An Evaluation of the Protein Mass of Particulate Matter

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Abstract

A comparison of ambient particulate matter (PM) mass concentrations with the total protein mass concentrations has not previously been performed in North Carolina (NC) and was the goal of this research. A study of PM10-2.5 (from <10 μm in aerodynamic diameter to PM2.5) and PM2.5 (<2.5 μm in aerodynamic diameter) fractions of ambient bioaerosols was undertaken for a six month period. The analysis of total protein mass was used as an all inclusive indicator of biologically based aerosols. These organic bioaerosols may have nucleated with inorganic non-biological aerosols, or be suspended in combination with inert aerosols. The source of these bioaerosol allergens may be any combination of pollen, mold, bacteria, insect debris, fecal matter, or dander, and can induce irritational, allergic, infectious, and chemical responses in exposed individuals. Ambient samples of PM2.5 and PM10-2.5 were analyzed for gravimetric mass and total protein mass. The results for 19 of 24 sample periods indicated that between 1 and 4% of PM10-2.5 and between 1 and 2% of PM2.5 mass concentrations were made of ambient protein bioaerosols (in 5 of 24 sample periods protein results were below detectable limits). This study provides the first characterization of protein to mass comparisons of ambient bioaerosols in a metropolitan area of NC.

Keywords: bioaerosols, allergens, total protein, PM.

Introduction

A correlation between exposure to particulate air pollution and severe adverse health effects have been shown to exist (Dockery and Pope 1994). The evaluation of total long-term particulate exposures is monitored by measurement of both PM10-2.5 (from <10 μm in aerodynamic diameter to PM2.5) and PM2.5 fraction (<2.5 μm in aerodynamic diameter) particulate matter (PM). Ambient PM exposure may occur from outdoor or indoor air. PM originating from ambient sources and penetrating into the indoor environment can expose individuals while being indoors (Samet and Spengler 1991).

Exposures to the environmental allergens in bioaerosols can cause immunological reactions and allergic disease (mediated by IgE antibodies), ranging in severity from contact urticaria, rhinitis, hypersensitivity pneumonitis, and asthma to anaphylaxis and death. Approximately 40% of the population have developed IgE antibodies in response to exposure to environmental allergens; of the general public 20% demonstrate upper respiratory symptoms typical of rhinitis (hay fever), and 10% show lower respiratory symptoms characteristic of asthma (Pope, Patterson, Burge, 1993). Respiratory effects like these are an allergenic response to the inhalation of airborne bioaerosols or particulate matter (PM) which are biologically derived proteins. These proteins are predominantly comprised of plant pollen and microbial metabolites (mold and bacteria) (Menetrez, Foarde and Ensor, 2000) and can induce allergic, toxic, and infectious responses in exposed individuals. In addition, the exposure of children to *Stachybotrys chartarum* is under investigation for an association with pediatric pulmonary hemorrhage (Levetin, 1995; Husman, 1996).

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) found

Cladosporium spores in ambient air at a concentration 1000 times the mass concentration of pollen grains. Aerometric sampling devices have collected spores from 20,000 to 40,000 species of fungi. 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. The size range of airborne bacteria spores and vegetative cells is from 0.5 to 2.0 μm (*Bacillus* spp., *Pseudomonas* spp., *Xanthomonas* spp. and *Arthrobacter* spp.), while many mold spores are significantly larger; 2.5 to 3.0 μm for *Aspergillus fumigatus*, 3.5 to 5.0 μm for Aspergillus niger, 3.0 to 4.5 for *Penicillium brevicompactum*, 7 to 17 μm by 5 to 8 μm for *Cladosporium macrocarpum*, 15.0 to 25 μm for *Epiccocum nigrum*, and 2.8 to 3.2 for *Trichoderma harizanum* (Samson and Hoekstra 1995). 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, Foarde and Ensor 2001). This biological component can be identified specifically by species as in Menetrez, Foarde and Ensor (2001), or collectively accounted for in the measurement of protein concentrations [primarily made up of whole and fragmented mold spores, bacteria (endotoxins), and pollen]. These fragments can remain toxic or allergenic depending upon the specific organism or organism component. These bioaerosols can cause allergic, asthma-like reactions or pulmonary disease upon exposure. 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 effects. The exact constituents of PM air pollution that cause disease and the mechanisms involved are unknown. Studies to determine the components of PM which contribute to airway inflamation and irritation have been attempted (Bonner, et al., 1998; Donaldson and MacNee 2001; Soukup and Becker 2001). Aerodynamic size fractions of PM have been studied including coarse (PM10-2.5) and fine (PM2.5), and recently fine and submicronmeter fractions have been studied (Pope 2000). 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).

Road dust present on the streets of Southern California was shown to consist of a significant portion of organic protein material. Allergens from at least 20 different sources were found including pollen, animal dander and mold. Up to 5 to 12% of the atmospheric total suspended particular matter samples consisted of biological protein allergens. The close proximity of abundant vegetation was presumed to be the source of allergens and the action of passing traffic was the force which caused biological allergens to be aerosolized (Miguel, et al., 1999).

The present study monitored ambient samples of PM2.5 and PM10-2.5 for mass and total protein. The PM data and protein mass data was then compared to evaluate the portion of PM which was composed of biological allergens. A time frame of six months was the period of sample collection. This study discusses the monitoring analysis and results of the bioaerosol content of ambient air in urban central North Carolina.

Materials and Methods

Filter Collection

Fine and coarse ambient particle samples were collected using a Dichotomous Partisol Plus 2025 sequential air sampler, manufactured by Rupprecht and Patashnick Company Inc. (R&P). The air samples were collected on Gilman Teflo 47 mm Cat. No. R2P-J047 filters. The size fractions ranged from 0.1 to 2.5 μm for the fine fraction and 2.5 to 10.0 μm for the coarse fraction. The dichotomous sampler was located on a roof-top platform (the intake was 10.0 meters above the surrounding ground level) on the EPA building on the campus of the University of North Carolina in Chapel Hill NC. The rate of flow of the dichotomous sampler was at a constant rate of 5.0 L/min. The rate of flow of the dichotomous sampler was constant at 16.7 L/min. The flow is split such that the fine fraction rate is 15 L/min and the coarse fraction is 1.7 L/min. The total volume of air sampled varied with the length of the collection period and ranged from 21.13 to 24.90 m^3, except for week 9 when only 12 m^3 was sampled.

Filter cassettes coming into contact with the Teflon filters were wrapped in pre-cleaned aluminum foil (rinsed with methylene chloride and allowed to air dry). The instruments were baked in a laboratory oven at 120 °C for a period of not less than 12 hours. The instruments were then allowed to cool to room temperature within the lab oven, removed and stored within a sterile environmental chamber (sprayed and wiped with 95% ethanol and allow to air dry). Sterilization of the 47 mm Teflon filters was performed by placing them (polyethylene ring side up) in plastic petri dishes (with caps removed and placed beside each dish) in a biological hood (Class II Type A/B3) and exposing them to UV light for 8 to12 hours. After exposure, the lids were placed on the petri dishes containing the exposed filters and wrapped in groups of 8 to 15 filters with aluminum foil.

A minimum of 12% of the total anticipated number of filters were prepared as blanks. Of these blanks, during each sample period one blank was processed through each R&P sampler sleeve with zero exposure time (field 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.

The filters were each removed from their cassette using sterilized stainless steel tweezers and placed into individually numbered and labeled sterilized plastic petri plates. The petri plates were placed into a controlled environmental chamber for a minimum of 24 hours undisturbed to allow the filters to equilibrate prior to gravimetric weighing and sample recording. The chamber was kept in the laboratory which was maintained at a temperature of 20-23 °C ± 2 °C, and relative humidity of 30-40% ± 5%.

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 was recorded. Filters were then weighed following a 24 hour equilibrium period, and their total mass load determined. Filter gravimetric change was recorded with a calibrated Mettler-Toledo MX-5 microbalance using the instruments 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 protein analysis was performed. 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 containers with 30 ml buffered detergent, and shaken vigorously for 30 minutes to elute. Protein was measured using the Nano-Orange Protein Quantification Kit (Molecular Probes, Eugene, OR). 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, heated to 950.0 0C for 10 minutes and then allowed to cool to room temperature. A fluorescent reaction was produced which was compared with a known standard of bovine serum albumin. The level of fluorescence was measured using a Turner Digital Fluorometer Model 450. Protein was used as a general indicator of biologically based PM.

Results and Discussion

Filter samples were collected from August 19, 2003 to January 28, 2004. During this time, 12 periods of sample collection were performed. These secessions represented continuous daily (24 hour) sample collection of 5 to 15 days duration. Each period consisted of the collection of from as many as 15 to as few as 5 sets of coarse and fine filter samples. Control samples were also collected during each time period. All PM10-2.5 and PM2.5 filter samples were weighed for mass (μg) and analyzed for protein (μg).

A total of 342 filter samples of ambient air were analyzed successfully for protein, this represented 151 coarse fraction, 151 fine fraction, and 40 control samples. All of the individual PM10-2.5 and PM2.5 mass data and volumetric sample air flow rate data were averaged for each of the 12 monitoring periods. Figures 1 through 4 show the results and standard deviation for mass and protein for the averaged 12 monitoring periods. In 5 of 24 sample periods protein results were below detectable limits. These results could have been attributed to low protein levels on the collection filters which exceeded the sensitivity of the analytical procedure. These results are listed in Figures 3 and 4 as being zero for the purpose of graphical representation.

Frequently, samples analyzed for protein with results below the detectable limit (BDL) of 100ng/ml were omitted from the calculation of the average concentration, but were listed as zero when all of the samples in that period were BDL.

The averaged mass measurements listed in Figures 1 and 2, indicated higher PM2.5 or fine masses and lower PM10-2.5 coarse. The ratio of coarse to fine PM may have been affected by the positioning of the sample collection intake, which was 10 m above ground level. Coarse (PM10-2.5) sample mass averaged 139.3 μg (5.96 μg/m3), and fine (PM2.5) sample mass averaged 260.0 μg (11.0μg/m3).

Figure 1 lists the coarse (PM10-2.5) and fine (PM2.5) sample mass weight in micrograms (μg). In Figure 1, the PM coarse fraction concentrations peaked during the period 9/10/2003 to 9/23/2003 at 216 μg, and reached a low concentration during the period of 12/6/2003 to 12/13/2003 of 41.0 μg. The fine fraction concentrations peaked during the period 8/19/2003 to 8/26/2003 at 469.0 μg, and reached a low concentration during the period of 12/6/2003 to 12/13/2003 of 119.0 μg.

Figure 2 lists the sample mass by volume of air sampled (μg/m3). In Figure 2, the coarse fraction concentrations peaked during the period 9/10/2003 to 9/23/2003 at 9.21μg/m3, and reached a low concentration during the period of 12/15/2003 to 12/19/2003 of 3.13 μg/m3. The fine fraction concentrations peaked during the period 8/19/2003 to 8/26/2003 at 19.58 μg/m3, and reached a low concentration during the period of 12/15/2003 to 12/19/2003 of 6.23 μg/m3.

Figure 3 lists both the coarse and fine sample mass of protein (μg). In Figure 3, the coarse protein mass peaked during the period 8/19/2003 to 8/26/2003 at 4.8 μg, and reached a low concentration BDL on 11/19/2003 to 12/ 2/2003 and 1/6/2004 to 1/13/2004. The fine protein fraction concentrations peaked during the period 1/15/2004 to 1/28/2004 at 4.7 μg, and reached a low concentration BDL on 12/6/2003 to 12/13/2003, 12/15/2003 to 12/19/2003, and 1/6/2004 to 1/13/2004. The average protein concentration for the coarse fraction was 2.66 μg, and for the fine fraction was 2.53 μg.

Figure 4 lists the protein mass by volume of air sampled (μg/m3). In Figure 4, the protein coarse fraction concentrations peaked during the period 8/19/2003 to 8/26/2003 at 0.20μg/m3, and reached a low concentration BDL on 11/19/2003 to 12/2/2003, and 1/6/2004 to 1/13/2004. The protein fine fraction concentrations peaked during the period 8/27/2003 to 9/9/2003 at 0.20 μg/m3, and reached a low concentration BDL on 12/6/2003 to 12/13/2003, 12/15/2003 to 12/19/2003, and 1/6/2004 to 1/13/2004. The average protein mass concentration for the coarse fraction was 0.125 μg/m3, and fine PM2.5 was 0.11 μg/m3

Figure 5 lists the dimensionless ratio of protein mass (μg) divided by total sample mass (μg) for both coarse and fine PM. In Figure 5, the ratio of coarse fraction concentrations peaked during the period 1/15/2004 to 1/28/2004 at 0.04, and reached a low concentration BDL on 11/19/2003 to 12/ 2/2003 and 1/6/2004 to 1/13/2004. The ratio of fine fraction concentrations also peaked during the period 1/15/2004 to 1/28/2004 at 0.02, and reached a low concentration BDL on 12/6/2003 to 12/13/2003, 12/15/2003 to 12/19/2003, and 1/6/2004 to 1/13/2004. The average ratio for the coarse fraction was 0.023, and for the fine fraction was 0.010.

Of the 12 sample periods listed in Figure 3, seven periods had higher protein levels for coarse samples and one sample had higher protein for the fine fraction (four samples had BDL sample results). Of the 12 sample periods listed in Figure 4, four periods had higher protein levels for coarse samples and four sample had higher protein for the fine fraction (four samples had BDL sample results). This revealed a weak general trend association that greater protein concentrations were in the coarse fraction. This occurrence may be affected by the presence of numerous BDL sample results and the technique used to account for these results. A strong correlation between endotoxin and coarse fraction (PM10-2.5) was found in a study of southern California urban air (Mueller-Anneling et al. 2004). However, endotoxin may make up a small portion of the total protein mass. The presence of protein within both size ranges tested was demonstrated repeatedly. These respirable allergens shown in Figures 3 and 4 were found to

make up ambient particulate matter at concentrations reaching as high as 4% in the coarse fraction and 2% in the fine fraction.

The content of protein may be any combination of pollen, mold, bacteria, insect debris, fecal matter, or dander in large intact particles such as pollen or mold spores, and the smaller fragment as well as nucleated combinations of these particles occupying the entire spectrum of PM10-2.5 and PM2.5 or the respirable size range (Verhoeff and VanWijnen,1992; FDA 1994; Liccorish, 1985; Targonski, Persky and Rameskrishnan, 1995). 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.

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). 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. 2002, 2003). A significant association has been found between endotoxin and the clinical severity of asthma (Michel, et al. 1996).

Environmental monitoring of endotoxins has been reported repeatedly in literature, mainly in occupational settings and indoor dust samples collected from homes (Heederik et al. 2003; Menetrez et al. 2001).

The coarse fraction PM10-2.5 concentrations peaked at 216 μg (9.21μg/m3), and the fine fraction PM2.5 concentrations peaked at 469.0 μg (19.58 μg/m3). The average mass concentrations of coarse PM10-2.5 was 139.3 μg (5.96 μg/m3), and fine PM2.5 was 260.0 μg (11.0 μg/m3). The coarse protein fraction PM10-2.5 concentrations peaked at 4.8 μg

(0.20μg/m3), and the fine protein fraction PM2.5 concentrations peaked at 4.7 μg (0.20 μg/m3). The average protein concentrations for the coarse fraction was 2.66 μg (0.125 μg/m3), and for the fine fraction was 2.53 μg (0.11 μg/m3).

Figures 6 and 7 list the total sample mass (μg), protein mass (μg), and dimensionless ratio of protein divided by mass for coarse (Figure 6) and fine (Figure 7) PM. The ratio of coarse fraction concentrations varies predominantly from 0 to 4 %, and peaked at 10 %. The ratio of fine fraction concentrations varies predominantly from 0 to 1.5 %, and peaked at 3.5 %.

A study of Mexico City (Rosas et al., 1995) examined the protein content of coarse fraction PM10-2.5 samples. The study found protein concentrations of 0.5 μg/m3 associated with airborne particle concentrations of 100 μg/m3, in an urban-industrial site. This protein concentration of 0.5% found in Mexico City is similar to the range of 1 to 4% that found in this study in Chapel Hill, North Carolina.

Conclusions

A total of 342 filter samples of ambient air were analyzed for mass and protein; this represented 151 coarse fraction, 151 fine fraction, and 40 control samples. PM2.5 and PM10-2.5 concentrations of these bioaerosols were reported as a function of PM mass and volume of air sampled. The coarse protein fraction concentrations peaked at 4.8 μg (0.20μg/m3), and the fine protein fraction concentrations peaked at 4.7 μg (0.20 μg/m3). The average protein concentrations for the coarse fraction was 2.66 μg (0.125 μg/m3), and for the fine fraction was 2.53 μg (0.11 μg/m3). The results indicated that between 1 and 4% of PM10-2.5 and between 1 and 2% of PM2.5 mass concentrations were made of ambient protein bioaerosols (in 5 of 24 sample periods protein results were below detectable limits).

The concentrations of ambient bioaerosols documented in this study can help to more fully understanding exposure to airborne biological allergens. This can be significant to asthmatics and those with impaired pulmonary function.

The data presented in this paper depicts the mass and protein content of ambient bioaerosols. That mass of PM2.5 and PM10-2.5 total protein is a direct measurement of the portion of total PM that is biological in origin, referred to as BioPM.

This article provides the first investigation of protein bioaerosol concentrations in urban North Carolina. Additional studies are needed to further characterize seasonal and yearly variations of bioaerosols, as well as the role they play in affecting respiratory conditions.

References

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

Dockery, D.W., Pope, C.A. III, Annual Rev. Public Health, 1994, 15, 107-32.

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

Douwes, J., Heederick, D., Does environmental endotoxin exposure prevent asthma?, Thorax, 57(1); 86-90.

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

Food and Drug Administration, Center for Biological Evaluation and Research. Docket No. 94N-0012. Federal Register 1994; 59 (No. 225); 60362-3.

Heederik, D., Biological agents monitoring and evaluation of bioaerosol exposure, International Modern Industrial Hygiene, Vol. 2, Biological Aspects, (Perkins, J. L., ed.), Cincinnati, OH, American Conference of Governmental Industrial Hygienists, 2003, 293-327.

Husman, T. Health Effects of Indoor-air Microorganisms. *Scand J Work Environ Health,* 1996; **22**:5-13.

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

Liccorish, K. et.al., Role of Alternaria and Penicillium Spores in the Pathogenesis of Asthma., Journal of Allergy and Clinical Immunology, 1985;76:819-825.

Menetrez, M.Y., Foarde, K.K., and Ensor, D.S., 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, 2000, 24-28.

Menetrez, M. Y., Foarde, K. K., and Ensor, D. S.,"An Analytical Method for the Measurement of Nonviable Bioaerosols," Journal of the Air & Waste Management Association, October 2001, 51:1436-1442.

Michel, O., Kips, J., Duchateau, J., Vertanger, F., Robert, L., Collet, H., et al., Severity of asthma is related to endotoxin in house dust, American Journal of Respiratory Critical Care Medicine, 1996, 154 (6 Pt. 1); 1641-1646.

Miguel, A. G., Cass, G. R., Glovsky, M. M., Weiss, J., Allergens in Paved Road Dust and Airborne Particles, Environmental Science and Technology, (1999) 33, 4159-4168.

Monn, C., Becker, S., Cytotoxicity and induction of proinflammatory cytokins from human monocytes exposed to fine (PM2.5) and coarse (PM10-2.5) in outdoor and indoor air, Toxical Appl Pharmacol, 1999, 155(3); 245-252.

Mueller-Anneling, L., Avol, E., Peters, J.M., Thorne, S., Ambient Endotoxin Concentration in PM10 from Souther California, Environmental Health Perspectives, April 2004, Volume 112, No. 51, pp 583-588.

Pope, A. M., Patterson, R., Burge, H., Indoor Allergens: assessing and controlling adverse health effects: Eds: National Academy Press, Washington, D.C. 1993.

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

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

Salvaggio, J., and L. Aukrust, Allergy and Clinical Immunology, Journal of Allergy and Clinical Immunology, November 1981, Vol. 68, No. 5.

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

Samson, R., Hoekstra, E., et al., Introduction to Food Borne Fungi., Centraalbureau voor Schimmelcultures, P.O. Box 273, 3740 AG BAARN, The Netherlands, 1995.

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

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

Targonski, P., Persky, V., and Rameskrishnan, V., Effect of Environmental Molds on Risk of Death from Asthma During the Pollen Season., Journal of Allergy and Clinical Immunology, 1995; 95:955-961.

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

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

Verhoeff, A. P., Van Wijnen, J. H., Presence of Viable Mold Propagules in Indoor Air in Relationship to House Damp and Outdoor Air, Allergy, 1992; 47:83-91.

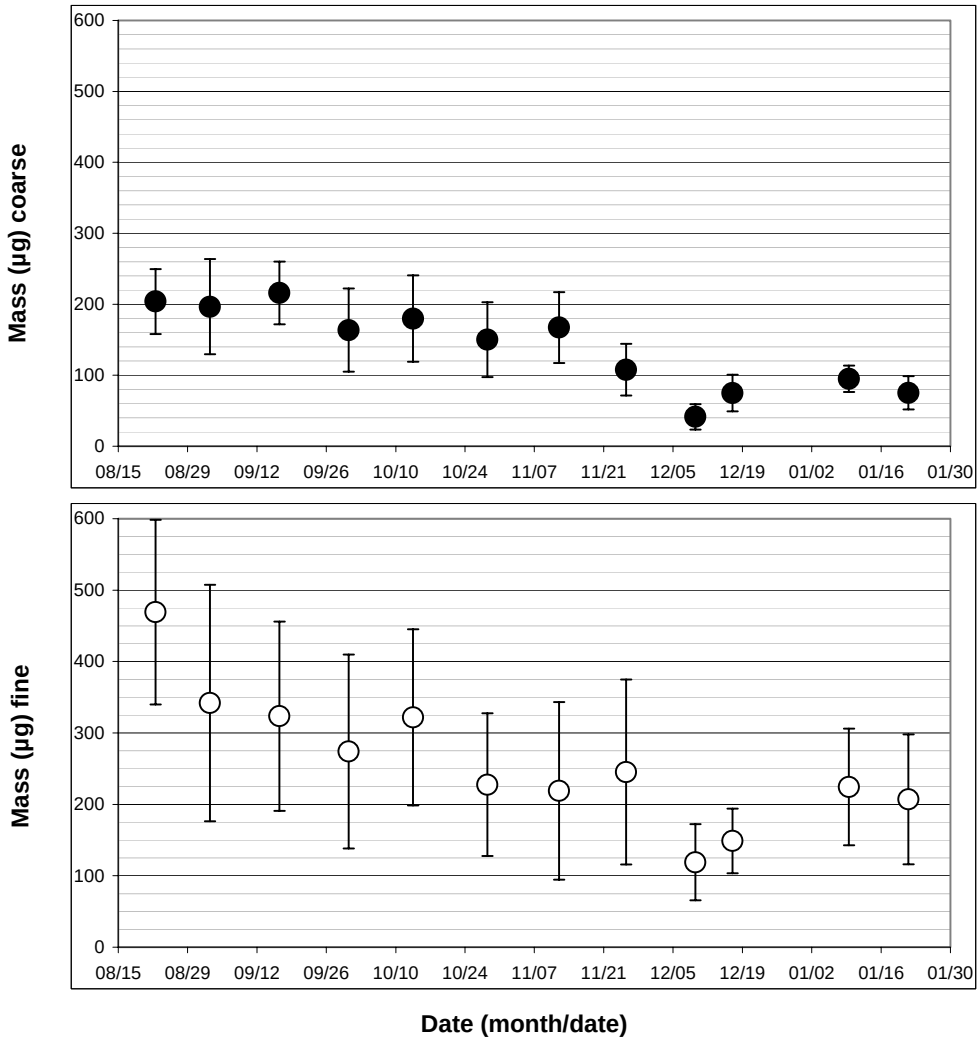

Figure 1, Mass (µg) of coarse $PM_{10-2.5}$ and fine $PM_{2.5}$

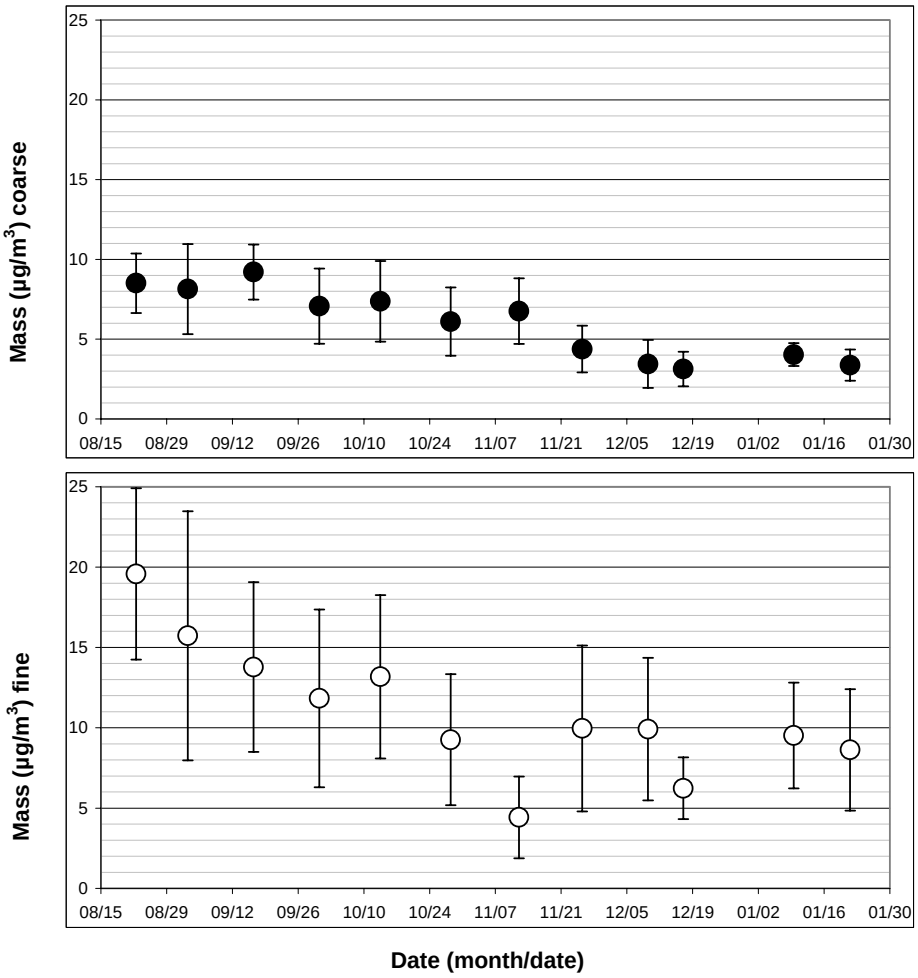

Figure 2, Mass ($\mu g/m^3$) of $PM_{10-2.5}$ and $PM_{2.5}$

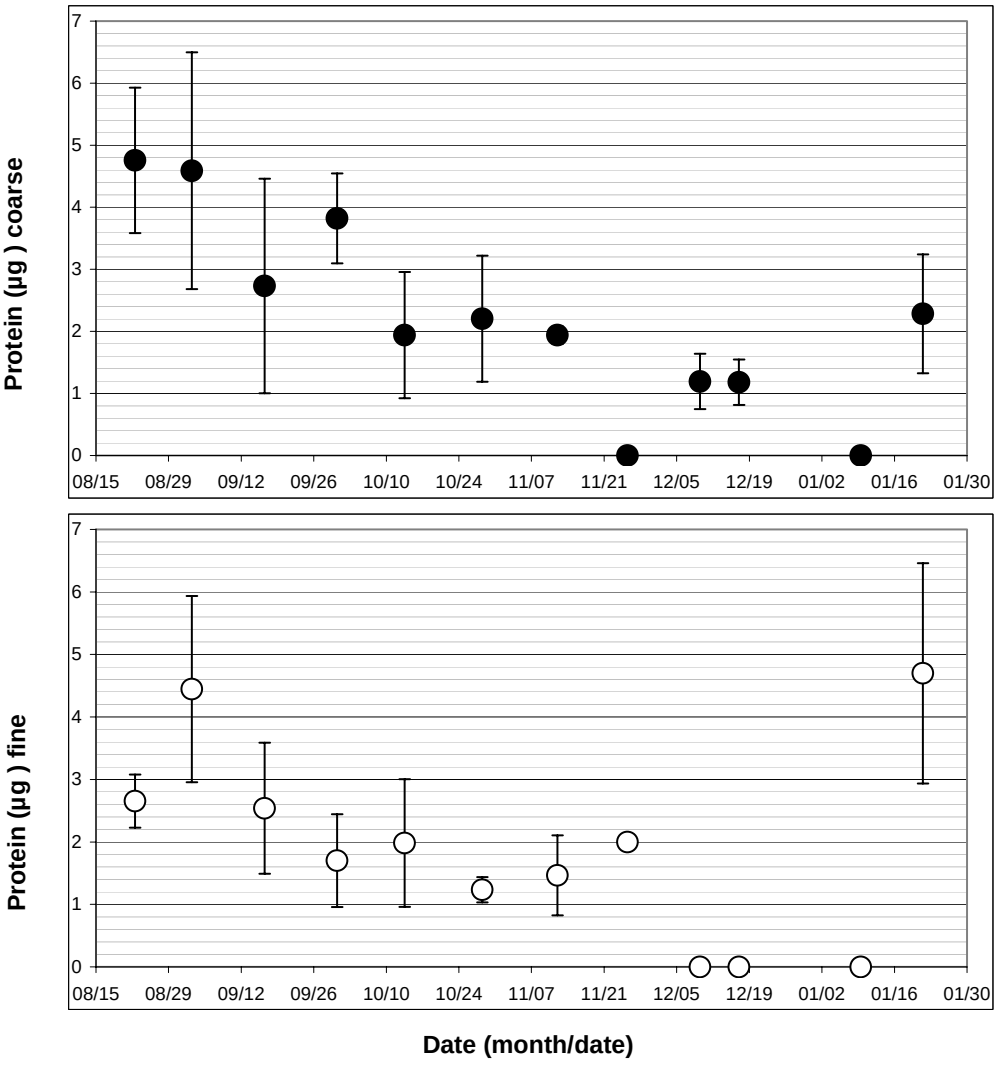

Figure 3, Protein (µg) of $PM_{10-2.5}$ and $PM_{2.5}$

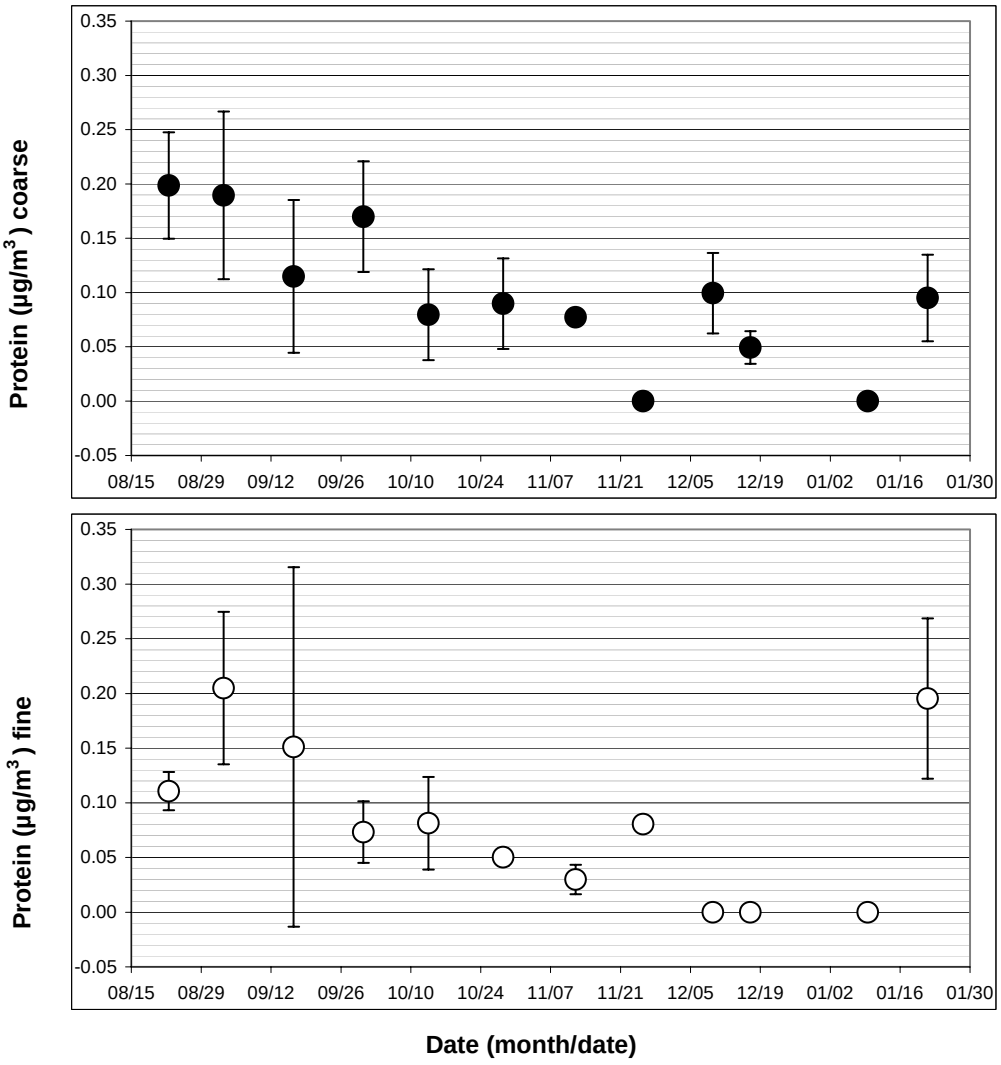

Figure 4, Protein ($\mu g/m^3$) of $PM_{10-2.5}$ and $PM_{2.5}$

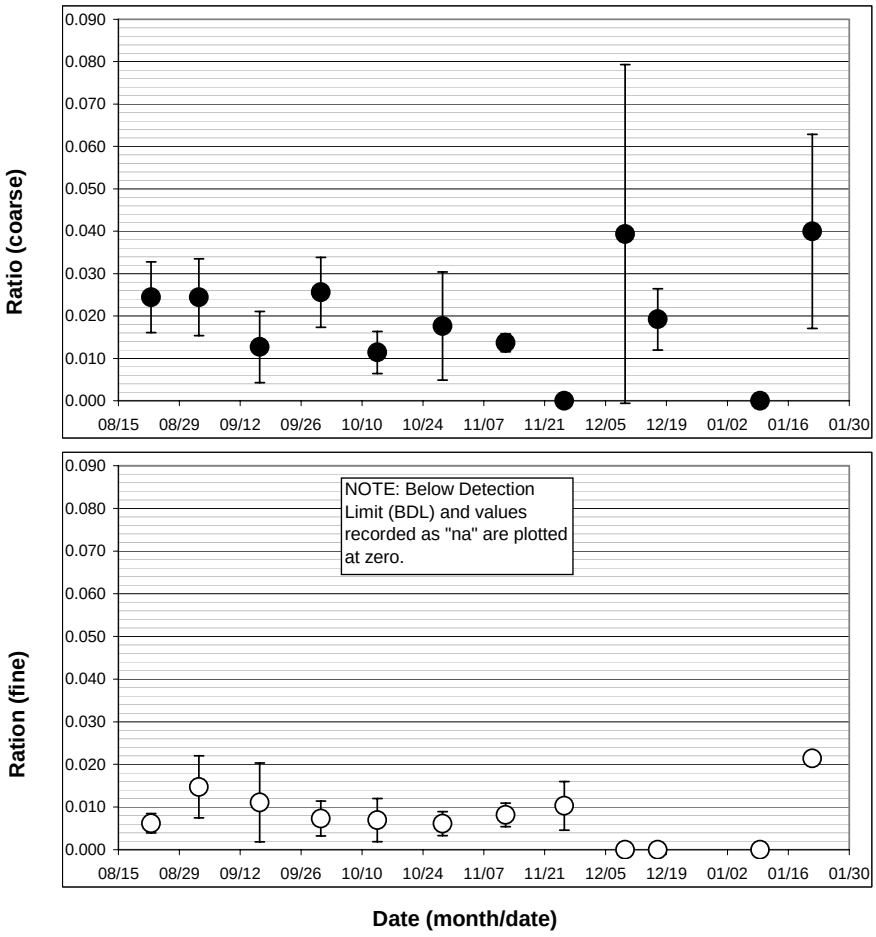

Figure 5, Ratio of protein/mass of $PM_{10-2.5}$ and $PM_{2.5}$

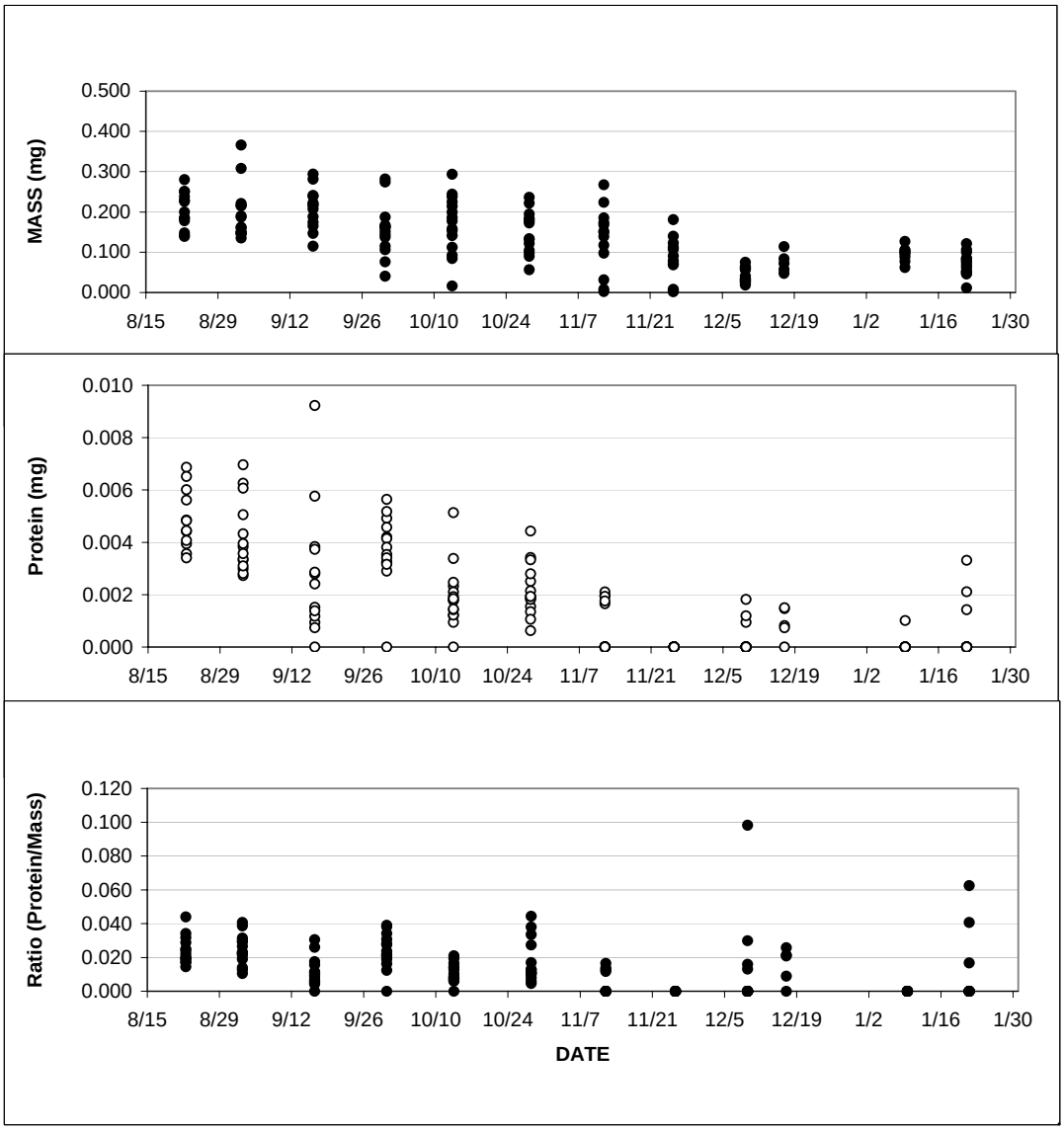

Figure 6, Mass (μg), Protein (μg), and Protein/Mass Ratio of coarse $PM_{10-2.5}$

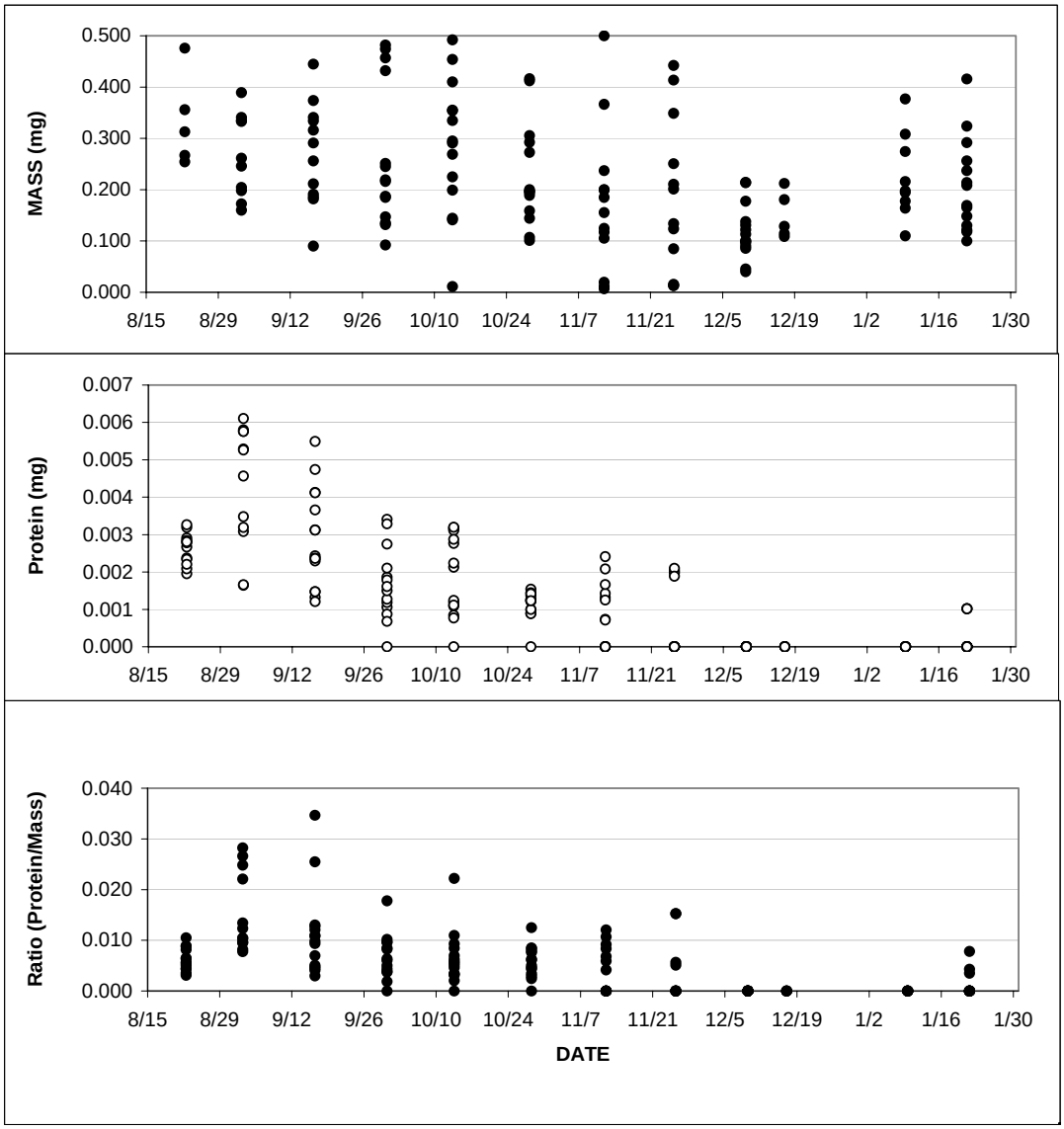

Figure 7, Mass (μg), Protein (μg), and Protein/Mass Ratio of fine $PM_{2.5}$