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Bioaerosols in the Midwestern United States : spatio-temporal variations, meteorological impacts and contributions to particulate matter

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BIOAEROSOLS IN THE MIDWESTERN UNITED STATES: SPATIO-TEMPORAL VARIATIONS, METEOROLOGICAL IMPACTS AND CONTRIBUTIONS TO PARTICULATE MATTER

by

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CERTIFICATE OF APPROVAL

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PH.D. THESIS

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This is to certify that the Ph.D. thesis of

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To my beloved parents and my loving husband

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ABSTRACT

When inhaled, bioaerosols exacerbate respiratory symptoms and diseases. Mitigating the negative health impacts of bioaerosols requires a robust understanding of the temporal and spatial dynamics of bioaerosols in the atmosphere as a function of their type (e.g., bacteria, fungal spores, plant pollens) and particle size, which determines their penetration into the respiratory tract. While it is known that bioaerosol concentrations vary by location, season and meteorological conditions, major gaps remain in understanding the co-occurrence of bioaerosols with one another, their size in the atmosphere, and their mass contributions to PM. Overall, research presented in this thesis advances the current knowledge about bioaerosols (including fungal spores, pollens, and bacteria) in following ways: 1) defining background and urban levels of bioaerosol concentrations in the Midwestern US across four seasons, 2) characterizing ambient bioaerosol and co-pollutant mixtures, 3) determining the influence of meteorology on their concentrations and size distributions, and 4) estimating bioaerosol contributions to PM mass.

The spatial analysis of respirable particulate matter (PM_{10}) across urban and background sites in Iowa demonstrated that urban areas are a source of fungal glucans, bacterial endotoxins and total proteins, which gives rise to significantly enhanced bioaerosols in urban locations compared to background sites. Similar urban enhancements in calcium—a crustal element—and its correlation with endotoxins suggested that wind-blown soil is likely the origin. Seasonally, fungal spores peaked in summer with temperature, while bacterial endotoxins peaked in autumn during the row crop harvesting season. Fungal spores, bacterial endotoxins, plant and animal detritus all

peaked during the growing season, such that maximum exposures to multiple bioaerosol types concurrently. Under the influence of rain chemical tracers of pollens peaked and decreased in size from coarse (2.5-10 μm) to fine particles (< 2.5 μm), likely due to the osmotic rupture of pollen grains upon wetting. While fine-sized fungal spores also increased during rain events, maximum spore levels were observed in coarse-sized particles post-rain. The comparison of spring to late summer measurements demonstrated these influences of precipitation on bioaerosols also occur during late summer, when fungal spore levels are high and ragweed is the dominant pollen source. The ability to apportion PM mass to bioaerosols was advanced through the development of chemical profiles of pollens and their integration with chemical mass balance (CMB) source apportionment modeling, for the first time. In late-April to early-May in 2013, pollens were estimated to contribute 0.2 - 38% of PM_{10} (0.04 – 0.8 μg m^{-3}) while fungal spores contributed 0.7 – 17% of PM_{10} (0.1 – 1.5 μg m^{-3}). Collectively, this thesis provides insight into spatial, seasonal and daily variations of bioaerosols, and shows elevated outdoor exposures to bioaerosols among urban populations, with maximum levels occurring during growing seasons, periods of high temperature, and during/immediately following rainfall.

PUBLIC ABSTRACT

Particles we breathe include plant pollens, fungal spores, bacteria and viruses that are referred to as bioaerosols. Their allergenic properties induce sneezing, coughing and wheezing, and can exacerbate respiratory diseases like asthma. This thesis serves to characterize the spatial, seasonal, and daily variations in bioaerosol levels and quantitatively defines their contributions to the mass of particles in outdoor air. Key findings of this research include: i) people in urban areas experience increased levels of bioaerosols compared to background areas, likely due to greater levels of wind-blown dust ii) bioaerosol reach their highest levels during periods of elevated temperature, such that bioaerosol co-vary and peak during summer, iii) fine-sized bioaerosol concentrations increase while raining and iv) bioaerosol contributions to PM increase associated with rain The impact of this research is significant as it provides insight to spatial, seasonal and daily variations of bioaerosols revealing outdoor exposures to bioaerosols are greater in urban areas, in growing seasons, and when conditions are warm and wet.

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CHAPTER 1

INTRODUCTION

1.1 Particulate matter

Airborne particulate matter (PM), also referred to as aerosols,[1] is defined as solid particles or liquid droplets that are suspended in the air.[2, 3] Atmospheric PM is a complex mixture of chemicals including sulfates, nitrates, ammonium, sodium, chloride, trace metals, crustal elements, carbonaceous material, water, and biological matter.[1] Natural PM sources include soil and rock debris, volcanic activities and sea spray, whereas anthropogenic PM sources include combustion processes, vehicular emissions, road and construction dust.[1, 3]

Exposures to higher levels of particles have been linked to increased morbidity and mortality around the world.[4-10] In particular, elevated PM levels exacerbate cardiovascular[8, 10] and respiratory diseases such as bronchitis and asthma.[4, 6] Ambient PM is ranked as one of the top 10 drivers of disease and premature deaths by the World Health Organization.[11] Understanding the sources and composition of PM will provide insight to strategies that may be used to reduce outdoor levels of PM, and mitigate its negative health impacts.

The size of PM is a key factor in its movement and lifetime in the atmosphere and the human respiratory tract.[2] PM is often categorized by its aerodynamic diameter, which is defined as the diameter of a sphere with a unit density (1 g cm^{-3}) that has the same settling velocity in air as the particle under consideration.[3, 12] PM with an aerodynamic diameter (d) less than 10 μm is referred to as as PM_{10}.[3] PM_{10} particles are further categorized as coarse ($10 > d > 2.5$ μm) and fine ($d < 2.5$ μm).[3, 1]

As PM enters the respiratory tract it may penetrate to different regions: head airways (nose, mouth, pharynx and larynx), lung airways (air ways from trachea to terminal bronchioles) and alveoli.[2] The fate of inhaled PM depends on the particle size, route of breathing, age, gender and activity level.[13] In general, larger particles ~10 μm mainly deposit in the upper airways[3] and only ~20% is predicted to reach beyond the larynx entering lung airways.[13] Smaller particles of 3 μm deposit efficiently in alveolar region while mouth breathing.[2] Consequently, increasing emphasis has been placed on the measurement of smaller aerosols that can penetrate deeper into the respiratory tract.

1.2 Bioaerosols

Bioaerosols are biologically originated particulate matter which include pollens, fungal spores, animal and plant detritus, bacteria and viruses.[2] These have been shown to account for a significant proportion of particle mass[14] and number[15] for coarse[16] and fine particles.[17] The size distributions of bioaerosols vary considerably by type: pollens are typically 5 - 100 μm, fungal spores are 1 - 30 μm, bacteria are 0.1 - 10 μm, and viruses are generally smaller than 0.3 μm.[17, 18] When airborne, bioaerosols may found as individual particles or agglomerates of particles.[18]

1.2.1 Health impacts

Exposures to inhalable (<100 μm) bioaerosols initiate airway symptoms and diseases. In particular, tree, grass and weed pollens shown to trigger respiratory symptoms: wheezing, runny nose and cough, and diseases: asthma and rhinitis.[19, 20] Moreover, exposure to fungal spores[21-23] and bacterial endotoxins exacerbates asthma,[24-26] a disease that affects 300 million people worldwide.[27, 28] Respiratory illnesses are predicted to increase in response to global trends of increasing carbon dioxide

concentrations[29, 30] and temperatures[31] which are shown to enhance allergenicity[29] and quantity[30] of airborne pollens.[31, 32] The protection of sensitive populations from bioaerosols requires understanding environmental exposures to bioaerosols as a function of type and size.

1.2.2 Climatic effects

Once released, bioaerosols in the atmosphere promote cloud and ice nucleation.[33-35] Particles that act as surfaces to condense water vapor at supersaturated conditions forming cloud droplets and ice crystals are called cloud condensation nuclei (CCN) and ice nuclei (IN), respectively.[1, 2] Intact birch, walnut and willow pollens are effective CCN,[33] with cytoplasmic pollen granules ranging from 0.05 - 0.3 μm being the most CCN active, due to their hygroscopicity and longer residence time.[36] Bacteria also are CCN, at relatively low supersaturations.[34, 35] Because of their ordered structures, bioaerosols are effective ice nuclei (IN), forming ice crystals at sub-cooled temperatures, and include intact pollens[37, 38] pollen extracts,[39] fungal spores,[40] and bacteria.[34, 41] Their ability to act as CCN and IN affects the earth's climate through increase of cloud albedo and alter precipitation cycles.[34, 37]

1.3 Biological and chemical tracers of bioaerosols

Bioaerosols in the atmosphere can be tracked by chemical or biological markers [42-45] that are unique to bioaerosols. These markers are typically present in large quantities, such as structural[46] or energy storage materials.[44, 47] Measurements of these markers have provided insights to the spatial and temporal variability in ambient air. Several examples of such markers and insights gained from them are discussed in the following sections 1.3.1 to 1.3.3.

1.3.1 Endotoxin

Endotoxin is a component of Gram-negative bacteria cell membranes that are also present in a few Gram-positive bacteria.[48] Outdoor endotoxin measurements are elevated during growing seasons of spring, summer and autumn compared to winter.[49-52] This seasonal variation has been attributed to increased vegetative surfaces (e.g. leaves) that provide substrates for bacterial growth.[53, 54] Taxonomic analysis of airborne bacteria in the summertime implicates vegetative detritus and soil as the primary origins.[55] In a highly agricultural setting in Iowa, peak endotoxin concentrations occurred in autumn when row-crops were harvested,[45] leading to suspensions of soil, dust, and detritus. Other sources associated with high endotoxin levels include compost facilities,[56] livestock operations,[57] crowded streets, and agricultural fields.[52] In the US, Sweden and China, higher levels of bacterial counts were documented in heavily populated urban areas compared to background locations, with this difference attributed to vehicular traffic that suspends road dust containing bacteria.[58-60] Consequently, outdoor exposures to endotoxins are strongly influenced by seasonal variability and proximity to point sources.

1.3.2 Water-soluble proteins

Water-soluble proteins have been assessed to determine levels of biological particles in the atmosphere in Mexico City,[61, 62] China,[63] and tropical forest area.[64] Proteins are relatively non-specific, having a multitude of sources including soil resuspension from traffic and construction, biomass burning, pollens, fungi and bacteria.[62, 63, 65] Previous studies have shown that ambient protein levels tend to be suppressed in wet periods that suppress soil resuspension containing proteinaceous material.[62, 63]

1.3.3 Fungal spore tracers

Airborne fungal spores may be assessed through direct spore counting, or chemical markers of fungal spores` including 1, 3-β-D-glucans and sugar alcohols. 1,3-β-D-glucans are polysaccharide components of fungal cell walls. They have been widely investigated in indoor environments[66] and often studied in ambient environments[45, 52, 67] and after flooding[68] to assess population exposures to fungi. Sugar alcohols—mannitol and arabitol are energy storage materials in fungi and are proposed as markers of airborne fungal spores.[44] Mannitol measurements have been used to estimate fungal spore PM mass contributions in tropical rain forests.[69, 70]

Strong seasonal variations in fungal spores have been established in prior studies. Outdoor measurements of glucans showed higher concentrations in warmer seasons of spring, summer and autumn compared to winter.[71, 72] Similarly sugar alcohols were elevated in ambient air in warm seasons.[42, 73-75] These temporal trends follow the general seasonal variation of fungal spore counts where fungal spores peak in the mid-summer and fall [76-78] when conditions are warmer and humid.[79] Fungal spores have also been shown to increase following rain events.[80-82] Within these general trends, the seasonal variation in fungal spores is species-dependent: peak levels of *Cladosporium* spores have been observed in early to mid-summer, while *Alternaria* is elevated in late-summer to early-autumn.[83, 84] This species-dependence is attributed to differences in how fungi respond to different meteorological factors (e.g. maximum temperature, minimum temperature and relative humidity).[84] Fungi that discharge spores under dry conditions (e.g. *Cladosporium*) do so when conditions are dry and warm, whereas others (e.g. many basidiospores and ascospores) release spores upon wet conditions and cooler

temperatures.[69] In Portugal higher spore levels of *Coprinus* and *Pleospora* were observed in urban areas compared to rural sites where *Alternaria, Penicillium*, and *Cladosporium* dominated.[85] Compared to urban sites, a rural excess in fungal spores was observed in Poland,[86] Portugal,[87] and Finland.[88] Meanwhile, suburban enhancements relative to urban locations have been documented in Taiwan[89] and Austria.[90] Taxonomic analysis suggests that enrichment in fungi at rural locations results from more vegetative surfaces that provide surfaces for fungi to grow.[86] Given the strong seasonal-dependence of fungal spores, human exposures are largely dependent on local fungal populations and time of year, with peak exposures during warmer months.

1.3.4 Pollen tracers

Sugars are measured in ambient PM to assess plant related particle releases, as they serve as main energy storage material in plants.[42, 43, 74] Prior studies showed seasonal differences of plant releases using glucose, fructose and sucrose where concentrations peaked in spring and summer following the seasonal growing cycle, compared to winter.[42, 43, 74] During springtime, sugars are mainly derived from pollens,[47] and can be used as chemical tracers of this bioaerosol source. Pollens are allergen particles that exacerbate respiratory illnesses.[91] Assessment of pollen levels in the atmosphere is important to protect sensitive populations, especially in growing seasons when pollen levels reach highest.

1.4 Gaps in current understanding of bioaerosols in the Midwestern US

Even though earlier work in the Midwestern US assessed bacterial endotoxins in close proximity to agricultural settings[45] and fungal glucans in flood damaged houses,[92] and within homes,[67] typical outdoor bioaerosol exposure levels were not known in the

Midwest. Considering impacts of bioaerosols on human health, examining outdoor bioaerosol exposure levels in different locations and their spatial variability is important to sensitive populations living in these areas. This thesis work defines urban and background outdoor levels of bioaerosols in Iowa. These measurements allowed assessing bioaerosol levels in Iowa against health-based threshold values and understanding spatial differences of bioaerosol exposure levels and their sources.

Most prior outdoor bioaerosol measurements have focused on assessing one bioaerosol type,[45, 52, 72, 93] while ambient exposures are to bioaerosol mixtures and other air pollutants. Understanding co-existing bioaerosols and pollutants are crucial as bioaerosols have been shown to agglomerate with other pollutants,[15, 94, 95] and those bioaerosol-pollutant mixtures may be more harmful than bioaerosols alone.[96] To expand the understanding of bioaerosols and co-pollutants, experiments in this work were designed to gain insight into bioaerosol mixtures in ambient air—including bacterial endotoxins, fungal spores, and water-soluble proteins—in addition to co-pollutants of crustal elements, biomass burning emissions, and secondary aerosols simultaneously and across four seasons. These measurements demonstrated co-existing bioaerosol-co-pollutant mixtures so that future studies could use these mixtures to assess real health implications of bioaerosols.

Bioaerosol particle concentrations and sizes are often determined utilizing online florescence-based techniques,[16, 77, 80] but these techniques lack the ability to distinguish bioaerosol types. Fluorescent-based measurements showed increase in fine-sized bioaerosols when raining which was explained as a result of bacteria or fungal spores released from mechanical agitation of vegetation by rain drops.[80] In the same work,

coarse-sized bioaerosols increased post-rain attributed to fresh releases from vegetation surfaces. All of these discussions on particle characterization are based on literature on bioaerosol sizes and releasing mechanisms.[80] This thesis work assesses the effect of rain on springtime and late summer bioaerosols utilizing their chemical and biological tracers, which can be used to relate measurements back to their sources. Hence, the results allowed characterizing bioaerosol types that increase with rain events providing essential information to persons who suffer from particular types of allergies.

Chemical mass balance (CMB) source apportionment model is used worldwide to assess contributions of different sources to PM,[97-99] but prior to this thesis had not been applied to pollens. To support the source apportionment of pollens, chemical profiles of prevalent Midwestern pollen types were developed. This allowed the apportionment of ambient PM to pollens for the first time, allowing for estimation of their contribution to PM mass.

1.5 Introduction to thesis chapters

The dissertation work presented herein mainly focuses on providing answers to where, when, and how bioaerosol exposures happen in the Midwestern US, which is essential information to mitigate human exposures to bioaerosols. Specifically, research presented in this thesis advances the current knowledge about bioaerosols in the following ways: 1) defining outdoor bioaerosol concentrations in the Midwestern US, and evaluating differences across urban-background locations, 2) characterizing ambient bioaerosol and co-pollutant mixtures, 3) determining the influence of meteorology on daily variation of bioaerosol concentrations and size distributions, and 4) estimating of bioaerosol contributions to PM mass.

A detailed description of sample collection, extraction and analysis methods, and the statistical analysis approach used in this thesis work is provided in Chapter 2. This chapter includes details of active sampling devices used to collect ambient PM, methods for determining PM mass gravimetrically, chemical speciation by high performance ion exchange chromatography (HPIEC), and bioassays for water-soluble proteins, endotoxins, and fungal glucans that were performed in the Pulmonary Toxicology Facility at the University of Iowa. Statistical methods for analysis evaluating significant differences and correlations among species are also described.

In Chapter 3, spatio-temporal variations of bioaerosols were assessed utilizing PM_{10} samples collected in 2012, at six sites in Iowa by Iowa Department of Natural Resources (IDNR). The seasonal variation was determined by assessing bioaerosol measurements made in January, April, July and October, representing seasons of winter, spring, summer and autumn, respectively. Spatial variation was determined by comparing bioaerosol tracer measurements among three urban and three background sites.

Chapter 4 presents a study that compare bioaerosols from 2013 springtime tree pollen season (mid-April to early-May) and the late summer ragweed season (late-August to early-September) in Iowa. Daily measurements were made for chemical tracers of pollens, fungal spores and Gram-negative bacterial endotoxins in fine and coarse PM. CMB source apportionment modelling and regionally-specific pollen profiles were used to apportion PM mass to pollens for the first time.

Chapter 5 presents results of preliminary work to develop tools to associate estimated pollen levels in Chapter 4 with asthma-related emergency room visits. This

chapter also describes microscopy-based studies conducted to develop tools to assess conditions that release fine pollen particles.

Chapter 6 is attributed to conclusions and future directions of the dissertation work presented here which includes descriptions of further studies that could be performed to advance the understanding of bioaerosols.

CHAPTER 2

EXPERIMENTAL METHODS

The research work presented in the following chapters was performed utilizing various experimental methods to collect PM, quantify its mass, and quantify its chemical constituents, particularly bioaerosol tracers. A detailed description of methods and techniques common across the studies that comprise this dissertation are discussed in this chapter, including collection of ambient PM samples, extraction of chemical components from filter substrates and analysis of these extracts for chemical tracers utilizing ion exchange chromatography. A brief description of biological assays conducted in the University of Iowa's Pulmonary Toxicology Facility is described. Specific details for each study (e.g. sampler siting) are provided in each corresponding chapter.

2.1 Collection of ambient particulate matter

Active sampling devices were used to obtain representative samples of PM suspended in the atmosphere. Particles are present in many shapes and sizes. During sampling, particles were collected based on aerodynamic diameter. Generally, a sampler consists of an inlet, a cyclone or impactor, a sample collection platform, a flow controller and a pump. Air is pulled through a vacuum pump at a constant flow rate through the inlet, which is regulated by the flow controller. The flow rate is measured at the beginning and end of sample collection utilizing a rotameter.

Particles entering the sampler are first separated based on their aerodynamic diameter by one of two methods: a cyclone or an impactor, both of which operate by the inertia possessed by particles in the moving air stream. A cyclone is a cylinder with a tangential inlet and central outlet. Inside a cyclone, a rotating high velocity flow is

created and particles are subjected to a centrifugal force. Larger particles that have more inertia also have difficulty following high velocity flow, thus they are removed from the air flow via impaction with the cyclone wall and are deposited onto the inner wall of the cyclone and finally collect in the dust cup located at the base of the cyclone. Smaller particles have less inertia, follow the high velocity air stream, exiting the cyclone and enter the sampling device and are then collected on to a filter. The cyclone geometry and the flow rate together determine the cut-off diameter of the particles that pass through the cyclone and reach the filter.[3,2] Impactors typically contain an impaction surface placed in the path of the air stream that will separate particles from the flowing air stream. Inside a virtual impactor, air is forced to flow through an accelerated jet and then impacted against a slowly pumped void with lower velocity than the initial air stream instead of a solid surface. With impaction, air flow is divided into two air streams; a minor and a major flow. Larger particles with higher inertia continue moving with the minor flow through void and slowly collected on to a filter down stream. Smaller particles with low inertia follow the major air flow which deviate 90˚ from the original direction after impacting with the virtual impactor, and deposit on a filter located in the main air flow downstream.[3,2,100]

Samples are typically collected over 24 hours in order to obtain enough material for chemical characterization. The sample collection time and flow rates are recorded to determine the total air volume gone through the filter. Sampling of PM_{10}, $PM_{10\text{-}2.5}$ and $PM_{2.5}$ in this thesis work used three different samplers and described in sections 2.1.1-2.1.3.

2.1.1 PM_{10} sampler

PM_{10} samples were collected by the Iowa Department of Natural Resources (IDNR) as part of a state-wide monitoring program using low volume federal reference method samplers (Thermo Fisher Scientific Partisol-Plus Model 2025 Sequential Air Sampler). The Partisol-Plus samplers used a PM_{10} cyclone inlet with a flow rate of 16.67 liters per minute (LPM). The flow controller of Partisol-Plus sampler was calibrated at a temperature of 0˚C and a pressure of 1 atm. The sampler system was capable of determining mass flow set point needed to obtain the flow rate of 16.67 LPM after measuring the atmospheric temperature and pressure. PM_{10} samples were collected on to 47-mm Teflon filters (Pall Corp.) using an automated filter changing system which had a capacity of 16 filter cassettes.[101]

2.1.2 $PM_{2.5}$ sampler

$PM_{2.5}$ samples were collected using a medium-volume sampler (URG Corp.), with a flow rate of 92 LPM. The flow rate was determined on each day before and after filter collection. A Teflon coated sharp cut Aluminum cyclone was used to filter-out particles with aerodynamic diameters larger than 2.5 μm.[102] $PM_{2.5}$ samples were collected on to 90 mm pre-cleaned quartz fiber filters (Pall Corp.).

2.1.3 Dichotomous sampler

An Anderson dichotomous sampler (Series 241) was used to collect $PM_{2.5}$ and $PM_{10\text{-}2.5}$ separately. The sampler consisted of an impactor-type PM_{10} inlet to separate particles less than 10 μm, and a virtual impactor to separate $PM_{2.5}$ and $PM_{10\text{-}2.5}$. Air flowed at a rate of 16.7 LPM through the inlet and the coarse flow rate maintained at 1.67 LPM.[103] PM samples were collected on to 37 mm Teflon filters (Pall Corp.).

2.1.4 Filter preparation

Quartz filter substrates were cleaned prior to sample collection. Aluminum foil covers that are used to store filter samples (Figure 2.1) were pre-baked at 550 ˚C for 5 hours. Quartz fiber filters were baked at 550 ˚C for 18 hours. Post-sampling filters were stored in aluminum lined petri dishes sealed with Teflon tape at -20˚C until extraction.

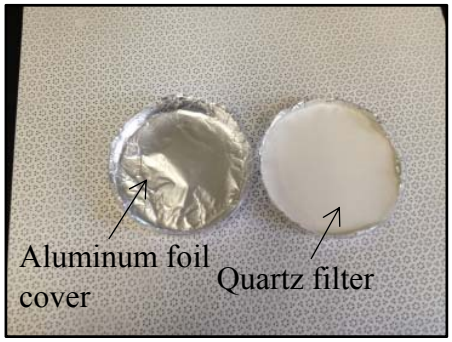

Figure 2.1: Blank Quartz filter used to collect samples (right), and pre-baked aluminum foil use to cover the filter (left).

2.1.5 Quality control in aerosol sampling

Field blanks were collected at a minimum rate of 1-in-5 samples, in order to determine background levels of analytes and account for any contamination that occurred during filter handling or storage. The field blank was handled similar to a sampled filter, placed in the sampler without pulling air through it, and stored at -20˚C until analysis.

2.2 Extraction of carbohydrates and ions from filter substrates

Carbohydrates and ions in PM were measured after extracting into Ultra-Pure (UP) water. All glassware was pre-baked at 500 ˚C for 5 hours and plastic vials used were pre-rinsed with UP water. One half of each Teflon filter was pre-wetted with 50 μL of isopropyl alcohol (Avantor Performance Materials, Center Valley, PA, US) and

extracted into 4.00 mL UP water with a specific resistance equal to or greater than 18.2 MΩ cm^{-1} (Barnstead EasyPure II, 7401). Extraction was assisted by rotary shaking for 10 min at 125 rpm, then sonication for 30 min at 60 Hz (Branson 5510, Danbury, CT, US), and rotary shaking for 10 min. Each extract was filtered through a 0.45 μm polypropylene syringe filter (GE Healthcare, UK) to remove insoluble particles. A second extraction with 15.0 mL of UP water with shaking for 12 h was performed to fully extract calcium (Ca^{2+}). One 1.5 mL aliquot of the extract was used for carbohydrate analysis and analyzed within 24 h. The remaining 2 mL was diluted by a factor of six with UP water, stored at 4 ˚C, and used for ion analysis within 28 days of extraction. When PM was collected on to quartz fiber filters same extraction protocol was followed with no pre-wetting.

2.3 Carbohydrate separation columns

Carbohydrates were separated by ion exchange chromatography. CarboPac columns used to separate carbohydrates contained a polymeric stationary phase composed of microbeads with anion exchange functional groups on the surface. Specifications for two columns (CarboPac MA1 and PA 20) utilized during this dissertation work are summarized in Table 2.1. Polymer based stationary phases provide stability over a wide range of pHs. Carbohydrates are weak acids and have pKa values around 12 (eg. pKa of glucose = 12.28, fructose = 12.03). At a very high pH carbohydrates become negatively charged, thereby the separation of carbohydrates in CarboPac columns are achieved via anion exchange. Specific conditions applied during carbohydrate separation with MA1 and PA20 columns are described in sections 2.3.1 and 2.3.2, respectively.

Table 2.1: Carbohydrate column specifications.

Column	**MA 1**[104]	**PA20**[105]
Polymer type	Vinylbenzyl chloride/divinylbenzene	Ethylvinylbenzene crosslinked with divinylbenzene
Resin particle size (μm)	7.5	6.0
Surface functional group	Alkyl quaternary ammonium group	Difunctional quaternary ammonium ion
Cross linking (%)	15	55
pH range	0-14	0-14
Temperature limit (˚C)	4-60	4-60
Pressure limit (psi)	2000	3500

2.3.1 MA1 column

Carbohydrates were separated using a high performance anion exchange chromatography system (HPAEC) (Dionex ICS 5000, Thermo Fisher, Sunnyvale, CA, USA), which consisted of an eluent organizer, dual pump, degasser, column compartment and AS-DV autosampler. A Dionex CarboPac MA1 analytical column (4 X 250 mm) with preceding guard column (4 X 50 mm) was used for the carbohydrate separation following Zhang et al (2013).[106] A 25 μL loop was used for sample injection. Isocratic separation of carbohydrates (erythritol, mannitol, levoglucosan, glucose, xylitol, arabinose [Sigma Aldrich], and arabitol, trehalose [Alfa Aesar]) (Figure 2.2) was achieved utilizing 480 mM sodium hydroxide (NaOH, Fisher Scientific) flowed at 0.4 mL min^{-1} which was stored under steady N_2 flow (Praxair).

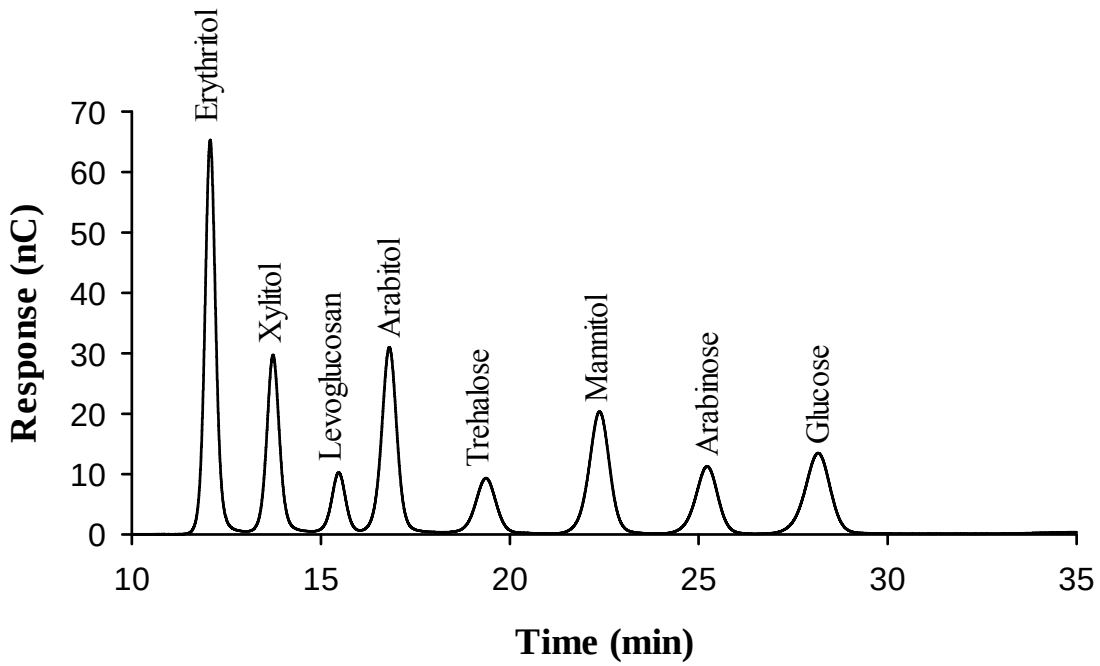

Figure 2.2*:* Separation of carbohydrates in CarboPac MA1 column.

2.3.2 PA20 column

Carbohydrates were separated using HPAEC as described above in section 2.3.1. A CarboPac PA20 analytical column (3 X 150 mm, Dionex) with a preceding guard column (3 X 30 mm) was used for the separation process with a 10 µl sample injection loop. Isocratic separation of carbohydrates (erythritol, levoglucosan, mannitol [Alfa Aesar], glucose, fructose, arabinose [Sigma Aldrich], and sucrose [Fisher Scientific]) (Figure 2.3) were achieved utilizing 10mM sodium hydroxide (NaOH, Fisher Scientific) as shown in Figure 2.3.

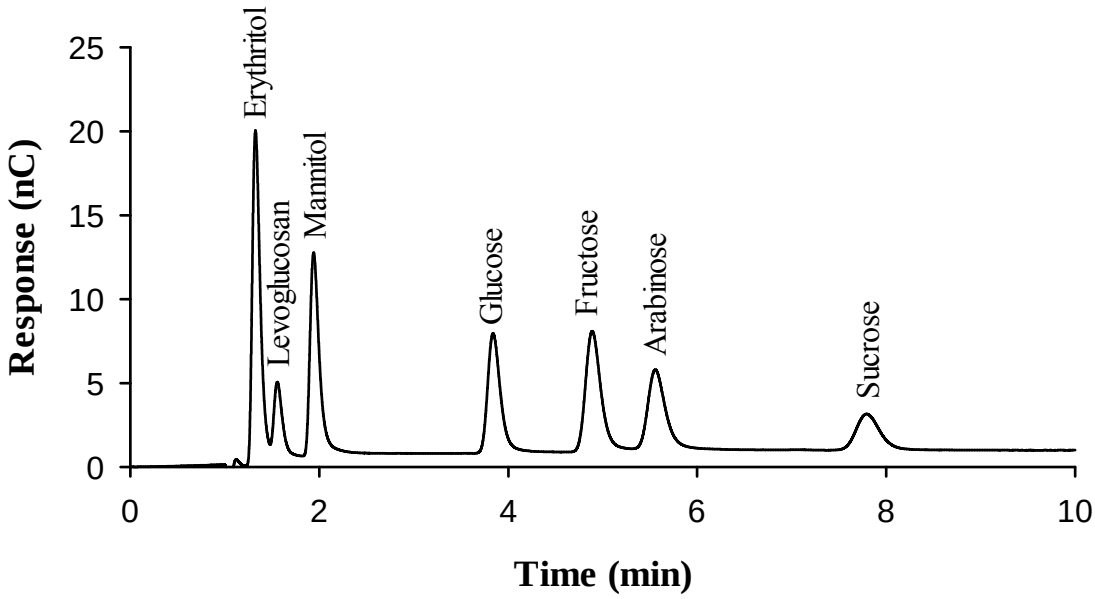

Figure 2.3*:* Separation of carbohydrates in CarboPac PA20 column.

2.4 Carbohydrate detection via pulsed amperometric detector (PAD)

Carbohydrates separated by the HPAEC system were detected by the PAD (ED 50, Thermo Fisher, Sunnyvale, CA, USA). The PAD cell contained a gold disposable working electrode (replaced nearly every 50 runs), to which quadruple waveform A (Figure 2.4) was applied relative to a pH-Ag/AgCl reference electrode.[107] In amperometric detection, carbohydrates are measured by the electrical current generated via oxidation of carbohydrates (E1) on the surface of the gold electrode. The products of the oxidation reaction poison the gold electrode. Therefore the gold surface is cleaned between measurements using a potential that desorbs oxidized carbohydrates (E2) and oxidizes the gold surface (E3). The electrode potential is then lowered (E4) to reduce gold on the electrode surface.[107]

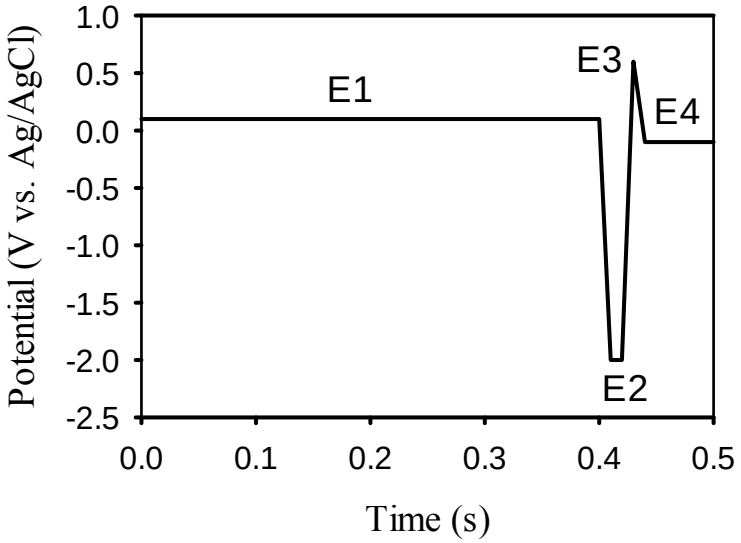

Figure 2.4: Schematic of waveform A, quadrupole waveform. E1 – oxidation of carbohydrates, E2 – desorption of carbohydrates, E3 – oxidation of gold, and E4 – reduction of gold.

2.5 Carbohydrate quantification

Chromeleon 7 software was used for instrumental control, data acquisition and analysis. Calibration curves were generated utilizing areas of carbohydrate peaks at seven different concentrations ranging from 0.0100 – 2.50 ppm. Carbohydrate peak areas were determined by integrating peaks in the chromatogram. Methyltetrols were identified using relative retention times in Zhang et al. (2013)[106] and semi-quantified using erythritol as a proxy standard.

2.6 Ion separation and detection

Anions and cations were analyzed by ion chromatography, with suppressed conductivity detection (ICS 5000, described above) following Jayarathne et al (2014).[108] Anion separation was achieved using an Ionpac AS22 analytical column (4 × 250 mm) preceded by a guard column, with an AERS 500 suppresser (Dionex). The anion mobile

phase was diluted from concentrate (Dionex AS22) in UP water to obtain 4.5 mM sodium carbonate, 1.4 mM sodium bicarbonate and flowed at 1.2 mL min^{-1}. Cation separation was obtained using an Ionpac CS12A analytical column (3 × 150 mm) preceded by a guard column, with a CERS 500 suppresser (Dionex). The cation mobile phase contained 20 mM methylsulfonic acid (Dionex CS12A eluent concentrate) and flowed at 0.5 mL min^{-1}. All mobile phases were maintained under N_2 headspace. Both columns were held at 35 ˚C and the injection volume was 25 µL. Seven-point calibration curves were prepared from Dionex Seven Anion Standard and Dionex Six Cation-II Standard over the range of 0.01 – 10 ppm.

2.7 Detection limits

Instrument and method detection limits were determined prior to sample analysis. Three times the standard deviation of the lowest detected concentration of each analyte through seven runs was taken as instrumental detection limit (IDL). Method detection limits (MDL) of analytes were obtained by performing seven spike recovery samples. To do this, seven blank filters were spiked with a standard solution which prepared targeting a final concentration which will be ~5 times higher than the IDL. The spiked filters were then extracted and analyzed for species concentrations. Similarly lab blanks were also done without spiking from the standard solution and these blank filter extracts accounted for any background analyte or contamination during sample extraction. Three times standard deviation of concentrations across seven extracts plus the average of blank levels were taken as the MDL.

2.8 Extraction efficiency

A check standard was analyzed daily to assess the validity of the calibration curve and if the results deviated beyond 100 ± 10% of the expected concentration, the instrument was recalibrated. Each analysis batch contained ten ambient samples, two field blanks, one lab blank and one spike recovery sample. For the spike, a blank filter was spiked with a known volume of a standard solution, and extracted in the same manner as ambient samples. The ratio of measured-to-spiked concentration is taken as the extraction efficiency. Average extraction efficiencies for carbohydrates ranged from 94 - 103% (Tables 2.2). Spike recoveries ranged from 96 - 103% for anions and 94 - 103% for cations (Table 2.3).

2.9 Determination of ambient concentrations

Ambient sampling filter extracts were analyzed and converted to mass concentrations per unit air volume (A_{AIR}) following eq: 1. Concentrations were first determined (A_S) in ng/ µL (ppm), subtracted by the mean concentration detected in field blanks (A_{FB}), multiply by the extraction volume (V_{AQ}) and divided by the air volume (V_{AIR}) passed through the extracted filter area.

$$[A_{AIR}]\, ng/m^3 = \left[A_S - A_{FB}\right] ng/\mu L * [V_{AQ}]\mu L * 1/[V_{AIR}]m^3 \qquad \textbf{[Eq: 1]}$$

Table 2.2. Method detection limits and average spike recoveries (± one standard deviation, $n = 12$) for carbohydrates.

	MDL (ppb)		Spike Recovery (%)	
Column	MA 1	PA 20	MA 1	PA 20
Erythritol	0.6	5.9	98 ± 3	99 ± 6
Levoglucosan	2.8	5.8	100 ± 4	102 ± 5
Xylitol	3.0	1.0	101 ± 4	ND
Arabitol	1.0	ND	98 ± 4	ND
Trehalose	3.0	1.0	101 ± 5	ND
Mannitol	2.3	1.7	99 ± 4	100 ± 6
Arabinose	3.8	3.3	100 ± 4	103 ± 5
Glucose	1.6	3.8	98 ± 3	101 ± 4
Fructose	ND	2.8	ND	100 ± 5
Sucrose	ND	3.7	ND	102 ± 6

MDL - Method detection limits
ND – not determined

Table 2.3. Method detection limits and average spike recoveries (± one standard deviation, $n = 12$) for ions.

	MDL (ppb)	Spike recoveries (%)		MDL (ppb)	Spike recoveries (%)
Anions			Cations		
Fluoride	5.0	96 ± 9	Lithium	3.8	94 ± 6
Chloride	7.8	103 ± 7	Sodium	5.3	96 ± 8
Nitrite	30.2	96 ± 4	Ammonium	40.0	103 ± 7
Bromide	22.9	96 ± 8	Potassium	30.4	94 ± 6
Nitrate	22.6	98 ± 4	Magnesium	14.9	96 ± 6
Phosphate	43.2	96 ± 4	Calcium	31.8	96 ± 7
Sulfate	60.0	96 ± 4			

MDL - Method detection limits
ND – not determined

2.10 Biological assays

Biological assays of glucans, endotoxins and proteins were done at the Pulmonary Toxicology Facility, University of Iowa by Nervana Metwali under the supervision of Prof. Peter S. Thorne.

2.10.1 Extraction of biological tracers

Teflon filters were extracted into 2 mL of sterile pyrogen-free (PF) water for 1 h at 22˚C, while shaking, then centrifuged (5 min at 600g at 4 °C), and the supernatant was divided into three portions to analyze for glucans, endotoxins and water-soluble proteins.

2.10.2 Analysis of biological tracers

For glucan analysis, one aliquot of the supernatant was transferred into a PF borosilicate tube, mixed with 10x PF phosphate buffered saline (PBS) containing 0.05% Tween-20 (a surfactant), subjected to 1 h of shaking, 1 h of autoclaving, 20 min shaking, and finally centrifuged for 5 min (600g at 4 ˚C). Fungal glucans were quantified in the supernatant by enzyme immunoassay as previously described in Blanc et al. (2005).[109] The reaction was stopped using 0.18M H_2SO_4 and measured at 450 nm (SpectraMax Plus 384; Molecular Devices, Sunnyvale, CA, USA). A twelve-point standard curve was generated using (1-3, 1-6)-β-D-glucan standard (*scleroglucan*), ranging from 3 - 5000 ng ml^{-1}.

For endotoxins, the second aliquot was subjected to the kinetic chromogenic *Limulus* amebocyte lysate assay (LAL) (Lonza, Inc., Walkersville, MD) as described in Thorne (2000).[110] A twelve-point calibration curve was generated utilizing endotoxin standard (*Escherichia coli* 055:B5), ranging from 0.024 - 50 Endotoxin Units (EU) ml^{-1},

and the absorbance was measured at 405 nm (SpectraMax M5, Molecular Devices, Inc., Sunnyvale, CA).

For analysis of water-soluble proteins, the third aliquot of the extracts was transferred into PF cryotubes, and then mixed with 10x PF PBS and vortexed for 5 min. A five point calibration curve was run in the range of 0.5 – 30 μg ml $^{-1}$ which is recommended by the manufacturer using the bovine serum albumin standard solution. The working reagents from the QuantiPro BCA assay kit (Sigma-Aldrich) were mixed according to the manufacturer's instructions. An aliquot of 150 μL was added to the plate and incubated for 16 h and assessed at 562 nm (SpectraMax Plus 384, Molecular Devices, Inc.).

2.11 Statistical analysis

Statistical analysis was performed for species detected in ≥75% of samples. Data points below detection limit were substituted with the limit of detection (LOD)/ √2 prior to further analysis.[111] For species detected in field blanks, the LOD is defined as the mean level in field blank plus three times standard deviation of the field blanks. For analytes that were not present in the field blanks, the LODs were calculated as three times the standard deviation of the instrument response for the lowest calibration standard across seven injections. All data sets were checked for normality and log-normality using the Anderson-Darling test in Minitab (version 16), with $p \geq 0.05$ signifying normally-distributed data sets. Most concentration data was not normally distributed, thus Spearman's rank order correlation for non-parametric comparisons (r_s) in SPSS (Statistical Package for the Social Sciences - 21) was utilized to investigate inter-species correlations. Resulting correlation coefficients were interpreted as follows: 0.0 - 0.3

negligible, 0.3 - 0.5 low, 0.5 – 0.7 moderate, 0.7 – 0.9 high, and 0.9 – 1.0 very high.[112] Correlations were considered significant when $p \leq 0.05$. Paired t-tests were conducted Minitab (version 16) to assess spatial differences of measured tracers in, and obtained results were taken significant when $p \leq 0.05$.

CHAPTER 3

URBAN ENHANCEMENT OF PM_{10} BIOAEROSOL TRACERS RELATIVE TO BACKGROUND LOCATIONS IN THE MIDWESTERN UNITED STATES[1]

3.1 Abstract

Bioaerosols are well-known immune-active particles that exacerbate respiratory diseases. Human exposures to bioaerosols and their resultant health impacts depend on their ambient concentrations, seasonal and spatial variation, and co-pollutants, which are not yet widely characterized. In this study, chemical and biological tracers of bioaerosols were quantified in respirable particulate matter (PM_{10}) collected at three urban and three background sites in the Midwestern United States across four seasons in 2012. Endotoxins from gram negative bacteria (and a few gram positive bacteria), water-soluble proteins, and tracers for fungal spores (fungal glucans, arabitol and mannitol) were ubiquitous and showed significant seasonal variation and dependence on temperature. Fungal spores were elevated in spring and peaked in summer, following the seasonal growing cycle, while endotoxins peaked in autumn during the row crop harvesting season. Paired comparisons of bioaerosols in urban and background sites revealed significant urban enhancements in PM_{10}, fungal glucans, endotoxins and water-soluble proteins relative to background locations, such that urban populations have a greater outdoor exposure to bioaerosols. These bioaerosols contribute, in part, to the urban excesses in PM_{10}. Higher bioaerosol mass fractions in urban areas relative to background sites indicate that urban areas serve as a source of bioaerosols. Similar urban enhancements in water-soluble calcium and its correlation with bioaerosol tracers point towards wind-blown soil as an important source of bioaerosols in urban areas.

3.2 Introduction

Ambient levels of airborne particulate matter (PM_{10}) in urban areas has been shown to exceed those in background locations, from smaller cities[113,114] to heavily urbanized megacities.[115,116] Urban excesses in PM have been linked to higher rates of morbidity and mortality in urban areas.[117] Elevated levels of PM_{10} have been connected to declines in lung function, respiratory diseases,[6] cardiovascular effects,[10] and asthma among children[118] at concentration levels that are often well below the Environment Protection Agency National Ambient Air Quality Standard (EPA NAAQS) for PM_{10}. Even short-term exposures to urban PM have resulted in increased respiratory symptoms and declined lung functions, increasing respiratory hospital admissions and mortality.[7,119]

Bioaerosols, inclusive of pollens, fungal spores, animal and plant detritus, bacteria and viruses, are associated with various health conditions such as pulmonary inflammation, asthma, allergenic responses, and respiratory infections.[120] When airborne, bioaerosols may be found as individual particles or agglomerates of particles.[18] The sizes of bioaerosols affect their atmospheric lifetime, transport, physical properties, and mechanisms of human contact.

Once in the air, bioaerosols agglomerate with other pollutants.[94,95,15] The combination of bioaerosols with PM from different sources can enhance their impact on respiratory morbidity, as shown by an *in vitro* study,[96] which suggests that bioaerosol mixtures are more harmful than single PM source types. In addition, the inflammatory endpoints of bioaerosols are enhanced when mixed with urban air pollution[121] and endotoxin in the presence of diesel exhaust particulate has been shown to have a synergistic effect on lung injury.[122] Pollutant-PM interactions can modify allergenicity.[123]

For instance, other pollutants such as ozone and nitrogen dioxide increase the permeability of airway membranes[124,125] enhancing the impact of allergens.[123] These findings underscore the need to understand the magnitude and chemical nature of PM from many sources simultaneously in order to accurately assess population exposure to bioaerosols and mitigate the health effects of ambient air pollution.

Given the importance of bioaerosols in contributing to negative health effects of ambient particulate matter, this study serves to define the seasonal and spatial trends in bioaerosols to which populations in the Midwestern US are exposed outdoors. To this end, samples of PM_{10} were analyzed from six sites in Iowa during spring, summer, autumn and winter by bioassays for endotoxins, fungal glucans, water-soluble proteins, and by chemical methods for tracers of fungal spores, biomass burning, secondary aerosol, and resuspended soil. All sites were part of the IDNR Ambient Air Monitoring Network. According to IDNR criteria, the three "background" sites were situated to establish background levels of pollutants.[126] Background sites were located in open fields immediately surrounded by forested areas that ranged in size from 4.0 – 6.7 km^2. Beyond the forests were agricultural fields, lakes, and rivers. The three "urban" sites were situated in cities with populations over 40,000 and were established to monitor outdoor pollutants to which surrounding populations would be exposed.[126] Urban sites were surrounded by relatively uniform land use on a spatial scale of 0.5 – 4.0 km, and common surroundings of the sampling sites were commercial, residential, and civic buildings. Data analysis centered on the following objectives: 1) evaluating the abundance and seasonal variation of bioaerosols in the Midwestern US, 2) assessing differences in ambient concentrations of bioaerosols across urban and background locations, and 3) determining which

bioaerosol types and pollutants co-vary. Statistical analyses were used to assess the significance of differences in bioaerosol concentrations and mass fractions across urban and background locations and to examine correlations among bioaerosols and other PM_{10} constituents.

3.3 Materials and methods

3.3.1 Sample collection

Samples were collected at six sites in Iowa (Figure 3.1), with urban and background being defined by the Iowa DNR as described in section 1.[126] Additional site characteristics are noted by site. The urban site in Council Bluffs, IA was located at Franklin School (41.26417,-95.89612). The urban site in Waterloo, IA (42.50154, -92.31602) was positioned near a water tower, railway, rail yard, and public park. The urban site in Davenport, IA was located at Adams School (41.55001, -90.60012) and was in the vicinity of agricultural land. The background sites were located at Viking Lake (40.96911,-95.04495), Lake Sugema (40.69508, -92.00632), and Backbone State Park (42.60083, -91.53833).

PM_{10} samples were collected by the IDNR as described in Chapter 2 section 2.1 using a Thermo Fisher Scientific Partisol-Plus Model 2025 Sequential Air Sampler. PM_{10} samples were collected over 24 h from midnight-to-midnight at an interval of 1-in-3 or 1-in-6 days following the EPA 2012 ambient air quality monitoring schedule. From these samples, filters from January, April, July and October were selected to represent winter, spring, summer, and autumn. Five samples were analyzed from the six sites for each of the four months; in total, 120 samples were analyzed. Field blanks were analyzed once

per month for each site; in total, 24 field blanks were analyzed. Meteorological data was accessed from the nearest airport weather stations (within 30 miles).

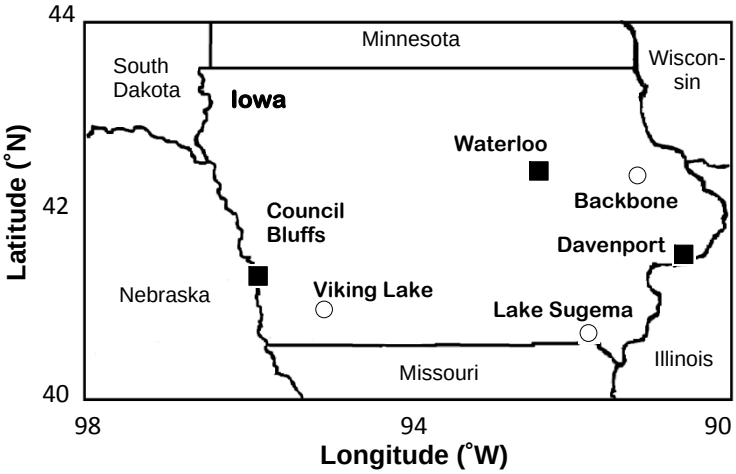

Figure 3.1: Map of DNR sampling sites, marking urban sites (filled squares) and background sites (open circles).

3.3.2 PM_{10} mass determination and filter preparation

PM_{10} mass concentration data were accessed from the Technology Transfer Network (TTN) Air Quality System (AQS) Data Mart.[127] Briefly, PM_{10} mass was determined gravimetrically as the difference between pre- and post-sampling filter weights using a Mettler Toledo AG microbalance (Model: XP6, Laboratory & Weighing Technologies, Switzerland) with a precision of 0.5 μg (standard deviation of 10 repeated measurements). The mass measurements were processed using an AH225 auto-handler produced by Measurement Technology Laboratories, Corp (MTL), with controlled temperature (20-23 ˚C) and relative humidity (30-40%) at the State Hygienic Laboratory at the University of Iowa. After mass measurement, PM_{10}-containing Teflon filters were cut in half using a pre-cleaned ceramic knife and scissors following a guided glass

surface in a laminar flow hood. One half was subjected to chemical analysis and the other half to bioassays.

3.3.3 Chemical analysis

One half of the Teflon filters were extracted in to 4.0 mL of UP water as described in Chapter 2, section 2.2. From the extract one portion was used to assess carbohydrates by HPAEC-PAD (Chapter 2, section 2.3). The other portion was assessed for cations and anions following Jayarathne et al. (2014)[108] by HPIEC-CD (Chapter 2, section 2.6).

3.3.4 Bioassays

One half of each Teflon filters loaded with PM_{10} was extracted in to 2 mL of PF water and analyzed for biological tracers as described in Chapter 2 section 2.9. Briefly, fungal glucans were assessed by enzyme immunoassay following Blanc et al. (2005)[109], bacterial endotoxins were analyzed via LAL assay following Thorne (2000)[110] and total proteins were measured using BCA assay.

3.3.5 Statistical analysis

Spearman's correlations (r_s) were used to assess relationships among measured tracers after examining for the normality as described in chapter 2, section 2.10. Analyte concentrations of each of the three background and urban sites were averaged by date (n = 20) and were log-normally distributed. Paired t-tests (by date) of the log-transformed data were conducted in Minitab (version 16).

3.4 Results and discussion

3.4.1 PM_{10} mass

3.4.1.1 Seasonal meteorology and trends in PM_{10}

The seasonal variation in the temperature reflected the temperate, continental environment of the Midwestern US. Daily mean temperatures in 2012 ranged from -8 to 28 ˚C and averaged 12 ˚C (Figure 3.2). For January (winter), April (spring), July (summer), and October (autumn), monthly average temperatures were 3 ˚C, 18 ˚C, 34 ˚C, and 17 ˚C, respectively. The daily relative humidity (RH) in 2012 ranged from 30 – 96% with monthly average of 70% in January, 62% in April, 63% in July, and 64% in October. Cumulative precipitation over the entire month varied across the 6 study sites, ranging from 0.3 - 11 mm in January, 4 - 61 mm in April, 0.1 – 15 mm in July, and 0 - 45 mm in October. Exceptional drought conditions occurred in the Midwest during summer of 2012, according to the US drought classification,[128] which affected samples collected in July.

Daily (24 h) PM_{10} concentrations throughout 2012 ranged from 2 - 68 μg m^{-3} with an average of 21.7 μg m^{-3} across the six study sites (Figure 3.2). PM_{10} levels did not exceed the 24-hr primary or secondary EPA NAAQS for PM_{10} set at 150 μg m^{-3}. Monthly average PM_{10} concentrations revealed minor seasonal variation, with maximum levels occurring in September (30 μg m^{-3}) and minimum concentrations in February (16 μg m^{-3}). These seasonal changes follow the greater national trend in the US with peak PM levels in the summer and minimum levels occurring in the winter.[129] The annual average PM_{10} concentration in this study (21 μg m^{-3}) was comparable to annual averages of

surrounding states (Wisconsin (14 μg m^{-3}), Illinois (29 μg m^{-3}), Minnesota (20 μg m^{-3}) and Missouri (25 μg m^{-3}) in 2012,[127] and earlier work in the Midwestern US.[45]

3.4.1.2 Spatial variation in PM_{10}

In comparing the background and urban sites in Iowa, an urban excess in PM_{10} was observed. On 52 of 61 sampling days in 2012, the average PM_{10} concentration across the three urban sites exceeded that of the background sites (Figure 3.2). PM_{10} concentrations at the three background locations averaged 19.3 μg m^{-3} (and ranged from 3.91–43.0 μg m^{-3}) compared to urban sites that averaged 22.2 μg m^{-3} (and ranged from 6.07–48.9 μg m^{-3}), as shown in Table 3.1. With daily average urban and background PM_{10} concentrations paired by date, a t-test comparison revealed a significant urban excess in PM_{10} ($p = 0.002$), averaging 3.0 μg m^{-3}. This excess is equivalent to a 15% increase of PM_{10} in urban areas relative to background locations. This demonstrates that, persons living in urban locations in Iowa are exposed to PM_{10} levels that are significantly greater than the regional background.

The urban enrichment of PM_{10} observed here has also being demonstrated previously when comparing urban PM_{10} with rural locations,[113] and suburban locations.[113,116] These variations were mostly attributed to enhanced anthropogenic activities in city areas such as soil resuspension by moving traffic.[113,116,130] The sources responsible for elevated PM_{10} levels in Iowa are discussed in the following sections in the context of bioaerosols and other source tracers.

3.4.2 Fungal spores

3.4.2.1 Seasonal variation in fungal spore tracers

The presence and abundance of fungal bioaerosols in ambient PM_{10} were assessed by fungal glucans and sugar alcohols. Glucans were ubiquitous components of PM_{10}, detected in 98% of PM_{10} samples and were quantified against (1-3, 1-6)-β-D-glucan standards (*scleroglucan*). For the 1-in-6 day samples, monthly average concentrations were lowest in January (8.2 ng m^{-3}), increased in April (19 ng m^{-3}), peaked in July (31 ng m^{-3}), and remained elevated in October (27.2 ng m^{-3}) (Figure 3.3a). Mannitol and arabitol were detected in 95% and 93% of samples, respectively. Mannitol concentrations also peaked in July (72 ng m^{-3}), were elevated in April (31 ng m^{-3}) and October (39 ng m^{-3}), and were largely undetected in January (Figure 3.3b).

The seasonal variations of fungal glucans and sugar alcohols are consistent with prior studies of glucans[71,72,131] and sugar alcohols,[42, 47, 75] in which fungal spore levels increase during warmer months characteristic of growing seasons that favor fungal growth and spore dispersal.[76,79] Accordingly, this work demonstrates that seasonal variations of fungal spores in Iowa follow those previously observed in different locations of the world.

Compared to other studies of PM_{10} sugar alcohols, peak mannitol levels in July were similar to summertime PM_{10} mannitol concentrations in Europe[132,133] and Beijing, China,[134] but were greater than Norway,[134] and Israel.[73] The elevated mannitol levels in autumn during this study were three times lower than a recent study from an agricultural town in California, US which captured the cotton harvesting season.[135]

In comparing glucans measurements to prior work in the US, it is necessary to note that prior studies utilized the LAL assay against a 1-3 -β-D-glucan standard,[44] while this study measures glucans with an enzyme immunoassay against (1-3, 1-6)-β-D-glucan standard (Chapter 2, section 2.9). Autumn glucan concentrations in Iowa were three times higher than those in an agricultural town in California during harvesting (9.6 ng m^{-3}),[135] with the opposite trend observed for mannitol. Differences in fungal species (with different mannitol-to-glucans ratios) impacted the different study sites and comparison between sites is confounded by measurement differences.

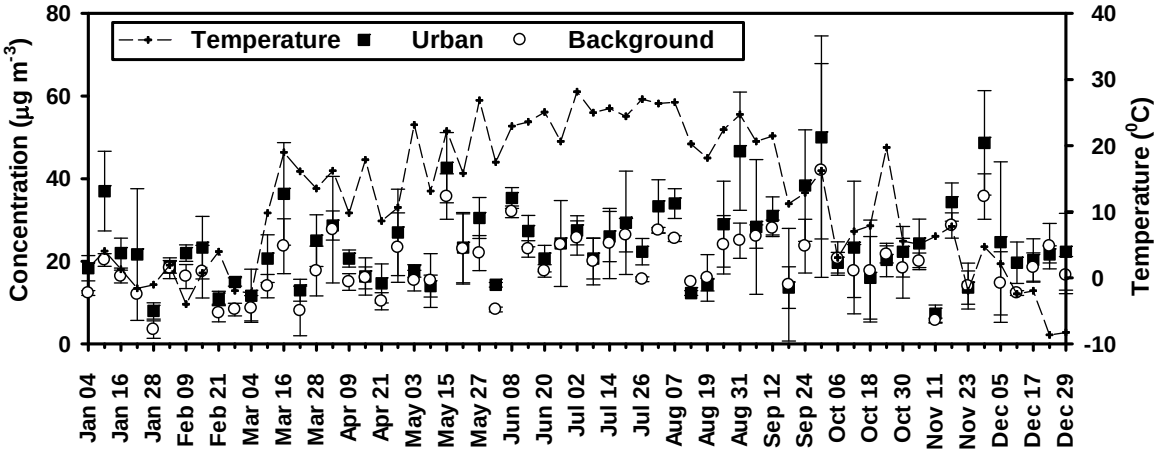

Figure 3.2: Average concentrations of PM_{10}, averaged across urban (n =3; filled squares) and background (n = 3; open circles) sites in Iowa, measured every 6th day in 2012.

Table 3.1: Summary statistics for PM_{10} and individual species concentrations measured at three urban and three background sites in Iowa in January, April, July and October 2012. The rightmost columns compare urban and background PM_{10} concentrations and mass fractions using paired t - tests (significantly different results are shown in bold) in Iowa for January, April, July, and October 2012.

Component	Unit	Freq. of detection (%)	*Urban*				*Background*				*Urban v. background* ***p*- value (n=20)**	
			Mean	±	Std. dev.	PM_{10} Mass fraction (%)	Mean	±	Std. dev.	PM_{10} Mass fraction (%)	Concen-tration	By mass fraction
PM10	($\mu g\ m^{-3}$)	100	22.2	±	8.72	NA	19.3	±	7.49	NA	**0.002**[a]	NA
Biomarkers												
Proteins	($\mu g\ m^{-3}$)	99	1.96	±	1.11	9.01[c]	1.51	±	0.96	8.12[c]	**<0.001**[a]	0.278[a]
Glucans	($ng\ m^{-3}$)	98	26.5	±	25.4	0.13[d]	16.4	±	17.1	0.09[d]	**<0.001**[b]	**<0.001**[b]
Endotoxins	($EU\ m^{-3}$)	99	0.26	±	0.24	1.36[e]	0.21	±	0.25	1.08[e]	**0.001**[b]	**0.030**[b]
Carbohydrates												
Arabitol	($ng\ m^{-3}$)	93	19.7	±	18	0.09	30.2	±	34.8	0.16	0.725[b]	0.372[b]
Mannitol	($ng\ m^{-3}$)	95	31.8	±	34.5	0.15	41.3	±	44.3	0.21	0.878[b]	0.096[b]
Glucose	($ng\ m^{-3}$)	92	32.7	±	32.9	0.15	44.1	±	47.1	0.23	0.936[b]	0.257[b]
Levoglucosan	($ng\ m^{-3}$)	98	49.9	±	51.5	0.23	49.1	±	78.8	0.26	0.973[b]	0.287[b]
Inorganic ions												
Calcium	($\mu g\ m^{-3}$)	100	1.94	±	1.31	8.28	1.75	±	1.31	8.76	**0.006**[a]	0.537[a]
Ammonium	($\mu g\ m^{-3}$)	100	0.67	±	0.73	3.14	0.58	±	0.49	3.28	0.259[b]	0.118[b]
Nitrate	($\mu g\ m^{-3}$)	99	1.21	±	1.61	5.35	0.91	±	0.91	5.17	**0.039**[b]	0.840[b]
Sulfate	($\mu g\ m^{-3}$)	99	1.5	±	1.2	6.99	1.7	±	1.82	9.05	0.941[b]	0.096[b]

[a] Paired t test performed on concentration data, [b]Paired t test performed on log-transformed concentration data, [c]µg of proteins measured relative to bovine serum albumin /µg of PM_{10}, [d]µg of glucans measured relative to (1-3, 1-6)-β-D-glucan standards (scleroglucan) /µg of PM_{10}, [e]µg of endotoxins measured relative to Escherichia coli 055:B5 /µg of PM_{10}, NA - Not applicable.

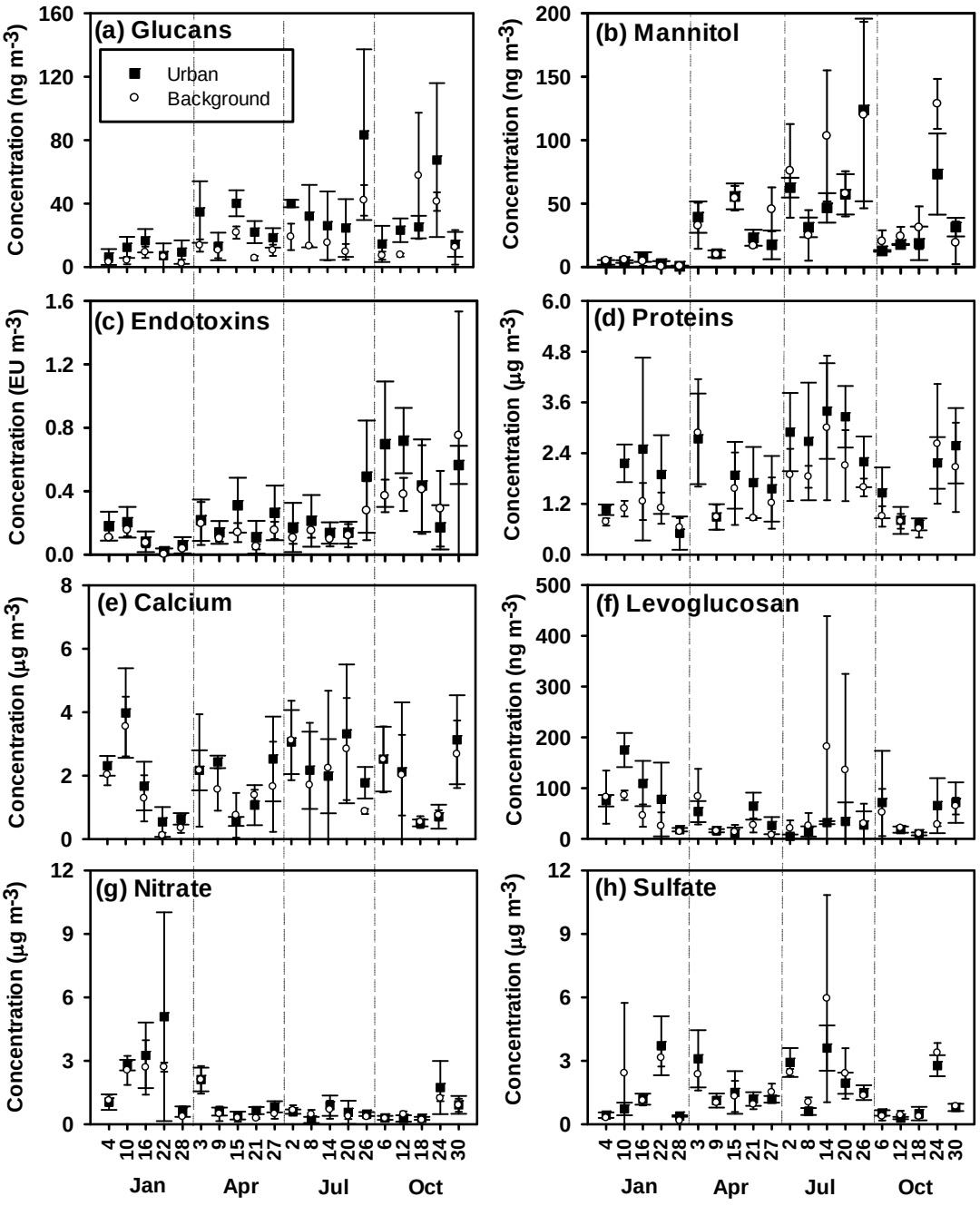

Figure 3.3: Average concentrations of PM_{10} bioaerosols (a-d) and other constituents (e-h), averaged across urban (n = 3) and background (n = 3) sites in Iowa (error bars represent one standard deviation), measured every sixth day during January, April, July, and October 2012.

3.4.2.2 Correlation of sugar alcohols and fungal glucans

Glucans and sugar alcohols generally showed consistent temporal trends, supporting their origins from the same source type. Arabitol and mannitol were highly correlated (r_s = 0.9, $p < 0.001$) as shown in Figure 3.4a; such a strong correlation is expected for chemical tracers originating from the same source that are measured by the same technique. Mannitol was consistently 1.5 times more abundant than arabitol. This result is consistent with Bauer et al. (2008)[44] who observed an average mannitol-to-arabitol ratio of 1.5 in ambient measurements. Mannitol and glucans were also positively correlated (r_s = 0.6, $p < 0.001$) as shown in Figure 3.4b; this is considered to be a relatively strong correlation across biological and chemical tracers, considering that glucans were measured by bioassay, while sugar alcohols were measured by HPAEC-PAD. When correlations of fungal spore tracers in background and urban data sets were assessed separately, equivalent correlations were obtained: for urban and background sites alike correlations were significant ($p < 0.001$) with $r_s \geq 0.9$ for mannitol and arabitol, and r_s = 0.6 for mannitol and glucans. The higher degree of scatter (lower r_s) when comparing glucans and mannitol could be due to non-fungal sources of either species. For instance, higher plants,[136] ragweed pollens,[46] and some bacteria[137,138] have 1,3-β-D-glucans in their structure. Biomass burning has been suggested as a source of ambient mannitol and arabitol.[139,140] In addition, mannitol has been shown to be present in higher plant species and algae. Alternatively, this scatter could arise from different fungal species with varying glucans-to-sugar alcohol levels, impacting urban and background sites to different extents.

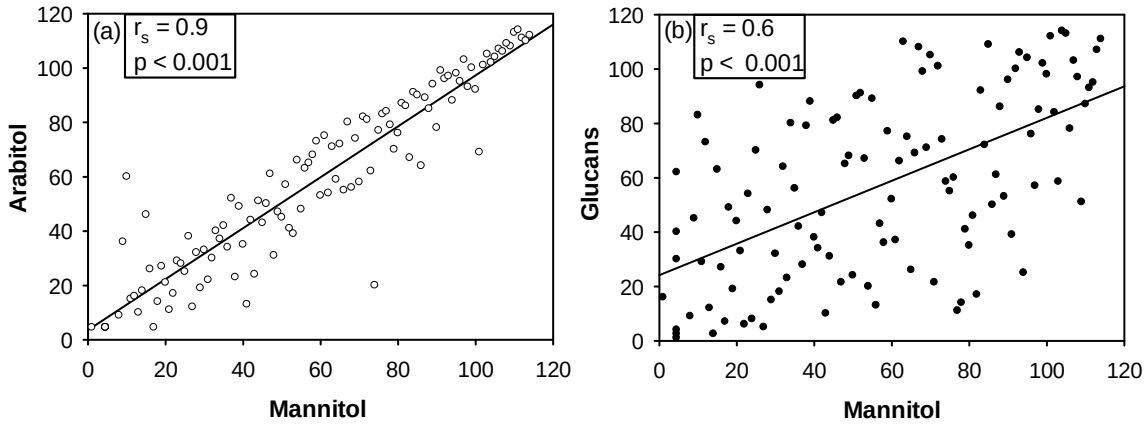

Figure 3.4: Spearman's correlation coefficient (r_s) of PM_{10} fungal spore markers when ranked tracer concentrations in ng m^{-3} are plotted for all six sites in Iowa across four seasons in 2012: (a) arabitol v. mannitol and (b) glucans v. mannitol.

3.4.2.3 Spatial variation of fungal spore tracers

The spatial comparison of fungal spore tracers revealed that urban sites had significantly higher PM_{10} fungal glucan concentrations (27 ng m^{-3}) compared to background sites (16 ng m^{-3}) ($p < 0.001$, Table 3.1). The observed urban excess in glucans concentrations indicates that urban populations have greater outdoor exposures to fungal glucans. Glucans accounted for a larger mass fraction of PM_{10} in urban areas (1.3 ng μg^{-1}) compared to background sites (0.86 ng μg^{-1}), with the urban enrichment also being statistically significant ($p < 0.001$). The urban enrichment of glucans mass in PM_{10} indicates that urban areas serve as a source of fungal glucans. Further, glucans are expected to contribute to the urban excess in PM_{10}, although the extent of this contribution cannot be quantified, because of the uncertainty of the mass of glucans introduced by the use of a proxy standard.

The urban enhancement in fungal spores, however, was not supported by measurements of mannitol and arabitol (Table 3.1). This result suggests differences in the

glucans-to-sugar alcohol ratios across urban and background sites, with the urban sites having greater glucans. Because glucans on spore surfaces vary among dominant fungal species (*Cladosporium, Aspergillus* and *Penicillium*),[46] it is expected that different fungal species impact the urban and background sites to some degree. Alternatively, there may be a non-fungal source of glucans or fungal spores that may impact the urban and background sites differently. Although, the urban excess in fungal spores was not supported by sugar alcohol measurements, the urban enhancement of glucans is significant because of its known health impacts.

3.4.2.4 Estimation of fungal spore counts

Ambient fungal spore counts allow for comparison to threshold values to protect sensitive populations and were estimated using mannitol, arabitol, and glucans. Estimations for sugar alcohols followed the method of Bauer et al.,[44] in which spores were found to contain 1.2 - 2.4 pg mannitol spore^{-1} (averaging 1.7 pg spore^{-1}), and 0.8 – 1.8 pg arabitol spore^{-1} (averaging 1.2 pg spore^{-1}). These conversion factors were developed from ambient air samples in Austria and in applying them to Iowa; it is assumed that the fungal populations had the same sugar alcohol-to-spore ratio. To convert glucans concentrations in to fungal spore counts the conversion factor of 3.1 pg glucans spore^{-1} was used[46] which applies to *Cladosporium*, one of the most abundant fungal spore types in the US.[79] In applying this conversion factor, it is assumed that *Cladosporium* is the main spore type in the atmosphere. Notably, the methods of glucans analysis in this study (enzyme immunoassay against (1-3, 1-6)-β-D-glucan standard [*scleroglucan*]; Chapter 2, section 2.9) differed from Foto et al. (2004)[46] who used the LAL assay an against 1-3 -β-D-glucan standard (*curdlan*). Such methodological

differences may lead to positive or negative biases in the fungal spore counts, depending on their relative response to the selected standard.

All three methods produced maximum spore counts in July and minimum spore counts in January; however, their magnitudes differed considerably (Table 3.2). Using glucans, estimates of monthly average spore counts ranged from 2,600-10,000 m^{-3}. Using mannitol and arabitol, estimates of monthly average spore counts ranged 2,200-43,000 m^{-3} and 2,100-41,000 m^{-3}, respectively. On average, the estimates of spore counts agreed well when using mannitol and arabitol, as marked by their significant and very high correlation ($r_s = 0.9$, $p < 0.001$) and slope of 0.91 (Figure 3.5a). By comparison, estimates of fungal spore counts via glucans were consistently lower (slope of 0.17, Figure 3.5b) and varied more widely ($r_s = 0.6$, $p < 0.001$). To assess which estimate was more accurate, data were compared to Dayton, Ohio, US, which was the nearest Midwestern site where fungal spore counts could be accessed for the study period, albeit 600 km east of Iowa. In Dayton, monthly mean fungal spore counts were 800 m^{-3} in January, 1400 m^{-3} in April, 10,000 m^{-3} in July, and 6000 m^{-3} in October.[141] These values agreed best with fungal spore estimates made via glucans, and suggested over-estimations of spore counts when using sugar alcohols. These estimated spore counts (using glucans) were in good agreement with other outdoor spore counts in the US, including Cincinnati, Ohio,[67] and Tulsa, Oklahoma.[142] However, spore counts were much lower than those observed at the onset of harvesting activities in an agricultural town in California (66,000 spores m^{-3}).[135] To validate estimates of spore counts using bioassays and chemical measurements in different regions, side-by-side spore counts and PM-based measurements are needed.

Numerical spore counts are a valuable metric for relaying fungal spore levels to sensitive populations. The National Allergy Bureau of the American Academy of Allergy Asthma and Immunology (NAB AAAAI) has predefined atmospheric spore counts as *low*: 1-6499 spores m^{-3}, *moderate*: 6500- 12,999 spores m^{-3}, *high*: 13,000-49,999 spores m^{-3} and *very high*: > 50,000 spores m^{-3}.[143] The best estimate of spore counts (using glucans) across the six sites in Iowa indicated low to moderate levels in January, low to high levels in April and low to high levels in July and October. The highest estimated fungal spore count was on July 26 at the Davenport site with 43,000 spores m^{-3}. Transitioning from summer to autumn, fungal spore counts decreased, except for October 24 when the estimated fungal spore count in Waterloo reached high levels with 32,000 spores m^{-3}. Notably, on this day the average temperature was 21 ˚C in Waterloo, which was an unseasonably warm day in autumn. Thus, peak levels in July and October are sufficiently high to have deleterious respiratory impacts, especially for sensitive populations.

Table 3.2: Monthly spore counts for the six study sites in Iowa in 2012, estimated using mass-to-spore conversion factors of glucans, mannitol, and arabitol.

	Estimated average monthly fungal spore counts (spores m^{-3})					Spore counts in Dayton, OH (spores m^{-3})[c]
	Via glucans[a]	Via mannitol[b]		Via arabitol[b]		
	Avg.	Range	Avg.	Range	Avg.	Avg.
January	2600	1600 - 3000	2200	2000 - 4700	2100	800
April	6200	13,000 – 25,000	18,000	10,000 – 24,000	16,000	1400
July	10,000	30,000 – 60,000	43,000	27,000 – 61,000	41,000	10,000
October	8800	16,000 – 32,000	23,000	15,000 – 34,000	23,000	6000

[a] The conversion factor of 3.1 pg glucans $spore^{-1}$ was applied assuming that spores were *Cladosporium*.[46]

[b] Estimations followed the method of Bauer et al.,[44] in which spores were found to contain 1.2 - 2.4 pg mannitol $spore^{-1}$ (averaging 1.7 pg $spore^{-1}$), and 0.8 – 1.8 pg arabitol $spore^{-1}$ (averaging 1.2 pg $spore^{-1}$).

[c] Spore counts were measured at the Dayton-Montgomery County Public Library in downtown Dayton, OH.[141]

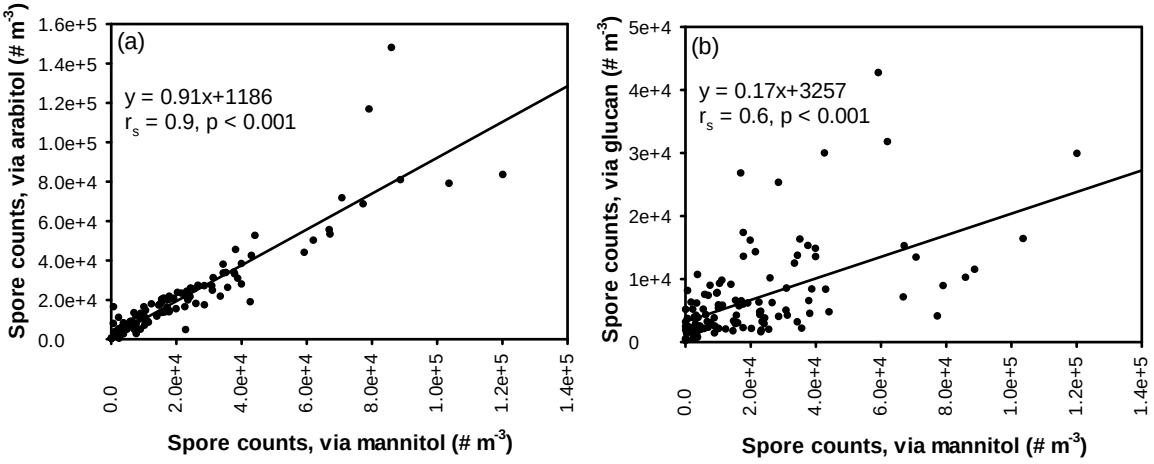

Figure 3.5. Comparison of fungal spore counts estimated by (a) arabitol and mannitol, and (b) glucans and mannitol.

3.4.3 Endotoxin

3.4.3.1 Temporal variation in endotoxin

Endotoxins were present in 99% of PM_{10} samples and concentrations were measured in endotoxin units (EU) against an *Escherichia coli* (055:B5) standard (Figure 3.3c). Endotoxin concentrations were log-normally distributed and the geometric means (GM) across all six sites were 0.10 EU m^{-3} in January, 0.17 EU m^{-3} in April, 0.19 EU m^{-3} in July, and 0.47 EU m^{-3} in October. The highest endotoxin levels were observed in October, reaching maximum daily values at Viking Lake (1.65 EU m^{-3}) and Council Bluffs (1.15 EU m^{-3}). During October, row crops are harvested in Iowa, which gives rise to large levels of resuspended dust and vegetative detritus. Endotoxin concentrations were lowest in winter due to the lack of vegetation coverage, with the lowest detected concentrations occurring on January 22 when snow fell at two sites. Even the maximum levels observed in this study are well below the health-based recommended threshold value of 50 EU/m^3 proposed by the National Health Council of the Netherlands.[144]

The obtained average endotoxin level in October was six times lower than a previously reported average autumn endotoxin level (2.63 EU m^{-3}) in Keokuk County, Iowa, which is in close spatial proximity to agricultural dust sources.[45] Importantly, these data show that the very high endotoxin levels that have been documented in agricultural areas of Iowa do not extend to background sites or urban areas. The four-month geometric mean endotoxin concentration (0.14 EU m^{-3}) in this study was consistently low in comparison to other studies in the US, including Keokuk County (1.93 EU m^{-3}),[45] urban areas in Southern California (0.44 EU m^{-3})[135] and Massachusetts (0.51 EU m^{-3}).[145] Meanwhile, the PM_{10}-normalized mass fraction of endotoxins is similar to Southern

California,[93] indicating a similar PM_{10} mass fraction of endotoxins across these two regions.

The minimum endotoxin levels in winter and elevated levels in the growing seasons are consistent with earlier studies in the US,[93,45] and Europe,[49,51,52] which attributed the difference to an increase of vegetation coverage that provide surfaces for bacterial growth.[53] However, the highest endotoxin levels in autumn were unique to Iowa,[45] with most other locations having maximum concentrations in spring or summer. Thus, maximum endotoxin exposures in autumn appear to be a characteristic of agriculture activities in the Midwestern US.

3.4.3.2 Spatial variation of endotoxin

Spatially, urban locations were found to have a statistically significant excess of bacterial endotoxins relative to background sites ($p = 0.001$). When normalized to the mass of PM_{10}, the urban sites (0.014 EU μg^{-1}) also had significant enrichment in bacterial endotoxins ($p = 0.03$), relative to background sites (0.010 EU μg^{-1}), demonstrating greater population exposures to airborne bacterial endotoxins in urban locations relative to background sites. This urban excess in Iowa is significant because endotoxins have been linked to multiple health impairments.[25] Endotoxins were significantly correlated with Ca^{2+} (see section 3.4.3) during January, April and July ($r_s = 0.4$, $p < 0.001$) suggesting that soil-derived bacteria are likely the source of airborne bacteria in this study.

Prior studies have rarely compared urban and background atmospheric endotoxin levels. A study in Massachusetts, US demonstrated 2 - 3 folds higher ambient endotoxin levels than the urban endotoxin concentrations of this study (Table 3.1), with elevated

levels in their urban site (GM = 0.5 EU m^{-3}) relative to the suburban location (GM = 0.4 EU m^{-3}).[145] Moreover, studies from urban sites of Europe showed similar outdoor endotoxin levels to the background endotoxin levels of this work, and showed no spatial differences within urban locations.[50,146,147] The urban excess in endotoxins observed in Iowa is not consistent across other studies, suggesting that spatial differences in airborne bacteria vary on local and regional scales.

3.4.4 Water-soluble proteins

Water-soluble proteins were measured relative to a bovine serum albumin standard using a bicinchoninic acid (BCA) assay. This assay is based on a re-dox reaction of copper (Cu) where Cu^{2+} forms Cu^{+} in the presence of proteins. Then Cu^{+} chelates with BCA forming a purple complex which allows for an indirect measurement of proteins in the sample.[148] The BCA assay can be interfered with high concentrations of strong reducing agents such as dithiothreitol.[148] Nonetheless, biochemists use BCA assay frequently with biological samples and showed less interferences and higher sensitivity compared to other available methods.[148] BCA assays have previously applied to quantify proteins in atmospheric PM_{10} samples.[63,65]

Water-soluble proteins were present in 99% of PM_{10} samples analyzed with levels ranging from < 0.08 – 5 μg m^{-3} relative to the bovine serum albumin standard (Figure 3.3d). Ambient protein concentrations in Iowa for the four months sampled were lowest in January (1.4 μg m^{-3}), rose in April (1.6 μg m^{-3}), peaked in July (2.5 μg m^{-3}), and decreased by October (1.5 μg m^{-3}).

Urban sites had significantly higher ambient concentrations of proteins ($p < 0.001$). The observed urban enhancement of proteins is likely associated with higher

levels of urban dust and resuspended soil, which was supported by the positive correlation with the crustal element Ca^{2+} in July ($r_s = 0.2$, $p = 0.03$). Winter protein levels are much higher than other bioaerosol tracers, indicating wintertime protein sources (Figure 3.3d). The correlation with levoglucosan in January revealed a significant, but low correlation ($r_s = 0.3$, $p \leq 0.001$) suggesting biomass burning as a source of proteins during winter. These suggested source categories are consistent with previous findings that implicated soil resuspension in summer and biomass burning in winter.

The protein concentrations observed in Iowa were comparable to levels measured in Rubidoux, CA, US, which had a mean concentration of 2.7 μg m^{-3} of proteins for a study conducted from October to May. Meanwhile, the observed levels in Iowa were much lower than those measured in Mexico City, where protein levels averaged 81 μg m^{-3} from July to December,[61] and Hefei, China where levels averaged 11 μg m^{-3} from June – February;[63] in these impacted environments, high protein levels were attributed to anthropogenic and/or crustal sources. Even though proteins are not measured widely in outdoor environments it is valuable to determine their ambient levels as an indicator of biologically derived material that is often connected with negative health impacts.[149-151]

3.4.5 Crustal elements

Calcium, which comprises a significant fraction of road and soil dust in the Midwestern United States[152] was used as a marker of suspended dust. The use of Ca^{2+} as a dust tracer is supported by a prior source apportionment study in Iowa that showed 75% of Ca^{2+} in ambient $PM_{2.5}$ is associated with dust.[153] In this study, PM_{10} Ca^{2+} was detected in all samples with monthly averages of 1.73 μg m^{-3} (January), 1.63 μg m^{-3} (April), 2.30 μg m^{-3} (July), and 1.73 μg m^{-3} (October). Its concentrations did not show a strong

seasonal dependence (Figure 3.3e), but were highest during summer. The summertime dust maximum was expected to have been enhanced by the drought conditions. By applying the Ca^{2+} to dust mass fraction observed for urban dust in St. Louis (0.143 g g^{-1}),[152] it is estimated that crustal materials accounted for approximately 55 - 62 % of PM_{10} in Iowa, making airborne dust the largest source of PM_{10}.

Spatial evaluation of PM_{10} Ca^{2+} measurements (Table 3.1) revealed significantly higher levels in urban areas compared to background locations ($p = 0.006$), indicating the greater impact of resuspended dust in urban environments. Previous work has shown the origin of urban resuspended dust from road traffic,[154,155] and the connection of resuspended PM_{10} with respiratory symptoms among children.[156] Moreover, Rogge et al. (1993)[157] assessed paved road dust and confirmed its ability to act as a repository for vehicle related hazardous particles which then become airborne upon passing traffic, suggesting higher exposure of traffic related harmful substances among urban populations in the presence of elevated levels of road dust.[157]

3.4.6 Biomass burning

Significant urban and background variation was not observed for the biomass burning tracer levoglucosan[158] (Figure 3.3f) during the study period. However, considering the days with the temperature below 10 ˚C a significant urban excess was obtained, suggesting more residential wood burning in urban sites.

3.4.7 Secondary aerosols

Secondary aerosol is a major contributor to fine particulate matter in the Midwest. Secondary ions, including ammonium (NH_4^+), nitrate (NO_3^-) and sulfate (SO_4^{2-}) were detected in ≥99% of PM_{10} samples and averaged 0.63 μg m^{-3}, 1.1 μg m^{-3} and 1.6 μg m^{-3},

respectively. Ammonium, NO_3^- and SO_4^{2-}, contributed 3.2%, 5.3%, and 8.0% of the PM_{10} mass, respectively. Ammonium nitrate was the dominant secondary aerosol compound during winter, with the highest monthly average NO_3^- concentration occurring in January (2.31 μg m^{-3}) (Figure 3.3g). Wintertime production of NH_4NO_3 in the Midwest is attributed to low temperatures, long nights and low boundary layer heights.[159] Meanwhile, sulfate (Figure 3.3h) and methyltetrols that are isoprene secondary organic aerosol (SOA) tracers[139] peaked in July, due to higher emissions of isoprene and elevated photochemical reaction rates at higher temperatures.[160]

The ambient concentrations of secondary ions are frequently determined in fine mode, and observed PM_{10} secondary ion levels were comparable with $PM_{2.5}$ levels observed in the Midwestern US.[153,161,162] In particular, the highest levels of SO_4^{2-} observed in July (2.4 μg m^{-3}) were similar to previously determined 3 year average summer level in $PM_{2.5}$ at Iowa (2.2 μg m^{-3}).[153] The winter levels of NH_4^+ (0.74 μg m^{-3}) and NO_3^- (2.31 μg m^{-3}) were lower than earlier determined $PM_{2.5}$ regional averages (~2 μg m^{-3} and 4.5 μg m^{-3}, respectively), which is likely due to differences in local sources such as traffic.

When comparing NO_3^- across urban and background locations, significant urban excess ($p = 0.04$) was observed. This result is consistent with previous studies in the US and is caused by urban combustion sources (e.g. mobile sources and power plants) that emit oxides of nitrogen, a precursor to NO_3^-.[163] Overall, both primary and secondary aerosol sources showed enhancements in urban areas, whereas, on average, none of the measured analytes were enhanced in background locations.

3.4.8 Correlation of bioaerosols with temperature

Non-parametric correlation analysis indicated significant correlations ($p < 0.001$) between bioaerosol markers and average and maximum temperatures as shown in Table 3.3. In particular, arabitol and mannitol, chemical tracers of fungal spores indicated high positive correlations with average temperature (with $r_s = 0.8$ for both tracers). Fungal glucans also showed moderate correlation with average temperature ($r_s = 0.5$). Endotoxins, however, did not have a significant correlation with temperature when all four months were considered. When October dates were eliminated, to exclude the influence of row crop harvesting on ambient concentration levels, a low positive correlation ($r_s = 0.4$) was observed. Water-soluble proteins also showed moderate correlations ($r_s = 0.5$) with temperature. Glucose is associated with vegetative emissions, and exhibited high positive correlations with temperature ($r_s = 0.8$). Associations between bioaerosols and temperature have been observed previously in diverse geographical regions of the world. The correlations of fungal spore markers with temperature observed in this study can be explained by higher temperatures (~23 - 27 ˚C) being favorable for fungal development that increase airborne fungal spores.[164] Similar significant correlations ($p < 0.05$) have been previously demonstrated in field studies from Colorado and Oklahoma in the US with *Cladosporium* spores.[165] In addition, a study from New Zealand indicated significant correlations of mean temperature with ascospores ($r_s = 0.3$, $p < 0.005$), and maximum temperature with basidiospores ($r_s = 0.5$, $p < 0.005$).[166] The dependence of endotoxin levels on temperature in this study is consistent with prior studies where this association is suggested as a result of enhanced bacteria growth in warmer temperatures.

Table 3.3: Spearman's correlation coefficient values (r_s) performed on concentration data from six sites in Iowa during January, April, July, and October, 2012, with color indicating the significance of the correlation.

Variable	Max Temp	Mean Temp	PM_{10}	Glucans	Arabitol	Mannitol	Proteins	Glucose	Endotoxins	Calcium	Levoglucosan	Ammonium	Nitrate
PM10	**0.4**	**0.4**	**x**										
Glucans	**0.4**	**0.5**	0.2	**x**									
Arabitol	**0.7**	**0.8**	**0.4**	**0.6**	**x**								
Mannitol	**0.8**	**0.8**	**0.4**	**0.6**	**0.9**	**x**							
Proteins	**0.5**	**0.5**	**0.6**	**0.4**	**0.5**	**0.5**	**x**						
Glucose	**0.7**	**0.8**	0.3	**0.5**	**0.8**	**0.8**	**0.4**	**x**					
Endotoxins	0.1	**0.4***	0.3	**0.4**	**0.4**	0.3	0.2	0.2	**x**				
Calcium	0.2	0.2	**0.7**	-0.1	0.1	0.1	0.2**	0.0	0.4*	**x**			
Levoglucosan	-0.2	-0.3	0.2	-0.2	-0.2	-0.2	0.3	-0.3	-0.1	0.2	**x**		
Ammonium	**0.4**	**0.4**	0.2	0.2	0.3	**0.4**	**0.6**	**0.4**	-0.3	-0.2	0.1	**x**	
Nitrate	-0.2	-0.3	0.3	-0.2	-0.3	-0.3	0.3	-0.2	-0.2	0.2	**0.5**	**0.4**	**x**
Sulfate	**0.5**	**0.5**	0.3	0.2	**0.4**	**0.5**	**0.5**	**0.5**	-0.3	0.0	0.1	**0.9**	**0.4**

p - value ≤ 0.05 and > 0.01
p - value ≤ 0.01 and > 0.001
p - value ≤ 0.001
Bold when $r_s \geq 0.4$

* Correlation value is calculated with January, April and July data.

** Correlation value is calculated with July data.

Bioaerosol tracers did not have strong correlations with RH ($r_s < 0.4$, $p < 0.01$) across the six study sites. In contrast, other studies have shown dependence of ambient fungal spore levels on RH [165, 167] because humid conditions facilitate active release of spores by wet discharge.[69] The lack of a stronger fungal spore tracer correlation with RH suggests that fungal spores released by dry discharge mechanisms such as *Cladosporium* and *Penicillium*[69] may dominate in the Midwestern US. The relationship between

bioaerosols and precipitation was not examined, because the 1-in-6 day sampling rate and drought-conditions in July gave low numbers of days with precipitation. This comparison is further confounded by the lag time between precipitation and the response of bioaerosols (e.g. fungal spores may be released 8 hours after the onset of rain).[80]

3.4.9 Co-variation of bioaerosols and PM_{10} constituents

Significant correlations were observed among bioaerosol tracers, indicative of their co-existence and simultaneous population exposure to many bioaerosol types. All three fungal spore tracers showed low - moderate correlation with water-soluble proteins and endotoxins (r_s ranged 0.3 - 0.5, $p < 0.001$), and moderate – high correlations with glucose (r_s ranged 0.5 - 0.8, $p < 0.001$). This reflects their co-variation with temperature and the growing season (discussed in section 3.4.8), leading to lowest concentrations in winter and the highest concentrations in warmer months. Thus, bioaerosols are coincident with one another, such that populations in the Midwest are simultaneously exposed to many bioaerosol types, including fungal spores, plant and animal detritus, and bacteria, especially in the summer and autumn.

The co-variation of bioaerosols with other PM_{10} constituents serves to define the multi-pollutant mixtures in which bioaerosols are present. The observed correlations among calcium and endotoxins ($r_s = 0.4$, $p < 0.001$, discussed in section 3.4.3) suggest windblown soil and dust serve as a source of endotoxins. Also, significant low to moderate correlations of mannitol, glucose and water-soluble proteins with ammonium and sulfate (r_s 0.4 - 0.6, $p < 0.001$) indicate that peak levels of fungal spores and pollens in warmer months are coincident with secondary inorganic aerosols such as $(NH_4)_2SO_4$, and biogenic SOA. In autumn, airborne bacteria peak along with fungal spores,

vegetative detritus and windblown dust. Although not represented by the measurements in this study, industrial and vehicular emissions are omnipresent in urban centers in Iowa[153] and are important bioaerosol co-pollutants that are likely to exacerbate bioaerosol health impacts.

3.5 Conclusions

Seasonal variations in bioaerosols were driven by temperature. Maximum levels of fungal spores and water-soluble proteins were observed during summer when temperatures peaked. Maximum bacterial endotoxin levels occurred in autumn, coinciding with agricultural harvesting activities, yet were well below health-based threshold values for endotoxins. Overall, temperature dependence of bioaerosols suggested higher population exposures to bioaerosols during warmer days.

Fungal glucans, bacterial endotoxins and water-soluble proteins were enhanced in PM_{10} in urban areas over background sites, revealing that these classes of bioaerosols contribute to the urban excess in PM_{10} and further indicating elevated bioaerosol exposures of urban populations. Further, the fungal glucans and bacterial endotoxins made up a greater fraction of PM_{10} mass in urban locations, consistent with these bioaerosol types originating in urban areas. Correlation analysis demonstrated the co-variation of fungal spores, bacterial endotoxins and plant detritus, indicating that human populations are exposed to complex mixtures of bioaerosols. In urban areas, exposures to bioaerosols is coincident with combustion pollutants, as evidenced by the significantly higher levels of NO_3^- in urban locations compared to background sites. The significant correlations of endotoxins and water-soluble proteins with Ca^{2+}, a marker of resuspended soil and dust, suggested that wind-blown soil may be an important source of bioaerosols

in urban locations in Iowa. Our measurements propose possible atmospheric bioaerosol and co-pollutant mixtures that need further investigations to determine potential health impacts of human exposures to ambient air. Overall, urban populations are exposed to higher concentrations of bioaerosol and co-pollutant mixtures that may cause greater health risks among sensitive populations.

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CHAPTER 4

INFLUENCE OF RAIN ON THE ABUNDANCE AND SIZE DISTRIBUTION OF BIOAEROSOLS

4.1 Abstract

Assessing the environmental, health and climate impacts of bioaerosols requires knowledge of their size and abundance. These two properties were assessed through daily measurements of chemical tracers for pollens (sucrose, fructose, and glucose), fungal spores (mannitol and glucans) and Gram-negative bacterial endotoxins in fine and coarse particulate matter and PM_{10} (combination of $PM_{2.5}$ and $PM_{10-2.5}$). This study captured 2013 springtime tree pollen season (mid-April to early-May) and late summer ragweed season (late-August to early-September) in the Midwestern US in 2013. Under dry conditions, pollen and fungal spore tracers were primarily in coarse PM (>75%), as expected for particles greater than 2.5 μm. Rainfall on May 2 corresponded to maximum atmospheric pollen tracer levels and a redistribution of pollen tracers to the fine PM fraction (>80%). Both changes were attributed to the osmotic rupture of pollen grains that led to the suspension of fine-sized pollen fragments. Fungal spore tracers peaked in concentration following spring rain events and decreased in particle size, but to a lesser extent than pollens. A short, heavy thunderstorm in late summer corresponded to an increase in endotoxin and glucose levels, with a simultaneous shift to smaller particle sizes. Chemical mass balance (CMB) source apportionment modelling and regionally-specific pollen profiles were used to apportion PM mass to pollens and fungal spores. Springtime pollen contributions to PM_{10} mass ranged from 0.04–0.8 μg m^{-3} (0.2-38%, averaging 4%), with maxima occurring on rainy days. Fungal spore contributions to PM_{10}

mass ranged from 0.1–1.5 μg m^{-3} (0.8–17%, averaging 5%), with maxima occurring after rain. Overall, this study defines changes to size distributions and concentrations of pollens, fungal spores, and endotoxins in response to rain in the Midwestern United States and advances the ability to apportion PM mass to pollens. These results have significant implications for population exposures to pollens, particularly during rain events.

4.2 Introduction

Ambient levels of pollens vary seasonally with growing cycles.[78, 168] Springtime in the Midwestern United States is generally characterized by high levels of tree pollens,[78] such as oak,[169] birch,[170] alder, and hazel.[171] Summertime has elevated concentrations of grass pollens (e.g., Timothy and Rye grass). During late summer, weed pollens, especially ragweed dominate.[78] Daily pollen levels are affected by temperature, with warmer conditions favoring pollen development, maturation, and active release.[172] Rainfall promotes the passive release of intact pollens by agitation.[173] In rainy conditions, pollen grains absorb water, osmotically rupture, and release cytoplasmic starch granules.[174] Microscopy studies have shown that intact birch pollens of 22 μm in size can rupture and release around 400 starch granules[175] ranging from 0.03 - 4 μm.[174] Consequently, human exposures to pollens in the atmosphere are highly dependent on pollen type, season, and local meteorology.

Fungal growth and spore release is also promoted by elevated temperatures[176] and wet conditions.[177] Fungi discharge spores via splash-induced emission, as is the case for *Cladosporium*, a prominent fungal genus[82, 85] that releases spores by mechanical shock and fast air currents produced by rain drops.[69, 178] Fungi that belong to the division

Ascomycetes disperse spores in moist conditions[18] leading to elevated spore levels that occur several hours after rain.[178, 179] The release of bioaerosols during and after rain events can trigger significant changes to ambient bioaerosol numbers[80, 180] and mass concentrations.[181]

Bacteria in the atmosphere are typically settled on soil or vegetative surfaces and are found in cell agglomerates.[18] The taxonomic analysis revealed that soil and plant surfaces serve as sources of bacteria in the Midwestern US.[55] Ambient bacterial levels increase with temperature[49] due to conditions that favor vegetation and bacterial habitat.[53, 54] In vegetation-covered areas, atmospheric bacterial concentrations observed a peak after approximately 1 h of rain relative to areas with bare soil.[182] This response to precipitation has been attributed to rain moving plants and aerosolizing bacteria.[18] With strong dependences on local meteorology, bacteria are likely to exhibit high temporal variability.

Atmospheric levels of bioaerosols can be assessed through measurements of specific chemical and biological tracers (Chapter 1, section 1.3). Glucose, fructose and sucrose are main energy storage material in plants, major contributors to pollen mass[47, 183] and have been used as pollen tracers in China and the United States.[43, 47, 74] Although not unique to pollens, these three sugars also comprise a minor fraction of suspended soil,[184] road dust[185] and biomass burning.[186] Measurement of these bioaerosol tracers allows for the evaluation of the atmospheric concentrations and size distributions of pollens, fungal spores, and Gram-negative bacteria.

Given the important role of bioaerosols in the health of sensitive populations and in atmospheric processes, a robust understanding of bioaerosol types and their response

to changing meteorological conditions is needed. Our central objectives were *i*) to assess temporal variations in pollens, fungal spores and endotoxins in the Midwestern United States during the spring and late summer, *ii*) determine the distribution of these bioaerosol types in fine and coarse, *iii*) chemically profile regionally-important pollen types (red oak, pin oak, cotton ragweed, giant ragweed and corn) for use in source apportionment, and *iv*) estimate pollen and fungal spore contributions to PM mass by way of chemical mass balance (CMB) modelling. The outcomes of this study include an improved understanding of changes in ambient concentrations and PM size distributions of bioaerosols in response to rain events and their contributions to PM mass.

4.3 Methods

4.3.1 Sample collection

Daily (24 h) PM samples in spring and late summer were collected from 17 April – 9 May, and 15 August – 04 September, respectively in 2013, from the University of Iowa air monitoring site in Iowa City, Iowa, US (+41.6647, – 91.5845). $PM_{2.5}$ and $PM_{10-2.5}$ were collected on 37-mm Teflon filters (Pall Corp.) using an Andersen dichotomous sampler (Series 241) that described section 2.1.3. An additional set of $PM_{2.5}$ samples were collected on to 90-mm quartz fiber filters (Pall Life Sciences) using a medium-volume sampler (URG Corp.) that described in section 2.1.2. The 24-h filter samples were changed at 08:00 local time.

To assess the representativeness of 2013 PM levels to typical conditions in Iowa, $PM_{2.5}$ and PM_{10} mass measurements were compared to measurements from 2010 - 2015 downloaded from the Technology Transfer Network (TTN) Air Quality System (AQS) Data Mart.[187] In this work PM_{10} was calculated by adding $PM_{2.5}$ and $PM_{10-2.5}$

measurements. The federal reference method (FRM) site for Johnson County, Iowa is located at Hoover Elementary School, (+41.6572, – 91.5035), 6.3 km east of the University of Iowa air monitoring site. $PM_{2.5}$ concentrations were compared to average levels over the sampling period calculated from hourly PM measurements while PM_{10} data were compared to filter measurements collected from midnight to midnight every three days.

4.3.2 PM mass measurements

PM mass was determined by the difference of pre- and post-sampling Teflon filter weights. Filter measurements made in a temperature (21.9 °C) and humidity controlled (25±5%) room using an analytical microbalance (Mettler Toledo XP26) after conditioning 48 hours. Standard deviations of triplicate measurements were used as the error associated with the mass measurement.

4.3.3 Analysis of carbohydrates and inorganic ions

Half of the Teflon filters that collected $PM_{10\text{-}2.5}$ was extracted for carbohydrates and ions after pre-wetting with 100 μL of acetone (Sigma Aldrich) following the extraction methodology described in section 2.2. Quartz filters that collected $PM_{2.5}$ extracted in the same manner without pre-wetting (section 2.2). Carbohydrate concentrations were determined by HPAEC-PAD described in section 2.3.2. Inorganic ion concentrations were determined using ion exchange chromatography with suppressed conductivity detection (ICS-5000, described in section 2.6) following Jayarathne et al.[108]

4.3.4 Analysis of biomarkers

The remaining half of the Teflon filter containing coarse PM and entire Teflon filters containing fine PM were extracted via shaking into 2 mL of sterile pyrogen-free water following the method described in section 2.10.1. Water extracts were then assessed for fungal glucans and bacterial endotoxins as detailed in section 2.10.2.

4.3.5 Collection and analysis of pollens

Oak pollens were harvested from pin and red oak trees in park areas surrounding Iowa City during the spring of 2013 into pre-cleaned aluminum lined bags. Cotton and giant ragweed pollens were collected in late-summer of 2015 from bushes near roadways in residential areas of Iowa City. Cotton ragweed and corn pollens were purchased (Polysciences Inc., Warrington, PA). Pollen images were taken using a Zeiss LSM 710 fluorescence microscope (Carl Zeiss Microscopy GmbH, 07745 Jena, Germany) following Pöhlker et al.[188] and IX-81 inverted microscope (Olympus Corporation, Tokyo, Japan). Prior to extraction and chemical analysis, pollens were desiccated overnight and weighed (Mettler Toledo XS204 and XP26 balances). Pollens (~0.005–0.015 g) were extracted similar methodology as for quartz filters.

4.3.6 Chemical mass balance (CMB) modelling

PM mass was apportioned to fungal spores and pollens using EPA-CMB model (version 8.2). PM mass during the springtime was apportioned in $PM_{2.5}$ and PM_{10} (combination of $PM_{2.5}$ and $PM_{10\text{-}2.5}$) using sucrose, glucose, fructose, and mannitol as fitting species. Input source profiles included one pollen profile selected from red oak, pin oak (this study), white birch, Chinese willow, or Peking willow[47] and one fungal

spore profile.[44] Sensitivity tests were conducted to assess the fit of different pollen profiles to ambient measurements, focusing on sampling days from 26 April – 9 May when pollen tracer levels were highest.

4.3.7 Statistical analysis

Species concentration measurements were not normally distributed, thus Spearman's rank order correlation was employed for non-parametric comparisons (r_s) in SPSS (Statistical Package for the Social Sciences–21). PM measurements were normally distributed thus t-tests comparing PM means from dry and rainy periods was conducted in Minitab (version 16). Significance was assessed at the 95% confidence interval ($p \leq 0.05$).

4.4 Results and discussion

4.4.1 Characterization of pollens common to the Midwestern US

Pollens common in the Midwestern United States including red oak, pin oak, corn, cotton ragweed and giant ragweed were chemically and physically characterized. Microscopy revealed average pollen diameters of approximately 30 μm for red oak, 25 μm for pin oak, 90 μm for corn, and 20 – 30 μm for cotton and giant ragweed (Table 4.1; Figure 4.1a-f). Glucose, fructose and sucrose accounted for an average of 5–14 % of pollen mass. Trace amounts of erythritol, arabinose, mannitol and rhamnose were quantified, while arabitol, xylitol, trehalose, fucose, mannose, xylose and ribose were below detection limits. Inorganic ions contributed 1.5% of oak pollen mass, 2.8% of corn and 1.1% of cotton ragweed (Polysciences). Potassium was consistently the most abundant cation and phosphate the most abundant anion, with smaller mass contributions

for ammonium, calcium, and chloride. Due to the relatively high abundance of glucose, fructose, and sucrose in pollens in the present and in prior studies[47, 183] these carbohydrates are the best candidates for assessing pollen contributions to ambient PM.

Among the similarities in major pollen constituents, carbohydrate measurements showed significant differences in their relative abundances. Sucrose was the most abundant carbohydrate in red oak, pin oak, and Polysciences cotton ragweed, while sucrose to fructose ratios varied (2.1, 1.6, and 4.4, respectively) and glucose to fructose ratios were nearly equivalent (1.2). Glucose was the most prominent carbohydrate in locally-collected cotton and giant ragweed pollens, while sucrose to fructose ratios were around 1.2 and glucose to fructose ratios were around 1.7. Meanwhile, fructose was the most abundant carbohydrate in corn pollens, with sucrose to fructose ratio of 0.5 and glucose to fructose ratio of 0.6. Differences in sucrose to fructose ratios across different pollen types may serve as a diagnostic ratio for identifying pollen types, for cases when a single pollen type dominates the ambient signal. Notably, the carbohydrate distributions in corn pollens differ from those previously reported,[183] with differences likely resulting from genetics[183] and environmental factors (e.g. temperature, availability of water, and CO_2 levels) that are known to affect the synthesis and storage of carbohydrates.[189-191] Further studies are needed to evaluate how the chemical composition of pollens varies across different species, during maturation, and in response to environmental factors.

Table 4.1: Pollen diameter and mass fractions of carbohydrates and ions with standard errors.

	Red Oak	Pin Oak	Corn	Cotton ragweed[a]	Cotton ragweed[b]	Giant ragweed[b]
n	5	5	5	5	3	3
Diameter (μm)[c]	30	30	80	20	35	35
Carbohydrates (μg mg^{-1})						
Glucose	41.1 ± 4.1	40.2 ± 3.5	15.2 ± 0.9	15.9 ± 1.6	43.3 ± 2.0	39.2 ± 2.8
Fructose	33.0 ± 1.8	33.9 ± 2.9	25.0 ± 1.1	13.5 ± 0.6	24.4 ± 1.1	22.9 ± 1.5
Sucrose	68.3 ± 4.5	55.2 ± 3.2	13.4 ± 1.7	59.4 ± 3.3	28.0 ± 1.4	27.9 ± 1.3
Erythritol	8.1 ± 3.1	8.7 ± 3.4	28.7 ± 3.2	NQ[d]	NQ[d]	NQ[d]
Mannitol	0.1 ± 0.01	0.2 ± 0.01	<0.001	<0.001	0.2 ± 0.01	0.8 ± 0.1
Rhamnose	0.1 ± 0.01	0.1 ± 0.01	<0.001	<0.001	<0.001	<0.001
Arabinose	0.3 ± 0.03	0.5 ± 0.1	0.9 ± 0.2	0.3 ± 0.03	1.2 ± 0.1	2.3 ± 0.2
Inorganic ions (μg mg^{-1})						
Sodium	0.25 ± 0.20	0.23 ± 0.01	0.30 ± 0.10	0.03 ± 0.002		
Ammonium	1.36 ± 0.11	1.11 ± 0.90	0.89 ± 0.16	1.33 ± 0.13		
Potassium	7.56 ± 0.81	6.43 ± 0.51	11.97 ± 0.16	5.22 ± 0.48	NA[e]	
Magnesium	0.03 ± 0.00	0.05 ± 0.01	0.88 ± 0.01	0.74 ± 0.06		
Calcium	0.07 ± 0.02	0.12 ± 0.01	0.37 ± 0.03	1.85 ± 0.12		
Chloride	0.40 ± 0.08	0.42 ± 0.05	2.10 ± 0.11	1.64 ± 0.18		
Nitrate	0.19 ± 0.04	0.31 ± 0.11	<0.019	<0.019		
Phosphate	3.94 ± 0.39	1.65 ± 0.41	10.5 ± 0.88	8.99 ± 0.87		
Sulfate	0.79 ± 0.29	0.46 ± 0.02	0.94 ± 0.12	0.25 ± 0.03		

[a] Purchased from Ploysciences

[b] Collected locally from Iowa City during late summer 2015

[c] Approximate diameters

[d] Not quantified (NQ) due to chromatographic interferences

[e] Not analyzed (NA)

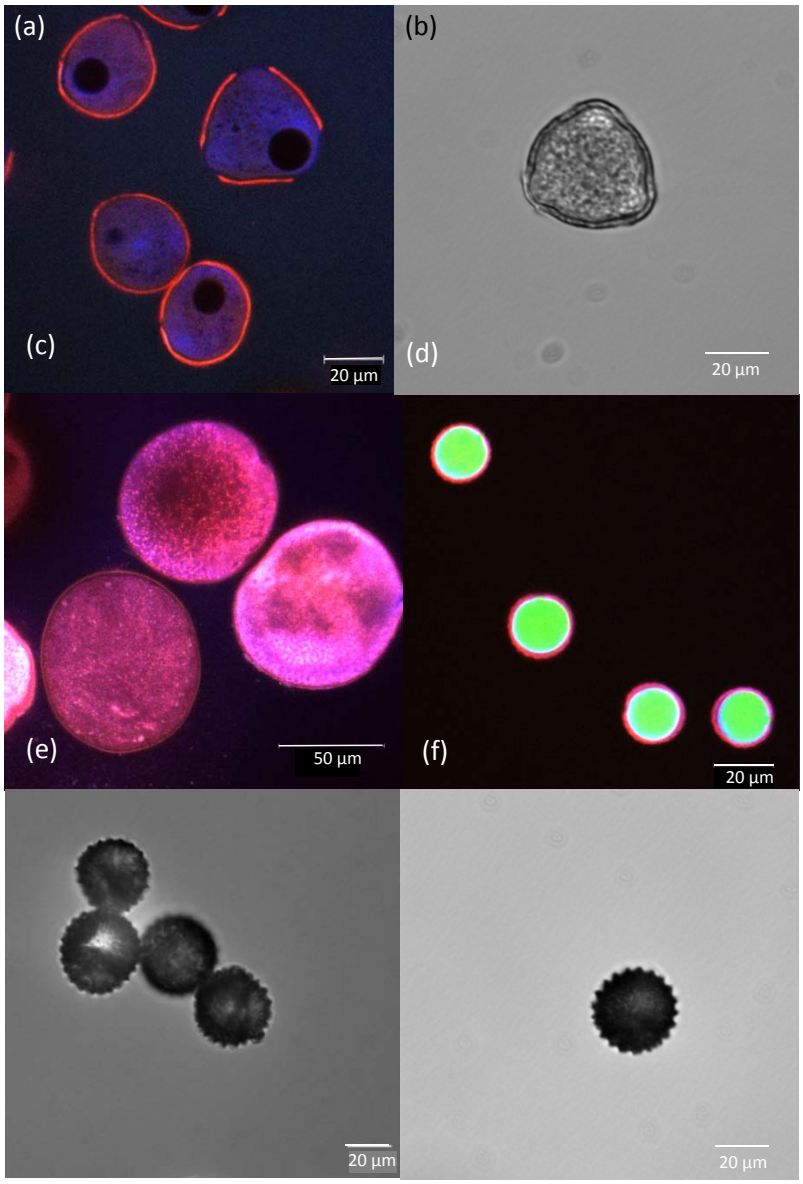

Figure 4.1: Microscope images of pollens from (a) red oak, (b) pin oak, (c) corn, (d) Polysciences cotton ragweed, locally collected (d) cotton ragweed and (e) giant ragweed. Images in color were captured by fluorescence microscope, while the others were captured by an inverted microscope.

4.4.2 Fine and coarse PM concentrations

4.4.2.1 Spring

From 17 April to 9 May, 2013, daily PM_{10} levels in Iowa City ranged from 2 – 32 μg m^{-3}, and fine PM ranged from 2 – 13 μg m^{-3}. PM levels did not exceed the 24 h average Environmental Protection Agency National Ambient Air Quality Standards (EPA NAAQS) of 150 μg m^{-3} and 35 μg m^{-3} for PM_{10} and $PM_{2.5}$, respectively.[192] Mean (± one standard deviation) PM_{10} and fine PM levels were 15 ± 8.9 μg m^{-3} and 7.1 ± 3.0 μg m^{-3}, respectively. On average fine PM accounted for 57 ± 17 % of PM_{10}.

For 15 of the 23 spring sampling days, conditions were dry and no rain occurred (Figure 4.2a). On the remaining 8 days, daily rainfall totaled 0.3 – 85 mm. Ambient PM levels were sensitive to rain, with rainfall corresponding to low PM_{10} levels (Figure 4.2b). On rainy days, fine PM levels averaged 4.7 ± 2.2 μg m^{-3}, compared to dry days of 8.3±2.6 μg m^{-3}. Meanwhile, on rainy days, coarse PM levels averaged 1.9 ± 1.5 μg m^{-3} relative to dry days of 10 ± 5.6 μg m^{-3}. The observed reduction in PM during rainy days was statistically significant ($p < 0.01$), and was driven by wet deposition of PM in both size mode. Furthermore, the distribution of particles between the fine and coarse modes was affected by rain; on dry days, 48 ± 11 % of PM_{10} was less than 2.5 μm compared to rainy days when 80 ± 13 % of PM_{10} was less than 2.5 μm. The shift in the PM size distribution of PM reflects that rain was more effective at scavenging and/or suppressing the release of coarse particles compared to fine particles.

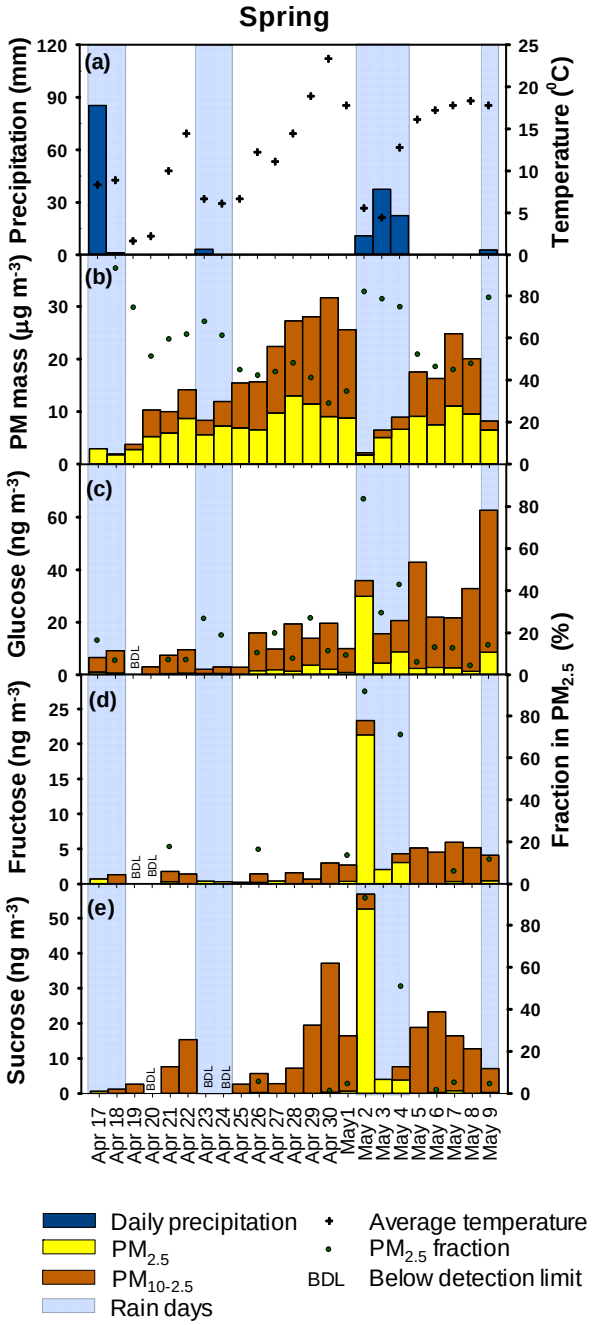

Figure 4.2: Temporal variation in precipitation and average temperature (a) in Iowa City, IA in the spring of 2013. Ambient concentrations of PM mass (b), glucose (c), fructose (d) and sucrose (e) in coarse and fine size fractions. The percent of PM and bioaerosol tracer mass in fine particles is shown on the right-axis for samples in which the analyte was detected in both size modes. During rain on 2 May, PM is suppressed while pollen tracers in the fine mode substantially increased.

The observed 2013 springtime PM levels were in good agreement with PM measurements at a nearby FRM site located 6.3 km to the east (Table 4.2). The mean PM_{10} levels in spring 2013, however, were slightly lower when compared to the average FRM level of 19 ± 9.0 μg m^{-3}, because the FRM measured PM in 2013 at a rate of 1-in-3 days and only captured one rain event, compared to our continuous daily measurements that captured seven rain events. The mean springtime $PM_{2.5}$ and PM_{10} levels were in good agreement with PM concentrations from 2010 - 2015 when mean concentration varied form 5.6 - 8.3 μg m^{-3} and 14 - 22 μg m^{-3} (Table 4.2), respectively. Overall, these comparisons indicate that the spring of 2013 represented typical springtime PM levels in Iowa City.

4.4.2.2 Late summer

From 15 August to 4 September, 2013, Iowa City daily PM_{10} levels ranged from 21 – 50 μg m^{-3} and fine PM levels ranged from 3 – 17 μg m^{-3} These levels were well below the 24 h average EPA NAAQS.[192] During late summer, PM_{10} and fine PM mass concentrations averaged 33 ± 8 μg m^{-3} and 12 ± 4 μg m^{-3}, respectively. On average, fine PM accounted for 39 ± 12 % of PM_{10}. The measured $PM_{2.5}$ and PM_{10} levels in 2013 agreed well with levels obtained at the nearby FRM site (Table 4.3).

During the late-summer period, meteorological conditions were much drier, with only one recorded rain event: a thunderstorm on August 22 providing 1.0 mm of rain between 10 – 11 am (Figure 4.3a). The conditions at Iowa City in late summer of 2013 were dryer compared to adjacent years (2010 – 2015), which had at least 4 rain events and cumulative rainfall ranging from 36–115 mm. PM levels were higher in Iowa City in

Table 4.2: Temperature, precipitation, PM concentrations and their size distribution in Iowa City from 17 April – 9 May, from 2010 to 2015.

				$PM_{2.5}$ (µg m^{-3})			**PM_{10} (µg m^{-3})**		
Year	Avg. temp. (˚C) ± Std. Dev.	Cumulative precipitation (mm)	Avg. ± Std. Dev.	Range	Avg. ± Std. Dev.	Range	Reference
2013	NA		7.1 ± 3.0	1.8 - 13	15 ± 8.9	2.0 - 32	This study
2010	13 ± 3.4	93	8.2 ± 6.2	3.2 - 25	20 ± 12	7.0 - 38	EPA AQS*
2011	10 ± 4.8	41	5.6 ± 2.3	2.5 - 11	14 ± 9.0	4.0 - 30	EPA AQS*
2012	14 ± 5.2	111	8.3 ± 5.1	3.4 - 26	21 ± 9.3	10 - 37	EPA AQS*
2013	12 ± 6.0	224	6.3 ± 2.6	1.2 - 10	19 ± 9.0	11 - 35	EPA AQS*
2014	13 ± 4.2	93	7.5 ± 4.3	1.1 - 15	18 ± 7.8	7.0 - 30	EPA AQS*
2015	14 ± 5.1	124	8.3 ± 4.6	2.3 - 18	22 ± 12	8.0 - 47	EPA AQS*

* $PM_{2.5}$ averages were calculated using daily concentrations, and PM_{10} averages were calculated using daily concentrations of 1-in-3 day sampling.
NA - not applicable

Table 4.3: Temperature, precipitation, PM concentrations and their size distribution in Iowa City from 15 August – 4 September, from 2010 to 2015.

				$PM_{2.5}$ ($\mu g\ m^{-3}$)			**PM_{10} ($\mu g\ m^{-3}$)**			
Year	Avg. temp. (˚C) ± Std. Dev.	Cumulative precipitation (mm)	Avg. ± Std. Dev.	Range	Avg. ± Std. Dev.	Range	Reference
2013	NA		12 ± 3.6	3.4 - 17	33 ± 8.3	21 - 49	This study
2010	23 ± 3.0	57	9.9 ± 4.2	4.3 - 19	27 ± 13	16 - 52	EPA AQS*
2011	23 ± 2.9	51	9.9 ± 4.7	4.9 - 27	26 ± 4.6	21 - 33	EPA AQS*
2012	23 ± 3.0	36	9.3 ± 3.3	4.0 - 17	28 ± 9.6	15 - 39	EPA AQS*
2013	24 ± 3.8	1.0	12 ± 2.9	7.5 - 17	31 ± 5.7	25 - 39	EPA AQS*
2014	23 ± 2.2	115	9.8 ± 4.0	4.6 - 18	23 ± 11	10 - 40	EPA AQS*
2015	21 ± 3.5	54	9.4 ± 4.9	2.2 - 19	24 ± 9.2	8 - 36	EPA AQS*

* $PM_{2.5}$ averages were calculated using daily concentrations, and PM_{10} averages were calculated using daily concentrations of 1-in-3 day sampling.
NA - not applicable

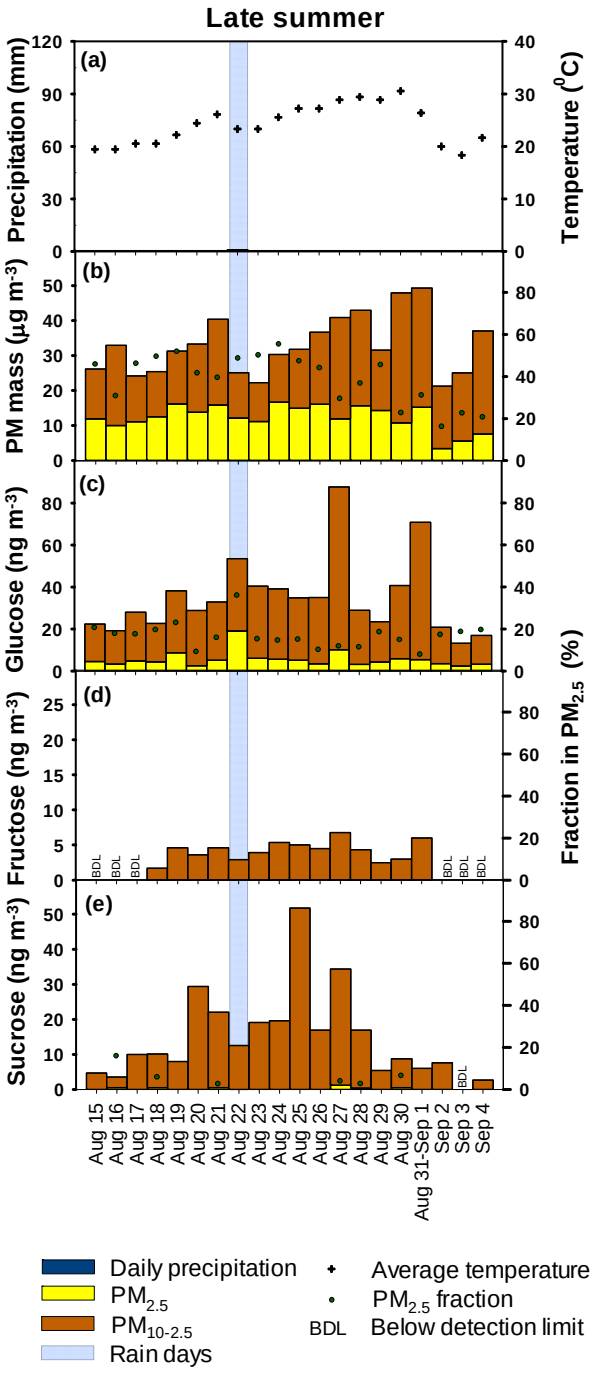

Figure 4.3: Temporal variation in precipitation and average temperature (a) in Iowa City, IA in the late summer of 2013. Ambient concentrations of PM mass (b), glucose (c), fructose (d) and sucrose (e) in coarse and fine size fractions. The percent of PM and bioaerosol tracer mass in fine particles is shown on the right-axis for samples in which the analyte was detected in both size modes. Glucose, a pollen tracer increased in fine particles during the 22 August rain event.

2013 (Figure 4.3b and Table 4.3) compared to adjacent years from 2010 – 2015 (Table 4.3), which may attributed to the dry weather conditions that reduced soil moisture and increased soil resuspension, and the lack of wet deposition.

4.4.3 Pollen tracers

4.4.3.1 Spring

The temporal variations of pollens were assessed utilizing the combination of glucose, fructose and sucrose as chemical tracers. From 17 – 25 April, lower temperatures (averaging 7 ˚C) and rainy conditions prevailed coinciding with relatively low levels of glucose, fructose and sucrose (Figure 4.2c-e). Warmer temperatures (averaging 15 ˚C) set on from 26 April marking the beginning of spring, the emergence of blooming plants, and leading to increased glucose, fructose and sucrose levels. During the springtime period of 26 April – 9 May, glucose, fructose and sucrose concentrations in fine PM averaged 5.2 ± 7.5 ng m^{-3}, 2.1 ± 5.6 ng m^{-3} and 4.6 ± 14 ng m^{-3} and in coarse PM they averaged 19 ± 13 ng m^{-3}, 2.7 ± 1.7 ng m^{-3} and 12 ± 9.6 ng m^{-3}, respectively. The correlations of daily temperature with coarse mode glucose and sucrose were statistically significant ($r_s \geq 0.8$, $p < 0.001$), reflecting that warmer temperatures promote the development, maturation, and release of pollens.

Atmospheric levels of pollen tracers in springtime were affected by temperature and precipitation. Maximum levels of fructose and sucrose were observed on 2 May and glucose peaked on 9 May (Figure 4.2c-e and Table 4.2); rain occurred on both of these days, following a dry period with relatively high

temperatures. It is expected that on these days, rain contributed to the passive release of pollen that had matured during periods of warmer temperatures.[170, 193] Early-spring rain events during cooler conditions with low atmospheric levels of pollen tracers (e.g., 17 – 18 and 23 – 24 April), did not trigger release of pollens, possibly because pollens had not sufficiently developed, as indicated by low levels of glucose, fructose, and sucrose on preceding days.

Notably, rain altered the size distribution of pollen tracers. On a typical dry day, more than 80% of pollen tracers were present in coarse PM, which is expected for pollen particles that have geometric diameters in the range of 5-100 μm[16] that are excluded from the fine PM fraction. However, when pollen markers peaked on 2 May, mass fractions of glucose, fructose and sucrose in the fine mode reached 83%, 91% and 93%, respectively (Figure 4.2c–e, right axis). With continued rainfall on 3 – 4 May, pollen markers remained elevated in the fine mode relative to coarse PM. Fructose and sucrose were not detected in coarse particles on 3 May, while more than 70% of fructose and 50% of sucrose were observed in the fine mode on 4 May. After the rain stopped, coarse mode pollens increased in concentration and resumed the typical size distribution by 5 May. On 9 May, a light rain occurred, causing glucose in fine and coarse PM to increase with 14% of these tracers in the fine mode. These size-resolved measurements indicate the passive release of pollen fragments less than 2.5 μm are released during some rain events (2–4 May) and the passive release of larger pollen particles ranging 2.5 – 10 μm during others (9 May). Notably, this is the first observation of the release of fine particle pollen fragments to the atmosphere

using chemical tracers. Most field measurements include analysis of either $PM_{2.5}$ or PM_{10}, but not both size modes that are required to capture this phenomenon.

The likely explanation for the increase in airborne pollens and simultaneous decrease in their size on May 2 is the rupturing of pollen walls as a result of the osmotic pressure that builds up inside the pollen due to absorbed moisture during rain.[194, 195] Osmotic shock has been previously demonstrated to cause rupturing of grass and birch pollens, releasing cytoplasmic constituents to their surroundings.[194-196] The daily averaged wind speeds varied from 1 - 19 mph, were not associated with fine pollen tracer levels (for glucose, $r_s = 0.2$, and $p = 0.5$) and suggested that daily average wind speed is not a good predictor fine pollen levels during spring of Iowa. However, gusty winds can loft pollen fragments[197] and may explain some of the observed ambient measurements. Strong gusty winds on 2 May (17 – 31 mph) are likely to have contributed to the elevated fine pollen levels. Other rain days, either had lower gust speeds (16 - 22 mph) as is the case for 3 may, or no gusts, as the case for 4 and 9 May. Thus, on rain days when fine pollen fragments form, gusty winds seem to be responsible for dispersal of the fine pollens to the atmosphere.

Differences in the size distributions of pollen tracers during the rain events on 2 May and 9 May are expected to result from different pollen types predominating. Pollen characterization in prior studies,[47, 183] described in section 4.4.1 shows that different plant species gave rise to pollens with different carbohydrate profiles. On 2 May, the relative ratios of glucose and sucrose (normalized to fructose) in fine PM were 1.4 and 2.5, respectively, consistent with

pin oak and red oak pollens. Meanwhile, on 9 May, these ratios in coarse PM were 3.6 and 1.4, respectively, which does not match any of the available pollen profiles. Consequently, it appears that some pollen types undergo osmotic rupturing, while others do not. Further studies are needed to identify the types of pollens that rupture and conditions required for this to occur.

The levels of pollen tracers observed in Iowa City were generally within an order of magnitude of measurements made previously at various locations worldwide, with notable geographic differences. The springtime average glucose concentration in the fine mode was twice as levels in Arizona, US,[43] but 1.3 - 3 folds less than Texas, US[74] and Po Valley, Italy.[198] Meanwhile, sucrose levels measured in fine PM of this work was ~2 times lower than Arizona and Dallas, Texas.[43, 74] The mean glucose level in PM_{10} in this study was 1.3 times higher than the glucose level of Jeju Island (in total suspended PM), however, fructose and sucrose levels were 2.3 and 15 times lower than levels of Jeju Island,[47] respectively. Other than the differences of ambient concentrations of pollen tracers, PM_{10} glucose:fructose:sucrose ratio of 5:1:3 in this study was different from Jeju Island (2:1:22),[47] and suggest differences of pollen types in the atmosphere (section 4.4.1). Therefore observed variations in ambient concentrations mainly can be attributed to the differences pollen types that dominate in different locations.

4.4.3.2 Late summer

From mid-August to early-September, glucose concentrations in fine PM averaged 5.6 ± 3.7 ng m^{-3}, and fructose and sucrose were scarcely detected

(Figure 4.3c-e). Glucose, fructose and sucrose in coarse PM averaged (± one standard deviation) 30 ± 16 ng m^{-3}, 3.2 ± 2.0 ng m^{-3} and 14 ± 12 ng m^{-3}, respectively. In late summer 83% of glucose mass concentration was found in coarse mode, (Figure 4.3c) consistent with typical intact pollens size.[16] The average temperature was moderately correlated with coarse mode glucose, fructose and sucrose ($r_s > 0.5$, $p < 0.02$), indicating that pollen levels were higher when temperatures were elevated.

Only one rain event occurred in late summer of 2013 on 22 August (section 4.4.2.2), when fine mode contributed to 34% of the measured glucose concentration compared to dry days that averaged 16% (Figure 4.3c). On the rain day, fine mode glucose concentration reached 19 ± 1 ng m^{-3}, which was 4 folds higher than fine mode glucose concentration of an average dry day (5 ± 2 ng m^{-3}), while fructose and sucrose were below detection limits. The increase in glucose concentrations and the increase in the fine fraction are consistent with the passive release of pollen fragments during rain events as observed in spring as described in section 4.4.3.1. However, with only one rain event occurring in the three-week late summer study, additional studies are needed to understand 1) how pollen tracer concentrations, and their size distributions in fine and coarse PM vary with late summer rain events, and 2) what types of pollens could be higher during late summer rain events.

The observed late summer glucose concentration in the fine mode agreed well with prior studies in the region. In particular, the average fine glucose concentration was 3 times higher compared to summer glucose levels of

Minnesota and Ohio, however, 1.5 times less than levels from Indiana.[42] When comparing observed PM_{10} pollen tracer ratios during late summer with prior work they varied widely. For instance, average glucose:fructose:sucrose concentration ratio in PM_{10} during late summer (10:1:4.2) was different from summertime ratios from Jeju Island (3:1:0.3),[47] and Norway (2:1:10).[134] These variations can be attributed to differences in pollen types predominating in the atmosphere.

4.4.3.3 Significance of the release of fine particle pollens during rain

Springtime measurements and to a lesser extent late summer measurements reveal that rain releases high amounts of pollen fragments less than 2.5 μm that has significant health implications and climatic effects.

1. Pollen allergens have been shown to be localized in the cytoplasm,[196, 199] such that the bursting of pollens results in a more direct exposure to aeroallergens.
2. In the form of smaller particles, aeroallergens penetrate deeper into the respiratory tract where they may trigger more severe allergenic responses.[195, 200]
3. Smaller particles have longer atmospheric lifetimes and are transported longer distances before deposition, and thus may affect populations downwind of the release of fine pollen fragments.
4. Intact pollens as well as pollen fragments are CCN [33, 36] and IN [37, 38, 201] active. Rupturing of a one pollen granule produces hundreds of fine-sized pollen particles,[196, 175, 174] significantly increasing the number of CCN and IN active particles in the atmosphere.

5. These released pollen fragments contain cytoplasmic macromolecules that are readily exposed to the outer environment making them more hygroscopic and efficient CCN active particles.[36]

Acute asthma epidemics associated with rain events, so-called "thunderstorm asthma," have been documented in Australia, Europe, Mexico and the US.[174, 202, 203] These asthma epidemics often reports during pollen seasons[123, 174] and shown to associate with ambient pollen counts.[181] In addition, thunderstorm asthma linked with ambient ozone concentrations,[204] that increase during lightning.[205] However, an epidemiological study in the US showed that thunderstorms alone (without rain) not cause asthma epidemics, suggesting that asthma exacerbations occur via a process that triggered by rainfall, supporting that osmotically ruptured pollen granules may be the cause of thunderstorm asthma epidemics.

Pollen forecasting models currently do not include mechanisms for the release of pollen in response to rain and instead assume that rain serves only as a sink of pollens, by means of droplet scavenging and wet deposition.[206] This erroneous assumption leads to predictions of low atmospheric pollen levels on days with rain, when exposure levels are shown here to be greatest and in smaller sized particle that lead to deeper lung deposition and more direct exposure to allergenic proteins. Predictions for this early-warning system must go beyond simply the co-occurrence of elevated pollen levels and thunderstorms, which are suggested to cause too many false alarms.[207] For accurate model parameterizations, a mechanistic and species-level understanding of this

phenomenon is needed and should include definitions of the pollen types, seasonality, and meteorological conditions that promote the release of pollen particles less than 2.5 μm to the atmosphere. In the meantime, persons suffering from pollen allergies should follow the recommendations of D'Amato et al. (2007),[174] "when asthmatic patients realize that a thunderstorm is approaching, the best thing for thing for them to do is to stay indoors, with windows closed."

4.4.4 Fungal bioaerosol tracers

4.4.4.1 Spring

Two markers of fungi were used to examine the size distribution and temporal variation of fungal spores: fungal sugar mannitol and the fungal cell wall component glucan. From 17 – 21 April, cooler temperatures prevailed (average temperature = 6 ˚C) and PM_{10} mannitol and glucan concentrations were at or below 8.8 ng m^{-3} and 14 ng m^{-3}, respectively. On 22 April an exceptionally high PM_{10} glucan level of 61 ng m^{-3} (Figure 3b) occurred when temperature elevated to 14 ˚C (Figure 4.2a), a warmer day in early-spring. From 23–25 April, temperature dropped to less than 7 ˚C, and mannitol and glucan remained less than 4.5 ng m^{-3} and 7.0 ng m^{-3}, respectively, in PM_{10}. From 26 April conditions became warmer (average temperature = 15 ˚C), and fungal spore tracer levels elevated in PM_{10} (Figure 4.4a and b) with average mannitol and glucan concentrations of 36 ± 27 ng m^{-3} and 23 ± 27 ng m^{-3}, respectively. During springtime, coarse mode mannitol and glucans demonstrated moderate positive correlation (r_s = 0.5, $p < 0.02$) signifying that glucans and mannitol originated from the same source. Furthermore both mannitol and glucans in coarse mode significantly correlated

with daily average temperature ($r_s > 0.4$, $p < 0.05$), consistent with warmer temperatures favoring fungal growth.

Springtime rain events significantly influenced ambient concentrations of fungal spore tracers (Figure 4.4a and b). The first peak in springtime mannitol concentrations occurred from 27–30 April (post-rain dry period) with an average mannitol level of 21 ± 5 ng m^{-3} in PM_{10}. Rainfall on 2 May caused a drop in PM_{10} mannitol concentration to 7 ng m^{-3}, reflecting fungal spore removal by wet deposition. For fungal glucans, there was

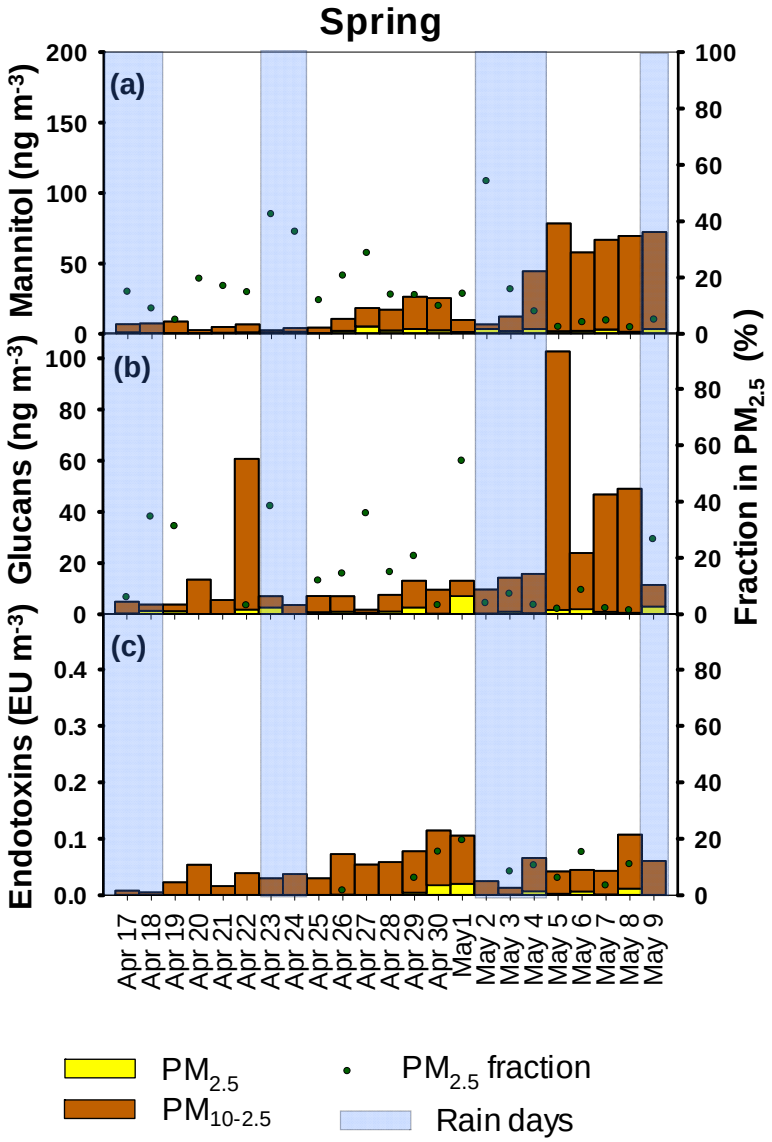

Figure 4.4: Ambient concentrations of mannitol (a), glucans (b), and endotoxins (c) in coarse and fine size fractions in Iowa City, IA during spring of 2013. The percent of PM and bioaerosol tracer mass in fine particles is shown on the right-axis for samples in which the analyte was detected in both size modes. Fungal spore tracers increased significantly after rain on 2-4 May.

no such pronounced decrease in ambient concentrations observed when transitioning from dry period to the rainy day; from 28 April–1 May PM_{10} glucans averaged 11±3 ng m^{-3} and on the rainy day of 2 May the glucan level was 9.7 ng m^{-3} (Figure 4.4b). On 3 and 4 May, PM_{10} mannitol and glucan concentrations increased up to 45 ng m^{-3} and 15 ng m^{-3}, respectively, in spite of continued rainfall, consistent with wet conditions following rain being favorable for fungal spore release.[208] The maximum PM_{10} mannitol (80 ng m^{-3}), and glucan (103 ng m^{-3}) levels occurred on 5 May immediately after three rainy days, which was attributed to freshly released fungal spores from wet surfaces. Overall, fungal spore tracers are elevated while atmospheric conditions are wet and peaked following rain events.

Fungal spores typically have diameters in the range of 1–30 μm.[18] During dry days, an average of 13% of PM_{10} fungal spore tracers were in the fine PM fraction (Figure 4.4a and b right axis). The fraction of mannitol in the fine mode reached local maxima on 23 April (42%), 24 April (36%), and 2 May (54%), with similar enhancement of glucans observed on 23 April (38%) (Figure 4.4a and b, right axis), all of which corresponded to rain days. The relative decrease in the size of fungal spores is attributed to a combination of 1) the release of fungal spores less than 2.5 μm via the passive discharge of fungal spores by rain splash and mechanical agitation of vegetative surfaces by rain drops [69, 80, 178], and 2) the removal of fungal spore particles in coarse PM by droplet scavenging. Compared to pollens (section 4.4.3.1), rain events impacted the size distribution of fungal spores to a much lesser extent.

The levels of mannitol observed in Iowa City from 17 April – 9 May, were consistent with prior springtime measurements elsewhere. In Iowa City, fine and coarse mode mannitol averaged 2.0 ± 1.3 ng m^{-3} and 23 ± 26 ng m^{-3}, while glucans averaged 1.4 ± 1.5 ng m^{-3} and 18 ± 24 ng m^{-3}, respectively. The observed PM_{10} mannitol concentration in this study was slightly higher (~1.3 times) than previously determined springtime levels in Iowa,[209] and Rome,[132] and ~1.5 folds higher than Israel.[73] The fine mode mannitol levels are similar to those observed in San Augustine, Texas[74] and were 2.8 folds less than San Pietro, Italy.[198] These slight differences of mannitol concentrations when comparing this work to previous studies could be attributed to variation of fungal spore levels in different locations.

Comparisons of glucan concentrations to previous work are limited, because outdoor springtime glucan measurements are scarce and differences among glucan analysis methods confound direct comparisons. Glucan measured in PM_{10} in this study was similar to previously observed spring concentrations in Iowa,[209] which both determined by enzyme immunoassay with a (1-3, 1-6)-β-D-glucan standard. However the average glucan in PM_{10} in this work was ~100 times greater than springtime glucan levels in North Carolina, US, which were assessed using a LAL assay against a 1-3-β-D-glucan standard.[210] Methodological differences may cause positive or negative biases in glucan levels, depending on their relative response to the selected standard, and are likely the main cause of the observed difference.

4.4.4.2 Late summer

Atmospheric concentrations of mannitol increased when temperature increased in late summer. From 15 – 20 August daily average temperatures were less than 24 ˚C and coincided with a relatively lower mean PM_{10} mannitol level of 49 ± 12 ng m^{-3}. An exceptionally high PM_{10} mannitol concentration occurred on 22 August (121 ng m^{-3}), the one rainy day captured in late summer (Figure 4.5a). From 24 August – 1 September temperatures ranged from 26 – 31 ˚C, and the mean mannitol level in PM_{10} was 89 ± 53 ng m^{-3}. Temperatures dropped below 22 ˚C from 2 – 4 September and mannitol concentrations also decreased to an average of 39 ± 17 ng m^{-3} in PM_{10}. The daily average temperature was moderately correlated with coarse mode mannitol (r_s = 0.5, p = 0.01), consistent with increased fungal growth with elevated temperatures.

Late summer rain influenced atmospheric mannitol concentrations. The local maxima (16 ng m^{-3}) of fine mode mannitol occurred on 22 August when rain fell. Similar enhancements of mannitol concentrations in particles less than 2.5 μm were observed during rain in springtime of this work (2 May, Figure 4.4a). Such enhancements are consistent with releasing fungal spores via rain splash and agitation of vegetative surfaces such as plant leaves by rain drops.[80] Coarse mode mannitol also increased on 22 August (105 ng m^{-3}) due to release of fungal spores upon post-rain wet conditions (section 4.4.4.1). The fine fraction accounted for an average of 9 ± 4 % of mannitol in late summer, and was not notably changed on the rainy day of 22 August (14%). The springtime rain caused the fraction of mannitol in fine PM to increase (section 4.4.4.1), and the likely explanation for

not observing the same during late summer is the short duration of rain on 22 August (1 h, section 4.4.2.2). Overall, mannitol, the chemical tracer of fungal spores increased when conditions are wet during seasons of spring and late summer.

The average mannitol level observed in late summer, was ~3 times higher compared to springtime, however was in good agreement with earlier observed summer levels. Similar enhancement of PM_{10} mannitol in summer relative to spring demonstrated previously in Iowa[209] and was attributed to the dependence of fungal growth on temperature. Fine mode average mannitol level of 5.8 ± 3.6 ng m^{-3} in this study was similar to East St. Louis, Illinois, and was slightly higher (by 1.6 – 3 folds) compared to Minnesota, Indiana and Ohio, US,[42] which all were measured in summer. Furthermore, the average coarse mode mannitol level of 59 ± 36 ng m^{-3} in this study was ~1.5 folds higher than summertime levels of Beijing, China,[75] and ~3.5 times higher than Norway[134] and Israel.[73] These differences are likely due to varying fungal spore levels and spore type across different locations.

Ambient glucan concentrations in late summer were neither associated with temperature, nor correlated with mannitol, which suggests an alternative source of glucans. From 15 August – 4 September glucans in fine mode averaged 4.3 ± 7.4 ng m^{-3}, while glucans in the coarse mode averaged 12 ± 12 ng m^{-3} (Figure 4.5b). The daily average temperature and coarse mode glucans were not correlated ($r_s = 0.01$, $p = 1$), but maximum fine mode glucan concentration (30 ng m^{-3}) occurred on 30 August, the day with highest temperature (31 ˚C) in late

summer. A poor correlation was observed among coarse mode glucans and mannitol (r_s = 0.2, p = 0.3). This lack of correlation suggested non-fungal sources of glucans in late summer. For instance, coarse mode glucans were correlated with pollen tracers, particularly glucose and sucrose (r_s= 0.4, p = 0.07, r_s = 0.5, p = 0.04, respectively), suggesting pollens as a source of glucans. In prior work ragweed pollens have been shown to contain glucans,[46] and ragweed is one of the most abundant pollen types in Iowa during the late summer. Moreover, coarse mode glucans correlated with bacterial endotoxins (r_s = 0.4, p = 0.1), indicating the possibility of bacteria to serve as a source of glucans. Some pathogenic bacteria such as *Agrobacterium spp.*, and *Rhizobium spp.* that grow on crops have shown to contain glucans in their structure.[138] Agricultural crops are abundant in Iowa during the growing season and the mechanical agitation of plant surfaces by wind can aerosolize surface bacteria. Although glucans appear to have been influenced by bacterial and pollen levels in addition to fungi, the assessment of their ambient concentrations remains important, because they are immunostimulants that negatively impact human health.[131, 211]

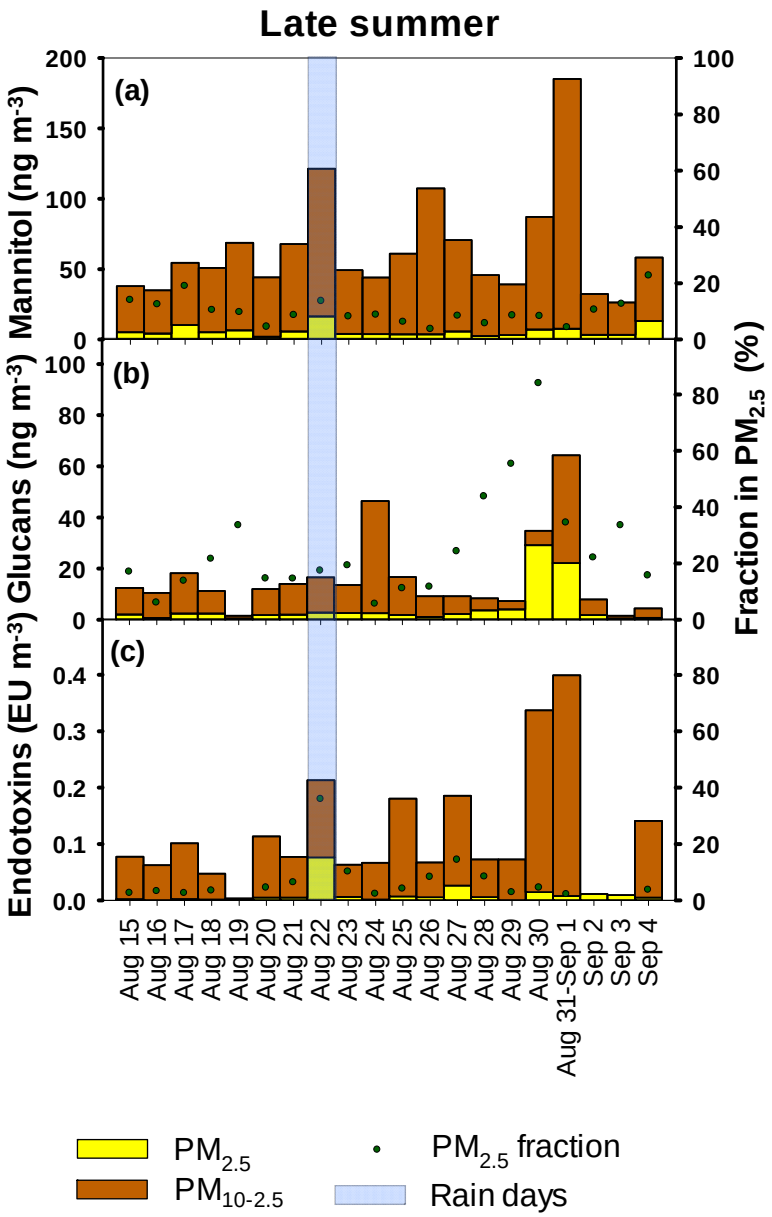

Figure 4.5: Ambient concentrations of mannitol (a), glucans (b), and endotoxins (c) in coarse and fine size fractions in Iowa City, IA during late summer of 2013. The percent of PM and bioaerosol tracer mass in fine particles is shown on the right-axis for samples in which the analyte was detected in both size modes. Mannitol, the chemical tracer for fungal spores, and endotoxins in fine mode increased on 22 August when it rained.

4.4.5 Bacterial endotoxins

4.4.5.1 Spring

Bacterial endotoxins were measured in endotoxin units (EU) against an *Escherichia coli* (055:B5) standard, and their ambient concentrations in springtime increased with temperature. From 17 - 25 April, atmospheric conditions were cold (temperature averaged 7 ˚C), and PM_{10} endotoxin levels were less than 0.056 EU m^{-3}. From 26 April to 1 May temperatures increased to the range of 11 - 23 ˚C, which coincided with increased PM_{10} endotoxin concentrations above 0.056 EU m^{-3}. On 2 and 3 May rain occurred and endotoxin concentrations in PM_{10} dropped to less than 0.025 EU m^{-3}. This suppression of bacterial endotoxins is likely an influence of rain which caused wet deposition and suppression of soil dust particles that bacteria are settled on. From 4 May temperatures were greater than 12 ˚C and endotoxin concentrations rose above 0.041 EU m^{-3} in PM_{10}. Coarse mode endotoxins were significantly correlated with daily average temperature ($r_s = 0.7$, $p < 0.001$), in agreement with prior ambient studies.[49, 212] The correlation of endotoxins with temperature indicated that warmer temperatures in spring promoted Gram-negative bacterial growth increasing ambient endotoxin concentrations.

In the spring, 92 ± 5 % of PM_{10} endotoxins were in the coarse mode (Figure 4c). The distribution of bacterial endotoxins towards larger particles has been observed previously, [52, 147] reflecting the association of bacteria plant parts, animal parts, soil, spores or pollen surfaces by agglomeration or rafting.[18] Coarse mode endotoxins demonstrated moderate positive correlation with calcium, the crustal element ($r_s = 0.7$, $p < 0.001$), which suggests soil resuspension as a source

of endotoxins in Iowa City, which has also been demonstrated previously in the Midwestern US.[55, 209]

In comparison to prior studies, the average PM_{10} endotoxin level in Iowa City (0.064 ± 0.030 EU m^{-3} for 26 April – 9 May) was relatively low. In particular, the average level was ~3 times lower than April 2012 levels across three urban and three background sites in Iowa.[209] The likely explanation for such a variation is the dependence of bacterial growth on ambient temperature; in fact the average temperature in spring of this work was 6 ˚C less compared to the prior work.[209] Likewise, the observed spring levels in this study were approximately one order of magnitude lower than levels measured in an agricultural town in Iowa[45] and urban and suburban locations in Boston,[145] also measured in spring. In addition to temperature differences, these temporal and spatial differences may be attributed to differing proximities to point sources of endotoxins such as agricultural activities[45, 213] and moving traffic,[58] which cause resuspension of soil that contain Gram-negative bacteria. The endotoxin in Iowa City during springtime was well below the health-based threshold value of 50 EU m^{-3} and temporary legal limit of 200 EU m^{-3} proposed by the National Health Council of the Netherlands.[25, 214]

4.4.5.2 Late summer

In late summer ambient endotoxin concentrations co-varied with temperature. From 15 - 19 August, temperature was less than 22 ˚C and PM_{10} endotoxins averaged 0.059 ± 0.036 EU m^{-3}. From 20 August – 1 September temperature was higher than 23 ˚C, and PM_{10} endotoxins averaged 0.154 ± 0.114

EU m^{-3}. Beginning from 2 September temperature decreased below 22 ˚C and endotoxins dropped to 0.056 ± 0.074 EU m^{-3} in PM_{10}. Daily average temperature showed positive moderate correlation with coarse mode endotoxins (r_s = 0.5, p = 0.02) similar to springtime (section 4.4.5.1).

During dry days endotoxins in fine mode averaged 0.007 ± 0.006 EU m^{-3}, and peaked on the one rainy day in late summer: 22 August (Figure 5.5c) when the fine mode endotoxin concentration reached a maximum of 0.076 EU m^{-3}. Endotoxin fraction in the fine mode also increased from an average of 5 % on dry days to 36% on 22 August. The maximum ambient concentration and decrease in particle size may be attributed to the dissemination of Gram-negative bacteria due to agitation of plant parts from rain drops,[18, 215] and/or aerosolization of Gram-negative bacteria settled on to fine sized fungal spores [18] by splashing rainwater. Because endotoxins induce inflammations in the respiratory tract, [24-26] further studies are necessary to understand changes in endotoxin concentrations and size distributions during rain.

Soil resuspension was suggested as an important source of bacterial endotoxins in spring (section 4.4.5.1), however coarse mode endotoxins were not correlated with calcium in late summer (r_s = 0.2, p = 0.33). Consequently, non-soil sources of bacteria were likely present, such as plant surfaces[54, 216] particularly crops,[217] which is highly relevant to the heavily agricultural state of Iowa. This link could be further explored by examining the co-occurrence of bacterial endotoxins with markers of plant waxes, but is beyond the scope of the present

study. Nonetheless, sources of bacterial endotoxins appear to differ across seasons in Iowa.

Late summer endotoxin levels are comparable with previous work and lower when compared to values measured in close proximity to point sources. The mean endotoxin concentration in PM_{10} during late summer (0.12 EU m^{-3}), comparable to previously observed summer concentrations in Iowa (19 EU m^{-3}),[209] ~1.6 times higher than springtime of this study (section 4.4.5.1), but ~10 folds higher than previously observed summer concentrations in an agricultural county in Iowa[45] attributed to the local agricultural activities.

4.4.6 Contributions of pollens and fungal spores to PM

CMB source apportionment modeling was applied to estimate mass contributions from pollens and fungal spores to PM_{10} and $PM_{2.5}$. This work extends the application of fungal spores tracer-to-mass ratios to estimate their contributions to PM mass[132, 218] to pollens for the first time. The CMB model requires representative source profiles for sources, which were drawn from the literature in the case of fungal spores[44] , birch and willow pollens[47], and from this study (section 4.4.1).

4.4.6.1 Source apportionment in spring

The pollen profiles that explained the greatest fraction of the variance in the ambient measurements (assessed by the CMB R^2 value) were pin oak and red oak (Figure 4.6). The resultant R^2 value further increased when fungal spores were added to the model (Figure 4.6). Birch and willow profiles, which showed an excess of sucrose,[47] explained a substantially lower fraction of the variance in

ambient data, where glucose and fructose concentrations outweighed sucrose. Hence, birch and willow pollen profiles were not considered further. Model results from using pin oak or red oak profiles in concert with the fungal spore profile produced consistent source contributions that were strongly correlated (Figure 4.7). Because red oak and pin oak fit ambient data to a comparable extent and both are sources of atmospheric pollens in Iowa, the best estimate of pollen contributions was calculated as the average contribution from red oak and pin oak.

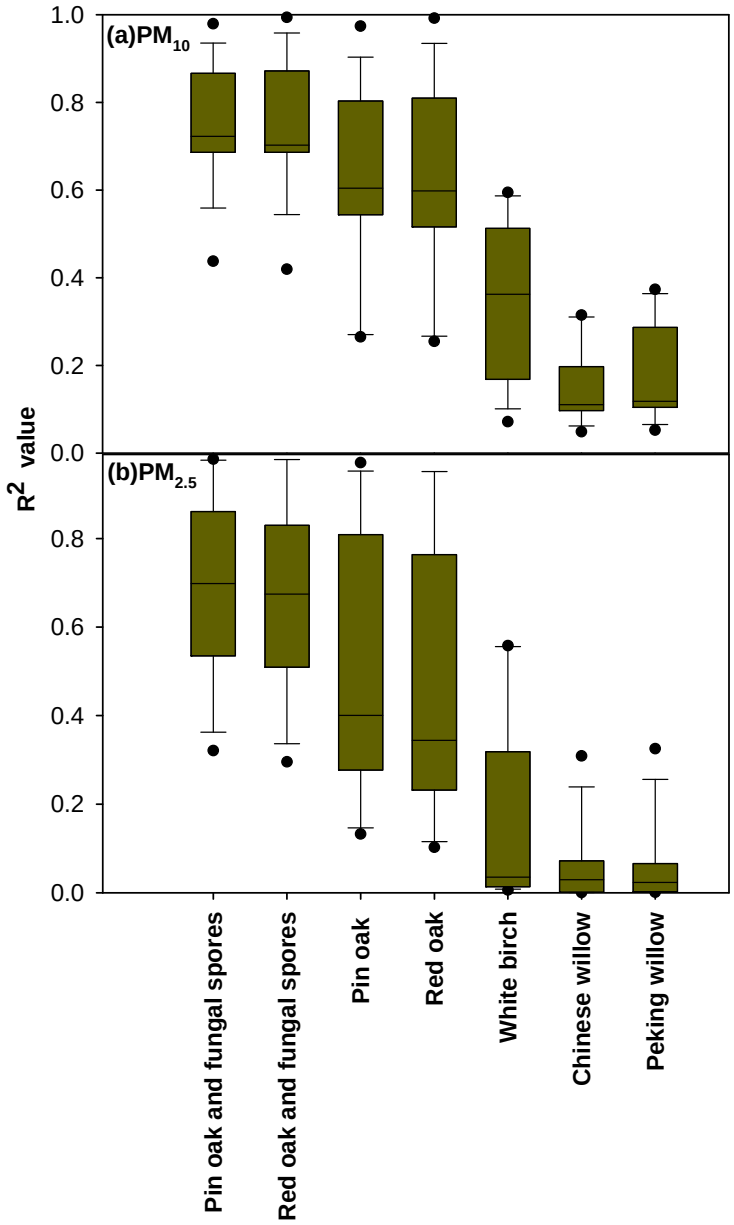

Figure 4.6: Comparison of CMB calculated R^2 values, which represent the fraction of the variance in the ambient measurements explained by the model, for PM_{10} (a) and $PM_{2.5}$ (b) for various pollen profiles. The comparison is limited to 26 April – 9 May, after the onset of the pollen season. Results indicated that the oak pollens fit the ambient data better than birch or willow, with the most variance explained by the combination of oak pollens and fungal spores.

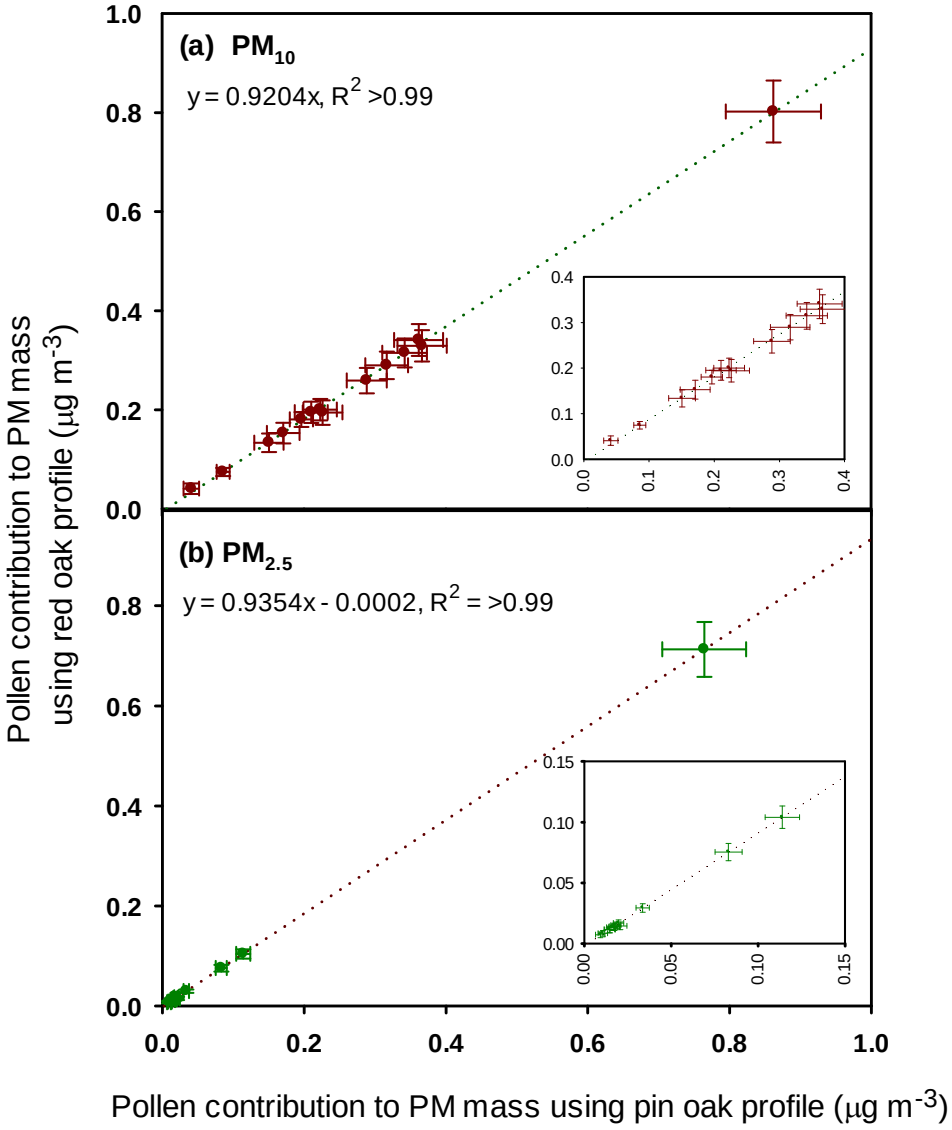

Figure 4.7: Comparison of pollen contributions to PM_{10} (a) and $PM_{2.5}$ (b) mass, estimated by the CMB model using pin oak and red oak pollen profiles. The comparison is limited to 26 April – 9 May, after the onset of the pollen season. The strong correlation (R^2 > 0.99) and numerical agreement (slopes close to 1) demonstrate the consistency in model results for when either profile is used. Consequently, source contributions from these two types were averaged to obtain the best estimate of pollen contributions to PM mass.

Pollen and fungal spore contributions to PM_{10} and $PM_{2.5}$ estimated by the CMB model are shown in Figure 4.8a and b, respectively. Overall, contributions to fine PM ranged from 0.01–0.7 μg m^{-3} for pollens and 0.03 – 0.1 μg m^{-3} for fungal spores, while contributions to PM_{10} were consistently higher at 0.04 – 0.8 μg m^{-3} for pollens and 0.13 –1.5 μg m^{-3} for fungal spores. Even though estimates of fungal spore contributions to PM mass have previously been estimated following Bauer et al. (2008),[44] these are the first estimates of pollen contributions to PM mass using CMB source apportionment modelling. These results demonstrate that pollens contribute significantly to PM in spring, particularly on days with rain.

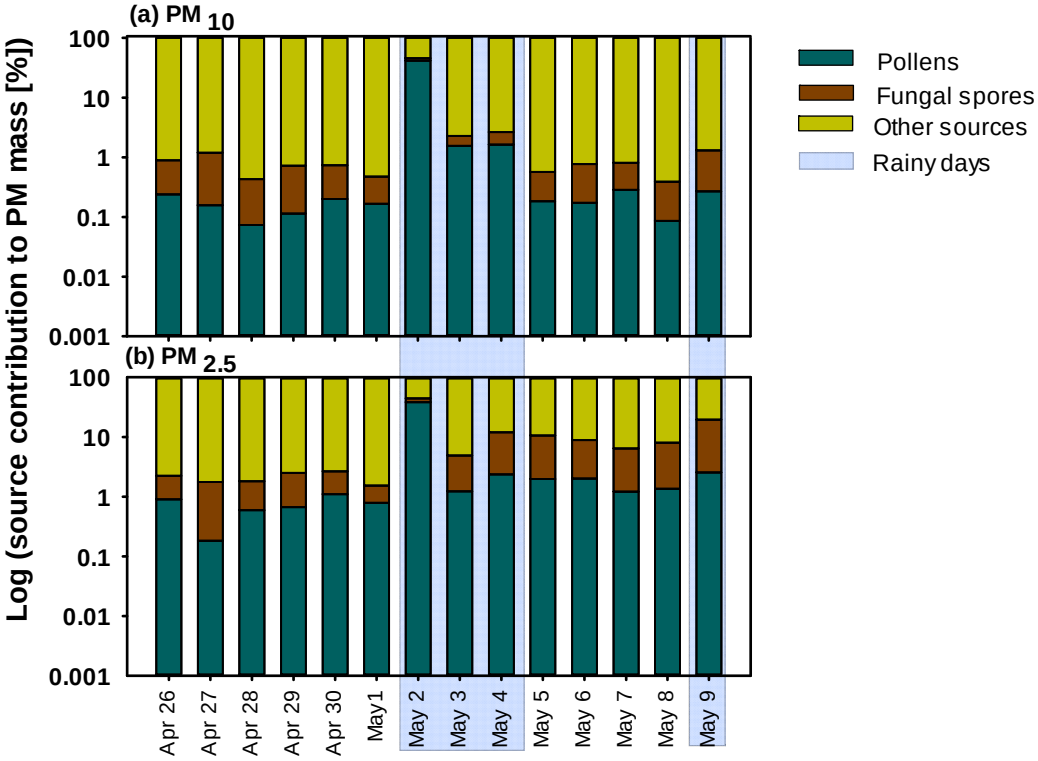

Figure 4.8: Apportionment of PM_{10} mass (a) and $PM_{2.5}$ mass (b) to pollens and fungal spores using chemical mass balance modeling. Pollen contributions to PM peaked on 2 May, a rainy day, while fungal spore contributions enhanced on 5 May, a post-rain day.

On dry days, pollens contributed an average of 0.17% of $PM_{2.5}$ and 3.3% of PM_{10}. On rainy days, pollen contributions to fine PM averaged 11% and reached a maximum of 42% on May 2. Fungal spore contributions to fine PM averaged 0.5% on dry days and 1.7% on days with rain. However, fungal spores had the greater contributions to PM_{10} mass on days following rain, reaching 8.7% on May 5. These source apportionment results demonstrate that bioaerosol contributions to PM mass are typically low (< 1%), but can be significantly greater on days with rain, when bioaerosols are released and PM is removed by wet deposition.

Bioaerosol contributions to PM in this study were relatively in good agreement with previous literature. The average fungal spore contribution to PM_{10} (5%) of this work was 1.6 times higher than suburban site of Vienna, Austria, and 1.6 times less than a tropical rainforest in China,[218] which were measured during springtime. Collectively, contributions from pollens (3.3%) and fungal spores (0.9%) to fine PM was ~2 times lower than contributions reported in US which were determined in summertime.[219] The slight variations of contributions could be attributed to the differences in ambient bioaerosol levels.

The distribution of bioaerosols in fine and coarse PM during spring is shown in Figure 4.9. For dry conditions, ~11% of pollens and fungal spores were observed in fine PM. However, during rainy days, 62% of pollen mass and 20% of fungal spore mass were observed in fine PM. These results indicate the importance of rain in shifting the size distribution of bioaerosols by affecting

release mechanisms (i.e. passive release by splashing, or osmotic rupture of pollens).

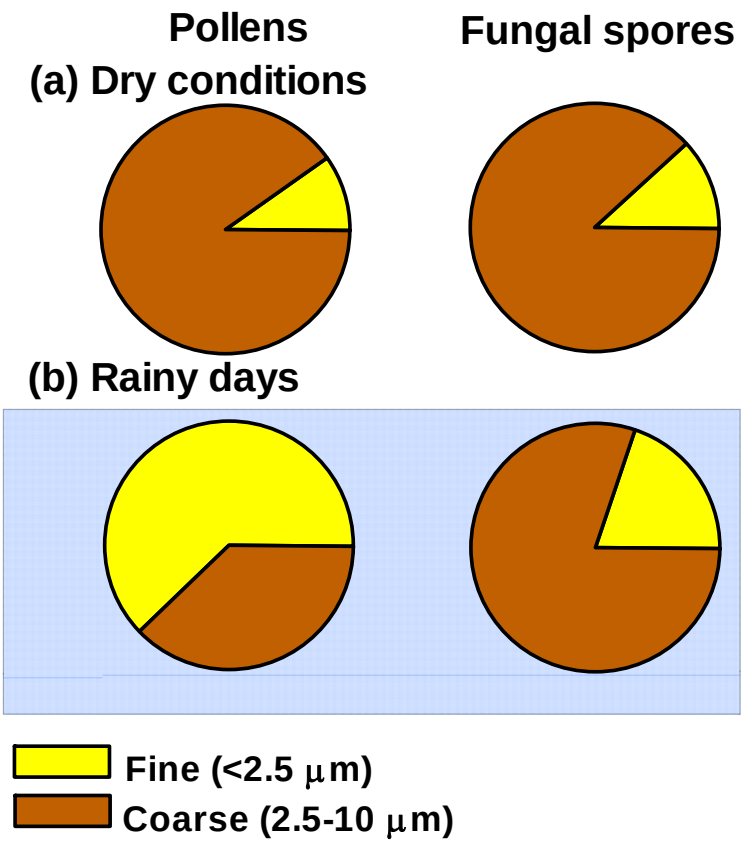

Figure 4.9: Distribution of pollen and fungal spore mass (apportioned by the CMB model) across fine and coarse PM during dry and rainy conditions. Fine sized pollens and fungal spores increased while raining, with more pronounced enhancement in pollens compared to fungal spores.

4.4.6.2 Source apportionment in late summer

Late summer ambient pollen tracer ratios showed poor agreement with these ratios for ragweed pollens, which is the predominant pollen type during this season in the Midwest. Among all the coarse samples from 15 August–4 September, fewer than 10% had sucrose to fructose ratios in the range of locally collected ragweed pollen profiles (1.1-1.6). While the ambient data showed moderate agreement with sucrose to fructose ratio of commercially purchased ragweed, they had significantly different glucose to sucrose ratios. Such differences may result from mixtures of pollen types in the atmosphere that cannot be explained utilizing carbohydrate ratios of a single pollen type, and/or other dominant pollen types during late summer (e.g. Timothy grass). Consequently, CMB source apportionment of PM mass to pollens was not performed with late summer ambient samples.

4.4.7 Fungal spore contribution in late summer

Fungal spore contribution to $PM_{10-2.5}$ mass was highest during 22 August, a day with ~1 h of precipitation in late summer. Fungal spore masses in PM (Table 4.4) were calculated using the average mannitol conversion factor of 1.7 pg mannitol $spore^{-1}$ (range from 1.2 - 2.4 pg mannitol $spore^{-1}$) and 33 pg $spore^{-1}$ in Bauer et al. (2008).[44] Estimated fungal spore mass contributions to $PM_{2.5}$ and $PM_{10-2.5}$ ranged from 0.04 - 0.31 μg m^{-3} and 0.45 - 3.44 μg m^{-3}, respectively. The contribution of fungal spores to $PM_{2.5}$ averaged 1% on dry days, and when rain occurred increased to 3%. Fungal spore contributions to $PM_{10-2.5}$ averaged 6% and

reached to 16% on 22 August possibly due to fungal spores released during post rain wet conditions (section 4.4.4.1). Even though this study does not capture heavy rain fall in late summer, atmospheric fungal spore contribution to PM impacted by rain similar to springtime (section 4.4.4.1) as a result of increasing fine sized fungal spores when raining, and coarse sized spores post-rain.[80, 81]

Fungal spore contributions to PM in late summer agreed with springtime (section 4.4.4.1) and previous work.[44, 219] The fungal spore mass in PM_{10} during springtime averaged 0.7 μg m^{-3}, and in late summer the contributions were higher with a mean concentration of 1.3 μg m^{-3}, consistent with seasonal trends of fungal spore levels in the Midwestern US with higher fungal spore counts during summer compared to spring.[209] The average contribution of fungal spores to PM_{10} remained consistent at 5% and 4% in spring and late summer, respectively. Similarly, fungal spore contribution to $PM_{2.5}$ averaged ~1% in spring and late summer seasons. These estimated fungal spore mass contributions to PM_{10} were consistent with prior work in urban Austria during the summertime when fungal spore contribution to PM_{10} averaged 4%.[44] The fungal spore contribution to $PM_{2.5}$ in this study was comparable with a study from north-eastern US where fungal spore contributions to fine PM averaged 3% in July and decreased to 0.4% in August. Overall, fungal spore concentrations are higher during late summer compared to spring; however their contributions to PM remain consistent.

Table 4.4 : Absolute and percent contributions of fungal spores to $PM_{2.5}$ and $PM_{10-2.5}$ mass during late summer in Iowa City, IA, USA estimated using the conversion factors in Bauer et al. (2008).[44] Uncertainties in bioaerosol contributions represent standard errors of the estimate.

Date in 2013	Start time (local)	End time (local)	$PM_{2.5}$ Fungal spores Mass ± Error ($\mu g\ m^{-3}$)	(%)	$PM_{10-2.5}$ Fungal spores Mass ± Error ($\mu g\ m^{-3}$)	(%)
15-Aug	8:29	8:44	0.10 ± 0.02	(0.85)	0.63 ± 0.11	(4.44)
16-Aug	9:47	9:46	0.08 ± 0.01	(0.82)	0.59 ± 0.10	(2.59)
17-Aug	10:18	9:04	0.20 ± 0.03	(1.80)	0.86 ± 0.15	(6.54)
18-Aug	9:20	8:32	0.10 ± 0.02	(0.81)	0.88 ± 0.15	(6.86)
19-Aug	8:55	8:08	0.13 ± 0.02	(0.78)	1.20 ± 0.21	(7.96)
20-Aug	8:14	7:17	0.04 ± 0.01	(0.27)	0.82 ± 0.14	(4.18)
21-Aug	7:22	7:57	0.11 ± 0.02	(0.70)	1.20 ± 0.21	(4.90)
22-Aug	8:01	7:21	0.31 ± 0.05	(2.57)	2.03 ± 0.35	(15.74)
23-Aug	7:24	8:11	0.08 ± 0.01	(0.68)	0.87 ± 0.15	(7.84)
24-Aug	8:20	8:31	0.07 ± 0.01	(0.44)	0.78 ± 0.14	(5.72)
25-Aug	8:41	7:17	0.07 ± 0.01	(0.47)	1.11 ± 0.19	(6.59)
26-Aug	7:20	7:27	0.07 ± 0.01	(0.44)	2.01 ± 0.35	(9.74)
27-Aug	7:33	7:22	0.11 ± 0.02	(0.95)	1.25 ± 0.22	(4.32)
28-Aug	7:26	7:27	0.05 ± 0.01	(0.31)	0.83 ± 0.15	(3.07)
29-Aug	7:32	7:29	0.06 ± 0.01	(0.44)	0.69 ± 0.12	(4.02)
30-Aug	7:32	6:24	0.14 ± 0.02	(1.28)	1.55 ± 0.27	(4.17)
31- Aug - 01-Sep	6:28	7:46	0.15 ± 0.03	(0.98)	3.44 ± 0.60	(10.11)
2-Sep	7:50	7:40	0.06 ± 0.01	(1.89)	0.56 ± 0.10	(3.14)
3-Sep	7:43	7:29	0.06 ± 0.01	(1.14)	0.45 ± 0.08	(2.31)
4-Sep	7:32	7:25	0.26 ± 0.04	(3.37)	0.87 ± 0.15	(2.97)

4.5 Conclusions

Daily concentrations of PM mass and bioaerosol tracers (including fructose, glucose, and sucrose for pollens, mannitol and glucans for fungal spores, and endotoxins from Gram-negative bacteria) demonstrated high day-to-day variability and meteorological influences. Warmer temperatures promoted pollen, fungal and bacterial growth leading to higher ambient levels of these bioaerosols

during both spring and late summer periods. Rain events of spring triggered the release of pollens, with maximum levels of pollen tracers occurring on May 2 and May 9, when rain occurred following a period of elevated temperatures in spring. Airborne fungal spore tracers, however, were suppressed by spring rain and increased in concentration following rain events. Source apportionment by CMB modeling in concert with Midwestern pollen profiles indicated significant contributions from bioaerosols to PM mass on rainy days during springtime. Importantly, the size distribution of pollen and fungal spore tracers shifted towards fine particles (<2.5 μm) during periods of rain. The fragmentation of pollens due to osmotic rupture, shown previously only through microscopy methods, is demonstrated in this study for the first time by way of chemical tracers. The release of finer-sized bioaerosols during rain events has important implications for human exposures, because finer particles may penetrate more deeply into the lung and be transported over longer distances.

A detailed level of understanding is needed to improve the accuracy of allergen prediction models that erroneously forecast low airborne allergen levels during periods of rain. Future research should focus on a more precise determination of the duration of heightened pollen levels during rain events with higher time resolution measurements. Similarly, measurements with higher PM size resolution should be employed to determine the specific size range of pollen fragments during these events. Additionally, further efforts are needed to more accurately define the fungal and floral species that release fine-sized bioaerosols

to the atmosphere and the mechanisms that trigger such release, to allow for models to reflect the predominant species in the surrounding geographic region.

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CHAPTER 5

TOOLS TO EVALUATE HEALTH IMPACTS OF FINE POLLEN PARTICLES AND IDENTIFICATION OF ENVIRONMENTAL CONDITIONS THAT TRIGGER FINE POLLEN RELEASE

5.1 Abstract

Asthma is a serious global health problem. Development of robust tools to identify environmental risk factors that exacerbate asthma is needed to mitigate their occurrences. This study focuses on developing an approach to evaluate the impact of fine-sized pollen particles released from pollen rupturing on asthma-related emergency room (ER) visits. To evaluate asthma exacerbation due to fine-sized pollen particles, pollen fraction in fine mode during spring 2013 in Iowa was assessed with asthma-related ER visits in central and eastern Iowa. ER visits were not elevated with increased fine pollen particle fraction; however the limited number of ER visits suggested expansion of data collection for asthma exacerbation to include other health care systems: primary health care providers and asthma medicine purchases from pharmacies. In addition, this work studies microscopy-based screening methods to identify pollen types that undergo osmotic rupture and determines size and number of pollen particles that are formed via rupturing. Microscopy-based observation of pollen bursting was facilitated by using the inverted microscope; but suitable fixation methods should be further investigated prior to more widespread screening and analysis.

5.2 Introduction

Asthma is recognized as a major public health concern in the US[220] and worldwide.[221, 27] Among all the chronic illnesses, asthma is categorized as one of the most widespread.[27] In the US, ~25 million people suffer from asthma, 25% of whom are children less than 18 years of age.[222] The number of people that suffer from asthma is increasing,[220, 27] and by 2025 it is predicted that approximately 400 million people worldwide will suffer from this disease.[27] Identification of environmental risk factors that trigger asthma is important to protecting sensitive populations by warning them when unsafe conditions are likely to occur, so that they may modify their behavior and mitigate health risks.[27, 223] The purpose of this chapter is to document progress in evaluating the epidemiological impact of the fine particle pollen release on May 2, 2013 and the environmental conditions that trigger the release of fine-sized pollen particles to the atmosphere (as described in Chapter 4).

Aeroallergens, such as pollens and fungal spores, can trigger asthma attacks and other chronic respiratory diseases. When allergens enter the human respiratory tract, the body responds by producing immunoglobulin E (IgE) antibodies against these antigens. IgE antibodies bind with mast cells in the respiratory system, and a subsequent inhalation of allergens triggers release of histamine from IgE-bound mast cells.[224] Histamine triggers runny nose, sneezing and coughing, also it can stimulate muscle oedema in lower airways resulting in wheezing and breathing trouble.[225] In order to protect sensitive populations from

such negative health impacts, it is important to identify environmental factors that could enhance atmospheric allergen levels.

Prior studies have shown increased emergency room (ER) visits related to asthma following thunderstorm events worldwide.[181, 203, 226-228] The largest asthma outbreak reported was in London during July, 1994 when 640 patients (~10 times higher than on a regular day) presented asthma symptoms within 30 h of thunderstorm.[226] Moreover, asthma outbreaks following thunderstorms have been observed in the US,[203] Canada,[227] Italy[228] and Australia.[181] Asthma epidemics during thunderstorms were coincided with increased pollen grain levels,[181] and many asthma patients that presented were sensitive to pollens[228, 226] suggesting enhanced pollen exposures during thunderstorms are likely a source of asthma exacerbation.

Intact pollens are large (~5 - 100 μm)[229, 18] and rarely reach deep into the respiratory tract,[174] however, rupturing of pollens creates much smaller particles (0.03 – 4 μm)[174] with cytoplasmic constituents that can reach the bronchi. Pollens release such smaller particles containing allergenic proteins[230] when contacted with water[231, 194] or higher humidity.[195, 194] For instance, rye grass pollens release ~700 smaller particles ranging from 0.6 – 2.5 μm via osmotic rupturing of one pollen grain.[196] Furthermore, birch pollens release ~400 starch granules per pollen with a size range of 0.03 – 4 μm.[175, 174] Collectively, bursting of pollen grains increases the number of allergenic particles in the atmosphere while decreasing allergen particle size,[196] thus facilitating their transportation to deeper airways.

Together, this may be the reason behind asthma outbreaks following rain events.[203]

Various microscopic techniques have been employed to observe the structure of different pollen types and their behavior when exposed to moisture. These techniques include photomicrography,[231] light microscopy,[195] transmission electron microscopy (TEM),[199] scanning electron microscopy (SEM)[199] and fluorescence microscopy (FM).[232] FM provides a sensitive means of characterizing pollens that contain fluorophores[188, 233] and has been used to both identify and characterize pollens.[234, 235] Photomicroscopy and light microscopy were used to visualize the mechanism of releasing cytoplasmic contents from cotton and rye grass pollens once exposed to water.[195, 231] Ruptured pollen particles after exposure to rain water[199, 236] and high relative humidity[194] have been studied in higher resolution with SEM.[199, 236] TEM has been used to evaluate the localization of allergenic proteins inside the cytoplasm of tree and grass pollens, which are released to the atmosphere via rupturing of the pollen wall.[199, 236] Overall, combination of microscopy techniques revealed that pollen wall rupture upon wetting releases inner cytoplasmic constituents to the environment.

During the spring rain of 2013, enhancement of pollen particles less than 2.5 μm were observed in Iowa City (Chapter 4). Chemical profiles of glucose, fructose and sucrose in ambient air on May 2, 2013 matched that of oak pollens. Combined with the knowledge that oak trees are prominent in Iowa and the springtime release of their pollens, it is likely that this is the pollen type having undergone rupture. The first objective of this chapter was to examine the extent to

which the May 2 release of fine pollen fragments impacted asthma-related hospital visits in eastern and central Iowa. The second objective was to develop microscopy-based screening methods to identify pollen types that undergo osmotic rupture to assess the size and number of pollen fragments that form. Both objectives involve new research directions that will support the long-term goal of evaluating the health effects of pollen rupturing events and protecting sensitive populations from exposures to bioaerosols. Preliminary findings from these two exploratory objectives are described within this chapter.

5.3 Method

5.3.1 Assessments of ER visits for asthma

ER presentations of asthma related diseases from April 13 – May 13, 2013 were accessed through the Wellmark Blue Cross and Blue Shield data warehouse, through the Center of Public Health Statistics (CPHS) of the University of Iowa. Wellmark Blue Cross and Blue Shield insurance covers 1.8 million Iowans, which is ~60% of Iowa's total population.[237] Asthma related ER visits were collected from hospitals located in central and eastern Iowa, inclusive of Iowa City, Des Moines, Waterloo, Cedar Rapids, Burlington, Dubuque and Davenport (Figure 5.1), all of which experienced heavy rainfall on May 2, 2013. ER presentations are categorized according to the International Classification of Diseases, ninth revision (ICD - 9) codes. The requested patient presentations were for those diagnosed with asthma related diseases having ICD – 9 codes ranging from 493.0 – 493.9 that include allergic asthma, non-allergic asthma, chronic obstructive asthma, other forms of asthma, and unspecified asthma. The received data sets

were organized according to patient age and zip codes, and contained subject-specific identifiers. All data presented within this chapter have been de-identified.

Epidemiological data assessment was planned to include: 1) comparing the time series of fine pollen particle fraction (calculated % fraction by dividing the pollen concentration in fine mode by the sum of pollen concentrations in fine and coarse modes, which were estimated in Chapter 4) and pollen tracer abundances, to asthma-related ER visits, and 2) establish the time lag between the release of fine pollens and hospital visits, by comparing daily visits on the same day, as well as 1, 2, 3 and 4 days prior to the visit, to account for the delay between a high pollen level day and the time of patient presentation at the ER. Initial assessment included the comparison of fine pollen particle fraction with the asthma-related ER visits was first assessed by plotting the data to visualize trends. T-tests comparing means used to assess differences of asthma-related ER visits before (April 17- 25) and after on-set of spring (April 26 – May 9) and with a lag time ranging from 1 to 4 days.

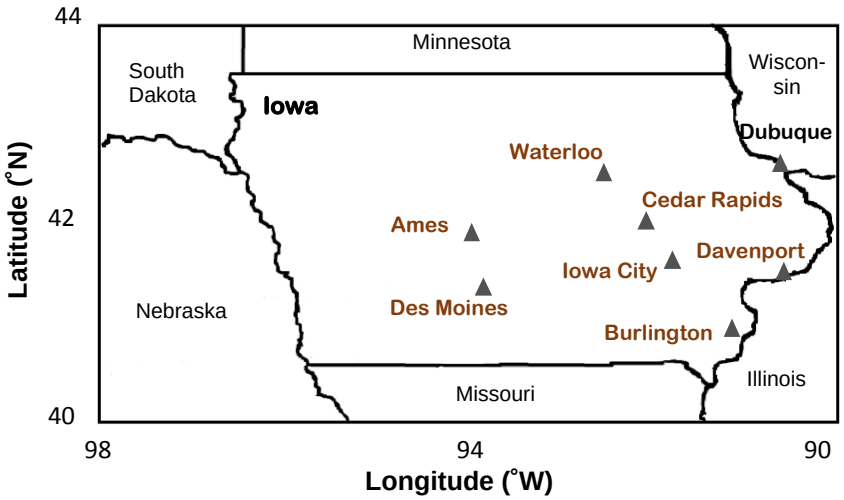

Figure 5.1: Data collection sites in Iowa.

5.3.2 Observation of pollen bursting via the inverted microscope

Poly-L-lysine was used as the binder to fix pollens on to a glass bottomed dish to prevent moving after adding water. One drop of poly-L-lysine (0.1% w/v in water, Sigma-Aldrich Corporation, St. Louis, MO, US) was placed in the middle of the glass bottom microwell dish (Mat Tec Corp. Ashland, MA, US), dried in the oven at 65˚C for 15 min, and additional solvent was removed via blotting with a filter paper (Figure 5.2a). Pollens were placed in the middle of the dried poly-L-lysine drop using a Blick size zero round brush as shown in Figure 5.2b. Slides were dried overnight and observed under an Olympus IX 81 inverted microscope (Olympus Corporation, Tokyo, Japan).

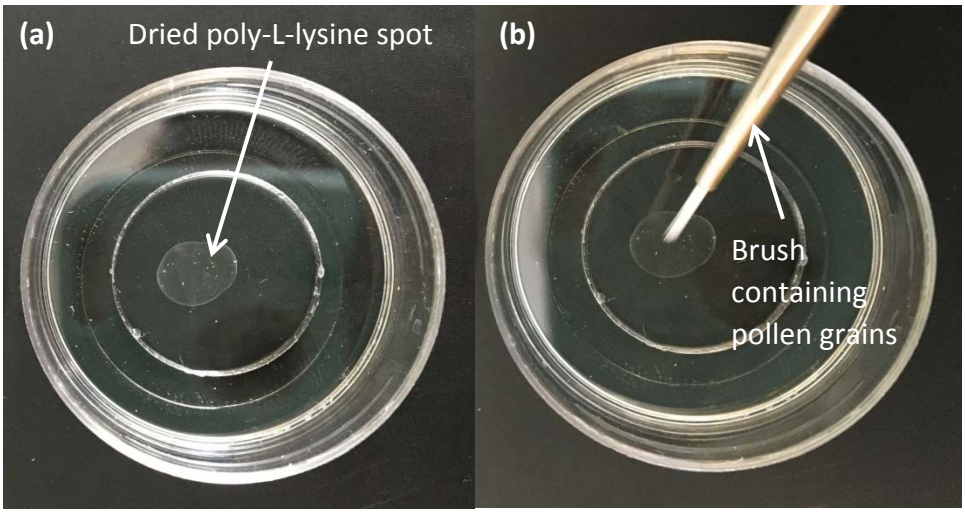

Figure 5.2: Dried poly-L-lysine spot on the well plate (a), and placing pollen grains using a brush on the poly-L-lysine spot (b).

A single pollen particle was located looking through the microscope prior to adding water. A glass-bottomed slide containing pollen particles was placed in the observing chamber which had a continuous carbon dioxide flow and a temperature around 35 ˚C. A clear image was obtained utilizing the bright light and 40X magnification. Tap-water (~20 μL) was added on to the pollen. Images were taken every 20 seconds for 45 min.

Aldehyde fixation was used to fix ruptured pollen particles on to the surface of the glass slide. The slide was dried overnight to evaporate additional water. About 0.5 mL of glutaraldehyde solution (2.5% glutaraldehyde, Electron Microscope Sciences, Hatfield, PA, USA) was added, dried for 1 h, and washed with 0.1 M cacodylate buffer with a pH of 7.2 (Electron Microscope Sciences, Hatfield, PA, US). The ruptured pollen was observed via the stereoscope to confirm that the pollen was fixed on the glass slide prior to applying Osmium treatment. Osmium tetroxide (Electron Microscope Sciences, Hatfield, PA, US)

was added, dried for 30 min, and washed with double distilled water, and a series of ethanol solutions (25 - 100%).

The glass dish containing ruptured pollen was mounted onto an aluminum stub to observe under SEM (Hitachi, S4800). The glass slide was separated from the dish, and mounted on to an aluminum stub using carbon tape, and the sample was then sputter-coated.

5.4 Results and discussion

5.4.1 Health data

Among 31 days from April 13 – May 13 twenty days presented asthma-related ER visits with a limited number of patients which totaled to 32 presentations. The age distributions of patients are shown in Table 5.1, and among all the age groups 11 (34%) patients were less than 10 years old. There were 12 rain days with rain ranging from 0.3 – 85 mm with thunderstorm events on April 17 - 18, and May 3. Asthma related ER visits during April 13 – May 13 ranged from 1-3 patients per day, and showed no enhancements with spring rain events (May 2, 3 and 4) in eastern and central Iowa in 2013 (Figure 5.3).

Within the small dataset that was generated, there was no significant difference among asthma-related ER visits before and after on-set of spring and when considering a time lag of 1 to 4 days ($p > 0.3$). In other words, the dataset revealed no significant association of asthma-related ER visits with either increased levels of pollen tracers or their particle size surrounding the May 2, 2013 rain event. Asthma outbreaks followed by rain events are suggested to occur via exposure to increased levels of allergenic pollen particles.[123] With only 32

asthma-related visits to ER further statistical analysis was not performed. These patient counts were very low when compared to previous work where larger patient data sets ranging from ~100 to ~5000 were used to link asthma-related ER visits with airborne pollen and meteorological measurements via performing the Poisson regression analysis.[238-240]

Ultimately, the Wellmark dataset produced a surprisingly low number of ER-visits related to asthma. There could be multiple likely reasons for low counts of asthma-related ER visits, such as seeking primary care for asthma exacerbation rather than visiting the ER, or the use of prophylactic therapy (particularly self-treatment) to avoid ER and hospital expenses.[241, 242] Prior work has shown that people in the US seek primary care when possible without attending to ER indicating that accounting only ER visits would underestimate real occurrences of illnesses.[243, 244] Prophylactic therapy includes identifying risk factors that exacerbate asthma, and being pro-active and taking prescribed medication prior to allergen exposures.[241] Prophylactic therapy requires less money compared to rescuing therapy, and is thus promoted by the health care system.[241] Consequently, real asthma exacerbations in spring 2013 may not have been accurately reflected by asthma-related ER visits alone.

Alternatively, environmental factors may explain this lack of association, such as little time spent outdoors during the peak release of pollen particles, or different allergenic potentials of different pollen species and their relationship to sthma.[225] Asthma symptoms depend on the content of allergenic proteins in pollen allergens, which varies among species having similar pollen types as well

as different pollen types.[225] For example, grass pollens are frequently connected with exacerbation of asthma in prior work,[181,174] and shown to contain higher content of proteins (>90% of protein mass) that can bind with IgE antibodies triggering asthma symptoms.[225] Collectively, the preliminary work presented here in suggest alternative approaches for future epidemiological research in Midwest which is described further in Chapter 6.

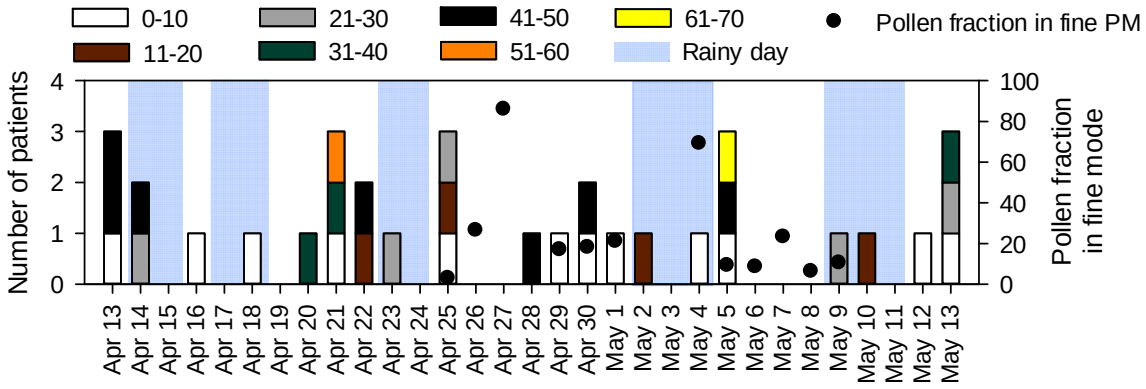

Figure 5.3: Emergency room presentations for asthma in Eastern Iowa during spring 2013. These presentations were not associated with the fraction of pollen tracers observed in the fine PM fraction (black circles).

Table 5.1: Categorization of asthma related emergency visits by patient age group from April 13 – May 13, 2013 in central and eastern Iowa.

Age group	Patients
Birth - 10	11
11 - 20	4
21 - 30	5
31 - 40	3
41 - 50	7
51 - 60	1
61 - 70	1

5.4.2 Microscopic analysis of pollens

Pin oak pollens collected from spring 2013 and cotton ragweed pollens collected in autumn 2015 (section 4.3.4) were used to observe pollen bursting once exposed to moisture. Some ragweed pollens ruptured once exposed to water, but not others. Prior to mounting the poly-L-lysine fixed pollens, free cotton ragweed pollens (~10 grains) were observed. Once exposed to water, some ragweed pollens ruptured readily. In fact, approximately one out of three pollens ruptured within 1 min of adding water (Figure 5.4a), while others remained intact even after 5 min of water exposure. Meanwhile, poly-L-lysine fixed pin oak pollen remained intact even after 45 min of water exposure.

Differences in the time required for pollens to burst after exposure vary with and across species. These observations are consistent with previous findings that different pollens of the same species have different hydration rates depending on pollen wall characteristics,[245, 246] where pollens with more exposed inner walls rupture rapidly relative to others. [245] In addition, previous observations with ragweed pollens indicated that there can be pollens in the same species that need more time to hydrate relative to others.[245, 246] Some pollen types burst within a few seconds, while others require hours.[194, 195, 199, 231, 236] We cannot rule out the possibility of sample preparation artifacts on our analysis, which may result from the poly-L-lysine layer used to fix pollens on to the glass surface acting as a barrier between pollen wall and water, thus delaying the rupturing process.

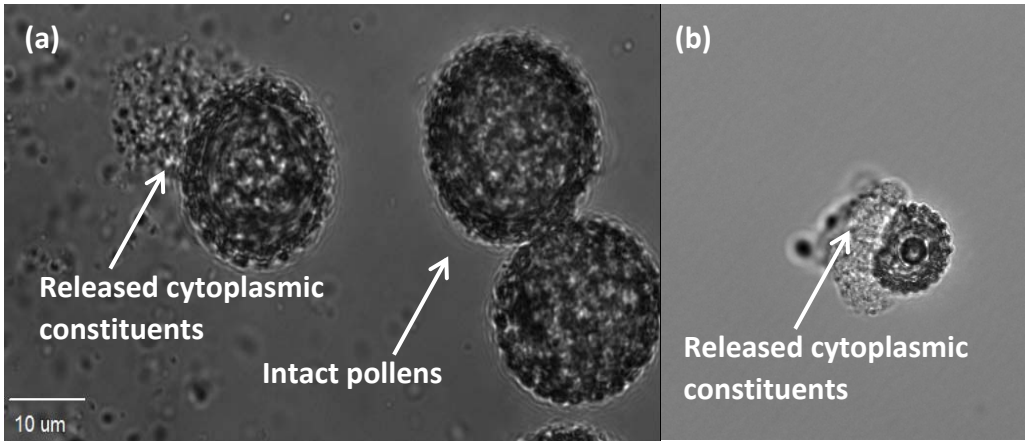

Figure 5.4: Inverted microscope images of releasing cytoplasmic constituents of cotton ragweed pollens once exposed to water, when observed as multiple free pollens (a), and single poly-L-lysine fixed pollen (b), which both taken approximately after 1 min of water exposure.

Pollen fragments were not readily observed by SEM. After exposing to water, ruptured pollen particles were immersed in water, thus, the sample had to dry before mounting on to a SEM stub. Moreover, the sample was fixed via glutaraldehyde fixation, so the sample would not be destroyed by high vacuum generated inside the SEM chamber. The observation of ruptured pollen particles under the SEM was not successful, possibly due to washing-out of the pollen particles during glutaraldehyde fixation.

5.5 Conclusions

While low counts of asthma-related ER visits precluded rigorous analysis of the epidemiological effect of the release of fine pollen fragments to the atmosphere in Eastern Iowa surrounding the May 2 rain event, this research has provided insight into how a future epidemiological study may be improved. First, epidemiological data collection should be expanded to include primary health care providers and medicinal purchases from pharmacies. Such data collections are expected to provide a more accurate representation of asthma exacerbations and increase the number of asthma presentations. Moreover, health studies be performed by a recruiting patient samples[247, 248] with asthma, where patients will log their daily activities and asthma symptoms which will then can be used to evaluate how fine-pollen releases impacted their lives. Second, the number of patients could be increased by encompassing a larger area with a larger population. Overall, having a larger number of asthma presentations would allow visualizing changes of asthma exacerbations associated with rain when plotted as in Figure 5.3.

While the inverted microscope is a powerful tool to qualitatively visualize pollen rupturing, additional research is needed in order to develop this into a quantitative tool for assessing time taken by pollens to rupture. This includes: 1) monitoring a statistically significant number of pollen grains to determine the time needed to rupture, which can be done by comparing average time taken to rupture when assessing different numbers of pollens ranging from 3 – 20, and 2) understanding the role of poly-L-lysine in absorbing water by pollens, which could be done by comparing time taken for pollen rupturing without poly-L-lysine and when fixed with varying amounts of poly-L-lysine ranging from 10 - 50 μL. SEM is a suitable technique to evaluate pollen size and number after pollen bursting,[199] however, fixation of the ruptured pollen to substrates is a challenge. In future, such pollen bursting can be examined directly after water evaporation[236] without additional fixation to understand the impact of vacuum created inside the SEM on the pollen sample. Overall, microscopy-based future studies should be further developed to implement robust methods to investigate the phenomenon of pollen bursting and the release of fine-sized pollen particles.

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CHAPTER 6

CONCLUSIONS AND FUTURE WORK

Evaluating negative health impacts of bioaerosols requires understanding of when, where and how exposures to bioaerosols happen. Spatial comparison of bioaerosols revealed significant enhancements of fungal glucans, bacterial endotoxins and water-soluble proteins and their sources in urban locations compared to background and co-located measurements of calcium suggest wind-blown dust as a main urban bioaerosol source. Seasonal analysis indicated increasing ambient fungal spore tracers, bacterial endotoxins and water-soluble proteins with temperature having maximum concentrations occurring in growing seasons. Overall, urban populations are at a higher risk of exacerbating respiratory illnesses triggered by bioaerosol mixtures relative to background populations in the Midwestern US, which are further enhanced in growing seasons when conditions are warmer.

Assessing bioaerosols in daily samples in spring and late summer indicated that a major fraction of pollen and fungal spore tracers were in coarse PM during dry days consistent with their sizes. When rain occurred pollen concentrations increased and shifted to fine particle size attributed to the osmotic rupture of pollen grains that released fine-sized pollen fragments to the atmosphere. Fungal spores also increased in fine mode during rain, but to a lesser extend compared to pollens, while maximum coarse mode fungal spores observed post-rain wet conditions. Collectively, these results indicated increased outdoor exposure levels to bioaerosols associated with rain, in particular, enhancement of

fine-sized pollen particles during rain, which should be further investigated to understand atmospheric conditions that trigger such releases and actual health implications of fine pollen particles.

Application of the CMB model for the first time with locally generated source profiles facilitated determining pollen and fungal spore contributions to PM and their significant increase associated with rain. In PM_{10}, mean pollen contribution of 4% increased to 38% while raining, and mean fungal spores contribution of 5% increased to 17% post-rain. This successful application of CMB model to apportion pollens to PM mass indicated the ability of sugar tracers to estimate pollen contributions when locally important pollen profiles are available. Future improvements include: investigation of potential changes of pollen sugar profiles after exposing pollens to the ambient conditions, introduce additional pollen profiles that could co-exist in the atmosphere to identify pollen types that contribute to PM mass, and incorporation of unique tracers to recognize pollen types.

The research work presented in this dissertation, opens-up different important paths to continue future work to improve the understanding of atmospheric bioaerosols. These main paths include: validation of using sugars as pollen tracers, combinations of sugar measurements with real-time bioaerosol measurements, determining conditions that trigger fine-sized bioaerosol release and their actual health implications, and further evaluation of the assumptions made in CMB model estimations of pollen contributions to PM. Such studies are summarized in the following discussion.

Measuring pollen tracers to estimate their atmospheric levels is an alternative approach compared to traditional direct counting method,[249] thus should be validated by doing side by side with actual pollen counting. For instance, fungal spore tracers, mannitol and arabitol validated previously using direct spore counts.[44] Similar assessments are needed for pollens. To so this, PM samples could be collected side by side with a Burkard sampler to trap pollens.[249] Pollen counts in 1 m^3 of air could be assessed against each sugar tracer (glucose, fructose and sucrose) mass in 1 m^3, the correlation values can be used to assess the trends, a correlation value >0.8 will be taken as a strong correlation[112] when validating sugars as tracers of airborne pollens. Such studies should be extended to different seasons and locations to understand how valid to use sugars as tracers of pollens even in the presence of other atmospheric sources of sugars such as soil,[184] road dust[185] and biomass burning.[186] These studies will provide strong evidence to validate using measurements of ambient glucose, fructose and sucrose as tracers of pollens.

Identification of residence time of pollen particles released via pollen bursting is important to understand to protect sensitive populations from fine-sized pollen exposures. To do that, chemical tracer measurements can be combined with real-time fluorescence-based measurements. Fluorescent based techniques are often used to understand real-time bioaerosol number concentrations as a function of their size.[16, 80] However, this technique lack the ability to distinguish among bioaerosol types. Combinations of fluorescent based measurements[16, 80] and tracer measurements (discussed in this thesis work) would

allow identification of bioaerosol types that shift in sizes with response to meteorology. For example, ambient bioaerosol samples can be collected in a higher size resolution using a micro-orifice uniform deposit impactor sampler (MOUDI)[3] which will include size fractions of 5 – 10 μm, 2.5 – 5 μm, 1.0 – 2.5 μm and <1 μm. These sampling filters can be analyzed for chemical tracer concentrations (section 2.3). Simultaneously with the MOUDI sampler, real-time measurements can be performed using the ultraviolet aerodynamic particle sizer which will provide number and size of fluorescent particles in the air.[80] Comparison of these two measurements with meteorology will allow understanding: i) conditions that increase atmospheric pollen particles, ii) exact sizes of pollen fragments that increase in response to meteorology, and iii) residence time of released pollen particles in the atmosphere. This information is important to develop future lab-based experiments and population exposure experiments.

Methods should be implemented to visualize fine-sized pollen release when atmospheric conditions trigger rupturing of pollen wall. At the same time it is important to assess the number and sizes of released pollen particles which are important parameters to measure to identify the extend of negative health implications. Preliminary work on such work is discussed in Chapter 5 and found that inverted microscopy facilitates observing pollen particle release when exposed to water. Pollen bursting also happens when conditions are humid,[194] and the exact conditions that trigger rupturing of pollen wall releasing fine-sized particles and the time taken to rupture can be investigated using the inverted

microscope. In future, pollen particles can be observed after exposing to different relative humidity ranging 75 – 100 % to understand what humidity conditions are needed for pollen rupturing. After pollen bursting the number of particles that formed by one pollen grain and their size distribution can determined using a microscope with higher magnifications, such as SEM[194, 236] to understand the extend of health implications of fine-sized pollen particles. Similar methodologies could be performed using different pollen types that are prominent in different regions and seasons. Such information is useful to improve current pollen prediction models in a way that they incorporate particle release by pollen bursting to forecast actual pollen exposures when rain occurs.

While sugar ratios of different pollen types indicated using sugar ratios as diagnostics to identify pollens (Chapter 4), it could be further investigated by profiling other pollen types co-exist in the season. For instance, daily variations of sugar ratios in springtime (May 2 vs May 9, section 4.4.3) indicated the need of characterizing more pollen types other than oak pollens that could dominate the airborne pollens in spring. To do this, pollens could be trapped by a Burkard sampler,[249] and can be identified by type using their morphology. The main pollen types can be harvested and profiled. Comparison of sugar ratios in different source profiles will show the reliability of identifying pollen types in the atmosphere based on their sugar ratios.

Co-existence of different pollen types is a challenge when estimating pollen contributions to PM using sugars as tracers, therefore, incorporation of unique compounds that facilitate identification of one pollen type from the other is

important. Pollens contain proteins which are unique to the species. For example, birch pollens has Bet v1 and Bet v2 proteins[171] while oak pollens has Que e1.[169] Both oak and birch co-exist in springtime, when estimating masses, proteins can be used as unique tracers, but methods should be developed to quantify these proteins in pollen samples as well as ambient sample collecting filters.[250] These studies will enable identification of multiple pollen types and contribution from each pollen type to PM.

When estimating pollen contribution to PM utilizing the CMB model it is assumed that species concentrations measured at receptor site remain same even after exposing to ambient conditions. To test this, pollens harvested from catkins can be spread equally in to two petri-dishes, one measured directly for tracers, the other one is analyzed after exposing to a chamber with ambient conditions as described in Taylor et al (2002).[195] Inside the chamber, in different experiments pollens can be exposed to various atmospheric conditions (temperatures and humidity levels). Pollen particles then measured for species concentrations; comparison of pollen tracer ratios after exposing to different conditions with directly harvested sample profiles will facilitate understanding if chamber conditions triggered any changes to tracer concentrations. Such studies would allow determining if pollen tracer concentrations vary after the pollen particle released to the atmosphere.

Similar chamber experiments as above could be extended to perform microscopy-based studies to identify physical changes that pollens undergo via exposing to co-pollutants. Previous ambient studies showed the ability of pollens

to agglomerate with other pollutants,[94, 95] thus pollen also act as carriers of other pollutants to the human body. To start with, atmospheric pollutants that were previously shown to interact with pollens such as, diesel exhaust particles[95] and dust particles[94] can be introduced to the pollen containing chamber with the air flow. Also, pollens can be exposed to other compounds including bioaerosol and co-pollutant mixtures identified in Chapter 3 that co-exist in different seasons. Pollen particles are then be observed by SEM to visualize particle agglomerations.[94] Overall, these studies will inform the ability of pollens to agglomerate with other bioaerosol and co-pollutants providing information to future exposure studies of what harmful particles could potentially enter the human respiratory tract with pollens.

Health implications of bioaerosol-co-pollutant mixtures are important to investigate to protect sensitive humans as prior studies have shown that bioaerosols-co-pollutant mixtures are more harmful than individual particles.[122] Pollen-co-pollutant mixtures identified in chamber experiments above could be used in animal studies to understand health implications.[122] Pollen and co-pollutant mixtures can be stimulated to agglomerate in the chamber (same as above), SEM could be used to confirm particle agglomerations, these particles can then be used for animal studies as in Takano et al (2002).[122] Such studies will provide information on potential negative health implications of pollen-co-pollutant mixtures.

Tools should be developed to assess actual asthma-related health impacts of fine-sized pollen particle release via pollen rupturing. Chapter 5 of this thesis

presents some preliminary results from a similar study and suggested collection of epidemiological data from a broader health care network to obtain a larger set of data without solely relying on ER visits for asthma. Hence in future, health data collection should expand to incorporate primary health care systems and entrees of medicine purchases from pharmacies in the area along with ER visits. Epidemiological studies could be expanded to cover a larger area and also could be conducted in highly populated areas. In addition, health studies can conduct using a larger set of recruiting patients[247, 248] who have asthma. The selected set of patients with higher response rates can be given a questionnaire to fill their daily activities and asthma symptoms. These logs will be then used to evaluate how fine-pollen releases impacted their life. Alterations of data collection will aid obtaining a larger set of health data. Such larger data set will allow visualizing trends, for instance, time taken after fine-sized pollen release (lag-time) to exacerbate respiratory symptoms. Furthermore, a larger data set could be divided based on type of respiratory symptoms and diseases, so health implications could be recognized as a function of pollen concentrations. Other than the springtime tree pollen season, conducting similar studies in summer grass pollen season and late-summer to autumn weed pollen season is also required to understand different types of pollens that impact human health.

Overall, all these research areas proposed as future work will allow better understanding of air surrounding humans to protect them from harmful exposures.

REFERENCES

1. Seinfeld, J. H.; Pandis, S. N., *Atmospheric chemistry and physics: from air pollution to climate change*. John Wiley & Sons: 2012.

2. Hinds, W. C., *Aerosol technology: properties, behavior, and measurement of airborne particles*. John Wiley & Sons: 2012.

3. Finlayson-Pitts, B. J.; Pitts Jr, J. N., *Chemistry of the upper and lower atmosphere: theory, experiments, and applications*. Academic press: 1999.

4. Pope III, C. A., Respiratory hospital admissions associated with PM_{10} pollution in Utah, Salt Lake, and Cache Valleys. *Archives of Environmental Health: An International Journal* **1991,** *46*, (2), 90-97.

5. Brunekreef, B.; Holgate, S. T., Air pollution and health. *The Lancet* **2002,** *360*, (9341), 1233-1242.

6. Pope III, C. A.; Dockery, D. W.; Spengler, J. D.; Raizenne, M. E., Respiratory health and PM_{10} pollution: a daily time series analysis. *Am. Rev. Respir. Dis.* **1991,** *144*, (3_pt_1), 668-674.

7. Pope III, C. A.; Thun, M. J.; Namboodiri, M. M.; Dockery, D. W.; Evans, J. S.; Speizer, F. E.; Heath Jr, C. W., Particulate air pollution as a predictor of mortality in a prospective study of US adults. *Am. J. Respir. Crit. Care Med.* **1995,** *151*, (3_pt_1), 669-674.

8. Dominici, F.; Peng, R. D.; Bell, M. L.; Pham, L.; McDermott, A.; Zeger, S. L.; Samet, J. M., Fine particulate air pollution and hospital admission for cardiovascular and respiratory diseases. *JAMA* **2006,** *295*, (10), 1127-1134.

9. Le Tertre, A.; Medina, S.; Samoli, E.; Forsberg, B.; Michelozzi, P.; Boumghar, A.; Vonk, J.; Bellini, A.; Atkinson, R.; Ayres, J., Short-term effects of particulate air pollution on cardiovascular diseases in eight European cities. *J. Epidemiol. Community Health* **2002,** *56*, (10), 773-779.

10. Schwartz, J., Air pollution and hospital admissions for heart disease in eight US counties. *Epidemiology* **1999,** *10*, (1), 17-22.

11. Lim, S. S.; Vos, T.; Flaxman, A. D.; Danaei, G.; Shibuya, K.; Adair-Rohani, H.; AlMazroa, M. A.; Amann, M.; Anderson, H. R.; Andrews, K. G., A comparative risk assessment of burden of disease and injury attributable to 67 risk factors and risk factor clusters in 21 regions, 1990–2010: a systematic analysis for the Global Burden of Disease Study 2010. *The lancet* **2013,** *380*, (9859), 2224-2260.

12. Biegalski, S.; Colbeck, I.; Holländer, W.; Koutrakis, P.; Landsberger, S.; Pacyna, J.; Pandis, S.; Pilinis, C.; Selin, E.; Sioutas, C., *Airborne particulate matter*. Springer: 2013; Vol. 4.

13. Brown, J. S.; Gordon, T.; Price, O.; Asgharian, B., Thoracic and respirable particle definitions for human health risk assessment. *Part. Fibre Toxicol.* **2013,** *10*, (1), 1-12.

14. Després, V. R.; Huffman, J. A.; Burrows, S. M.; Hoose, C.; Safatov, A. S.; Buryak, G.; Fröhlich-Nowoisky, J.; Elbert, W.; Andreae, M. O.; Pöschl, U., Primary biological aerosol particles in the atmosphere: a review. *Tellus B* **2012,** *64*.

15. Pöschl, U.; Shiraiwa, M., Multiphase Chemistry at the Atmosphere–Biosphere Interface Influencing Climate and Public Health in the Anthropocene. *Chem. Rev.* **2015,** *115*, (10), 4440-4475.

16. Huffman, J.; Treutlein, B.; Pöschl, U., Fluorescent biological aerosol particle concentrations and size distributions measured with an Ultraviolet Aerodynamic Particle Sizer (UV-APS) in Central Europe. *Atmos. Chem. Phys.* **2010,** *10*, (7), 3215-3233.

17. Womiloju, T. O.; Miller, J. D.; Mayer, P. M.; Brook, J. R., Methods to determine the biological composition of particulate matter collected from outdoor air. *Atmos. Environ.* **2003,** *37*, (31), 4335-4344.

18. Jones, A. M.; Harrison, R. M., The effects of meteorological factors on atmospheric bioaerosol concentrations—a review. *Sci. Total Environ.* **2004,** *326*, (1–3), 151-180.

19. White, J. F.; Bernstein, D. I., Key pollen allergens in North America. *Ann. Allergy Asthma Immunol.* **2003,** *91*, (5), 425-435.

20. D'Amato, G.; Spieksma, F. T. M.; Liccardi, G.; Jäger, S.; Russo, M.; Kontou-Fili, K.; Nikkels, H.; Wüthrich, B.; Bonini, S., Pollen-related allergy in Europe*. *Allergy* **1998,** *53*, (6), 567-578.

21. Salvaggio, J.; Seabury, J.; Schoenhardt, E. A., New Orleans asthma: V. Relationship between Charity Hospital asthma admission rates, semiquantitative pollen and fungal spore counts, and total particulate aerometric sampling data. *J. Allergy Clin. Immunol.* **1971,** *48*, (2), 96-114.

22. Newson, R.; Strachan, D.; Corden, J.; Millington, W., Fungal and other spore counts as predictors of admissions for asthma in the Trent region. *Occup. Environ. Med.* **2000,** *57*, (11), 786-792.

23. Rosas, I.; McCartney, H.; Payne, R.; Calderon, C.; Lacey, J.; Chapela, R.; Ruiz-Velazco, S., Analysis of the relationships between environmental factors (aeroallergens, air pollution, and weather) and asthma emergency admissions to a hospital in Mexico City. *Allergy* **1998,** *53*, (4), 394-401.

24. Dales, R.; Miller, D.; Ruest, K.; Guay, M.; Judek, S., Airborne endotoxin is associated with respiratory illness in the first 2 years of life. *Environ. Health Perspect.* **2006**, 610-614.

25. Liebers, V.; Raulf-Heimsoth, M.; Brüning, T., Health effects due to endotoxin inhalation (review). *Arch. Toxicol.* **2008,** *82*, (4), 203-210.

26. Thorne, P. S.; Mendy, A.; Metwali, N.; Salo, P.; Co, C.; Jaramillo, R.; Rose, K. M.; Zeldin, D. C., Endotoxin Exposure: Predictors and Prevalence of Associated Asthma Outcomes in the US. *Am. J. Respir. Crit. Care Med.* **2015,** *192(11)*, 1287-1297.

27. Masoli, M.; Fabian, D.; Holt, S.; Beasley, R., The global burden of asthma: executive summary of the GINA Dissemination Committee report. *Allergy* **2004,** *59*, (5), 469-478.

28. GAN, *Global Asthma Network,The Global Asthma Report, http://www.globalasthmareport.org/resources/Global_Asthma_Report_2014.pdf, acessed June* **2014**.

29. Singer, B. D.; Ziska, L. H.; Frenz, D. A.; Gebhard, D. E.; Straka, J. G., Research note: Increasing Amb a 1 content in common ragweed (Ambrosia artemisiifolia) pollen as a function of rising atmospheric CO2 concentration. *Funct. Plant Biol.* **2005,** *32*, (7), 667-670.

30. Ziska, L. H.; Caulfield, F. A., Rising CO2 and pollen production of common ragweed (Ambrosia artemisiifolia L.), a known allergy-inducing species: implications for public health. *Funct. Plant Biol.* **2000,** *27*, (10), 893-898.

31. Beggs, P. J., Impacts of climate change on aeroallergens: past and future. *Clin. Exp. Allergy* **2004,** *34*, (10), 1507-1513.

32. Beggs, P. J.; Bambrick, H. J., Is the global rise of asthma an early impact of anthropogenic climate change? *Ciência & Saúde Coletiva* **2006,** *11*, (3), 745-752.

33. Pope, F., Pollen grains are efficient cloud condensation nuclei. *Environ. Res. Lett.* **2010,** *5*, (4), 044015.

34. Sun, J.; Ariya, P. A., Atmospheric organic and bio-aerosols as cloud condensation nuclei (CCN): A review. *Atmos. Environ.* **2006,** *40*, (5), 795-820.

35. Franc, G. D.; Demott, P. J., Cloud activation characteristics of airborne Erwinia carotovora cells. *J. Appl. Meteorol.* **1998,** *37*, (10), 1293-1300.

36. Steiner, A. L.; Brooks, S. D.; Deng, C.; Thornton, D. C.; Pendleton, M. W.; Bryant, V., Pollen as atmospheric cloud condensation nuclei. *Geophys. Res. Lett.* **2015**.

37. Diehl, K.; Quick, C.; Matthias-Maser, S.; Mitra, S.; Jaenicke, R., The ice nucleating ability of pollen: Part I: Laboratory studies in deposition and condensation freezing modes. *Atmos. Res.* **2001,** *58*, (2), 75-87.

38. Diehl, K.; Matthias-Maser, S.; Jaenicke, R.; Mitra, S., The ice nucleating ability of pollen:: Part II. Laboratory studies in immersion and contact freezing modes. *Atmos. Res.* **2002,** *61*, (2), 125-133.

39. Augustin, S.; Wex, H.; Niedermeier, D.; Pummer, B.; Grothe, H.; Hartmann, S.; Tomsche, L.; Clauss, T.; Voigtländer, J.; Ignatius, K., Immersion freezing of birch pollen washing water. *Atmos. Chem. Phys.* **2013,** *13*, (21), 10989-11003.

40. Murray, B.; Ross, J.; Whale, T.; Price, H.; Atkinson, J.; Umo, N.; Webb, M., The relevance of nanoscale biological fragments for ice nucleation in clouds. *Sci. Rep.* **2015,** *5*.

41. Pouleur, S.; Richard, C.; Martin, J.-G.; Antoun, H., Ice nucleation activity in Fusarium acuminatum and Fusarium avenaceum. *Appl. Environ. Microbiol.* **1992,** *58*, (9), 2960-2964.

42. Sullivan, A.; Frank, N.; Kenski, D.; Collett, J., Application of high-performance anion-exchange chromatography–pulsed amperometric detection for measuring carbohydrates in routine daily filter samples collected by a national network: 2. Examination of sugar alcohols/polyols, sugars, and anhydrosugars in the upper Midwest. *J. Geophys. Res. Atmos.* **2011,** *116*, (D8).

43. Jia, Y.; Clements, A. L.; Fraser, M. P., Saccharide composition in atmospheric particulate matter in the southwest US and estimates of source contributions. *J. Aerosol Sci.* **2010,** *41*, (1), 62-73.

44. Bauer, H.; Claeys, M.; Vermeylen, R.; Schueller, E.; Weinke, G.; Berger, A.; Puxbaum, H., Arabitol and mannitol as tracers for the quantification of airborne fungal spores. *Atmos. Environ.* **2008,** *42*, (3), 588-593.

45. Pavilonis, B. T.; Anthony, T. R.; O'Shaughnessy, P. T.; Humann, M. J.; Merchant, J. A.; Moore, G.; Thorne, P. S.; Weisel, C. P.; Sanderson, W. T., Indoor and outdoor particulate matter and endotoxin concentrations in an intensely agricultural county. *J. Expo. Sci. Environ. Epidemiol.* **2013,** *23*, (3), 299-305.

46. Foto, M.; Plett, J.; Berghout, J.; Miller, J. D., Modification of the Limulus amebocyte lysate assay for the analysis of glucan in indoor environments. *Anal. Bioanal. Chem.* **2004,** *379*, (1), 156-162.

47. Fu, P.; Kawamura, K.; Kobayashi, M.; Simoneit, B. R., Seasonal variations of sugars in atmospheric particulate matter from Gosan, Jeju Island: Significant contributions of airborne pollen and Asian dust in spring. *Atmos. Environ.* **2012,** *55*, 234-239.

48. Yoshimura, A.; Lien, E.; Ingalls, R. R.; Tuomanen, E.; Dziarski, R.; Golenbock, D., Cutting edge: recognition of Gram-positive bacterial cell wall components by the innate immune system occurs via Toll-like receptor 2. *J. Immunol.* **1999,** *163*, (1), 1-5.

49. Carty, C. L.; Gehring, U.; Cyrys, J.; Bischof, W.; Heinrich, J., Seasonal variability of endotoxin in ambient fine particulate matter. *J. Environ. Monit.* **2003,** *5*, (6), 953-958.

50. Heinrich, J.; Pitz, M.; Bischof, W.; Krug, N.; Borm, P. J., Endotoxin in fine ($PM_{2.5}$) and coarse ($PM_{2.5–10}$) particle mass of ambient aerosols. A temporo-spatial analysis. *Atmos. Environ.* **2003,** *37*, (26), 3659-3667.

51. Hetland, R. B.; Cassee, F. R.; Lag, M.; Refsnes, M.; Dybing, E.; Schwarze, P. E., Cytokine release from alveolar macrophages exposed to ambient particulate matter: heterogeneity in relation to size, city and season. *Part. Fibre Toxicol.* **2005,** *2*, (4).

52. Madsen, A. M., Airborne endotoxin in different background environments and seasons. *Ann. Agric. Environ. Med.* **2006,** *13*, (1), 81-86.

53. DeLucca, A.; Palmgren, M., Mesophilic microorganisms and endotoxin levels on developing cotton plants. *Am. Ind. Hyg. Assoc. J.* **1986,** *47*, (8), 437-442.

54. Romantschuk, M., Attachment of plant pathogenic bacteria to plant surfaces. *Annu. Rev. Phytopatho.l* **1992,** *30*, (1), 225-243.

55. Bowers, R. M.; Sullivan, A. P.; Costello, E. K.; Collett, J. L.; Knight, R.; Fierer, N., Sources of bacteria in outdoor air across cities in the midwestern United States. *Appl. Environ. Microbiol.* **2011,** *77*, (18), 6350-6356.

56. Pankhurst, L.; Deacon, L.; Liu, J.; Drew, G.; Hayes, E. T.; Jackson, S.; Longhurst, P.; Longhurst, J.; Pollard, S.; Tyrrel, S., Spatial variations in airborne microorganism and endotoxin concentrations at green waste composting facilities. *Int. J. Hyg. Environ. Health* **2011,** *214*, (5), 376-383.

57. Thorne, P. S.; Ansley, A. C.; Perry, S. S., Concentrations of bioaerosols, odors, and hydrogen sulfide inside and downwind from two types of swine livestock operations. *J. Occup. Environ. Hyg.* **2009,** *6*, (4), 211-220.

58. Bovallius, A.; Bucht, B.; Roffey, R.; Anäs, P., Three-year investigation of the natural airborne bacterial flora at four localities in sweden. *Appl. Environ. Microbiol.* **1978,** *35*, (5), 847-852.

59. Fang, Z.; Ouyang, Z.; Zheng, H.; Wang, X.; Hu, L., Culturable airborne bacteria in outdoor environments in Beijing, China. *Microb. Ecol.* **2007,** *54*, (3), 487-496.

60. Shaffer, B. T.; Lighthart, B., Survey of culturable airborne bacteria at four diverse locations in Oregon: urban, rural, forest, and coastal. *Microb. Ecol.* **1997,** *34*, (3), 167-177.

61. Gutiérrez-Castillo, M.; Olivos-Ortiz, M.; De Vizcaya-Ruiz, A.; Cebrián, M., Chemical characterization of extractable water soluble matter associated with PM_{10} from Mexico City during 2000. *Chemosphere* **2005,** *61*, (5), 701-710.

62. Rosas, I.; Yela, A.; Salinas, E.; Arreguin, R.; Rodriguez-Romero, A., Preliminary assessment of protein associated with airborne particles in Mexico City. *Aerobiología* **1995,** *11*, (2), 81-86.

63. Kang, H.; Xie, Z.; Hu, Q., Ambient protein concentration in PM 10 in Hefei, central China. *Atmos. Environ.* **2012,** *54*, 73-79.

64. Jaenicke, R., Abundance of cellular material and proteins in the atmosphere. *Science* **2005,** *308*, (5718), 73-73.

65. Miguel, A. G.; Cass, G. R.; Glovsky, M. M.; Weiss, J., Allergens in paved road dust and airborne particles. *Environ. Sci. Technol.* **1999,** *33*, (23), 4159-4168.

66. Douwes, J.; Thorne, P.; Pearce, N.; Heederik, D., Bioaerosol health effects and exposure assessment: progress and prospects. *Ann. Occup. Hyg.* **2003,** *47*, (3), 187-200.

67. Crawford, C.; Reponen, T.; Lee, T.; Iossifova, Y.; Levin, L.; Adhikari, A.; Grinshpun, S. A., Temporal and spatial variation of indoor and outdoor airborne fungal spores, pollen, and (1→ 3)-β-D-glucan. *Aerobiologia* **2009,** *25*, (3), 147-158.

68. Adhikari, A.; Jung, J.; Reponen, T.; Lewis, J. S.; DeGrasse, E. C.; Grimsley, L. F.; Chew, G. L.; Grinshpun, S. A., Aerosolization of fungi,(1→ 3)-β-d glucan, and endotoxin

from flood-affected materials collected in New Orleans homes. *Environ. Res.* **2009,** *109*, (3), 215-224.

69. Elbert, W.; Taylor, P.; Andreae, M.; Pöschl, U., Contribution of fungi to primary biogenic aerosols in the atmosphere: wet and dry discharged spores, carbohydrates, and inorganic ions. *Atmos. Chem. Phys.* **2007,** *7*, (17), 4569-4588.

70. Zhang, Z.; Engling, G.; Zhang, L.; Kawamura, K.; Yang, Y.; Tao, J.; Zhang, R.; Chan, C.-Y.; Li, Y., Significant influence of fungi on coarse carbonaceous and potassium aerosols in a tropical rainforest. *Environ. Res. Lett.* **2015,** *10*, (3), 034015.

71. Hwang, S. H.; Yoon, C. S.; Park, J. B., Outdoor (1→3)-β-D-glucan Levels and Related Climatic Factors. *J. Prev. Med. Public Health* **2014,** *47*, (2), 124-128.

72. Madsen, A. M.; Frederiksen, M. W.; Allermann, L.; Peitersen, J. H., (1→ 3)-β-d-glucan in different background environments and seasons. *Aerobiologia* **2011,** *27*, (2), 173-179.

73. Burshtein, N.; Lang-Yona, N.; Rudich, Y., Ergosterol, arabitol and mannitol as tracers for biogenic aerosols in the eastern Mediterranean. *Atmos. Chem. Phys.* **2011,** *11*, (2), 829-839.

74. Jia, Y.; Bhat, S.; Fraser, M. P., Characterization of saccharides and other organic compounds in fine particles and the use of saccharides to track primary biologically derived carbon sources. *Atmos. Environ.* **2010,** *44*, (5), 724-732.

75. Liang, L.; Engling, G.; He, K.; Du, Z.; Cheng, Y.; Duan, F., Evaluation of fungal spore characteristics in Beijing, China, based on molecular tracer measurements. *Environ. Res. Lett.* **2013,** *8*, (1), 014005.

76. Manninen, H. E.; Bäck, J.; Sihto-Nissilä, S.-L.; Huffman, J. A.; Pessi, A.-M.; Hiltunen, V.; Aalto, P. P.; Hidalgo Fernández, P. J.; Hari, P.; Saarto, A., Patterns in airborne pollen and other primary biological aerosol particles (PBAP), and their contribution to aerosol mass and number in a boreal forest. *Boreal Environ. Res.* **2014,** *19(B)*, 383–405.

77. Schumacher, C.; Pöhlker, C.; Aalto, P.; Hiltunen, V.; Petäjä, T.; Kulmala, M.; Pöschl, U.; Huffman, J., Seasonal cycles of fluorescent biological aerosol particles in boreal and semi-arid forests of Finland and Colorado. *Atmos. Chem. Phys.* **2013,** *13*, (23), 11987-12001.

78. Targonski, P. V.; Persky, V. W.; Ramekrishnan, V., Effect of environmental molds on risk of death from asthma during the pollen season. *J. Allergy Clin. Immunol.* **1995,** *95*, (5), 955-961.

79. Shelton, B. G.; Kirkland, K. H.; Flanders, W. D.; Morris, G. K., Profiles of airborne fungi in buildings and outdoor environments in the United States. *Appl. Environ. Microbiol.* **2002,** *68*, (4), 1743-1753.

80. Huffman, J. A.; Pöhlker, C.; Prenni, A.; DeMott, P.; Mason, R.; Robinson, N.; Fröhlich-Nowoisky, J.; Tobo, Y.; Després, V.; Garcia, E., High concentrations of biological aerosol particles and ice nuclei during and after rain. *Atmos. Chem. Phys.* **2013,** *13*, (1), 6151-6164.

81. Hjelmroos, M., Relationship between airborne fungal spore presence and weather variables: Cladosporium and Alternaria. *Grana* **1993,** *32*, (1), 40-47.

82. Troutt, C.; Levetin, E., Correlation of spring spore concentrations and meteorological conditions in Tulsa, Oklahoma. *Int. J. Biometeorol.* **2001,** *45*, (2), 64-74.

83. Nikkels, A.; Terstegge, P.; Spieksma, F. T. M., Ten types of microscopically identifiable airborne fungal spores at Leiden, The Netherlands. *Aerobiologia* **1996,** *12*, (2), 107-112.

84. Stępalska, D.; Wołek, J., Variation in fungal spore concentrations of selected taxa associated. *Aerobiologia* **2005,** *21*, (1), 43-52.

85. Oliveira, M.; Ribeiro, H.; Delgado, J.; Abreu, I., The effects of meteorological factors on airborne fungal spore concentration in two areas differing in urbanisation level. *Int. J. Biometeorol.* **2009,** *53*, (1), 61-73.

86. Kasprzyk, I.; Worek, M., Airborne fungal spores in urban and rural environments in Poland. *Aerobiologia* **2006,** *22*, (3), 169-176.

87. Oliveira, M.; Ribeiro, H.; Delgado, J.; Abreu, I., Seasonal and intradiurnal variation of allergenic fungal spores in urban and rural areas of the North of Portugal. *Aerobiologia* **2009,** *25*, (2), 85-98.

88. Kaarakainen, P.; Meklin, T.; Rintala, H.; Hyvärinen, A.; Kärkkäinen, P.; Vepsäläinen, A.; Hirvonen, M. R.; Nevalainen, A., Seasonal variation in airborne microbial concentrations and diversity at landfill, urban and rural sites. *CLEAN–Soil, Air, Water* **2008,** *36*, (7), 556-563.

89. Pei-Chih, W.; Huey-Jen, S.; Chia-Yin, L., Characteristics of indoor and outdoor airborne fungi at suburban and urban homes in two seasons. *Sci. Total Environ.* **2000,** *253*, (1), 111-118.

90. Bauer, H.; Schueller, E.; Weinke, G.; Berger, A.; Hitzenberger, R.; Marr, I. L.; Puxbaum, H., Significant contributions of fungal spores to the organic carbon and to the aerosol mass balance of the urban atmospheric aerosol. *Atmos. Environ.* **2008,** *42*, (22), 5542-5549.

91. D'Amato, G.; Spieksma, F. T. M., Allergenic pollen in Europe. *Grana* **1991,** *30*, (1), 67-70.

92. Hoppe, K. A.; Metwali, N.; Perry, S. S.; Hart, T.; Kostle, P. A.; Thorne, P. S., Assessment of airborne exposures and health in flooded homes undergoing renovation. *Indoor Air* **2012,** *22*, (6), 446-456.

93. Mueller-Anneling, L.; Avol, J. M. P.; Thorne, P. S., Ambient endotoxin concentrations in PM_{10} from Southern California. *Environ. Health Perspect.* **2004,** *112*, (5), 583.

94. Behrendt, H.; Becker, W.; Friedrichs, K.; Darsow, U.; Tomingas, R., Interaction between aeroallergens and airborne particulate matter. *Int. Arch. Allergy Immunol.* **1992,** *99*, (2-4), 425-428.

95. Knox, R.; Suphioglu, C.; Taylor, P.; Desai, R.; Watson, H.; Peng, J.; Bursill, L., Major grass pollen allergen Lol p 1 binds to diesel exhaust particles: implications for asthma and air pollution. *Clin. Exp. Allergy* **1997,** *27*, (3), 246-251.

96. Imrich, A.; Ning, Y. Y.; Koziel, H.; Coull, B.; Kobzik, L., Lipopolysaccharide priming amplifies lung macrophage tumor necrosis factor production in response to air particles. *Toxicol. Appl. Pharmacol.* **1999,** *159*, (2), 117-124.

97. Schauer, J. J.; Rogge, W. F.; Hildemann, L. M.; Mazurek, M. A.; Cass, G. R.; Simoneit, B. R., Source apportionment of airborne particulate matter using organic compounds as tracers. *Atmos. Environ.* **1996,** *30*, (22), 3837-3855.

98. Harrison, R. M.; Smith, D.; Luhana, L., Source apportionment of atmospheric polycyclic aromatic hydrocarbons collected from an urban location in Birmingham, UK. *Environ. Sci. Technol.* **1996,** *30*, (3), 825-832.

99. Querol, X.; Alastuey, A.; Rodriguez, S.; Plana, F.; Ruiz, C. R.; Cots, N.; Massagué, G.; Puig, O., PM10 and PM2. 5 source apportionment in the Barcelona Metropolitan area, Catalonia, Spain. *Atmos. Environ.* **2001,** *35*, (36), 6407-6419.

100. EPA(1999), Environmental Protection Agency, Sampling of ambient air for PM10 using an andersen dichotomous sampler,https://www3.epa.gov/ttnamti1/files/ambient/inorganic/mthd-2-2.pdf, accessed May 2016.

101. Thermo Scientific, Partisol-Plus 2025 Sequential Air Sampler, http://www.thermoscientific.com/content/dam/tfs/ATG/EPD/EPD%20Documents/Product%20Manuals%20%26%20Specifications/Air%20Quality%20Instruments%20and%20Systems/Particulate/D19985~.pdf, accessed May 2016.

102. URG, Medium-volume manual sampling system, http://www.urgcorp.com/library/catalogs/URG%20Systems%20Overview.pdf, accessed May 2016.

103. Andersen Dichotomous Sampler , Sampling of Ambient Air for PM10 using an Andersen Dichotomous Sampler,https://www3.epa.gov/ttnamti1/files/ambient/inorganic/mthd-2-2.pdf, accessed May 2016.

104. CarboPac MA1 column product manual, Thermo Scientific, http://www.dionex.com/en-us/webdocs/115483-Man-CarboPac-MA1-065546-01.pdf, 2013, accessed April 2016.

105. CarboPac PA20 column product manual, Thermo Scientific, http://www.dionex.com/en-us/webdocs/4378-Man-031884-05-CarboPac-PA20-Jul11.pdf, 2011, accessed April 2016.

106. Zhang, Z.-S.; Engling, G.; Chan, C.-Y.; Yang, Y.-H.; Lin, M.; Shi, S.; He, J.; Li, Y.-D.; Wang, X.-M., Determination of isoprene-derived secondary organic aerosol tracers (2-methyltetrols) by HPAEC-PAD: Results from size-resolved aerosols in a tropical rainforest. *Atmos. Environ.* **2013,** *70*, 468-476.

107. Rocklin, R. D.; Clarke, A. P.; Weitzhandler, M., Improved long-term reproducibility for pulsed amperometric detection of carbohydrates via a new quadruple-potential waveform. *Anal. Chem.* **1998,** *70*, (8), 1496-1501.

108. Jayarathne, T.; Stockwell, C. E.; Yokelson, R. J.; Nakao, S.; Stone, E. A., Emissions of fine particle fluoride from biomass burning. *Environ. Sci. Technol.* **2014,** *48*, (21), 12636-12644.

109. Blanc, P. D.; Eisner, M. D.; Katz, P. P.; Yen, I. H.; Archea, C.; Earnest, G.; Janson, S.; Masharani, U. B.; Quinlan, P. J.; Hammond, S. K., Impact of the home indoor environment on adult asthma and rhinitis. *J. Occup. Environ. Med.* **2005,** *47*, (4), 362-372.

110. Thorne, P. S., Inhalation toxicology models of endotoxin-and bioaerosol-induced inflammation. *Toxicology* **2000,** *152*, (1), 13-23.

111. Hewett, P.; Ganser, G. H., A comparison of several methods for analyzing censored data. *Ann. Occup. Hyg.* **2007,** *51*, (7), 611-632.

112. Mukaka, M., A guide to appropriate use of Correlation coefficient in medical research. *Malawi Med. J.* **2012,** *24*, (3), 69-71.

113. Hueglin, C.; Gehrig, R.; Baltensperger, U.; Gysel, M.; Monn, C.; Vonmont, H., Chemical characterisation of $PM_{2.5}$, PM_{10} and coarse particles at urban, near-city and rural sites in Switzerland. *Atmos. Environ.* **2005,** *39*, (4), 637-651.

114. Monn, C.; Braendli, O.; Schaeppi, G.; Schindler, C.; Ackermann-Liebrich, U.; Leuenberger, P.; Team, S., Particulate matter< 10 μm (PM_{10}) and total suspended particulates (TSP) in urban, rural and alpine air in Switzerland. *Atmos. Environ.* **1995,** *29*, (19), 2565-2573.

115. Mugica, V.; Maubert, M.; Torres, M.; Muñoz, J.; Rico, E., Temporal and spatial variations of metal content in TSP and PM_{10} in Mexico City during 1996–1998. *J. Aerosol Sci.* **2002,** *33*, (1), 91-102.

116. Wei, F.; Teng, E.; Wu, G.; Hu, W.; Wilson, W.; Chapman, R.; Pau, J.; Zhang, J., Ambient concentrations and elemental compositions of PM_{10} and $PM_{2.5}$ in four Chinese cities. *Environ. Sci. Technol.* **1999,** *33*, (23), 4188-4193.

117. Dockery, D. W.; Pope, C. A.; Xu, X.; Spengler, J. D.; Ware, J. H.; Fay, M. E.; Ferris Jr, B. G.; Speizer, F. E., An association between air pollution and mortality in six US cities. *N. Engl. J. Med.* **1993,** *329*, (24), 1753-1759.

118. Weinmayr, G.; Romeo, E.; De Sario, M.; Weiland, S. K.; Forastiere, F., Short-term effects of PM_{10} and NO_2 on respiratory health among children with asthma or asthma-like symptoms: a systematic review and meta-analysis. *Environ. Health Perspect.* **2010,** *118*, (4), 449-57.

119. Schwartz, J.; Slater, D.; Larson, T. V.; Pierson, W. E.; Koenig, J. Q., Particulate air pollution and hospital emergency room visits for asthma in Seattle. *Am. Rev. Respir. Dis.* **1993,** *147*, (4), 826-831.

120. DellaValle, C. T.; Triche, E. W.; Leaderer, B. P.; Bell, M. L., Effects of ambient pollen concentrations on frequency and severity of asthma symptoms among asthmatic children. *Epidemiology (Cambridge, Mass.)* **2012,** *23*, (1), 55.

121. Wagner, J. G.; Morishita, M.; Keeler, G. J.; Harkema, J. R., Divergent effects of urban particulate air pollution on allergic airway responses in experimental asthma: a comparison of field exposure studies. *Environ. Health* **2012,** *11*, (1), 45.

122. Takano, H.; Yanagisawa, R.; Ichinose, T.; Sadakane, K.; Yoshino, S.; Yoshikawa, T.; Morita, M., Diesel exhaust particles enhance lung injury related to bacterial endotoxin through expression of proinflammatory cytokines, chemokines, and intercellular adhesion molecule-1. *Am. J. Respir. Crit. Care Med.* **2002,** *165*, (9), 1329-1335.

123. D'Amato, G.; Cecchi, L.; Bonini, S.; Nunes, C.; Annesi-Maesano, I.; Behrendt, H.; Liccardi, G.; Popov, T.; Van Cauwenberge, P., Allergenic pollen and pollen allergy in Europe. *Allergy* **2007,** *62*, (9), 976-990.

124. Jenkins, H. S.; Devalia, J. L.; Mister, R. L.; Bevan, A. M.; Rusznak, C.; Davies, R. J., The effect of exposure to ozone and nitrogen dioxide on the airway response of atopic asthmatics to inhaled allergen: dose-and time-dependent effects. *Am. J. Respir. Crit. Care Med.* **1999,** *160*, (1), 33-39.

125. Jörres, R.; Nowak, D.; Magnussen, H., The effect of ozone exposure on allergen responsiveness in subjects with asthma or rhinitis. *Am. J. Respir. Crit. Care Med.* **1996,** *153*, (1), 56-64.

126. DNR(2014), Department of Natural Resources, Iowa Ambient Air Monitoring Site, http://www.iowadnr.gov/Portals/idnr/uploads/air/insidednr/monitoring/network_plan_2012.pdf, accessed December 2014.

127. USEPA, Technology Transfer Network, National Ambient Air Quality Standards (NAAQS), http://www.epa.gov/ttn/naaqs/standards/pm/s_pm_index.html, accessed. In 2013.

128. Mallya, G.; Zhao, L.; Song, X.; Niyogi, D.; Govindaraju, R., 2012 Midwest Drought in the United States. *J. Hydrol. Eng.* **2013,** *18*, (7), 737-745.

129. Malm, W. C.; Sisler, J. F.; Huffman, D.; Eldred, R. A.; Cahill, T. A., Spatial and seasonal trends in particle concentration and optical extinction in the United States. *J. Geophys. Res. Atmos.* **1994,** *99*, (D1), 1347-1370.

130. Padoan, E.; Malandrino, M.; Giacomino, A.; Grosa, M. M.; Lollobrigida, F.; Martini, S.; Abollino, O., Spatial distribution and potential sources of trace elements in PM_{10} monitored in urban and rural sites of Piedmont Region. *Chemosphere* **2016,** *145*, 495-507.

131. Thorn, J., Seasonal variations in exposure to microbial cell wall components among household waste collectors. *Ann. Occup. Hyg.* **2001,** *45*, (2), 153-156.

132. Di Filippo, P.; Pomata, D.; Riccardi, C.; Buiarelli, F.; Perrino, C., Fungal contribution to size-segregated aerosol measured through biomarkers. *Atmos. Environ.* **2013,** *64*, 132-140.

133. Pashynska, V.; Vermeylen, R.; Vas, G.; Maenhaut, W.; Claeys, M., Development of a gas chromatographic/ion trap mass spectrometric method for the determination of levoglucosan and saccharidic compounds in atmospheric aerosols. Application to urban aerosols. *J. Mass Spectrom.* **2002,** *37*, (12), 1249-1257.

134. Yttri, K.; Dye, C.; Kiss, G., Ambient aerosol concentrations of sugars and sugar-alcohols at four different sites in Norway. *Atmos. Chem. Phys.* **2007,** *7*, (16), 4267-4279.

135. Chow, J. C.; Yang, X.; Wang, X.; Kohl, S. D.; Hurbain, P. R.; Chen, L. A.; Watson, J. G., Characterization of Ambient PM10 Bioaerosols in a California Agricultural Town. *Aerosol Air Qual. Res.* **2015,** *15*, 1433-1447.

136. Chen, X.-Y.; Kim, J.-Y., Callose synthesis in higher plants. *Plant Signal Behav.* **2009,** *4*, (6), 489-492.

137. Lipke, P. N.; Ovalle, R., Cell wall architecture in yeast: new structure and new challenges. *J. Bacteriol.* **1998,** *180*, (15), 3735-3740.

138. McIntosh, M.; Stone, B.; Stanisich, V., Curdlan and other bacterial (1→ 3)-β-D-glucans. *Appl. Microbiol. Biotechnol.* **2005,** *68*, (2), 163-173.

139. Claeys, M.; Graham, B.; Vas, G.; Wang, W.; Vermeylen, R.; Pashynska, V.; Cafmeyer, J.; Guyon, P.; Andreae, M.; Artaxo, P., Formation of secondary organic aerosols through photooxidation of isoprene. *Science* **2004,** *303*, (5661), 1173-1176.

140. Yang, Y.; Chan, C.-Y.; Tao, J.; Lin, M.; Engling, G.; Zhang, Z.; Zhang, T.; Su, L., Observation of elevated fungal tracers due to biomass burning in the Sichuan Basin at Chengdu City, China. *Sci. Total Environ.* **2012,** *431*, 68-77.

141. RAPCC(2016), Reigional Air Pollution Control Center, http://www.rapca.org/daily/pollenmoldsum12.htm, accessed February 2016.

142. Sterling, M.; Rogers, C.; Levetin, E., An evaluation of two methods used for microscopic analysis of airborne fungal spore concentrations from the Burkard Spore Trap. *Aerobiologia* **1999,** *15*, (1), 9-18.

143. NAB(2015) National Allergy Bureau,http://www.aaaai.org/global/nab-pollen-counts.aspx, accessed November 2014.

144. Liebers, V.; Brüning, T.; Raulf-Heimsoth, M., Occupational endotoxin-exposure and possible health effects on humans (review). *Am. J. Ind. Med.* **2006,** *49*, (6), 474-491.

145. Park, J.-H.; Spiegelman, D. L.; Burge, H. A.; Gold, D. R.; Chew, G. L.; Milton, D. K., Longitudinal study of dust and airborne endotoxin in the home. *Environ. Health Perspect.* **2000,** *108*, (11), 1023.

146. Morgenstern, V.; Carty, C. L.; Gehring, U.; Cyrys, J.; Bischof, W.; Heinrich, J., Lack of spatial variation of endotoxin in ambient particulate matter across a German metropolitan area. *Atmos. Environ.* **2005,** *39*, (36), 6931-6941.

147. Nilsson, S.; Merritt, A.; Bellander, T., Endotoxins in urban air in Stockholm, Sweden. *Atmos. Environ.* **2011,** *45*, (1), 266-270.

148. Noble, J. E.; Bailey, M. J., Quantitation of protein. *Methods Enzymol.* **2009,** *463*, 73-95.

149. Madsen, A. M.; Zervas, A.; Tendal, K.; Nielsen, J. L., Microbial diversity in bioaerosol samples causing ODTS compared to reference bioaerosol samples as measured using Illumina sequencing and MALDI-TOF. *Environ. Res.* **2015,** *140*, 255-267.

150. Puc, M., Characterisation of pollen allergens. *Ann. Agric. Environ. Med.* **2003,** *10*, (2), 143-150.

151. Schäppi, G. F.; Suphioglu, C.; Taylor, P. E.; Knox, R. B., Concentrations of the major birch tree allergen Bet v 1 in pollen and respirable fine particles in the atmosphere. *J. Allergy Clin. Immunol.* **1997,** *100*, (5), 656-661.

152. Rutter, A. P.; Snyder, D. C.; Schauer, J. J.; Sheesley, R. J.; Olson, M. R.; DeMinter, J., Contributions of resuspended soil and road dust to organic carbon in fine particulate matter in the Midwestern US. *Atmos. Environ.* **2011,** *45*, (2), 514-518.

153. Kundu, S.; Stone, E. A., Composition and sources of fine particulate matter across urban and rural sites in the Midwestern United States. *Environ. Sci. Process Impacts* **2014,** *16*, (6), 1360-1370.

154. Almeida, S.; Pio, C.; Freitas, M.; Reis, M.; Trancoso, M., Source apportionment of atmospheric urban aerosol based on weekdays/weekend variability: evaluation of road re-suspended dust contribution. *Atmos. Environ.* **2006,** *40*, (11), 2058-2067.

155. Amato, F.; Pandolfi, M.; Viana, M.; Querol, X.; Alastuey, A.; Moreno, T., Spatial and chemical patterns of PM_{10} in road dust deposited in urban environment. *Atmos. Environ.* **2009,** *43*, (9), 1650-1659.

156. Tiitanen, P.; Timonen, K.; J Ruskanen, J.; Mirme, A.; Pekkanen, J., Fine particulate air pollution, resuspended road dust and respiratory health among symptomatic children. *Eur. Respir. J.* **1999,** *13*, (2), 266-273.

157. Rogge, W. F.; Hildemann, L. M.; Mazurek, M. A.; Cass, G. R.; Simoneit, B. R., Sources of fine organic aerosol. 2. Noncatalyst and catalyst-equipped automobiles and heavy-duty diesel trucks. *Environ. Sci. Technol.* **1993,** *27*, (4), 636-651.

158. Simoneit, B. R.; Schauer, J. J.; Nolte, C.; Oros, D. R.; Elias, V. O.; Fraser, M.; Rogge, W.; Cass, G. R., Levoglucosan, a tracer for cellulose in biomass burning and atmospheric particles. *Atmos. Environ.* **1999,** *33*, (2), 173-182.

159. Alexander, B.; Hastings, M.; Allman, D.; Dachs, J.; Thornton, J.; Kunasek, S., Quantifying atmospheric nitrate formation pathways based on a global model of the oxygen isotopic composition (Δ 17 O) of atmospheric nitrate. *Atmos. Chem. Phys.* **2009,** *9*, (14), 5043-5056.

160. Khoder, M., Atmospheric conversion of sulfur dioxide to particulate sulfate and nitrogen dioxide to particulate nitrate and gaseous nitric acid in an urban area. *Chemosphere* **2002,** *49*, (6), 675-684.

161. Jayarathne, T.; Rathnayake, C. M.; Stone, E. A., Local source impacts on primary and secondary aerosols in the Midwestern United States. *Atmos. Environ.* **2015**.

162. Malm, W. C.; Schichtel, B. A.; Pitchford, M. L.; Ashbaugh, L. L.; Eldred, R. A., Spatial and monthly trends in speciated fine particle concentration in the United States. *J. Geophys. Res. Atmos.* **2004,** *109*, (D3).

163. Hand, J.; Schichtel, B.; Pitchford, M.; Malm, W.; Frank, N., Seasonal composition of remote and urban fine particulate matter in the United States. *J. Geophys. Res. Atmos.* **2012,** *117*, (D5).

164. Harvell, C. D.; Mitchell, C. E.; Ward, J. R.; Altizer, S.; Dobson, A. P.; Ostfeld, R. S.; Samuel, M. D., Climate warming and disease risks for terrestrial and marine biota. In *Science*, 2002; Vol. 296, pp 2158-2162.

165. Katial, R.; Zhang, Y.; Jones, R. H.; Dyer, P. D., Atmospheric mold spore counts in relation to meteorological parameters. *Int. J. Biometeorol.* **1997,** *41*, (1), 17-22.

166. Hasnain, S. M., Influence of meteorological factors on the air spora. *Grana* **1993,** *32*, (3), 184-188.

167. Ho, H.-M.; Rao, C. Y.; Hsu, H.-H.; Chiu, Y.-H.; Liu, C.-M.; Chao, H. J., Characteristics and determinants of ambient fungal spores in Hualien, Taiwan. *Atmos. Environ.* **2005,** *39*, (32), 5839-5850.

168. Galán, C.; Emberlin, J.; Domínguez, E.; Bryant, R. H.; Villamandos, F., A comparative analysis of daily variations in the Gramineae pollen counts at Córdoba, Spain and London, UK. *Grana* **1995,** *34*, (3), 189-198.

169. Wallner, M.; Erler, A.; Hauser, M.; Klinglmayr, E.; Gadermaier, G.; Vogel, L.; Mari, A.; Bohle, B.; Briza, P.; Ferreira, F., Immunologic characterization of isoforms of Car b 1 and Que a 1, the major hornbeam and oak pollen allergens. *Allergy* **2009,** *64*, (3), 452-460.

170. Emberlin, J.; Detandt, M.; Gehrig, R.; Jaeger, S.; Nolard, N.; Rantio-Lehtimäki, A., Responses in the start of Betula (birch) pollen seasons to recent changes in spring temperatures across Europe. *Int. J. Biometeorol.* **2002,** *46*, (4), 159-170.

171. Niederberger, V.; Pauli, G.; Grönlundc, H.; Fröschla, R.; Rumpold, H.; Kraft, D.; Valenta, R.; Spitzauer, S., Recombinant birch pollen allergens (rBet v 1 and rBet v 2) contain most of the IgE epitopes present in birch, alder, hornbeam, hazel, and oak pollen: a quantitative IgE inhibition study with sera from different populations. *J. Allergy Clin. Immunol.* **1998,** *102*, (4), 579-591.

172. van Vliet, A. J.; Overeem, A.; De Groot, R. S.; Jacobs, A. F.; Spieksma, F. T., The influence of temperature and climate change on the timing of pollen release in the Netherlands. *Int. J. Climatol.* **2002,** *22*, (14), 1757-1767.

173. Taylor, P. E.; Jonsson, H., Thunderstorm asthma. *Curr. Allergy. Asthma Rep.* **2004,** *4*, (5), 409-413.

174. D'Amato, G.; Liccardi, G.; Frenguelli, G., Thunderstorm-asthma and pollen allergy. *Allergy* **2007,** *62*, (1), 11-16.

175. Staff, I.; Schäppi, G.; Taylor, P., Localisation of allergens in ryegrass pollen and in airborne micronic particles. *Protoplasma* **1999,** *208*, (1-4), 47-57.

176. Corden, J. M.; Millington, W. M., The long-term trends and seasonal variation of the aeroallergen Alternaria in Derby, UK. *Aerobiologia* **2001,** *17*, (2), 127-136.

177. Pasanen, A.-L.; Kasanen, J.-P.; Rautiala, S.; Ikäheimo, M.; Rantamäki, J.; Kääriäinen, H.; Kalliokoski, P., Fungal growth and survival in building materials under fluctuating moisture and temperature conditions. *International Biodeterioration & Biodegradation* **2000,** *46*, (2), 117-127.

178. Allitt, U., Airborne fungal spores and the thunderstorm of 24 June 1994. *Aerobiologia* **2000,** *16*, (3-4), 397-406.

179. Packe, G.; Ayres, J., Asthma outbreak during a thunderstorm. *The Lancet* **1985,** *326*, (8448), 199-204.

180. Knox, R., Grass pollen, thunderstorms and asthma. *Clin. Exp. Allergy* **1993,** *23*, (5), 354-359.

181. Marks, G.; Colquhoun, J.; Girgis, S.; Koski, M. H.; Treloar, A.; Hansen, P.; Downs, S.; Car, N., Thunderstorm outflows preceding epidemics of asthma during spring and summer. *Thorax* **2001,** *56*, (6), 468-471.

182. Robertson, B.; Alexander, M., Mode of dispersal of the stem-nodulating bacterium, Azorhizobium. *Soil Biol. Biochem.* **1994,** *26*, (11), 1535-1540.

183. Speranza, A.; Calzoni, G.; Pacini, E., Occurrence of mono-or disaccharides and polysaccharide reserves in mature pollen grains. *Sex. Plant Reprod.* **1997,** *10*, (2), 110-115.

184. Rogge, W. F.; Medeiros, P. M.; Simoneit, B. R., Organic marker compounds in surface soils of crop fields from the San Joaquin Valley fugitive dust characterization study. *Atmos. Environ.* **2007,** *41*, (37), 8183-8204.

185. Simoneit, B. R.; Elias, V. O.; Kobayashi, M.; Kawamura, K.; Rushdi, A. I.; Medeiros, P. M.; Rogge, W. F.; Didyk, B. M., Sugars dominant water-soluble organic compounds in soils and characterization as tracers in atmospheric particulate matter. *Environ. Sci. Technol.* **2004,** *38*, (22), 5939-5949.

186. Medeiros, P. M.; Simoneit, B. R., Source profiles of organic compounds emitted upon combustion of green vegetation from temperate climate forests. *Environ. Sci. Technol.* **2008,** *42*, (22), 8310-8316.

187. USEPA, Technology Transfer Network, http://aqsdr1.epa.gov/aqsweb/aqstmp/airdata/download_files.html#Daily, accessed December **2013**.

188. Pöhlker, C.; Huffman, J.; Pöschl, U., Autofluorescence of atmospheric bioaerosols–fluorescent biomolecules and potential interferences. *Atmos. Meas. Tech.* **2012,** *5*, (1), 37-71.

189. Aloni, B.; Peet, M.; Pharr, M.; Karni, L., The effect of high temperature and high atmospheric CO_2 on carbohydrate changes in bell pepper (Capsicum annuum) pollen in relation to its germination. *Physiol. Plantarum* **2001,** *112*, (4), 505-512.

190. Yoshida, M.; Abe, J.; Moriyama, M.; Kuwabara, T., Carbohydrate levels among winter wheat cultivars varying in freezing tolerance and snow mold resistance during autumn and winter. *Physiol. Plantarum* **1998,** *103*, (1), 8-16.

191. Vesprini, J. L.; Nepi, M.; Cresti, L.; Guarnieri, M.; Pacini, E., Changes in cytoplasmic carbohydrate content during Helleborus pollen presentation. *Grana* **2002,** *41*, (1), 16-20.

192. USEPA, National Ambient Air Quality Standards (NAAQS), http://www3.epa.gov/ttn/naaqs/criteria.html, accessed December 2013. **2013**.

193. Emberlin, J.; Mullins, J.; Corden, J.; Millington, W.; Brooke, M.; Savage, M.; Jones, S., The trend to earlier birch pollen seasons in the UK: a biotic response to changes in weather conditions? *Grana* **1997,** *36*, (1), 29-33.

194. Taylor, P.; Flagan, R.; Miguel, A.; Valenta, R.; Glovsky, M., Birch pollen rupture and the release of aerosols of respirable allergens. *Clin. Exp. Allergy* **2004,** *34*, (10), 1591-1596.

195. Taylor, P. E.; Flagan, R. C.; Valenta, R.; Glovsky, M. M., Release of allergens as respirable aerosols: a link between grass pollen and asthma. *J. Allergy Clin. Immunol.* **2002,** *109*, (1), 51-56.

196. Suphioglu, C.; Singh, M. B.; Taylor, P.; Knox, R.; Bellomo, R.; Holmes, P.; Puy, R., Mechanism of grass-pollen-induced asthma. *The Lancet* **1992,** *339*, (8793), 569-572.

197. Wallis, D. N.; Webb, J.; Brooke, D.; Brookes, B.; Brown, R.; Findlay, A.; Harris, M.; Hulbert, D.; Little, G.; Nonoo, C., A major outbreak of asthma associated with a thunderstorm: experience of accident and emergency departments and patients' characteristics. *BMJ* **1996,** *312*, (7031), 601-604.

198. Pietrogrande, M. C.; Bacco, D.; Visentin, M.; Ferrari, S.; Casali, P., Polar organic marker compounds in atmospheric aerosol in the Po Valley during the Supersito campaigns—Part 2: Seasonal variations of sugars. *Atmos. Environ.* **2014,** *97*, 215-225.

199. Grote, M.; Vrtala, S.; Niederberger, V.; Wiermann, R.; Valenta, R.; Reichelt, R., Release of allergen-bearing cytoplasm from hydrated pollen: a mechanism common to a variety of grass (Poaceae) species revealed by electron microscopy. *J. Allergy Clin. Immunol.* **2001,** *108*, (1), 109-115.

200. Wilson, A. F.; Novey, H. S.; Berke, R. A.; Surprenant, E. L., Deposition of inhaled pollen and pollen extract in human airways. *N. Engl. J. Med.* **1973,** *288*, (20), 1056-1058.

201. Hader, J.; Wright, T.; Petters, M., Contribution of pollen to atmospheric ice nuclei concentrations. *Atmos. Chem. Phys.* **2014,** *14*, (11), 5433-5449.

202. Dales, R. E.; Cakmak, S.; Judek, S.; Dann, T.; Coates, F.; Brook, J. R.; Burnett, R. T., The role of fungal spores in thunderstorm asthma. *CHEST Journal* **2003,** *123*, (3), 745-750.

203. Grundstein, A.; Sarnat, S. E.; Klein, M.; Shepherd, M.; Naeher, L.; Mote, T.; Tolbert, P., Thunderstorm associated asthma in Atlanta, Georgia. *Thorax* **2008,** *63*, (7), 659-660.

204. Anderson, W.; Prescott, G.; Packham, S.; Mullins, J.; Brookes, M.; Seaton, A., Asthma admissions and thunderstorms: a study of pollen, fungal spores, rainfall, and ozone. *QJM* **2001,** *94*, (8), 429-433.

205. Griffing, G. W., Ozone and oxides of nitrogen production during thunderstorms. *J. Geophys. Res.* **1977,** *82*, (6), 943-950.

206. Zhang, R.; Duhl, T.; Salam, M. T.; House, J. M.; Flagan, R. C.; Avol, E. L.; Gilliland, F. D.; Guenther, A.; Chung, S. H.; Lamb, B. K., Development of a regional-scale pollen emission and transport modeling framework for investigating the impact of climate change on allergic airway disease. *Biogeosciences (Online)* **2013,** *10*, (3), 3977.

207. Newson, R.; Strachan, D.; Archibald, E.; Emberlin, J.; Hardaker, P.; Collier, C., Acute asthma epidemics, weather and pollen in England, 1987-1994. *European Respiratory Journal* **1998,** *11*, (3), 694-701.

208. Rodriguez Rajo, F. J.; Iglesias, I.; Jato, V., Variation assessment of airborne Alternaria and Cladosporium spores at different bioclimatical conditions. *Mycol. Res.* **2005,** *109*, (4), 497-507.

209. Rathnayake, C. M.; Metwali, N.; Baker, Z.; Jayarathne, T.; Thorne, P. S.; O'Shaughnessy, P. T.; Stone, E. A., Urban enhancements of bioaerosol tracers in the Midwestern United States. *J. Geophys. Res. Atmos.* **2016,** *121*.

210. Menetrez, M.; Foarde, K.; Esch, R.; Schwartz, T.; Dean, T.; Hays, M.; Cho, S.; Betancourt, D.; Moore, S., An evaluation of indoor and outdoor biological particulate matter. *Atmos. Environ.* **2009,** *43*, (34), 5476-5483.

211. Bonlokke, J. H.; Stridh, G.; Sigsgaard, T.; Kjærgaard, S. K.; Löfstedt, H.; Andersson, K.; Bonefeld-Jørgensen, E. C.; Jayatissa, M. N.; Bodin, L.; Juto, J.-E., Upper-airway inflammation in relation to dust spiked with aldehydes or glucan. *Scand. J. Work. Environ. Health* **2006**, 374-382.

212. Guan, T.; Yao, M.; Wang, J.; Fang, Y.; Hu, S.; Wang, Y.; Dutta, A.; Yang, J.; Wu, Y.; Hu, M., Airborne endotoxin in fine particulate matter in Beijing. *Atmos. Environ.* **2014,** *97*, 35-42.

213. Olenchock, S. A.; May, J. J.; Pratt, D. S.; Piacitelli, L. A.; Parker, J. E., Presence of endotoxins in different agricultural environments. *Am. J. Ind. Med.* **1990,** *18*, (3), 279-284.

214. Spaan, S.; Wouters, I. M.; Oosting, I.; Doekes, G.; Heederik, D., Exposure to inhalable dust and endotoxins in agricultural industries. *J. Environ. Monit.* **2006,** *8*, (1), 63-72.

215. Constantinidou, H.; Hirano, S.; Baker, L.; Upper, C., Atmospheric dispersal of ice nucleation-active bacteria: The role of rain. *Phytopathology* **1990,** *80*, (10), 934-937.

216. Jeter, C.; Matthysse, A. G., Characterization of the binding of diarrheagenic strains of E. coli to plant surfaces and the role of curli in the interaction of the bacteria with alfalfa sprouts. *Mol. Plant. Microbe Interact.* **2005,** *18*, (11), 1235-1242.

217. Lindemann, J.; Constantinidou, H. A.; Barchet, W. R.; Upper, C. D., Plants as sources of airborne bacteria, including ice nucleation-active bacteria. *Appl. Environ. Microbiol.* **1982,** *44*, (5), 1059-1063.

218. Zhang, T.; Engling, G.; Chan, C.-Y.; Zhang, Y.-N.; Zhang, Z.-S.; Lin, M.; Sang, X.-F.; Li, Y.; Li, Y.-S., Contribution of fungal spores to particulate matter in a tropical rainforest. *Environ. Res. Lett.* **2010,** *5*, (2), 024010.

219. Coz, E.; Artíñano, B.; Clark, L. M.; Hernandez, M.; Robinson, A. L.; Casuccio, G. S.; Lersch, T. L.; Pandis, S. N., Characterization of fine primary biogenic organic aerosol in an urban area in the northeastern United States. *Atmos. Environ.* **2010,** *44*, (32), 3952-3962.

220. Redd, S. C., Asthma in the United States: burden and current theories. *Environ. Health Perspect.* **2002,** *110*, (Suppl 4), 557.

221. To, T.; Stanojevic, S.; Moores, G.; Gershon, A. S.; Bateman, E. D.; Cruz, A. A.; Boulet, L.-P., Global asthma prevalence in adults: findings from the cross-sectional world health survey. *BMC Public Health* **2012,** *12*, (1), 1.

222. CDC(2014), Center for Disease Control adn Prevention, http://www.cdc.gov/nchs/fastats/asthma.htm, accessed May 19, 2016.

223. Gilmour, M. I.; Jaakkola, M. S.; London, S. J.; Nel, A. E.; Rogers, C. A., How exposure to environmental tobacco smoke, outdoor air pollutants, and increased pollen burdens influences the incidence of asthma. *Environ. Health Perspect.* **2006**, 627-633.

224. Knox, B.; Suphioglu, C., Environmental and molecular biology of pollen allergens. *Trends Plant Sci.* **1996,** *1*, (5), 156-164.

225. Bachert, C., Histamine-a major role in allergy? *Clin. Exp. Allergy* **1998,** *28*, (6), 15-19.

226. Venables, K.; Allitt, U.; Collier, C.; Emberlin, J.; Greig, J.; Hardaker, P.; Highham, J.; LATNG-MORTON, T.; Maynard, R.; Murray, V., Thunderstorm-related asthma–the epidemic of 24/25 June 1994. *Clin. Exp. Allergy* **1997,** *27*, (7), 725-736.

227. Villeneuve, P. J.; Leech, J.; Bourque, D., Frequency of emergency room visits for childhood asthma in Ottawa, Canada: the role of weather. *Int. J. Biometeorol.* **2005,** *50*, (1), 48-56.

228. Losappio, L.; Heffler, E.; Contento, F.; Cannito, C.; Rolla, G., Thunderstorm-related asthma epidemic owing to Olea Europaea pollen sensitization. *Allergy* **2011,** *66*, (11), 1510-1511.

229. Matthias-Maser, S.; Jaenicke, R., The size distribution of primary biological aerosol particles with radii> 0.2 µm in an urban/rural influenced region. *Atmos. Res.* **1995,** *39*, (4), 279-286.

230. Bacsi, A.; Choudhury, B. K.; Dharajiya, N.; Sur, S.; Boldogh, I., Subpollen particles: carriers of allergenic proteins and oxidases. *J. Allergy Clin. Immunol.* **2006,** *118*, (4), 844-850.

231. Burke, J. J., Moisture sensitivity of cotton pollen. *Agron. J.* **2002,** *94*, (4), 883-888.

232. Pöhlker, C.; Huffman, J. A.; Förster, J.-D.; Pöschl, U., Autofluorescence of atmospheric bioaerosols: spectral fingerprints and taxonomic trends of pollen. *Atmos. Meas. Tech.* **2013,** *6*, (12), 3369-3392.

233. Andrade-Eiroa, Á.; Canle, M.; Cerdá, V., Environmental applications of excitation-emission spectrofluorimetry: an in-depth review II. *Appl. Spectrosc. Rev.* **2013,** *48*, (2), 77-141.

234. Pöhlker, C.; Huffman, J.; Förster, J.-D.; Pöschl, U., Autofluorescence of atmospheric bioaerosols-spectral fingerprints and taxonomic trends of native pollen. *Atmos. Meas. Tech. Discuss.* **2013,** *6*, 5693-5749.

235. Ronneberger, O.; Schultz, E.; Burkhardt, H., Automated pollen recognition using 3D volume images from fluorescence microscopy. *Aerobiologia* **2002,** *18*, (2), 107-115.

236. Grote, M.; Valenta, R.; Reichelt, R., Abortive pollen germination: a mechanism of allergen release in birch, alder, and hazel revealed by immunogold electron microscopy. *J. Allergy Clin. Immunol.* **2003,** *111*, (5), 1017-1023.

237. Shield, W. B. c. a. B., News release,https://www.wellmark.com/AboutWellmark/Newsroom/Releases/_PDF/2009092870thAnniversaryIA.pdf, accessed June 2016. **2006**.

238. Celenza, A.; Fothergill, J.; Kupek, E.; Shaw, R. J., Thunderstorm associated asthma: a detailed analysis of environmental factors. *BMJ* **1996,** *312*, (7031), 604-607.

239. Tobias, A.; Galan, I.; Banegas, J.; Aranguez, E., Short term effects of airborne pollen concentrations on asthma epidemic. *Thorax* **2003,** *58*, (8), 708-710.

240. Anderson, H.; de Leon, A. P.; Bland, J.; Bower, J.; Emberlin, J.; Strachan, D., Air pollution, pollens, and daily admissions for asthma in London 1987–92. *Thorax* **1998,** *53*, (10), 842-848.

241. Barnes, P.; Jonsson, B.; Klim, J., The costs of asthma. *Eur. Respir. J.* **1996,** *9*, (4), 636-642.

242. GINA, Pocket guide for asthma managemint adn prevention,http://ginasthma.org/wp-content/uploads/2016/01/GINA_Pocket_2015.pdf, accessed June 2016. **2015**.

243. Fishe, J. N.; Luberti, A. A.; Master, C. L.; Robinson, R. L.; Grady, M. F.; Arbogast, K. B.; Zonfrillo, M. R., After-hours call center triage of pediatric head injury: outcomes after a concussion initiative. *Pediatr. Emerg. Care* **2016,** *32*, (3), 149-153.

244. Arbogast, K. B.; Curry, A. E.; Pfeiffer, M. R.; Zonfrillo, M. R.; Haarbauer-Krupa, J.; Breiding, M. J.; Coronado, V. G.; Master, C. L., Point of health care entry for youth with concussion within a large pediatric care network. *JAMA Pediatrics* **2016**, e160294.

245. Heslop-Harrison, J.; Heslop-Harrison, Y., Germination of stress-tolerant Eucalyptus pollen. *J. Cell Sci.* **1985,** *73*, (1), 135-157.

246. Gilissen, L., The influence of relative humidity on the swelling of pollen grains in vitro. *Planta* **1977,** *137*, (3), 299-301.

247. Junghans, C.; Feder, G.; Hemingway, H.; Timmis, A.; Jones, M., Recruiting patients to medical research: double blind randomised trial of “opt-in” versus “opt-out” strategies. *BMJ* **2005,** *331*, (7522), 940.

248. Tell, G. S.; Fried, L. P.; Hermanson, B.; Manolio, T. A.; Newman, A. B.; Borhani, N. O., Recruitment of adults 65 years and older as participants in the Cardiovascular Health Study. *Ann. Epidemiol.* **1993,** *3*, (4), 358-366.

249. Frenz, D. A., Comparing pollen and spore counts collected with the Rotorod Sampler and Burkard spore trap. *Ann. Allergy Asthma Immunol.* **1999,** *83*, (5), 341-349.

250. Holmquist, L.; Vesterberg, O., Quantification of birch and grass pollen allergens in indoor air. *Indoor Air* **1999,** *9*, (2), 85-91.