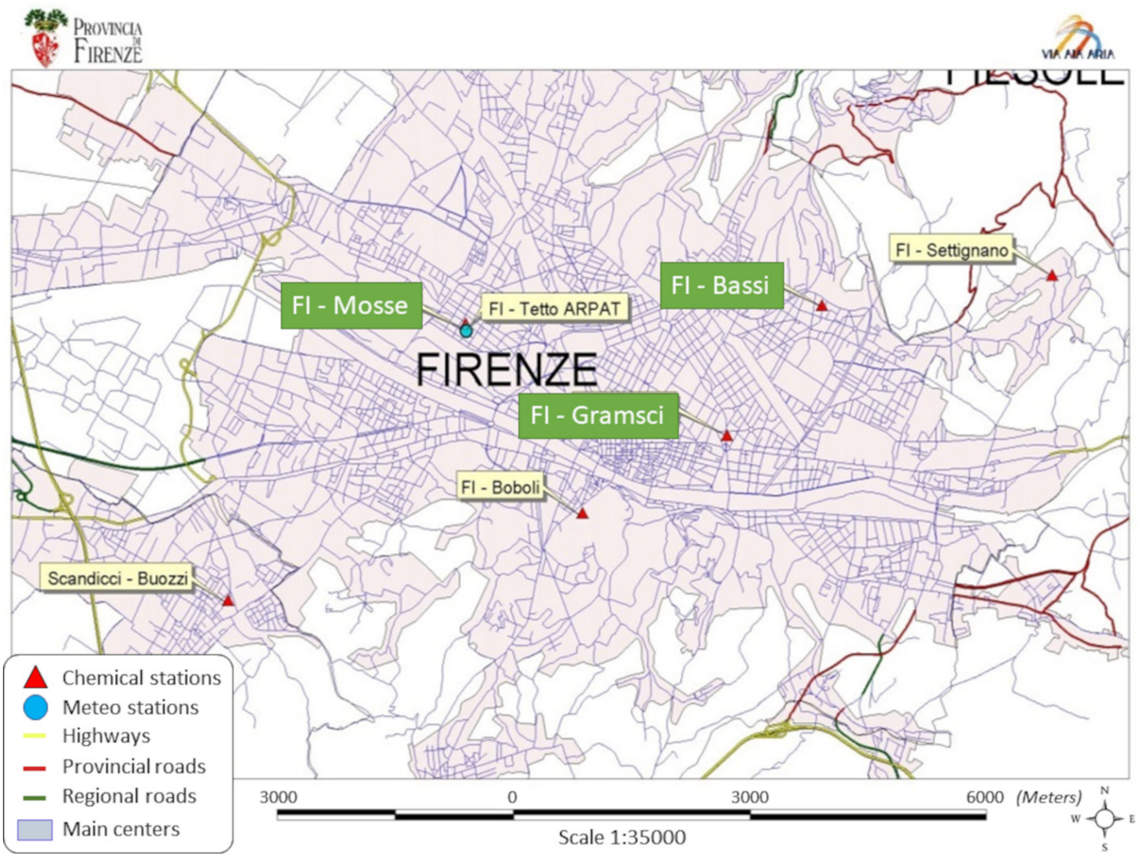


**Figure 1.** Distribution of air monitoring stations in the city of Florence. The planimetric map displays the location of the air quality monitoring stations inside the area of Florence. Symbols and colors depict the type of monitoring stations indicated in the planimetric map. White text in green background rectangles denotes the investigated air monitoring stations.

extracted according to the same procedure and used as control sample. Protein concentration in the supernatants was measured by Bradford protein assay (Bio-Rad Laboratories, Hercules, CA, USA).

**One-dimensional gel electrophoresis and immunoblotting.** SDS-poliacrilamide gel electrophoresis (SDS-PAGE) was performed with 12% (w:v) polyacrylamide under reducing conditions with a Mini-Protean apparatus (Bio-Rad Laboratories; 1000 AlfredNobel Drive, Hercules (CA), USA). The gel was then stained with 0.05% Comassie Brillant Blue (Sigma-Aldrich Chemie GmbH, Taufkirchen, Germany) in water/methanol/acetic acid (50:40:10). SDS-PAGE and immunoblotting experiments were carried out, as previously described [22, 23]. After electrophoresis (10 µg protein/well), proteins extracted from *Cs* pollen grains collected in the different city sites (Bassi, Mosse, Gramsci) were transferred onto nitrocellulose membranes (Schleicher & Shuell, Dassel, Germany).

Membranes were blocked with 3% (w:v) gelatin in PBS, then incubated overnight at room temperature (r.t) with serum from a *Cs* allergic patient [PlasmaLab International, Everett (WA), USA] diluted in 0.05% Tween 20 in PBS (PBS-T). Human IgE were detected by peroxidase-conjugated goat anti-human IgE antibody [KPL, Gaithersburg (MD), USA]. The 3,9-diaminobenzidine peroxidase substrate (Sigma-Aldrich, Milan, Italy) was then added to develop the colourimetric reaction.

**Gel and nitrocellulose images digitization and semi-quantitative determination of allergens expression.** SDS-PAGE gel and nitrocellulose sheets containing *Cs* pollen proteins were scanned for documentation and analysis by an UVIdoc scanner (Cambridge, UK). Photographs were acquired in TIFF format for further analysis by using the ImageJ software (https://imagej.net/Welcome), developed by the National Institutes of Health [Bethesda (MD), USA].

The original SDS-PAGE images were then used to compute the associated density profile images. A standard curve was constructed by plotting the log molecular weights of each protein marker (Prestained Protein SHARPMASS™ VI, Cod. EPS025500 Euroclone SpA, Italy) on y-axis against their relative forward (Rf) values on x-axis. Subsequently, the standard curve regression was used to compute the estimated molecular weight (MW) of the unknown *Cs* bands on each density profile.

The amounts of the electrophoretically separated fractions (Cup s-39 kDa, Cup s-47.8 kDa and Cup s-97.8 kDa) were determined by densitometric analysis of SDS-PAGE band intensities and width using the ImageJ software. These amounts were expressed as integrated density area (IDarea) values. Protein expression levels determined for each *Cs* allergen were normalized to bovine serum albumin (BSA) and reported as arbitrary units (A.U.), calculated as:

$$A.\,U. = (ID\ area\ of\ allergen\ band/ID\ area\ of\ 250\ ng\ loaded\ BSA\ band) \times 10^3$$

**Air quality data**. Data of mean annual $NO_2$ concentrations were collected for the sampling year 2011 from the Tuscan Regional Agency for Environment Protection (ARPAT) database (http://sira.arpat.toscana.it/sira/) from the nearest air monitoring stations. $NO_2$ monitoring stations placed in Gramsci and Mosse are classified as 'Traffic', the monitoring station located in Bassi is classified as 'Background'. 'Traffic' means that the station is located in such a position that the level of pollution is mainly influenced by traffic emissions from neighbouring roads with medium-high traffic intensity. 'Background' means that the station is located in a site where the level of pollution is not mainly influenced by emissions from specific sources (industry, traffic, residential heating, etc.). The coordinates (Gauss Boaga projection) of $NO_2$ monitoring stations are: Mosse – N:4850406 – E:1679502, Gramsci – N:4849080 – E:1682817, Bassi – N:4850623 – E:1684020.

**Statistical and regression analysis.** To compare mean values of atmospheric $NO_2$ concentration and pollen allergens expression at the city sites, One-way ANOVA and *post-hoc* Tukey test for multiple comparisons were performed. Previous reports [15], [16] showed a functional correlation between pollen grains protein expression and $NO_2$ air concentration in plants exposed to this gas throughout the entire growing season. Based on this, for Cupressus plants exposed to $NO_2$ for a long time, mean annual $NO_2$ concentration value was considered as the nearest to real exposure concentration. A non-linear regression analysis was conducted to determine the better fitting curve.

## RESULTS

$NO_2$ mean annual concentration values at the three sites were significantly different ($p<0.0001$) in the range of 38–103 µg/m³ (at #1-Bassi and #3-Gramsci, respectively). Moreover, data indicate that site #3 (Gramsci) was more polluted than site #1(Bassi), with site #2 (Mosse) showing intermediate values (Tab. 1, Fig. 2B). The PM10 values are indicative of vehicular traffic being the main $NO_2$ source at these sites. Analysis of the SDS-PAGE results reveals a polypeptide profile of proteins extracted from *Cs* pollen grains composed of several bands with molecular weights ranging between 100 kDa and 17 kDa (Fig. 3A). Serum IgE recognized the most relevant allergenic components of 39 and 47.8 kDa, corresponding respectively to the Cup s 1 and Cup s 2 allergens [24] and the 97.8 kDa component in the *Cs* pollen extract (Fig. 3B).

**Table 1.** Air pollutants and pollen sampling sites

| Pollen code | City site | Site classification | PM10 (µg/m³) | $NO_2$ (µg/m³) |
|---|---|---|---|---|
| #1 | Bassi | Urban Background | 23 | 38 |
| #2 | Mosse | Urban Traffic | 38 | 67 |
| #3 | Gramsci | Urban Traffic | 38 | 103 |

Site name, classification of air monitoring stations (according to http://data.europa.eu/eli/dir/2008/50/oj) and mean annual air concentration of $NO_2$ and PM10 in the sampling year 2011.

The amount of all three major *Cs* allergens changed significantly in relation to the site from which they were collected. In particular, the highest values ($p<0.0001$) were observed at the #2-Mosse site, both for the 97.8 kDa (82.77 ± 1.904 A.U.) and the 39 kDa (128.14 ± 1.780 A.U.) allergens. On the contrary, the 47.8 kDa allergen was highly

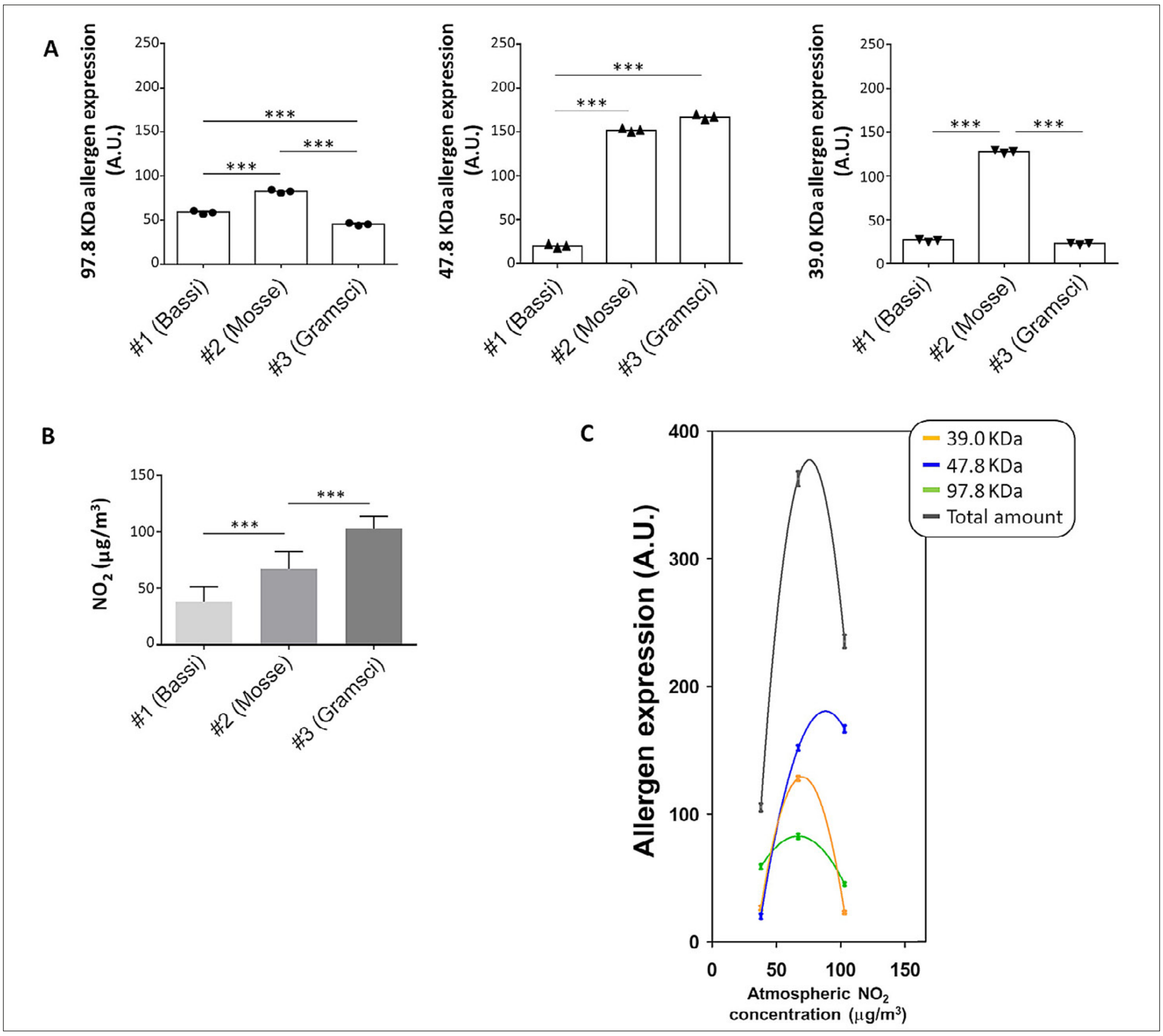


**Figure 2.** Model for $NO_2$-allergens expression relationship. **A.** Changes of Cs allergens expression (mean ± S.D. of arbitrary units determined by three SDS-PAGE replicates). *** P< 0.001. **B.** Changes of $NO_2$ air concentration (mean ± S.D. of μg/m³). **C.** Graphs describe allergen amount changes in relation to $NO_2$ air concentration

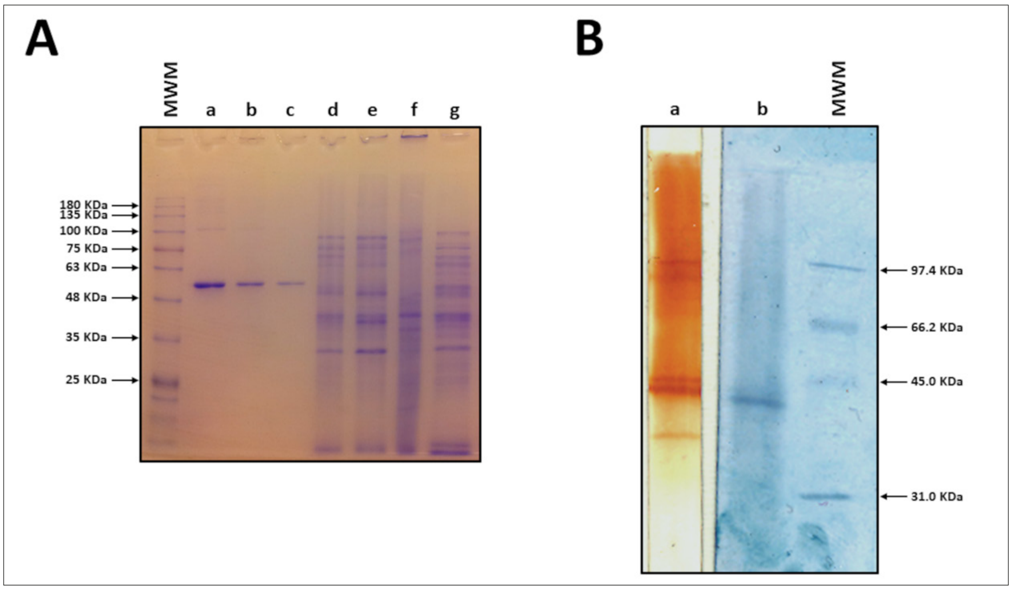


**Figure 3.** Representative Cs pollen extracts SDS-PAGE and immunoblot. **A.** Lowercase letters represent lanes of the SDS-PAGE. Lanes a-c: BSA 500, 250 and 125 ng, respectively; Lane d, Cs#1; Lane e, Cs#2; Lane f, extract from commercial Cs pollen; Lane g, Cs#3. **B.** Immunoblot of Cs#2 sample with serum IgE derived from an allergic subject. MWM, molecular weight markers. Arrows in each panel indicate the values associated to each MWM band

**Table 2.** Pollen allergen amount changes in relation to $NO_2$ air concentration, measured at the different pollen sampling sites

| Pollen sample | 97.8 kDa MW allergen (A.U.) | 47.8 kDa MW allergen (A.U.) | 39 kDa MW allergen (A.U.) | Total amount of major allergens (A.U.) | Atmospheric $NO_2$ concentration§ (μg/m³) |
|---|---|---|---|---|---|
| #1 | 58.83 ± 1.850 | 19.98 ± 1.990 | 26.67 ± 1.528 | 105.48 ± 3.051 | 38.22 ± 13.20 |
| #2 | 82.77 ± 1.904 | 151.93 ± 2.046 | 128.14 ± 1.780 | 362.84 ± 5.728 | 67.50 ± 15.08 |
| #3 | 45.36 ± 1.350 | 166.97 ± 2.800 | 23.13 ± 1.172 | 235.46 ± 5.114 | 103.2 ± 10.34 |

97.8 kDa, 47.8 kDa and 39 kDa allergenic fractions amount ± S.D., calculated as in Materials and Method. Three replicated SDS-PAGE of pollen extracts were considered for calculating the indicated values. A.U., arbitrary units; §2011 annual mean values.

expressed at the #3-Gramsci site (166.97 ± 2.8 A.U.; $p<0.0001$) (Tab. 2, Fig. 2A). Considering the total amount of the three major allergens, the highest allergenic expression (362.84 A.U. ± 5.728) was observed at # 2-Mosse (Tab. 2, Fig. 2C). Among the different sites, the amount of each single allergen expressed in pollens displayed a variation between 2 and 8-fold (Tab. 2). Furthermore, it was observed that protein expression level of all three major *Cs* pollen allergens increased non-linearly with increasing $NO_2$ concentration. In particular, the obtained curves show an inverse 2$^{nd}$ order polynomial trend for all three allergens (Fig. 2C, Tab. 3). It was not possible to evaluate the effect of PM2.5 and $O_3$ as fundamental air concentration data were not entirely available for the three city sites. The mean amount of pollen grains produced per square meter of cypress crown was significantly different in the three sites ($p=0.0075$): lower in Bassi (14.7 g/m$^2$), higher in Gramsci (27.45 g/m$^2$, while in Mosse showed intermediate values (18.3 g/m$^2$).

**Table 3.** Regression analysis data. Statistical parameters and coefficients from the nonlinear regression computed for each allergen are reported in the indicated column groups

| | Grouping factors | | | |
|---|---|---|---|---|
| Coeffcient values * | 97.8 kDa | 47.8 kDa | 39.0 kDa | Total amount |
| A | -45.56 | -314.6 | -357.5 | -717.7 |
| B | 3.837 | 11.22 | 13.86 | 28.92 |
| C | -0.02868 | -0.063554 | -0.09868 | -0.1909 |
| Standard deviations | | | | |
| A | 5.313 | 7.133 | 4.678 | 14.74 |
| B | 0.1673 | 0.2246 | 0.1473 | 0.4641 |
| C | 0.001167 | 0.001567 | 0.001027 | 0.003237 |
| **$R^2$** | 0.9918 | 0.9992 | 0.9994 | 0.9986 |

* Referred to the 2$^{nd}$ order polynomial function $Y = A + BX + CX^2$

## DISCUSSION

As widely reviewed by Reinmuth-Selzle et al. [25], atmospheric pollutants may have the following direct effects on pollen: (a) modifications to their biological and reproductive functions, (b) alteration of the physicochemical characteristics of the pollen surface, (c) change in the allergenic potential, and (d) adjuvant effect increasing their potential health hazards.

The present study evaluated the effect of outdoor atmospheric $NO_2$ gas pollutant on *Cs* pollen allergenic potential. Both proteic and allergenic patterns of *Cs* pollen extracts were in line with previous findings by Barletta et al [26], who reported that at least nine bands corresponding to gel-resolved *Cs* proteins were clearly bound by a rabbit antiserum against the *Cs* pollen extract. Interestingly, the same authors showed that components of about 100, 43 and 39 kDa molecular weight, respectively, were also recognized by human IgE of pooled *Cs* allergic sera. A similar immunoblotting IgE reactivity was also showed by Shahali et al. [27], particularly when Italian patients' sera were used, unlike French patients' sera that mainly recognized low molecular weight components. Although the minimal dose to induce the allergic sensitization or respiratory symptoms is unknown [28–30], in the current study it is assumed that increasing amounts of allergenic proteins in the pollen grains may represent a risk factor for allergic patients [31–33].

Data reported in this study indicate that in the city of Florence, the amount of pollen allergens can differ significantly, depending on the site where the *Cs* trees were located. Notably, the correlation model describing $NO_2$ air concentrations and Cs allergens expression relationship complies with a recent regulatory directive on non-linear models established by the United States Environmental Protection Agency [34]. Indeed, all four curves displayed a 2$^{nd}$ order polynomial trend, where the peak of allergens expression is reached at the intermediate value of mean annual $NO_2$ air concentration (67 μg/m$^3$). Interestingly, this value is close to the concentration limit value for the protection of human health (40 μg/m$^3$) (European Union Directive 2008/50/EC). This evidence suggests that 67 μg/m$^3$ mean annual $NO_2$ concentration, an apparently low risk value, should be regarded instead as being additionally harmful for sensitive subjects in relation to allergic respiratory reaction risks. Investigating on the detailed mechanisms of $NO_2$ modulation is beyond the scope of this study. However, it might be speculated that there are similar to that indicated by Zhao et al. [16], $NO_2$ gas behaves like a reactive nitrogen species that induce stress-related change in allergen genes expression.

It is important to consider the real allergenic content of pollen as a key parameter to be evaluated. With this in mind, it will be possible to shed new light on the issues of both pollen allergens exposure peak and clinical thresholds [35]. The importance of quantitative and qualitative determination of the seasonal allergen exposure peak has been underlined in a recent EAACI position paper [36], with the aim of correctly evaluating the efficacy of clinical trials for specific immunotherapy, as required by European Medicines Agency directives (CHMP/EWP/18504/2006).

## CONCLUSIONS

The study shows that intra-urban $NO_2$ variations do affect the expression of *Cs* pollen major allergens. The findings emphasize the need for a constant monitoring of the pollen allergens amount in parallel with assessment of the chemical pollutant levels. This will reflect on more comprehensive air quality policies, particularly within urban areas with increased motor vehicle density.

**Funding**
This study did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

## REFERENCES

1. Marcellusi A, Viti R, Incorvaia C, Mennini FS. [Direct and indirect costs associated with respiratory allergic diseases in Italy. A probabilistic cost of illness study]. Recenti Prog Med. 2015; 106(10): 517–527. https://doi.org/10.1701/2032.22086
2. Ishizaki T, Koizumi K, Ikemori R, Ishiyama Y, Kushibiki E. Studies of prevalence of Japanese cedar pollinosis among the residents in a densely cultivated area. Ann Allergy. 1987; 58(4): 265–270.
3. Renz H, Holt PG, Inouye M, Logan AC, Prescott SL, Sly PD. An exposome perspective: Early-life events and immune development in a changing world. J Allergy Clin Immunol. 2017; 140(1): 24–40. https://doi-org.iss.clas.cineca.it/10.1016/j.jaci.2017.05.015
4. Barberini S, Della Rocca G, Danti R, Zanoni D, Mori B, Ariano R, et al. Different allergenicity of pollen extracts of three Mediterranean cypress species accounted for cytological observations. Eur Ann Allergy Clin Immunol. 2015; 47(5): 149–155.
5. Charpin D, Pichot C, Belmonte J, Sutra JP, Zidkova J, Chanez P, et al. Cypress Pollinosis: from Tree to Clinic. Clin Rev Allergy Immunol. 2019; 56(2): 174–195. https://doi-org.iss.clas.cineca.it/10.1007/s12016-017-8602-y
6. Italian Association of Aerobiology. An epidemiological survey of Cupressaceae pollenosis in Italy. J Investig Allergol Clin Immunol. 2002; 12(4): 287–292.
7. Scala E, Alessandri C, Bernardi ML, Ferrara R, Palazzo P, Pomponi D, et al. Cross-sectional survey on immunoglobulin E reactivity in 23,077 subjects using an allergenic molecule-based microarray detection system. Clin Exp Allergy. 2010; 40(6): 911–921. https://doi-org.iss.clas.cineca.it/10.1111/j.1365-2222.2010.03470.x
8. Della Rocca G, Danti R, Intini M. Il cipresso: Cupressus sempervirens L. In: Il cipresso: dalla leggenda al futuro; IPSP-CNR, PA, Ed.; Italy, 2007. p. 119–132.
9. Grossoni P, Bruschi P, Bussotti F, Selvi F. Trattato di botanica forestale: 1. Parte speciale e gimnosperme; Italia, WK, Ed.; Italy, 2018. p. 286–291.
10. Asero R, Ceriotti V, Bonini M. Cypress pollen allergy in Milan: the story of an ongoing growth. Eur Ann Allergy Clin Immunol. 2020; 53: 209–213. https://doi-org.iss.clas.cineca.it/10.23822/eurannaci.1764-1489.155.
11. Schiavoni G, D'Amato G, Afferni C. The dangerous liaison between pollens and pollution in respiratory allergy. Ann Allergy Asthma Immunol. 2017; 118(3): 269–275. https://doi-org.iss.clas.cineca.it/10.1016/j.anai.2016.12.019
12. Ackaert C, Kofler S, Horejs-Hoeck J, Zulehner N, Asam C, von Grafenstein S, et al. The impact of nitration on the structure and immunogenicity of the major birch pollen allergen Bet v 1.0101. PLoS One. 2014; 9(8): e104520. https://doi-org.iss.clas.cineca.it/10.1371/journal.pone.0104520
13. Cuinica LG, Abreu I, Esteves da Silva J. Effect of air pollutant $NO_2$ on Betula pendula, Ostrya carpinifolia and Carpinus betulus pollen fertility and human allergenicity. Environ Pollut. 2014; 186: 50–55. https://doi-org.iss.clas.cineca.it/10.1016/j.envpol.2013.12.001
14. Ouyang Y, Xu Z, Fan E, Li Y, Zhang L. Effect of nitrogen dioxide and sulfur dioxide on viability and morphology of oak pollen. Int Forum Allergy Rhinol. 2016; 6(1): 95–100. https://doi-org.iss.clas.cineca.it/10.1002/alr.21632
15. Zhao F, Elkelish A, Durner J, Lindermayr C, Winkler JB, Ruëff F, et al. Common ragweed (Ambrosia artemisiifolia L.): allergenicity and molecular characterization of pollen after plant exposure to elevated NO2. Plant Cell Environ. 2016; 39(1): 147–164. https://doi-org.iss.clas.cineca.it/10.1111/pce.12601
16. Zhao F, Durner J, Winkler JB, Traidl-Hoffmann C, Strom TM, Ernst D, et al. Pollen of common ragweed (Ambrosia artemisiifolia L.): Illumina-based de novo sequencing and differential transcript expression upon elevated NO2/O3. Environ Pollut. 2017; 224: 503–514. https://doi.org/10.1016/j.envpol.2017.02.032
17. Honour SL, Bell JN, Ashenden TW, Cape JN, Power SA. Responses of herbaceous plants to urban air pollution: effects on growth, phenology and leaf surface characteristics. Environ Pollut. 2009; 157(4): 1279–86. https://doi.org/10.1016/j.envpol.2008.11.049
18. Anenberg SC, Mohegh A, Goldberg DL, Kerr GH, Brauer M, Burkart K, et al. Long-term trends in urban NO2 concentrations and associated paediatric asthma incidence: estimates from global datasets Lancet Planet Health. 2022; 6(1): e49-e58. https://doi.org/10.1016/S2542-5196(21)00255-2
19. Bottalico F, Travaglini D, Chirici G, Garfì V, Giannetti F, De Marco A, et al. A spatially-explicit method to assess the dry deposition of air pollution by urban forests in the city of Florence, Italy. Urban Forestry & Urban Greening. 2017; 27: 221–234. http://dx.doi.org/10.1016/j.ufug.2017.08.013
20. Hidalgo PJ, Galán C, Domínguez E. Pollen production of the genus Cupressus. Grana. 1999; 38(5): 296–300. http://dx.doi.org/10.1080/001731300750044519
21. Aboulaich N, Bouziane H, Kadiri M, Trigo M, Riadi H, Kazzaz M, et al. Pollen production in anemophilous species of the Poaceae family in Tetouan (NW Morocco). Aerobiologia 2009;25:27–38. http://dx.doi.org/10.1007/s10453-008-9106-2
22. Afferni C. Role of carbohydrate moieties in IgE binding to allergenic components of Cupressus arizonica pollen extract. Clin Exp Allergy. 1999; 29(8): 1087–1094. https://doi-org.iss.clas.cineca.it/10.1046/j.1365-2222.1999.00590.x
23. Alisi C, Afferni C, Iacovacci P, Barletta B, Tinghino R, Butteroni C, et al. Rapid isolation, characterization, and glycan analysis of Cup a 1, the major allergen of Arizona cypress (Cupressus arizonica) pollen. Allergy. 2001; 56(10): 978–984. https://doi-org.iss.clas.cineca.it/10.1034/j.1398-9995.2001.103125.x
24. Charpin D, Ramadour M, Lavaud F, Raherison C, Caillaud D, de Blay F, et al. Climate and Allergic Sensitization to Airborne Allergens in the General Population: Data from the French Six Cities Study. Int Arch Allergy Immunol. 2017; 172(4): 236–241. https://doi-org.iss.clas.cineca.it/10.1159/000471511
25. Reinmuth-Selzle K, Kampf CJ, Lucas K, Lang-Yona N, Fröhlich-Nowoisky J, Shiraiwa M, et al. Air Pollution and Climate Change Effects on Allergies in the Anthropocene: Abundance, Interaction, and Modification of Allergens and Adjuvants. Environ Sci Technol. 2017; 51(8): 4119–4141. https://doi-org.iss.clas.cineca.it/10.1021/acs.est.6b04908
26. Barletta B, Afferni C, Tinghino R, Mari A, Di Felice G, Pini C. Cross-reactivity between Cupressus arizonica and Cupressus sempervirens pollen extracts. J Allergy Clin Immunol. 1996; 98(4): 797–804. https://doi-org.iss.clas.cineca.it/10.1016/s0091-6749(96)70129-x
27. Shahali Y. Complementarity between microarray and immunoblot for the comparative evaluation of IgE repertoire of French and Italian cypress pollen allergic patients. Folia Biol (Praha). 2014; 60(4): 192–201.
28. Caillaud DM, Martin S, Segala C, Besancenot JP, Clot B, Thibaudon M, et al. Nonlinear short-term effects of airborne Poaceae levels on hay fever symptoms. J Allergy Clin Immunol. 2012; 130(3): 812–814.e1. https://doi-org.iss.clas.cineca.it/10.1016/j.jaci.2012.04.034
29. Bastl K, Kmenta M, Jäger S, Bergmann K-C, Berger U, Ean. Development of a symptom load index: enabling temporal and regional pollen season comparisons and pointing out the need for personalized pollen information. Aerobiologia. 2014; 30(3): 269–280. http://dx.doi.org/10.1007/s10453-014-9326-6
30. Caillaud D, Martin S, Segala C, Besancenot JP, Clot B, Thibaudon M, et al. Effects of airborne birch pollen levels on clinical symptoms of seasonal allergic rhinoconjunctivitis. Int Arch Allergy Immunol. 2014; 163(1): 43–50. https://doi-org.iss.clas.cineca.it/10.1159/000355630
31. Buters JT, Weichenmeier I, Ochs S, Pusch G, Kreyling W, Boere AJ, et al. The allergen Bet v 1 in fractions of ambient air deviates from birch pollen counts. Allergy. 2010; 65(7): 850–8. https://doi-org.iss.clas.cineca.it/10.1111/j.1398-9995.2009.02286.x
32. Galan C, Antunes C, Brandao R, Torres C, Garcia-Mozo H, Caeiro E, et al. Airborne olive pollen counts are not representative of exposure to the major olive allergen Ole e 1. Allergy. 2013; 68(6): 809–812. https://doi-org.iss.clas.cineca.it/10.1111/all.12144
33. Buters J, Prank M, Sofiev M, Pusch G, Albertini R, Annesi-Maesano I, et al. Variation of the group 5 grass pollen allergen content of airborne pollen in relation to geographic location and time in season. J Allergy Clin Immunol. 2015; 136(1): 87–95.e6. https://doi-org.iss.clas.cineca.it/10.1016/j.jaci.2015.01.049
34. Agathokleous E, Anav A, Araminiene V, De Marco A, Domingos M, Kitao M, et al. Commentary: EPA's proposed expansion of dose-response analysis is a positive step towards improving its ecological risk assessment. Environ Pollut. 2019; 246: 566–570. https://doi-org.iss.clas.cineca.it/10.1016/j.envpol.2018.12.046
35. Cecchi L. From pollen count to pollen potency: the molecular era of aerobiology. Eur Respir J. 2013; 42(4): 898–900. https://doi-org.iss.clas.cineca.it/10.1183/09031936.00096413
36. Pfaar O, Bastl K, Berger U, Buters J, Calderon MA, Clot B, et al. Defining pollen exposure times for clinical trials of allergen immunotherapy for pollen-induced rhinoconjunctivitis – an EAACI position paper. Allergy. 2017; 72(5): 713–722. https://doi-org.iss.clas.cineca.it/10.1111/all.13092