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Correlation of ambient inhalable bioaerosols with particulate matter and ozone: A two-year study

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Synergistic effects of these pollutants may increase incidence of respiratory health problem.

Abstract

In this study, we have examined the relationships between the concentrations of ambient inhalable airborne fungi and pollen with PM_{10}, $PM_{2.5}$, ozone, organic carbon, selected trace metals (cadmium, copper, lead, and zinc), temperature, and relative humidity. The database was collected in Cincinnati, Ohio, USA, during two consecutive years. Measurements of all environmental variables were performed at the same site continuously 5 days a week except during winter months. The airborne concentrations of biological and non-biological pollutants ranged as follows: total fungi: 184–16 979 spores m^{-3}; total pollen: 0–6692 pollen m^{-3}; PM_{10}: 6.70–65.38 μg m^{-3}; $PM_{2.5}$: 5.04–45.02 μg m^{-3}; and ozone: 2.54–64.17 ppb. Higher levels of total inhalable fungi and particulate matter were found during fall and summer months. In contrast, total pollen concentration showed elevated levels in spring. Peak concentrations of ozone were observed during summer and beginning of fall. Our study concluded that several types of inhalable airborne fungi and pollen, particulate matter, and ozone could be positively correlated as a result of the atmospheric temperature influence.

Keywords: Inhalable bioaerosol; Aeroallergen; PM_{10} and $PM_{2.5}$; Ozone; Air pollutants

1. Introduction

There is a lack of investigations on ambient inhalable bioaerosols and little attention has been paid on the interrelationships between allergenic inhalable bioaerosols, ambient air pollutants relevant to respiratory health, and meteorological factors. A significant portion of atmospheric aerosol is of biological origin. Approximately 24% of the count of total atmospheric particles and 5–10% of the total suspended particulate mass were reported to be contributed by bioaerosols (Matthias-Maser and Jaenicke, 1995; Glikson et al., 1995). Based on the concentration of phospholipids, Womiloju et al. (2003) reported that cell materials of fungi and pollen could contribute 4–11% of the total $PM_{2.5}$ mass and 12–22% of organic carbon in fine particulate matter.

Among different bioaerosol components, airborne fungi and pollen grains are associated with respiratory allergic diseases and asthma (D'Amato et al., 1998;

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Chapman, 1999; Solomon, 2002). Various studies around the world have investigated the ambient airborne fungi and pollen in relation to respiratory allergies (Caiola et al., 2002; Myszkowska et al., 2002; Adhikari et al., 2004; Hasnain et al., 2004). The health relevant inhalable fractions of the total airborne fungi and pollen, however, were investigated in a few studies. Toivola et al. (2002) compared the inhalable bioaerosol concentration levels using personal and stationary sampling approaches in home and work environments. Mitakakis et al. (2000) compared the airborne pollen and spore concentrations measured with the personal IOM samplers and the stationary Burkard sampler. Our previous study has shown that the Button Personal Inhalable Aerosol Sampler is efficient for the collection of airborne fungi and pollen in outdoor environments (Adhikari et al., 2003). Using this inhalable sampling method, we have conducted long-term (two-year) monitoring of airborne fungi and pollen in the Greater Cincinnati metropolitan area.

Particulate matter (PM) in general, and especially PM_{10} ($PM \leq 10$ μm in diameter) and $PM_{2.5}$ ($PM \leq 2.5$ μm), are associated with respiratory allergic diseases, asthma (D'Amato, 2000), and mortality (Dockery et al., 1993). PM can act as a carrier of allergens (Knox et al., 1997; Ormstad et al., 1998). Diesel exhaust particles can act as an adjuvant with bioaerosols enhancing IgE production (Parnia et al., 2002) and thus facilitating allergic sensitization. Intact pollen (>10 μm) cannot reach the small airways, however, pollen allergens present in $PM_{2.5}$ can easily penetrate there (Monn, 2001). Thus, the separate effects of bioaerosols and PM as well as their synergistic effects can aggravate respiratory allergy and other pulmonary diseases. A previous study performed in the Cincinnati area showed that high concentration of PM_{10} was synergistic with the airborne pollen concentration levels for predicting daily asthma visits (Lierl and Hornung, 2003). On the other hand, trace metal constituents of fine particulate matter can deposit on vegetation and may induce some toxic effects to phyllosphere fungi and flowering plants. Gingell et al. (1976) found that the higher levels of zinc, lead, and cadmium on the leaf surfaces significantly reduced the levels of foliar microbes. Excess accumulation of trace metals, particularly lead, cadmium, zinc, and copper may reduce the decomposition rates in the forest floor (Jackson et al., 1978) indicating reduced microbial activity. Hence, the adverse effects of these trace metals on the airborne mycoflora and plants may not be immediate but delayed by several days, months or years. Furthermore, different air pollutants may interact with forest trees to reduce pollen production and flower or cone initiation and may decrease the pollen size (Smith, 1990a; Emberlin, 1995). Previous studies reported that PM could bind with airborne pollen (Behrendt et al., 1992; Risse et al., 2000) and fungal spores (Glikson et al., 1995) altering their morphology. The PM presence on the surface of bioaerosol particles may change the dispersal pattern of bioaerosols in ambient air by altering the particle aerodynamic properties. Air pollutants may ultimately increase the bioavailability of pollen allergens by modifying their surface (Monn, 2001).

Among various gaseous air pollutants, association of ozone with adverse respiratory health effects is well reported (Jorres et al., 1996; Kehrl et al., 1999). Exposure to ozone may increase the allergic response to aeroallergens by increasing the permeability of the airways and also by altering the immune response to allergens (Parnia et al., 2002). Ross et al. (2002) found that simultaneous exposure to both ozone and pollen may have adverse effects on respiratory health. Tropospheric ozone is a strongly phytotoxic oxidant (Tiedemann and Firsching, 2000), possibly altering the production and allergenic content of pollen (D'Amato, 2000). Tiedemann and Firsching (2000) reported that the pathogenicity of rust fungi could be increased by ozone. Thus, besides causing respiratory health hazards, ozone may also influence the sources of ambient bioaerosols.

Temperature and relative humidity cyclic and fluctuating variations may alter the airborne concentration of bioaerosols (Li and Kendrick, 1995; Gioulekas et al., 2004). Long-term changes in temperature can influence pollen production (Beggs, 2004) and temperature can affect concentrations of particulate matter (Martuzevicius et al., 2004; Rajsic et al., 2004) and ozone (Sartor et al., 1997; Ross et al., 2002). Mirme and Ruuskanen (1996) found that ambient relative humidity could decrease airborne particulate mass. To summarize, bioaerosols can contribute to particulate matter and different PM components can influence bioaerosol generation and dispersal; bioaerosols, PM and ozone are associated with common respiratory health effects; and all three are influenced by temperature and/or relative humidity. The interrelationship of these factors is complex (a possible schematic is presented in Fig. 1) and deserves a comprehensive research.

The objective of this study was to examine the relationships between ambient inhalable airborne fungi and pollen (represented by their total concentrations, as well as individual concentrations of the prevalent genera) with PM_{10}, $PM_{2.5}$, ozone, organic carbon, and selected trace metals as well as temperature and relative humidity during two consecutive years. A non-parametric statistical correlation and simple as well as multiple linear regression analyses were used for this purpose. Since the effects of toxic trace metals and ozone on the flowering plants and aeromycoflora may be delayed, our study also aimed to examine the relationships using 3-day and 6-day lagged airborne fungi and pollen concentration data.

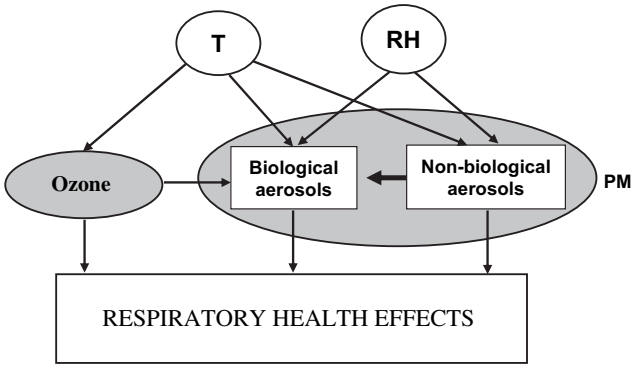

Fig. 1. Schematic representation of the complex relationships between biological and non-biological components of PM, ozone, temperature (T), relative humidity (RH), and respiratory health effects.

2. Materials and methods

2.1. The monitoring site

The monitoring site chosen for the ambient environmental monitoring was located in the Cincinnati metropolitan area (southwestern part of the Ohio state, USA). The average annual temperature of the area is 11.8 °C and the climate is primarily continental. Prevalent wind direction is from south-southwest. The downtown Cincinnati is located on the Ohio River bank and extends over two hills reaching about 120 m above the river valley. With a rich vegetation and farmlands around, the area is also characterized by intense motor traffic through the city centers. It is a home for several large industrial companies and several hundreds small industrial facilities. Overall, the area has considerable biological and non-biological air pollutant sources.

A rooftop of a two-storied office building about 3 miles north of the downtown Cincinnati was selected for the measurement of all environmental factors. The height of the rooftop was about 7 m, vegetation was sparse and there were no tall buildings in the vicinity allowing free movement of wind and spatially uniform intensity of solar radiation.

2.2. Measurement of inhalable bioaerosols

Ambient airborne fungi and pollen were collected using the Button Personal Inhalable Aerosol Samplers (SKC, Inc., Eighty-four, PA, USA). Airborne fungi were collected with the sampler oriented facing the prevalent wind direction (southwest). For collecting airborne pollen, two samplers were used: one facing the southwest and another northeast. This sampling strategy was based on our previous study showing that for fungi, the concentrations measured by two Button Samplers, one facing the prevalent wind and the other towards the opposite wind, were similar, while, for larger pollen grains, statistically significantly different concentrations were observed (Adhikari et al., 2003). Hence, the use of one Button Sampler, facing the prevalent wind, was sufficient for fungi, while for pollen, the average concentration was calculated from the data obtained with the two samplers oriented toward opposite directions. The samplers were operated for five consecutive 24-h periods (9:00 A.M.–9:00 A.M.) every week between March 1 and October 31 during two years. Two heavy-duty pumps (Model 1531-107-0288, Gast Corp., Benton Harbor, MI, USA) were used to provide a constant sampling flow rate of 4 L min^{-1}. The flow rate of the pumps was calibrated before and after each sample collection by a DryCal® DC-Lite Calibrator (SKC, Inc., Eighty-four, PA, USA).

The Button Sampler was selected in our study as it follows the ACGIH/CEN/ISO inhalable sampling convention (CEN, 1993; ISO, 1995; ACGIH, 1999). The elucidation of the personal inhalable sampling in assessing the exposure to bioaerosols has been described previously (Adhikari et al., 2003).

In the present study, mixed cellulose ester (mixture of cellulose acetate and cellulose nitrate) membrane filter of 1.2 μm pore size (Millipore Corp., Bedford, MA, USA) was used for the collection of fungi and pollen. Each Button Sampler was covered with a clean cap immediately after the sampling and carried to the laboratory in a horizontal position using a sterile box. In the laboratory, the filter was cleared by acetone vapor using a modified instant acetone-vaporizing unit (Model: Quickfix, Environmental Monitoring Systems, Inc., Charleston, SC, USA) on a glass slide. The cleared filter was mounted with glycerin jelly (gelatin: 20 g, phenol crystals: 2.4 g, glycerol: 60 mL, water: 70 mL) mixed with Calberla's stain and covered with a square 25 × 25 mm cover glass. Once the mounting glycerin jelly became solidified, the edge of the cover glass was sealed using transparent nail enamel.

2.3. Microscopic analyses of fungi and pollen

Forty randomly selected microscopic fields were analyzed in each sample using a Nikon high-resolution light microscope (Model: Labophot 2, Nikon Corp., Japan). In each field, pollen grains and fungal spores were counted and identified to the genus/class/family level. For fungi, the magnification of 400× was used, except when spores were not identifiable or the deposition was dense, necessitating a higher magnification (1000×). To identify unpigmented hyaline spores phase contrast objectives were utilized. For pollen identification, 100× and 400× microscopic magnifications were used. The concentrations of total fungi or pollen were

calculated from the microscopic counts following the protocols described by Adhikari et al. (2003).

2.4. Measurement of PM_{10}, $PM_{2.5}$, and $PM_{2.5}$ constituents (trace metals and organic carbon)

The particulate matter concentration was determined for two size ranges: PM_{10} and $PM_{2.5}$. A real-time mass concentration measurement method was used to obtain hourly PM concentrations, and values were then averaged for the 24 h matching the PM concentration data to the bioaerosol concentration data. Only the same time periods, for which the bioaerosol data were available, were included in this study. Due to instrument malfunctioning, the PM_{10} data collected between May 17–July 10, 2002, March, 2003, and July–October, 2003, were not used for the analyses. Two Tapered Element Oscillating Microbalances (TEOM Series 1400a, Rupprecht & Patashnick Co., East Greenbush, NY, USA) were used as PM measurement devices. One instrument was configured with the 10 μm inlet and measured PM_{10} concentrations. Another instrument had both the PM_{10} inlet and the sharp-cut 2.5 μm cyclone for collecting $PM_{2.5}$. The total sampling flowrate was 16.67 L min^{-1}, of which 3 L min^{-1} was passed through TEOM microbalance, and the remaining 13.67 L min^{-1} was bypassed directly to a pump.

To obtain concentrations of several $PM_{2.5}$ constituents, the data from a speciation sampler (SASS, Met One Instruments Inc., Grants Pass, OR, USA) were utilized. This sampler was operated by using every sixth-day, midnight-to-midnight measurement schedule. The effects of these trace elements are not likely to be immediate and thus we assumed that the 9 h difference in the measurement schedule with the bioaerosols is acceptable. The SASS sampler had a set of two cyclones for a parallel collection of ambient particles on filters with a flow rate of 16.67 L min^{-1}. Teflon filters were used for gravimetric measurement (particulate mass concentration) following an analysis for trace elements using X-ray fluorescence (XRF) technique. The data on the concentration of four selected trace elements – cadmium, copper, lead, and zinc – were used in this study. Quartz filters were utilized for measuring organic and elemental carbon concentrations in the particles by thermal-optical transmittance (TOT) technique. The analyses of the filter media for the chemical speciation were performed in the Research Triangle Institute (Research Triangle Park, NC, USA).

Monthly flow checks and quarterly flow audits were performed on all PM samplers. The monthly flow checks were conducted by the operators of each unit using flow devices, which were calibrated annually to the National Institute of Standards and Technology (NIST, Gaithersburg, MD, USA) traceable standards. The quarterly flow audits were conducted by the Ohio Environmental Protection Agency (EPA).

2.5. Measurement of ozone

An ultraviolet photometric ozone analyzer (Model: 1008 PC, Dasibi Environmental Corp., Glendale, CA, USA) was utilized for the ozone measurement. This unit provides an updated reading every 10 s. These readings were averaged to achieve the hourly and 24-h average. The 24-h averaged ozone concentration value (9 A.M.–9 A.M.) was determined in conjunction with bioaerosol and PM measurement schedules. The quality control procedures included the unit calibration performed at the time of installation prior to the beginning of the major ozone season (April 1). After that, the unit was calibrated every third month. The Ohio EPA audited the instrument each quarter. Zero/span checks were performed each week.

2.6. Measurement of temperature and relative humidity

Temperature and relative humidity were measured using a temperature/relative humidity probe (Model 083D, Met One instruments). Hourly readings were averaged over a 24-h period (9 A.M.–9 A.M.), which was used for the analyses to study the relationships between temperature, relative humidity, and other environmental variables.

2.7. Statistical analyses

Non-parametric Spearman's correlation coefficients were calculated between different environmental variables. Non-parametric method was employed because the data were not normally distributed. Linear regression models were developed to understand the relationships amongst environmental variables using after log transformation or square root transformation for the dependent variables. We developed linear regression models between variable pairs in order to understand these dual relationships more precisely. However, since the strong relationship between ozone and temperature is well known (Sartor et al., 1997; Ross et al., 2002), additionally we performed stepwise multiple regression analysis to understand the combined or individual influence of temperature and ozone on different environmental variables. All statistical tests were performed using the SPSS 11.0 for Windows software (SPSS Inc., Chicago, IL, USA).

3. Results and discussion

3.1. Inhalable bioaerosols

A total of 28 fungal genera/groups and 20 pollen genera/families were recorded during this two-year

study. Predominant airborne fungi and their corresponding percentages relative to the total airborne fungal load during the entire sampling period were: *Aspergillus/Penicillium* group (41.6%), *Cladosporium* (28.4%), Ascospores (10.6%), Basidiospores (9.8%), smut spores (2.6%), *Alternaria* (1.4%), *Epicoccum* (0.7%), and rust spores (0.2%). The dominant pollen types and corresponding percentages with respect to the total airborne pollen load were determined at the time of their seasonal occurrence in the air (only these specific periods were selected because different pollen showed different seasonal patterns). The following contributions were found: *Ambrosia* (Ragweed) [88.0%]; *Quercus* (Oak) [51.3%]; *Juniperus* (Juniper, Cedar) [11.5%], *Ulmus* (Elm) [8.8%], *Acer* (Maple) [8.0%], Pinaceae (Pine, Fir, Spruce) [4.8%], and Poaceae (Grass) [3.3%]. Mean, median, standard deviation, and inter-quartile range of the concentrations of predominant fungi and pollen types are presented in Table 1.

Monthly variation patterns of the airborne fungi and pollen during the two-year period are presented in Figs. 2 and 3, respectively. The figures show that the peaks for the total concentrations of airborne fungi (496–16 980 spores m^{-3}) were observed during fall of both years (September–October). For total pollen, however, the peaks (0–6692 pollen m^{-3}) were found in spring (March–May). Two most prevalent fungi showed different seasonal peaks. Concentration of *Aspergillus/Penicillium* was highest during summer (June–August); however, *Cladosporium* showed maximum concentration in fall. Ascospores, Basidiospores, and rust spores did not reveal any clear seasonal patterns during the two sampling years. *Alternaria*, *Epicoccum*, and smut spores demonstrated peak concentrations during fall months. Among different tree pollen, peaks of *Quercus*, *Juniperus*, and Pinaceae were observed during spring and beginning of summer. *Ulmus* showed a sharp peak in spring. Poaceae demonstrated peaks in summer, while *Ambrosia* was present only during fall (September–October).

The concentrations of inhalable airborne fungi were compared with the similar data available to the public from the local news media. Those are based on the measurement by a conventional stationary Rotorod sampler (described in detail in our previous study: Adhikari et al., 2003) operating as a part of Cincinnati weather station. We consistently found higher concentration of *Aspergillus/Penicillium* in our samples likely related to the small aerodynamic sizes of these spores (Adhikari et al., 2003) leading to possible underestimation of concentration by conventional impaction-based sampling methods (in contrast to filter-based methods). As spores of *Aspergillus* and *Penicillium* are well-recognized aeroallergens (Horner et al., 1995) our observations strengthen the application of Button Sampler for the measurement of outdoor bioaerosols.

Table 1
Variable distribution of bioaerosols, meteorological factors, ozone, particulate matter, trace elements, and organic carbon

Variables	*n*	Mean	Median	SD	IQR
Inhalable bioaerosols					
Total fungi (spores m^{-3})	256	4229	3910	2458	3188
Aspergillus/Penicillium	256	1775	1488	1218	1435
Alternaria	256	59	23	98	80
Ascospores	256	459	337	466	428
Basidiospores	256	417	335	369	456
Cladosporium	256	1171	630	1543	1085
Epicoccum	256	25	11	38	34
Rust spores	256	6	0	17	0
Smut spores	256	105	34	260	79
Total pollen (pollen m^{-3})	316	224	22	737	88
Ambrosia (Ragweed)	102	24	4	37	37
Acer (Maple)	122	35	0	133	6
Pinaceae (Pine, Fir, Spruce)	175	16	2	38	16
Poaceae (Grass)	232	6	2	12	5
Quercus (Oak)	127	211	2	793	65
Juniperus (Juniper, Cedar)	51	48	6	167	19
Ulmus (Elm)	87	52	5	165	31
Air pollutants					
Ozone (ppb)	306	28.31	28.37	11.74	16.02
PM_{10} ($\mu g\ m^{-3}$)	181	23.99	22.03	10.73	14.22
$PM_{2.5}$ ($\mu g\ m^{-3}$)	303	17.20	15.07	8.87	11.46
Cadmium ($\mu g\ m^{-3}$)	39	0.0029	0.0003	0.0061	0.0041
Copper ($\mu g\ m^{-3}$)	39	0.0045	0.0035	0.0069	0.0058
Lead ($\mu g\ m^{-3}$)	22	0.0125	0.0050	0.0256	0.0101
Zinc ($\mu g\ m^{-3}$)	39	0.0260	0.0148	0.0362	0.3082
Organic carbon ($\mu g\ m^{-3}$)	35	5.26	4.81	2.14	2.72
Meteorological parameters					
Temperature (°C)	316	18.49	19.81	7.13	10.36
Relative humidity (%)	195	65.83	66.79	12.59	15.58

Abbreviations: *n* = number of observations; SD = standard deviation; IQR = inter-quartile range.

While performing microscopic analyses, we occasionally found pollen grains covered with black particulate matter. This finding was observed in many cases for *Ambrosia* suggesting that the spiny surface may facilitate adherence of particulate matter.

Among the most prevalent fungal genera, *Cladosporium, Aspergillus/Penicillium*, and *Alternaria* are strongly associated with allergic respiratory disease and asthma (Horner et al., 1995; Douwes et al., 2003). *Epicoccum* (Bisht et al., 2002) and smuts of common cereal grains and grasses (McDevitt et al., 1977) are also important aeroallergens. Among different dominant pollen types, *Ambrosia* and Poaceae were reported as highly allergenic, whereas *Quercus, Ulmus*, and *Juniperus* are recognized as

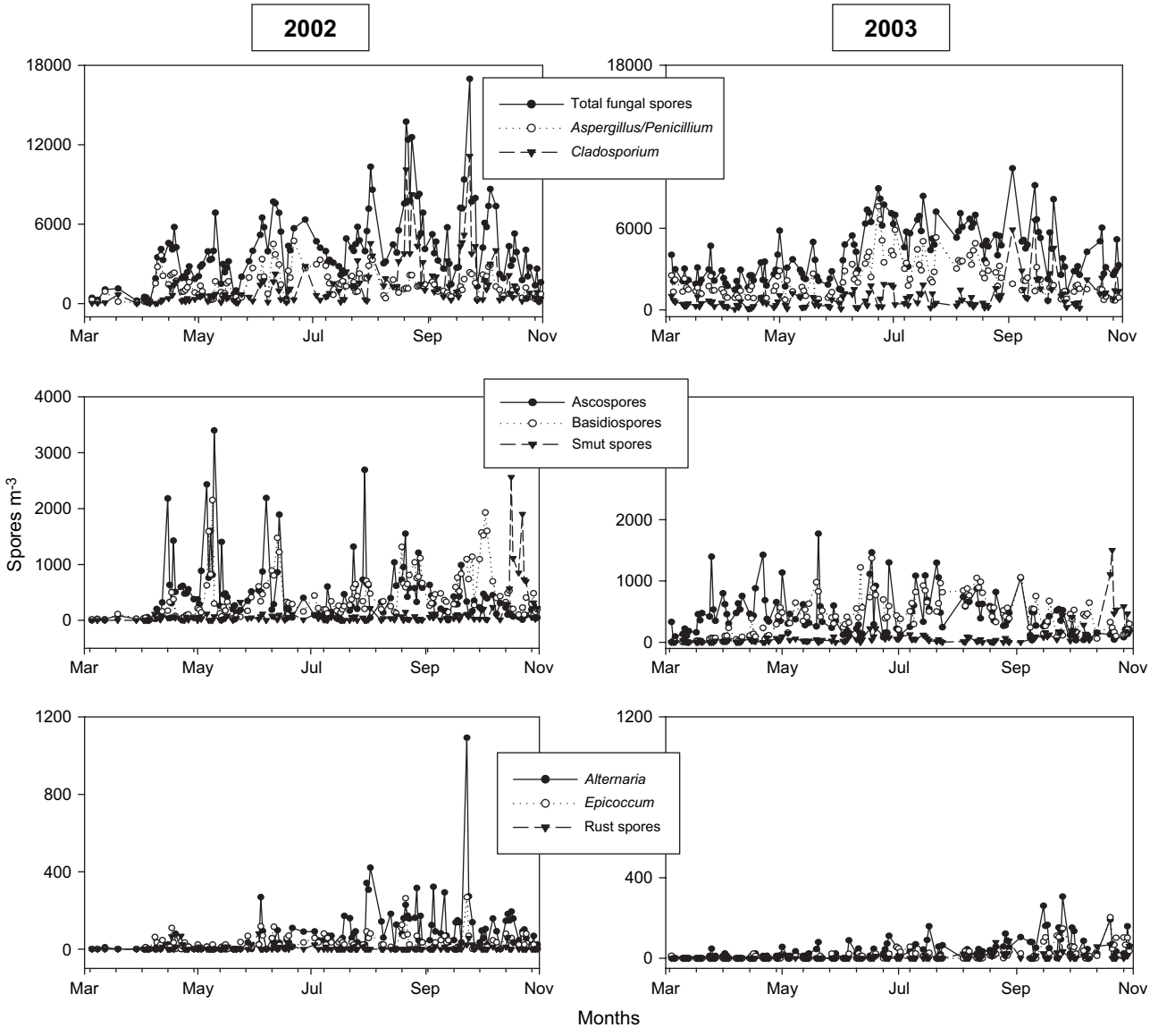

Fig. 2. Monthly variation patterns of the airborne concentrations of prevalent airborne fungi determined during two years of the study.

moderately allergenic, and Pinaceae is referred to as mildly allergenic or of indeterminate allergenic strength (Smith, 1990b). Since the inhalable concentration levels have not been reported earlier for these aeroallergens, we believe that these results will be very useful to investigate the relationship between the impact of aeroallergens on allergy and asthma symptoms as well as asthma hospital visits.

3.2. Airborne particulate matter

The 24-h average airborne mass concentration of PM_{10} and $PM_{2.5}$ ranged from 6.70 to 65.38 $\mu g\,m^{-3}$ ($n = 181$) and 5.04 to 45.02 $\mu g\,m^{-3}$ ($n = 303$), respectively. Mean, median, standard deviation, and interquartile range data on the PM_{10} and $PM_{2.5}$ are presented in Table 1. Both mean PM_{10} (23.99 $\mu g\,m^{-3}$) and $PM_{2.5}$ (17.2 $\mu g\,m^{-3}$) concentration levels were well below the US ambient air quality standard (US E.P.A., 2004), which is 150 $\mu g\,m^{-3}$ for PM_{10} and 65 $\mu g\,m^{-3}$ for $PM_{2.5}$ (24-h average). We found that the concentration levels of both PM_{10} and $PM_{2.5}$ were higher during summer and fall months, similar to fungi and unlike pollen (see also Section 3.6). We concluded from the significant correlations of the concentration patterns that the interaction between mold (fungal) aeroallergens and particulate matter is plausible and should be further investigated. In Cincinnati metropolitan area, the airborne PM_{10} and $PM_{2.5}$ are greatly influenced by regional secondary aerosol formation processes, which are largely dependent on temperature. Even though the inhalable bioaerosols were not fully addressed in the literature, a study from Australia has reported inverse correlation between ambient fungi and pollen, and PM_{10}

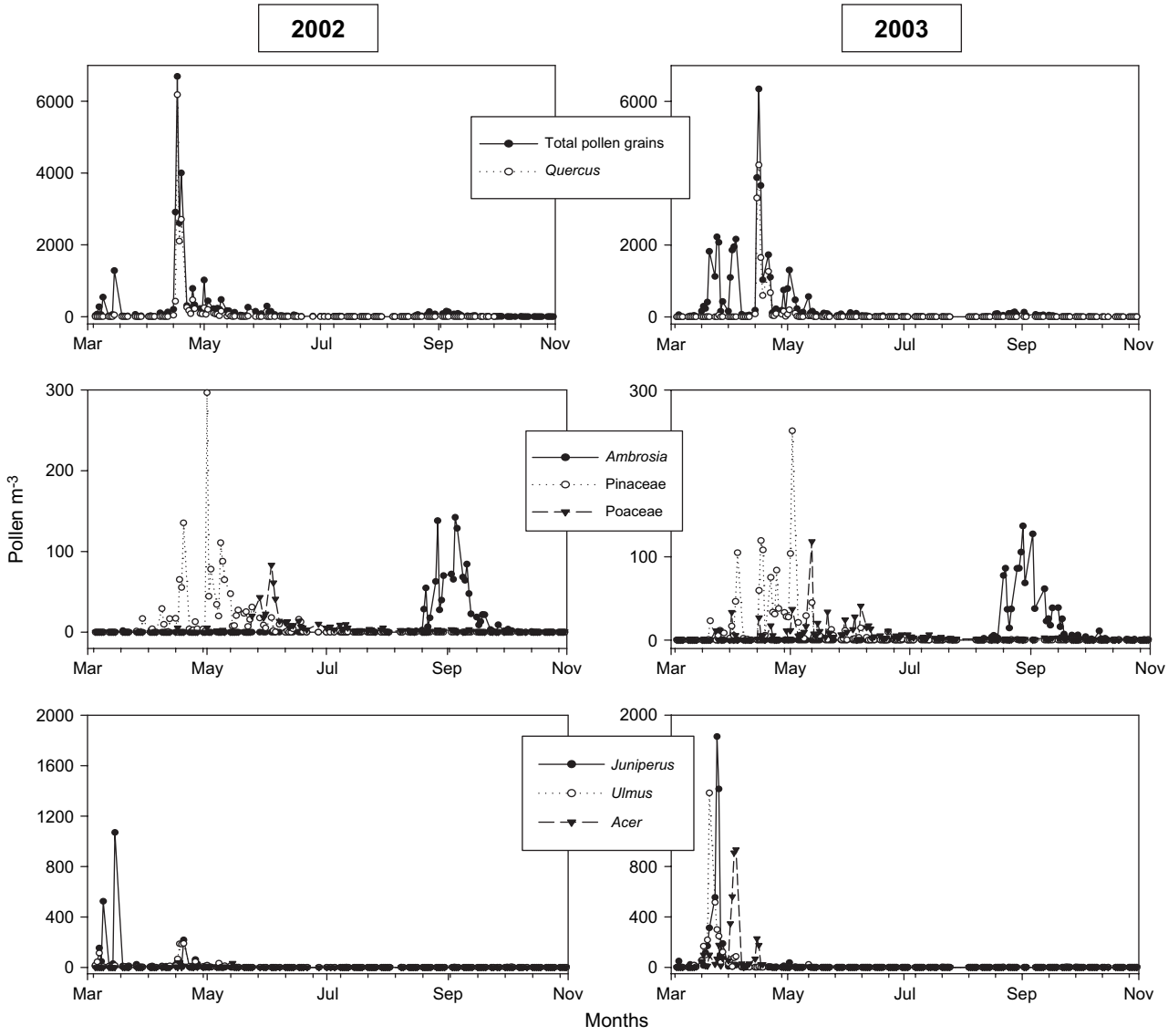

Fig. 3. Monthly variation patterns of the airborne concentrations of prevalent airborne pollen grains determined during two years of the study.

(Glikson et al., 1995). The comparison of that study results with our findings may not be fully warranted because of differences in climate, bioaerosol sources, and dispersal patterns; in addition, the sources of secondary aerosols and temperature levels were likely different in the Australian study.

3.3. Atmospheric ozone

The daily average concentration of ozone ranged between 2.88 and 60.46 ppb ($n = 306$) (Table 1). Highest ozone concentration was found during summer months followed by spring months. This seems reasonable because tropospheric ozone is produced by the reaction of solar radiation on nitrogen oxides (Eliasson et al., 2003). When the hourly data (not shown) were compared with the US ambient air quality standard (US E.P.A., 2004), which is 0.12 ppm for hourly ozone concentration, we found that only in one sampling day (August 10, 2002) ozone concentration was close to the standard and in most of the days it was well below it. However, a synergistic health effect of ozone with aeroallergens may be expected even at low ozone concentration. Indeed, Ross et al. (2002) found synergistic effect of ozone and pollen on the respiratory health of asthmatics when the ozone concentration was in a range of 8.9–78.3 ppb. To our knowledge, correlation between low ozone levels with ambient mycoflora and flowering of plants has not previously been explored. Since ozone is strong phytotoxic oxidant (Tiedemann and Firsching, 2000), we hypothesized that an inverse correlation exists between the ozone concentration and bioaerosols. Therefore, an immediate, 3-day lagged, and 6-day lagged relationships of ozone with airborne fungi and pollen were examined as described in Section 3.6.

3.4. Airborne trace metals and organic carbon in $PM_{2.5}$

Concentration ranges of four selected trace metals were as follows: cadmium: 0–0.035 μg m^{-3} ($n = 39$); copper: 0–0.041 μg m^{-3} ($n = 39$); lead: 0–0.123 μg m^{-3} ($n = 22$); and zinc: 0.001–0.187 μg m^{-3} ($n = 39$). Zinc concentration was the highest among the four trace metals. Unlike the particulate matter and ozone, the trace metals did not show any consistent seasonal periodicity during the two years of monitoring. Although these trace metals are known to have adverse effects on leaf and soil mycoflora, their interactions with ambient airborne mycoflora and flowering of plants have not yet been adequately explored. We hypothesized that a negative correlation exists between these toxic trace metals and airborne fungi and pollen. An immediate, 3-day lagged, and 6-day lagged relationships were investigated as described in Section 3.6. Concentration of organic carbon ranged from 2.16 to 13.33 μg m^{-3} ($n = 35$). No clear seasonal periodicity was observed for the organic carbon. Mean, median, standard deviation, and inter-quartile range values for the trace metals and organic carbon are presented in Table 1.

3.5. Temperature and relative humidity

During this two-year study the daily average temperature ranged from −2.7 to 30.2 °C ($n = 316$), and the relative humidity range was 30.8–92.0% ($n = 195$). Number of observations for the relative humidity was lower than that for the ambient temperature because the relative humidity data were not consistently collected during a period of March through September, 2002. A clear temperature peak was found during summer months (June–August); however, the relative humidity levels did not show any seasonal peaks. Monthly variation patterns of temperature and ozone were found to be very similar (not shown). Mean, median, standard deviation, and inter-quartile range values of the temperature and relative humidity are presented in Table 1.

3.6. Non-parametric correlation between environmental variables

Results of the non-parametric correlation analyses are presented in Tables 2–4. Table 2 shows the Spearman's correlation coefficient values for the airborne fungi concentration with respect to the ambient temperature, relative humidity, ozone, PM, trace elements, and organic carbon. The same coefficients for correlation between the pollen concentrations with all of the above-mentioned factors (measured simultaneously) are presented in Table 3. Table 4 shows how temperature and ozone correlate with PM, trace metals, and organic carbon.

Statistically significant positive correlations were found in the following cases:

1. Temperature versus most of the fungi (total fungi, *Aspergillus/Penicillium, Alternaria*, Ascospores, Basidiospores, *Cladosporium*, and *Epicoccum*), *Ambrosia, Ulmus* (Tables 2 and 3), $PM_{2.5}$, PM_{10}, ozone, and organic carbon (Table 4).
2. $PM_{2.5}$ and PM_{10} versus total fungi, *Aspergillus/Penicillium, Alternaria*, and Basidiospores (Table 2).
3. Ozone versus total fungi, *Aspergillus/Penicillium*, Ascospores (Table 2), total pollen, *Ambrosia*, Poaceae (Table 3), $PM_{2.5}$, and PM_{10} (Table 4).
4. $PM_{2.5}$ versus *Ambrosia* and Poaceae (Table 3).
5. Relative humidity versus Basidiospores (Table 2).

Statistically significant inverse correlations were found in the following cases:

1. Temperature versus *Acer* and Pinaceae (Table 3).
2. Relative humidity versus *Acer* (Table 3).
3. $PM_{2.5}$ versus Pinaceae (Table 3).
4. Copper versus Pinaceae (Table 3).

Table 2
Spearman's correlation coefficients between the concentrations of airborne fungi and simultaneously measured meteorological factors, ozone, particulate matter, four selected trace elements, as well as organic carbon

Variables	Temperature ($n = 256$)	Relative humidity ($n = 156$)	Ozone ($n = 286$)	PM_{10} ($n = 165$)	$PM_{2.5}$ ($n = 294$)	Cadmium ($n = 39$)	Copper ($n = 39$)	Lead ($n = 22$)	Zinc ($n = 39$)	Organic carbon ($n = 35$)
Total fungi	0.579**	0.047	0.176**	0.217**	0.306**	0.024	−0.194	0.018	0.023	0.197
Aspergillus/Penicillium	0.558**	0.011	0.271**	0.470**	0.396**	−0.185	−0.022	−0.042	0.028	0.280
Alternaria	0.422**	−0.064	0.131*	0.288**	0.223**	0.031	−0.209	−0.044	0.181	0.179
Ascospores	0.204**	0.156	0.139**	−0.064	0.016	−0.037	0.019	0.010	−0.168	0.034
Basidiospores	0.495**	0.288**	0.028	0.240**	0.308**	−0.040	0.193	0.195	0.094	0.143
Cladosporium	0.321**	−0.030	0.088	0.036	0.082	0.032	−0.244	−0.325	0.030	−0.041
Epicoccum	0.252**	−0.107	0.059	0.181*	0.071	0.110	−0.168	0.191	0.212	0.160
Rust spores	0.095	−0.062	−0.039	−0.081	0.035	0.134	−0.123	−0.012	0.256	0.214
Smut spores	0.012	−0.093	−0.133*	0.097	−0.027	0.078	−0.199	0.007	0.155	−0.183

*$P < 0.05$; **$P < 0.01$.

Table 3
Spearman's correlation coefficients between the concentrations of airborne pollen and simultaneously measured meteorological factors, ozone, particulate matter, four selected trace elements, as well as organic carbon

Variables	Temperature	Relative humidity	Ozone	PM_{10}	$PM_{2.5}$	Cadmium	Copper	Lead	Zinc	Organic carbon
Total pollen	−0.044	−0.117	0.266**	−0.041	−0.011	−0.052	0.202	−0.051	−0.210	0.098
	($n = 316$)	($n = 195$)	($n = 306$)	($n = 181$)	($n = 303$)	($n = 37$)	($n = 37$)	($n = 21$)	($n = 37$)	($n = 37$)
Ambrosia (Ragweed)	0.566**	0.201	0.554**	0.176	0.404**	0.004	0.004	0.643	0.378	0.189
	($n = 101$)	($n = 78$)	($n = 101$)	($n = 40$)	($n = 101$)	($n = 19$)	($n = 19$)	($n = 7$)	($n = 19$)	($n = 17$)
Acer (Maple)	−0.362**	−0.405**	0.092	−0.073	−0.096	0.167	0.272	0.018	0.270	0.212
	($n = 122$)	($n = 46$)	($n = 133$)	($n = 63$)	($n = 78$)	($n = 13$)	($n = 13$)	($n = 11$)	($n = 13$)	($n = 13$)
Pinaceae (Pine, Fir, Spruce)	−0.377**	−0.107	0.035	0.032	−0.251**	−0.324	−0.624*	0.200	−0.354	−0.331
	($n = 175$)	($n = 80$)	($n = 147$)	($n = 96$)	($n = 142$)	($n = 13$)	($n = 13$)	($n = 10$)	($n = 13$)	($n = 13$)
Poaceae (Grass)	0.126	−0.118	0.236**	0.185*	0.133**	−0.369	−0.005	−0.458	−0.338	0.050
	($n = 232$)	($n = 137$)	($n = 245$)	($n = 155$)	($n = 245$)	($n = 23$)	($n = 23$)	($n = 15$)	($n = 23$)	($n = 24$)
Quercus (Oak)	0.128	−0.129	0.153	0.084	−0.129	−0.390	−0.073	0.198	−0.049	0.203
	($n = 127$)	($n = 65$)	($n = 123$)	($n = 84$)	($n = 117$)	($n = 8$)	($n = 8$)	($n = 7$)	($n = 9$)	($n = 9$)
Juniperus (Juniper, Cedar)	−0.057	−0.034	−0.130	0.018	−0.087	0.185	−0.088	−0.551	−0.377	0.116
	($n = 103$)	($n = 52$)	($n = 93$)	($n = 82$)	($n = 88$)	($n = 6$)	($n = 6$)	($n = 6$)	($n = 6$)	($n = 6$)
Ulmus (Elm)	0.233**	−0.224*	0.054	−0.049	−0.038	−0.094	0.087	−0.638	−0.227	0.055
	($n = 87$)	($n = 120$)	($n = 84$)	($n = 86$)	($n = 72$)	($n = 15$)	($n = 15$)	($n = 6$)	($n = 15$)	($n = 15$)

*$P < 0.05$; **$P < 0.01$. (For individual pollen, data between 1 day before and after the detection limit were used).

In most cases, insignificant correlation coefficients between ozone and trace metals, and 3-day lagged and 6-day lagged concentrations of airborne fungi and pollen (all data are not shown) were observed. However, significant positive correlations were observed in the following cases: ozone versus 3-day lagged Ascospores ($r = 0.19$, $P < 0.05$), total pollen ($r = 0.20$, $P < 0.01$), *Ambrosia* ($r = 0.15$, $P < 0.05$), Poaceae ($r = 0.18$, $P < 0.05$); and ozone versus 6-day lagged total fungi ($r = 0.16$, $P < 0.05$), *Aspergillus/Penicillium* ($r = 0.15$, $P < 0.05$), Ascospores ($r = 0.20$, $P < 0.01$), and *Ambrosia* ($r = 0.16$, $P < 0.05$). Significant negative correlations were observed in the cases of: ozone versus 3-day lagged smut spores ($r = -0.33$, $P < 0.01$); ozone versus 6-day lagged smut spores ($r = -0.15$, $P < 0.05$) and *Juniperus* ($r = -0.25$, $P < 0.05$); and zinc versus 6-day lagged Basidiospores ($r = -0.32$, $P < 0.01$) and *Juniperus* ($r = -0.97$, $P < 0.05$).

Among different test variables, ambient temperature was clearly the most important factor, which showed positive correlation with most of the biological and non-biological air pollutants. For fungi, this finding is consistent with the observations of Li and Kendrick (1995), who reported statistically significant relationships between most airborne fungal taxa and temperature. In contrast to their observation, we found a strong positive correlation of *Aspergillus/Penicillium* with temperature. However, Li and Kendrick used an impaction-based sampling method (Samplair-MK1 particle sampler), which may not be efficient for the collection of relatively small *Aspergillus/Penicillium* spores. Similar to our previous study carried out in the Cincinnati metropolitan area (Martuzevicius et al., 2004), which revealed statistically significant correlation of temperature with $PM_{2.5}$, the present investigation demonstrates statistically significant positive correlations between temperature and both $PM_{2.5}$ and PM_{10}. Strong correlation of temperature with ozone was consistent with observations reported by Sartor et al. (1997) and Ross et al. (2002).

We expected positive correlations between particulate matter and fungi since most of the fungal spores were <10 μm size. However, as described below in Section 3.9, we found much lower contribution of fungi to PM_{10} than previously reported. For ozone we found positive correlations with several fungi and pollen, which contradicted our hypothesis on the negative correlations. To understand this result, we performed stepwise multiple regression using both ozone and temperature as independent variables (see Section 3.8).

The positive correlation of relative humidity with Basidiospores seems reasonable because release of Basidiospores in Hymenomycetes (the largest order of Basidiomycetes) depends on the activity of swollen spore bearing structures that require moisture (Jones and Harrison, 2004).

Table 4
Spearman's correlation coefficients between temperature and ozone, on one hand, and particulate matter, four selected trace metals, and organic carbon on the other hand

Variables	Ozone	PM_{10}	$PM_{2.5}$	Cadmium	Copper	Lead	Zinc	Organic carbon
Temperature	0.597** ($n = 303$)	0.685** ($n = 152$)	0.701** ($n = 256$)	0.001 ($n = 30$)	−0.094 ($n = 30$)	0.293 ($n = 20$)	0.095 ($n = 30$)	0.601** ($n = 30$)
Ozone	1	0.425** ($n = 160$)	0.478** ($n = 250$)	0.103 ($n = 32$)	0.179 ($n = 32$)	−0.004 ($n = 19$)	0.034 ($n = 32$)	0.302 ($n = 30$)

**$P < 0.01$.

Fungi and pollen did not show strong correlation with organic carbon (Tables 2 and 3). This observation raises two questions:

1. Is the contribution of bioaerosols to organic carbon lower than previously reported? Or,
2. Is there a substantial spatial variability in the composition of organic carbon so that previous reports (Womiloju et al., 2003) did not reflect a ubiquitous general phenomenon?

During the microscopic analyses, we found various large plant parts, such as trichomes, which may produce fragments <2.5 μm, and thus may contribute to a large portion of the organic carbon mass. Further studies should be conducted to explore this issue and answer the above questions.

When 3-day and 6-day lagged bioaerosol data were tested against trace metal data, inverse correlations were found in many cases, although, the relationships were mostly not significant. However, zinc has a statistically significant inverse 6-day lagged influence on the concentration of airborne *Juniperus* pollen and Basidiospores. Concentration of zinc was higher compared to other three trace metals and thus showed some stronger influence. Further studies with more trace metal data may improve our understanding on the relationship between ambient trace metals and bioaerosols.

3.7. Linear regression models

We developed linear regression models for the pairs of variables that showed statistically significant correlations. Statistically significant linear relationships were observed for the following cases:

Temperature versus transformed values of total fungi, *Aspergillus/Penicillium*, *Ambrosia*, ozone, PM_{10}, $PM_{2.5}$, and organic carbon;
Ozone versus transformed values of *Ambrosia*, *Aspergillus/Penicillium*, PM_{10}, and $PM_{2.5}$;
Total fungi versus transformed values of $PM_{2.5}$;
Aspergillus/Penicillium versus transformed values of $PM_{2.5}$ and PM_{10}.

Regression equations, P values, adjusted r^2 values, and F values for the above listed relationships are presented in Table 5. For different relationships adjusted r^2 ranged between 0.062 and 0.442 and P values for all relationships were <0.001. Since our numbers of observations (n) for all relationships were always >100 (except organic carbon), which increased the strength of tests, we anticipate that the regression models can be used in the future for predicting the levels and interrelationship between ambient inhalable bioaerosols, other pollutants, and temperature in the Greater Cincinnati area.

Table 5
Regression models of several significant relationships between ambient bioaerosols, meteorological factors, ozone, particulate matter, trace elements, and organic carbon

Relationship	Regression equation	P	Adjusted r^2	F
1. Temperature versus total fungi ($n = 256$)	Log total fungi = 3.08 + 0.02 × temperature	<0.001	0.299	109.99
2. Temperature versus *Aspergillus/Penicillium* ($n = 256$)	$\sqrt{Aspergillus/Penicillium}$ = 19.41 + 1.07 × temperature	<0.001	0.281	100.73
3. Temperature versus *Ambrosia* ($n = 101$)	Log *Ambrosia* = 0.078 × temperature − 0.82	<0.001	0.301	44.51
4. Temperature versus PM_{10} ($n = 152$)	Log PM_{10} = 1.007 + 0.0182 × temperature	<0.001	0.426	113.07
5. Temperature versus $PM_{2.5}$ ($n = 256$)	Log $PM_{2.5}$ = 0.783 + 0.0209 × temperature	<0.001	0.442	203.29
6. Temperature versus Ozone ($n = 251$)	Ozone = 0.958 × temperature + 10.29	<0.001	0.328	148.45
7. Temperature versus organic carbon ($n = 30$)	Log organic carbon = 0.422 + 0.0149 × temperature	<0.001	0.342	16.06
8. Total fungi versus $PM_{2.5}$ ($n = 294$)	Log $PM_{2.5}$ = 1.06 + 0.00002 × total fungi	<0.001	0.062	20.39
9. *Aspergillus/Penicillium* versus $PM_{2.5}$ ($n = 294$)	Log $PM_{2.5}$ = 1.02 + 0.00008 × *Aspergillus/Penicillium*	<0.001	0.199	73.92
10. *Aspergillus/Penicillium* versus PM_{10} ($n = 165$)	Log PM_{10} = 1.25 + 0.00005 × *Aspergillus/Penicillium*	<0.001	0.102	19.68
11. Ozone versus *Aspergillus/Penicillium* ($n = 286$)	$\sqrt{Aspergillus/Penicillium}$ = 30.65 + 0.333 × ozone	<0.001	0.078	25.00
12. Ozone versus *Ambrosia* ($n = 101$)	Log *Ambrosia* = 0.034 × ozone − 0.099	<0.001	0.255	35.52
13. Ozone versus PM_{10} ($n = 160$)	Log PM_{10} = 1.129 + 0.0071 × ozone	<0.001	0.187	37.60
14. Ozone versus $PM_{2.5}$ ($n = 250$)	Log $PM_{2.5}$ = 0.917 + 0.0092 × ozone	<0.001	0.245	81.97

3.8. Stepwise regression analyses performed for understanding the effects of temperature and ozone on bioaerosols and particulate matter

Statistically significant positive correlations were observed for total fungi, *Aspergillus/Penicillium*, *Alternaria*, Ascospores, Ambrosia, $PM_{2.5}$, and PM_{10} with both the ambient temperature and ozone. Since temperature demonstrated a statistically significant positive correlation with ozone, stepwise regression analyses were performed while selecting both as independent variables to understand their effects. Ozone was dropped off from the models for *Aspergillus/Penicillium*, Ascospores, *Ambrosia*, and PM_{10}, clearly indicating that temperature is the main influencing factor. Although for total fungi, *Alternaria*, and $PM_{2.5}$ both variables were included in the multiple stepwise regression models, no major increase in the adjusted r^2 value (0.007–0.04) was observed in two models to allow establishing an independent effect of ozone. These observations indicate that temperature is the main influencing factor while ozone has a confounding role.

3.9. Contribution of spore mass of Aspergillus/Penicillium and Cladosporium to PM_{10}

Aspergillus/Penicillium and *Cladosporium* were the most prevalent fungi in our study contributing 41.6% and 28.4%, respectively, to the total fungal concentration. The spore aerodynamic sizes for almost all identified fungi are <10 μm. For example, aerodynamic diameter (d_a) of *Aspergillus/Penicillium* is 3.7 μm, $d_a = 5.6$ μm for Ascospores, $d_a = 6.8$ μm for Basidiospores, $d_a = 8.1$ μm for *Cladosporium*, and $d_a = 9.7$ μm for smut spores (Lee et al., in press). Therefore, we estimated the mass contributions of *Aspergillus/Penicillium* and *Cladosporium* to the total PM_{10} mass assuming density $= 1\ g\,cc^{-1}$. For *Aspergillus/Penicillium*, the average spore mass percentage contribution was $0.17 \pm 0.13\%$ and for *Cladosporium* this contribution was $0.95 \pm 1.63\%$. These small percentage values indicate that statistically significant correlation and linear relationships between particulate matter and bioaerosols may not be necessarily due to the large contribution of bioaerosol particles to PM, but could be caused by some environmental parameters, such as temperature, which influences both variables.

4. Conclusions

We found that particulate matter, ozone, and several types of inhalable airborne fungi and pollen were positively correlated likely as a result of the atmospheric temperature influence. Temperature has a statistically significant effect on the concentrations of most airborne fungi. For pollen, the effect is genera specific. Ambient bioaerosols might not be a significant contributor to PM_{10} and $PM_{2.5}$ (by mass); however, temperature might act as a common influencing factor for both bioaerosols and PM. Inverse correlations were found between the toxic trace metals and inhalable bioaerosols, but in most cases the relationships were not statistically significant. Stepwise multiple regression analyses showed that although both temperature and ozone demonstrated statistically significant positive correlations with several bioaerosol types and particulate matter, the ambient temperature was the main influencing factor while ozone was a confounding factor. Regression models from this study can be used in the future for predicting the levels and interrelationship between ambient inhalable bioaerosols, ozone, particulate matter, and ambient temperature. The synergistic effects of all these pollutants may cause increased incidence of respiratory health symptoms.

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