



Effect of O₃ and NO₂ atmospheric pollutants on *Platanus x acerifolia* pollen: Immunochemical and spectroscopic analysis

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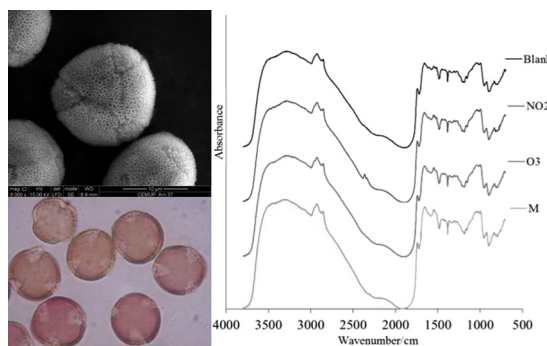
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HIGHLIGHTS

- Airborne pollen and pollutants interaction may modify pollen composition.
- Immunoblotting, infrared and X-ray photoelectron spectroscopy techniques were used.
- Modifications occurred on pollen' allergenicity and surface elemental composition.
- Changes are pollutant dependent and occur at levels below the regulated thresholds.

GRAPHICAL ABSTRACT



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ABSTRACT

In the present study, the effects of two important oxidizing atmospheric pollutants (O₃ and NO₂) on the allergenic properties and chemical composition of *Platanus x acerifolia* pollen were studied. Pollen samples were subjected to O₃ and/or NO₂ under in vitro conditions for 6 h at atmospheric concentration levels (O₃: 0.061 ppm; NO₂: 0.025 ppm and the mixture of O₃ and NO₂: 0.060 and 0.031 ppm respectively). Immunoblotting (using Pla a 1 and Pla a 2 antibodies), infrared and X-ray photoelectron spectroscopy techniques were used. Immunochemical analysis showed that pollen allergenicity changes were different according to the pollutant tested (gas or mixture of gasses) and that the same pollutant gas may interact in a different manner with each specific allergen. The spectroscopy results showed modifications in the FTIR spectral features of bands assigned to proteins, lipids, and polysaccharides of the pollen exposed to the pollutants, as well as in the XPS spectra high-resolution components C 1s, N 1s, and O 1s. This indicates that while airborne, the pollen wall suffers further modifications of its components induced by air pollution, which can compromise the pollen function.

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1. Introduction

Air pollutants emission increase in the past decades, particularly related to industrial growth, led to a worldwide environmental problem. In industrialized countries nitrogen dioxide (NO₂), ozone (O₃) and particulate matter (PM) are the most abundant aerosol pollutants,

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with a marked impact on human health such as in increased airway responsiveness and airway inflammation or changes in lung function (D'Amato et al., 2015; WHO, 2013).

As a consequence of anemophilous plants pollination process, large amounts of pollen grains are released into the atmosphere, becoming a seasonal biogenic constituent of the particulate matter fraction of the aerosol (PM). Allergenic airborne pollen size varies between 10 and 58 μm with their outermost layer, the exine, ranging between 1 and 5 μm (Erdtman, 1969).

Pollen grains are allergen-carriers since they contain in their composition many allergen molecules, found in the outer wall as well as in the cytoplasm that in susceptible individuals can trigger respiratory allergic diseases when entering people's airways (D'Amato et al., 2015; Linskens and Cresti, 2000; Sun et al., 2016). Pollen is mainly deposited in the upper respiratory airways, but while in contact with the respiratory mucosa releases allergens that elicit IgE-mediated allergic reactions (Rouvinen et al., 2010; Traidl-Hoffmann et al., 2009).

In Europe, airborne pollen concentration tends to be lower in urban areas than in rural ones (D'Amato et al., 2013), nevertheless, people living in more urbanized environments are the most affected by pollen-induced respiratory allergies (D'Amato et al., 2013). Typically airborne pollen monitoring and quantification are performed by roof-level samplers to avoid distortion due to local emissions, reflecting a regional trend, and architectural barriers such as high buildings (Maya-Manzano et al., 2016; Oteros et al., 2017; Rojo et al., 2015; Scheifinger et al., 2013). In fact, Oteros et al. (2015) using a concentric ring method (CRM) to model the relationship between the emission source and the total amount of airborne pollen showed that most of the pollen could come from long and regional distances transport just because most of the sources are located at these distances. Thus, the airborne pollen samplers are less able to detect important intra-urban concentration variations which are significant, within an urbanized environment, to determine the real human exposure risk to allergenic pollen at the breathing level (Davies et al., 2015; García-Mozo et al., 2016; Hjort et al., 2016; Rojo et al., 2015). Furthermore, the concomitant human exposure to airborne pollen and mixtures of primary and secondary air pollutants greatly enhance the occurrence of allergenic respiratory disorders (D'Amato et al., 2015).

The airborne pollen and air pollutants interaction is complex and many hypotheses have been formulated how this interplay may modify pollen allergenicity. It has been described that gaseous pollutants can alter airborne pollen properties, particularly at the biochemical level, which can enhance the risk of atopic sensitization and lead to an exacerbation of allergic symptoms in susceptible individuals (D'Amato et al., 2015; Frank and Ernst, 2016; Kanter et al., 2013; Ribeiro et al., 2014; Zhao et al., 2016). It has been shown that NO_2 and O_3 can promote the nitration of pollen proteins, with post-translational modification leading to increased or decreased allergenicity (Gruijthuisen et al., 2006; Karle et al., 2012; Shiraiwa et al., 2012).

Also, airborne pollen and air pollutants interaction can lead to changes in pollen general chemistry that can have an important impact in pollen's primary task that is the reproduction (Senechal et al., 2015; Sousa et al., 2012). Variations in the pollen lipids, proteins, carbohydrates and sporopollenin surface components have been reported after pollen exposure to different gaseous pollutants (Kanter et al., 2013; Zhao et al., 2016).

Therefore, in order to study the effects of air pollutants on pollen after being released into the atmosphere, pollen from randomize samples collected from a batch of *P. x acerifolia* trees located in an urbanized environment was in vitro subjected to gaseous pollutants using concentrations around the atmospheric levels. The effects of O_3 , NO_2 and the mixture of both pollutants in *Platanus* pollen allergenic properties using Pla 1 and Pla 2 antibodies were evaluated. Also, near surface and surface pollen modifications were studied by infrared and X-ray photoelectron spectroscopy.

2. Material and methods

2.1. Pollen samples

Platanus x acerifolia belongs to the Platanaceae family and is widely planted as an ornamental tree across the Porto's urban area (41°11' N, 8°39' W). The pollen collection was performed from trees in a public garden, situated in a residential area, near the Douro River and Atlantic ocean and near to a highway road with high road traffic. The sampling was conducted according to the methodology previously described in Ribeiro et al. (2013). Afterwards, the material collected was brought to the laboratory, dried (24 °C) in a laboratory stove and the pollen was separated using sieves and stored at –20 °C as a single sample.

2.2. Pollen exposure to the gaseous pollutants

Pollen was exposed to O_3 , NO_2 and to both gasses simultaneously under in vitro conditions (Table 1), during 6 h, in an environmental chamber system, already detailed in Sousa et al. (2012). The chamber is composed by a Solar Simulator (Newport Oriel 96,000 150 W) to simulate the daylight, fans (SUNON SF23080AF) to homogenize the air, temperature and relative humidity sensors (EBI20 sensor) and gas sensors (AEROQUAL Series 500 sensors; O_3 sensor: 0–0.5 ppm, NO_2 sensor: 0–0.2 ppm). The gas concentration and meteorological conditions were not constant inside the environmental chamber in order to imitate what happens in the atmosphere along the diurnal cycle.

Individual pollen samples (150 mg) were placed in Falcon tubes (50 ml) with both edges open but coated by a mesh with a pore opening size of 23 μm (SEFAR PET 1000). The pollen formed a very thin layer at the bottom covering the mesh. The tube was then positioned over a fan that enables pollen dispersion inside, permitting a homogeneous contact between the pollen grains and the gas and also mimics what occurs in the atmosphere with airborne pollen.

Exposure conditions reproducibility has been demonstrated in Ribeiro et al. (2013).

A A2ZS-1GLAB ozone system connected to a timer (OMRON H3DK-S1) to control the gas injection was used to generate ozone. The NO_2 was obtained in a sealed bottle through the chemical reaction between concentrated nitric acid (to ensure the production of NO_2 , otherwise we would have a mixture of NO and NO_2) and solid copper mixed, at stoichiometric amounts.

A blank pollen sample (150 mg), not exposed to the gasses but subjected to the same procedure, was used as the control.

2.3. Protein extraction and estimation

To conduct the immunoblots experiments, pollen protein extracts were obtained by suspending, with continuous stirring, the pollen (50 mg) in phosphate buffered saline (1:20 w/v) at pH 7.4 for 4 h at 4 °C. Afterward, the suspension was centrifuged (16,100 g for 30 min at 4 °C) and the supernatant filtered (0.45 μm Millipore filter) and centrifuged again. The Bradford's method (Bradford, 1976) was used to estimate the soluble protein content of all pollen extracts by means of a colorimetric reaction with the Coomassie Protein Assay Reagent

Table 1

– Average and standard deviation of gas concentration, temperature and relative humidity registered along the exposure of *Platanus x acerifolia* pollen to O_3 , NO_2 and to both gasses simultaneously under in vitro conditions during 6 h in an environmental chamber system.

	[Gas] ppm	Temperature °C	Relative humidity %
Blank	–	25.2 ± 1.5	44.6 ± 0.8
O_3	0.061 ± 0.018	22.8 ± 1.5	65.5 ± 0.8
NO_2	0.025 ± 0.004	24.1 ± 1.3	56.1 ± 1.3
Mixture (O_3 + NO_2)	0.060 ± 0.009 0.031 ± 0.007	26.8 ± 0.7	58.3 ± 1.0

(Pierce). Different BSA concentrations (0–2000 µg/ml) were used to estimate a standard curve for protein calibration.

2.4. Immunoblots

Proteins from pollen extracts (20 µg per lane) were separated under reducing conditions in 12.5% (w/v) polyacrylamide gels and were electroblotted (TE22 Mighty small transphor unit - GE Healthcare) onto nitrocellulose membranes (Protran, Whatman® Schleicher & Schuell, Germany) in a solution of 192 mM glycine, 25 mM Tris and 20% methanol (transfer buffer) during 2 h at 200 mA. The membranes were saturated during 1 h in a blocking solution (5% non-fat dry milk and 0.1% goat serum in TBS-T (20 mM Tris, 150 mM NaCl with 0.1% Tween-20). Afterwards were incubated overnight at 4 °C with *Platanus x acerifolia* pollen antibodies (polyclonal antisera raised against Pla a 1 and Pla a 2 purified allergens, produced by immunizing New Zealand white rabbits (Asturias et al., 2002; Ibarrola et al., 2004), diluted 1:400 in blocking solution. After washing, bond specific to Pla a 1 and Pla a 2 allergens were detected using a secondary antibody (goat anti-Rabbit IgG (H + L) - HRP diluted in TBS-T (1:10,000). Our detection system was an ECL solution (Luminata™ Crescendo, Western HRP substrate). The reactive bands of the immunoblots were revealed in a Chemidoc™ XRS + system (Bio-rad laboratories) and quantified using the ImageLab™ v5.2 software. The changes in the reactivity to the different pollen extracts were evaluated through the optic density values of the reactive bands.

2.5. Spectroscopic analysis

In order to study the impact of the gaseous air pollutants on the pollen surface components (pollen coating and exine) near-surface and surface techniques of analysis such as Infrared spectroscopy - FTIR (analysis depths of about 1–2 µm) and as X-ray photoelectron spectroscopy - XPS (analysis depths of about 8–10 nm) respectively were performed.

2.5.1. Infrared spectroscopy of pollen

FTIR allows characterizing the signatures of the overall biochemical composition of the pollen surface through specific spectral features of proteins, lipids, carbohydrates and cell wall biopolymers (e.g. sporopollenin) (Zimmermann, 2010; Zimmermann and Kohler, 2014). With this technique, it is aimed to compare the main spectral variations of bands between unexposed and pollutants exposed pollen.

A JASCO FT-IR-460 Plus spectrometer, using a diffuse reflectance (DRIFT) accessory was used to perform the Fourier transform infrared spectroscopy (FT-IR).

All pollen samples FT-IR spectra were acquired by measuring mixtures of several thousand intact pollen grains plus potassium bromide (KBr). Assay samples were prepared by mixing 1 µ-spatula of pollen and 3 µ-spatula of KBr. One IR spectrum was obtained for each sample recorded with a total of 60 scans and since we were working with solid samples a 4 cm⁻¹ resolution was chosen. The spectral background was acquired using KBr. The obtained data were treated with the acquisition software of the spectrometer. The formation of HNO₃ was not detected in the FTIR spectra.

2.5.2. X-ray photoelectron spectroscopy

XPS is an ultrahigh-vacuum surface analysis technique that allows characterizing the surface elemental and chemical structures of materials (McArthur et al., 2014). In our study, this technique was used to analyze the surface composition of the pollen wall in order to detect chemical changes in the high-resolution components of the XPS spectra (carbon - C 1s, nitrogen - N 1s and oxygen - O 1s) in response to the exposure to the gaseous pollutants.

For the analysis, 1 µ-spatula of pollen for each assay was used. The pollen samples were dried, overnight, at 40 °C in a laboratory stove

and afterwards gently compressed in a pellet, to allow a homogenous fixation of the intact pollen into an adhesive surface which supports the samples during the analysis.

A Kratos AXIS Ultra HAS, equipped with the VISION software for the acquisition of the data and with a CASAXPS software for the analysis of the data was used.

The samples were analyzed with a monochromatic Al Ka X-ray source (1486.7 eV) operating with a pass energy of 80 eV, an energy step of 1.0 eV and 200 ms dwell time per data point for survey spectra. For the regions-of-interest, it was applied a pass energy of 40 eV, with energy step of 0.1 eV and dwell time per data point for carbon, nitrogen, and oxygen 1 s high-resolution spectra of 600, 600 and 2000 ms respectively. The acquisition of the data was performed with very short times (0.6 s) to avoid beam damage, a pressure lower than 1 × 10⁻⁶ Pa, and using a charge neutralization system. The surface charge correction for binding energy scale calibration was referenced to the carbon 1s core level photoemission peak with a binding energy of 285 eV.

Spectral deconvolution was performed in peak fitting, by applying the Gaussian-Lorentzian peak shape and the Shirley-type background subtraction. Also, the same constraint for the shape and full width at half maximum (FWHM) for all peaks was considered for sample comparison.

3. Results and discussion

3.1. Immunochemical analysis

The results obtained from the probed 1-DE immunoblots with the antibodies against Pla a 1 and Pla a 2 revealed several dissimilarities between the blank and the pollutant exposed samples.

The 1-DE immunoblotting with Pla a 1 and Pla a 2 Abs showed a tendency towards greater reactivity to protein extracts from pollen exposed to NO₂, at average concentration levels found in the atmosphere of Porto, and to the O₃-NO₂ mixture (Fig. 1). This point out that modifications occur on the major *P. x acerifolia* pollen allergens even at an

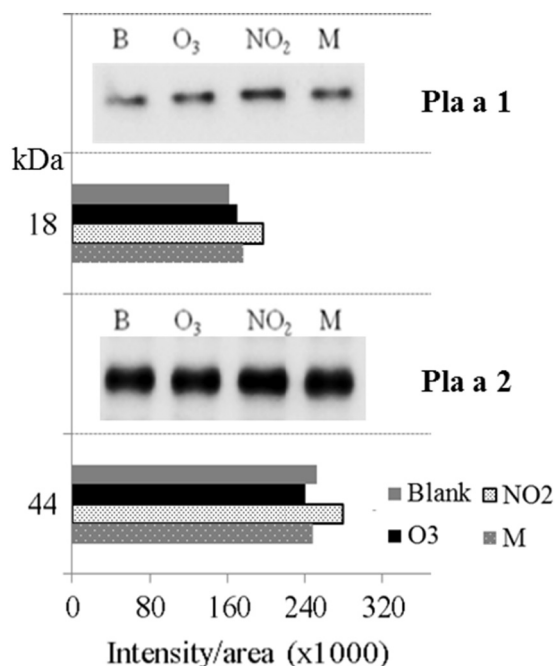


Fig. 1. Immunoblots and values optic densities (intensity/area) of reactive bands of *Platanus x acerifolia* pollen protein extracts exposed to distinct gasses and assayed with Pla a 1 (18 kDa) and Pla a 2 (44 kDa) antibodies. Above each lane and in the legend the letters represents the Blank (B), O₃, NO₂ and the pollen exposed simultaneously to O₃-NO₂ mixture (M). Bands molecular weights are expressed in kDa.

atmospheric concentration of these pollutants close and below the limit value for the human health protection in Europe.

Protein nitration upon exposure to O_3 and NO_2 (two step reaction with the formation of reactive oxygen intermediates (ROIs), such tyrosine radicals) has been pointed out as one of the mechanisms behind the increase in pollen allergenic potential in air polluted environments (Franze et al., 2005; Gruijthuisen et al., 2006; Reinmuth-Selzle et al., 2014; Shiraiwa et al., 2012). It was also reported that the NO_3 radicals formed upon reaction of NO_2 with O_3 play an important role in the nitration process of proteins present in organic aerosol particles, such as pollen (Chapleski et al., 2016; Franze et al., 2005; Ghiani et al., 2016).

Nonetheless, in our study, the pollen exposed to the gas mixture induced a Pla a 1 and Pla a 2 reactivity always lower than the one observed when pollen was only exposed to NO_2 . These results may indicate that in the case of the *P. x acerifolia* pollen an antagonistic effect between O_3 and NO_2 on its allergenicity may occur. Protein oxidation and nitration posttranslational modification (PTM) have been reported during oxidative stress, inflammation and chemical aging under environmental or physiological conditions (Reinmuth-Selzle et al., 2014). In the living cell that is pollen, oxidative PTM induced by O_3 can change the protein's function resulting in inhibition, gain or no change (Shiraiwa et al., 2012). In the case of *P. x acerifolia*, an hypothesis could be that its sensitivity to O_3 is a consequence of its pollen protein structure and its susceptibility to oxidation processes (Golebski et al., 2013; Gunawan et al., 2008) that can lead to an inhibition of the allergenic protein function and mechanical loss of allergens or even affect allergen recognition by IgE, promoting therefore a decrease in the pollen allergenicity (Rogerieux et al., 2007). Furthermore in the presence of O_3 , amino acids such as tyrosine are oxidized which can induce proteins denaturation by the introduction of changes in their secondary and tertiary structure (Cataldo, 2003), and/or sulphur containing amino acids such as cysteine produces cysteine-sulfenic acid that further reacts with air pollutants or form disulfide (Enami et al., 2009). So, when *P. x acerifolia* pollen is exposed to the gas mixture (O_3 - NO_2) it can be speculated that the oxidation step may possibly interfere in the nitration process. In fact, under field conditions, the allergenicity of ragweed pollen, collected along high-traffic roads, was correlated with air pollution measurements and it was shown a positive correlation with NO_2 concentrations but no correlation with O_3 levels (Ghiani et al., 2012).

Considering the effect of O_3 alone, a different behavior was observed when compared with the blank experiment. For Pla a 1 Abs an increase in reactivity was observed while for Pla a 2 Abs a decrease was observed. In a previous publication, Ribeiro et al. (2014) used 10 different patient's sera to probe IgE modifications when *P. x acerifolia* pollen was exposed to half, equal and four times the limit value of O_3 for the human health protection in Europe. These authors reported a general tendency towards a decrease in most IgE reactive bands with increasing gas concentration, contrary to what was observed for *Acer negundo* or *Quercus robur*. However, focusing only on the reactive band with a similar molecular weight as the Pla a 1 (18 kDa) reported by Ribeiro et al. (2014), compared with the blank experiment 5 out of the 10 patient sera tested presented higher reactivity when pollen was exposed at gas concentrations similar to our study. Furthermore, in the 10 patient sera tested, when the molecular weight band of around 44 kDa was present revealed always lower reactivity. In a study with pollen from birch trees exposed to environmental conditions, it was observed that elevated O_3 concentrations revealed less immune modulatory but higher immune stimulatory potential (Beck et al., 2013).

Therefore, our results suggest that the same gas pollutant may interact in a different manner with each specific pollen allergen, increasing or decreasing its allergenicity.

3.2. Spectroscopic analysis

In our study on both the FTIR and XPS results were observed modifications on the spectra of pollen exposed to the pollutants compared with the blank pollen sample. These results point out that modifications induced by gaseous pollutants, even at levels below the currently regulated thresholds, occur on the pollen wall that might have important physiological implications namely in pollen–stigma interactions or in pollen germination.

Fig. 2 shows the recorded FT-IR spectra of the pollen samples with the representative bands present in pollen grains already mentioned in the literature (Bağcıoğlu et al., 2015; Gottardini et al., 2007; Mularczyk-Oliwa et al., 2012; Pappas et al., 2003; Zhao et al., 2016; Zimmermann, 2010; Zimmermann and Kohler, 2014). Two regions could be observed in the FT-IR spectra, one between 3800 and 2600 cm^{-1} characterized by a broad band around 3400 cm^{-1} that corresponds to the stretching of –OH bands (Mularczyk-Oliwa et al., 2012), followed by two strong bands at 2930 and 2850 cm^{-1} that are assigned to the out-of-phase CH_2 and in-phase CH_3 stretching respectively (Mularczyk-Oliwa et al., 2012), characteristic of hydrocarbon chains in lipids (Kanter et al., 2013; Zhao et al., 2016).

The second region from 1800 to 800 cm^{-1} is considered as a fingerprint region of each pollen taxon (Gottardini et al., 2007; Mularczyk-Oliwa et al., 2012). It contains specific vibrational bands assigned to the main constituents present in the pollen grains as i) proteins [two strong broad bands 1658 cm^{-1} (amide I: C=O stretch) and 1546 cm^{-1} (amide II: NH deformation and C–N stretch)], ii) lipids [a strong band 1741 cm^{-1} (C=O stretch of methyl-esters), a weaker band at 1454 cm^{-1} (CH_2 deformation) and a broad band at 1166 cm^{-1} (P=O stretch of phospholipids)], iii) carbohydrates [broad absorption spectral region in 1200–900 cm^{-1} represented by several vibrational bands associated with C–O–C stretch, C–OH stretch, COH deformation, COC deformation] and iv) sporopollenin constituents [1602, 1515, 1168, 850, 833 and 811 cm^{-1} bands associated with the vibration of aromatic rings] (Bağcıoğlu et al., 2015; Gottardini et al., 2007; Kanter et al., 2013; Mularczyk-Oliwa et al., 2012; Pappas et al., 2003; Zhao et al., 2016; Zimmermann, 2010; Zimmermann and Kohler, 2014).

In our study modifications between the FT-IR spectral features of the blank *Platanus* pollen and the ones from the pollen exposed to the distinct gasses can be observed (Fig. 2 B). The vibrational band associated to the amide II (1533 cm^{-1}) increased in the NO_2 -exposed pollen while in the O_3 - NO_2 mixture flattened (Fig. 2-1). The duplet band 1462 and 1453 cm^{-1} , this last one assigned to the CH_2 deformation in lipids, is presented as one shoulder in the spectra of the pollen exposed to O_3 and to the O_3 - NO_2 mixture (Fig. 2-2). In the NO_2 -exposed pollen, a small peak (1291 cm^{-1}) between the bands 1306 and 1272 cm^{-1} can be observed but is absent from all the other spectra (Fig. 2-3). Also, the band at 833 cm^{-1} , assigned to sporopollenin constituents, presents a different shape in the NO_2 exposed pollen spectrum (Fig. 2-5). Finally, in the carbohydrates spectral region some variations between the spectra can be detected (Fig. 2-4). These observed modifications has also been demonstrated for ragweed pollen collected from plants exposed over the entire vegetation period to elevated levels of O_3 and of NO_2 in separated experiments (Kanter et al., 2013; Zhao et al., 2016) and in hazel pollen collected in areas of heavy air pollution compared with pollen from less-polluted areas (Depciuch et al., 2016). So in regions with elevated air pollution levels besides its influence on the pollen wall composition along pollen formation and development inside the anther (Depciuch et al., 2016; Kanter et al., 2013; Zhao et al., 2016), our results point out that while airborne the pollen wall still suffers further modifications of its components induced by air pollution. It could be interesting to know if these changes can significantly affect pollen–stigma interactions or pollen germination in the stigma.

In the XPS analysis, it was observed no significant quantitative differences in the XPS spectra high-resolution components C 1s, N 1s, and O 1s between the blank samples and the exposed ones.

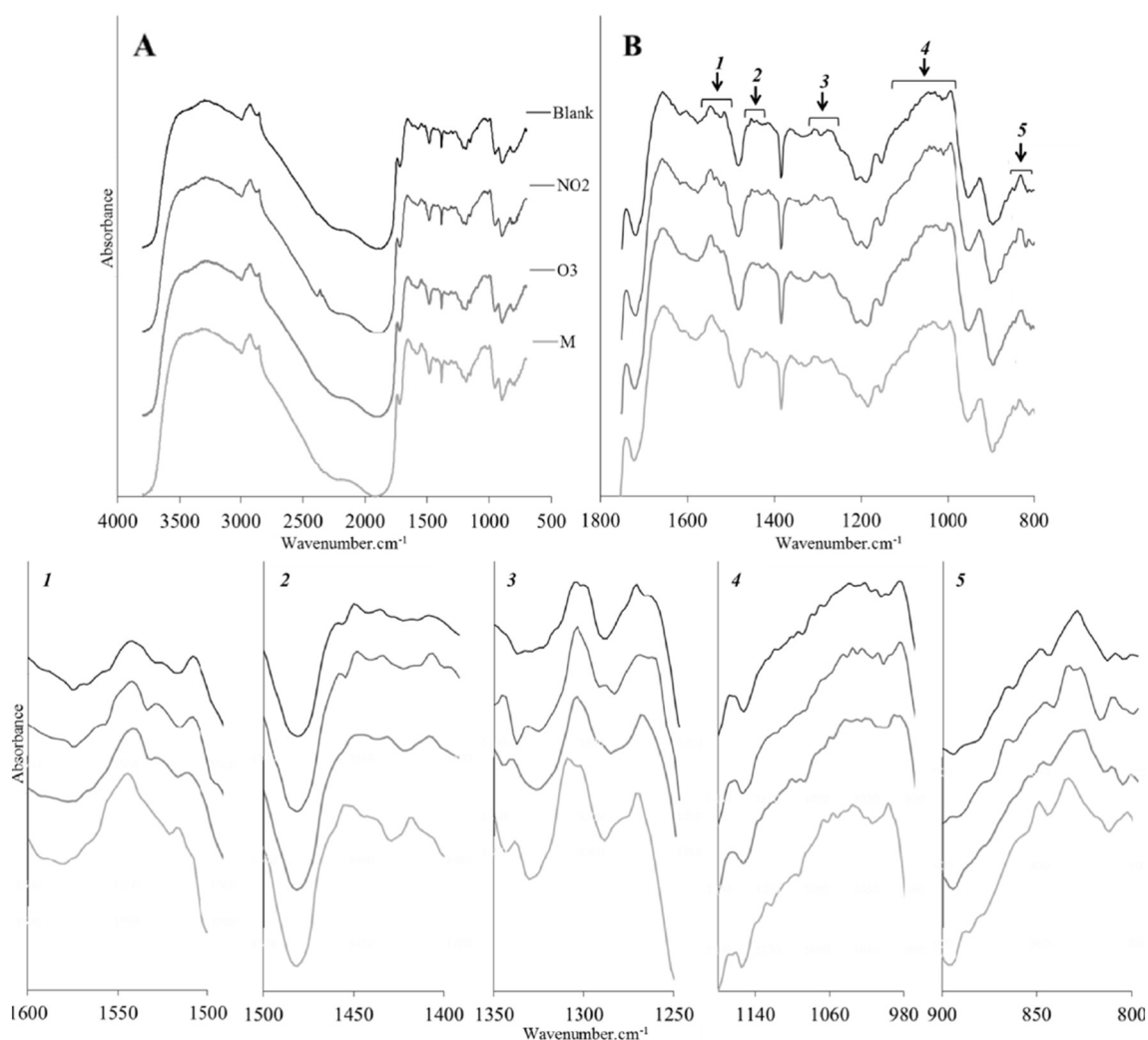


Fig. 2. Fourier transform infrared (FT-IR) spectra of pollen of *Platanus x acerifolia* exposed to distinct gases (A): Blank, O₃, NO₂ and the pollen exposed simultaneously to O₃ and NO₂ (M). Detailed spectra in the band range 1800 to 800 cm⁻¹ (B) with most prominent modifications observed (arrows) and highlighted (1–5).

Nevertheless, an increase in the chemical bonds heterogeneity (broader bands which can either reveal changes in the number of bonds or of different groups) on the C 1s and N 1s XPS core level spectra (Fig. 3A and B respectively) has been observed in the pollen samples exposed to pollutants.

The C 1s core level spectrum of the blank sample can be decomposed into five contributions at approximately 285 eV (carbon bond to carbon and hydrogen; C—(C,H)), 286.5 eV (amine group: C—N bond), 287.5 eV (not assigned), 288 eV (amide group: O—C—N bond) and 289 eV (carboxylate and carboxyl groups in proteins) (McArthur et al., 2014). In the spectra of the exposed samples, it was observed a shift in the binding energy of similar peaks compared with the blank sample: O₃, NO₂ and O₃/NO₂ mixture samples in the amide group peak and for all other secondary peaks in the O₃/NO₂ mixture sample. Differences in the FTIR vibrational band associated to the amide II were also observed for the NO₂ and O₃/NO₂ mixture samples. This points out to change in the protein's conformation of the pollen wall after pollutants exposure.

The N 1s core level spectrum revealed that the nitrogen bonding became more heterogeneous after exposure to the gaseous pollutants. Since the XPS is a surface-sensitive technique, the results obtain correspond mainly to the outermost layer of pollen particularly to the sporopollenin and to the pollen coat (McArthur et al., 2014). Therefore, exposure to the gaseous pollutants at the tested low concentrations may not be sufficient to obtain significant changes in the amount of C,

N or O of the airborne pollen outermost layer. However, they were sufficient to induce an increase in the chemical bonds heterogeneity, which can compromise the pollen function.

4. Conclusions

Our immunochemical analysis not only suggests that *P. x acerifolia* airborne pollen allergenicity modifications occur after being exposed to the gaseous pollutants but also that are different according to the pollutant gas or mixture of gases tested. Furthermore, the same gas pollutant may interact in a different manner with each specific pollen allergen, increasing or decreasing its allergenicity, which we think is a step forward to what is known until now.

Modifications between the FTIR spectral features of bands assigned to proteins, lipids, polysaccharides and sporopollenin of the blank *Platanus* pollen and the ones from the pollen exposed to the distinct gases were observed.

No significant quantitative differences in the XPS spectra high-resolution components C 1s, N 1s, and O 1s were observed between the blank sample and the exposed ones. However, it was observed an increase in the chemical bonds heterogeneity on the C 1s and N 1s core level spectra of the pollen samples exposed to pollutants.

The modifications induced by the gaseous pollutants occur even at levels below the limit value for the human health protection in Europe.

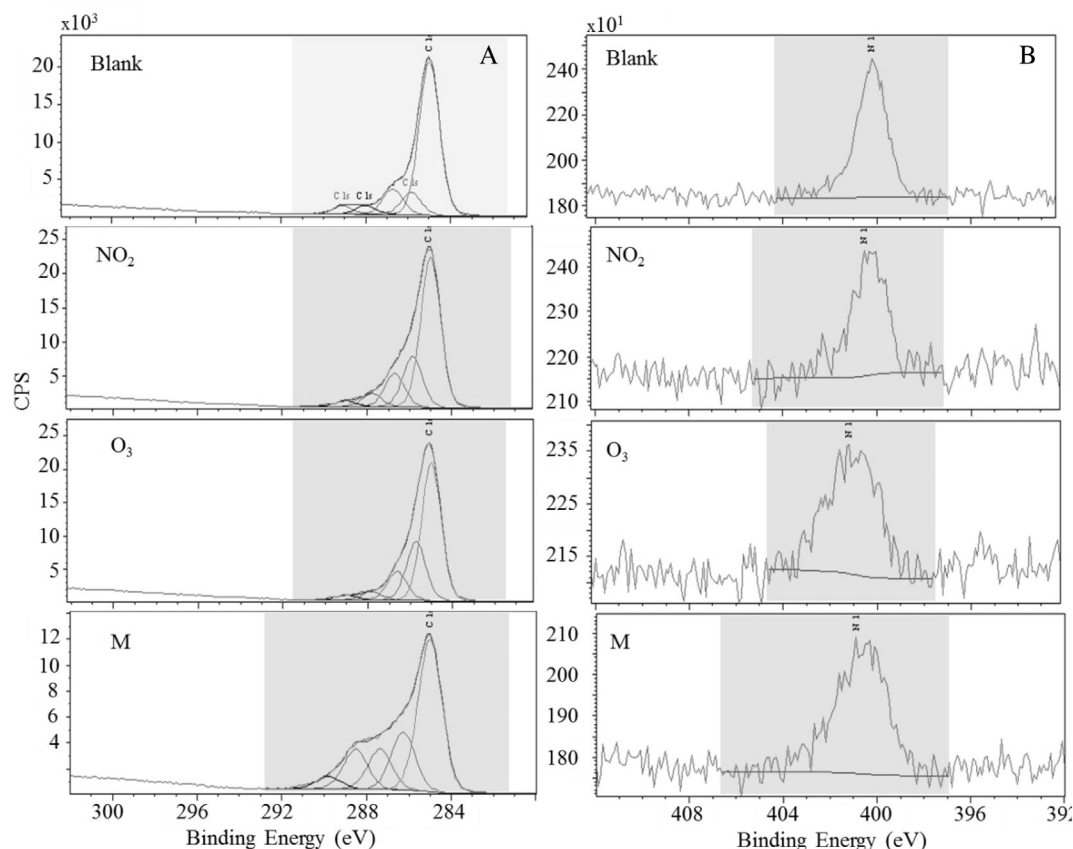


Fig. 3. - C 1s (A) and N 1s (B) core level XPS spectra of pollen of *Platanus x acerifolia* exposed to distinct gases: Blank, O₃, NO₂ and the pollen exposed simultaneously to O₃/NO₂ mixture (M).

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