



Impact of urban air pollution on the allergenicity of *Aspergillus fumigatus* conidia: Outdoor exposure study supported by laboratory experiments



Naama Lang-Yona^{a,1}, Timor Shuster-Meiseles^a, Yinon Mazar^a, Oded Yarden^b, Yinon Rudich^{a,*}

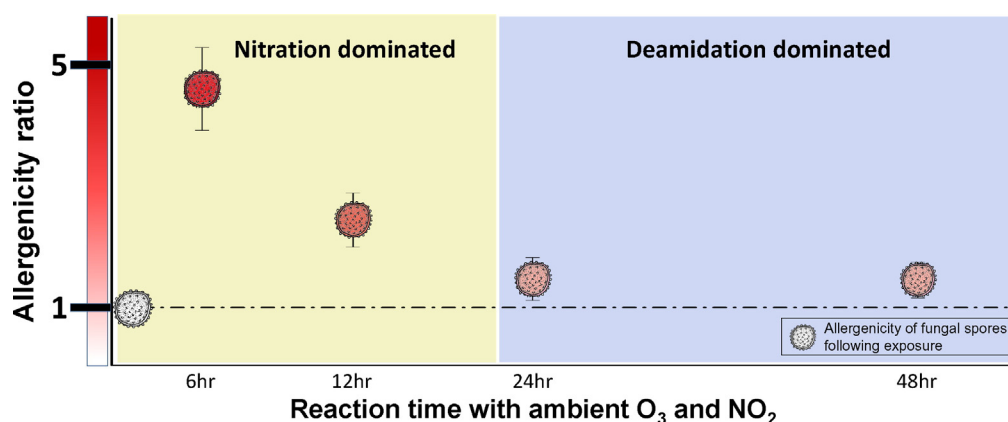
^a Department of Earth and Planetary Sciences, Weizmann Institute of Science, Rehovot 76100, Israel

^b Department of Plant Pathology and Microbiology, The Robert H. Smith Faculty of Agriculture, Food and Environment, The Hebrew University of Jerusalem, Rehovot 76100, Israel

HIGHLIGHTS

- Short-term exposure to urban air pollution increases *A. fumigatus* allergenicity.
- The effect is most significant during the first hours due to nitration of proteins.
- At longer exposure time allergenicity reduces, possibly due to protein deamidation.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:

Received 28 July 2015

Received in revised form 10 September 2015

Accepted 13 September 2015

Available online xxxx

Editor: D. Barcelo

Keywords:

Allergenicity
Urban air pollution
Fungal spore

ABSTRACT

Understanding the chemical interactions of common allergens in urban environments may help to decipher the general increase in susceptibility to allergies observed in recent decades. In this study, asexual conidia of the allergenic mold *Aspergillus fumigatus* were exposed to air pollution under natural (ambient) and controlled (laboratory) conditions. The allergenic activity was measured using two immunoassays and supported by a protein mass spectrometry analysis. The allergenicity of the conidia was found to increase by 2–5 fold compared to the control for short exposure times of up to 12 h (accumulated exposure of about 50 ppb NO₂ and 750 ppb O₃), possibly due to nitration. At higher exposure times, the allergenicity increase lessened due to protein deamidation. These results indicate that during the first 12 h of exposure, the allergenic potency of the fungal allergen *A. fumigatus* in polluted urban environments is expected to increase. Additional work is needed in order to determine if this behavior occurs for other allergens.

© 2015 Elsevier B.V. All rights reserved.

Abbreviation: PTM, post-translational modifications; HLA-DR, human leukocyte antigen class II; PDA, potato-dextrose agar; PBS-T, phosphate buffered saline with 0.05% tween 20; MCE, mixed cellulose esters; HEPA, high efficiency particulate air; BCA, bicinchoninic acid assay; SAFS, severe asthma with fungal sensitizations; ABPA, allergic bronchopulmonary aspergillosis; RH, relative humidity; DA, deamidation.

* Corresponding author.

E-mail address: Yinon.rudich@weizmann.ac.il (Y. Rudich).

¹ Multiphase Chemistry Department, Max Planck Institute for Chemistry, Mainz 55128, Germany.

1. Introduction

Prevalence of allergies in children and adults is increasing worldwide (Asher et al., 2006; Nicolaou et al., 2005; Sicherer and Sampson, 2014; Wong et al., 2013; Zhang and Zhang, 2014). Several recent studies have linked the increased occurrence of allergies and asthma cases to higher susceptibility due to increased exposure to urban pollutants (Gruzdeva et al., 2012; Kim et al., 2011; Kim et al., 2013; Ko et al., 2007). The globally mounting urbanization is accompanied by increased traffic-related air pollution that is characterized by high concentration of pollutants such as nitrogen oxides, ozone, sulfate, and particulate matter. Some of these pollutants can adversely affect the immune system, and modulate airway diseases such as asthma by increasing the release of inflammatory mediators from bronchial epithelial cells (Bayram et al., 2001; Chuang et al., 2007).

An additional hypothesized pollution effect is the induction of increased prevalence of allergies and asthma, through chemical alteration of the airborne allergens during their transport in the lower troposphere (Franze et al., 2005; Gruijthuisen et al., 2006; Karle et al., 2012; Untersmayr et al., 2010), possibly by post-translational modifications (PTM). Previous studies have shown that Bet v 1.0101, an allergenic protein originated from birch pollen, undergoes nitration by mixtures of NO₂ and O₃ (Franze et al., 2003; Gruijthuisen et al., 2006; Walcher et al., 2003). This was further supported by kinetic studies (Shiraiwa et al., 2011) and by a report on the effect of surface-to-volume ratio on this process (Kampf et al., 2015). These allergenic proteins were then shown to increase allergen-derived human leukocyte antigen class II (HLA-DR)-associated peptides in human dendritic cells (Karle et al., 2012). In a later work, it was shown that an increase in the level of nitration (produced by tetranitromethane) leads to changes in the molecular structure of the protein (Ackaert et al., 2014).

Fungal molds are known to be one of the asthma inducing factors (Denning et al., 2013; Karjalainen et al., 2001). According to global models, over 6.5 million people have severe asthma with fungal sensitizations (SAFS) and about 4.8 million adults have allergic bronchopulmonary aspergillosis (ABPA), both caused frequently by *Aspergillus fumigatus* (Chaudhary and Marr, 2011; Denning et al., 2014; Denning et al., 2013).

In this study we investigated whether exposure to urban air pollution affects the common fungal allergen, *A. fumigatus* (Chaudhary and Marr, 2011; Simon-Nobbe et al., 2008), and studied the changes in the allergenicity of its asexual conidia (spores), which contain at least 23 potential allergenic proteins (IUIS, 2014). We hypothesize that urban air pollution can induce changes in the allergenic potency of common fungal allergens, on the basis of PTM. Using a unique ELISA bioassay that evaluates the efficiency of the IgE-antigen interaction (Low et al., 2011), we show that exposure of *A. fumigatus* spores to ambient air pollution significantly increases their allergenicity during the first hours of exposure to ambient urban air pollution. The effect weakens after longer exposure periods, and higher cumulative gas exposure. We support this finding with laboratory experiments and protein mass spectrometry analysis and discuss possible mechanisms to explain these results.

2. Materials and methods

2.1. Fungal growth and sample preparation

A. fumigatus (ATCC, #1022) was cultivated on potato-dextrose agar (PDA, Difco) amended with 100 mg/l chloramphenicol (Sigma-Aldrich), and incubated at 25 °C in the dark. After 10 days of growth, spores were

harvested using flocked, nylon fiber tipped swabs (Copan Diagnostics, Brescia, Italy), and re-suspended in 50 ml phosphate buffered saline with 0.05% tween 20, pH = 7.4 (PBS-T, Sigma-Aldrich). The conidial suspension concentration was determined using a hemocytometer. Conidial samples were prepared by pipetting 2 ml of the conidial suspension (2×10^8 conidia/ml) on Mixed Cellulose Esters (MCE) sterile filters (Millipore, Billerica, MA, USA), using a vacuum system, and stored at 4 °C under low pressure conditions until required.

2.2. Exposure to air pollution

Outdoor exposure was conducted in central Israel, in the city of Modi'in (35.0092N, 31.9082E) at a certified and calibrated governmental air quality monitoring station. This area is downwind from the metropolitan area of Tel Aviv and experiences relatively high levels of secondary pollutants such as O₃ and NO₂ (Boersma et al., 2009). Permission to sample in this location was provided from the Israel Ministry of Environmental Protection <http://www.sviva.gov.il>.

The experimental setup, detailed in Fig. 1A, is based on pumping ambient air onto the samples using an environmental pump (AirCon-2, Sensidyne Gilian, FL, USA) located at the end of the flow line, with a controlled flow rate of 300 cm³ min⁻¹. After removal of particles using high efficiency particulate air (HEPA) cap filters (Whatman, WA, USA), the air was directed into a chamber in which the spore-containing filters were placed, allowing reactive gases to interact with the spores. Air was then directed through a carbon-cap filter (Whatman, WA, USA), adsorbing the reactive gases, and then into a control chamber, in which duplicate spore-containing filters were placed. Experimental chambers were placed in a dark box, preventing light from entering. To reduce pressure fluctuations, a bulb was placed between the controlled chamber and the pump. It was verified that the pollutants were absorbed and that the control sample was not exposed to the ambient pollution.

The outdoor concentrations of ambient pollutants for the sampling period were obtained from the Israel Ministry of Environmental Protection (<http://www.svivaqm.net/Default.rtl.aspx>) and are shown in Fig. 2 and Table S1.

Three complementary experiments were conducted in the laboratory to expose the spores in four exposure treatments: 1) exposure to synthetic air composed of pure N₂ and O₂ (98% purity); 2) exposure to O₃; 3) exposure to NO₂; 4) exposure to synthetic air containing both O₃ and NO₂, as described in Fig. 1B. Ozone was produced from O₂ using a UV-light source (Ozone Generator, 500 Jelight Company Inc., Irvine, CA, USA), and diluted with N₂ to 50 ± 8 ppb (see Table 1). The concentration was measured using a commercial O₃ monitor (Model 1180, Dasibi Environmental Corp., Glendale, CA, USA). Nitrogen dioxide (86 ppm, Linde) was diluted to 47 ± 3 ppb with N₂. To simulate tropospheric conditions, additional O₂ was added to the flow, maintaining an O₂:N₂ ratio of 21:79%. Each experiment (both outdoor and laboratory) was conducted at 4 time-intervals of exposure: 6, 12, 24 and 48 h with triplicates. In control laboratory experiments, exposure only to the synthetic air did not lead to any significant difference between the samples in the exposure chamber compared to the control chamber.

2.3. Protein extraction procedure

After exposure to air pollution or controlled gas flow, proteins were extracted from the spores as described by Low et al. (2011). Briefly, glass beads were added to 2 ml PBS-T (Sigma Aldrich) suspensions and spores were lysed by bead-beating (Mini-Beadbeater-16, BioSpec)

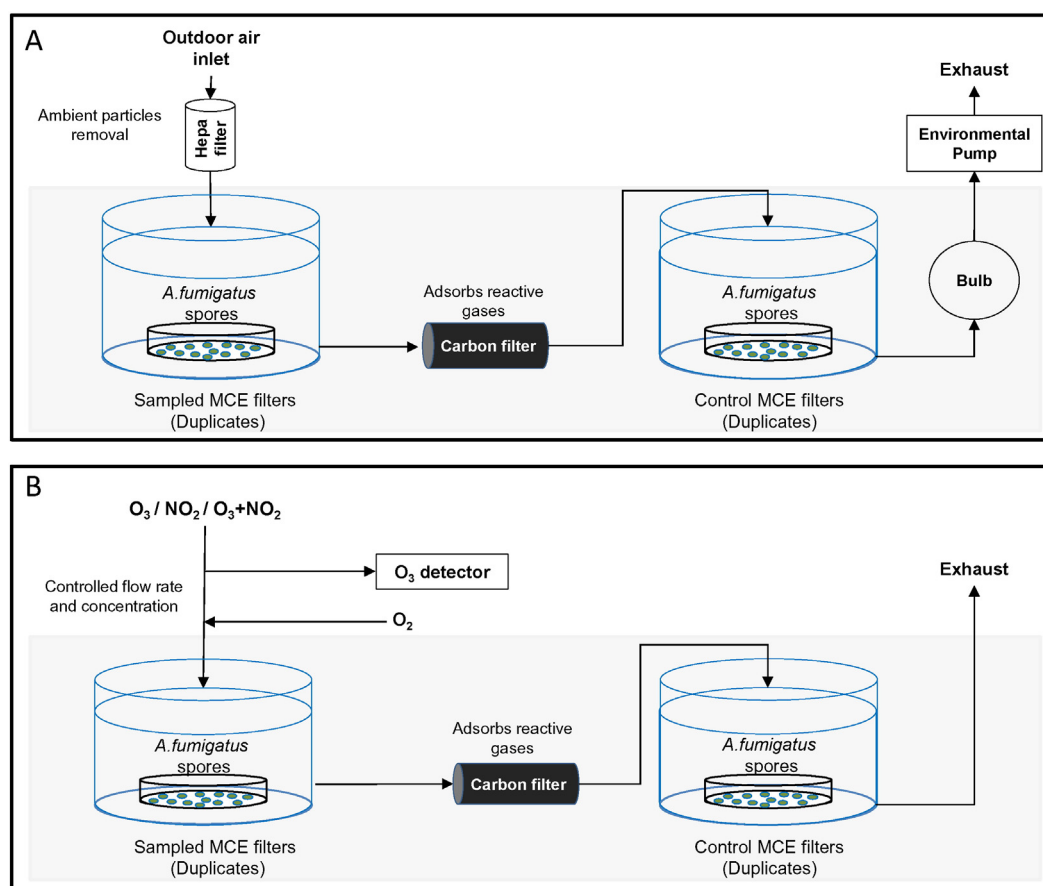


Fig. 1. Experimental setup for exposing *A. fumigatus* spores to outdoor air pollution (A) and the laboratory setup for exposure to NO_2 and Ozone (B). Gray area indicates a dark environment.

for 30 s. Lysed cells were centrifuged and the protein-containing supernatant was collected and stored at -80°C until further analysis. Protein content was determined using the bicinchoninic acid assay (BCA, ThermoFisher Scientific) in accordance with the manufacturer's protocol and averaged for three replicates.

2.4. Immunoassays

Both immunoassays were performed on sub-samples from the same sample-extracted proteins.

2.4.1. Relative allergenicity

The relative allergenicity immunoassay was performed as described by Low et al. (2011), with modifications as described by Lang-Yona et al. (2013). Briefly, a dilution series of allergenic protein extracts in triplicates were allowed to bind to IgE from *A. fumigatus* sensitized patients' pooled plasma (see Table S2). The mixtures were then transferred to high-binding ELISA plates and coated with *A. fumigatus* spore-extracted protein from cultures grown under standard conditions. Following the binding of the free IgE to the coated plate, a secondary IgG antibody (Mouse anti Human IgE (Fc)-HRP clone HP6029, Southern Biotech) was applied and the plates were subjected to colorimetric reading at 450 nm (Synergy HT Multi-Mode Microplate Reader, BioTek). At least two biological repeats for each exposure experiment were conducted, with no significant difference between them ($P < 0.05$). The allergenicity per spore value was determined by plotting the observed allergenicity versus the spore concentration for each treatment.

2.4.2. Asp f1 concentration

The concentration of the major allergenic protein Asp f1 per spore was determined by immunoassay using a commercial ELISA kit (Indoor Biotechnology Ltd., UK), according to the manufacturer's protocol. In this assay, the extracted allergenic proteins were bound to Asp f1-specific monoclonal IgE. The binding signal was measured colorimetrically after the addition of enzyme-labeled IgG antibody, at 405 nm. The Asp f1 concentration was retrieved from a standard curve produced from monoclonal Asp f1 standard, and normalized to *A. fumigatus* spore number for each treatment.

2.5. Mass spectrometry analysis

Two biological repeats of the three laboratory exposures and control treatments were analyzed with the MS analysis for 6 and 48 h of exposure. Preparation included overnight dialysis against 100 mM Ammonium Bicarbonate using micro Spectra/Por Float-A-Lyzer G2 dialysis system (MWCO 3.5–5 kD, 100–200 μl , Spectrum Labs, CA) to remove tween residues. A BCA analysis was carried out to determine the protein concentration. Proteins were analyzed for post-translational modifications (e.g. nitration, de/oxidation and deamidation) using LC-MS/MS. Analysis was performed at the Mass Spectrometry Unit of the Weizmann Institute of Science. Preparations included overnight Tryptic digestion, reduction with DTT and alkylation using iodoacetamide. Peptide separation was performed using an in-house produced fused-silica capillary column with 3 μm ReproSil-Pur C18AQ media (Dr. Maisch GmbH). Raw data files were searched with MASCOT (Matrix Science London, UK) against a Uniprot *A. fumigatus* database, and further analyzed with Scaffold Proteome software (Portland, Oregon). No significant differences were found between the biological repeats.

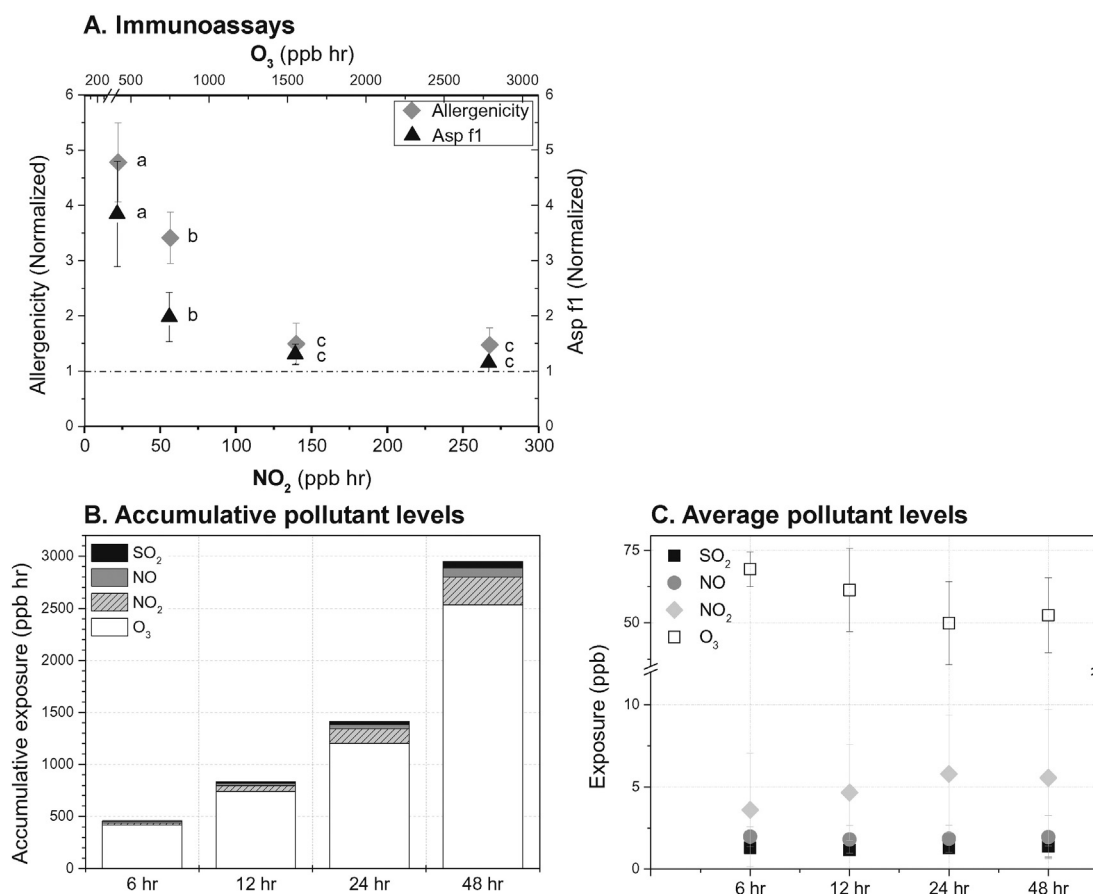


Fig. 2. The effect of ambient pollution exposure on the allergenicity of *A. fumigatus* spores. Relative allergenicity (Dark gray rhombus) and Asp f1 concentration (Black triangle) of spores, normalized to the control sample (A), as a function of accumulating NO₂ levels (Lower axis) and ozone (Upper axis). Accumulative pollutant levels (B) as well as their average concentrations (C), as detected during the experiments. Exposure experiments were performed twice, in triplicate.

2.6. Statistical analysis

For the ELISA assays, comparison between groups within the same treatment was performed using One-Way ANOVA, with a Tukey post-hoc test using the OriginPro 9.1 software (OriginLab). Significance between different treatments was determined by Two-Way ANOVA, with the Tukey post-hoc test. Comparing between relative allergenicity and the Asp f1 assay for the same treatment was carried out using a two sample t-test for independent groups with no equal variance using the OriginPro 9.1 software (OriginLab). Protein PTM distributions of treatments and controls were compared with the binomial distribution

test, using Microsoft Excel 2010 software. The differences between biological repeats were analyzed using an independent two sample T-test.

3. Results and discussion

We investigated the hypothesis that outdoor air pollution can affect the allergenic activity of the abundant fungal spore, *A. fumigatus*. Our results suggest that the exposure to ambient pollution increases the allergenicity of the spore in a time-dependent manner, i.e.; at shorter exposure times or lower integrated exposure (<12 h, <60 ppb h NO₂, <750 ppb h ozone, Fig. 2A).

Table 1
NO₂ and O₃ exposure levels in laboratory experiments.

Treatment	NO ₂			O ₃		
	Exposure time, h	Accumulated exposure, ppb h	Concentration ^a , ppb	Accumulated exposure, ppb h	Concentration, ppb	Std
NO ₂	6	312	52			
	12	624	52			
	24	1248	52			
	48	2496	52			
NO ₂ + O ₃	6	312	52	365.7	56.3	19.2
	12	624	52	750.7	54.6	1.4
	24	1248	52	1362.7	57.4	22.3
	48	2496	52	2670	55.6	18.1
O ₃	6			260.5	43.4	6.6
	12			532.5	46.3	12.4
	24			1675	68.4	15.4
	48			3076.5	64.1	16.3

^a ± 3 ppb.

We observed a significant 2–5 fold enhancement in the allergenicity after exposure to ambient levels of pollutants between 20 to 100 ppb h NO₂ and 415 to 1000 ppb h of ozone (6–15 h under our experimental conditions). However, following additional exposure, allergenicity levels decreased with no significant changes found upon exposure from approximately 140 to 270 ppb h NO₂ and 1200 to 2530 ppb h O₃ (24 and 48 h.). Nevertheless, even under these conditions, enhanced allergenicity (by a factor of about 1.5) was still observed, compared to the blank. These observations were consistent in both the Asp f1 and total allergenicity assays. Temperature and relative humidity (RH) at the sampling site are described in Fig. S1, showing stable RH levels of about 10% at the first 27 h of exposure, and a later increase up to levels of around 70% at the late stages of sampling (after about 45 h). The temperature range was between 17 and 33 °C, where the temperature decline corresponded to the increase in RH. Such changes could affect the chemistry of the pollutants (Finlayson-Pitts and Pitts, 2000). Nevertheless, no significant change was observed in the NO₂ or O₃ levels at the sampling site.

The relative allergenicity assay includes all allergenic proteins bound to the sensitized patients' IgE. This point may explain the significantly higher impact of this assay compared to the Asp f1 assay ($P_{\text{value}} = 0.004$), which estimates only the major allergen – Asp f1. The similar trend implies that the net effect of air pollution on the allergenic protein population examined in the allergenicity assay is corresponded to the effect on the single protein Asp f1.

To further investigate the allergenicity trend observed in the case of outdoor exposure, we conducted laboratory experiments where similar spore preparates were exposed to a single pollutant (ozone or NO₂) or to both (Fig. 3).

When exposed to NO₂ alone (Fig. 3A), the activity of the allergens significantly increased with accumulating exposure, reaching higher

allergenicity levels when compared to those observed in the outdoor experiment (up to about 21 fold in total allergenicity). In this experiment, a difference in the results between the two immunoassays was observed ($P_{\text{value}} = 0.0038$). The increased activity could be attributed to either direct modification of the allergenic proteins, as was shown previously (Ackaert et al., 2014) or indirect modification of other molecules serving as adjuvants or as a de-novo-produced allergy-inducing species. These hypotheses, however, have not yet been proven, and need to be further investigated.

We deduce that different allergenicity-modifying mechanisms are involved following exposure to NO₂ alone, compared to urban air pollution, since the allergenicity trends behave differently with increased exposure time. Moreover, it seems that the main change that had occurred following NO₂ exposure is not predominantly mediated by the Asp f1 protein.

When testing ozone influence on the allergenic characteristics of *A. fumigatus* spores (Fig. 3B), no significant difference was observed, when compared to the non-exposed samples, at all exposure levels. These results are supported by the protein PTM MS analysis, presenting no statistically significant difference between treatments and controls (Table 2). This observation may be linked to the conidial resistance mechanism against atmospheric ozone oxidation (Liu et al., 2014; Ozkan et al., 2011).

Nevertheless, the combined exposure to NO₂ and O₃ (Fig. 3C) resulted in a similar trend to the one observed in the outdoor exposure experiment, yet with lower allergenicity values ($P_{\text{value}} = 6.8 \times 10^{-4}$). In this experiment the initial NO₂ concentration (NO₂(i) = 47 ppb) was higher than the measured outdoor levels. However, as discussed in previously published papers, NO₂ may react with O₃ and OH[−] (formed at ambient relative humidity) in the reaction chamber, leading to the formation of HNO₃[−], HONO, and other possible derivatives, and hence to a

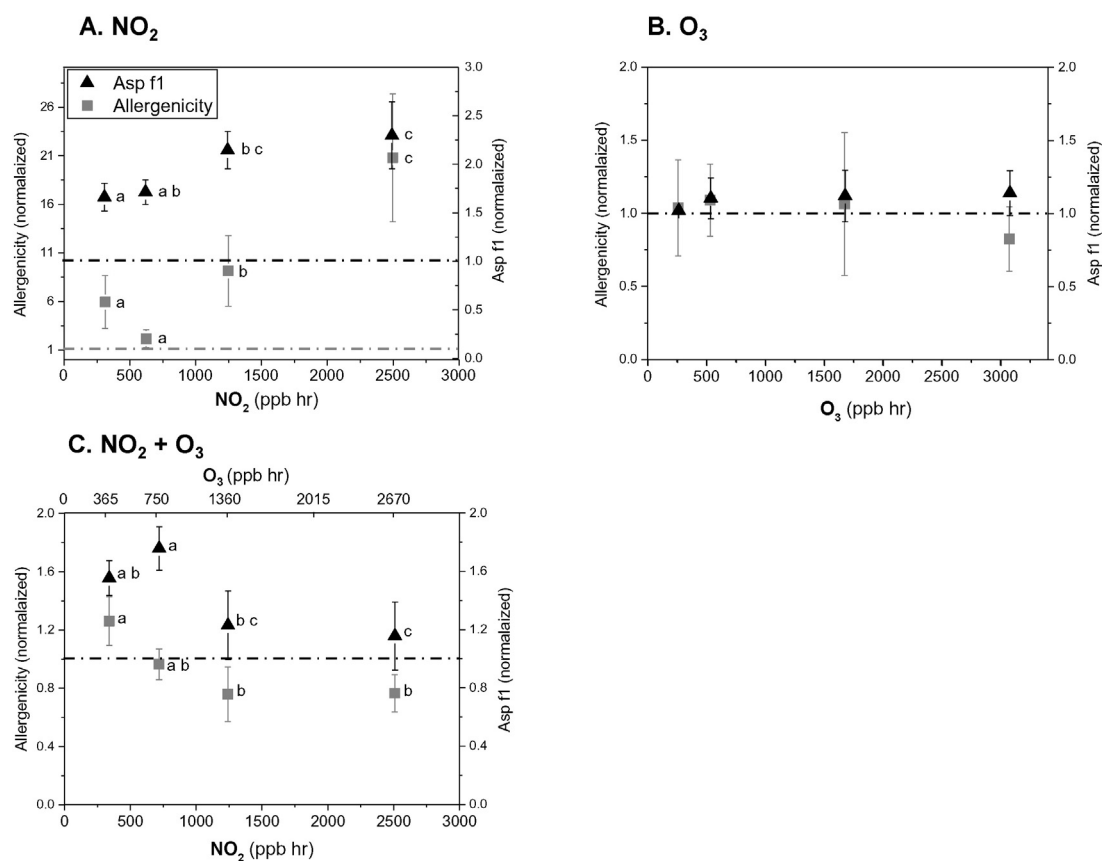


Fig. 3. The effect of laboratory exposure of NO₂ (A), O₃ (B), and NO₂ + O₃ (C) on the allergenicity of *A. fumigatus* spores. Relative allergenicity (gray squares) and Asp f1 concentration (Black triangles) of spores, normalized to the control sample, are shown as a function of accumulating NO₂ levels and ozone. Exposure experiments were performed twice, in triplicate. Different letters indicate statistical significance at $P_{\text{value}} < 0.05$.

Table 2Significance of protein post-translational modification after exposed to NO₂ and O₃ pollutants.^a

Exposure time	6 h		48 h		48 vs. 6 h ^b
Treatment	Control	Sample	Control	Sample	
A. Nitration modification					
O ₃	0.433	0.772	0.399	0.922	
NO ₂	0.246	0.924	0.064	0.991	
NO ₂ + O ₃	0.264	0.900	0.021 [*]	0.999	0.109
B. Oxidation modification					
O ₃	0.972	0.090	0.578	0.578	
NO ₂	0.903	0.259	0.884	0.271	
NO ₂ + O ₃	0.090	0.972	0.884	0.271	
C. Deamidation modification					
O ₃	0.199	0.851	0.944	0.086	
NO ₂	0.776	0.289	0.412	0.658	
NO ₂ + O ₃	0.814	0.242	0.002 ^{**}	0.999	0.000 ^{**}

^a P-value of the binomial distributions.^b Performed only for significant P-values.

* Significant with 95%.

** Significant with 99%.

reduction in the NO₂ levels and the spore reaction rates (Colussi et al., 2013; Crutzen, 1988; Finlayson-Pitts and Pitts, 2000; Song et al., 2011; Spicer et al., 1993).

Proteomic analysis for PTM present in the laboratory-exposed proteins revealed significant modifications following long exposure duration (48 h) to NO₂ + O₃. Deamidation (DA) of a variety of proteins (Table 2) is significantly affected by NO₂ and O₃ exposure ($P_{\text{value}} = 0.002$) compared to the non-exposed treatment, as well as with time ($P_{\text{value}} = 0.000$). Among 105 DA-modified proteins detected, 3 were allergenic proteins (see Table S3, Fig. 4A).

Deamidation was previously shown to reduce allergenicity of the wheat allergen gliadin (Kumagai et al., 2007), indicative of the cause leading to the reduction in their activity with exposure time. Deamidation leads to an alteration of asparagine and glutamate residue to a mixture of isoaspartate and aspartate or isoglutamate and glutamate, respectively (Robinson and Rudd, 1974). Such changes most probably impact the characteristics of the proteins, leading to the observed reduction in allergenic activity.

Nitration is also significantly affected by NO₂ and O₃ ($P_{\text{value}} = 0.021$). However, no significant change was observed for different exposure times ($P_{\text{value}} = 0.109$). As shown in Table S4 and Fig. 4B, the amount of nitrated proteins was significantly lower than that of the deamidified proteins, implying the DA mechanism as more pronounced under such experimental conditions.

Although a similar trend was detected for the laboratory NO₂ + O₃ exposure and the outdoor exposure, outdoor air may contain other pollutants that may affect the allergenicity of exposed spores. Previous studies showed protein modifications due to exposure to SO₂ (Greene et al., 2002; Jeong et al., 2011) as well as to NO (Derakhshan et al., 2007), but to the best of our knowledge, their effect on allergenicity have not been reported to date.

Based on our findings, we propose that the NO₂/O₃ pollution in urban environments has a dual effect on the allergenic activity of *A. fumigatus* spore. Initial exposure of spores to urban pollution leads to substantial increase in the allergenicity. We attribute this behavior to nitration of the allergy-inducing proteins. Over time, their degradation rate increases (possibly due to reactive oxidant species), and DA of the proteins significantly increases, leading to a decrease in the potency of the allergens. At high concentrations of reactive species, a significant decrease in the allergenic activity of the fungal spores is expected, due to further oxidation and degradation. This mechanism is probably not specific, indicated by the randomly modified proteins observed in the MS analysis. Therefore other allergenic proteins are

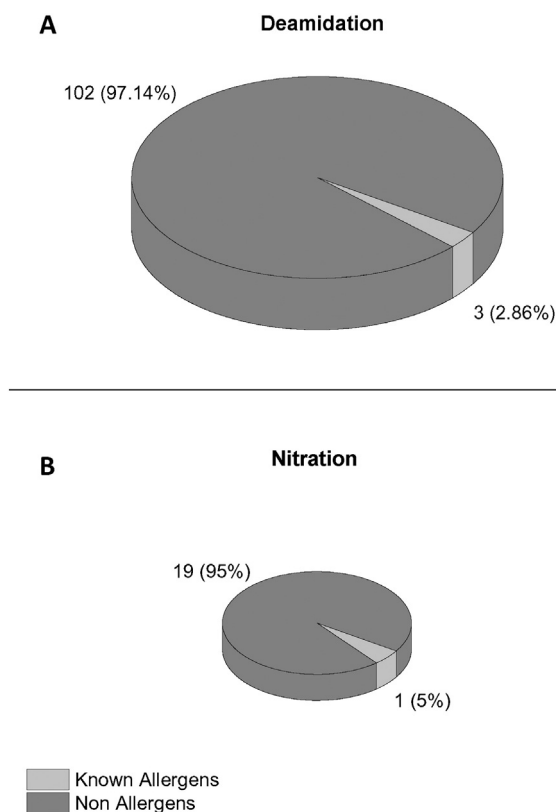


Fig. 4. The actual number, as well as percent values of allergenic and non-allergenic proteins modified with deamidified (A) and nitrated (B) PTM as a result of NO₂ + O₃ exposure. Exposure experiments were performed in duplicates.

expected to be affected in a similar manner in urban and downwind urban centers.

The outdoor NO₂ levels measured in this study are an order of magnitude lower than those measured in highly polluted areas (Goldberg et al., 2013; Guo et al., 2013; Spataro et al., 2013). Based on our observations, we conclude that the allergenicity of fungal spores emitted in urban environments would be significantly enhanced in the first few hours after their emission following exposure to NO₂ and ozone pollution.

4. Conclusions

In this study we showed that exposure of fungal spores to urban air pollution affects their allergenicity. We suggest that upon exposure to air pollution, a complex change in allergenicity occurs. Initial modification involves protein nitration that leads to increased allergenicity. Additional detected process of protein deamidation leads to decreased potency with increased exposure, moderating the increase in allergenicity due to nitration. The similarity of the results for a specific allergenic protein and for a mixture of allergenic proteins calls for further investigation on the universality of the allergenicity-modification mechanism.

Our results suggest that in and downwind polluted urban environments, people may be exposed to allergens of higher potency, mostly during the first 12 h of allergen exposure to urban air pollution.

Appendix A Supplementary data

Supplementary material.

Acknowledgments

This research was support by the Israeli Science Foundation (grant # 913/12). The authors thank Dror Barzilay for technical assistance; Yoav Barak for instrumentation and experimental consultancy; Nir Bluvshstein, Michal Levin and Michel Flores for laboratory assists and support. We also gratefully acknowledge the expert assistance of Tevie Mehlman and Dalit Merhav from the Mass Spectrometry Unit, Weizmann Institute of Science.

References

- Ackaert, C., Kofler, S., Horejs-Hoeck, J., Zulehner, N., Asam, C., von Grafenstein, S., et al., 2014. The impact of nitration on the structure and immunogenicity of the major birch pollen allergen bet v 1.0101. *PLoS One* 9, e104520.
- Asher, M.I., Montefort, S., Bjorksten, B., Lai, C.K., Strachan, D.P., Weiland, S.K., et al., 2006. Worldwide time trends in the prevalence of symptoms of asthma, allergic rhinoconjunctivitis, and eczema in childhood: ISAAC phases one and three repeat multicountry cross-sectional surveys. *Lancet* 368, 733–743.
- Bayram, H., Sapsford, R.J., Abdelaziz, M.M., Khair, O.A., 2001. Effect of ozone and nitrogen dioxide on the release of proinflammatory mediators from bronchial epithelial cells of nonatopic nonasthmatic subjects and atopic asthmatic patients in vitro. *J. Allergy Clin. Immunol.* 107, 287–294.
- Boersma, K.F., Jacob, D.J., Trainic, M., Rudich, Y., DeSmedt, I., Dirksen, R., et al., 2009. Validation of urban NO₂ concentrations and their diurnal and seasonal variations observed from the SCIAMACHY and OMI sensors using in situ surface measurements in Israeli cities. *Atmos. Chem. Phys.* 9, 3867–3879.
- Chaudhary N, Marr KA. Impact of *Aspergillus fumigatus* in allergic airway diseases. *Clinical and Translational Allergy* 2011; 1: 4–4.
- Chuang, K.-J., Chan, C.-C., Su, T.-C., Lee, C.-T., Tang, C.-S., 2007. The effect of urban air pollution on inflammation, oxidative stress, coagulation, and autonomic dysfunction in young adults. *Am. J. Respir. Crit. Care Med.* 176, 370–376.
- Colussi, A.J., Enami, S., Yabushita, A., Hoffmann, M.R., Liu, W.G., Mishra, H., et al., 2013. Tropospheric aerosol as a reactive intermediate. *Faraday Discuss.* 165, 407–420.
- Crutzen, P., 1988. Tropospheric ozone: an overview. In: ISA, I. (Ed.), *Tropospheric Ozone*. 227. Springer, Netherlands, pp. 3–32.
- Denning, D.W., Pashley, C., Hartl, D., Wardlaw, A., Godet, C., Del Giacco, S., et al., 2014. Fungal allergy in asthma-state of the art and research needs. *Clin Transl Allergy* 4, 14.
- Denning, D.W., Pleuvry, A., Cole, D.C., 2013. Global burden of allergic bronchopulmonary aspergillosis with asthma and its complication chronic pulmonary aspergillosis in adults. *Med. Mycol.* 51, 361–370.
- Derakhshan, B., Hao, G., Gross, S.S., 2007. Balancing reactivity against selectivity: the evolution of protein S-nitrosylation as an effector of cell signaling by nitric oxide. *Cardiovasc. Res.* 75, 210–219.
- Finlayson-Pitts, B.J., Pitts Jr., J.N., 2000. Chapter 1 – overview of the chemistry of polluted and remote atmospheres. In: Pitts, B.J.F.-P.N. (Ed.), *Chemistry of the Upper and Lower Atmosphere*. Academic Press, San Diego, pp. 1–14.
- Franze, T., Weller, M.G., Niessner, R., Poschl, U., 2003. Enzyme immunoassays for the investigation of protein nitration by air pollutants. *Analyst* 128, 824–831.
- Franze, T., Weller, M.G., Niessner, R., Poschl, U., 2005. Protein nitration by polluted air. *Environ. Sci. Technol.* 39, 1673–1678.
- Goldberg, M.S., Burnett, R.T., Stieb, D.M., Brophy, J.M., Daskalopoulou, S.S., Valois, M.F., et al., 2013. Associations between ambient air pollution and daily mortality among elderly persons in Montreal, Quebec. *Sci. Total Environ.* 463–464, 931–942.
- Greene, S.N., Chang, C.H., Richards, N.G., 2002. The role of post-translational modification in the photoregulation of Fe-type nitrile hydratase. *Chem Commun (Camb)* 2386–7.
- Grujthuisen, Y.K., Grieshuber, I., Stockinger, A., Tischler, U., Fehrenbach, T., Weller, M.G., et al., 2006. Nitration enhances the allergenic potential of proteins. *Int. Arch. Allergy Immunol.* 141, 265–275.
- Gruzdeva, O., Bellander, T., Eneroth, K., Kull, I., Melen, E., Nordling, E., et al., 2012. Traffic-related air pollution and development of allergic sensitization in children during the first 8 years of life. *J. Allergy Clin Immunol* 129, 240–246.
- Guo, Y., Li, S., Tian, Z., Pan, X., Zhang, J., Williams, G., 2013. The burden of air pollution on years of life lost in Beijing, China, 2004–08: retrospective regression analysis of daily deaths. *BMJ* 347, f7139.
- IUIS. Allergen Nomenclature Sub-Committee ALLERGEN NOMENCLATURE <http://www.allergen.org/index.php>, 2014.
- Jeong, J., Jung, Y., Na, S., Jeong, J., Lee, E., Kim, M.S., et al., 2011. Novel oxidative modifications in redox-active cysteine residues. *Mol. Cell. Proteomics* 10 (M110.000513).
- Kampf, C.J., Liu, F., Reinmuth-Selzle, K., Berkemeier, T., Meusel, H., Shiraiwa, M., et al., 2015. Protein cross-linking and oligomerization through dihydroxylation formation upon exposure to ozone. *Environ. Sci. Technol.*
- Karjalainen, A., Kurppa, K., Martikainen, R., Klaukka, T., Karjalainen, J., 2001. Work is related to a substantial portion of adult-onset asthma incidence in the Finnish population. *Am. J. Respir. Crit. Care Med.* 164, 565–568.
- Karle, A.C., Oostingh, G.J., Mutschlechner, S., Ferreira, F., Lackner, P., Bohle, B., et al., 2012. Nitration of the pollen allergen bet v 1.0101 enhances the presentation of bet v 1-derived peptides by HLA-DR on human dendritic cells. *PLoS One* 7, e31483.
- Kim, B.J., Kwon, J.W., Seo, J.H., Kim, H.B., Lee, S.Y., Park, K.S., et al., 2011. Association of ozone exposure with asthma, allergic rhinitis, and allergic sensitization. *Ann. Allergy Asthma Immunol.* 107, 214–219 e1.
- Kim, K.-H., Jahan, S.A., Kabir, E., 2013. A review on human health perspective of air pollution with respect to allergies and asthma. *Environ. Int.* 59, 41–52.
- Ko, F.W., Tam, W., Wong, T.W., Lai, C.K., Wong, G.W., Leung, T.F., et al., 2007. Effects of air pollution on asthma hospitalization rates in different age groups in Hong Kong. *Clin. Exp. Allergy* 37, 1312–1319.
- Kumagai, H., Suda, A., Sakurai, H., Kumagai, H., Arai, S., Inomata, N., et al., 2007. Improvement of digestibility, reduction in allergenicity, and induction of oral tolerance of wheat gliadin by deamidation. *Biosci. Biotechnol. Biochem.* 71, 977–985.
- Lang-Yona, N., Levin, Y., Dannemiller, K.C., Yarden, O., Peccia, J., Rudich, Y., 2013. Changes in atmospheric CO₂ influence the allergenicity of *Aspergillus fumigatus*. *Glob. Chang. Biol.* (n/a–n/a).
- Liu, J., Zhou, L., Chen, J.H., Mao, W., Li, W.J., Hu, W., et al., 2014. Role of ozone in UV-C disinfection, demonstrated by comparison between wild-type and mutant conidia of *Aspergillus niger*. *Photochem. Photobiol.* 90, 615–621.
- Low, S.Y., Dannemiller, K., Yao, M., Yamamoto, N., Peccia, J., 2011. The allergenicity of *Aspergillus fumigatus* conidia is influenced by growth temperature. *Fungal Biology* 115, 625.
- Nicolau, N., Siddique, N., Custovic, A., 2005. Allergic disease in urban and rural populations: increasing prevalence with increasing urbanization. *Allergy* 60, 1357–1360.
- Ozkan, R., Smilanick, J.L., Karabulut, O.A., 2011. Toxicity of ozone gas to conidia of *Penicillium digitatum*, *Penicillium italicum*, and *Botrytis cinerea* and control of gray mold on table grapes. *Postharvest Biology and Technology* 60, 47–51.
- Robinson, A.B., Rudd, C.J., 1974. Deamidation of glutamyl and asparaginyl residues in peptides and proteins. *Curr. Top. Cell. Regul.* 8, 247–295.
- Shiraiwa, M., Sosodova, Y., Rouvière, A., Yang, H., Zhang, Y., Abbatt, J.P.D., et al., 2011. The role of long-lived reactive oxygen intermediates in the reaction of ozone with aerosol particles. *Nat. Chem.* 3, 291–295.
- Sicherer, S.H., Sampson, H.A., 2014. Food allergy: epidemiology, pathogenesis, diagnosis, and treatment. *J. Allergy Clin Immunol* 133, 291–307 quiz 308.
- Simon-Nobbe, B., Denk, U., Poll, V., Rid, R., Breitenbach, M., 2008. The spectrum of fungal allergy. *Int. Arch. Allergy Immunol.* 145, 58–86.
- Song, F., Shin, J.Y., Jusino-Atresino, R., Gao, Y., 2011. Relationships among the springtime ground level NO_x, O₃ and NO₃ in the vicinity of highways in the US east coast. *Atmospheric Pollution Research* 2, 374–383.
- Spataro, F., Ianniello, A., Esposito, G., Allegrini, I., Zhu, T., Hu, M., 2013. Occurrence of atmospheric nitrous acid in the urban area of Beijing (China). *Sci. Total Environ.* 447, 210–224.
- Spicer, C.W., Kenny, D.V., Ward, G.F., Billick, I.H., 1993. Transformations, lifetimes, and sources of NO₂, HONO, and HNO₃ in indoor environments. *Air Waste* 43, 1479–1485.
- Untersmayr, E., Diesner, S.C., Oostingh, G.J., Selzle, K., Pfaller, T., Schultz, C., et al., 2010. Nitration of the egg-allergen ovalbumin enhances protein allergenicity but reduces the risk for oral sensitization in a murine model of food allergy. *PLoS One* 5, e14210.
- Walcher, W., Franze, T., Weller, M.G., Poschl, U., Huber, C.G., 2003. Liquid- and gas-phase nitration of bovine serum albumin studied by LC–MS and LC–MS/MS using monolithic columns. *J. Proteome Res.* 2, 534–542.
- Wong, G.W., Leung, T.F., Ko, F.W., 2013. Changing prevalence of allergic diseases in the Asia-Pacific region. *Allergy Asthma Immunol Res* 5, 251–257.
- Zhang, Y., Zhang, L., 2014. Prevalence of allergic rhinitis in China. *Allergy Asthma Immunol Res* 6, 105–113.