Biological and Chemical Air Pollutants in an Urban Area of Central Europe: Co-exposure Assessment

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ABSTRACT

Synergistic interactions between biological and chemical air pollutants, enhanced by the effect of meteorological factors, may increase the risk of respiratory disease. Therefore, to accurately evaluate the impact of air pollution on human health, the concomitant behaviors of various air pollutants should be investigated. In this study, the peculiarities of the temporal co-existence of allergenic pollen (alder, birch, grass, and mugwort), fungal spores (*Alternaria* and *Cladosporium*), and hazardous air pollutants (ground-level ozone and particulate matter, PM₁₀) collected in Poznań (western Poland) from 2005 to 2016 were analyzed with particular attention to their relation with air temperature. The results of the statistical analysis showed that the daily concentrations of certain airborne particles (pollen, fungal spores, and ozone) significantly increased on days with high mean temperatures. However, high temperatures occurring during earlier stages of development for grass and mugwort, prior to pollen release, decreased the overall quantity of pollen produced and released during the season. Furthermore, the daily concentration of PM₁₀ decreased with increasing temperature. As a result, the co-exposure of alder pollen and PM₁₀ was limited to a narrow temperature range (4–10°C) and mainly recorded during February and March. In most cases, a characteristic pattern was observed: The co-occurrence of air pollutants increased with the temperature. When birch and grass pollen co-occurred with other air pollutants, the temperature was significantly higher (by 2.0 to 8.0°C) than when only pollen grains were observed. In general, high temperatures favored the simultaneous occurrence of pollen grains, fungal spores, and ozone, which was most pronounced during hot days in June and August. Such conditions should therefore be considered the most hazardous for people suffering from allergic airway diseases.

Keywords: Bioaerosols; Allergens; Ozone; PM₁₀; Respiratory health.

INTRODUCTION

Air pollution is considered a prominent factor responsible for an increase in exacerbation and prevalence of allergic airway diseases (Saxon and Diaz-Sanchez, 2005; Bartra *et al.*, 2007). Important components of these mixtures include anthropogenic (chemical) airborne particles, e.g., particulate matter (PM), ozone and carbon monoxide concentrations, and natural (biological) particles, such as pollen and fungal spores. PM triggers innate immunity inflammation, oxidative stress and autophagy resulting in pathological changes in allergic respiratory diseases (Wu *et al.*, 2018). Daily variation in the concentration of PM has been closely correlated, in

many cities worldwide, with daily fluctuations in death rates (from respiratory and cardiovascular diseases), and these effects occur even at low concentrations (20–200 µg m⁻³) (Smith and Meeker, 2011). Elevated ozone concentrations were associated with current wheezing, allergic rhinitis, and increased risk of admission for respiratory disease, especially among children (Kim *et al.*, 2011; Luong *et al.*, 2018). Pollen and fungal spores, by inducing allergy reactions, have a remarkable clinical impact. The symptoms of hay fever can significantly affect a person's quality of life and can have high socioeconomic costs (Smith *et al.*, 2009). Allergenic plant species, e.g., grasses, many weeds, and trees, are predominantly wind-pollinated, and their pollen grains are small, light, and dry and are released in high abundance during the flowering season (Swoboda *et al.*, 2008).

However, the impact of certain man-made and natural air pollutants on human health should not be considered separately. Allergic patients are often polysensitized (react

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to more than one allergenic proteins, i.e., allergens), and the pollen seasons of certain allergic plants might overlap with fungal spore seasons. As a result, exacerbated allergic symptoms might be due to fungal allergy or pollen allergy (Twaroch *et al.*, 2015). The combined exposure to chemical and biological air pollutants may also strengthen (both synergistically and additively) allergic reactions (Baldacci *et al.*, 2015). Importantly, this effect is nonspecific and may involve different types of air pollutants. For instance, it was shown that acute effects of pollen were greater when the concentration of the airborne particulate matter was higher (Konishi *et al.*, 2014). Moreover, the association between pollen concentration and daily clinic visits was enhanced by high PM levels and its specific composition (e.g., non-sea-salt Ca^{2+}) (Phosri *et al.*, 2017). Similarly, grass pollen acts as a cofactor in patients with *Alternaria* spore allergy by lowering the threshold at which severe acute asthma takes place (Pulimood *et al.*, 2007). Also, alder pollen may act as a primer and make allergic people more sensitive to birch pollen—it may evoke stronger reactions during the birch pollen season, and allergic reactions may occur at lower thresholds of birch pollen concentrations (Emberlin *et al.*, 1997). The same phenomenon has been proposed for birch and grass pollen (de Weger *et al.*, 2011). Recently, the importance of bacterial endotoxins detected on mugwort pollen grains has been announced as the essential factor for inducing lung inflammation and allergic sensitization (Oteros *et al.*, 2018). Importantly, particulate pollution can induce alveolar macrophage death and interleukin-1 α release in the lung by a series of events (i.e., formation of inducible bronchus-associated lymphoid tissue) causing respiratory illnesses (including asthma) (Kuroda *et al.*, 2016). In addition, air pollutants may directly interact with airborne pollen grains, affecting their morphological features and altering allergenic properties (Behrendt and Becker, 2001). For instance, the elevated ozone concentrations increased the amount of allergens in pollen (Masuch *et al.*, 1997; Eckl-Dorna *et al.*, 2010; Beck *et al.*, 2013; Garcia-Gallardo *et al.*, 2013), decreased the pollen viability and germination rates (Cuinica *et al.*, 2013) and modified the structure of lipids, proteins and polysaccharides of pollen (Ribeiro *et al.*, 2017). Airborne pollen has the ability to adsorb particulate matter that in turn may affect the pollen wall structure, e.g., by breaking and thinning of the exine (Surekha and Jaiswal, 2014; Ribeiro *et al.*, 2015; Azzazy, 2016). Therefore, a multi-pollution approach should be assessed to properly estimate the effects of air pollution on human health. The need to consider both pollen and pollutant contents for epidemiologic evaluation of environmental determinants in respiratory allergies has been recently announced (Schiavoni *et al.*, 2017).

In response to these expectations, we have investigated the temporal co-occurrence of chemical (PM_{10} and ground-level ozone) and biological (pollen grains and fungal spores) air pollutants in a medium-sized city in Central Europe. The process of pollutant release depends strongly on temperature (Sofiev *et al.*, 2013); thus, we focused on this weather parameter and estimated its impact on the co-existence of

selected particles in the air. Furthermore, temperature is often used as a proxy for other meteorological parameters. For example, a clear negative relationship between air humidity and temperature is well documented (Hage, 1975). Many complex meteorological variables can be reliably calculated using only air temperature, e.g., vapor pressure deficit, solar radiation and reference evapotranspiration (Stöckle *et al.*, 2004; Bogawski and Bednorz, 2014). From an allergenic point of view, evidence shows that the occurrence of allergic rhinitis in spring is significantly correlated with mean daily temperature (Chappard *et al.*, 2004).

METHODS

Study Site

Air monitoring was conducted over 12 years (2005–2016) in the center of Poznań ($52^{\circ}18'–52^{\circ}30'N$, $16^{\circ}48'–17^{\circ}04'E$), the largest city in western Poland (Fig. S1). On a European scale, Poznań is considered to be a “medium-sized” city—it extends over 261.3 km² and is home to a population of ~550,000 (GUS, 2015). Poznań is located on the Warta River at an elevation of 50 to 154 m a.m.s.l. (Kondracki, 1998). The climate is temperate with both Atlantic Maritime and continental influences. The mean January and July temperatures in Poznań are -1.0°C and 18.0°C , respectively, and the mean annual precipitation is approximately 550 mm (1971–2000 average) (Lorenc, 2005).

Air Quality Monitoring

The following biological particles have been monitored in the air: birch (*Betula* sp.), alder (*Alnus* sp.), grass (Poaceae) and mugwort (*Artemisia* sp.) pollen, and *Alternaria* and *Cladosporium* spores. These taxa have been chosen based on their allergenic properties and clinical importance in Central Europe (Burbach *et al.*, 2009). Airborne pollen grains and fungal spores were collected by a 7-day volumetric trap using the Hirst design (Hirst, 1952). The Hirst-type trap was specifically designed to record the atmospheric concentration of pollen grains and fungal spores as a function of time (Mandrioli *et al.*, 1998). The trap was located at roof level (33 m) in the Poznań city center ($52^{\circ}24'N$, $16^{\circ}53'E$) (Fig. S1). The particles were collected on adhesive tape moving at 2 mm hour⁻¹. After one week, the tape was divided into 7 segments corresponding to 24-hour periods. The preparation and counting methods were performed according to the recommendation of the European Aerobiology Society (Galán *et al.*, 2014). Daily average (00:00–23:00) pollen and fungal spore counts were expressed as pollen m⁻³ and spores m⁻³, respectively. Note: *Alternaria* and *Cladosporium* spore data were available from 2005–2014 and in 2016.

Mean hourly and daily air pollution data (ground-level ozone and PM_{10} concentrations, respectively) were obtained from two monitoring stations belonging to the Provincial Inspectorate for Environmental Protection in Poznań. Particulate matter measurements were performed by the automatic analyzer in accordance with the European Committee for Standardization norm EN 12341 (Czernecki *et al.*, 2017). Both stations are located within 5.0 km from

the pollen/spore monitoring trap. The concentrations of other anthropogenic air pollutants, e.g., sulfur dioxide or carbon monoxide, were not included in the analysis, as their concentrations in the air very rarely exceeded the air quality thresholds (AQTs) recommended by the European Environmental Agency (EEA, 2014). Daily mean temperature data were obtained from the main official meteorological station situated at the Poznań Ławica airport (app. 5.0 km from the pollen/spore monitoring trap, 3.0 km from the ozone monitoring station, and 10 km from PM monitoring station) (Fig. S1).

Statistical Analysis

The collected datasets were screened to select days with mean daily particle concentrations above certain AQTs, i.e., birch (> 20 pollen m^{-3}), grass (> 20 pollen m^{-3}), mugwort (> 30 pollen m^{-3}), alder (> 50 pollen m^{-3}), *Alternaria* sp. (> 100 spores m^{-3}), *Cladosporium* sp. (> 3000 spores m^{-3}), PM_{10} (> 50 $\mu\text{g m}^{-3}$), and ozone (maximum daily 8-hour mean > 120 $\mu\text{g m}^{-3}$). The AQTs have been chosen according to the recommendation of the EEA (2014) and based on clinical studies linking allergy symptoms with daily pollen and fungal spore concentrations (de Weger *et al.* (2013) and references therein). All data (except of ozone) was extracted with daily resolution (24-hour). The ozone data was extracted with hourly resolution. The hourly ozone data was used to calculate the maximum daily 8-hour mean.

The AQTs were used to determine the limits of pollutant seasons (start and end dates). Such defined periods were used in statistical analysis to determine the impact of (bi)monthly/daily temperatures on (1) daily mean particle concentration, (2) the first day in the year with particle levels $>$ AQT, and (3) the number of days per year with particle levels $>$ AQT. To reduce the skewness of data and high interannual difference in particle concentrations, the daily air pollution levels were logarithmically transformed ($\log_{10}$). The direction and strength of temperature–pollution relationships were determined by a linear regression model. The Kruskal-Wallis test and Dunn's procedure for multiple pairwise comparisons were used to compare the temperature during days with and without concomitant air pollutants in the air. *p*-values were adjusted using Benjamini-Hochberg correction. The co-occurrence level of pollen with other air pollutants was analyzed using an area-proportional Euler diagram in eulerAPE software (Micallef and Rodgers, 2014) and a bivariate Gaussian kernel density estimator with default smoothing bandwidth (Silverman, 1986). The statistical significance of all results was determined by comparing the *p*-value with the significance level ($\alpha = 0.05$). The statistical analysis was performed using R statistical software version 3.5.1 (R Core Team, 2018).

RESULTS

The main characteristics of the pollutant seasons are presented in Table 1. PM_{10} and alder pollen were the first particles recorded in the air (9th day of the year (DOY)/9 January and 65 DOY/6 March, respectively). The distribution of PM_{10} concentration was characterized by

Table 1. Main characteristics of selected pollutant seasons during 2005–2016 in Poznań.

Pollutant	Air Quality Threshold (AQT)	Mean start day of the season (DOY/date)	Mean end day of the season (DOY/date)	Mean duration of the season (days)	Mean number of days per year with particle level $>$ AQT
Alder pollen	$> 50 \text{ P m}^{-3}$	65/06 March	84/25 March	20	15
Birch pollen	$> 20 \text{ P m}^{-3}$	99/09 April	130/10 May	33	27
Grass pollen	$> 20 \text{ P m}^{-3}$	143/23 May	201/20 July	59	44
Mugwort pollen	$> 30 \text{ P m}^{-3}$	213/01 August	227/15 August	15	10
<i>Alternaria</i> spores	$> 100 \text{ S m}^{-3}$	174/23 June	282/09 October	108	64
<i>Cladosporium</i> spores	$> 3000 \text{ S m}^{-3}$	135/15 May	304/31 October	170	85
PM_{10} (first peak)	$> 50 \mu\text{g m}^{-3}$	9/09 January	125/05 May	117	35
PM_{10} (second peak)	$> 50 \mu\text{g m}^{-3}$	258/15 September	351/17 December	94	25
Ozone	$> 120 \mu\text{g m}^{-3}$	131/11 May	218/06 August	88	15

two distinct peaks (first peak between 9 and 125 DOY (9 January–5 May) and second peak between 258 and 351 DOY (15 September–17 December)) (Fig. S2). Later in the season, the following pattern of particle appearance was observed: birch pollen (99 DOY/9 April), ozone (131 DOY/11 May), *Cladosporium* spores (135 DOY/15 May), grass pollen (143 DOY/23 May), *Alternaria* spores (174 DOY/23 June) and mugwort pollen (213 DOY/01 August). The longest particle seasons were recorded for *Cladosporium* spores and PM₁₀ (170 and 211 days, respectively), while the highest mean number of days per year with particle levels > AQT was determined for *Cladosporium* spores (85 days year⁻¹), *Alternaria* spores (64 days year⁻¹) and PM₁₀ (60 days year⁻¹). In contrast, the pollen season of mugwort was the shortest (15 days), with the lowest number of days with particle levels > AQT (10 days year⁻¹).

High mean (bi)monthly temperature accelerated the beginning of pollen seasons of alder, birch and grasses, spore seasons of *Alternaria* and *Cladosporium*, and ozone (Fig. 1). In the case of PM₁₀, and mugwort pollen seasons, the mean monthly temperatures (January and July, respectively) delayed the time when high concentrations of air particles were recorded in the air. Daily concentrations of all monitored air pollutants (except PM₁₀) were positively associated with daily mean temperature. Only PM₁₀ showed a negative correlation with temperature, i.e., the PM₁₀ level increased with decreasing daily mean temperature (Fig. 2). The number of days with particle concentrations > AQT per year was positively and significantly correlated with mean (bi)monthly temperature for alder and birch pollen, *Alternaria* spore, and ozone. For instance, the number of days (per year) with elevated levels of alder pollen was

positively related to the mean February temperature ($r^2 = 0.466$, $p < 0.05$). Opposite relationships were observed with respect to PM₁₀, grass and mugwort pollen and *Cladosporium* spores (however, the last relationship was not statistically significant) (Fig. 3).

Among selected air pollutants, the co-existence of alder pollen and other air pollutants was almost entirely limited to PM₁₀ (by 29.1% of all days with pollen concentrations > AQT), while birch pollen was limited to PM₁₀ and ozone (by 10.0% and 6.4%, respectively). When alder pollen and PM₁₀ were recorded in the air, the mean daily air temperature varied from 5 to 10°C. The mean daily air temperature recorded on days with only birch pollen was significantly lower than that on days when birch pollen co-occurred with ozone and PM. A similar phenomenon was detected when grass pollen was recorded in the air. Grass pollen co-occurred predominantly with *Cladosporium* spores (65.3%, mean temperature: 20.4°C) and *Alternaria* spores (28.9% and 21.4°C), especially in the second part of the grass pollen season, and ozone (14.3% and 23.0°C) in the first part of the grass season. During days when only grass pollen was recorded in the air, the mean air temperature reached 16.9°C (3rd quartile never exceeded 19°C), while when two or three air pollutants were simultaneously observed, the temperature increased to 25°C (1st quartile always exceeded 19°C). Mugwort pollen almost completely overlapped with fungal spores (*Cladosporium* spores by 81.6% of the days and *Alternaria* spores by 93.0% of the days with mugwort pollen level > AQT) and to a lesser extent with ozone (16.5%). The highest mean daily temperature was recorded when mugwort pollen co-occurred with ozone (25.5°C) (Figs. 4–6).

Fig. 1. Correlation between (bi)monthly mean air temperature (°C) and the first day with particle concentrations above air quality thresholds (day of the year, DOY). The following pairs are presented: alder pollen-February temp., birch pollen-March temp., ozone-March temp., PM₁₀-January temp., grass pollen-April–May temp., mugwort pollen-July temp., *Alternaria* spore-May–June temp., and *Cladosporium* spores-April temp. All correlations are statistically significant at $p < 0.05$, except for *Alternaria* spore-May–June temp. ($p = 0.07$). Grey area represents the 95% confidence intervals.

Fig. 2. Correlation between daily mean air temperature ($^{\circ}\text{C}$) and daily particle concentration (pollen m^{-3} , spores m^{-3} , $\mu\text{g m}^{-3}$). All correlations are statistically significant at $p < 0.05$. Grey area represents the 95% confidence intervals.

Fig. 3. Correlation between (bi)monthly mean air temperature ($^{\circ}\text{C}$) and the number of days per year with particle concentrations above air quality thresholds. The following pairs are presented: alder pollen–February temp., birch pollen–March and May temp., ozone–June and August temp., PM_{10} –January–February temp., grass pollen–May–July temp., mugwort pollen–June–July temp., *Alternaria* spore–May–June temp., and *Cladosporium* spores–July–August temp. All correlations are statistically significant at $p < 0.05$, except for birch pollen–March and May temp. ($p = 0.08$) and *Cladosporium* spores–July–August temp. ($p = 0.32$). Grey area represents the 95% confidence intervals.

DISCUSSION

Concomitant exposure to natural and man-made air pollutants was the most pronounced during summer months (June–August), when pollen seasons of highly allergenic grass and mugwort pollen co-occurred with elevated concentrations of fungal spores and ozone. It should be stressed that the elevated ozone concentrations

increased the allergen contents of pollen released by various grass species, e.g., *Lolium perenne* and *Secale cereale* (Masuch et al., 1997; Eckl-Dorna et al., 2010). In addition, exposure of *Phleum pratense* pollen to increasing ozone concentrations resulted in a significant increase in the naturally released pollen cytoplasmic granules, which are also known to contain allergens (Motta et al., 2006). A similar effect, i.e., higher allergen contents and larger wheal

Fig. 4. The mean seasonal level (2005–2016) of co-occurrence (%) of investigated pollen grains with other air pollutants. The ellipse areas within certain groups are proportional to the number of days per year with particle levels > AQT (see Table 1).

and flare sizes in patients pricked with pollen extracted from high ozone stands, has also been observed in relation to birch pollen (Beck *et al.*, 2013). In Poznań, however, the co-occurrence of ozone and birch pollen was a relatively rare phenomenon (on average 3 days per year; Fig. S3), and therefore should have a limited clinical importance. Nevertheless, considering the possible interaction between ozone and pollen allergens (especially during warm summer months), in some European countries, e.g., Denmark, the ambient ozone concentrations were recommended for consideration when assessing pollen exposure (Ørby *et al.*, 2015).

The concomitant occurrence of grass and mugwort pollen and other air pollutants was not, however, homogenous during the whole pollen seasons. For instance, high concentrations of ozone were limited to only the first part of the grass pollen season (May–June), while *Cladosporium* and *Alternaria* spores were recorded in the second part (July–August). In this context, it is worth mentioning that the severity of symptoms of seasonal allergic rhinitis may differ along the season. For instance, in the Netherlands allergic symptoms to grass pollen were more severe in the early than in the late flowering season (de Weger *et al.*, 2011). Several explanations were proposed for this clinical phenomenon, including the down regulation of allergic response (decrease in allergic inflammation) after repeated allergen exposure (de Bruin-Weller *et al.*, 1999; de Weger *et al.*, 2011), higher pollen allergenicity of early flowering grass species (Buters *et al.*, 2015) as well as the overlapping effect of pollen released by different plants. It was

suggested that patients co-sensitized to birch and grass pollen may respond in the overlapping season to the presence of birch pollen, while grass pollen concentrations are still low (de Weger *et al.*, 2011). Possibly, the effect of anthropogenic air pollutants, such as ozone, should also be taken into account in this process. For instance, it was shown that high ozone levels increase the bronchial responsiveness to pollen allergens in atopic asthmatic subjects (Molfino *et al.*, 1991). In addition, ozone increased the permeability of the bronchial epithelial layer and inhibited the ciliary beat frequency, leading to delayed clearance of inhaled particles, e.g., allergens released from pollen grains (Schiavoni *et al.*, 2017). However, more studies are required in this topic, as the comprehensive epidemiological data is still limited.

Our study showed that the co-occurrence of certain air pollutants was related to daily mean temperature. During the summer months, the concomitant occurrence of grass pollen, fungal spores and ozone was the most pronounced at temperatures above 20°C. The mean daily levels of all selected biological air pollutants were positively correlated with daily mean temperature. These results concur with previous findings showing that the release of pollen and spores of many fungal species is facilitated by high temperatures and low air humidity (Diaz de la Guardia *et al.*, 1998; Zink *et al.*, 2013; Kasprzyk *et al.*, 2016). According to Harvey (1970), dry conditions favor sporulation and may double the number of *Cladosporium* spores in the air. The positive effect of air temperature on pollen level has been shown for both herbaceous and hardwood plants. For

Fig. 5. The mean seasonal level (2005–2016) of co-occurrence of two air pollutants expressed by bivariate Gaussian kernel density estimator with respect to daily mean temperature (°C) and day of the year. Levels reflect the likelihood of a co-occurrence of two air pollutants at a certain temperature $\times$ day of the year grid. Colors and shapes of the points represent different air pollutants: blue dots—pollen grains, green triangle—fungal spores, and black diamond—chemical pollutants.

Fig. 6. Comparison of daily mean temperature during days with only pollen in the air and with the occurrence of other air pollutants. Statistical significance is marked by “***” ($p < 0.01$) and “****” ($p < 0.001$). The black dots represent outliers. Black diamonds represent mean. As the mugwort pollen grains almost completely co-occurred with fungal spores (see Fig. 4), the comparison analysis for this pollen was not performed.

instance, in London, UK, the maximum daily temperatures (within the range 21.1–25°C) were associated with the highest daily grass pollen concentrations (Norris-Hill, 1997), while the rate of birch pollen release in Poznań, Poland, was significantly correlated with daily maximum temperature (Grewling *et al.*, 2012a). Similarly, a positive linear relationship has been typically observed between ground-level ozone concentration and temperature (Vukovich and Sherwell, 2003; Jacob and Winner, 2009). Temperature and sunlight directly influence chemical kinetic rates and the mechanism pathway, i.e., reaction of oxides of nitrogen (NO_x) and volatile organic compounds (VOCs) in the generation of ozone (Steiner *et al.*, 2010). As a result, in summertime during sunny and dry days, many clinically important air pollutants (both natural and anthropogenic) exceeded high concentrations in the air. Such weather conditions should therefore be considered the most hazardous for people suffering from pollen and fungal spore allergies.

On the other hand, high temperatures recorded before pollen seasons may decrease the number of days with high pollen concentrations later in the season. Such a phenomenon was noted in Poznań for grass and mugwort pollen. In addition, high temperature significantly delayed the moment when high levels of mugwort pollen were recorded in the air. High temperature has been suggested to limit the water available to plants, resulting in slower and weaker plant development and, in turn, lower pollen production (Cockshull and Kofranek, 1994; Grewling *et al.*, 2012b; Bogawski *et al.*, 2014; Bogawski *et al.*, 2016). The negative impact of high temperature recorded weeks before pollination on pollination intensity has been previously observed for mugwort (Munuera Giner *et al.*, 1999; Bogawski *et al.*, 2014) and grasses (Smith and Emberlin, 2006). In addition, a distinct effect of drought on the decrease in the annual concentration of pollen was

recorded in Spain for many other non-arboreal plant species, such as sorrel, plantain, sunflower and sedges (Gonzalez Minero *et al.*, 1998). As a result, although high air temperature recorded simultaneously with certain air pollutants will presumably increase their daily concentrations, the prolonged effect of high temperature recorded before plant/fungi development may decrease the overall number of produced and released pollen/spores.

In contrast to previously described air pollutants, the daily concentration of PM_{10} significantly decreased with increasing temperature, which was related to home-heating emission, which was most intense at low temperatures (Cichowicz *et al.*, 2017). Thus, the concomitant occurrence of PM_{10} and biological air pollutants is mainly limited to a certain temperature range within which the process of pollen and fungal spore release is still possible. In Poznań, these temperature ranges should be defined as follows: 4–10°C for alder pollen and PM_{10} , 8–18°C for birch and PM_{10} (during spring), and 8–22°C for fungal spores and PM_{10} (during autumn). At lower temperatures, the release of pollen and fungal spores is hindered, while at higher temperatures, the concentration of PM_{10} markedly decreases. Notably, due to the very low temperature requirements of alder (one of the earliest flowering trees in Europe, January–March) (Smith *et al.*, 2007; Rodríguez-Rajo *et al.*, 2009), its pollen might be observed in the air even during very cold days ($< 0^\circ\text{C}$). The co-occurrence of alder and PM_{10} is not a rare phenomenon; in almost 30% of all days with high alder pollen levels, the concentrations of PM_{10} exceeded health threshold levels ($> 50 \mu\text{g m}^{-3}$). In the case of birch, which pollinates mainly in April, this value is lower (9.6%).

The presented multi-pollution pattern is characterized for Poznań, a medium-sized city in western Poland. Based on the plant and fungi distribution, this pattern can be

assumed to be similar at least in Central European cities. For instance, in Copenhagen, Denmark, the tendency for coincidence of high grass pollen and ozone concentrations was also identified (Ørby *et al.*, 2015), while in Malmö and Gothenburg, southern Sweden, the co-exposure of birch pollen and PM was emphasized as an important health risk factor (Grundström *et al.*, 2017). In addition, similar to Poznań, the high probability of becoming exposed to elevated concentrations of grass pollen and *Alternaria* and *Cladosporium* spores has been observed in Rzeszów, a city located 500 km away from Poznań (Kasprzyk, 2008). It is important to note that more than half of the *Alternaria*-sensitized patients might be also sensitized to grass allergens (Katotomichelakis *et al.*, 2012), and *Alternaria* spores activate the innate immune system enhancing lung inflammation induced by grass pollen (Kim *et al.*, 2014). As Katotomichelakis *et al.* (2012) stressed, due to the common polysensitization phenomenon, it is wise to search for sensitization to other allergens simultaneously present with *Alternaria* spores. Our research clearly indicates which natural and anthropogenic air pollutants and to what extent co-occur with fungal spores; therefore, such information could be useful in clinical practice.

In more bioclimatologically distinct European regions, the multi-pollution pattern might be of course different, as shown for the Netherlands, where the birch pollen season overlapped with the grass pollen season (de Weger *et al.*, 2011) (a phenomenon not observed in Poznań). In Stockholm, Sweden, the peak in ozone concentrations was recorded between April–May (thus earlier than in Poznań) co-occurring with the period of birch pollination (Olstrup *et al.*, 2019). In addition, in large urban agglomerations commonly found in Western Europe, the impact of other anthropogenic air pollutants such as SO₂ or NO₂ could be more pronounced. According to the EEA report (EEA, 2017), in many cities in western Germany, the annual mean NO₂ levels exceeded values (> 40–50 µg m⁻³) not observed in Poznań. The multi-pollution peculiarities of certain sites should therefore be determined to better understand the impact of air pollutants on local populations. For instance, a recent study from Sweden has shown that areas with simultaneously high birch pollen and PM concentrations resulted in higher sales of antihistamines than in areas with high birch pollen alone (Grundström *et al.*, 2017). Unfortunately, currently, the airborne behavior of certain inhalant natural pollutants is predominantly investigated without considering other concomitant particles. For effective prevention strategies of allergic disease, this single-particle approach should be replaced by integrated air quality monitoring, which includes simultaneous records of both natural and man-made air pollutants. Currently, with the fast development of new real-time pollen monitoring devices, e.g., Plair PA-300 (Crouzy *et al.*, 2016) and Hund BAA500 (Oteros *et al.*, 2015), such an integrated approach is more accessible than ever before.

CONCLUSIONS

Temperature affects the rate of plant and fungal

development, including the process of pollen and spore release, and, at the same time, influences pollution-causing human activities, mainly the usage of domestic heating. As a result, significant correlations between the temperature, and the starting date and intensity of seasonal particle pollution, as well as the daily particle concentration, have been observed. The health risk from multi-pollution exposure increases with the temperature, peaking in summer when grass and mugwort pollen co-occur with fungal spores and high concentrations of ozone. During other periods of the year, mixtures of PM-alder/birch pollen (in spring) and PM-fungal spores (in autumn) are predominant in the air. As co-exposure to various, simultaneously occurring air pollutants is an important health risk factor (due to its possible synergistic and additive effects), we recommend measuring both biological and chemical air pollutants during air quality monitoring.

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DISCLAIMER

The authors report no conflicts of interest. The authors alone are responsible for the content and the writing of the paper.

SUPPLEMENTARY MATERIAL

Supplementary data associated with this article can be found in the online version at <http://www.aaqr.org>.

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