Fine Bioaerosols in Outdoor Air – Characterization, Influence of Seasonal Variation and Chemical Co-pollutants in Urban and Rural Airsheds

by
Pearl M. Nathan

A dissertation submitted in partial fulfillment
of the requirements for the degree of
Doctor of Philosophy
(Environmental Health Sciences)
In the University of Michigan
2018

Doctoral Committee:

Associate Professor J. Tim Dvonch, Chair
Professor Jack Harkema, Michigan State University
Professor Marie S. O'Neill
Associate Professor Alexander H. Rickard
Professor Chuanwu Xi

Pearl M. Nathan

pnathan@umich.edu

ORCID iD 0000-0002-4959-3404

Dedication

To my family

Acknowledgements

My journey as a doctoral student began thanks to my advisor Dr. Tim Dvonch who accepted me as his graduate student and gave me the opportunity to pursue my research in air quality. I am grateful to him for providing me with mentorship, guidance and immense support needed to successfully complete my dissertation. I am very grateful to my cognate member, Dr. Alexander H. Rickard, for his guidance, support and assistance with microbiology laboratory techniques and analysis crucial for the interdisciplinary nature of bioaerosol research. I would like to thank Dr. Chuanwu Xi for his guidance in helping me with DNA quantification analysis and for the use of his lab. I thank Dr. Marie O'Neill and Dr. Jack Harkema for their support and insightful comments.

Special thanks to Ting Luo, Kirtana Ramadugu, Jianfeng Wu and Rebecca Brewster for their support and assistance with the sequence analysis and qPCR analysis; this research would not be complete without their help. Thanks to Dr. Scot Dowd for processing and analyzing the bioaerosol samples using pyrosequencing techniques.

This research would not have been possible without the support and assistance from the team of people who made the intensive sample collection and analysis possible – special thanks to Claire Kwaitkowski, Wendy Qi, Tommy Beard, Chenye Liu and Taylor

Mann. I would also like to thank Mary Lynam, Jim Barres and Naima Hall for their encouragement and support throughout my time as a doctoral student.

This research is a part of the Great Lakes Air Center for Integrated Environmental Research (GLACIER), funded by the U.S. EPA (R83479701) STAR Clean Air Research Centers program. I am also grateful to my advisor Dr. Tim Dvonch for additional support to complete the sample analysis. I am thankful for the Rackham One Term Dissertation Award and Rackham Travel Grant that allowed me to travel and present my work at various conferences.

Finally, I would like to thank my family, to my parents for always having faith in me and encouraging me to pursue my dreams. To my brother, who has always believed in me and supported my academic pursuits. To my in-laws, who have always been there for me. To my daughter, who helped me be patient and perseverant during the writing phase of my dissertation. And most special thanks to my husband, Steve, whose immense support, patience and encouragement made it possible for me to complete this dissertation.

Table of Contents

List of Figures

List of Tables

Abstract

Particulate matter (PM) pollution has been studied in great depth with respect to its chemical composition. Outdoor air is made up of chemical as well as biological components. However, bioaerosols in PM2.5 (particles equal to or less than 2.5 micro-meters in aerodynamic diameter) have not been fully explored to understand their composition and interactions in different airsheds. This dissertation describes the findings of three studies investigating sampling methods, characterization and impact of environmental and chemical variables on fine bacterial bioaerosols in outdoor air.

The first study developed and evaluated a sampling methodology to collect fine bioaerosols in outdoor air using gelatin membrane and black polycarbonate filters, to collect total fine bioaerosols present, both culturable and non-culturable. The analysis involved a thorough characterization of fine bioaerosols using culture based assays, pyrosequencing and epifluorescent microscopy techniques to characterize the species present and also to understand both the viable and non-viable components. Gelatin membrane filters were found to be better than polycarbonate filters at maintaining the viability of bacterial cells over the sampling and transportation times. GM filters were also found to be better than PC filters at collecting a larger number of total bacterial

bioaerosols as well as a truer representation of the given ambient bacterial microbiome and better suited for quantitative characterization.

The second study investigated the fine bacterial bioaerosols composition in urban (Dearborn, MI) and rural (Dexter, MI) airsheds in Michigan. The urban site is located downwind of several steel processing plants, meat-processing facilities and near a large municipal wastewater treatment facility. The rural site is located significantly upwind of any large anthropogenic point sources. Both airsheds were found to have similar overall bacterial concentrations. However, a significant difference was observed in the bacterial community composition within the urban airshed as compared to the rural airshed with greater bacterial diversity observed at the urban site. Bacterial families identified in both airsheds appear to be influenced by the possible emission sources within each local vicinity.

The third study examined the role of seasonal variability, meteorological parameters and chemical co-pollutants on fine bacterial bioaerosol composition. Samples were collected in 25-min intervals each day over a two-week period each season from Summer 2013 until Summer 2014 to account for seasonal variability. Results indicate that bacterial compositions were influenced by changes in seasons. The differences due to changes in season were greater between the urban and rural airsheds in the winter, spring and summer seasons. Relative humidity, temperature, wind speed and ozone all appeared to have an influence on fine bacterial bioaerosol composition in outdoor air.

Particulate air pollution is a critically important public health concern, not only the total mass concentration but the particle composition as well, including bioaerosols. With an increasing number of residential communities downwind of facilities that are potential sources of microbial loading, the findings of this research will be informative to public health at large.

CHAPTER 1 Background

Introduction

Particulate matter (PM) is a criteria air pollutant as regulated by the United States Environmental Protection Agency [1]. PM in the air exists as a complex mix of chemical and biological components. The biological component of PM also known as bioaerosols consists of all particles of biological origin including bacteria, fungi, viruses, pollen, plant cells and fragments. For regulatory purposes PM is classified as coarse particles (PM10 ≤ 10 μm in aerodynamic diameter, >2.5 μm) and fine particles (PM2.5 fraction ≤ 2.5 μm in aerodynamic diameter) based on the aerodynamic diameter of the particle. However, although composition of PM is complex it is still regulated on a mass only basis. The current regulations need to consider the toxicological significance of the constituents of PM. There has been a lot of research investigating the chemical components of PM but very little into understanding bioaerosols in outdoor air. Most of the research on bioaerosols has been in the context of indoor air quality within residential and occupational exposure environments. Bioaerosol research in outdoor air has mainly focused on coarse size fraction consisting of pollen and other aeroallergens [2,3,4]. Fine bioaerosols need to be investigated since they are respirable and capable of being

inhaled deep into the lungs, where they may enter the bloodstream and be disseminated to other organ systems.

This dissertation focuses on the investigation of bacteria present in fine bioaerosols in outdoor air. This chapter provides a background on sampling and analytical methods used, impact of environmental variables and chemical co-pollutants on bioaerosols in the scientific literature. It also states the aims and hypothesis of this dissertation.

Chapter two describes the evaluation of a method for fine bioaerosol collection and analysis. The study provides for a comparison between gelatin membrane filters and black polycarbonate filters for sampling of bacterial bioaerosols. The methods used were evaluated for filter ability to capture a comprehensive and representative sample of the outdoor bacterial bioaerosol composition.

Chapter three details the characterization of fine bioaerosols within an urban and rural airshed as it relates to possible sources in the vicinity. It provides a description of the urban and rural airsheds, analytical methods used, analysis of bacterial bioaerosol community composition between both airsheds and discussion of results in relation to possible bacterial emission sources.

Chapter four investigates the influence of seasonal variation on fine bacterial bioaerosol composition between urban and rural airshed. This chapter also examined the influence

of meteorological parameters and certain chemical co-pollutants on fine bioaerosol concentrations and compositions within an urban airshed.

Chapter five concludes this thesis with a summary of findings, strengths and limitations and recommended future research.

Bioaerosol Sampling Methods

The methods used to sample bioaerosols are based on impaction, impingement, filtration, gravity, electrostatic precipitation, cyclone, thermal precipitator and condensation techniques [5]. Of these, the most common methods involve principles of impaction, impingement and filtration. Impaction and filtration methods can be used for sampling of bioaerosols by size fraction [6]. However, impaction methods used for collection of bacteria mainly require impaction onto agar plates. This only permits for estimation of viable bacterial cells that are capable of colony growth on specific agar media chosen. Impingement methods mostly collect bacterial bioaerosols into a liquid, which may then be analyzed using culture based methods or culture independent methods [5]. However, liquid impingement does not allow for investigation of bioaerosols in different size fractions. Filtration based methods can be used for the collection of bioaerosols in different size fractions by using a size selective inlet such as a cyclone. This method may need to be modified depending on the goals of the study by choice of size selective instruments used, types of filters used and need for sterilization of apparatus and materials used.

Very few studies have sought to investigate bacterial bioaerosols within size fractions in outdoor air [7,8]. Those that have done so, are still limiting in their ability to comprehensively describe the bacterial communities present in terms of sampling methods and analytical techniques used.

Currently, there is no gold standard for methods of bioaerosol sample collection whether in indoor or outdoor environments [9]. This is not only due to the complex nature of bioaerosols but also due to the limited research in this area and also because the sample collection method implemented depends on the goals of a particular study. This includes the type of organisms being studied whether fungi, bacteria or other bioaerosols, the interest in the viability of the organism studied and bioaerosols present in different size fractions.

Bioaerosol Characterization Methods

The methods used to enumerate bacteria can be broadly classified as culture based and culture independent methods. Bacterial bioaerosols in outdoor air have been severely underestimated till date especially due to the use of classical culture based methods [10]. The classical approach only yields results for bacteria that are viable and can grow on the agar media and conditions used in the given study [11]. Certain viable bacteria require optimal growth conditions which maybe dependent on factors such as temperature, aerobic or anaerobic conditions and presence of certain nutrients, absence of any of these factors would result in no growth of such bacteria even when present in viable form [12, 13, 14]. Also, several bacteria are capable of existing in a

viable but non-culturable state, but which are still capable of causing infection or allergic responses [15]. Such studies have been able to account for only about 10% of total bacteria present in the air [16,17].

Culture independent methods that have been employed include epifluorescence microscopy, flow cytometry, PCR and next generation sequencing (NGS) techniques [5,18]. These methods allow for the characterization of both viable and nonviable bacterial bioaerosols. While PCR and NGS technologies are able to characterize total bacteria present in an air sample, they aren't able to distinguish between viable and nonviable cells. So if the goal of the study were to identify cell viability, epiflourescence microscopy would be better suited since stains to distinguish viability of cells based on cell wall integrity can be used [19]. In addition quantitative PCR (qPCR) is another method that can used to measure bacterial bioaerosol concentrations.

Impact Of Sources On Bioaersosol Composition

Bioaerosols in the outdoor air are a complex mix of biological particles that maybe from natural and anthropogenic sources. The natural sources include re-suspended soils, plant based microbes or particles such as pollen, animal sources and bioaerosols from aquatic environments. Occupational exposure studies have revealed high concentrations of bacterial bioaerosols within wastewater treatment plants, animal rearing operations and slaughter houses [20,21,22]. Studies have also shown high concentrations of bacterial bioaerosols in the air outside of these operations with concentrations decreasing with increasing distance from the source [23, 24, 25]. Land

use changes that include facilities capable of bioaerosols emissions such as wastewater and sewage treatment plants also influence the overall microbial load in the outdoor air [26,27].

Influence of Environmental Variables and Chemical Co-Pollutants

The bioaerosol composition and concentration in outdoor air is subject to variation not only due to possible microbial emission sources but also due to changes in meteorological factors such as temperature, relative humidity, wind speed, wind direction, precipitation and solar radiation [28,29]. Periods of rainfall have been found to have fewer viable fungal spores in the air whereas cloudy conditions were found to carry a larger number of viable fungal spores suggesting that the rain may have washed out the spores [30]. The concentrations of outdoor bioaerosols may also be affected by changes in temperature and relative humidity [31,32]. Changes in temperature may also affect concentrations of chemical co-pollutants in the air such as PM and ozone [33,34,35]. Also, individual components of PM could also impact bioaerosol compositions and concentrations and also distribution in the air. Seasonal changes have also been shown to have an effect on bioaerosol compositions and concentration in the air. Higher concentrations of bacteria and fungi have been observed in the warmer seasons mainly due to presence of plant related bioaerosols [36,37,38].

There is a lack of substantial research investigating outdoor bioaerosols from a public health perspective especially in the respirable size fractions. Most of the studies are limited in their methods of sample collection and analysis. The research conducted for

this thesis, provides for a comprehensive investigation to better understand the fine bacterial bioaerosols in the outdoor air. The work presented here, includes evaluation of a sampling and analytical method to allow for collection of total bacterial bioaerosols present in PM2.5 – both culturable and non-culturable. It also provides a characterization of fine bacterial bioaerosol composition and concentrations in two distinct airsheds- urban and rural. The influence of seasonal changes, environmental parameters and chemical co-pollutants were also examined.

Aims and Hypothesis of the Study

Aim 1: To develop and evaluate a method to sample fine bacterial bioaerosols both viable and non-viable organisms.

- Hypothesis 1: The use of gelatin membrane (GM) filters with PM2.5 cyclones would be able to better capture the bacterial communities in outdoor air both qualitatively and quantitatively as compared to black polycarbonate filters (PC).
- Hypothesis 2: Gelatin membrane filters would provide a low blank collection approach as compared to polycarbonate filters.

Aim 2: To characterize fine bacterial bioaerosol composition within an urban and rural airshed.

- Hypothesis 3: Fine bacterial bioaerosols within the urban airshed will exhibit a greater bacterial diversity including pathogenic/opportunistic pathogenic species as compared to the rural airshed.

Aim 3: To investigate the influence of seasonal variation and chemical co-pollutants on fine bacterial bioaerosol composition.

- Hypothesis 4: Presence of higher concentrations of certain chemical co-pollutants will be associated with changes in fine bacterial bioaerosol concentration and composition.
    a) Meteorological parameters such as temperature, relative humidity, wind speed and wind direction will have an influence on bacterial DNA concentrations.
    b) Higher concentrations of co-pollutants like ozone and PM2.5 will be associated with reduction in bacterial DNA concentrations.
- Hypothesis 5: Bacterial composition of outdoor air will change with seasonal variations.

References

1. Criteria Air Pollutants, United States Environmental Protection Agency. Accessed online https://www.epa.gov/criteria-air-pollutants.

2. Salvaggio J, and Aukrust L, 1981. Allergy and Clinical Immunology. Journal of Allergy and Clinical Immunology; 68(5).

3. Levetin E, 1995. Fungi. In Bioaerosols, Burge, H. (ed.). CRC Press, Boca Raton, FL.

4. Husman T, 1996. Health Effects of Indoor-Air Microorganisms. Scandinavian Journal of Work, Environment & Health; 22: 5–13.

5. Mandal J, Brandl H, 2011. Bioaerosols in Indoor Environment –A review with special reference to residential and occupational locations. The Open Environmental & Biological monitoring Journal; 4:83-96.

6. Ghosh B, Lal H, Srivastava A, 2015. Review of bioaerosols in indoor environments with special reference to sampling, analysis and control mechanisms. Environmental International; 85: 254-272.

7. Li M, Qi J, Zhang H, Huang S, Li L, Gao D, 2011. Concentration and size distribution of bioaerosols in an outdoor environment in the Qingdao coastal region. Science of the Total Environment; 409: 3812-3819.

8. Franzetti A, Gandolfi I, Gaspari E, Ambrosini R, Bestetti G, 2011. Seasonal variability of bacteria in fine and coarse urban air particulate matter. Applied Microbiology Biotechnology; 90: 745-753.

9. Grinshpun SA, Buttner MP, Willeke K, 2007. Sampling for airborne microorganisms. In Manual of Environmental Microbiology. Edited by C.J. Hurst, R.M. Crawford, J.L. Garland, D.A. Lipson, A.L. Mills, and L.D. Stetzenbach, ASM Press, Washington, DC. 939–951.

10. Lange JL, Thorne PS, Lynch N, 1997. Application of flow cytometry and fluorescent in situ hybridization for assessment of exposures to air- borne bacteria. Applied Environmental Microbiology; 63: 1557-63.

11. Douwes J, Thorne P, Pearce N, Heederik D, 2003. Bioaerosol health effects and exposure assessment: Progress and Prospects. The Annals of Occupational Hygiene; 47 (3): 187-200.

12. Droffner ML, Brinton WF, Evans E, 1995. Evidence for the prominence of well characterized mesophilic bacteria in thermophilic (50–70 °C) composting environments. Biomass Bioenergy; 8 (3): 191–195.

13. Pillai SD, Ricke SC, 2002. Bioaerosols from municipal and animal wastes: background and contemporary issues. Canadian Journal of Microbiology; 48: 681–696.

14. Neidhart FC, Ingraham JL, Schaechter M, 1990. Physiology of the Bacterial Cell: A Molecular Approach. Sinauer Associates, Inc., Sunderland.

15. Oliver, JD, 2005. The viable but nonculturable state in bacteria. The Journal of Microbiology; 43: 93–100.

16. Heidelberg JF, Shahamat M, Levin M, Rahman I, Stelma G, Grim C, Colwell RR, 1997. Effect of aerosolization on culturability and viability of Gram-negative bacteria. Applied Environmental Microbiology; 63 (9): 3585–3588.

17. Torsvik V, Goksoyr J, Daae FL, Sorheim R, Michalsen J, Salte K, 1994. Use of DNA analysis to determine the diversity of microbial communities. In: Ritz, K., Dighton, J., Giller, K.E. (Eds.), Beyond the Biomass: Compositional and Functional Analysis of Soil Microbial Communities. John Wiley & Sons Ltd., Chichester, UK, 39–48.

18. Cartwright C, Horrocks S, Kirton J, Crook B, 2009. Review of Methods to Measure Bioaerosols from Composting Sites. Publ. By Environment Agency, Rio House, Waterside Drive, Aztec West, Almondsbury, Bristol, BS32 4UD.

19. Kildeso J, Nielsen BH, 1997. Exposure assessment of airborne microorganisms by fluorescence microscopy and image processing. Annals of Occupational Hygiene; 41 (2): 201–207.

20. Lues JFR, Theron MM, Venter P, Rasephei MHR, 2007. Microbial composition in bioaerosols of a high-throughput chicken slaughtering facility. Poultry Science; 86: 142-149.

21. Millner PD, 2009. Bioaerosols associated with animal production operations. Bioresource Technology; 100: 5379-5385.

22. Karra S, Katsivela E, 2007. Microorganisms in bioaerosol emissions from wastewater treatment plants during summer at a Mediterranean site. Water Research; 41 (6): 1355-1365.

23. Taha MPM, Pollard SJT, Sarkar U, and Longhurst P, 2005. Estimating fugitive bioaerosol releases from static compost windrows: Feasibility of a portable wind tunnel approach. Waste Management; 25: 445 – 450.

24. Green CF, Gibbs SG, Tarwater PM, Mota LC, and Scarpino PV, 2006. Bacterial plume emanating from the air surrounding swine confinement operations. Journal of Occupational and Environmental Hygiene; 3: 9–15.

25. Low SY, Paez-Rubio T, Baertsch C, Kucharski M, and Peccia J, 2007. Off-site exposure to respirable aerosols produced during the disk-incorporation of class B biosolids. Journal of Environmental Engineering; 133: 987–994.

26. Calderon C, Lacey J, McCartney A, and Rosas I, 1997. Influence of urban climate upon distribution of airborne Deuteromycete spore concentrations in Mexico City. International Journal of Biometeorology; 40: 71–80.

27. Kasprzyk I, Worek M, 2006. Airborne fungal spores in urban and rural environments in Poland, Aerobiologia; 22: 169–176.

28. Troutt C, Levetin E, 2001. Correlation of spring spore concentrations and meteorological conditions in Tulsa, Oklahoma. International Journal of Biometeorology; 45 (2): 64-74.

29. Chandra Mouli P, Venkata Mohan S, Jayaramma Reddy S, 2005. Assessment of microbial (bacteria) concentrations of ambient air at semi-arid urban region: Influence of meteorological factors. Applied Ecology and Environmental Research; 3(2): 139-149.

30. Maldonado-Ramirez SL, Schmale DGI, Shields EJ, Bergstrom GC, 2005. The relative abundance of viable spores of Gibberellazeae in the planetary boundary layer suggests the role of long-distance transport in regional epidemics of Fusarium head blight, Agricultural and Forest Meteorology; 132, 20–27.

31. Li D-W, Kendrick B, 1995. A year-round study on functional relationships of airborne fungi with meteorological factors. International Journal of Biometeorology; 39: 74-80.

32. Gioulekas D, Balafoutis C, Damialis C, Papakosta D, Gioulekas G, Patakas D, 2004. Fifteen years' record of airborne allergenic pollen and meteorological parameters in Thessaloniki, Greece. International Journal of Biometeorology; 48: 128-136.

33. Martuzevicius D, Grinshpun SA, Reponen T, Gorny R, Shukla R, Lockey J, Hu S, McDonald R, Biswas P, Kliucininkas L, LeMasters G, 2004. Spatial and temporal variations of PM2.5 concentration and composition throughout an urban area with high freeway density - the Greater Cincinnati study. Atmospheric Environment; 38: 1091-1105.

34. Rajsic SF, Tasic MD, Novakovic VT, Tomasevic MN, 2004. First assessment of the PM10 and PM2.5 particulate level in the ambient air of Belgrade city. Environmental Science and Pollution Research International; 11: 158-164.

35. Ross MA, Persky VW, Scheff PA, Chung J, Curtis L, Ramakrishnan V, Wadden RA, Hryhorczuk DO, 2002. Effect of ozone and aeroallergens on the respiratory health of asthmatics. Archives of Environmental Health; 57: 568-578.

36. Levetin E, Dorsey K, 2006. Contribution of leaf surface fungi to the air spora. Aerobiologia; 22(1): 3–12.

37. Mitakakis TZ, Clift A, McGee PA, 2001. The effect of local cropping activities and weather on the airborne concentration of allergenic Alternaria spores in rural Australia, Grana; 40: 230–239.

38. Tong Y, Lighthart B, 2000.The annual bacterial particle concentration and size distribution in the ambient atmosphere in a rural area of the Willamette Valley, Oregon, Aerosol Science and Technology; 32: 393–403.

CHAPTER 2 Development and evaluation of a sampling method for fine bacterial bioaerosols in outdoor air

Abstract

Background: Fine bioaerosols represent an important portion of total fine particulate matter (PM2.5- particles with an aerodynamic diameter ≤ 2.5μm) mass in outdoor air. However, to date, only a few studies have employed sampling methods that allow characterization of fine bacterial bioaerosols in outdoor air, both viable and non-viable components. This study used and evaluated a method using two types of filters to sample and analyze the bacterial composition of PM2.5.

Hypotheses: The hypotheses of this study were:

1. The use of gelatin membrane (GM) filters with PM2.5 cyclones would be able to better capture the bacterial microbiome in outdoor air both qualitatively and quantitatively as compared to black polycarbonate filters (PC).
2. Gelatin membrane filters would provide a low blank collection approach as compared to polycarbonate filters.

Methods: The sampling method was developed to collect the total biological particles in PM2.5 by employing GM filters and black PC filters along with size-selective cyclone inlets. Culture-based and culture-independent techniques – microscopy and molecular –

were used to characterize the species present while also providing insight into the total microbial presence, both viable and non-viable.

Results: GM filters were found to be better than polycarbonate filters at maintaining the viability of cells over the sampling and transportation times. GM filters were also superior in comparison to PC filters at collecting a larger number of total bacteria - magnitude of total cells as well as a more accurate representation of the bacterial species present in the given ambient microbiome - and were better suited for quantitative characterization. However, GM filters appeared to be constrained in their ability to provide a low blank approach as compared to PC filters.

Conclusions: An understanding of the presence of bacteria in outdoor air and its ability to cause illness is of public health concern. This study provides insight into the methodology for field collection of bacteria in PM2.5.

Introduction

Bioaerosols in ambient air are an important component of particulate matter (PM) pollution [1]. They have been studied mainly as indoor air pollutants primarily in occupational and residential settings or within controlled laboratory experiments [2,3,4]. Evaluation of total long-term exposures to both coarse particles - PM10−2.5 (≤ 10 μm in aerodynamic diameter, >2.5 μm) and fine particles - PM2.5 fraction (≤ 2.5 μm in aerodynamic diameter) of particulate matter should ideally include exposures to bioaerosols, which are a major component of PM [1]. This is important because as the particle size decreases it can be inhaled into the lungs and be retained there for long

periods of time leading to respiratory disorders and cardiovascular effects such as hypertension [5, 6].

Numerous sources contribute to PM pollution, and although the components are complex, PM is currently still regulated on a mass only basis [7]. The components of PM are physically, chemically and biologically diverse and the complexity of this mixture needs to be understood especially in the context of their toxicological significance. The research to characterize the physical and chemical components is ongoing for both the coarse and fine fractions. In studies that have examined the role of bioaerosols in outdoor air, the focus has mainly been on larger particles (coarse) of biological origins such as pollen and fungal spores, which are aeroallergens that cause respiratory inflammation and lung function impairment [8,9,10]. However, very few studies have explored the bacterial bioaerosol composition in fine PM in outdoor air [11,12,13]. Fine particulates are specifically capable of being inhaled deep into the lungs and entering the bloodstream [14].

A detailed understanding of the bacterial composition of fine PM is an important knowledge gap in the scientific literature, because, in recent times land use changes and urban planning have resulted in closer proximity of residential communities to industrial operations such as sewage treatment, animal rendering, food processing plants and agricultural activities which contribute to emissions of microorganisms into the air.

Outdoor bioaerosol sampling methods

Previous exposure characterization and assessment methods for bioaerosols have been designed based on the goals of the particular research study [15]. Some of the key issues with exposure characterization of bacterial bioaerosols are the sampling times, suitable sampling medium to maintain bacterial cell viability and appropriate methods of analysis for identification of specific organisms. Bioaerosol sampling is ideally carried out over shorter time intervals, between 1 and 30 minutes [16,17], especially if the goal is to maintain cell viability. Longer sampling times may result in desiccation of cells due to environmental conditions [18]. Sampling time is also dependent on the sampling method used. Several studies that have conducted sampling of outdoor bacterial bioaerosols have carried out sampling through impaction directly onto agar plates [13,19,20,21]. This method, however, only allows for the sampling of culturable bioaerosols and is not able to provide sufficient insight on the bacteria that may be present. Filtration is another method that employs filters or membrane filters. However, in most studies using filters, the aim is to understand the total microbial flora present without the need to ascertain the fraction of viable and non-viable cells [22,23]. These experiments have used polytetrafluoroethylene (PTFE), mixed cellulose ester and polycarbonate filters which do not maintain cell viability over the sampling time and transport time before analysis [24,25]. However, these filters are a suitable medium for collection and analysis of total fine bioaerosols. The study described here used pre-sterile gelatin membrane (GM) filters, which are similar in structure to PTFE filters, but have a high moisture content of 46-49% that allow for sampling for viable and non-viable species over the sampling period [26]. This study

was also designed to compare the performance of gelatin filters with black polycarbonate filters. Black polycarbonate filters were chosen instead of white polycarbonate filters since the smooth, black background provides a zero autofluorescence background for microscopic viewing, i.e., producing better images.

Methods of bioaerosol analysis

The factors influencing the exposure characterization of bioaerosols include the selection of analytical and identification methods. Most studies over the past several decades relied mainly on culture-based techniques using different growth media to enumerate and isolate microbes from air samples [27, 28]. These methods are limiting since only a small fraction of microbes found in the environment are culturable and this may lead to an underestimation of the total microbial diversity present when using culture-based methods [29]. However, these can be valuable methods to include when trying to ascertain the viable and culturable fraction present, as these fractions may be capable of eliciting pathogenicity. Several culture-independent methods are available and have been used in studies in more recent times but do not differentiate based on culturability. The use of molecular techniques, such as polymerase chain reaction (PCR) based technologies and pyrosequencing has opened new avenues for detection and speciation regardless of whether the organisms are culturable. The selection of identification methods is dependent on the goals of the study. This study compared gelatin membrane filters and black polycarbonate filters by carrying out an extensive characterization of fine bioaerosols using a combination of microscopy, culture, and DNA sequencing methods.

Materials and Methods

Sample collection

The sample collection method was designed to investigate the bacterial composition of fine particulate matter (PM2.5) in outdoor air. Air samples were collected on 4 individual days spread over a two-week period in July 2014 in Dearborn, Michigan (Fig.2.1) Fine bioaerosol samples were collected onto 47mm pre-sterile gelatin membrane filters, pore size 3µm (Sartorius, Germany) and 47 mm black polycarbonate filters (EMD Millipore Isopore, USA), pore size 0.2µm, using Teflon coated aluminum cyclone inlets (University Research Glassware, USA) to exclude larger particles upstream of the filter. The black polycarbonate filters were sterilized via autoclave prior to field sampling.

Samples of 25-minute durations were collected over a 4-hour period each day, over a total of 4 individual days (total n=30 for each filter type) by drawing air through the cyclone samplers at a flow rate of 16.7 liters/minute using vacuum pumps. Dry test meters were aligned between the vacuum pump and sample inlets to determine the volume of air drawn through each sampling interval. To make sure the flow rate of 16.7 lpm was maintained during each sampling interval, a calibrated rotameter was used at the start and end of every sampling interval.

Field blanks for each filter type were also collected for each day of sampling. Field blanks used in this study were sterile filters for each filter type that were handled and placed in the filtration unit identical to sample filters but with no air passing through the

filter. Thus, the filter was subjected to identical handling as the sample filters to check for contamination on filters during the handling and transport of filters. Collected filters were then stored in sterile Petri dishes sealed with Teflon tape and stored on ice during transport to the laboratory. In the laboratory, filters were then stored in a cold room at 4^0C until extraction. To minimize the loss of viable cells captured all filters were extracted within 24 hours of sample collection.

All filters were handled in a sterile environment both in the field and the laboratory. Extraction of filters and analysis were performed in biosafety hoods. Instruments and supplies used for sample collection, including the cyclone sampler inlets and filter pack screens, were sterilized either in an autoclave or with dry heat and disinfected with a 70% ethanol solution. Sample extracts were stored at -80^0C until all molecular analysis was completed.

Sample analysis

The sample analysis included a comprehensive approach to analyze the strengths and limitations of each filter type for bacterial bioaerosol characterization. Samples collected on gelatin membrane filters were extracted in 1X phosphate buffered saline (PBS) for microscopic, molecular and culture-based analysis. The black polycarbonate filters were cut into halves for microscopic and molecular analysis.

Epifluorescence microscopy for enumeration of viable and non-viable bacteria

To distinguish and enumerate bacterial cells in the collected samples, LIVE/DEAD BacLight Bacterial Viability Kit (Molecular Probes, USA) was applied to stain samples collected on both filter types. The use of BacLight stain to detect bacterial cells has been used in several laboratory and outdoor experiments [30,31,32]. BacLight stain is composed of two fluorescent stains; SYTO9 and propidium iodide, and distinguishes between viable and non-viable cells based on the whether the cell membrane is intact or damaged. Therefore, cells stained fluorescent green were assumed to be viable or live bacterial cells and cells stained fluorescent red were assumed to be non-viable or dead bacterial cells. A stock solution of BacLight stain was prepared using equal portions of Syto9 and propidium Iodide. This was then mixed with Nanopure water to create a 1X stain solution and was stored at -20^{o}C. For samples collected on gelatin membrane filters, 2ul of stain solution was added to the 18ul of sample extract and stored for 20min in the dark at room temperature. Then 4ul of the stained sample was placed on a clean glass slide and covered with a cover glass for viewing under the epifluorescent microscope (OLYMPUS BX51) using a 60x, 1.25 numerical aperture, oil immersion objective lens. For samples collected on black polycarbonate filters, one-half of the filter was placed on a glass side and was stained with 4ul of the stain solution. The filter was then covered with a cover glass and incubated in the dark for 30 minutes before epifluorescent microscopic viewing with a 20x, 0.50 numerical aperture, objective lens. Images were obtained using a 3.3 megapixel charge-coupled camera (Tucsen; Fuzhou, Fujian, China). An average of cell counts from three viewing fields per sample was used. Field blank samples were prepared and analyzed using the same method.

DNA extraction

The gelatin membrane filter extracts were homogenized and used at 400ul into PowerSoil DNA isolation kit (MoBio Laboratories, Carlsbad, CA, USA). Additional steps for the DNA extraction were carried out according to the manufacturer's instructions.

bTEFAP sequencing

The bTEFAP® was carried out by Molecular Research LP (MR DNA, Shallowater, TX). 16S universal Eubacterial primers (27Fmod AGRGTTTGATCMTGGCTCAG and 519Rmod GTNTTACNGCGGCKGCTG) were used to amplify the 300bp region of 16s rRNA genes. The HotStarTaq Plus Master Mix Kit (Qiagen, Valencia, CA) was used to perform a 30-cycle PCR under the following settings: 94^{o}C for 3 minutes, followed by 28 cycles of 94^{o}C for 30 seconds; 53^{o}C for 40 seconds and 72^{o}C for 1 minute; after these steps a final elongation step at 72^{o}C for 5 minutes was carried out. After PCR completion, all amplicon products from different samples were combined in equal concentrations and purification was carried out using Agencourt Ampure beads (Agencourt Bioscience Corporation, MA, USA). The samples were then sequenced using Roche 454 FLX titanium instruments and reagents as per manufacturer's instructions.

Downstream analysis

Microbiome analysis was conducted with QIIME 1.9.0 and the resultant operational taxonomic unit (OTU) table was analyzed with the R package Phyloseq 1.10.0. First, raw sequences were demultiplexed with the split_library.py script where sequences are

assigned to a sample based on its sequenced barcode. An open-reference OTU-picking strategy using the GreenGenes 13_8 database was used to generate the OTU table. A post-processing step was applied where OTUs constituting less than .05% of the total reads was excluded. Taxonomic summaries and alpha diversities of the samples by polycarbonate filters and gelatin membranes were generated with Phyloseq 1.10.0 after importing the OTU-table into R.

Culture-based assays for the enumeration of viable bacteria

Culture-based assays have been traditionally employed in many studies investigating bacterial bioaerosols. This study includes a comprehensive approach and therefore chose to use the culture-based methods for method comparison. Only samples collected onto gelatin membrane filters were analyzed since gelatin membrane filters would be capable of retaining viable cells due to its higher moisture content over the transport time. Agar media chosen were Mannitol Salt Agar for Gram-positive bacteria, Eosine Methylene Blue Agar for Gram-negative bacteria and R2A agar as a general growth media. The gelatin membrane filter extracts were used to prepare serial dilutions, and the Miles & Misra plating method was employed to maximize laboratory resources and time so that samples could be plated within 24 hours of sample collection. All plates were wrapped in parafilm to prevent dehydration of media and were incubated at 30^0C for a period of 3 days.

Data analysis

Epifluorescence microscopy images were used to identify and distinguish cells inferred as viable (live) and non-viable (dead) cells. Manual cell counts were performed and the mean values of viable cell counts and total cell counts for each sampling day were used to determine ratios of cell viability for each filter type using Microsoft Excel. Field blank images were also analyzed similarly to assess the ability of each filter type to provide a low blank approach.

Alpha diversity represents the diversity within samples or within groups. Diversity within samples was estimated using Shannon's index. This metric describes the relative abundance of operational taxonomic unit (OTU) rather than plain presence. The Kruskal-Wallis nonparametric test was performed to test for community differences in the mean number of observed OTU by groupings of interest. The significance threshold was set at an α-value of 0.05. Ecological investigation and visualization were carried out using R.

Results and Discussion

Gelatin membrane filters appear to be better than polycarbonate filters at collecting a larger magnitude of total bacterial cells, almost 10 fold greater on some sampling days and also a larger portion of viable cells [Figure 2.2, Table 2.1]. The higher ratio of captured viable cells could be attributed to the high moisture content (46-49%) of the gelatin membrane filters and the shorter sampling time, transportation and storage conditions chosen. The high moisture content enables the viable cells to remain intact

longer and not suffer desiccation due to environmental parameters such as temperature, relative humidity, and solar radiation. The difference in the magnitude of total cells collected on the filters could be explained due to the basic structural difference between the two filter types. The black polycarbonate filters have a capillary pore structure whereas the gelatin membrane filters have a more complex membrane structure which allows for an overall larger surface area for collection of particles or in this case bacteria [33,34]. Though the pore size of gelatin membrane filters used was 3um, as compared to pore size 0.2um for the black polycarbonate filters, the gelatin membrane filters appear to perform better for quantitative characterization of total bacterial cells. Other membrane filters such as PTFE filters commonly used do not perform well at capturing viable cells that may be present due to lack of moisture to avoid cell desiccation and damage [35].

Ecological diversity is measured in terms of species richness and evenness. Alpha diversity described by the Shannon diversity index explains both species richness and evenness. The microbial diversities of the air samples collected as measured using Shannon's index, were significantly different for samples collected on gelatin membrane filters (3.1, n=19) and on black polycarbonate filters (2.4, n=30) (Fig. 2.4, Kruskal-Wallis, $p = 0.001$). The diversity in bioaerosol samples collected using gelatin membrane filters was greater than the diversity in bioaerosol samples collected using the polycarbonate filters. The two filters also greatly differ in their ability to capture the relative abundance of bacterial families present in the air samples [Fig.2.5]. Gelatin membrane filters appear to have captured a much more diverse group of bacterial families. With sample collection conditions being the same for both filter types, this difference could be

explained by the structural difference between the two filters with gelatin membrane filters able to provide a larger surface area for capture. The methods used to determine relative abundance analyze total bacterial DNA present therefore; the viability of cells is not of consequence in this case. Since gelatin membrane filters structurally allow for the collection of a higher magnitude of bacterial cells, the higher number of cells could possibly also be bringing additional diversity with it. Also, another factor may be the effect of bacterial cell shape and how it is collected onto the filters [36]. The bacteria captured by both filters comprise of cocci (spherical) or bacilli (rod-shaped) cells. The bacteria collected on the black polycarbonate filters are mainly rod-shaped cells whereas; the gelatin membrane filters collected all types of bacteria cells.

Culture-based methods did not yield any colony forming units for any of the samples collected. Based on the results from the microscopic and sequence analysis, there appear to be plenty of viable bacterial cells whose composition is very diverse, yet no growth was observed using the culture method. So the limiting factors could be either the growth media chosen or the possibility that the viable or live cells observed may not have been easily capable of growth under the given experimental conditions i.e. they may be in a viable but non-culturable state [37]. The culture-based method was severely limiting to this study in estimating the total bacteria and its composition in outdoor air as it solely depends on the viable cells present, the growth medium and conditions selected.

Analysis of field blanks was carried out using mean values of total cell count data from the epifluorescence microscopy images for each filter type. The gelatin membrane filters produced a much higher field blank as compared to the black polycarbonate filters (Fig.2.3, Table 2.2). This does not support the proposed second hypothesis that the gelatin membrane filter would provide a low, artifact-free blank. From a quality control aspect of method development and evaluation, a high field blank is a drawback for the gelatin membrane filter and would need to be accounted for while choosing to use these filters. However, for this study, the gelatin membrane filters were still the better option since although the field blank accounted for less than half the total bacterial cells collected, the gelatin membrane filters still collected up to 10 times more bacterial cells than the black polycarbonate filters while also being able to capture higher bacterial diversity. Overall the gelatin membrane filters were better at capturing a complete bacterial composition both quantitatively and qualitatively.

Conclusions

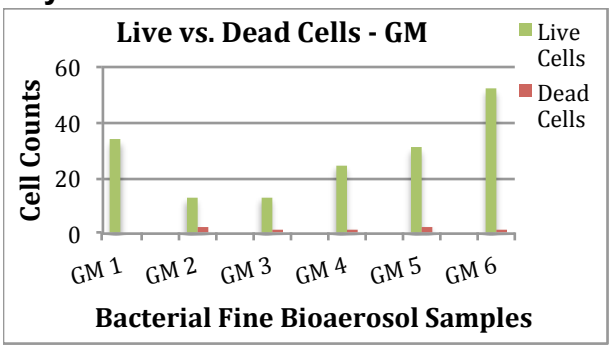
This study developed and evaluated a sampling method using gelatin membrane and black polycarbonate filters to investigate fine bacterial bioaerosols in outdoor air. The study findings conclude that gelatin membrane filters collected a more comprehensive sample of the outdoor bacterial microbiome as compared to polycarbonate filters both quantitatively and qualitatively. The gelatin membrane filters were also able to capture a larger portion of viable/live bacterial cells in the outdoor air. This is important since live bacterial cells may be capable of growth and multiplication given optimal conditions and

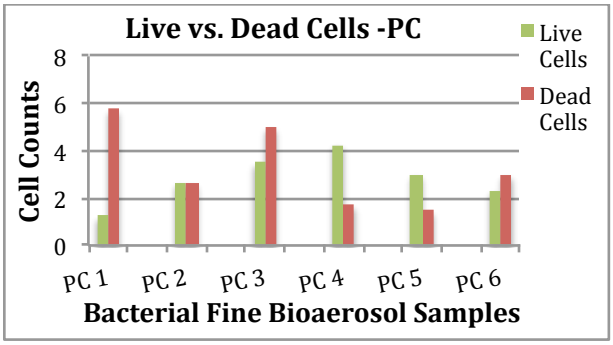
could pose a public health concern depending on the pathogenic nature of the organism. The investigation of particulate matter air pollution routinely uses size selective inlets such as cyclones and different types of filters to collect air samples. This study utilized these methods to study both viable and nonviable biological components of particulate matter in outdoor air. One limitation of using filters in this study to investigate the viable components of bioaerosols was the short sampling duration (25 minutes), which required a manual setup for each sample collection interval. However, this approach of using filters in combination with size-selective inlets has proved promising for further study of the outdoor microbiome and improved insight into fine bioaerosol composition and its potential role in airborne particulate exposures and impacts on human health.

Figures

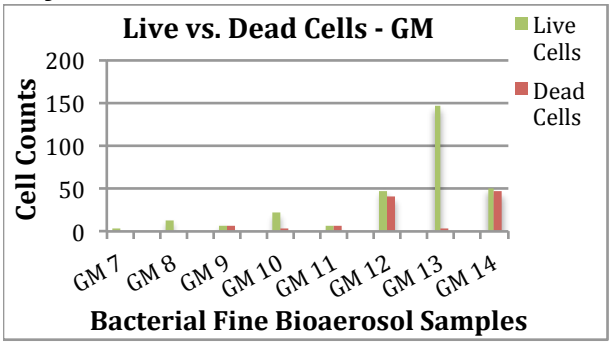
Figure 2.1: Sampling site at Dearborn, Michigan (A Michigan Department of Environmental Quality (MDEQ) air monitoring site)

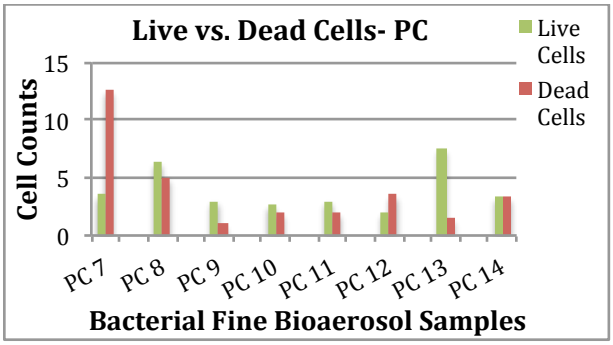
Day 1:



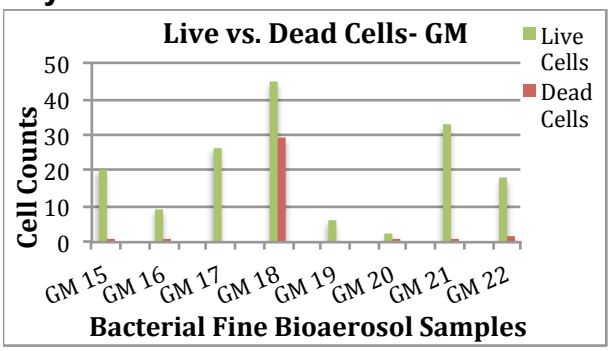


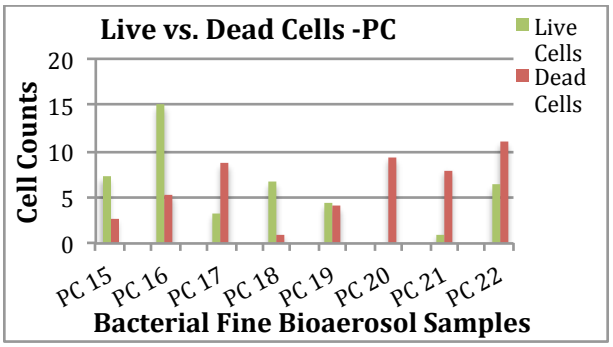
Day2:



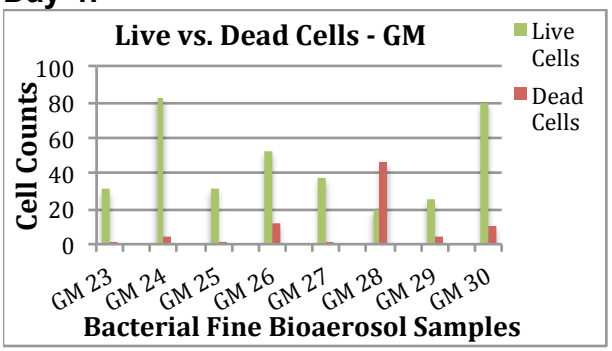


Day 3:





Day 4:



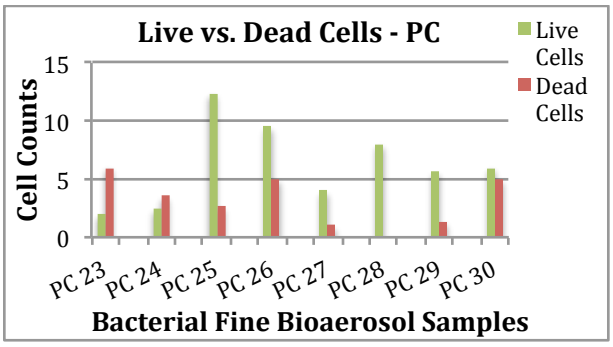


Figure 2.2: Manual counts of cells inferred to be live (viable) cells versus dead cells (non-viable) on both gelatin membrane (GM) and black polycarbonate (PC) filters, determined during each sampling interval (July 2014).

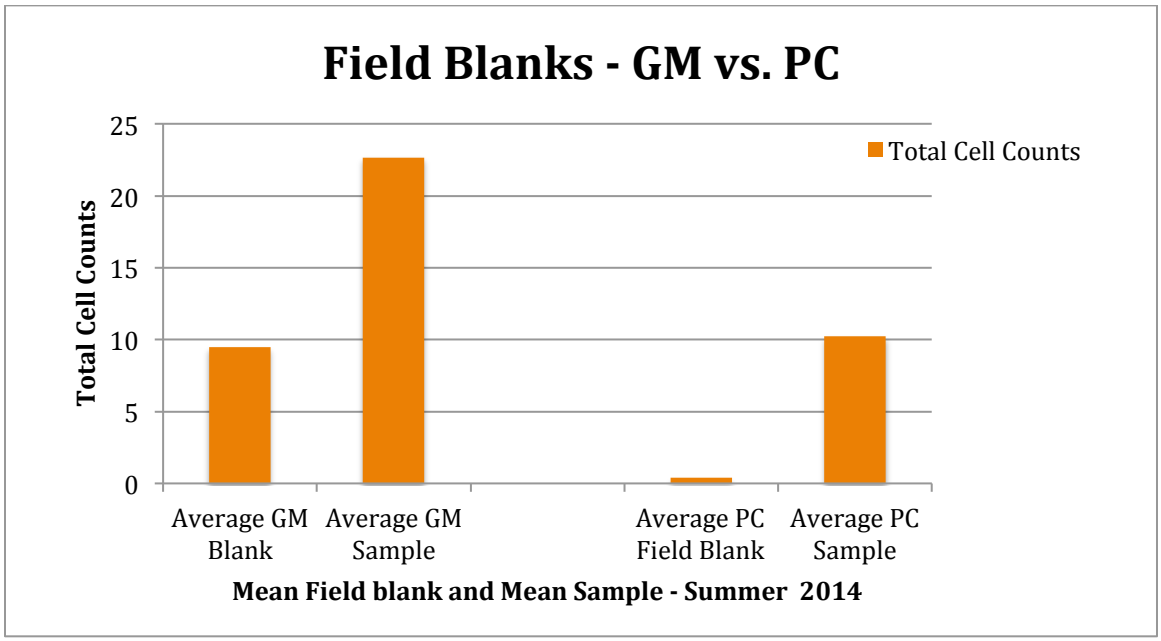


Figure 2.3: Analysis of field blanks for gelatin membrane (GM) and black polycarbonate (PC) filters.

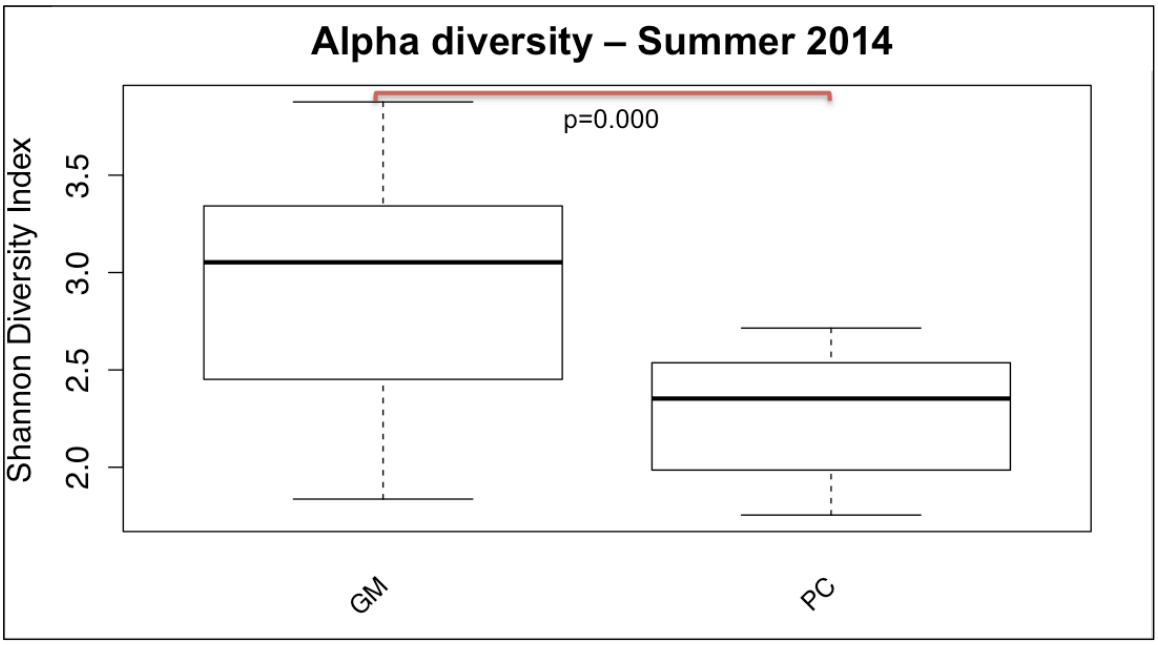


Figure 2.4: Shannon diversity index (Alpha diversity) to estimate the bacterial diversity collected on gelatin membrane (GM) and black polycarbonate (PC) filters at the urban airshed (DBN) in July 2014.

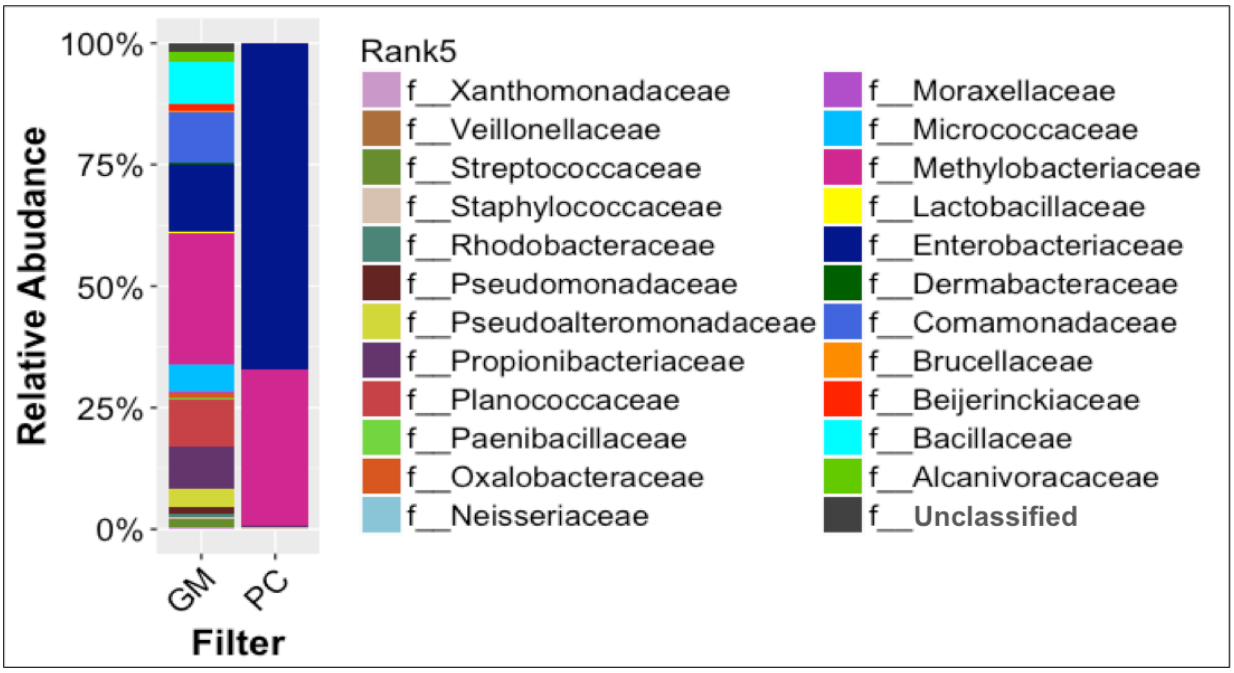


Figure 2.5: Relative abundance of bacterial families captured by each filter type – gelatin membrane (GM) and black polycarbonate (PC)

Tables

Table 2-1: Ratio of bacterial cell viability captured by each filter type.

Filter Type	Average Sample (Total Cell count)	Average Sample (Viable/live cell count)	Ratio of viable/live cells captured (%)
Gelatin Membrane	39.398	32.389	82.210
Black Polycarbonate	8.736	4.622	52.911

Table 2-2: Table showing field blank percentages as compared to samples collected on each filter type.

Filter Type	Average Sample (Total Cell count)	Average Field Blank (Total Cell count)	Percent Field Blank/Sample
Gelatin Membrane	22.667	9.500	41.912
Black Polycarbonate	10.250	0.375	3.659

References

1. Menetrez MY, Foarde KK, Esch RK, Dean TR, Betancourt DA, Moore SA, Svendsen ER, Yeatts K, 2007. The Measurement of Ambient Bioaerosol Exposure, Aerosol Science and Technology; 41:9, 884-893.

2. Thorne PS, Kiekhaeffer MS, Whitten P, Donham KJ, 1992. Comparison of Bioaerosol Sampling Methods in Barns Housing Swine. Applied and Environmental Microbiology; Vol.58, No.8, p.2543-2551.

3. Ross MA, Curtis L, Scheff A, Hryhorczuck DO, Ramakrishnan V, Waden RA, Persky VW, 2000. Association of asthma symptoms and severity with indoor bioaerosols. Allergy; 55:705-711.

4. Dybwad M, Skogan G, Blatny JM, 2014. Comparative Testing and Evaluation of Nine Different Air Samplers: End-to-End Sampling Efficiencies as Specific Performance Measurements for Bioaerosol Applications. Aerosol Science and Technology; 48:282-295.

5. Pope CA, III, Dockery DW, 2006. Health effects of fine particulate air pollution: lines that connect. Journal of the Air & Waste Management Association; 56:709–742.

6. Rivero DH, Soares SR, Lorenzi-Filho G, Saiki M, Godleski JJ, Antonangelo L, 2005. Acute cardiopulmonary alterations induced by fine particulate matter of São Paulo, Brazil. Toxicological Sciences; 85:898–905.

7. EPA, 2016. National Ambient Air Quality Standards. U.S.Environmental Protection Agency. https://www.epa.gov/criteria-air-pollutants/naaqs-table Accessed September 28, 2017.

8. Salvaggio J, and Aukrust L, 1981. Allergy and Clinical Immunology. Journal of Allergy and Clinical Immunology; 68(5).

9. Levetin E, 1995. Fungi. In Bioaerosols, Burge, H. (ed.). CRC Press, Boca Raton, FL.

10. Husman T, 1996. Health Effects of Indoor-Air Microorganisms. Scandinavian Journal of Work, Environment & Health; 22:5–13.

11. Franzetti A, Gandolfi I, Gaspari E, Ambrosini R, Bestetti G, 2011. Seasonal variability of bacteria in fine and coarse urban air particulate matter. Environmental Biotechnology; 90:745-753.

12. Haas D. Galler H, Luxner J, Zarfel G, Buzina W, Friedl H, Marth E, Habib J, Reinthaler F.F, 2013. The concentrations of culturable microorganisms in relation to particulate matter in urban air. Atmospheric Environment; 65:215-222.

13. Raisi L, Aleksandropoulou V, Lazaridis M, Katsivela E, 2012. Size distribution of viable, cultivable, airborne microbes and their relationship to particulate matter concentrations and meteorological conditions in a Mediterranean site. Aerobiologia; DOI 10.1007/s10453-012-9276-9.

14. Marshall J, 2013. PM 2.5. Proceedings of the National Academy of Sciences, USA; 110 (22): 8756.

15. Jensen PA, Shaffer MP, 1994. Sampling and Characterization of bioaerosols. NIOSH Manual of Analytical Methods. Available online at http://www.cdc.gov/niosh/docs/2003-154/pdfs/chapter-j.pdf

16. Wang Z, Reponen T, Grinshpun SA, Gorny RL, Willeke K, 2001. Effect of sampling time and air humididty on the bioefficiency of filter samplers for bioaerosol collection. Journal of Aerosol Science; 32:661-674.

17. Mainelis G, Tabayoyong M, 2010. The Effect of Sampling Time on the Overall Performance of Portable Microbial Impactors, Aerosol Science and Technology, 44:1, 75-82.

18. Li C-S, Hao M-L, Lin W-H, Chang C-W, Wang C-S, 1999. Evaluation of Microbial Samplers for Bacterial Microorganisms. Aerosol Science and Technology; 30:100–108.

19. Chih-Shan Li (1999). Sampling Performance of Impactors for Bacterial Bioaerosols, Aerosol Science and Technology, 30:3, 280-287

20. Mouli CP, Mohan S, Reddy JS, 2005. Assessment of Microbial (Bactteria) Concentrations of Ambient Air at Semi-Arid Urban Region:Influence of Meteorological Factors. Applied Ecology and Environmental Research; 3(2): 139-149.

21. Go`rny RL, Dutkiewicz J, Krysi`nskap-Traczyk E, 1999. Size distribution of bacterial and fungal bioaerosols in indoor air. Annals of agricultural and environmental medicine: AAEM; **6:**105–113.

22. Maki T, Puspitasari F, Hara K, Yamada M, Kobayashi F, Hasegawa H, Iwasaka Y, 2014. Variations in the structure of airborne bacterial communities in a downwind area during an Asian dust (Kosa) event. Science of the Total Environment; 488-489(1): 75-84.

23. Martin E, Dziurowitz N, Jackel U, Schafer J, 2015. Detection of airborne bacteria in a duck production facility with two different personal air sampling devices for an exposure assessment. Journal of Occupational and Environmental Hygiene; Vol. 12, Iss. 2: 77-86.

24. Wang C-H, Chen BT, Han B-C, Liu AC-Y, Hung P-C, Chen C-Y, Chao HJ, 2015. Field Evaluation of Personal Sampling Methods for Multiple Bioaerosols. PLoS ONE 10(3): e0120308. doi:10.1371/journal. pone.0120308.

25. Droogenbroeck CV, Risseghem MV, Braeckman L, Vanrompay D, 2009. Evaluation of bioaerosol sampling techniques for the detection of *Chlamydophila psittaci* in contaminated air. Veterinary Microbiology; 135 (1-2), pp.31-37

26. SKC Inc. Gelatin Filters Operating Instructions. Air Sampling Filters and Accessories. http://www.skcinc.com/catalog/pdf/instructions/40060.pdf Accessed September 29, 2017.

27. Predicala BZ, Urban JE, Maghirang RG, Jerez SB, Goodband RD, 2002. Assessment of bioaerosols in swine barns by filtration and impaction. Current Microbiology; 44(2):136-140.

28. Kalogerakis N, Paschali D, Lekaditis V, Pantidou A, Eleftheriadis K, Lazaridus M, 2005. Indoor air quality – bioaerosol measurements in domestic and office premises. Journal of Aerosol Science; 36(5-6):751-761.

29. Rinsoz T, Duquenne P, Greff-Mirguet G, Oppliger A, 2008. Application of real-time PCR for total airborne bacterial assessment: Comparison with epifluorescence microscopy and culture-dependant methods. Atmospheric Environment; 42(28):6767-6774.

30. Hara K, Zhang D, 2011. Bacterial abundance and viability in long-range transported dust. Atmospheric Environment; 47:20-25.

31. Grey BE, Steck TR, 2001. The viable but nonculturable state of Ralstonia sol-anacearum may be involved in long-term survival and plant infection. Applied and Environmental Microbiology; 67:3866-3872.

32. Janssen PH, Yates PS, Grinton BE, Taylor PM, Sait M, 2002. Improved culturability of soil bacteria and isolation in pure culture of novel members of the divisions Acidobacteria, Actinobacteria, Proteobacteria, and Verrucomicrobia. Applied and Environmental Microbiology; 68 :2391-2396.

33. Lindsley WG, 2016. Filter Pore Size and Aerosol Sample Collection. NIOSH Manual of Analytical Methods, 5th Edition. https://www.cdc.gov/niosh/docs/2014-151/pdfs/chapters/chapter-fp.pdf

34. Burton NC, Grinshpun SA, Reponen T, 2007. Physical collection efficiency of filter materials for bacteria and viruses. The Annals of Occupational Hygiene; 51(2): 143-151.

35. Willeke K, Macher JM, 1999. Air sampling. In: J.M. Macher (Ed.), Bioaerosols: Assessment and control. Cincinnati, OH: American Conference of Governmental Industrial Hygienists; (pp. 11-1-11-25)

36. Wang Y, Hammes F, Duggelin M,Egli T, 2008. Influence of size, shape and flexibility on bacterial passage through micropore membrane filters. Environmental Science and Technology; 42(17): 6749-6754.

37. Oliver JD, 2005. The viable but nonculturable state in bacteria. The Journal of Microbiology; 43 (S): 93-100.

CHAPTER 3 Characterization of fine bacterial bioaerosols present in urban and rural airsheds

Abstract

Background: The presence of bacterial bioaerosols in fine Particulate Matter (PM2.5) in outdoor air has not been fully investigated to date. This is an important knowledge gap in the scientific literature, as land use changes and urban planning have often resulted in close proximity of residential communities to industrial operations such as sewage treatment, animal rendering, food processing plants and agricultural activities which contribute to emissions of microorganisms into the air. This study investigated the bacterial bioaerosol compositions in fine PM2.5 within a rural and an urban airshed to understand the impact of possible emission sources in these vicinities.

Hypothesis: Fine bacterial bioaerosols within the urban airshed will exhibit a greater bacterial diversity including pathogenic/opportunistic pathogenic species as compared to the rural airshed.

Methods: Fine bioaerosol samples were collected on sterile gelatin membrane filters using size selective cyclone inlets. The bacterial DNA concentrations were measured using quantitative PCR. The relative abundance and variation in bacterial community

compositions within each airshed was determined using 16s rRNA barcoded pyrosequencing methods.

Results: A significant difference was observed in the bacterial community composition within the urban airshed as compared to the rural airshed (p=0.007).

Conclusion: Bacterial families identified in both airsheds appear to be influenced by the possible emission sources within each local vicinity.

Introduction

Bioaerosols, or the biological component of particulate matter, are comprised of natural occurring components such as pollen, fungal spores, bacteria and parts of plant matter. Bioaerosols arise from anthropogenic activities such as farming practices, animal rearing activities and certain industrial activities. The presence of bacteria in fine bioaerosols as it relates to possible emission sources needs to be better investigated as it impacts human health mainly by affecting the respiratory system through inhalation [1,2]. The presence of certain pathogenic bacteria or their cell components could have adverse impacts on human health either by causing infection or allergic responses.

Bacterial bioaerosols have mainly been studied with regards to indoor air quality [3,4,5]. These studies included exposure within occupational settings, whether conducted at animal feeding operations, composting facilities, wastewater treatment plants, biosolids application sites, or wastewater spray irrigations sites. The general trend observed in these studies was that the airborne microorganism concentrations decreased with

distance from the source [6,7,8]. However, as human populations have grown, land use changes have occurred and cities have expanded, this has led to closer proximity of residential communities to sources of bacterial bioaerosol emissions.

Some studies, which have attempted to study the role of outdoor bioaerosols, have found large variations in the diversity and concentrations of bioaerosols based on location of the sampling site [9,10]. These differences were seen in urban versus rural areas and were also influenced by temperature, wind speeds and relative humidity. However, sources responsible for these differences were not reported.

Bioaerosols are known to be emitted during various stages of sewage and wastewater treatment [11,12,13]. These emissions can also contain pathogenic bacteria, which are known to be harmful to human health [14,15,16]. Also, due to their small particle size, they can be transported over long distances and can possibly affect larger populations mainly through inhalation [17]. In a study at a swine operation, the average bacterial concentrations within the barns were 1.8 × 104 cfu.m−3, and although the outside air concentration decreased with distance from the facility, at 150 m downwind the bacterial concentration was still 2.5-fold greater (208 cfu.m−3) than at the upwind location [18]. These findings suggest that even with current ventilation and filtration methods in place, facilities such as these are a source of microbial loading in the surrounding outdoor air. These studies investigating the role of bacteria in outdoor air are often limited either by the use of classical culture based methods that underestimate the bacteria in air or by focusing only on larger particulate matter.

Our study focused on bacteria present in PM2.5 as those particles are easily respirable and can directly impact human health. This study has implemented quantitative PCR along with 16s rRNA barcoded pyrosequencing methods to allow for a comprehensive understanding of the bacterial bioaerosols present in outdoor air. The choice of a rural and urban airshed provided for the investigation of contribution from natural and anthropogenic sources of bacterial bioaerosols.

Study Airsheds

Dearborn, MI: An existing exposure study location in Dearborn, MI (42.3063° N, 83.1497° W) was utilized as the monitoring site [Fig.3.1a, 3.1b, 3.1c]. This site has been used in the past by the University of Michigan Air Quality Laboratory (UMAQL) for PM exposure studies. The site is located on the grounds of Salina Elementary School, and also utilizes an existing Michigan DEQ routine air monitoring site. This site also represents some of the highest measured PM2.5 levels in the state of Michigan [19]. The Dearborn site is located within 5km of iron/steel production facilities, a coke oven, oil refinery and a coal-fired power plant. There are also residential communities in the immediate vicinity. This site was chosen specifically to investigate the role of fine bioaerosols in this community, as it is also located immediately downwind of several meat processing facilities and the largest wastewater treatment facility in the United States.

Dexter, MI: The Dexter monitoring site (42.416874^{o} N, 83.902230^{o} W) is located in a rural area located approximately 35km northwest of Ann Arbor and the University of Michigan (UM) campus [Fig.3.1a, 3.1.d, 3.1e]. The site is located on UM research property and has been operated as a long-term air monitoring site by the UMAQL, as well as serving as a site for the Clean Air Status and Trends Network (CASTNET). The Dexter airshed is located significantly upwind of any large anthropogenic point source and serves as an ideal site to assess levels of regionally transported pollutants. The possible sources of bioaerosol emissions at this location include mostly natural sources such as dense vegetation, soil, water bodies such as lakes and presence of woodland animals.

Materials and Methods

Sample collection

The sample collection method was designed to investigate the bacterial composition of fine particulate matter (PM2.5) at an urban (Dearborn, MI) and rural airshed (Dexter, MI). The sampling period was over 4 days spread out over a two-week period (through June and July) in Summer 2014 at each airshed respectively. Air samples were collected on 47mm pre-sterile gelatin membrane filters having a pore size of 3µm (Sartorius, Germany) and using size selective Teflon coated aluminum cyclone inlets (University Research Glassware, USA) drawing air at a flow rate of 16.7 liters/minute using vacuum pumps. Samples were collected over 25 minute intervals. A total of 30

samples were collected at each airshed site. All samples were collected, handled and stored until analysis as described in the methods section in Chapter 2.

Sample analysis

To estimate the total bacterial concentrations in each given airshed, the gelatin membrane filters were processed and analyzed using quantitative PCR. Bacterial communities and their relative abundance within samples from each airshed were identified and assessed using 16S rRNA barcoded pyrosequencing.

Quantitative PCR (qPCR)

Total bacterial quantification was performed using real-time PCR according to Einen *et al*. [20]. 20 µL PCR reaction mixtures were prepared containing 500 nM of primer Eu338 (5'-ACTCCTACGGGAGGCAGCAG-3') and Eu518 (5'-ATTACCGCGGCTGCTGG-3') [20], about 100 pg of template DNA, and 10 µL of 2 × SYBR Green PCR Master Mix (Applied Biosystems, Foster City, CA). The PCR program was as follows: 95 ^{o}C for 15 min, 40 cycles of denaturing (15 seconds at 94 ^{o}C), annealing (30 seconds at 61 ^{o}C), and extension (30 seconds at 72 ^{o}C). The cycling was followed by a final extension at 72 ^{o}C for 7 min and a melting curve analysis from 65–95 ^{o}C, with a plate read every 0.5 ^{o}C.

DNA extraction

The gelatin membrane filter extracts were homogenized and used at 400ul into PowerSoil DNA isolation kit (MoBio Laboratories, Carlsbad, CA, USA). Additional steps for the DNA extraction were carried out according to the manufacturer's instructions.

bTEFAP sequencing

The bTEFAP® was carried out by Molecular Research LP (MR DNA, Shallowater, TX). 16S universal Eubacterial primers (27Fmod AGRGTTTGATCMTGGCTCAG and 519Rmod GTNTTACNGCGGCKGCTG) were used to amplify the 300bp region of 16s rRNA genes. The HotStarTaq Plus Master Mix Kit (Qiagen, Valencia, CA) was used to perform a 30-cycle PCR under the following settings: 94^{o}C for 3 minutes, followed by 28 cycles of 94^{o}C for 30 seconds; 53^{o}C for 40 seconds and 72^{o}C for 1 minute; after these steps a final elongation step at 72^{o}C for 5 minutes was carried out. After PCR completion, all amplicon products from different samples were combined in equal concentrations and purification was carried out using Agencourt Ampure beads (Agencourt Bioscience Corporation, MA, USA). The samples were then sequenced using Roche 454 FLX titanium instruments and reagents as per manufacturer's instructions.

Downstream analysis

Microbiome analysis was conducted with QIIME 1.9.0 and the resultant operational taxonomic unit (OTU) table was analyzed with the R package Phyloseq 1.10.0. First, raw sequences were demultiplexed with the split_library.py script where sequences are assigned to a sample based on its sequenced barcode. An open-reference OTU-picking

strategy using the GreenGenes 13_8 database was used to generate the OTU table. A post-processing step was applied where OTUs constituting less than .05% of the total reads was excluded. Taxonomic summaries and alpha diversities of the samples by polycarbonate filters and gelatin membranes were generated with Phyloseq 1.10.0 after importing the OTU-table into R.

Data analysis

Bacterial DNA concentrations were calculated using the raw qPCR results as the number of genome copies per cubic meter of air. The visualization of results was carried out using Microsoft Excel.

The bacterial diversity between samples or between groups is represented by beta diversity. Diversity between samples was assessed using the Euclidean distances. This metric takes describes the relative abundance of operational taxonomic unit (OTU) rather than only presence. The Mann Whitney nonparametric test was performed to test for community differences in the mean number of observed OTU by groupings of interest. The significance threshold was set at an α-value of 0.05. Ecological investigation was were carried out using R.

Results and Discussion

The overall bacterial DNA concentrations at both airsheds did not appear to differ greatly [Fig.3.2]. The total bacterial DNA concentrations observed in both airsheds were consistent with other studies describing background bacterial bioaerosol concentrations [21]. It is interesting to note that the urban airshed had similar bacterial DNA concentrations even though it does not share the same natural sources - abundance of vegetation, soil, presence of water bodies and animals found in the rural airshed. So, the bacterial bioaerosols present in the urban airshed can be assumed to be mainly from the anthropogenic sources of bioaerosol emissions in the vicinity. These anthropogenic sources that include wastewater treatment facilities and meat processing facilities could be possible sources of bacterial communities in the air that may be capable of causing illness. When investigating the presence of potentially pathogenic bacterial bioaerosols, estimating bacterial bioaerosol concentration is an important consideration. However, the composition of bacterial communities i.e. identification of the type of bacteria – pathogen or an opportunistic pathogen is fundamental when the goal is to safeguard human health. Therefore, it is important to understand the bacterial composition in these two airsheds, as the sources of bioaerosol emissions are different.

The degree of difference between the bacterial community compositions within each airshed can be explained by the beta diversity. The beta diversity calculated in this study revealed that the bacterial community composition at the urban airshed was significantly diverse as compared to the bacterial communities at the rural airshed

[Fig.3.3 MannWhitney, p=0.007]. This higher variation in bacterial species diversity could be due to the urban site having a more diverse group of emission sources as compared to the rural site comprising of mainly leaf and soil bacteria. This would be expected from the urban site due to the varied mix of anthropogenic activity in the area such as industrial operations, meat processing plants, wastewater treatment plant and other human activities. The bacterial presence as a whole including its concentration and diversity at this urban site is informative especially since it exists along with other conventional air pollutants such as PM2.5, nitrogen oxides and ozone which may have previously been thought to negatively impact bacteria in air, especially in its ability to remain viable [22,23].

The identified bacterial families and their relative abundance within each airshed are seen in Fig. 3.4. A greater variation in bacterial families was observed within the urban airshed. The bacterial communities at the urban airshed appear to be more diverse as compared to the bacterial communities at the rural airshed. The bacterial families found at the urban airshed are also related to water, animal and human gut microbial communities, which maybe due to the presence of the wastewater treatment facility and meat processing faciltities in the area. The urban airshed contained a larger portion of certain bacterial families such as Streptococcaceae (urban = 2.64%, rural = 0.45%), Pseudomonadaceae (urban = 19.48%, rural = 8.08%), Micrococcaceae (urban = 4.14%, rural = 0.58%) and Enterobacteriaceae (urban = 11.78%, rural = 0.34%), which are known to be pathogens and opportunistic pathogens [Table 3.1]. The rural site was rich with bacteria, mainly from vegetation and soil that are abundant in the surrounding area

considering it is mostly wooded land with very few anthropogenic emission sources. The bacterial bioaerosol composition results presented in this study are consistent with findings from previous studies that investigated bacterial bioaerosol composition in outdoor airsheds, where the bacterial composition was found to differ by geographical location that included different possible sources [10,24]. The bacterial bioaerosol concentration results from this study are also consistent with other studies that have examined ambient outdoor air using similar analytical methods [24]. However, these studies are few, most outdoor bioaerosol studies have focused on studying emissions at a given source and have therefore found concentrations to be higher when compared to airsheds without such anthropogenic point sources [9,10]. Also, the sampling duration and analytical methods used to estimate these parameters in most studies are either too short or limited to classical culture-based methods to calculate bacterial concentrations in air. Also, as this study was conducted during the summer season, it would be interesting to examine if the bacterial bioaerosol concentrations at both airsheds are similar during winter time periods when there would be a shift in the source of bacterial emissions in the rural airshed as it consists of mainly wooded land.

Conclusion

The bacterial community composition at the urban airshed is significantly diverse as compared to the bacterial communities at the rural airshed. The urban airshed also contains a higher portion of pathogenic and opportunistic pathogenic bacteria that appear to be related to the possible sources in the area. It needs to be further

investigated to understand if this is due to the specific presence of the meat processing and waste water treatment plants in the vicinity.

The results of this study highlight the difference in bacterial communities present in fine bioaerosols based on sources in the vicinity. This possibility for additional microbial loading into the air of communities from nearby anthropogenic sources should be considered while carrying out land use planning.

From a public health perspective, an airshed simply exhibiting a greater microbial diversity does not signal cause for concern as presence of certain bacteria in the environment and in the human body is essential for survival and well being. As mentioned earlier, information on the type of bacteria present, its concentrations and its ability to cause infection or an allergic response is what's critical to be investigated.

Figures

Figure 3.1a: Study airsheds in Southeast Michigan.

Figure 3.1b: Urban airshed and MI DEQ Air monitoring site at Dearborn, MI

Figure 3.1c: Urban airshed with possible bioaerosol emission sources, Dearborn, MI

Figure 3.1d: Sample collection setup at Rural airshed- Dexter, MI

Figure 3.1e: Rural airshed, no large anthropogenic emission sources, Dexter, MI.

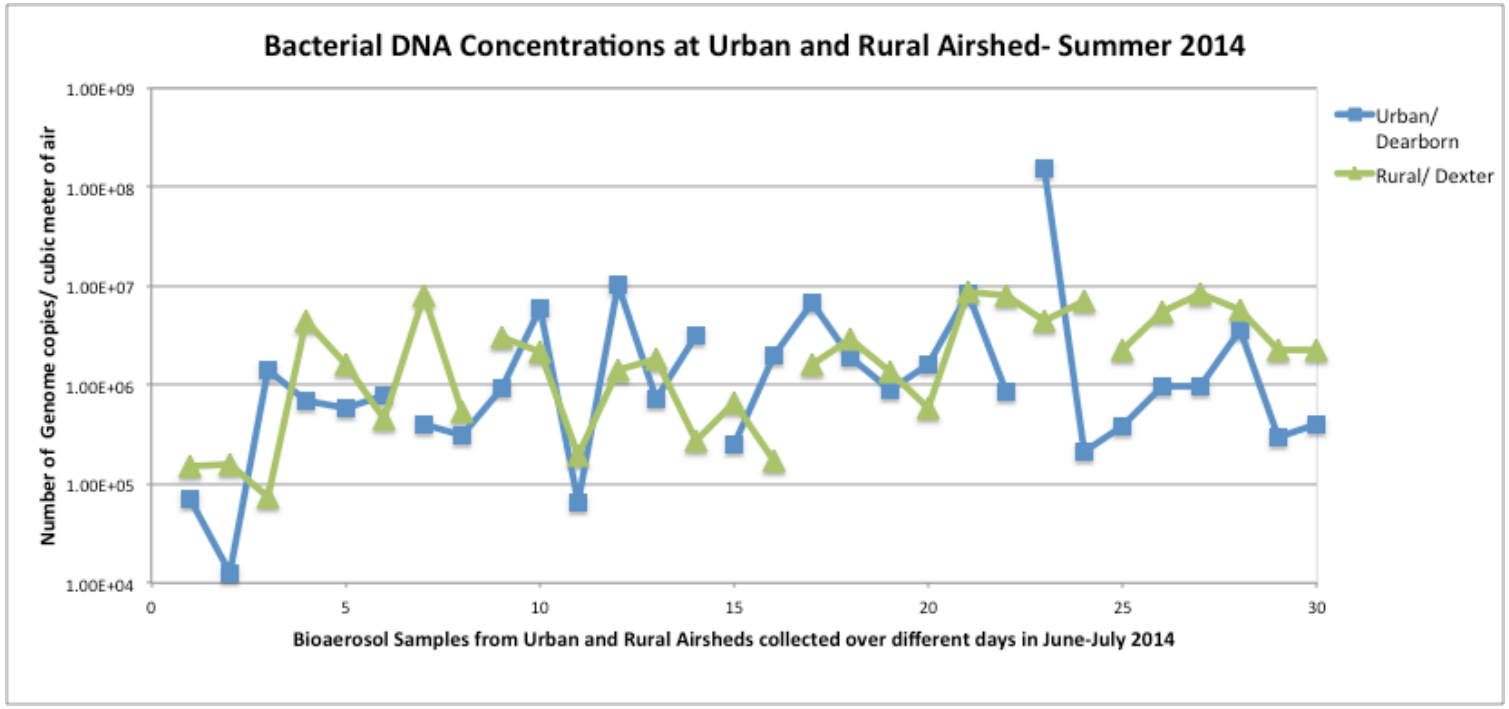


Figure 3.2: Comparison of Bacterial DNA concentrations in the urban and rural airsheds. Samples were collected over different time periods over the summer at each airshed; breaks in the scatterplot represent samples collected on different days.

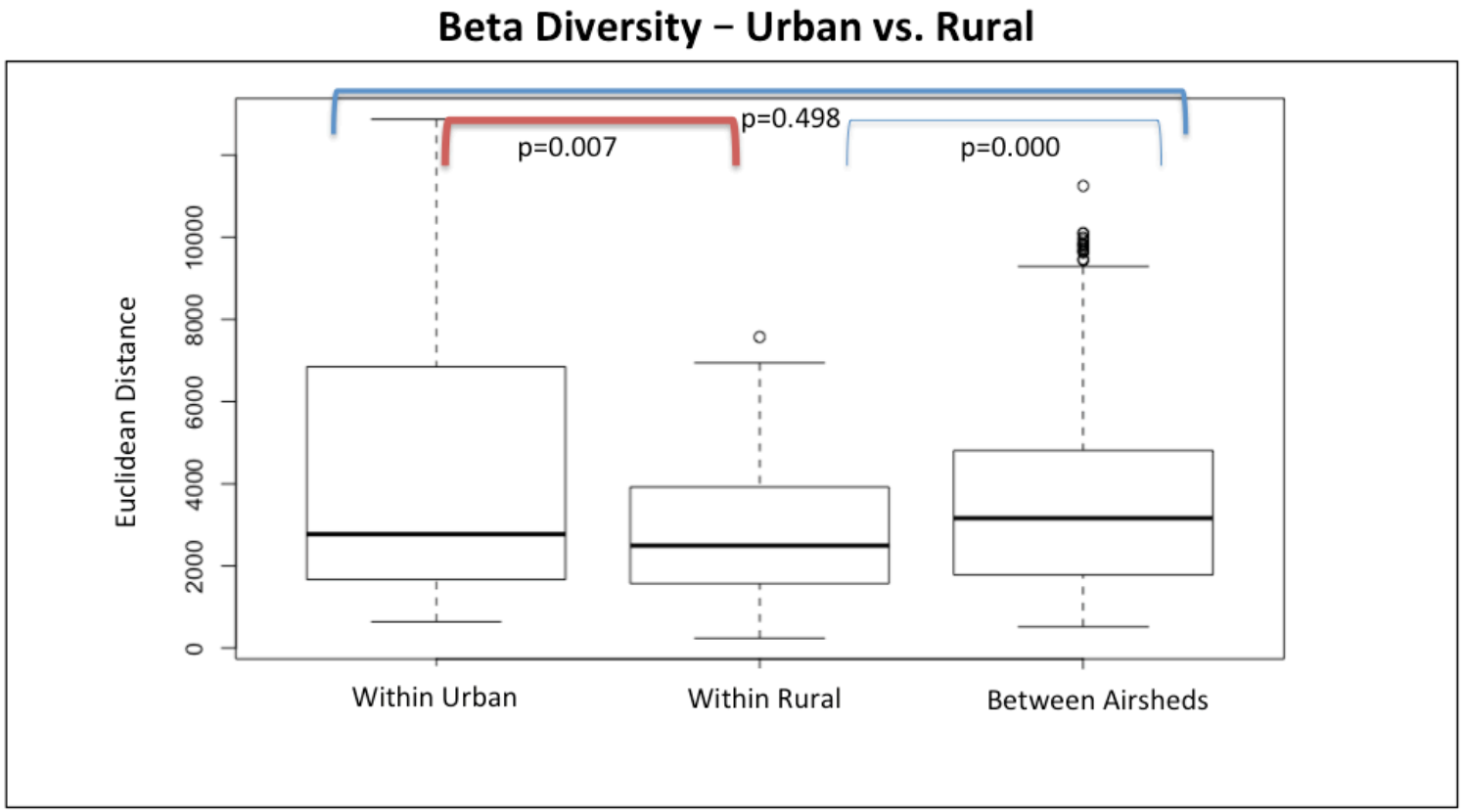


Figure 3.3: Euclidean distances within and between samples at the urban and rural airsheds in Summer 2014 collected on GM filters.

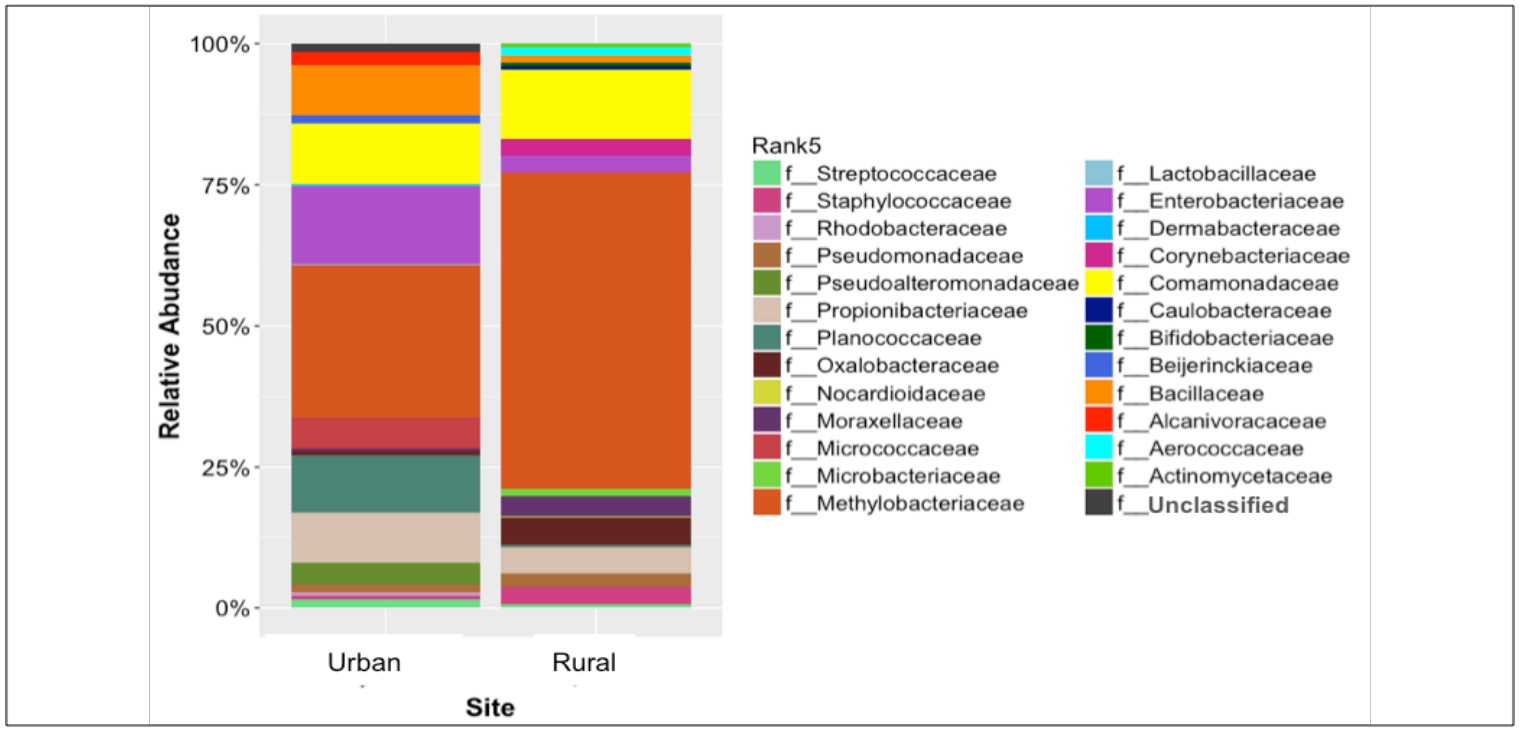


Figure 3.4: Relative abundance of bacterial families within each airshed in Summer 2014.

Tables

Table 3-1: Summary table highlighting the pathogenic species and percentages at each airshed.

Bacterial Family	Urban (%)	Rural (%)
Streptococcaceae	2.64	0.45
Staphylococcaceae	1.06	2.62
Pseudomonadaceae	19.48	8.08
Micrococcaceae	4.14	0.58
Methylobacteriaceae	23.19	60.83
Enterobacteriaceae	11.78	0.34
Comamonadaceae	1.05	1.09

References

1. D'Amato G. 2002. Environmental urban factors (air pollution and allergens) and the rising trends in allergic respiratory diseases. Allergy; 57:30–33.

2. Pope CA, III, Dockery DW, 2006. Health effects of fine particulate air pollution: lines that connect. Journal of the Air & Waste Management Association; 56:709–742.

3. Meretrez MY, Foarde KK, Esch RK, Schwartz TD, Dean, TR, Hays MD, et al.,2009. An evaluation of indoor and outdoor biological particulate matter. Atmospheric Environment; 43: 5476–5483.

4. Parat S, Perdrix A, Mann S, Baconnier P, 1999. Contribution of particle counting in assessment of exposure to airborne microorganisms. Atmospheric Environment; 33: 951–959.

5. Kalogerakis N, Paschali D, Lekaditis V, Pantidou A, Eleftehriadis K, & Lazaridis M, 2005. Indoor air quality - bioaerosol measurements in domestic and office premises. Journal of Aerosol Science; 36: 751–761.

6. Taha MPM, Pollard SJT, Sarkar U, and Longhurst P, 2005. Estimating fugitive bioaerosol releases from static compost windrows: Feasibility of a portable wind tunnel approach. Waste Management; 25:445 – 450.

7. Green CF, Gibbs SG, Tarwater PM, Mota LC, and Scarpino PV, 2006. Bacterial plume emanating from the air surrounding swine confinement operations. Journal of Occupational and Environmental Hygiene; 3:9–15.

8. Low S Y, Paez-Rubio T, Baertsch C, Kucharski M, and Peccia J, 2007. Off-site exposure to respirable aerosols produced during the disk-incorporation of class B biosolids. Journal of Environmental Engineering; 133:987–994.

9. Chandra Mouli P, Venkata Mohan S, Jayaramma Reddy S, 2005. Assessment of microbial (bacteria) concentrations of ambient air at semi-arid urban region: Influence of meteorological factors. Applied Ecology and Environmental Research; 3(2):139-149.

10. Di Giorgio C, Krempff A, Guiraud H, Binder P, Tiret C, Dumenil G, 1996. Atmospheric pollution by airborne microorganisms in the city of Marseilles. Atmospheric Environment; 30 (1): 155-160.

11. Z Filipkowska, 2003.Sanitary and Bacteriological Aspects of Sewage Treatment, Acta Microbiologica Polonica: 52 (Suppl.), 57– 66.

12. Fernando NL, Fedorak PM, 2005. Changes at an Activated Sludge Sewage Treatment Plant Alter the Numbers of Airborne Aerobic Microorganisms, Water Research; 39 (19): 4597– 4608.

13. Patentalakis N, Pantidou A, Kalogerakis N, 2008. Determination of Enterobacteriaceae in Air and Wastewater Samples from a Wastewater Treatment Plant by Epi-fluorescence Microscopy, Water Air Soil Pollution, 8 (1): 107– 115.

14. Rylander R, 1999. Health Effects among Workers in Sewage Treatment Plants, Occupational and Environmental Medicine; 56 (5): 354– 357.

15. Laitinen S, Kangas J, Husman K, Susitaival P, 2001. Evaluation of Exposure to Airborne Bacterial Endotoxins and Peptidoglycans in Selected Work Environments, Annals of Agricultural and Environmental Medicine; 8(2): 213–219.

16. Carducci A, Tozzi E, Rubulotta E, Casini B, Cantiani L, Rovini E, Muscillo M, et al., 2000. Assessing Airborne Biological Hazard from Urban Wastewater Treatment, Water Research, 34 (4): 1173– 1178.

17. Gotkowska-Płachta A, Filipkowska Z, Korzeniewska, E, Janczukowicz W, Dixon, B, Gołaś I and Szwalgin D, 2013. Airborne Microorganisms Emitted from Wastewater Treatment Plant Treating Domestic Wastewater and Meat Processing Industry Wastes. Clean Soil Air Water, 41: 429–436. doi:10.1002/clen.201100466

18. Green CF, Gibbs SG, Tarwater PM, Mota LC, and Scarpino PV, 2006. Bacterial plume emanating from the air surrounding swine confinement operations. Journal of Occupational and Environmental Hygiene; 3:9–15.

19. Michigan Department of Environmental Quality webpage, 2016. Available at http://www.michigan.gov/documents/deq/deq-aqd-amu 2016_Annual_Air_Quality_Report_579259_7.pdf

20. Einen J, Thorseth IH, Ovreas L, 2008. Enumeration of Archae and Bactria in seafloor basalt using real-time quantitative PCR and fluorescence microscopy. FEMS Microbiology Letters; 282 (2): 182-7.

21. Lighthart, B. 2000. Mini-review of the concentration variations found in the alfresco atmospheric bacterial populations. Aerobiologia 16:7–16.

22. Raisi L, Aleksandropoulou V, Lazaridis M, Katsivela E, 2012. Size distribution of viable, cultivable, airborne microbes and their relationship to particulate matter concentrations and meteorological conditions in a Mediterranean site. Aerobiologia; DOI 10.1007/s10453-012-9276-9.

23. Garrison, V. H., Foreman, W. T., Genualdi, S., Griffin, D. W., Kellogg, C. A., Majewski, M. S., et al. 2006. Saharan dust—a carrier of persistent organic pollutants, metals and microbes to the Caribbean? Revista de Biologia Tropical (International Journal of Tropical Biology and Conservation), 54(3): 9–21.

24. Bowers, RM, McLetchie S, Knight R, and Fierer N, 2011. Spatial variability in airborne bacterial communities across land-use types and their relationship to the bacterial communities of potential source environments. The ISME Journal, 5(4): 601–612. http://doi.org/10.1038/ismej.2010.167.

CHAPTER 4 Influence of seasonal variation and chemical co-pollutants on fine bacterial bioaerosols in outdoor air

Abstract

Background: Fine bioaerosols are an important part of particulate matter pollution in outdoor air. This study was designed to examine the impact of chemical co-pollutants like fine particulate matter (PM2.5) mass and ozone on bacterial bioaerosol concentration and composition in outdoor air as well as the effects of seasonal variation.

Hypotheses:

1. Presence of higher concentrations of certain chemical co-pollutants will be associated with changes in fine bacterial bioaerosol concentration and composition.
    - Meteorological parameters such as temperature, relative humidity, wind speed and wind direction will have an influence on bacterial DNA concentrations.
    - Higher concentrations of co-pollutants like ozone and PM2.5 mass will be associated with reduction in bacterial DNA concentrations.
2. Bacterial composition of outdoor air will change with seasonal variations.

Methods: Bacterial DNA concentrations were quantified using quantitative PCR. Bacterial community composition was identified using 16s rRNA barcoded pyrosequencing. Redundancy analysis was performed to analyze the influence of environmental parameters on bacterial community composition. Beta diversity metric was assessed to understand the impact of seasonal changes on bacterial composition.

Results: Relative humidity, temperature, wind speed and ozone all appeared to have a significant influence on fine bacterial bioaerosol composition in outdoor air. However, the findings may be limited due to the short duration of the study period. Bacterial compositions were also influenced by changes in seasons. The differences due to changes in season were greater between the urban and rural airsheds in the winter, spring and summer seasons.

Conclusions: Seasonal variations and meteorological parameters do impact bacterial bioaerosol composition in outdoor air.

Introduction

The atmosphere has been suggested to serve primarily as a transport medium for microorganisms and not as reservoir for growth [1]. The diversity of microorganisms in the air depends primarily on the characteristics of the given environment, the type of microbe and on the transmission and transformation processes [2]. Bacterial concentrations are mostly governed by the sources of bacterial emissions – these could be natural or anthropogenic, with temporal influences such as season and time of day [1]. Meteorological parameters such as temperature and relative humidity in the air also

influence the concentrations of outdoor airborne microorganisms [3,4,5]. Of these parameters, temperature, relative humidity and wind speed appeared to have a significant impact on bacterial concentrations [5]. Temperature and relative humidity are often dependent on seasons. Most studies looking at the impact of seasonal changes in bacteria have been conducted in indoor environments [6,7,8]. The studies that have examined the effect of seasonal variation in outdoor environments have found bacterial compositions to be influenced by changes in temperature corresponding to seasonal changes [9].

Bacterial cell viability in the outdoor air can also be compromised by environmental factors such as solar radiation, ozone and other chemical co-pollutants [1,10]. In urban or industrial areas, air pollution is further exacerbated due to emissions from industrial processes, which include gases such as carbon monoxide, hydrocarbons, nitrogen oxides, sulfur dioxide and other trace elements. These additional chemical pollutants may also influence the survival and presence of bacteria in air.

Several studies have looked at the effect of chemicals on microorganisms present in different media such as water and soil. However, there are few studies looking at their effects on airborne microbes but even fewer have studied the effects of chemical air pollutants on microbial air pollutants. Ozone is known to be an effective disinfectant at higher concentrations and is used as standard disinfecting procedure in water treatment plants. The bactericidal effect of atmospheric ozone was tested on *Escherichia coli* and *Staphylococccus aureus* at concentrations between 300-1500ppm [11]. Death rates in

excess of 99.99% were achieved for both species; the mechanism of inactivation accorded with the predictions of first and second order kinetics suggesting that the disinfection action of ozone in air compares to its action in water. Another study subjected aerosolized bacteria – *Micrococcus luteus* in a laboratory experiment to ozone concentrations of 0.05, 0.1 and 2.0 ppm for an hour and observed significant log reductions of the aerosolized bacteria after the hour-long exposure [8]. These findings from studies conducted under controlled conditions suggest that similar associations might be observed in outdoor air between fine bioaerosols and gaseous pollutants. However, these associations have not been fully explored in ambient air. Also, in measurement sites with proximity to manufacturing facilities using combustion processes with sources of chemical pollutants, it will be informative to explore the associations between chemical co-pollutants in the airshed and fine bacterial bioaerosol concentrations and compositions.

This first part of this study investigated the influence of meteorological parameters such as temperature, relative humidity, wind speed and wind direction as well as the impact of chemical co-pollutants like PM2.5 mass and ozone on bacterial community composition and concentrations within an urban airshed. The second part of this study examined the influence of seasonal variation on bacterial community composition between a rural and an urban airshed.

Materials and Methods

Sample collection

Fine bioaerosol samples were collected on pre-sterile gelatin membrane filters (pore size 3.0um) over a two-week period across each of the four seasons from Fall 2013 through Summer 2014 at both the urban airshed located in Dearborn, MI and the rural airshed in Dexter, MI to account for seasonal variability. The sample collection, handling and storage conditions are described in Chapter 2. The urban and rural airsheds are described in Chapter 3.

Only fine bioaerosol samples collected on pre-sterile gelatin membrane filters in Summer 2014 from the urban airshed were used to study the influence of meteorological parameters and chemical co-pollutants.

The monitoring data for the four meteorological parameters – relative humidity, temperature, wind speed and wind direction were obtained from the Michigan Department of Environmental Quality for the urban airshed. Chemical co-pollutant monitoring data for PM2.5 mass and ozone were also obtained from the Michigan Department of Environmental Quality for the urban airshed.

Sample analysis

The fine bioaerosol samples collected over each season from both airsheds were processed and analyzed using 16s rRNA barcoded pyrosequencing methods to identify

bacterial community compositions. DNA extraction, sequencing and downstream analysis were followed as described in Chapter 2.

The bacterial DNA concentrations of all the Summer 2014 bioaerosol samples from the urban airshed were estimated by quantitative PCR as described previously in Chapter 3.

Data analysis

Constrained ordination Redundancy Analysis (RDA) was performed using R to understand influence of meteorological parameters and chemical co-pollutants on bacterial composition. Through RDA the main parameters or co-pollutants driving bacterial composition variation can be assessed [12]. Forward selection was used to identify the significant meteorological parameters and chemical co-pollutants driving the bacterial composition variation.

Bacterial DNA concentration data was plotted against the individual meteorological parameters- relative humidity, temperature, wind speed and wind direction to observe individual correlation patterns. Similar plots were created for chemical co-pollutant data using Microsoft Excel.

The beta diversity metric was used to evaluate the differences in bacterial community composition between the urban and rural airshed for each season. The analysis was

carried as described in Chapter 3. The significance threshold was set at an α-value of 0.05. Ecological analysis was carried out using R.

Results and Discussion

Influence of meteorological parameters and chemical co-pollutants on bacterial community composition

The influence of meteorological parameters, PM2.5 mass and ozone on the bacterial community composition in the summer of 2014 at the urban airshed was evaluated using Redundancy analysis. The RDA plot axes were able to explain 39.13% and 25.69% of variation in the sample OTUs composition respectively. Meteorological parameters such as relative humidity, temperature, wind speed and wind direction appeared to have a strong driven effect on the variation in bacterial composition [Fig.4.3 ,Table 4.1] Relative humidity, temperature and wind speed were observed to also have an influence on bacterial DNA concentrations [Fig.4.1a-4.1c]. As these parameters increased in magnitude a decrease in bacterial DNA concentrations was observed. However, as temperatures went higher than 30^0C a decrease in bacterial DNA concentrations was observed [Fig.4.1b].

Co-pollutants such as ozone and PM2.5 mass were also found to have a significant effect on the spread of the bacterial composition [Fig.4.3]. However, the effect of PM2.5 on the bacterial composition is not conclusive as the data collected for PM2.5 was an average daily value versus specifically for each sampling interval. Ozone levels over the

sampling intervals did seem to have an influence on bacterial composition as well as bacterial DNA concentrations [Fig.4.3, Fig.4.2a]. It was observed that as ozone levels went up corresponding bacterial DNA concentrations went down. This finding does seem to be in agreement with studies that have studied the antimicrobial effects of ozone in controlled environments [11]. However, it is important to note that this was an outdoor air study and that there were other meteorological parameters and co-pollutants to create a more complex mixture. So the specific role of ozone on bacterial DNA concentrations cannot be easily ascertained in an uncontrolled outdoor setting. The samples analyzed were collected over a period of only four days. Additional data collection in future studies over a longer time period would provide for further exploration of the correlation between such environmental variables and bacterial composition in the air.

Influence of seasonal variation on bacterial community composition at urban and rural airsheds

The effect of seasonal variation on bacterial community composition was investigated at both the urban and rural airshed. Fine bioaerosol samples were collected over fall, winter, spring and summer seasons from June 2013- July 2014 at each airshed. The beta diversity metric was used to understand the differences in bacterial community composition between the two airsheds. There were significant differences observed during the winter, spring and summer seasons [Fig.4.4b, 4.4c, 4,4d]. No significant difference was observed in the fall season. The urban airshed showed a greater bacterial diversity in the winter and summer seasons as compared to the rural airshed.

The rural airshed consisted mainly of wooded land and was completely covered in snow for almost the entire winter season and therefore had almost no local sources of bacterial emissions. The urban airshed comprising of an industrial and urban residential setting had more anthropogenic activity and therefore more sources of bacterial emissions. In the springtime, however, the rural airshed exhibited greater bacterial community diversity. This could be explained by the onset of plant growth, which results in a sudden surge in plant related bacteria along with re-suspension of soil bacteria into the air. However, in summer once the vegetation at the rural airshed had bloomed, the urban airshed once again exhibited greater bacterial community diversity. This could be due to the slightly higher (22-28^{0}C) summer temperatures along with higher relative humidity levels (40-60%) that might have been more conducive to the growth of bacteria. With a diverse set of emission sources, the urban airshed showed a significantly more diverse bacterial composition for most of the year. Therefore, seasonal variation did have an influence between airsheds.

The impact of seasonal variation was also pronounced during the sample analysis stage. Although the same number of total samples were collected every season (n=30 from each site per season) only a fraction of the samples from each season produced results using the 16s rRNA barcoded pyrosequencing method. The lowest number of samples capable of DNA amplification came from fall (urban=9, rural=3) and winter (urban=8,rural=13) seasons. This implies that most of the bioaerosol samples collected in these periods did not have enough bacterial DNA present suggesting that the bacterial load in the air at the time was low as compared to the spring (urban=14,

rural=19) and summer (urban=19, rural=24) seasons when the temperature and other parameters could favor bacterial growth and presence. Additional investigation looking at the variation in bacterial concentrations over different seasons and different airshed locations would provide further insight on how seasonal changes impact bacteria in fine bioaerosols.

Conclusions

Meteorological parameters such as relative humidity and wind speed and co-pollutants like ozone appear to have a strong influence at higher concentrations on bacterial composition. Though this study was limited in its duration of sample collection, the study findings warrant additional investigation in future studies to fully quantify their effect on bacterial composition and concentrations.

Seasonal variations did have an impact on overall bacterial bioaerosol community composition. The differences were significant between the urban and rural airsheds. Therefore, once again suggesting that the sources of bacterial emissions are important to understand the presence of bacterial bioaerosols in outdoor air, especially the anthropogenic sources that can be regulated. Further research is needed to understand public health implications from prolonged exposure to fine bacterial bioaerosols.

Figures

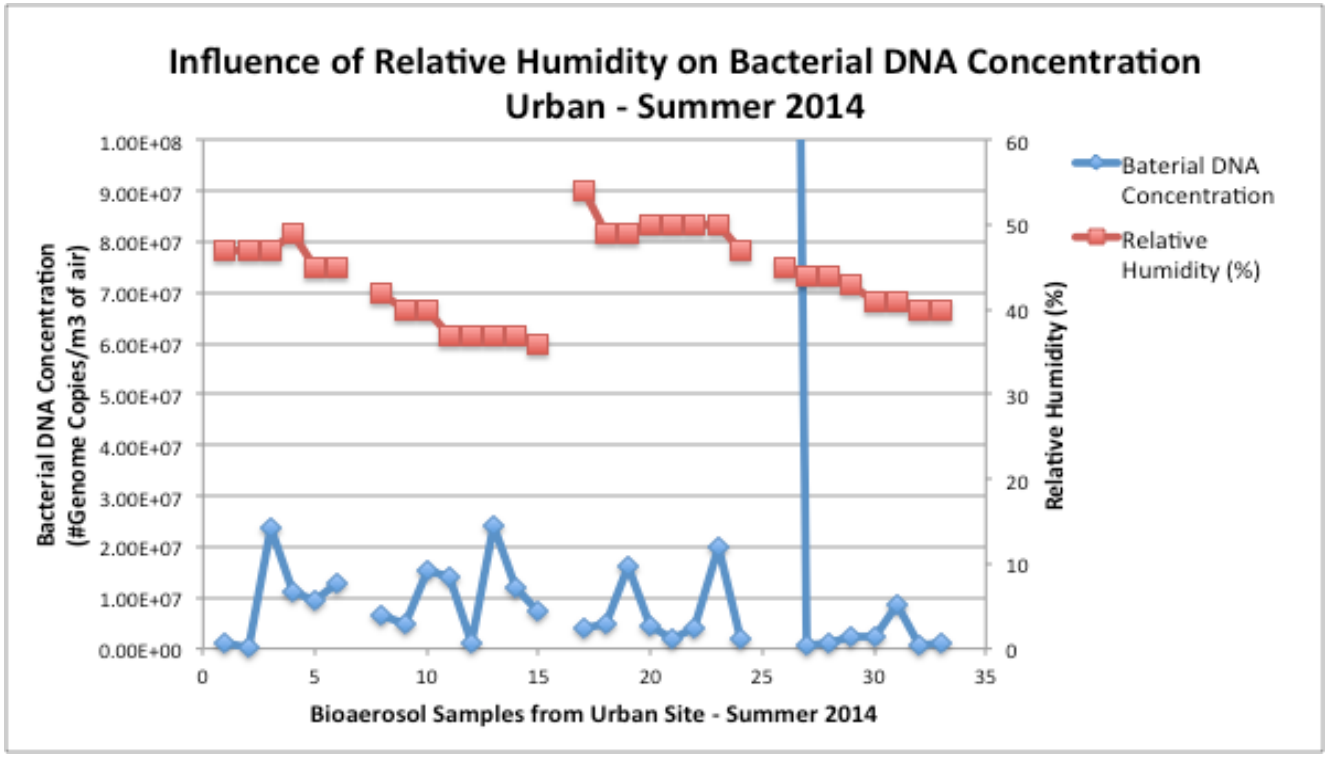


Figure 4.1a: Influence of relative humidity on bacterial DNA concentrations in the urban airshed.

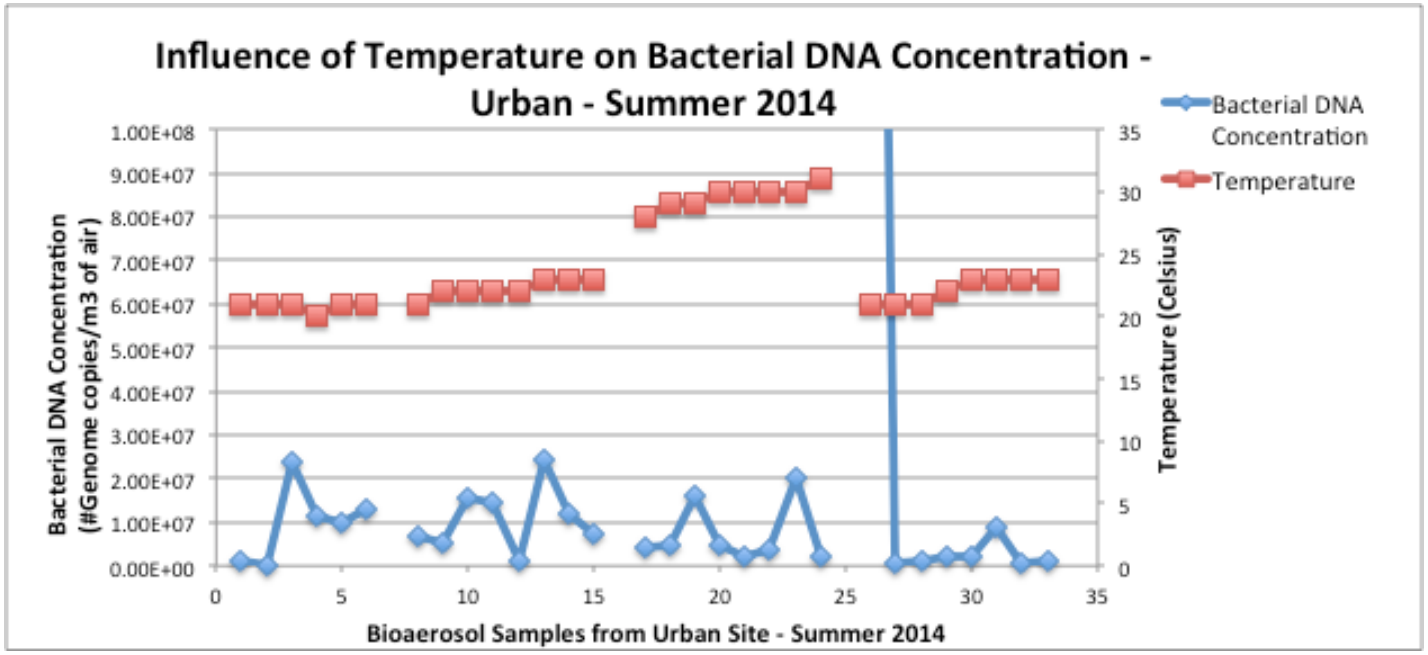


Figure 4.1b: Influence of temperature on bacterial DNA concentrations in the urban airshed.

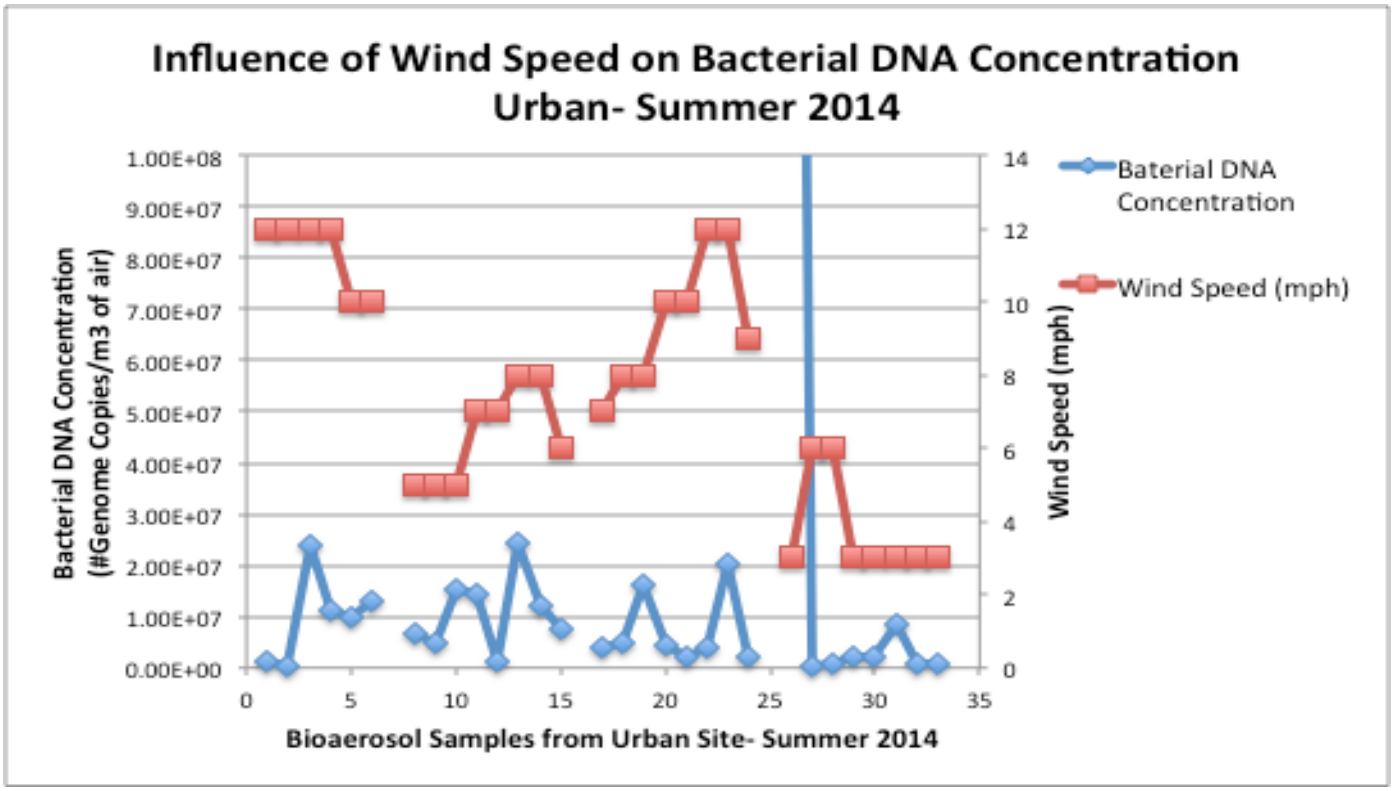


Figure 4.1c: Influence of wind speed on bacterial DNA concentrations in the urban airshed.

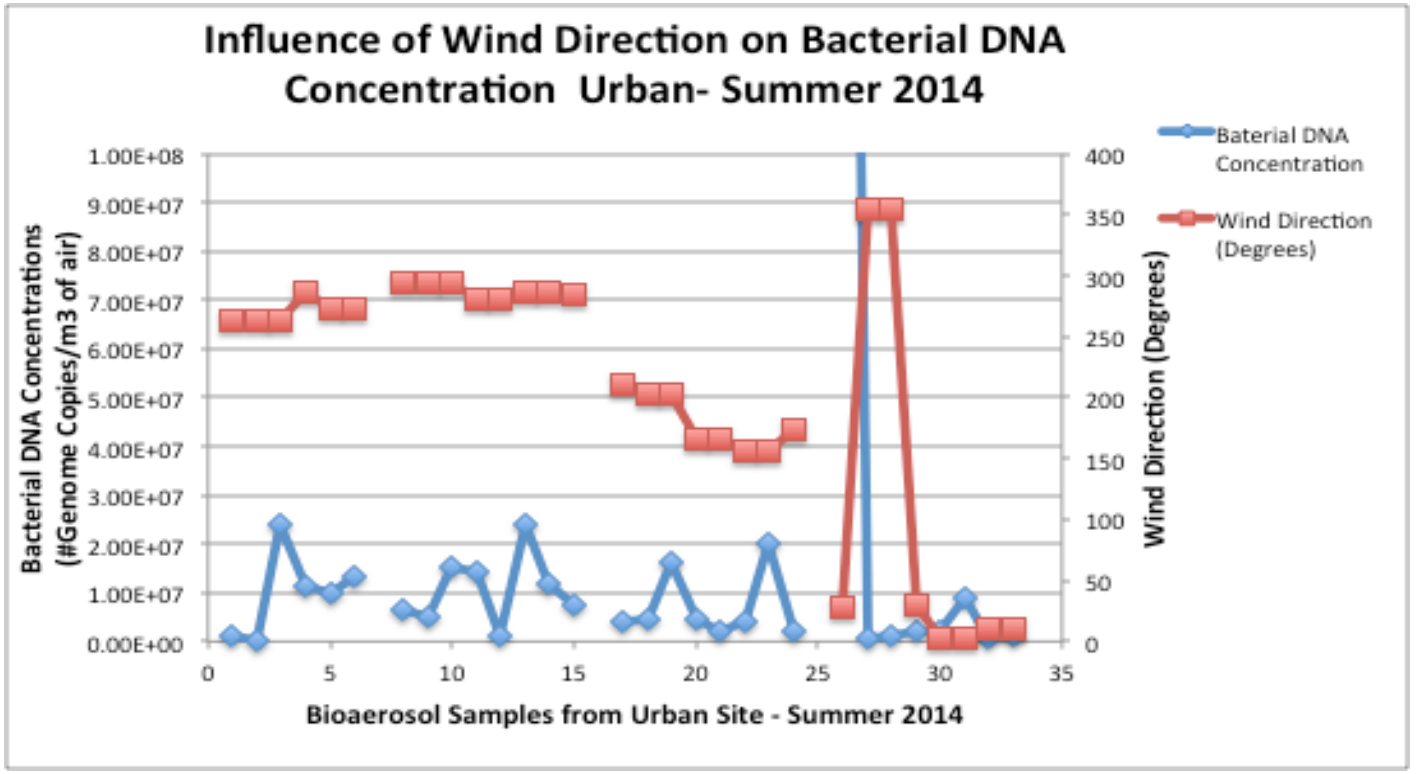


Figure 4.1d: Influence of wind direction on bacterial DNA concentrations in the urban airshed.

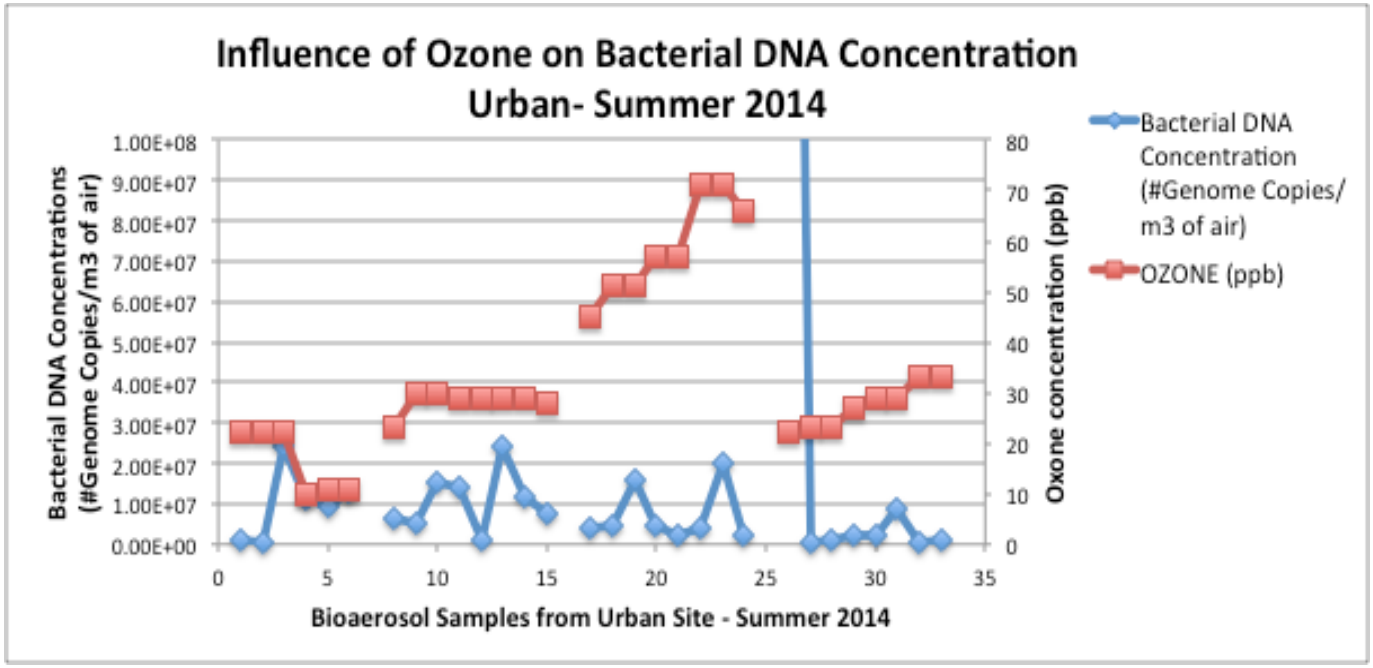


Figure 4.2a: **Influence of ozone levels on bacterial DNA concentrations.**

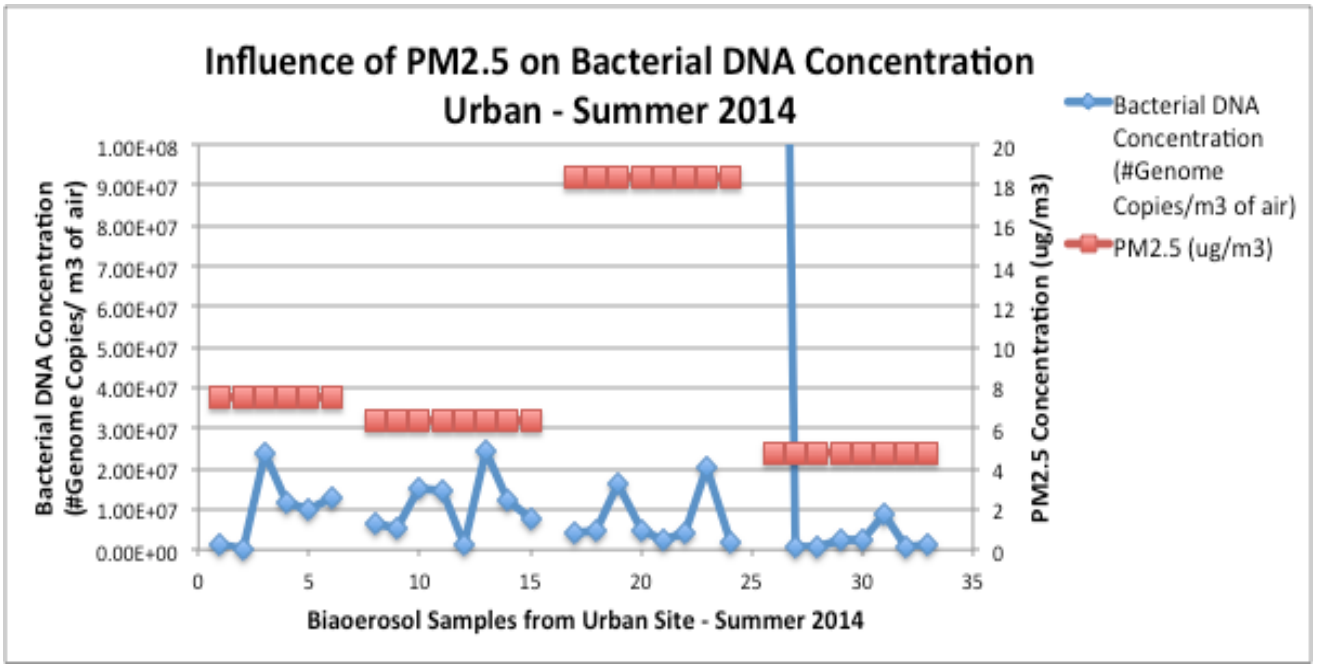


Figure 4.2b: Influence of PM2.5 levels on bacterial DNA concentrations.

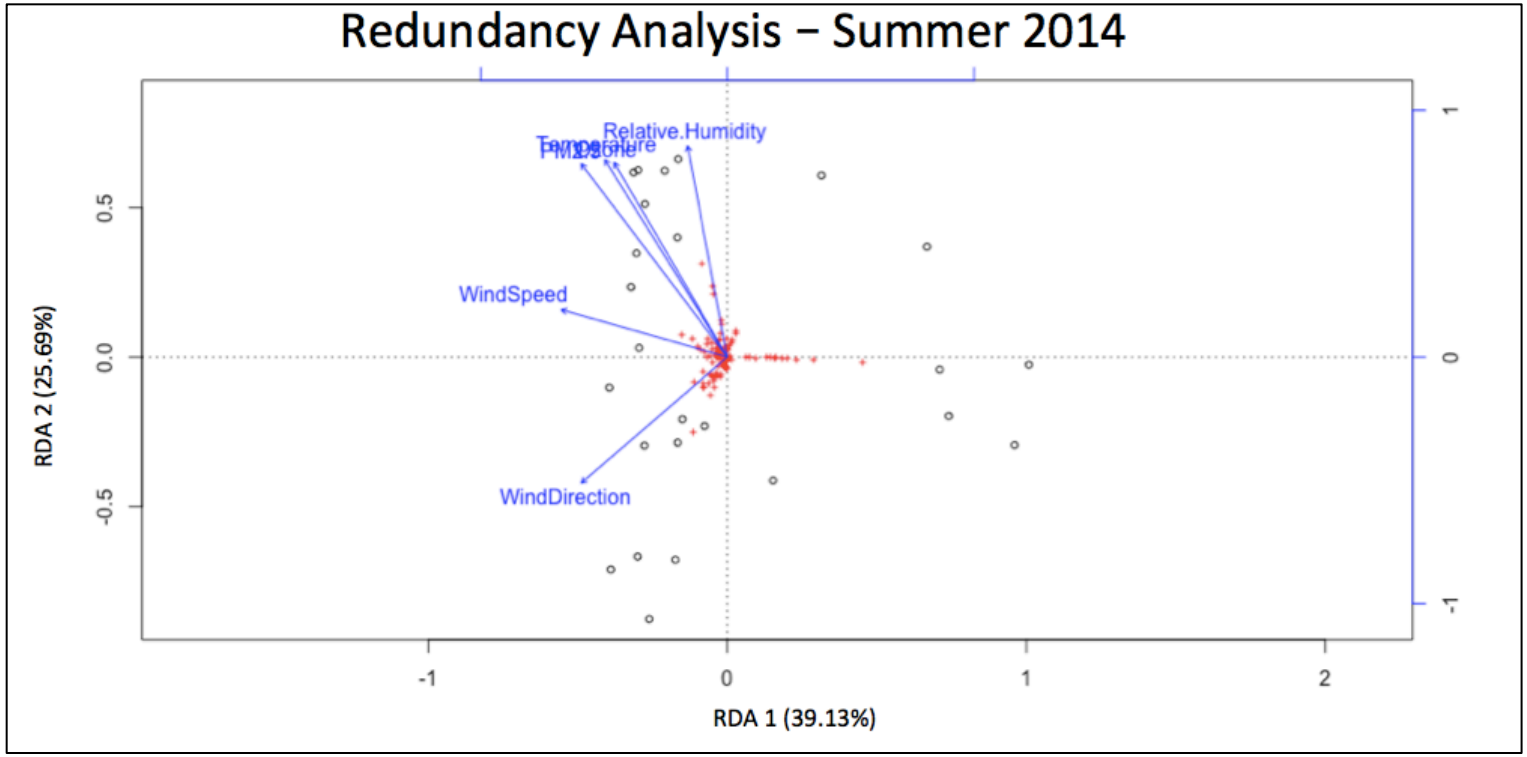


Figure 4.3: Redundancy Analysis plot showing the influence of environmental variables on bacterial composition of samples at the urban airshed. Percentage values displayed on axes indicate the total percentage variation described by consecutive axes.

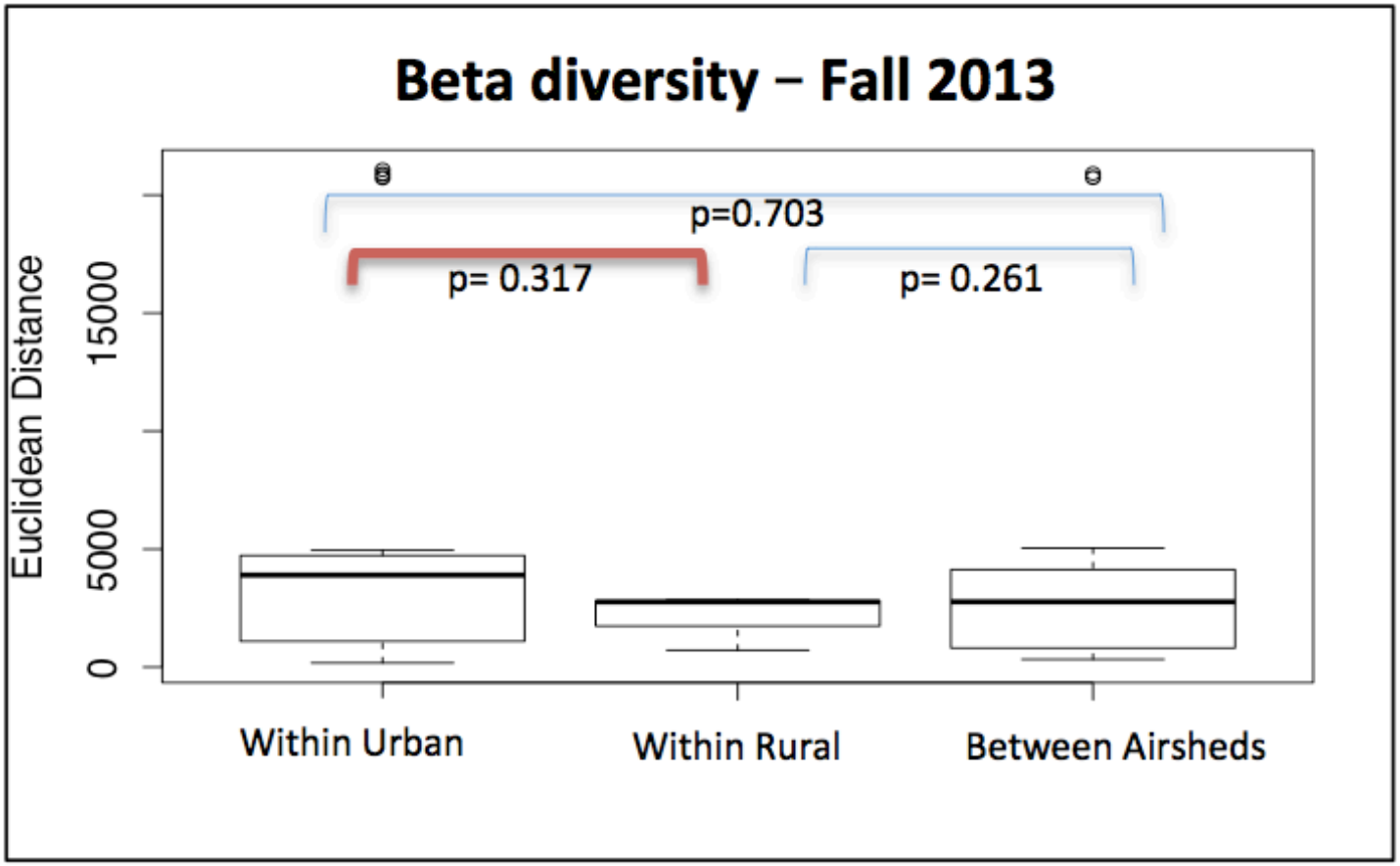


Figure 4.4a: Euclidean distances within and between samples at the urban and rural airsheds for Fall 2013 collected on GM filters.

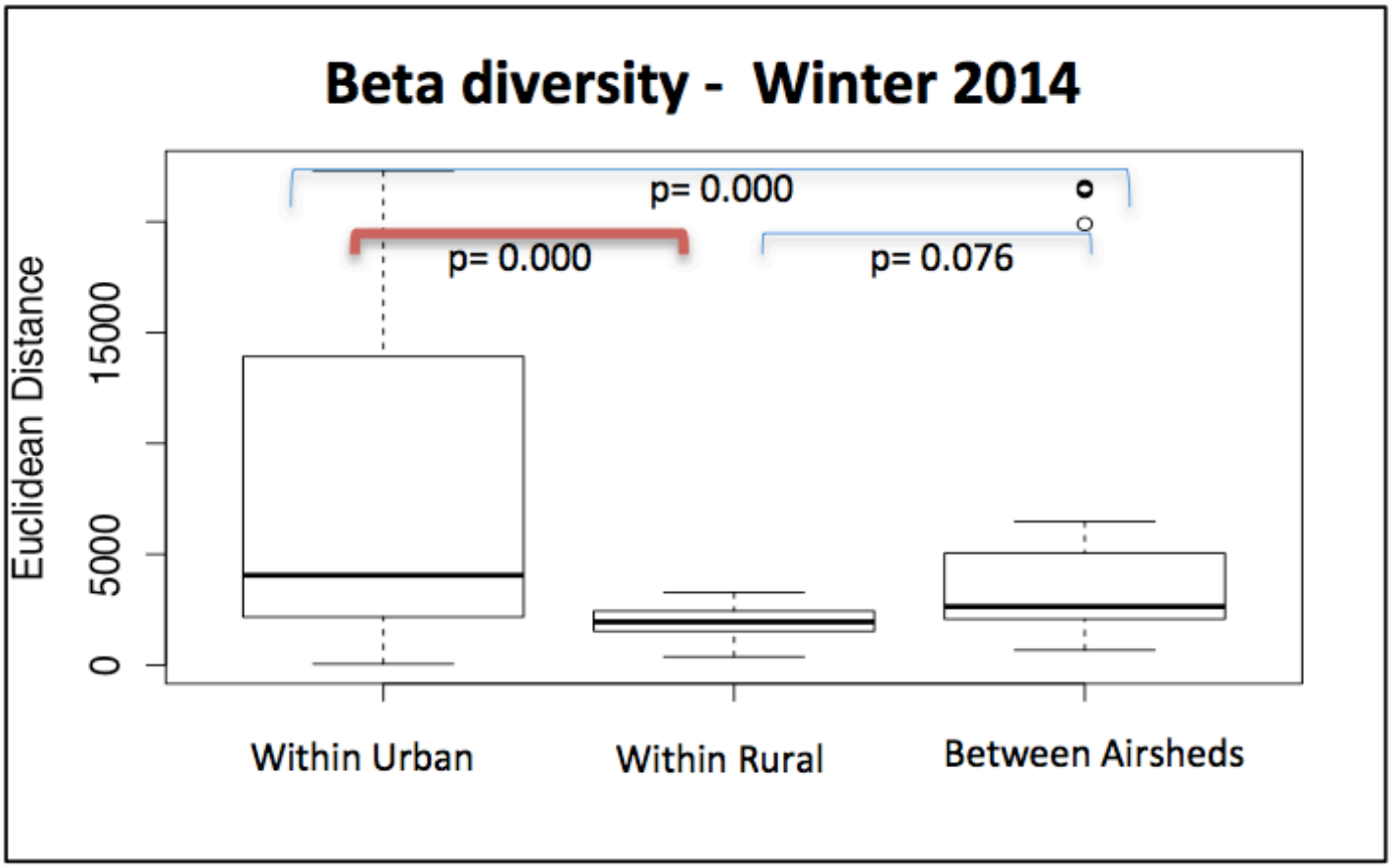


Figure 4.4b: Euclidean distances within and between samples at the urban and rural airsheds for Winter 2014 collected on GM filters.

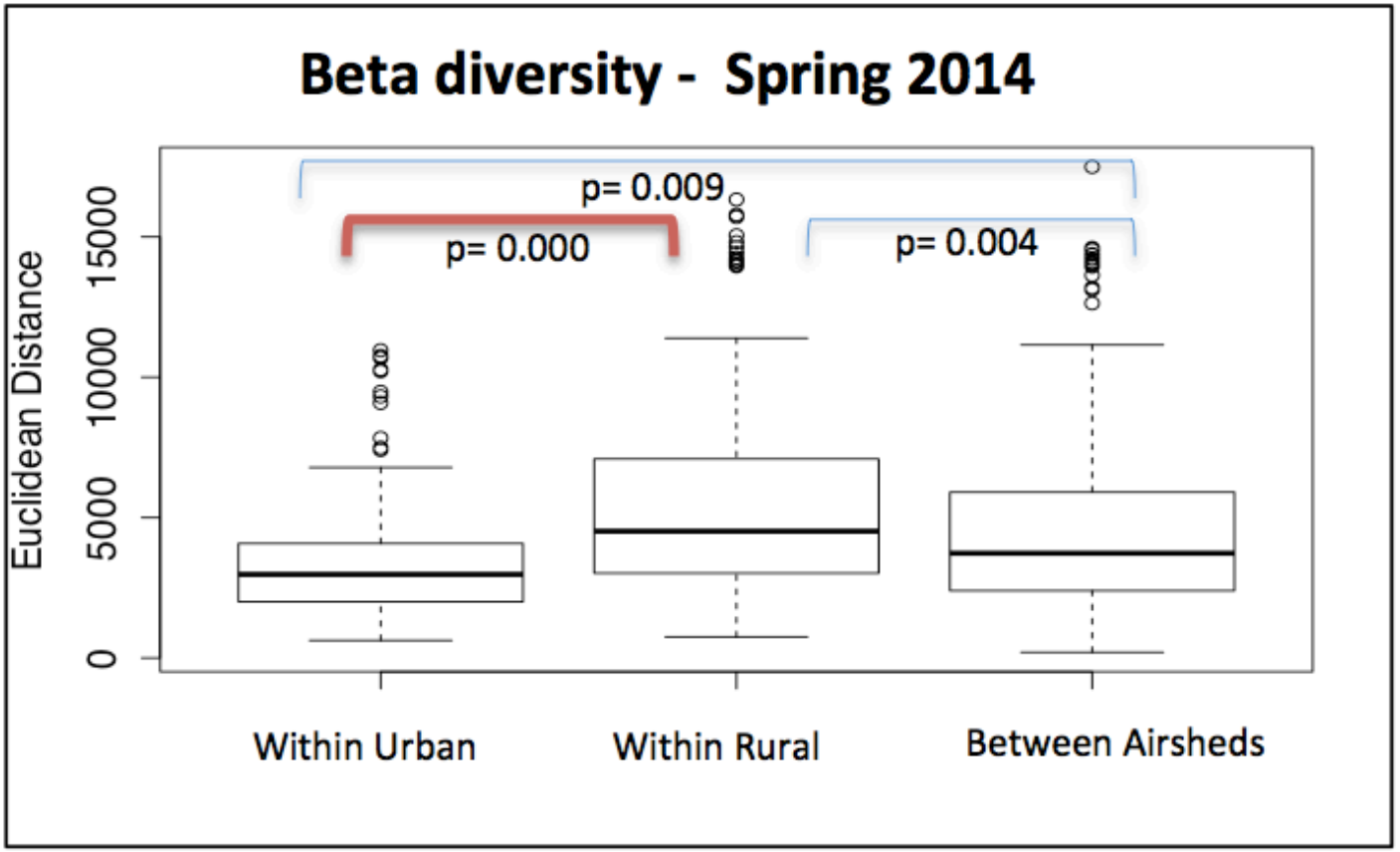


Figure 4.4c: Euclidean distances within and between samples at the urban and rural airsheds for Spring 2014 collected on GM filters.

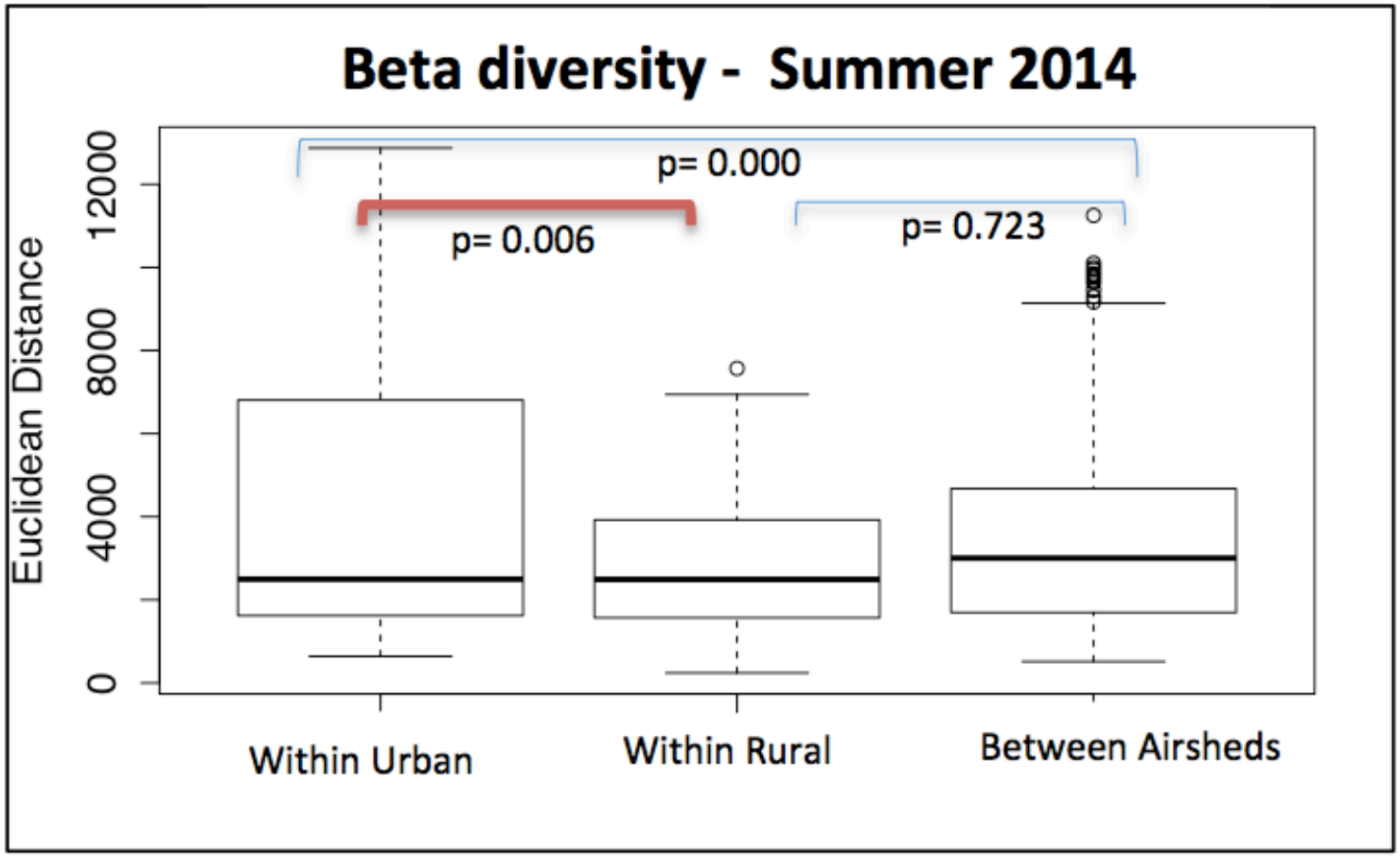


Figure 4.4d: Euclidean distances within and between samples at the urban and rural airsheds for Summer 2014 collected on GM filters.

Tables

Table 4-1: Forward Selection on Environmental variables.

	Df	AIC	F	Pr(>F)
PM2.5	1	-11.191	3.707	0.005
Temperature	1	-10.829	3.323	0.005
Ozone	1	-10.703	3.191	0.005
Relative Humidity	1	-10.344	2.817	0.005
Wind Speed	1	-10.176	2.644	0.005
Wind Direction	1	-10.123	2.5898	0.005

References

1. Haas D, Galler H, Luxnre J, Zarfel G, Buzina W, Friedl H, Marth E, Habib J, Reinthaler FF, 2013. The concentrations of culturable microorganisms in relation to particulate matter in urban air. Atmospheric Environment; 65: 212-222.

2. Lighthart B, Stetzenbach LD, 1994. Distribution of microbial bioaerosol. In: Lighthart Bruce, Jeff Mohr, Alan (Eds.), Atmospheric Microbial Aerosols. Theory and Applications. Chapman and Hall, New York, London, pp. 68-98.

3. Troutt C, Levetin E, 2001. Correlation of spring spore concentrations and meteorological conditions in Tulsa, Oklahoma. International Journal of Biometeorology 45 (2): 64-74.

4. Nasir ZA, Colbeck I, 2010. Assessment of bacterial and fungal aerosol in different residential settings. Water, Air, and Soil Pollution 211: 367-377.

5. Chandra Mouli P, Venkata Mohan S, Jayaramma Reddy S, 2005. Assessment of microbial (bacteria) concentrations of ambient air at semi-arid urban region: Influence of meteorological factors. Applied Ecology and Environmental Research; 3(2):139-149.

6. Rintala H, Pitkäranta M, Toivola M, Paulin L, Nevalainen A, 2008. Diversity and seasonal dynamics of bacterial community in indoor environment. BMC Microbiology; 8:56. doi:10.1186/1471-2180-8-56

7. Frankel M, Beko G, Timm M, Gustavsen S, Hansen EW, Madsen AM, 2012. Seasonal variation of indoor microbial exposures and their relations to temperature, relative humidity and air exchange rates. Applied Environmental Microbiology. doi:10.1128/AEM.02069-12

8. Fracchia L, Pietronave S, Rinaldi M, Martinotti MG, 2006. The assessment of airborne bacterial contamination in three composting plants revealed site-related biological hazard and seasonal variations. Journal of Applied Microbiology, 100: 973–984. doi:10.1111/j.1365-2672.2006.02846.x

9. Kaarakainen P, Meklin T, Rintala H, Hyvarinen A, Karkkainen P, Vepsalainen A, Hirvonen M-R, Nevalainen A, 2008. Seasonal variation in airborne microbial concentrations and diversity at landfill, urban and rural sites.

10. Flannigan B, 2001. Microorganisms In Home and Indoor Work Environments.Taylor & Francis, London and New York, p. 17.

11. Kowalski WJ, Bahnfleth WP, Whittam TS, 1998. Bactericidal effects of high airborne ozone concentrations on Escherichia coli and Staphylococcu aureus. Ozone Science and Engineering; 20:205-221.

12. Wang X, Cui H, Shi J, Zhao X, Zhao Y, Wei Z, 2015. Relationship between bacterial diversity and environmental parameter during composting of different raw materials. Bioresource Technology 198:395-402.

CHAPTER 5 Conclusions

Summary of findings

This dissertation investigated fine bacterial bioaerosols in outdoor air. The first study developed and evaluated an outdoor sampling method for the collection of fine bacterial bioaerosols both culturable and non-culturable using gelatin membrane and black polycarbonate filters. Gelatin membrane filters were found to be better at collection of a comprehensive sample of the outdoor bacterial microbiome and were also better at capturing a larger portion of viable bacterial cells as compared to black polycarbonate filters. The extensive characterization methods used also allowed for a better understanding of bacteria present in PM2.5.

The second study investigated the fine bacterial bioaerosol composition and concentration in two distinct airsheds, urban and rural, to understand the impact of sources of bacterial loading in the vicinity. The results of this study observed that the urban airshed, which has a mix of biological and chemical emission sources had a significantly diverse bacterial composition as compared to the rural airshed, which consists of predominantly natural sources. The urban airshed also presented a higher portion of pathogenic and opportunistic pathogenic bacteria. The findings of this study

are informative to public health as the urban area also has several residential communities.

In the third study, the influence of seasonal variations, meteorological parameters and co-pollutants such as PM2.5 and ozone were examined. Seasonal changes were found to have a significant impact on fine bacterial bioaerosol composition between the urban and rural airshed. Meteorological parameters such as relative humidity and wind speed as well as co-pollutants like ozone appeared to have an influence on fine bacterial bioaerosol composition and concentration.

Strengths and Limitations

Bioaerosol research is interdisciplinary and requires an understanding of air quality principles and the research methods involved as well as fundamentals of microbiology and analytical methods used. The main strength of this dissertation was to recognize this and approach the various studies and the goals in a collaborative manner. The next strength of this dissertation was the study airsheds chosen. Both the urban and rural airshed were an ideal setup to study spatio-temporal differences in fine bacterial bioaerosol composition based on their location and sources of both microbial and anthropogenic emissions. The other unique strength of this dissertation was the comprehensive design of the studies investigating the fine bacterial bioaerosol composition within the two distinct airsheds. The work presented here examined various aspects of bacterial bioaerosol research – size fraction, viable and non-viable cells,

culture based and culture independent methods, bacterial abundance and concentrations, seasonal variation, influence of environmental factors and chemical co-pollutants.

However, this research isn't without its limitations. Due to the extensive analytical methods employed, the completion of laboratory analysis took a long time to complete. One of the main limitations was the short sampling time (25 minutes) and quick processing of filters involved to retain viability of cells. The short sampling time required for sample collection to be a manual process and was labor intensive both in the field and laboratory when collecting samples over seasons. The third study was also limited in its study design, specifically in the duration of sample collection, greater number of samples collected over a longer period of time and not just limited to one season. Also, the limited availability of continuous monitoring data for the chemical co-pollutants did not allow for a more robust examination of a correlation of the chemical characteristics of the anthropogenic components of PM2.5 with the bacterial bioaerosols. This led to some of the results being more exploratory in nature rather than conclusive.

Recommendations for Future Research

Additional investigation is required to fully understand if the differences in the bacterial composition between the urban and rural airsheds are due to the possible microbial emission sources in the vicinity. Also, in addition to existing literature on worker exposures to bioaerosols within indoor exposure settings in facilities such as

wastewater treatment facilities, poultry and animal rearing operations, it would be informative to understand the contribution of such facilities to ambient bioaerosol concentrations if future studies could estimate bioaerosol concentrations at each type of facility and at distances upwind and downwind away from these sources.

Future research on bioaerosols must go beyond the current focus of regulated bioaerosols mainly aeroallergens such as pollen in PM10. These current regulations exist since there has been significant research investigating the human health effects to naturally occurring bioaerosols such as pollen and endotoxins. The role of bioaerosols - both from natural and anthropogenic sources in outdoor air including bacteria, fungi, viruses and their cell components capable of producing illness or allergic responses needs to be investigated in PM10, PM2.5 and ultrafine particles. There is also a need to expand the current knowledge base on effects on human health through inhalation of bioaerosols, more specifically research on health outcomes from inhalation of viable, non-viable cells and its components. The future approach needs to include not only bioaerosols from natural sources but also from the anthropogenic sources, which can be regulated to safeguard public health.

The airsheds studied in this dissertation both the urban and rural, are similar to many other locations across the United States and across the world, in terms of human populations, existing and developing infrastructure, geographical location and meteorology. Therefore, the findings, strengths and limitations of this comprehensive study can be applied to other airsheds as well. These findings would be particularly

useful with land use planning in rapidly developing cities to be mindful to account for bioaerosol exposures to surrounding neighborhoods. With ever increasing human populations, conventional landfills are struggling to cope with the increasing waste produced per person. An increasingly popular environmentally sustainable option is to compost a lot of this waste. However, a lot of these open composting facilities may lead to an increased presence of bioaerosol emissions including pathogens from outdoor windrows much like waste water treatment facilities. Future research should include efforts to address and regulate the emissions from such sources. Also, the findings and limitations from Chapter 4 which investigated the influence of seasonal variation and meteorological parameters should be considered in land use planning and bioaerosol regulation especially in tropical locations where the temperatures are warmer and have higher relative humidity making it conducive for increased year round bioaerosol presence and exposure. The results of such future studies will help greatly to safeguard public health while planning land use in rapidly expanding urban areas.

Also, to fully understand the impact of meteorological parameters and chemical co-pollutants on bacterial bioaerosol compositions, sample collection periods should include more number of continuous sampling intervals and also have access to corresponding monitoring data for the environmental and chemical components. Future research should also include a similar comprehensive investigative approach to understanding other bioaerosols components both in the fine and coarse fraction.

Conclusion

Urban planning in many areas has brought residential communities into the proximity of commercial facilities. These facilities often include sources of conventional particulate air pollution such as power plants, industrial operations or sources of bioaerosol emissions such as animal rearing facilities, agricultural operations or waste treatment facilities thereby modifying the atmosphere and its particulate matter components to which people including vulnerable populations are exposed. As land use changes increase with expanding cities, examination of the quality of air we breathe becomes imperative, especially the biaerosols present. The findings of this dissertation research are informative to public health to understand how this modified environment impacts public health.