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Abstract: The incidences of allergies, allergic diseases and asthma are increasing world wide. Just as global climate change is likely to alter the plants and animals, bacteria, fungi and other microbial populations will also be impacted. The World Health Organization, U.S. Environmental Protection Agency, U.S. Department of Agriculture, U.S. Department of Health and Human Services, and the Intergovernmental Panel on Climate Change cite increased allergic reactions due to climate change as a growing concern. Monitoring of indoor and ambient particulate matter (PM) and the characterization of the content for biological aerosol concentrations has not been extensively performed. Samples from urban and rural North Carolina (NC), and Denver (CO), very different geographical locations, were collected and analyzed as the goal of this research. A study of PM10 (<10 μm in aerodynamic diameter) and PM2.5 (<2.5 μm in aerodynamic diameter) fractions of ambient bioaerosols was undertaken for a six month period to evaluate the potential for long-term concentrations. These airborne bioaerosols can induce irritational, allergic, infectious, and chemical responses in exposed individuals. Three separate sites were monitored, samples were

collected and analyzed for mass and biological content (endotoxins, (1,3)-b-D-glucan and protein). Concentrations of these bioaerosols were reported as a function of PM size fraction, mass and volume of air sampled. The results indicated that higher concentrations of biologicals were present in PM10 than were present in PM2.5 ambient bioaerosols, except when near-roadway conditions existed. Improving our understanding of ambient bioaerosol concentrations can allow for appropriate exposure avoidance actions to be taken by asthmatics. This study provides the characterization of ambient bioaerosol concentrations in a variety of areas and conditions.

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Air Pollution Prevention
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Dr. H. Singh
Journal of Atmospheric Environment
Editorial Office

Dear Dr. Singh:

I have attached the manuscript, An Evaluation of Indoor and Outdoor Biological Particulate Matter, for publication in the Journal of Atmospheric Environment.

I look forward to hearing your response.

Sincerely yours,

Marc Y. Menetrez, Ph.D.
Environmental Engineer

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An Evaluation of Indoor and Outdoor Biological Particulate Matter

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Abstract

The incidences of allergies, allergic diseases and asthma are increasing world wide. Just as global climate change is likely to alter the plants and animals, bacteria, fungi and other microbial populations will also be impacted. The World Health Organization, U.S. Environmental Protection Agency, U.S. Department of Agriculture, U.S. Department of Health and Human Services, and the Intergovernmental Panel on Climate Change cite increased allergic reactions due to climate change as a growing concern. Monitoring of indoor and ambient particulate matter (PM) and the characterization of the content for biological aerosol concentrations has not been extensively performed. Samples from urban and rural North Carolina (NC), and Denver (CO), very different geographical locations, were collected and analyzed as the goal of this research. A study of PM_{10} (<10 µm in aerodynamic diameter) and $PM_{2.5}$ (<2.5 µm in

aerodynamic diameter) fractions of ambient bioaerosols was undertaken for a six month period to evaluate the potential for long-term concentrations. These airborne bioaerosols can induce irritational, allergic, infectious, and chemical responses in exposed individuals. Three separate sites were monitored, samples were collected and analyzed for mass and biological content (endotoxins, (1,3)-β-D-glucan and protein). Concentrations of these bioaerosols were reported as a function of PM size fraction, mass and volume of air sampled. The results indicated that higher concentrations of biologicals were present in PM_{10} than were present in $PM_{2.5}$ ambient bioaerosols, except when near-roadway conditions existed. Improving our understanding of ambient bioaerosol concentrations can allow for appropriate exposure avoidance actions to be taken by asthmatics. This study provides the characterization of ambient bioaerosol concentrations in a variety of areas and conditions.

Keywords: particulate matter, PM, bioaerosols, endotoxin, protein, (1,3)-β-D-glucan.

Introduction

The incidence of asthma and allergic disease is increasing world wide (Beggs, et al 2005; Breton et al., 2006; Williams, 2005). The increasing exposure to allergens is one consequence of global climate change which is being studied by many research organizations such as the World Health Organization, U.S. Environmental Protection Agency, U.S. Department of Agriculture, U.S. Department of Health and Human Services, and the Intergovernmental Panel on Climate Change (Pawankar, 2008; Naik, 2007). As temperatures increase plant distribution changes in latitude and elevation, and the growing season lengthens in which airborne pollen is produced

(Confalonieri, et al. 2007; Gala, et al. 2005; Garcý´a-Mozo, et al. 2006; Laaidi, 2001; Moorcroft, et al. 2006; Schneiter, et al. 2002; Truong, et al. 2007). Increased concentrations of carbon dioxide can produce increased rates of pollen production (Emberlin, et. al. 2003; Naik, 2007; Petee, 2000; Steinman, et al. 2003; Stach, et al. 2007). An example of increased pollen production found in urban environments is ragweed pollen concentration which can increase to five times of that found in rural environments (Naik, 2007; Wayne, et al. 2002; Ziska, et al. 2003). Areas that experience increased humidity can also have an increase in airborne mold spores (Pawankar, 2008; Puc, 2002; Weryzko-Chmielewska, 2006). Exposure to the increasing burden of allergens increases the incidence of asthma (Gilmour, et al. 2006). This impact to human health and global climate change make the monitoring of allergens an important and necessary parameter.

The study of exposure to both ambient and indoor aerosolized particulate matter has been the subject of intense activity. Associations between morbidity and population exposures to airborne particles are the subject of analytical models (Knight, 2002; Thorne, 2000; Wilson and Spengler, 1996). Airborne bioaerosols are a major component for evaluating total long-term personal exposures to both PM_{10} (<10 μm in aerodynamic diameter) and $PM_{2.5}$ fraction (<2.5 μm in aerodynamic diameter) particulate matter (PM)(Samet and Spengler, 1991). Exposure to ambient PM may occur from outdoor and indoor air. PM originating from ambient sources and penetrating into the indoor environment can expose individuals while being indoors (Samet and Spengler, 1991).

Bioaerosols or particulate matter (PM) which are biological in origin (BioPM) are predominantly comprised of plant pollen and microorganisms (mold and bacteria) or microbial metabolites

(Menetrez, et al. 2000; Menetrez, et al. 2001) and can induce allergic, toxic, and infectious responses in exposed individuals. Symptoms of exposed individuals include coughing, wheezing, runny nose, irritated eyes or throat, skin rash, diarrhea, aggravation of asthma, headache, and fatigue. Immunological reactions can include asthma, allergic rhinitis, and hypersensitivity pneumonitis (Husman, 1996; Levetin, 1995; Schwartz, et al. 1995; Targonski, et al. 1995). It is also well established that exposure to bioaerosol can result in adverse health effects in a large percentage of the population (Husman, 1996; Levetin, 1995; Schwartz, et al. 1995; Targonski, et al. 1995). Data suggests that biological sources of PM account for between 5 to 10% of the urban and rural aerosol composition (Menetrez, et al. 2007a; Menetrez, et al. 2007b; Monn, 2001; Womiloju, et. al. 2003).

Particulate matter of biological origin has been shown by Salvaggio and Aukrust (1981) to be made up of fungi, bacteria, plant pollen, and spore material, all of which have been linked to allergic symptoms. Although pollen is widely studied as an aeroallergen, comparably little is known about ambient concentrations of fungal spores. Salvaggio and Aukrust (1981) indicated *Cladosporium* spores outnumbered pollen spores in ambient air at a ratio of approximately 1000 to one. Aerometric sampling devices have collected spores from 20,000 to 40,000 species of fungi, bacteria, and actinomycetes. Of these, four major groups have been identified as being potential allergens: Phycomycetes, Ascomycetes, Basidiomycetes, and Deuteromycetes (Salvaggio and Aukrust, 1981).

Bacteria and fungi are important components of outdoor, or atmospheric, aerosols in addition to being important components of indoor aerosols. Desiccated non-viable fragments of microbial organisms are also common. These fragments have been identified in the sub-micron size range.

Studies indicated that a sizable fraction of both coarse and fine PM in both indoor and outdoor samples were of biological origin (Menetrez, et al. 2000; U.S. EPA, 2005). The biological component can be identified specifically by type or component (endotoxins) as in Menetrez, et al. (2000), or collectively accounted for in the measurement of protein concentrations [primarily made up of whole and fragmented mold spores, bacteria, and pollen]. Endotoxins are part of the outer membrane of the cell wall of gram-negative bacteria. Endotoxin is associated with gram-negative bacteria whether the organisms are pathogenic or not. Fragments can remain toxic or allergenic depending upon the specific organism or organism component. Although these bioaerosols have been identified, they have not been extensively studied for their prevalence in PM.

Exposure to ambient PM has been associated with adverse health effects. The exact constituents of PM air pollution that cause disease and the mechanisms involved are unknown. Studies to determine the components or characteristics of PM which contribute to airway inflamation and irritation have been attempted (Bonner, et al.1998; Donaldson and MacNee, 2001; Gilmour, et al. 2006; Ghio, et al. 2000; Soukup and Becker, 2001). Endotoxin, mold, and allergens produce the health effects associated with asthma triggers and are linked to adverse health effects (Mueller-Anneling, et al. 2004). However, one study found endotoxin exposure to be more significantly associated with the clinical severity of asthma (Michel, et al.1996). Aerodynamic size fractions of PM have been studied including coarse ($PM_{10-2.5}$) fine ($PM_{2.5}$), and submicrometer fractions have been studied (Pope, 1999). Yet the coarse PM fraction remains recognized as being associated with significant adverse effects on the bronchiolar region of conducting airways which remains the primary site of asthma and associated airway inflammation (Monn and

Becker, 1999; Soukup and Becker, 2001).

The goal of the study was to monitor and compare the levels of BioPM present in a variety of different environments. An urban site in Denver, CO, was monitored for indoor and outdoor $PM_{2.5}$, and the filters analyzed for mass, endotoxin and (1,3)-β-D-glucan (an indicator of mold); a rural site in Orange County, NC, was monitored for outdoor $PM_{2.5}$ and PM_{10}, and analyzed for mass, endotoxins, (1,3)-β-D-glucan and protein; and lastly, an urban site in Raleigh, NC, in close proximity to an interstate highway was monitored with a 15 stage particle impactor and analyzed for mass and protein. The data were reported as a function of PM mass and volume of air. This paper discusses the analysis and results of measurement of these bioaerosol parameters.

Materials and Methods

This study collected PM filter samples in three locations that are discussed individually below. Filters were equilibrated and weighed for comparison with exposed filters for recording mass gain. The unused filters were stored in the plastic petri dishes until needed.

A minimum of 12% of the total anticipated number of filters were prepared as blanks. One blank was carried to the field and returned with the technician (transport blank) and one blank remained in the laboratory hood (laboratory blank). The remaining blanks remained in the environmental chamber in the laboratory and were not removed. In order to control for contaminants, the blank filters were analyzed in the same manner as the air sample filters.

Filter Analysis

After samples were collected, filters were placed in a petri dish, the dish was labeled and the date and volume of air sampled were recorded. Filters were then weighed following a 24 hour equilibration period, and their total mass load determined. Filter gravimetric change was recorded with a calibrated Mettler-Toledo MX-5 microbalance using the instrument's internal calibration procedure. Documentation of the filter weight and volume of air filtered through each filter was archived. After weighing, each filter was placed back into the petri dish, and put back into the environmental chamber until analysis. At the time of analysis the filter was suspended in sterile, pyrogen-free, glucan-free water and analyzed for total protein. Each filter sample was placed into a separate sterile container with 30 mL buffered detergent, and shaken vigorously for 30 minutes to elute.

Sample Collection

Denver, CO (Indoor/Outdoor $PM_{2.5}$)

Filter samples were collected from January 23, 2001 through March 22, 2001 (59 days). The 18 indoor samples were produced from samplers located within a primary school facility while the 25 outdoor samples were produced from samplers located just outside the school facility. The $PM_{2.5}$ ambient particle samples were collected using custom-fabricated 2 L/min field-ready particulate sampling pumps (RTI International, RTP, NC) coupled with Personal Environmental Monitors (PEM™) manufactured by MSP (Shoreview, MN). The air samples were collected on 37 mm Poly-Tetra-Fluoro-Ethylene (PTFE) filters (Pall Corporation, East Hills, NY, Teflo 37 mm, Cat. No. R2P-J037). The rate of flow of the samplers varied between 1.94 and 2.05 L/min., generating air sample volumes ranging from 2.73 to 2.95 m^3.

Rural North Carolina (Orange County) Site (Outdoor PM_{10} and $PM_{2.5}$)

Filter samples were collected from March 18, 2007 through May 16, 2007 (59 days). The rural North Carolina (Orange County) site is 4 miles from downtown Hillsborough and 8 miles from downtown Carrboro, 200 feet from a private gravel road in a mixed hardwood-pine forest (80 feet from a two-story residence). PM_{10} and $PM_{2.5}$ ambient particle samples were collected using field-ready particulate sampling units and corresponding cyclones manufactured by URG (Carrboro, NC). The rate of flow of the samplers was constant at 16.7 L/min. The air samples were collected on 47 mm PTFE filters (Pall Corporation, East Hills, NY, Teflo 47 mm, Cat. No. R2P-J047). The samplers were located in a wooded area approximately 1 meter from the ground. The total volume of air sampled varied with the length of the collection period and ranged from 10.3 to 24.4 m^3. Blanks were carried to the field and loaded into the sampling filter holders in the same manner used for other filters, but received no air flow from the device. All PM_{10} and $PM_{2.5}$ filter samples were analyzed for mass (μg), (1,3)-β-D-glucan (pg), endotoxin (EU), and protein (ng).

City of Raleigh Site (Outdoor 15 stage impactor)

A site in Raleigh, NC, provided an area with air quality which was heavily influenced by an adjacent major road, specifically U.S. Interstate 440 (I-440 is a limited-access highway supporting approximately 125,000 vehicles per day) (Baldauf, et al., 2008). Filter samples were collected in Raleigh, NC, on July 27, 2006 through July 28, 2006 (1 day). At the time of the filter samples collected for this study, this site was also monitored as part of a larger study of

ambient air quality and near-roadway impacts (Baldauf, et al. 2008). The air samples were collected using a DEKATI Low-Pressure Impactor (Particle Instruments LLC, Vadnais Heights, MN). The filters were 25#mm PTFE membrane filters (Pall Corporation, East Hills, NY, Teflo 25 mm, Gelman Cat. No. 60048). The rate of flow of the sampler was 29.3 L/m to 29.9 L/m (29.6 L/m average) for a period from 14:50 to13:15 over a 22 hour 35 min. period (1,355 min. period and 40,108 L of total flow). Instrument pressure and rate of flow were calibrated before and after sampling. Samples were collected by EPA personnel and delivered for laboratory analysis. Filter samples were analyzed for mass (μg), and protein (ng). With the exception of the highway, no other major air pollution sources were identified within several kilometers of the study site.

Gravimetric Analysis

Prior to ambient air sampling, each filter was removed from the manufacturer's packaging using flame-sterilized forceps and placed into individually labeled sterile polystyrene Analyslide containers (Pall, East Hills N.Y.). The Analyslide containers were then placed into a controlled environmental chamber for approximately 48 hours undisturbed to allow the filters to equilibrate prior to gravimetric weighing and sample recording. The chamber was maintained at a temperature of 20 to 23 ^{o}C ± 2 ^{o}C, and relative humidity of 30 to 40% ± 5%.

After samples were collected, each filter was returned directly to its Analyslide , and the date and volume of air sampled were recorded. Filters were then weighed, again following a 48 hour equilibration period, and their total mass load determined. Filter gravimetric change was measured with a Mettler-Toledo UMX-2 microbalance using the instrument's internal calibration

procedure. Documentation of the filter weight and volume of air drawn through each filter was archived. After weighing, each filter was placed back into the Analyslide until biological analysis was performed.

BioPM Analysis

One of the goals of the study was to utilize a single filter to quantify mass and to conduct all biological analyses. Thus, contents on the filters used for gravimetric analysis were extracted simultaneously for analysis of endotoxin, (1,3)-β-D-glucan, and protein. Each filter from the rural North Carolina (Orange County) and the city of Raleigh site was placed into a separate sterile container with 5 mL of pyrogen-free 0.01% triethylamine and shaken vigorously in a Vibrax VXR platform shaker (IKA Works Inc., Wilmington, NC) for 30 minutes to elute the collected particles. Each filter from the Denver site was extracted by shaking with 2.5 mL of pyrogen-free 0.01% triethylamine. Extracts were removed to duplicate depyrogenated borosilicate glass tubes and stored at -20 ^{o}C. Endotoxin and β-1,3 glucan analyses were performed before the samples were frozen.

Endotoxin Analysis

Endotoxin levels were quantified using a kinetic chromogenic *Limulus* amebocyte lysate (LAL) assay (Associates of Cape Cod, Woods Hole, MA) following the manufacturer's instructions. To avoid detection of β-glucans present, the lysate was resuspended in Glucashield buffer supplied by the manufacturer. The level of endotoxin activity in a sample was determined by the reaction of endotoxins in the specimen with the lysate and a substrate, producing a color change over time, and comparing rate of color change to similar reactions of known endotoxin reference

standards in 0.01% triethylamine. The level of color change was quantified by measuring the absorbance at 405 nm using a BioTek ELx808 microplate reader and Gen5 software package (BioTek Instruments Inc., Winooski, VT). This assay is sensitive to endotoxin activity as low as 0.005 EU/mL. When the maximum interpretable color development rate was exceeded, the extract was diluted and reanalyzed.

β- 1,3 Glucan Analysis

To assess the viable and non-viable mold content of sample mass the (1,3)-β-D-glucan levels were quantified using a chromogenic β-glucan-specific *Limulus* amebocyte lysate (LAL) assay (Associates of Cape Cod, Woods Hole, MA.) following the manufacturer's instructions. To facilitate specific detection of β-glucans present, this assay employs a modified, Glucatell, lysate formulation. Otherwise this assay was conducted as the endotoxin assay described above with the exception that the rate of color development measured by absorbance at 405 nm is compared to β-glucan standards, diluted in 0.01% triethylamine, to determine quantities of β-glucan in extracted samples.

Protein Analysis

As a general indicator of the biological content of PM, protein was measured. For the protein analysis, aliquots from each eluted sample were analyzed using the Nano-Orange Protein Quantification Kit (Molecular Probes, Eugene, OR) following the manufacturer's instructions. Quantification of the level of protein in a sample was obtained by the reaction of protein in the specimen with a diluted Nano-Orange agent in a microtiter plate. Following addition of samples

and reagents, the plate is sealed with foil tape, heated to 95.0 ^{o}C for 10 minutes, allowed to cool to room temperature, and then centrifuged to restore any condensate to the corresponding wells. A fluorescent reaction was produced which was compared with those produced by bovine serum albumin standards in 0.01% triethylamine. The level of fluorescence was measured using a Genios Multilabel Plate Reader (Tecan US, RTP, NC).

Results

A total of 71 filter samples of outdoor and indoor air were analyzed. Table 1 provides a summary of some of the key study parameters from each of the three sample sites. Figures 1 through 5 displays more detailed results for the monitoring periods specified above. Both the Figures and Table 1 illustrates the results of these bioaerosols reported as a function of the volume of air sampled.

Denver, CO *(Indoor/Outdoor $PM_{2.5}$)*

Figure 1 illustrates the results of both indoor and outdoor samples analyzed for PM mass in micrograms per cubic meter (µg/m^3). In Figure 1, the indoor concentrations peaked during the 2/26/2001 sample at 28.23 µg/m^3, and reached a low concentration during the 3/6/2001 sample at 3.47 µg/m^3 (mean 8.52 µg/m^3, standard deviation 6.47 µg/m^3 and n=18). In Figure 1, the outdoor concentrations peaked during the 2/14/2001 sample at 22.01 µg/m^3, and reached a low concentration during the 3/1/2001 sample at 6.21 µg/m^3 (mean 12.80 µg/m^3, standard deviation 5.44 µg/m^3 and n=25).

Figure 2 illustrates the results of both indoor and outdoor samples analyzed for PM endotoxin in

endotoxin units per cubic meter (EU/m^3). In Figure 2, the indoor concentrations peaked during the 3/7/2001 sample at 0.0129 EU/m^3, and reached a low concentration during the 1/24/2001 sample at 0.0022 EU/m^3 (mean 0.0092 EU/m^3, standard deviation 0.0069 EU/m^3, and n=18). In Figure 2, the outdoor concentrations peaked during the 3/7/2001 sample at 0.0190 EU/m^3, and reached a low concentration on occasions during the 1/24/2001, 2/6/2001, 2/7/2001, 2/14/2001 and 2/26/2001 during which the results were below the limit of detection or BDL (mean 0.0057 EU/m^3, standard deviation 0.0059 EU/m^3 and n=20). The five BDL sample results were listed as zero in Figure 2, but were excluded from the calculation of mean and standard deviation.

Figure 3 illustrates the results of both indoor and outdoor samples analyzed for (1,3)-β-D-glucan levels in picograms per cubic meter (pg/m^3). In Figure 3, the indoor concentrations peaked during the 1/24/2001 sample at 29.58 pg/m^3, and reached a low concentration during the 1/24/2001 and 3/5/2001 sample at 0.26 pg/m^3 (mean 5.84 pg/m^3, standard deviation 6.91 pg/m^3 and n=18). In Figure 3, the outdoor concentrations peaked during the 2/14/2001 sample at 33.61 pg/m^3, and reached a low concentration during 15 samples that were below the limit of detection (mean 8.87 pg/m^3, standard deviation 10.61 pg/m^3, and n=10).

Rural North Carolina (Orange County) Site (Outdoor PM_{10} and $PM_{2.5}$)

Figure 4 shows the results of both PM_{10} and $PM_{2.5}$ outdoor samples analyzed for PM mass in micrograms per cubic meter (µg/m^3), (1,3)-β-D-glucan levels (pg/m^3), endotoxin (EU/m^3), and protein (µg/m^3). In Figure 4 the PM_{10} mass concentrations peaked during the 3/19/2007 sample at 32.50 µg/m^3, and reached a low concentration during the 3/29/2007 sample at 7.70 µg/m^3

(mean 18.20 μg/m^3, standard deviation 7.40 μg/m^3 and n=10). The $PM_{2.5}$ mass concentrations peaked during the 5/11/2007 sample at 12.10 μg/m^3, and reached a low concentration during the 5/14/2007 sample at 7.50 μg/m^3 (mean 9.30 μg/m^3, standard deviation 2.40 μg/m^3 and n=3).

In Figure 4 the PM_{10} (1,3)-β-D-glucan levels peaked during the 4/2/2007 sample at 87.20 pg/m^3, and reached a low concentration during the 3/18/2007 sample at 7.90 pg/m^3 (mean 33.60 pg/m^3, standard deviation 24.50 pg/m^3 and n=10). The $PM_{2.5}$ (1,3)-β-D-glucan levels peaked during the 5/11/2007 sample at 60.10 pg/m^3, and reached a low concentration during the 5/13/2007 sample at 29.70 pg/m^3 (mean 40.00 pg/m^3, standard deviation 17.40 pg/m^3 and n=3).

In Figure 4 the endotoxin concentrations peaked during the 3/20/2007 sample at 0.080 EU/m^3, and reached a low concentration during the 3/29/2007 sample at 0.024 EU/m^3 (mean 0.051 EU/m^3, standard deviation 0.016 EU/m^3 and n=10). The $PM_{2.5}$ endotoxin concentrations peaked during the 5/11/2007 sample at 0.037 EU/m^3, and reached a low concentration during the 5/13/2007 sample at 0.010 EU/m^3 (mean 0.023 EU/m^3, standard deviation 0.014 EU/m^3 and n=3).

In Figure 4 the PM_{10} protein concentrations peaked during the 3/31/2007 sample at 0.043 μg/m^3 (accounting for 4% of the sample mass), and reached a low concentration during the 4/9/2007 sample which was below the limit of detection (mean 0.274 μg/m^3, standard deviation 0.078 μg/m^3 and n=9). The $PM_{2.5}$ protein concentrations peaked during the 5/13/2007 sample at 0.014 μg/m^3 (accounting for 1.3% of the sample mass), and reached a low concentration during the

5/14/2007 sample which was below the limit of detection (mean 0.095 μg/m^3, standard deviation 0.064 μg/m^3 and n=2).

All four parameters indicated higher biological content for PM_{10} than for $PM_{2.5}$.

City of Raleigh Site (Outdoor 15 stage impactor)

Results of the 15 stage [from 0.011 to 10 μm aerodynamic diameter (AD)] filter samples taken on July 27, 2006 through July 28, 2006, were analyzed and are depicted in Figure 5 for PM mass (μg/m^3), and protein (μg/m^3). Figure 5 illustrates the sample mass peaking at 275 μg, at the 7 μm AD impactor size stage. However, the peak protein concentration was 40.85 μg, at the 1 μm AD size stage, which accounted for 27.6% of the 148 μg sample mass at this stage. This protein peak is less dramatic but also apparent in both adjoining size stages (8% at 0.6 μm AD, and 6 % at 1.75 μm AD). This percent of protein mass was greater than the peak of 4% protein found at the rural North Carolina (Orange County) site.

Of the 15 sample stages, the protein analyses of six were below the limit of detection. These were predominantly for sample stages below 0.5 μm AD, where sample mass was less than 100 μg.

Discussion

The sources of protein may be any combination of pollen, mold, bacteria, insect debris, fecal matter, or dander in large nucleated particles (such as PM_{10} or greater), or small respirable fragments ($PM_{2.5}$) (Verhoeff, et al.1992; Targonski, et al. 1995). Proteins are one of the most

potentially antigenic biological materials to which the immune system can respond. However, not all proteins are allergenic. Some components of protein PM when inhaled stimulate alveolar macrophages and respiratory epithelial tissue to release cytokines or chemattractants that initiate an inflammatory cascade (Thorne, 2000). The N.C. Department of Environmental and Natural Resources data base lists elevated pollen concentrations from 3/26/2007 to 4/8/2007, part of the sampling period during which samples were collected in rural North Carolina (Orange County) (NCDENR, 2008). Additional monitoring in a comprehensive study would be necessary to understand the impact of season pollen variation, or changing trends in pollen concentration, and seasonal and spacial distribution. Although seasonal variations of bioaerosols would affect the concentrations and constituent ratios of ambient BioPM, documenting the effects of seasonal variation was beyond the purpose of this study.

A study of Mexico City (Rosas, et al. 1995) examined the protein content of coarse fraction $PM_{10-2.5}$ samples. The study found protein concentrations of 2.54 $\mu g/m^3$ associated with airborne particle concentrations from 70.2 to 108.9 $\mu g/m^3$, in an urban-industrial site. The protein concentration found in Mexico City was similar to the range of 1 to 4% that was found by Menetrez, et al. (2007a, and 2007b) in studies in Chapel Hill, NC. This protein concentration also was in agreement with the peak PM_{10} protein content of 4% found at the rural Orange County site. However, the peak protein concentrations of 27.6%, 8% and 6% for samples within the range of $PM_{2.5}$ found at the Raleigh near-roadway site were significantly greater than the Orange County findings.

The seasonal variations in bioaerosols can possibly account for part of the differences in protein

data. The rural North Carolina (Orange County) site data being collected during March to May and the City of Raleigh site data being collected in July would be expected to have inherent differences. In addition, the near roadway impact of the City of Raleigh site may have had some effect on the high concentrations of protein found within a smaller than expected size range. Motor vehicles can influence the temporal and spatial patterns of PM. Emissions from motor vehicle operations near major roads have led to elevated concentrations of air pollutants (Baldauf, et al. 2008).

Studies have singled out endotoxin as the most significant component associated with the development and progression of airway disease. Endotoxin is recogized as an occupational hazard in agricultural and manufacturing industries (Schwartz, et al.1995; Douwes, et al. 2003, 2003). A significant association has been found between endotoxin and the clinical severity of asthma (Michel, et al. 1996).

The airborne $PM_{2.5}$ endotoxin concentrations measured at the Denver, CO site and the rural North Carolina (Orange County) site differed with Denver being lower. The rural North Carolina levels were similar to those reported in Menetrez, et al. (2007a). Undoubtably the seasonal difference was important. The PM_{10} in rural North Carolina were higher than the $PM_{2.5}$. Somewhat higher levels of endotoxin of 0.64 EU/m^3 have been reported from airborne total particulate (no size fraction) samples from polycarbonate filters (Milton, 2000).

Glucans are glucose polymers which are structural components of most fungal cell walls and are an indicator of the presence of mold (Burge and Ammann, 1999). In the bioassay results of outdoor samples from the rural North Carolina (Orange County) site (1,3)-β-D-glucan levels

peaked at 87.20 pg/m^3. This level is considerably lower in comparison to results of (1,3)-β-D-glucan levels recorded in a mold remediated indoor environment of 1,400 pg/m^3 (Rylander and Lin, 2000), but similar to 100 to 200 pg/m^3 measured in non-problem indoor environments (Li and Wan, 1999).

Conclusions

This study monitored and compared three environments; an urban site in Denver, CO, for indoor and outdoor $PM_{2.5}$, and analyzed for mass, endotoxin and (1,3)-β-D-glucan; a rural site in Orange County, NC, for outdoor $PM_{2.5}$ and PM_{10}, and analyzed for mass, endotoxins, (1,3)-β-D-glucan and protein; and an urban near-roadway site in Raleigh, NC, and analyzed for mass, and protein. PTFE filters were universally used at each of three sites, but used different collection devices for sampling.

While there were a number of similarities between the sites, the differences may be more instructive. Results for endotoxin, (1,3)-β-D-glucan, and protein outdoor $PM_{2.5}$ were quite different between the Denver and rural NC study sites; however, the mass numbers were similar. The results for protein concentrations in rural Orange County, NC, were in agreement with the peak $PM_{10-2.5}$ protein content of 1 to 4% found by Menetrez, et al. (2007a; 2007b), and Rosas (1995). However, samples obtained at the Raleigh near-roadway site exhibited peak protein concentrations of 27.6%, 8% and 6% within the range of $PM_{2.5}$ and were significantly greater than previous findings in that geographic area for ambient protein bioaerosols. The seasonal or near roadway impacts of the City of Raleigh site may have effected the high concentrations of protein found within a smaller than expected size range. Seasonal variation in North Carolina

(especially in March-April) can account for significant fluctuations in airborne pollen which could affect protein measurements such as those taken in Orange County, NC.

Studies have suggested that aeroallergens may be statistically associated with hospital admissions for asthma and may act as confounding factors in epidemiologic studies (Rosas, et al.1998). The concentrations of ambient bioaerosols documented in this study can help to more fully understand exposure to airborne biological allergens. These allergens can be significant for asthmatics and those with impaired pulmonary function.

Understanding the concentrations and distribution of airborne allergens can assist in our ability to cope with the reality of global warming. While measures are being taken to reduce and reverse the debilitating environmental effects of global warming, monitoring airborne allergens can help improve public health awareness and serve as a spatial indicator of climate change. A comprehensive monitoring study of seasonal protein variation and changing trends would contribute to the understanding of airborne BioPM. Comprehensive PM monitoring programs should incorporate protein as a measurement parameter as a general indicator of now much of the PM is biological in origin.

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trade names or commercial products does not constitute endorsement or recommendation for use.

References

Baldauf, R, Thoma, E., Isakov, V., Long, T., Weinstein, J., Gilmour, I., Cho, S., Khlystov, A., Chen, F., Kinsey, J., Hays, M., Seila, R., Snow, R., Shores, R., Olson, D., Gullett, B., Kimbrough, S., Watkins, N., Rowley, P., Bang, J., Costa, D. (2008). Traffic and Meteorological Impacts on Near Road Air Quality: Summary of Methods and Trends from the Raleigh Near Road Study, Journal of Air and Waste Management, 58; 7. 865, July 2008.

Beggs, P. J., Bambrick, H. J. (2005). Is the global rise of asthma an early impact of anthropogenic climate change? Environmental Health Perspectives, 113:915Y919.

Breton, M. C., Garneau, M., Fortier, I., Guay, F, Louis, J. (2006) Relationship between climate, pollen concentrations of Ambrosia and medical consultations for allergic rhinitis in Montreal, 1994Y2002. Science of the Total Environment, 370:39Y50.

Bonner, J. C., Rice, A. B., Lindroos, P. M., O'Brian, P. O., Dreher, K. L. and Rosas, I. (1998). Introduction of the lung myofibroblast PDGF receptor system by urban ambient particles from Mexico City, American Journal of Respir. Cell. Mol. Biology, 19(4); 672-680.

Burge, H. A. and Ammann, H. M. (1999). Fungal toxins and (1,3)-β-D-glucan; Bioaerosols: Assessment and Control, ACGIH, Chapter 24, 24.7.1.

Confalonieri, U., Menne, B., Akhtar, R., Ebi, M., Hauengue, R. S., Kovats, B. (2007). Human

health. In: Parry ML, Canziani OF, Palutikof JP, van der Linden PJ, Hanson CE, eds. Climate Change 2007: Impacts, Adaptation and Vulnerability. Contribution of Working Group II to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change, Cambridge, UK: Cambridge University Press; 2007:391Y431.

Donaldson, K. and MacNee, W. (2001). Potential mechanism of adverse pulmonary and cardiovascular effects of particulate air pollution (PM10), International Journal of Hygiene and Environmental Health, 203(5-6); 411-415.

Douwes, J., Thorne, P., Pearce, N. and Heederick, D. (2003). Bioaerosol health effects assessment progress and prospects, Ann. Occupational Hygiene, 47(3); 187-200.

Emberlin, J., Detandt, M., Gehrig, R., Jaeger, S., Nolard, N, Rantio-Lehtima¨ki, A. (2002). Responses in the start of Betula (birch) pollen seasons to recent changes in spring temperatures across Europe, International Journal of Biometeorol, 2002;46:159Y170.

Gala´n, C., Garcý´a-Mozo, H., Va´zquez, L., Ruiz, L., de la Guardia, C.D., Trigo, M. M. (2005). Heat requirement for the onset of the *Olea europaea L.* pollen season in several sites in *Andalusia* and the effect of the expected future climate change, International Journal of Biometeorol, 49:184Y188.

Garcý´a-Mozo, H., Gala´n, C., Jato, V., Belmonte, J., Diaz de la Guardia, C., Fernandez, D. (2006). Quercus pollen season dynamics in the Iberian Peninsula: response to meteorological

parameters and possible consequences of climate change, Ann Agricultural Environment Med. 13:209Y224.

Gilmour, M. I., Jaakkola, M. S., London, S. J, Nel, A. E., Rogers, C. A.(2006). How exposure to environmental tobacco smoke, outdoor air pollutants, and increased pollen burdens influences the incidence of asthma. Environmental Health Perspectives,114:627Y633.

Ghio, A. J., Kim, C., Devlin, R. B. (2000). Concentrated ambient air particles induce mild pulmonary inflammation in healthy human volunteers, American Journal of Respiratory Critical Care Medicine, Sep;162 (3 Pt 1):981-8.

Husman, T. (1996). Health effects of indoor-air microorganisms. *Scand. Journal of Work Environ. Health,* 22:5-13.

Knight, J. (2002). Statistical error leaves pollution data up in the air. Nature, 417, 677.

Laaidi, K. (2001). Predicting days of high allergenic risk during Betula pollination using weather types. International Journal of Biometerology, 45:124Y132.

Levetin, E. (1995). Fungi, *Bioaerosols*, Burge, H., Editor, CRC Press, Boca Raton, FL, 1995.

Li, C. and Wan G. (1999, May 1). Indoor endotoxin and glucan in association with airway inflammation and systemic symptoms, Articles of Environmental Health.

Menetrez, M.Y., Foarde, K.K. and Ensor, D.S. (2000). Fine biological PM: Understanding size fraction transport and exposure potential (Extended Abstract). The Air and Waste Management Association Specialty Conference, PM2000: Particulate Matter and Health- The Scientific Basis for Regulatory Decision-making, Charlestown, SC. Jan. 24-26.

Menetrez, M. Y., Foarde, K. K. and Ensor, D. S. (2001). An analytical method for the measurement of nonviable bioaerosols, Journal of the Air & Waste Management Association, 51:1436-1442.

Menetrez, M. Y., Foarde, K. K., Webber, T. D., Dean, T. R. and Betancourt, D. A. (2007a). An evaluation of the protein mass of particulate matter, Atmospheric Environment, doi10.1016/j.atmoseny,2007.06.021.

Menetrez, M. Y., Foarde, K. K., Webber, T. D., Dean, T. R., Betancourt, D. A., Moore, S. A., Svendsen, E. R. and Yeatts, K. (2007b). The measurement of ambient bioaerosol exposure. Aerosol Science and Technology, 41; 884-893.

Michel, O., Kips, J., Duchateau, J., Vertongen, F., Robert, L., Collet, H., Pauwels, R. and Sergysels, R. (1996). Severity of asthma is related to endotoxin in house dust. Am. Journal of Respir. Crit. Care Med., 154(6, Pt. 1): 1641-1646.

Milton, D. K. (1999). Endotoxin and other bacterial cell-wall components; Bioaerosols:

Assessment and Control, ACGIH, Chapter 23, 23.1.2.

Monn, C. and Becker, S. (1999). Cytotoxicity and induction of proinflammatory cytokins from human monocytes exposed to fine ($PM_{2.5}$) and course (PM_{10}) in outdoor and indoor air, Toxicol. Appl. Pharmacol.,155(3); 245-252.

Monn, C. (2001). Exposure assessment of air pollutants: a review on spatial heterogeneity and indoor/outdoor/personal exposure to suspended particulate matter, nitrogen dioxide and ozone, Atmospheric Environment, 35, 1-32.

Moorcroft, P. R., Pacala, S. W., Lewis, M. A. (2006). Potential role of natural enemies during tree range expansions following climate change. Journal of Theoretical Biology, 241:601Y616.

Mueller-Anneling, L., Avol, E., Peters, J.M., and Thorne, S. (2004). Ambient endotoxin concentration in PM_{10} from Southern California. Environmental Health Perspectives, 112: 51, 583-588.

Naik, G. (2007). Global Warming May Be Spurring Allergy, Asthma, The Wall Street Journal.

NCDENR, Data, Statistics, Reports, and Maps (http://www.enr.state.nc.us/htmr/data.html), 2008.

Pawankar, R, Baena-Cagnani, C.E., Bousquet, J., Canonica, G.W., Cruz, A.A., Kaliner, M.A., and Lanier, B.Q. (2008). State of World Allergy Report 2008: Allergy and Chronic Respiratory

Disease, Journal of World Allergy Organization, S4-S17.

Peteet, D (2000). Sensitivity and rapidity of vegetational response to abrupt climate change. Proceedings of the National Academy of Science U S A., 97:1359Y1361.

Pope, C. A. (1999). Mortality and air pollution associations persist with continued advances in research methodology. Environmental Health Perspectives, 107: 613-614.

Puc, M., Wolski, T. (2002). Betula and Populus pollen counts and meteorological conditions in Szczecin, Poland, Ann Agricultural Environment Med., 9:65Y69.

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

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

Rylander, R. and Lin, R. (2000). (1,3)-β-D-Glucan relationship to indoor air-related symptoms, allergy and asthma, Journal of Toxicology, 152;1-3, 2, 47-52.

Salvaggio, J. and Aukrust, L. (1981). Mold-Induced Asthma, Journal of Allergy and Clinical

Immunology, 68, 5.

Samet, J.M. and Spengler, J. D. (1991). *Indoor Air Pollution - A Health Perspective*, Johns Hopkins University Press, Baltimore, MD.

Schneiter, D., Bernard, B., Defila, C., Gehrig, R. (2002). Effect of climatic changes on the phenology of plants and the presence of pollen in the air in Switzerland, Journal of Allergy and Immunology (Paris),34:113Y116.

Schwartz, D. A., Thorne, P. S., Yagla, S. J., Burnmeister, L. F., Denchuck, S. A. and Watt, J. L. (1995). The role of endotoxin in grain dust induced lung disease, American Journal of Respir. Crit. Care Medicine, 152(2); 503-600.

Soukup, J. M. and Becker, S. (2001). Human alveolar macrophage responses to air pollution particulates are associated with insoluble components of course material, including particulate endotoxin. Toxicol. Appl. Pharmacol., 171(1): 20-26.

Stach, A., Garcý´a-Mozo, H., Prieto-Baena, J. C., Czarnecka-Operacz, M., Jenerowicz, D., Silny, W. (2007). Prevalence of Artemisia species pollinosis in western Poland: impact of climate change on aerobiological trends, 1995Y2004, Journal of Investig Allergol Clinical Immunology, 17:39Y47.

Steinman, H., Donson, H., Kawalski, M,, Toerien, A., Potter, P. C. (2003). Bronchial hyper-

responsiveness in urban, periurban and rural South African children, Journal of Pediatric Allergy and Immunology, 14:383Y393.

Targonski, P., Persky, V. and Rameskrishnan, V. (1995). Effect of environmental molds on risk of death from asthma during the pollen season. Journal of Allergy and Clinical Immunology, 95: 955-961.

Thorne, P. S. (2000). Inhalation toxicology models of endotoxin and bioaerosol induced inflammation. Toxicology, 152(1-3):13-23.

Truong, C., Palme´, A. E., Felber, F. (2007) Recent invasion of the mountain birch *Betula pubescens ssp. tortuosa* above the treeline due to climate change genetic and ecological study in northern Sweden, Journal of Evolutionary Biology, 20:369Y380.

US Environmental Protection Agency (2005). Indoor Air Quality Home Page, Sources of Indoor Air Pollution.

Verhoeff, A. P., Van Wijnen, J. H., Brunekreef, B., Fisher, P., Reenen-Horkstra, E. S. and Samsom, R. A. (1992). Presence of viable mold propagules in indoor air in relationship to house damp and outdoor air. Allergy, 47: 83-91.

Wayne, P., Foster, S., Connolly, J., Bazzaz, F., Epstein, P. (2002). Production of allergenic pollen by ragweed (*Ambrosia artemisiifolia L.*) is increased in CO_2-enriched atmospheres. Ann

Allergy, Asthma and Immunology, 88:279Y282.

Weryszko-Chmielewska, E., Puc, M., Piotrowska, K. (2006). Effect of meteorological factors on Betula, Fraxinus and Quercus pollen concentrations in the atmosphere of Lublin and Szczecin, Poland, Ann Agricultural Environment Med., 13:243Y249.

Williams, R. (2005). Climate change blamed for rise in hay fever. Nature, 434:105.

Wilson, R. and Spengler, J. (1996). Particles in our air, Harvard University Press, Cambridge, MA, 259.

Womiloju, T. O., Miller, J. D., Mayer, P. M. and Brook, J. R. (2003). Methods to determine the biological composition of particular matter collected from outdoor air, Atmospheric Environment, 37: 4335-4344.

Ziska, L. H., Gebhard, D. E., Frenz, D. A., Faulkner, S., Singer, B. D., Straka, J. G. (2003). Cities as harbingers of climate change: common ragweed, urbanization, and public health. Journal of Allergy Clinical Immunology,111:290Y295.

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Figure 4. Rural Orange County, N.C., PM_{10} & $PM_{2.5}$ Outdoor Mass ($\mu g/m^3$), (1,3)-β-D-glucan (pg/m^3), Endotoxin (EU/m^3), Protein ($\mu g/m^3$).

Figure 5. Raleigh, NC, 15 Stage (range from 0.01 to 10 μg),Outdoor Mass ($\mu g/m^3$), Protein ($\mu g/m^3$), Protein/Mass Ratio.

Table

Click here to download Table: Indoor-Outdoor-BioPM-Table3.doc

Study Parameter	Sample Site		
	Denver, CO	**Orange County, NC**	**Raleigh, NC**
Sampling Period	1/23/2001 to 3/22/2001	3/18/2007 to 5/16/2007	7/27/2006 to 7/28/2006
Sampling Location	Urban Denver CO at primary school	Rural Orange County NC in wooded area	Urban Raleigh NC, Next to highway I- 440
Number of Samples	18/25 (inside/outside)	3/10 ($PM_{2.5}/PM_{10}$)	1 per 15 stage
PM Sampler	RTI-made pump with MSP PEM impactor	URG sampler and cyclone	DEKATI low-pressure impactor
PM Filter	37-mm PTFE	47-mm PTFE	35 mm PTFE
PM Size Fraction	$PM_{2.5}$	$PM_{2.5}/PM_{10}$	15-stage (range from 0.01 to 10 µm)
PM Concentration	Indoor 3.5-28.2 µg/m^3 Outdoor 6.2-22.0 µg/m^3	$PM_{2.5}$ 7.5- 12.1 µg/m^3 PM_{10} 7.7- 32.5 µg/m^3	6.9 µg/m^3 at 7.0 µm max 0.007 µg/m^3 at 0.01 µm min
Sample Volume	2.73 to 2.95 m^3	10.3 to 24.4 m^3	40.1 m^3
Sample Flow Rate	2 L/min	16.7 L/min	29.6 L/min
Mass Analysis	Indoor mean 8.5 µg/m^3 std. dev. 6.5 µg/m^3 Outdoor mean 12.8 µg/m^3 std. dev. 5.4 µg/m^3	$PM_{2.5}$ mean 9.3 µg/m^3 std. dev. 2.4 µg/m^3 PM_{10} mean 18.2 µg/m^3 std. dev. 7.4 µg/m^3	4.0 µg/m^3 at 10.0 µm 6.9 µg/m^3 at 7.0 µm 1.0 µg/m^3 at 4.0 µm 0.7 µg/m^3 at 2.5 µm 2.6 µg/m^3 at 1.75 µm 3.7 µg/m^3 at 1.0 µm 3.0 µg/m^3 at 0.2 µm 3.2 µg/m^3 at 0.175 µm 1.6 µg/m^3 at 0.11 µm 0.8 µg/m^3 at 0.10 µm 0.1 µg/m^3 at 0.02 µm
Endotoxin Analysis	Indoor mean 9.2 EU^{-3}/m^3 std. dev. 6.9 EU^{-3}/m^3 Outdoor mean 5.7 EU^{-3}/m^3 std. dev. 5.9 EU^{-3}/m^3	$PM_{2.5}$ mean 23.0 EU^{-3}/m^3 std. dev. 14.0 EU^{-3}/m^3 PM_{10} mean 51.0 EU^{-3}/m^3 std. dev. 16.0 EU^{-3}/m^3	No
Glucan Analysis	Indoor mean 5.8 pg/m^3 std. dev. 6.9 pg/m^3 Outdoor mean 8.9 pg/m^3 std. dev. 10.6 pg/m^3	$PM_{2.5}$ mean 33.6 pg/m^3 std. dev. 24.5 pg/m^3 PM_{10} mean 40.0 pg/m^3 std. dev. 17.4 pg/m^3	No
Protein Analysis	Yes (most BDL)	$PM_{2.5}$ mean 0.095 µg/m^3 std. dev. 0.064 µg/m^3 PM_{10} mean 0.274 µg/m^3 std. dev. 0.078 µg/m^3	0.07 µg/m^3 at 7.0 µm 0.09 µg/m^3 at 4.0 µm 0.04 µg/m^3 at 2.5 µm 0.15 µg/m^3 at 1.75 µm 1.02 µg/m^3 at 1.0 µm 0.19 µg/m^3 at 0.6 µm

Table 1. Summary of Sample Locations, Data Collection and Analysis

Figure

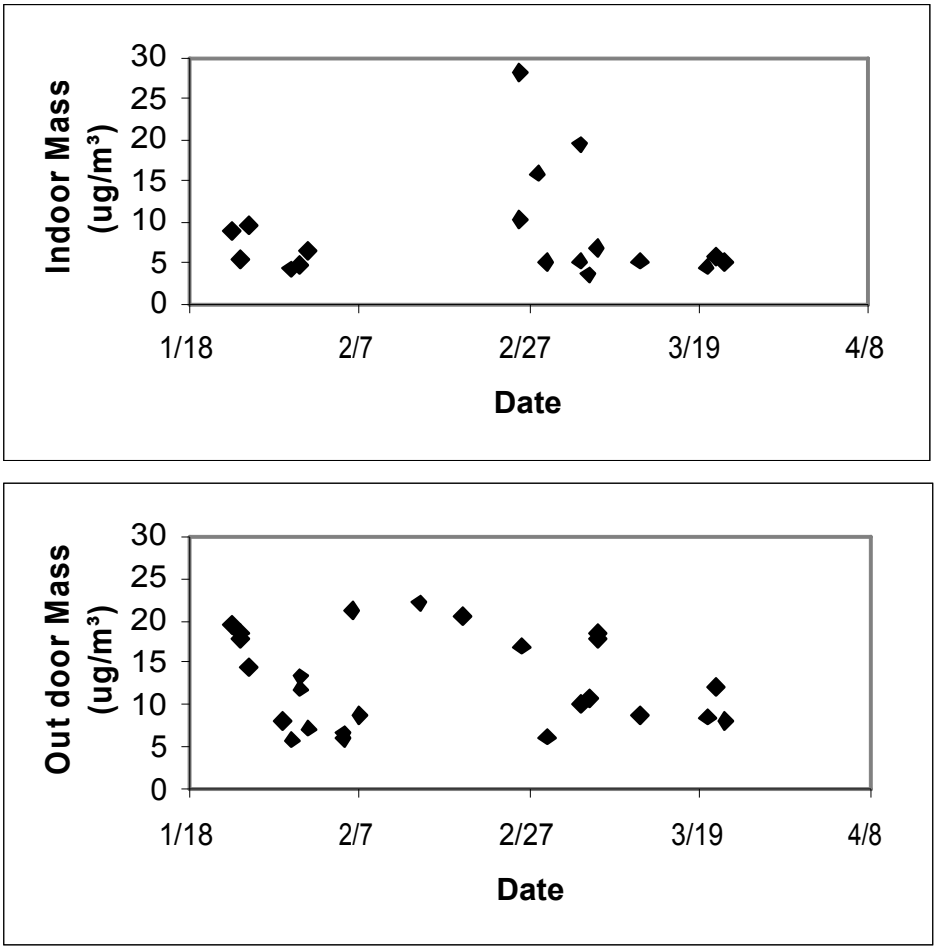
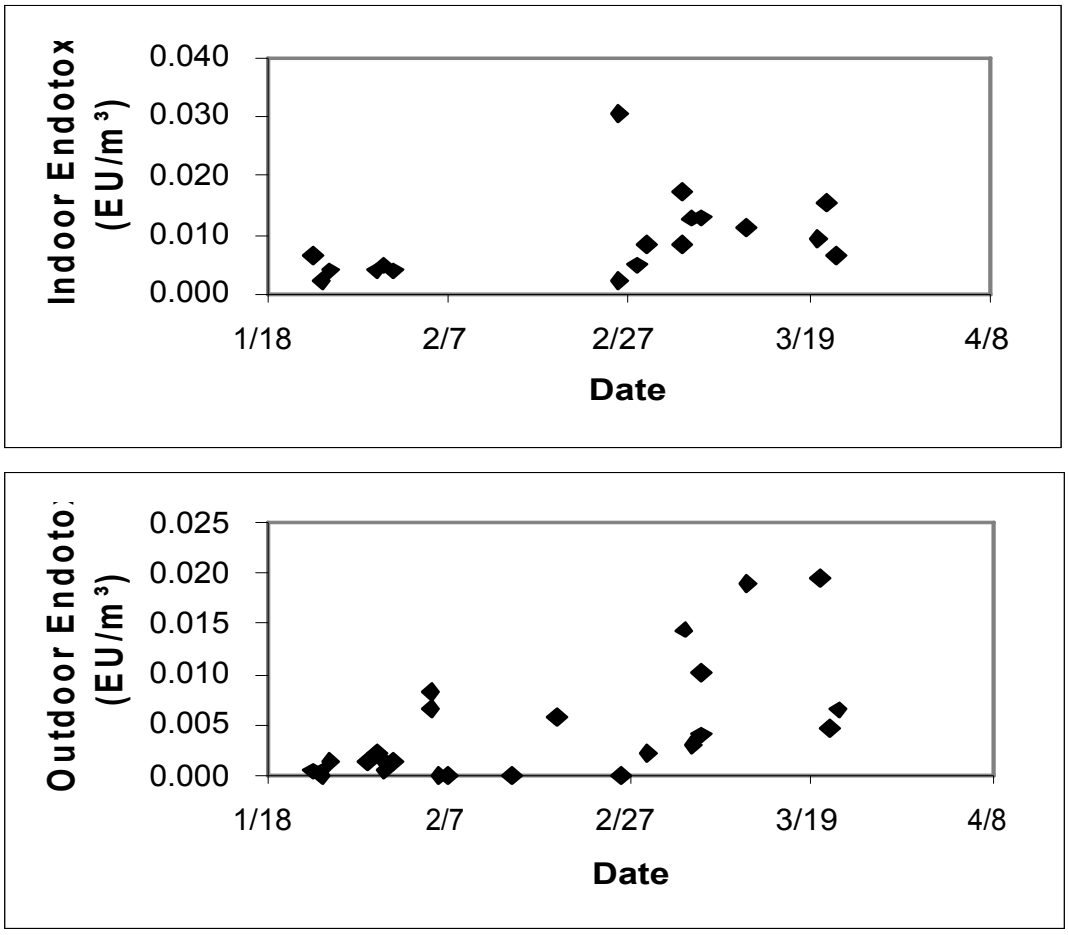


Figure 1. Denver, CO, $PM_{2.5}$ Indoor/Outdoor Mass ($\mu g/m^3$).

Figure

Figure 2. Denver, CO, $PM_{2.5}$ Indoor/Outdoor Endotoxin (EU/m^3).



Figure

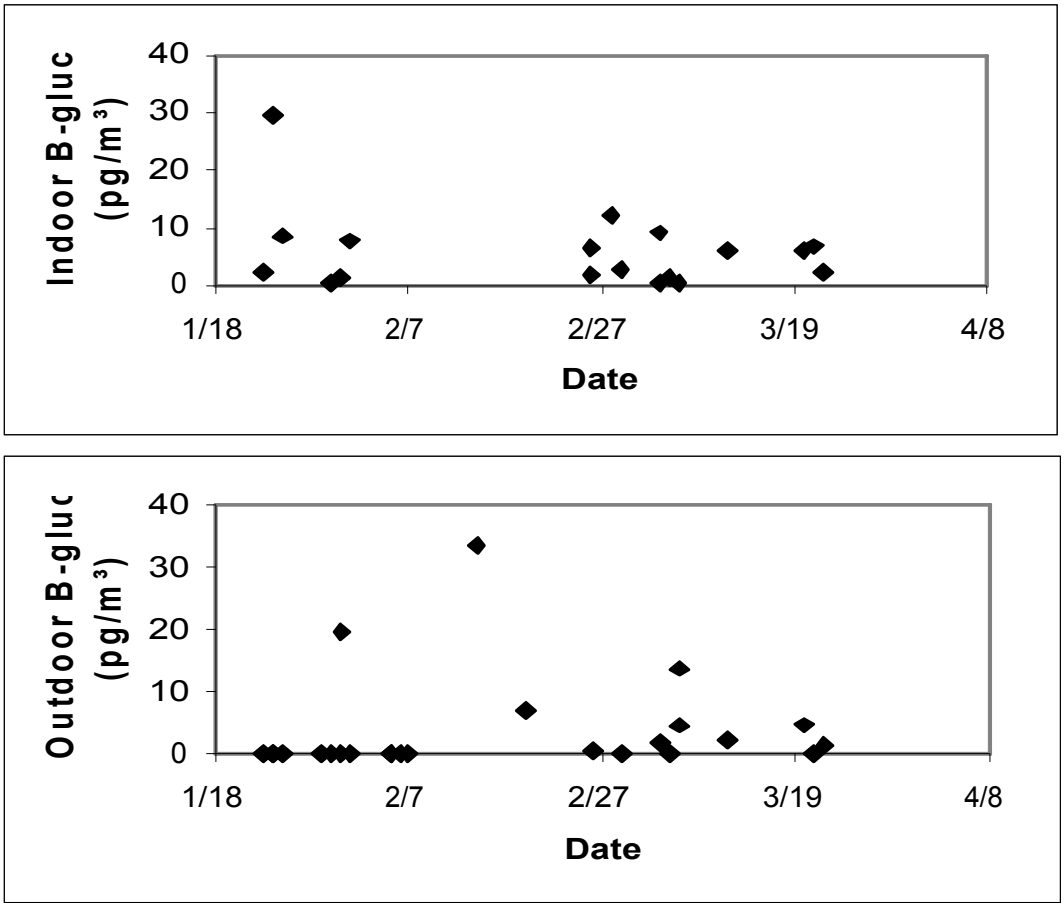


Figure 3. Denver, CO, $PM_{2.5}$ Indoor/Outdoor (1,3)-β-D-glucan (pg/m^3).

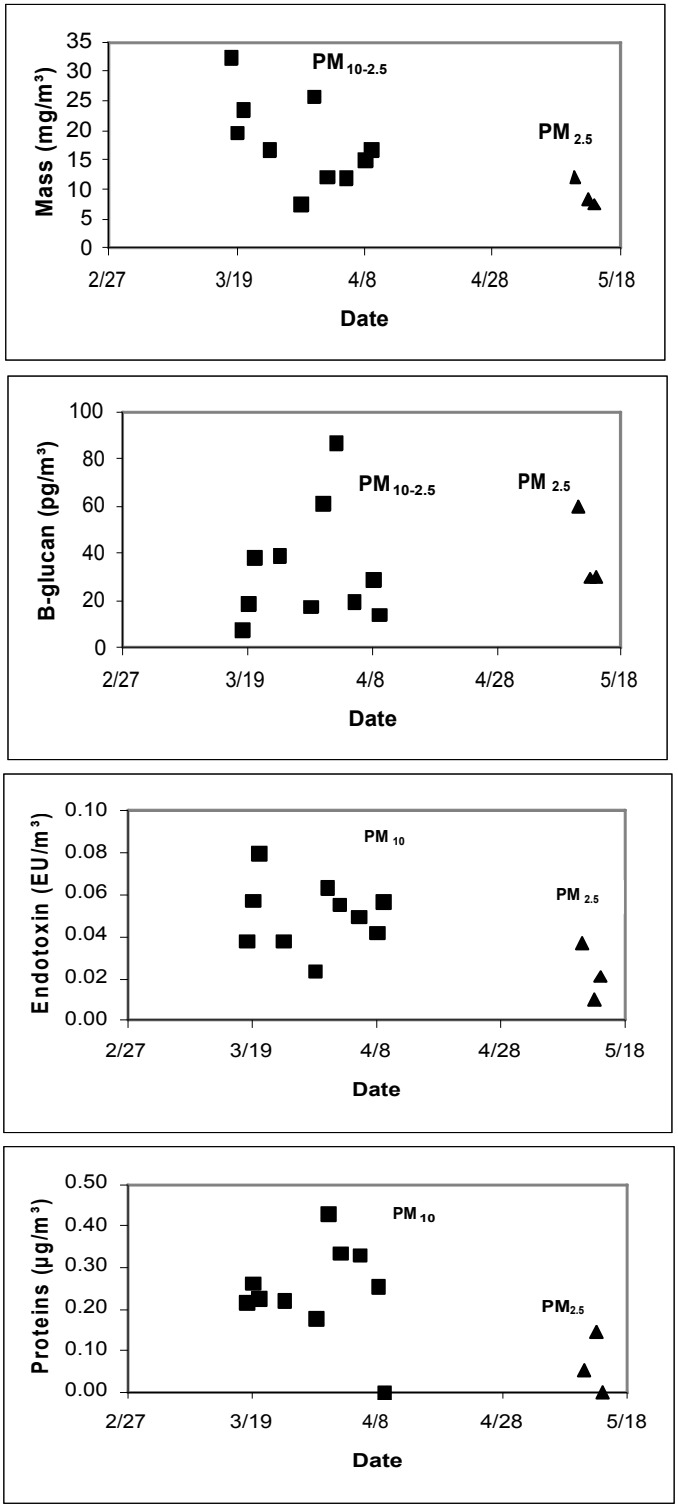
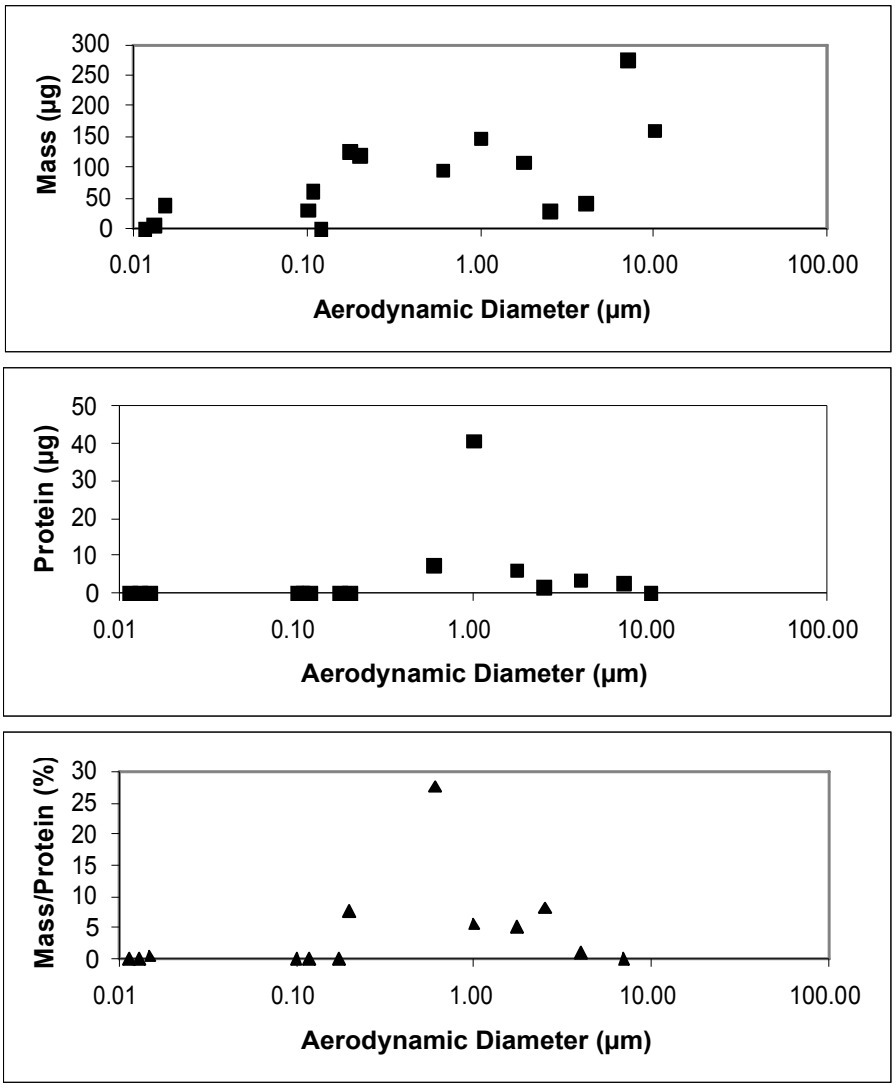


Figure 4. Rural Orange County, N.C., $PM_{10-2.5}$ & $PM_{2.5}$ Outdoor Mass ($\mu g/m^3$),



(1,3)-β-D-glucan (pg/m^3), Endotoxin (EU/m^3), Protein (μg/m^3).

Figure 5. Raleigh, NC, 15 Stage (range from 0.01 to 10 μg),Outdoor Mass (μg/m^3), Protein (μg/m^3), Protein/Mass Ratio.