Chemical and Biological Characterization of Air Particulate Matter in Japan, New Zealand and Rwanda as case studies

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Abstract

Particulate matter in the air is recognized as the largest environmental cause of premature human mortality worldwide. Their health risk arises largely from carcinogenic chemical components (polycyclic aromatic hydrocarbons (PAHs) and their nitro-derivatives (NPAHs)), and the allergenic and pathogenic effects of the biological components that are bound to inhalable particulate matter (PM), all of which can affect the human respiratory system. The synergistic effects that both of these components have to one another and to human health are currently poorly understood. Given the potential health effects of PM and the scarcity of information about atmospheric levels and components of PM in some parts of the world, monitoring is necessary to evaluate mitigation strategies. The aim of this thesis was to characterize chemical and biological composition of PM in Japan, New Zealand and Rwanda. PM with aerodynamic diameters of ≤ 2.5 μm and ≤ 10 μm ($PM_{2.5}$ and PM_{10}, respectively) was collected using high volume air samplers at three locations (urban roadside, urban background and rural sites) in Rwanda and $PM_{2.5}$ samples were also collected in two cities and rural locations in Japan and New Zealand. PAHs and NPAHs in these three countries were characterized using high-performance liquid chromatography. Microbial community structure (estimated using bacterial and fungal rRNA gene sequences) were characterized, and relationships were investigated using multivariate statistics. The mean concentrations of ΣPAHs and ΣNPAHs followed similar distribution profiles in all three countries, with higher concentrations in urban than in rural sites. Source identification using diagnostic ratio analysis and principal component analysis revealed diesel and gasoline-powered vehicles in the urban locations and wood/coal burning in rural locations were the major sources of PAHs and NPAHs in all three countries. Back trajectory analyses showed that high levels of PAHs detected in Japan and Rwanda were the result of long-range transport from neighbouring countries in close proximity, which was not observed in New Zealand due to its isolation from other countries. The analyses demonstrated that PM concentrations and lifetime cancer risks resulting from inhalation exposure to PM-bound PAHs and NPAHs exceeded the World Health Organization safe limits in Rwanda. Microbial analysis revealed that the diversity and composition of the airborne bacterial and fungal communities in Rwanda varied by site, PM size

fraction, and season. The majority of the biological material originated from dust and plants. Redundancy analysis indicated that PAH and NPAH species were significantly negatively correlated with microbial communities. Overall, this study provides insights into aerosol PM, PAHs and NPAHs in African, Japanese and New Zealand cities and rural areas with added microbial insight in Rwanda, and their possible threats to respiratory health. Exposure to toxic PAHs and NPAHs and to pathogenic microorganisms associated with PM air pollution is a global problem, and the research presented in this thesis has produced findings that may be relevant world-wide.

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Attestation of Authorship

I hereby declare that this submission is my own work and that, to the best of my knowledge and belief, it contains no material previously published or written by another person (except where explicitly defined in the acknowledgements), nor material which to a substantial extent has been submitted for the award of any other degree or diploma of a university or other institution of higher learning.

Signed:

Egide Kalisa

Date: 22.07.2019

Authors' contributions

The thesis is comprised of five peer-reviewed articles (Chapter 2, Chapter 3, Chapter 4, Chapter 5, and Chapter 6). Chapters 2, 4, and 5 have been published while Chapters 3 and 6 are in preparation and under review, respectively. Egide Kalisa, as the first author, had an overall contribution of 85% to each peer-reviewed manuscript. He conceived and designed the experiments, collected, analysed and interpreted the data, and wrote the manuscript. All other co-authors contributed equally to the analysis and discussion of the results and writing the final version of the manuscripts.

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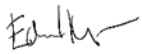
Edward G. Nagato	Kanazawa University, Japan	
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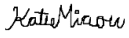
Katie King-Miaow	AUT, New Zealand	
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Dedication

I dedicate this thesis in memory of my mother (Melanie MUKARUBUGA) and my father (Anastase MUNYAKAZI) who always believed in my ability to succeed in the academic arena. They have gone, but their belief in me has made this journey possible and successful.

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Special thanks to the Rwandan community in New Zealand, who welcomed me and helped me to settle in New Zealand on my arrival. I cannot forget to thank my friend Bonaventure Gasana, who taught me how to drive in New Zealand. I am grateful to the Rubaduka family (Emmanuel and Angella) who have always called me their son and who have never forgotten to surprise me on my birthday. I thank Dr Gabriel Habiyaremye, Marie Laure Rurangwa and Dr Emmanuel Ndashimye for their daily encouragement; it has been a pleasure to know them. I am also very thankful to my brothers Dr Joseph Rwagitinywa, Mr Dieudonne Bunduki, Moise Bizimana and my sister Choisie Mukakalisa for their invaluable advice and opinions. I also thank all those whose names may have escaped attention here but have in one way or the other supported me during this PhD journey.

Chapter 1 - General Introduction and Thesis Framework

1. Introduction

Clean air is one of the basic needs for human life and wellbeing; however, a recent World Health Organization (WHO) air quality assessment confirms that 92% of the world's population lives in places where air pollution levels exceed the WHO guideline limits, resulting in the deaths of seven million people every year (World Health Organization, 2016a). Worldwide, air pollution is the largest environmental cause of premature human deaths, with nearly 90% of the mortality worldwide occurring in low- and middle-income countries (World Health Organization, 2016a).

This has the potential to get significantly worse due to ongoing population growth, multi-sectoral economic growth and rapid urbanization. All of these contribute to the increase in human activities that release different kinds of pollutants into the atmosphere, including particles and gases that can reach harmful concentrations in both the outdoor (ambient) and indoor environment. The United States Environmental Protection Agency (US EPA) has established national ambient air quality standards for six of the most common air pollutants (criteria pollutants), which include carbon monoxide, ozone, lead, nitrogen dioxide, sulfur dioxide and particulate matter (PM), to protect public health and the environment (US EPA, 2015). Among these air pollutants, PM has been extensively studied because of the widespread sources of emissions, toxic composition, and large effects on both human health and the environment. Recent studies have revealed PM is not a single pollutant but a mixture of particulate, chemical and biological fractions (Kalisa *et al.*, 2019a). The relationship between these properties and their risk to humans is currently poorly understood. Given the health effects of these chemicals and biological components of PM and the scarcity of information on their level and sources in some parts of the world, especially the African continent, monitoring of these compounds is necessary to evaluate mitigation strategies.

1.1 Particulate matter and human health

Particulate matter (PM) is generally defined as a complex mixture of solid particles and liquid droplets that remain individually dispersed in the air (Pöschl, 2005). PM is usually characterized by the aerodynamic diameter measured in micrometres. Particles with a diameter of 10 microns or less, ($\leq PM_{10}$) can penetrate and lodge deep inside the lungs, but the more health-damaging particles are those with a diameter of 2.5 microns or less, ($\leq PM_{2.5}$) that can penetrate the lung barrier and enter the blood system. By way of comparison, these particles are up to 70 times smaller than the thickness of a human hair (Figure 1.1). Atmospheric PM can be emitted by a wide variety of sources that influence its physical properties (size, surface area, density), chemical composition, biological compositions and size distribution (Després *et al.*, 2012). Sources of PM comprise both direct emissions, chemical transformations of precursor gases emitted from power plants, automobiles, wood burning, forest and agricultural fires, and natural sources (e.g., dust, volcanoes, vegetation) (World Health Organization, 2000). However, sources of PM are diverse, and their concentrations vary according to size, seasons and location. Chronic exposure to particles contributes to the risk of developing cardiovascular and respiratory diseases, as well as lung cancer (Dominici *et al.*, 2006). Exposure to high concentrations of small particulates (PM_{10} and $PM_{2.5}$) can also cause mortality or morbidity (World Health Organization, 2018a). Ambient PM remains the world's largest environmental health risks and a major cause of premature deaths worldwide (World Health Organization, 2016a). Thus, understanding their sources and their associated chemical and biological compositions can provide insight on exposure and possible mitigation strategies.

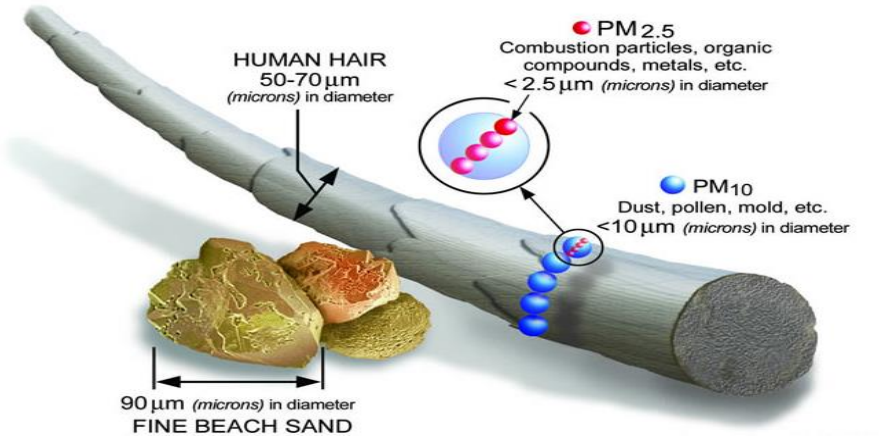

Figure 1. 1. Comparison of PM to human hair (US EPA, 2016a)

1.2 Polycyclic aromatic hydrocarbons

Atmospheric PM is a complex mixture of solid particles and liquid droplets found in the air. PM is composed by chemical components (sulfates, nitrates, polycyclic aromatic hydrocarbons (PAHs), nitro-polycyclic aromatic hydrocarbons (NPAHs), heavy metals and minerals) and biological components (viruses, bacteria and fungi) (Figure 1.2). The chemical compositions of PM, such as PAHs and NPAHs, are mostly studied because of their carcinogenic and mutagenic effects on humans (Barale *et al.*, 1991; Hayakawa, 2016). Detailed information (including definitions, sources and health effects) about the PAHs and NPAHs associated with PM is provided in Chapter 2. Chemical components of PM such PAHs and NPAHs have been characterised in Asia, Europe and the United States. However, the atmospheric behavior of these organic compounds is almost unknown for the Sub-Saharan African region (Kalisa *et al.,* 2019a).

1.3 Biological composition of particulate matter

Bioaerosols are composed of biologically derived matter, living or dead. Their behavior in the atmosphere is intertwined with that of non-biological (chemical and mineral) PM. Biological aerosols range in size from 0.1 mm to 100 mm in diameter and contain viruses (0.01–0.3 µm), bacteria (0.1–10 µm), and fungi (10-100 µm) (Després *et al.*, 2012; Huffman *et al.*, 2013).Viable microorganisms are particularly important in understanding aerosols' effects on human health. It is expected that the dynamics of biological particles in the air is governed mainly by the physical characteristics, of which the size and concentration of the particles are the most important

(Morawska *et al.*, 1999). Biological compositions of aerosols, including airborne bacteria and fungi, are widely studied because they are important to human and agricultural health (Ren *et al.*, 2006; Bowers *et al.*, 2012; Cao *et al.*, 2014). Detailed information on airborne microorganisms (bacteria, fungi and virus) associated with PM is provided in Chapter 2 (Kalisa *et al.*, 2019a). While several studies on atmospheric aerosols have focused on the chemical components of PM and associated health effects, there is a wealth of studies that holistically investigate the role of biological components of PM in different size fractions and the associated health outcomes.

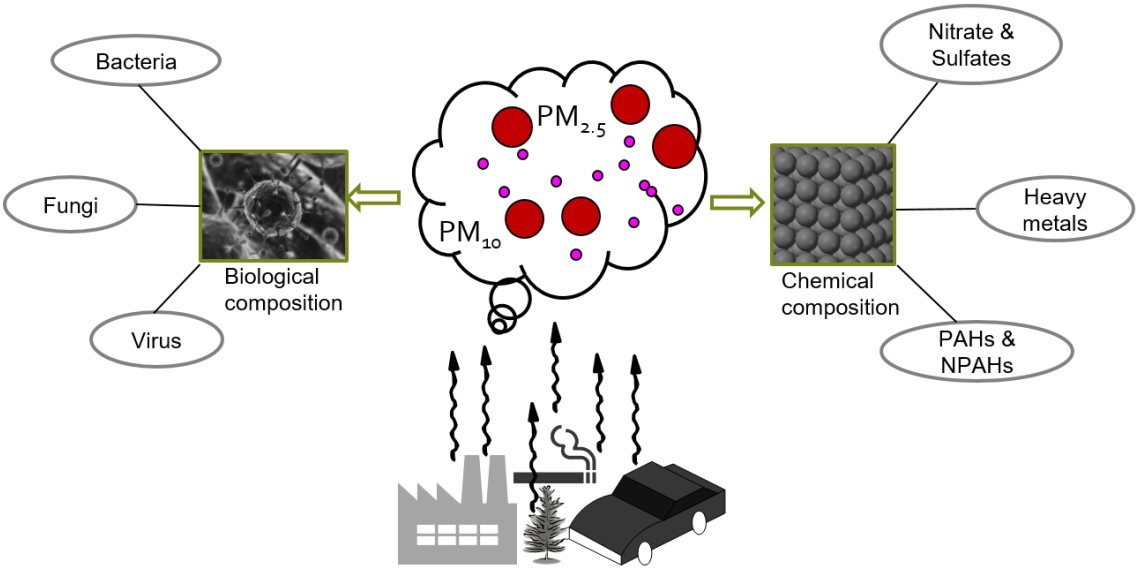

Figure 1. 2. Chemical and biological composition of particulate matter

1.4 Sampling of organic molecules

Over the past few decades, new techniques for analysing aerosol organic molecules in PM have been developed. At present, the most commonly used method is filter collection of ambient aerosols, followed by laboratory analyses. The commonly used filters are glass fiber filters (Riccio *et al.*, 2016), quartz fiber filters (Wang *et al.*, 2016), Teflon fiber filters (Bootdee, Chantara and Prapamontol, 2016) and polytetrafluoroethylene membrane filters (Garcia *et al.*, 2014). Several air-sampling devices are used to collect ambient aerosols for the analysis of chemical components. These are high volume air samplers (HVAS), low volume air samplers (LVAS) and personal cascade impactor air samplers (PCIAS). An HVAS is often used because of its ability to collect large volumes quickly and to enable the determination of chemical components of aerosols such

as organic and elemental carbon and trace elements in different particle size fractions. The most widely used method for analysing complex mixtures of organic compounds is high-resolution capillary gas chromatography with mass spectrometric detection (GC/MS) and high -performance liquid chromatography (HPLC) (Bates *et al.*, 2008; Hayakawa, 2016a). However, HPLC with fluorescence detection (HPLC-FLD) is widely used for analyzing PAHs in airborne PM samples, because of strong fluorescence detection (Hayakawa, 2016a, 2018a). Although GC/MS is also used for PAH analysis in particulate air samples, this analytical tool is not as sensitive in identifying PAHs with six rings (Hayakawa, 2016a, 2018a).

NPAHs, which exist at concentrations lower than those of PAHs, cannot be determined by GC/MS because of its very low sensitivity in determining trace levels of NPAHs. HPLC with chemiluminescence detection (CLD) is the most efficient technique for determining NPAHs in environmental samples (Hayakawa, 2018a; Kalisa *et al.,* 2018a).

1.5 Analysis of bioaerosols

There are a number of challenges in the sampling and analysis of airborne microorganisms. Although a wide variety of bioaerosol sampling and analytical techniques have been used, several problems remain unsolved. For example, there is no universal method suitable for the collection and analysis of all types of bioaerosols associated with PMs and no standard protocols are currently available (Grinshpun *et al.*, 2016). In addition, bioaerosols have very low biomass in the air, which is a limiting factor for DNA extraction and analysis (Luhung *et al.*, 2015). The collection of bioaerosol samples has been performed for decades and has mostly involved active air sampling (Burge and Solomon, 1987; Napoli *et al.*, 2012), which removes and collects biological particles in the air by speeding up the air and capturing the particles in various ways (Burge and Solomon, 1987). A wide variety of bioaerosol samplers has been used and new methods are being developed. However, each method has some advantages and disadvantages, as discussed in a previous review paper (Després *et al.*, 2012). The three main bioaerosol collection methods are impaction, impingement, and filtration (Yoo *et al.*, 2017). Among these methods, filtration is commonly used for collecting airborne dust, pollen, bacterial and fungal spores. Filtration involves the separation of airborne particles by passing the air through a porous

medium. Here the collection of the particles depends on their physical properties (size, density, shape), the filter pore size and the airflow rate (Li, 1999; Sippula *et al.*, 2013; Gao *et al.*, 2015). After sampling, the collected airborne microorganisms can be extracted directly from the filter for subsequent analyses. Filtration sampling is adaptable to a variety of assays such as polymerase chain reaction (PCR) and cultivation (Lundholm, 1982; Yao and Mainelis, 2007). Furthermore, microorganisms collected on the filter may be assessed in several different and complementary ways (Aizenberg *et al.*, 2000). Additionally, filtration methods have been found to meet several of the criteria for an "ideal bioaerosol sampler and can achieve high physical collection efficiency over a wide range of particle sizes" (Macher and Macher, 1997; Aizenberg *et al.*, 2000). A wide variety of techniques have been used to characterize bioaerosols. Traditionally, airborne microorganisms have been analysed by culture-based methods and microscopic total count determinations (Burge and Solomon, 1987). However, these methods suffer from many limitations, such as poor characterization of microbial diversity and composition. Therefore, culture-independent techniques such as biochemical essay and DNA sequencing have been developed that have increased the sensitivity and accuracy of bioaerosols analysis (Yamamoto *et al.*, 2012; Cao *et al.*, 2014; Dannemiller *et al.*, 2014).

1.6 Air pollution in Japan

Japan is an island country located in East Asia and is one of the most densely populated countries in the world. In 1960, after the Second World War, Japan was classified as among the most polluted countries in the world due to the emissions caused by industrialization and weak enforcement of environmental laws (Harada and Glasby, 2000). For example, Tokyo was at that time one of the cities with the worst air quality among the world's megacities (Hara *et al*., 2013). In the 1970s, Japan implemented environmental controls by setting up several countermeasures analogous to the US Clean Air Act passed in 1978 (Harada and Glasby, 2000; Hayakawa *et al*., 2018b). As a result, there was significant reduction in pollution over the next decade. Several air quality monitoring measures have reported that $PM_{2.5}$ has declined between 1990 and 2012 (Wakamatsu *et al.*, 2013). It has been reported that the mean concentration of $PM_{2.5}$ has decreased to 35 $\mu g/m^3$ (daily guideline) and 15 $\mu g/m^3$ (annual mean guideline) (Yorifuji *et al*., 2005; Hara

et al., 2013). Despite the noticeable decrease in PM concentration, a recent study has indicated that there are still at least 60,000 premature deaths resulting from air pollution in Japan every year (Cohen *et al*., 2017). Long-range transport of PAHs from neighboring countries by westerly winds have been reported (Tang *et al*., 2002; Yang *et al*., 2007, 2009; Hayakawa *et al*., 2014). While PAHs and NPAHs have been well studies in Japanese cities, there is limited information on the spatio-temporal variation in these compounds.

1.7 Air pollution in New Zealand

New Zealand has been classified among the top ten countries for air quality based on particulate matter ($PM_{2.5}$ and PM_{10}) (World Health Organization, 2015). The good air quality observed over New Zealand has been associated with the limited amount of heavy industry, the coastal location of the main centres, the strong winds that disperse pollutants, and its distance to other continents and sources of transboundary pollution (Fisher *et al.,* 2007). However, epidemiological studies on air pollution in New Zealand have indicated that there are times the main cities do not meet national air quality standards or WHO guidelines (Fisher *et al.,* 2007). This is primarily due to large emissions from wood burning used for heating during the winter months (Ministry for the Environment & Stats NZ, 2018). Vehicle emission was also found to contribute to poor air quality in New Zealand (Kalisa *et al*., 2019 b). The effects of air pollution associated with PM in in New Zealand include a 4.3% increase in annual mortality per 10 $\mu g/m^3$ of PM_{10} from all air pollution sources (vehicle, industry and domestic), in the 30+ age group, and 1,079 cases of premature mortality (people dying earlier than they would have if they had not been exposed to air pollution) (Fisher *et al.,* 2007). Further, there were 1,544 extra cases of bronchitis and 703 extra hospital admissions due to respiratory (465) and cardiac illnesses (238) (Fisher *et al.,* 2007). PM was the major cause of 1,921,000 restricted-activity days, defined as days on which people cannot do the things they might otherwise have done if air pollution was not present (Fisher *et al.,* 2007).

The chemical components of PM have been characterized in New Zealand air (Reche *et al.,* 2012; Trompetter *et al.,* 2013). However, little is known about organic air pollutants, including PAHs and NPAHs. The individual PAH compound Benzo[a]pyrene (BaP) has been classified by the United States Environmental Protection Agency (US EPA) as a Group 1 carcinogen for humans

and is the most widely studied PAH marker for carcinogenic risk levels. New Zealand has recognized BaP as an epidemiological health hazard indicator and the atmospheric standard for BaP is already set at 0.3 ng/m^3 annually (Ministry for the Environment and Statistics New Zealand, 2015). To date, there has been no information on spatio-temporal variation of atmospheric carcinogenic and mutagenic PAHs and NPAHs compounds in New Zealand. While most reports show that levels of PM are generally below the WHO regulatory limits (Ministry for the Environment & Stats NZ, 2018), the chemical compositions may still cause problems to vulnerable populations and increased attention is warranted for these toxic organic pollutants.

1.8 Air pollution in Rwanda

Africa currently has the fastest growing population in the world, which is projected to increase by more than 200% between 2010 and 2050 (Figure 1.3). It is estimated that over the next 40 years, air pollution (Lacey *et al.,* 2017) and climate change (Silva *et al.,* 2017) in Africa will further increase mortality, overtaking unsafe water and poor sanitation (OECD, 2012). In 2015, a study published by Nature indicated that exposure to PM in sub-Saharan Africa led to 400,000 deaths (Heft-Neal *et al.,* 2018). Despite the associated health risks, air quality programmes have been discontinued in recent years in Africa. The main problems are the severe lack of air pollution knowledge and inadequate funding to install air-monitoring stations. Available air data in Africa are sparse but indicate that levels of ambient air pollutants such as particulate matter (PM_{10}) are substantially higher than WHO air quality guidelines. There is limited air quality monitoring data available across Africa, particularly for particulate matter ($PM_{2.5}$ and PM_{10}) (Figure 1.4).

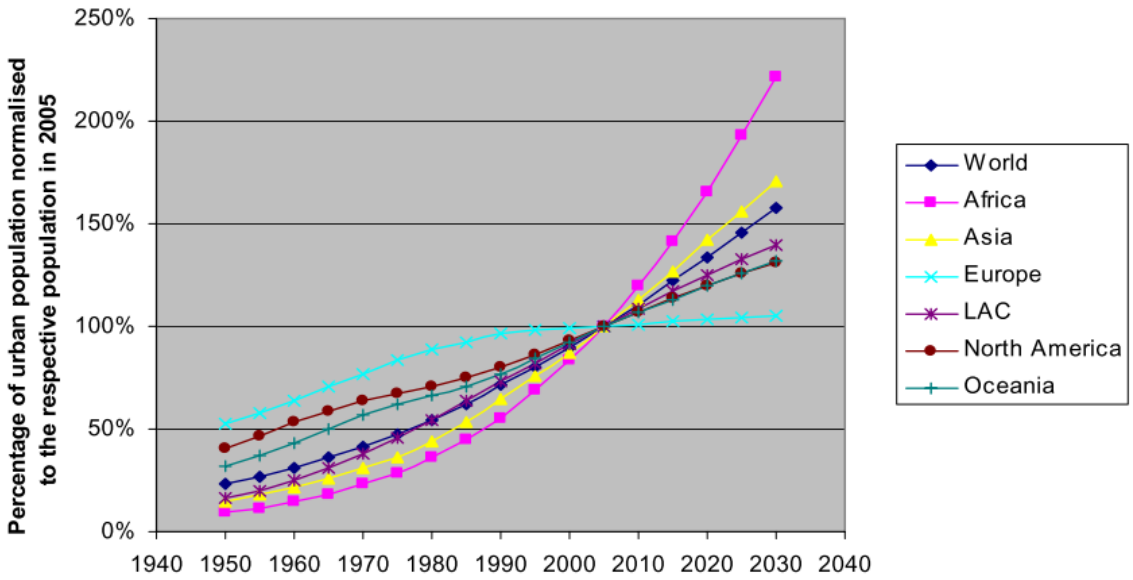

Figure 1. 3. World urban population growth rates from 1940 to 2040 (Schwela, 2012).

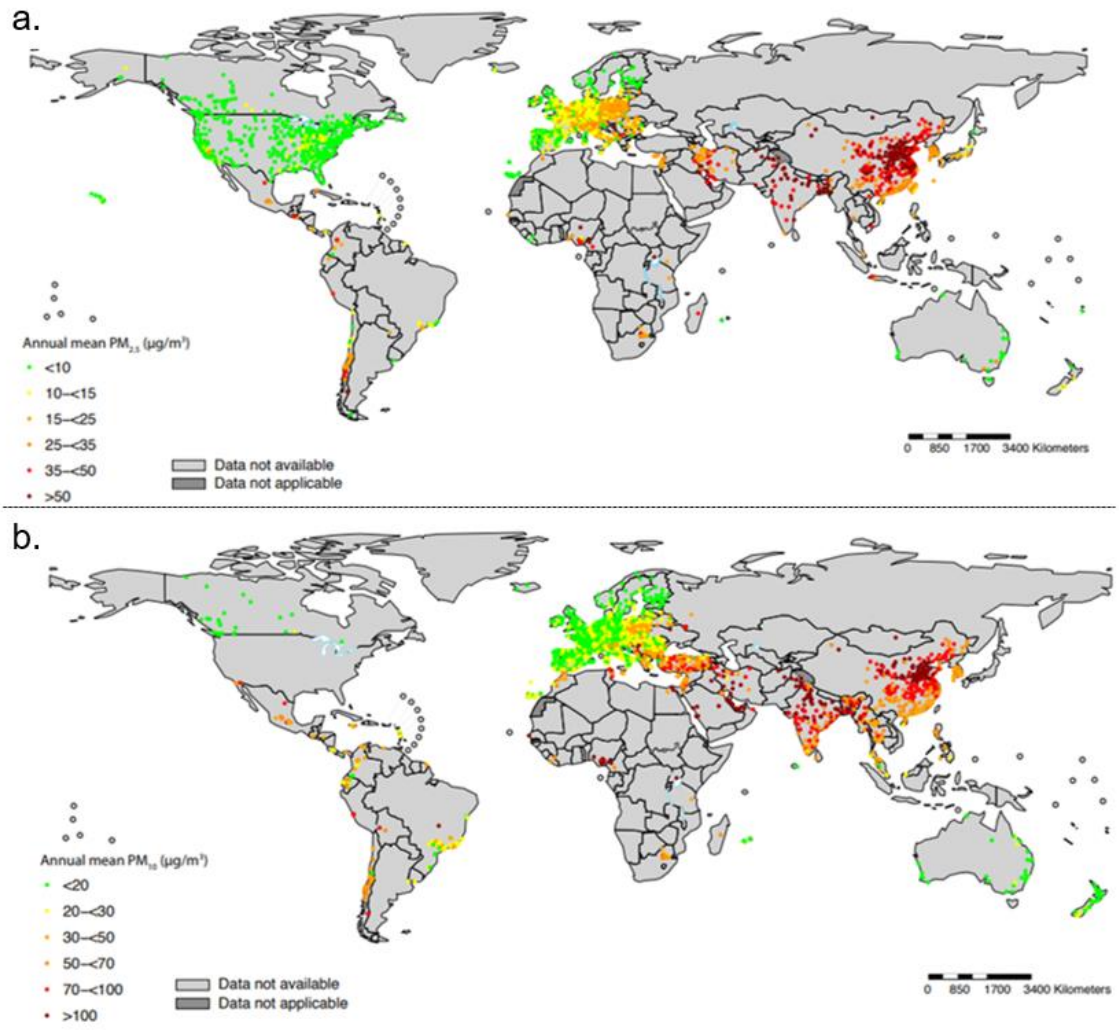

Figure 1. 4. Map of air pollution levels: $PM_{2.5}$ (a) and PM_{10} (b) measured during the years 2010–2016 (World Health Organization, 2019).

Rwanda is a landlocked country located in East Africa (population 12 million in 2017), which has experienced a rapid population growth and urbanization, but there is limited air quality data and a lack of air quality standards. Satellite estimate data published by the WHO indicated that about 3000 deaths were caused by ambient air quality in 2016 (Brauer *et al.*, 2012; World Health Organization, 2018b). However, there has been no more recent study to validate these estimated exposures based on ground ambient air quality measurement.

PM contains several toxic chemical components such as PAHs and NPAHs as well as pathogenic microorganisms. However, the composition of PM has never been investigated in Rwanda, despite the associated health risks. The Rwandan Environmental Management Authority

(REMA), responsible for air quality compliance and formulation of policies for environmental protection, has recently launched an air quality regulation to effectively control air pollution emissions. However, controlling these emissions alone will not achieve effective improvement in the air quality. Integrated control of all sources of air pollution is needed via a robust regulatory framework based on rigorous scientific evidence. Such a framework is not currently possible due to lack of knowledge of the sources, PM composition, causes and impacts of air pollution.

1.9 Why Japan, New Zealand and Rwanda?

The overall aim of this thesis was to characterize the chemical and biological components of particulate matter in Japan, New Zealand (two developed countries) and Rwanda (a developing country). These three locations were not selected for direct comparison based on their geographic locations and seasons. However, findings from this thesis provided an opportunity to characterize the emission effects of typical energy sources utilised by developed countries (fuel and oil) and developing counties (firewood). The atmospheric levels, source and health risk assessment in Japan, New Zealand and Rwanda were investigated using the same methodology. This was the first study to investigate the atmospheric behavior of PAHs and NPAH in airborne particulate in Rwanda and New Zealand. Given the health effects of these organic compounds, the measurement of these compounds is the first step towards reducing air pollution. Furthermore, follow-up monitoring of PAHs and NPAHs is needed to make accurate predictions in the rapidly changing world. The data obtained from this thesis have a positive impact in the scientific community as a result of the differentiation of pollution sources and the elevated concentrations of pollutants in a developing country compared with corresponding studies performed in more developed countries.

1.10 Thesis aims

The overall aim of this thesis was to characterize the atmospheric composition of particulate matter (PM) in Japan, New Zealand and Rwanda. The specific aims were to:

1. Generate a comprehensive literature review of the chemical and biological composition of PM and associated health outcomes in Africa.
2. Develop methods to simultaneously analyse atmospheric PAHs/NPAHs and airborne microorganisms associated with PM samples.
3. Determine the PAH and NPAH concentrations, identify their sources and conduct risk assessment in Japanese urban and rural sites.
4. Determine the PAH and NPAH concentrations, identify their sources and conduct risk assessment in New Zealand urban and rural sites.
5. Determine the PAH and NPAH concentrations, identify their sources and conduct risk assessment in Rwandan urban, roadside and rural sites.
6. Describe the spatio-temporal variation of the total microbial community structure (bacteria and fungi) recovered from the airborne PM size ($PM_{2.5}$ and PM_{10}) in Rwanda.
7. Determine the relationships between the total microbial communities (bacteria and fungi) and chemical components (PAHs and NPAHs) associated with $PM_{2.5}$ and PM_{10} in Rwanda.
8. Identify pathogenic microorganisms (bacteria and fungi) associated with $PM_{2.5}$ and PM_{10} in Rwanda.
9. Identify the effect of meteorological factors (temperature and relative humidity) on chemical and biological composition of PM in Rwanda.

1.11 Thesis structure

This thesis consists of the following chapters: literature review (Chapter 2), methods development and experimental design (Chapter 3), three experimental case studies (Chapters 4-6), and final discussion and conclusion (Chapter 7).

Chapter 3 is divided into three general themes to demonstrate the techniques of sampling and analysis of the chemical and biological compositions of aerosols.

- **Chapter 3A** - Evaluation of high-volume and low-volume air samplers for chemical and biological speciation of airborne particulate matter in Rwanda.
- **Chapter 3B** -Effect of sampling duration and filter sample size on the measurement of $PM_{2.5}$ and PM_{10} particulate-bound PAHs and NPAH.
- **Chapter 3C** - Efficiency of sampling duration for DNA-based analysis of bioaerosols.

The experimental chapters consist of three case studies to demonstrate the chemical and biological composition of PM, sources and risk assessments in different sampling locations.

- **Chapter 4** - Pollution characteristics and risk assessment of ambient $PM_{2.5}$-bound PAHs and NPAHs in typical Japanese and New Zealand cities and rural sites.
- **Chapter 5** - Characterization and risk assessment of atmospheric $PM_{2.5}$ and PM_{10} particulate-bound PAHs and NPAHs in Rwanda, Central-East Africa
- **Chapter 6** - Simultaneous chemical and microbial characterization of atmospheric PM_{10} and $PM_{2.5}$ particulate from three land-use types in Rwanda, Central-East Africa

Chapter 7 provides the key findings, limitations, future directions and conclusions of this thesis.

1.12 Thesis significance

This thesis assessed the chemical composition of PMs (PAHs and NPAHs) in Japan, New Zealand and Rwanda and the biological composition of PMs (bacteria and fungi) in Rwanda. The knowledge gap with relation to these PM compositions needs to be filled to address health concerns in these three countries. Overall, this thesis provides useful data for epidemiological studies and on the nature and interaction of biological and chemical aerosol composition loading in airborne particulate samples. This comprehensive dataset will be a key tool for policy makers in providing vital information for conducting epidemiological health studies, for setting up an

effective exposure limit for the PM composition metric rather than the existing limit of the total mass and ultimately for designing effective PM control strategies. This thesis provides insight into aerosol PM, PAHs and NPAHs in African, Japanese and New Zealand cities and rural areas and possible threats to respiratory health. These studies provided hard data with which to generate models of exposure and led to a comparative analysis of air quality in emerging versus developed urban and rural environments, and the data will have strong relevance at the science-policy interface. The data can be applied in epidemiological studies to develop policies on the monitoring and management of particulate air pollution and the protection of public health. This study has provided quantitative information on the sources of PM, PAHs, NPAHs and airborne microorganisms, allowing a more comprehensive assessment of the risk of human exposure to PM in Rwanda. Information on the abundance of potential pathogens in bio-aerosols and their variation across PM sizes will be useful in the prevention of respiratory diseases and in preventing the long-range transport of respiratory pathogens. These datasets can be used to explore different intervention and mitigation strategies to reduce exposure to air pollution in developing countries, leading to the development of clear policy recommendations and scenarios to guide decision-making and recommended actions. Airborne biological concentration data will be particularly useful for various stakeholders such as governments, local authorities, environmental agencies, city councils, and health departments to develop air quality strategy from bioaerosols loading on PM in urban settings where the PM levels are significantly higher and the chance of infection is exacerbated by a higher population density.

1.13 Research output arising from the thesis

1.13.1 Peer-reviewed journal publications

Chapter 2

Egide Kalisa, Stephen Archer, Edward Nagato, Elias Bizuru, Kevin Lee, Ning Tang, Stephen Pointing, Kazuichi Hayakawa, Donnabella Lacap-Bugler. Chemical and Biological Components of Urban Aerosols in Africa: Current Status and Knowledge Gaps (A review). *Int. J. Environ. Res. Public. Health*. **2019**;16(6):E941.doi:10.3390/ijerph16060941. This document can be found online at https://www.mdpi.com/1660-4601/16/6/941 and at the appendix 1.

Chapter 3

Part 3A - Egide Kalisa, Stephen Archer, Donnabella Lacap-Bugler. Evaluation of high-volume and low-volume air sampler for chemical and biological speciation of airborne particulate matter in Rwanda. Manuscript in preparation.

Part 3B - Egide Kalisa, Stephen Archer, Kevin Lee, Donnabella Lacap-Bugle. Effect of sampling duration and filter sample size on the measurement of $PM_{2.5}$ and PM_{10} particulate-bound PAHs and NPAH. Manuscript in preparation.

Part 3C - Egide Kalisa, Stephen Archer, Kevin Lee, Donnabella Lacap-Bugler. Efficiency of sampling duration for DNA-based analysis of bioaerosol. Manuscript in preparation

Chapter 4

Egide Kalisa; Edward Nagato; Elias Bizuru; Kevin Lee; Ning Tang; Stephen Pointing; Kazuichi Hayakawa; Stephen Archer; Donnabella Lacap-Bugler. Pollution characteristics and risk assessment of ambient $PM_{2.5}$-bound PAHs and NPAHs in typical Japanese and New Zealand cities and rural sites. *Atmos. Pollut. Res.* **2019**. doi:10.1016/J.APR.2019.03.009. This paper can be found at http://doi.org/10.1016/j.apr.2019.03.009 and at the appendix 2.

Chapter 5

Egide Kalisa, Edward Nagato, Elias Bizuru, Kevin Lee, Ning Tang, Stephen Pointing, Kazuichi Hayakawa, Stephen Archer, and Donnabella Lacap-Bugler. Characterization and Risk

Assessment of Atmospheric $PM_{2.5}$ and PM_{10} Particulate-Bound PAHs and NPAHs in Rwanda, Central-East Africa. *Environ. Sci. Tech.* **2018**; 52(21):12179-12187. This paper can be found at https://pubs.acs.org/doi/10.1021/acs.est.8b03219 and in the appendix 3.

Chapter 6

Egide Kalisa, Edward Nagato, Elias Bizuru, Katie King-Miaow, Tim Lawrence, Kevin Lee, Stephen Pointing, Kazuichi Hayakawa, Stephen Archer, Donnabella Lacap-Bugler. Simultaneous chemical and microbial characterization of atmospheric PM_{10} and $PM_{2.5}$ particulate matter from three land-use types in Rwanda, Central-East Africa. **2019**. Submitted to the Journal of Environmental International and under review.

1.13.2 Conference presentations

Egide Kalisa, Edward Nagato, Stephen Archer, Kevin Lee, Stephen Pointing, Kazuichi Hayakawa, Donnabella Lacap-Bugler. Spatial Temporal Variability of $PM_{2.5}$ in Urban Areas in Rwanda. **2017**. Presented 10 October at the International Collaboration Research Base for Reaction of Atmosphere Marine Ecosystem Caused by Aerosol, Noto Peninsula, Japan.

Egide Kalisa, Edward Nagato, Stephen Archer, Kevin Lee, Stephen Pointing, Kazuichi Hayakawa, Donnabella Lacap-Bugler**. 2017**. Effective sampling duration in determining PAHs and NPAHs concentration in $PM_{2.5}$ and PM_{10} glass filters. Presented 8 November at the Annual Aerosol Science Conference. The Aerosol Society. University of Birmingham, United Kingdom.

Egide Kalisa, Stephen Archer, Kevin Lee, Donnabella Lacap-Bugler. Sampling Homogeneities in Aerosol Sampling. **2017**. Presented on 23 November at the Annual meeting of the New Zealand Microbiological Society at Auckland University of Technology, New Zealand.

Egide Kalisa, Dieudonné Sebashongore, Jimmy Gasore, Ange Sabine Ingabire. Temperature and Air Pollution Relationship in Kigali, Rwanda. **2017**. Presented 14 June at the International Scientific Conference on Rebranding Research for Sustainable Development. University of Rwanda. Kigali, Rwanda.

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C. Pook, E. McKenzie, N. McKenzie, H. Devaraj, S. Shinde, C. Higgins, G. Valmonte, D. Lacap-Bugler, **E. Kalisa**, F. Zhang, M. Yoo. **2019**. Volatile profiling of biological samples using Thermal Desorption Gas Chromatography with Mass Spectrometry (TD-GC-MS). The Australian and New Zealand Society for Mass Spectrometry (ANZSMS). Presented 3 February, Auckland, New Zealand.

1.13.3 Other forms of publication (Press coverage)

Jamie Morton. Auckland traffic to blame for worrying levels of air pollutant. Science Reporter, NZ Herald. 3 June **2019.**

https://www.nzherald.co.nz/nz/news/article.cfm?c_id=1&objectid=12236907.

Jamie Morton. Tragedy spurs young researcher's air pollution fight. Science Reporter, NZ Herald. 12 September **2017**.

https://www.nzherald.co.nz/nz/news/article.cfm?c_id=1&objectid=11924869.

Chapter 2 - Literature Review: Chemical and Biological Components of Aerosol

The content of this chapter has been published as:

Egide Kalisa, Stephen Archer, Edward Nagato, Elias Bizuru, Kevin Lee, Ning Tang, Stephen Pointing, Kazuichi Hayakawa, Donnabella Lacap-Bugler. Chemical and Biological Components of Urban Aerosols in Africa: Current Status and Knowledge Gaps (A review). *Int. J. Environ. Res. Public. Health*. **2019**;16(6):E941.doi:10.3390/ijerph16060941.

2.1 Prelude

Despite hundreds of aerosol studies to date there are still key knowledge gaps that need addressing, particularly those involving the role of chemical and biological compositions of aerosols in disease associations. While the chemical components of aerosols have been well characterised with direct linkages to emission sources, the biological components are still far from being understood. The majority of work to date has been done in developed countries and there is almost no information available for developing countries such as those in the African continent. Additionally, little work has been done to determine the interactions between chemical and biological components loading in aerosol size fractions, despite mounting evidence of their synergistic significance. This is the first comprehensive review that summarizes the global evidence of health outcomes following exposure to the association of chemical and biological components attributed to particulate matter. This chapter will inform policy in the establishment of standard guidelines for the biological and chemical constituents of particulate matter. This review of literature shows that there is no standard protocol for collection of aerosols and emphasises the need to analyse the chemical and biological components of particulate matter simultaneously.

2.2 Introduction

Africa has the fastest growing population in the world and this is predicted to more than double between 2017 and 2050 (United Nations, 2017). This rapid population growth is associated with greater industrialization, motorization, and urbanization, creating dense urban centers. As a result, emissions from internal combustion engines, domestic cooking, and open fires contribute to worsening air quality. Air pollution is the single largest environmental cause of premature human mortality worldwide. Annually, approximately 4 million people die prematurely from illnesses attributable to household biomass smoke, such as pneumonia, chronic obstructive pulmonary disease (COPD), and lung cancer (World Health Organization, 2018c). The impacts disproportionately affect the world's poorest, most vulnerable populations. This is of particular concern in sub-Saharan Africa, where people are still heavily reliant on biomass fuel for cooking and heating, with a very low proportion (5%) of people using clean fuel as their primary source of energy (Rajendra *et al.*, 2018). In 2016, the World Health Organization (WHO) reported that household air pollution in Africa contributed to almost 739,000 deaths (World Health Organization, 2018c). According to the Organization for Economic Co-operation and Development (OECD), air pollution in Africa will be the highest cause of environmentally related deaths by 2050, overtaking unsafe water and poor sanitation (OECD, 2012). While these figures are alarming, there is little data on air quality and health-related problems. The extent to which an individual is harmed by air pollution depends on their total exposure to pollutants, including a measure of the duration of exposure, the concentration of pollutants, and population vulnerability. Thus, the evaluation of particulate air pollution for a country is typically measured by $PM_{2.5}$ (particulate matter with an aerodynamic diameter less than 2.5 micrometers) that can enter the lungs and PM_{10} (particulate matter with aerodynamic diameter less than 10 micrometers) that is trapped in the nasopharyngeal tract (World Health Organization, 2013). In 2013, ambient air pollution by PM was classified by the International Agency for Research Cancer (IARC) as a group I carcinogen (IARC-International Agency for Research on Cancer, 2013). Exposures to high levels of $PM_{2.5}$ and PM_{10} have been identified as causes of cancer, asthma, pulmonary

fibrosis, oxidative stress, altered immune responses, and chronic obstructive pulmonary disease (Falcon-Rodriguez *et al.*, 2016).

The development of coal-fired industries and increased automobile use have overlapped, which has resulted in emissions of a complex mix of air contaminants (Han *et al.*, 2018). Recent evidence suggests that PM is a mixture of chemical and biological origin (Sippula *et al.*, 2013; Yoo *et al.*, 2017). The total PM includes biological organisms (e.g., bacteria, fungi, and viruses), organic compounds (e.g., polycyclic aromatic hydrocarbons (PAHs) and their nitro-derivatives (NPAHs)), nitrates, sulfates, metals (e.g., iron, copper, nickel, zinc, and vanadium), dust, soot or smoke and elemental carbon (Morakinyo *et al.*, 2016). These components vary substantially according to time, location, season and climate, which results in spatial–temporal variation in characteristics, concentration, and toxicity (Harrison and Yin, 2000; Brodie *et al.*, 2007; Maki *et al.*, 2015; Gou *et al.*, 2016). PM-bound PAHs and NPAHs are the most studied components as they were found to be carcinogenic and enhance mutagenic properties (Dubey *et al.*, 2015; Bootdee, Chantara and Prapamontol, 2016; Masala *et al.*, 2016; Mohammed *et al.*, 2016).

A review of PAHs and their association with cancer revealed that there was an increase in lung cancer (relative risk of 1.2–1.4) and bladder cancer (relative risk of 2.2) in occupationally exposed subjects (40 years of exposure) (Mastrangelo *et al.*, 1996). Previous studies demonstrated that almost a quarter of the total airborne PM above land surfaces is made up of biological material (Womiloju *et al.*, 2003; Jaenicke, 2005). Another study indicated that the chemical composition of PM could provide insight into a variety of problems related to PM emissions (Matti Maricq, 2007). As particles of both biological and chemical origin are transported together with air currents in the atmosphere, PM can be used as a carrier of both pathogenic microorganisms such as bacteria, fungi, and viruses (Kellogg and Griffin, 2006) and carcinogenic compounds of organic aerosols (Després *et al.*, 2012; Morakinyo *et al.*, 2016). Depending on their concentration and meteorological factors (Jaenicke, 2005), inhalation of these mixtures can have significant effects on the health of the population (Figure 2.1).

The health effects of airborne PM have been linked to its chemical and biological components (Morawska *et al.*, 1999), while its interaction with regard to composition is influenced by meteorological conditions (long range transport, temperature, and relative humidity) and the physical properties of the PM (Figure 2.1) (Adhikari *et al.*, 2004). Recent evidence suggests that there may be an association between chemical and biological components in PM size fractions that results in increased negative health outcomes (Morakinyo *et al.*, 2016). Boreson *et al*. (2004) and Skóra *et al.* (2016) indicated that toxic chemical particles could be used as carriers of other pathogenic microorganisms and such interactions would have serious implications, as biological components could conceivably be penetrating deeper into the lungs than would have been expected. Consequently, health effects, such as COPD, asthma, and lung cancer (Figure 2.2), may be enhanced when biological and chemical components in PM are combined together (Boreson *et al.*, 2004). However, the association of these factors is complex and requires comprehensive research.

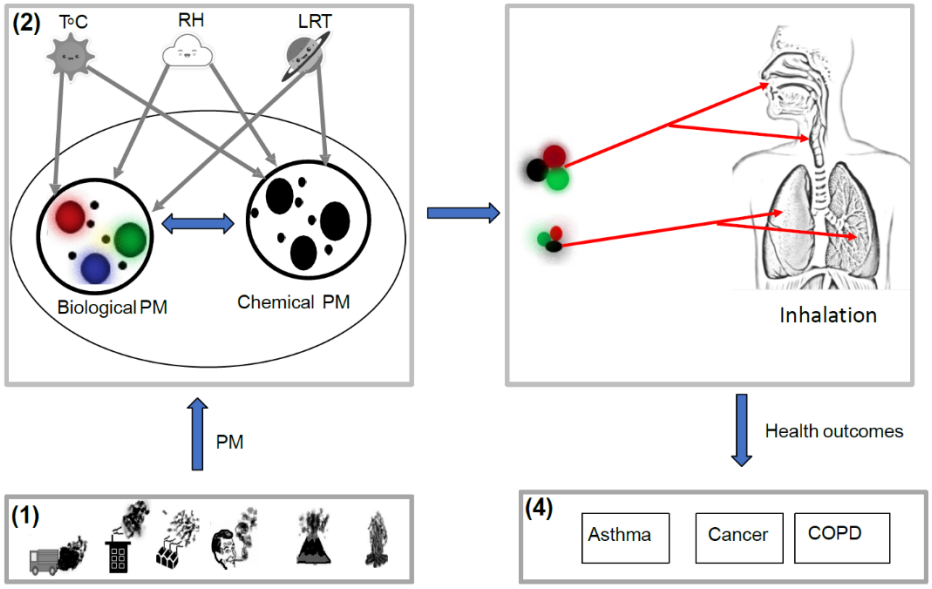

Figure 2. 1. A schematic representation of the complex relationships between biological and chemical components of particulate matter (PM). (1) The sources of airborne PM; (2) the interaction of chemical and biological components of PM through the influence of T °C (temperature), RH (relative humidity), and LRT (long range transport); (3) routes of exposure to the mixture of $PM_{2.5}$ (particulate matter with aerodynamic diameter less than 2.5 micrometers) that can enter the lungs and PM_{10} (particulate matter with aerodynamic diameter less than 10 micrometers) that are trapped in the nasopharyngeal from chemical and biological origins; and (4) possible health outcomes (Chronic Obstructive Pulmonary Diseases (COPD), asthma, and cancer).

In Africa, the available data on ambient PM levels are generally above the WHO's annual and 24-h mean guideline values for $PM_{2.5}$ and PM_{10} (Petkova *et al.*, 2013; Naidja *et al.*, 2017). A study indicated that PM encompasses many different chemical and biological components, which have been cited as major contributors to its toxicity (Kelly and Fussell, 2012). However, there are still limited studies in Africa on the characterization of chemical and biological components of PM. The few studies that have assessed the chemical components of ambient PM in Africa have demonstrated that PM concentrations and lifetime cancer risks resulting from inhalation exposure to PM chemical composition exceeded WHO safe limits and provide clear evidence that an immediate development of emission control measures is required (Kalisa *et al.*, 2018a). The available data from biological composition associated with atmospheric PM comes mostly from Asia (Woo *et al.*, 2013; Watanabe *et al.*, 2016; Yan *et al.*, 2016), Europe (Sippula *et al.*, 2013), and the United States (Bowers *et al.*, 2013). However, data on microorganisms associated with PM are scarce for Africa. Understanding both components of PM is crucial, as the relative harm of each component may differ by concentration or composition and the combination of both chemical and biological components may be more harmful than their individual components (Deguillaume *et al.*, 2008; Morakinyo *et al.*, 2016). Evidence suggests that the composition of emissions is more important than merely controlling the absolute sources (Briggs, 2003; Kelly and Fussell, 2012). As such, an inclusive control of all sources of the most toxic air pollutants is called for, together with a robust regulatory framework based on scientific evidence. This review summarizes the association between biological and chemical components of PM and their associated health outcomes, with an emphasis on the Environmental Protection Agency's (EPA) 16 priority-listed polycyclic PAHs (US EPA, 2008), their NPAHs, and pathogenic microorganism loadings in the PM size fractions.

A literature search was conducted in academic online databases, such as Web of Science, Google Scholar, American Chemical Society (ACS), PubMed, ProQuest, and Science Direct. Due to the paucity of air quality data in Africa, there was no restriction in the literature search in terms of study period and publication date. We searched literature using the MESH terms "PM", "particulate matter", "air pollution", "urban air quality", "fine particles", "coarse particles",

"PM_{10}", "$PM_{2.5}$", "Bioaerosols loading in PM", "Bacteria associated PM", "Fungi loading in PM", chemical composition of PM, "Polycyclic aromatic hydrocarbon in PM", "PAH", "nitro-polycyclic aromatic hydrocarbon in PM", "PAH", "NPAH", "carcinogenic PAH" "Organic carbon loading in PM", and "Human Health effects of PM". These MESH terms were sometime combined with names of African region or African countries. For chemical composition of PM, we selected studies conducted in ambient air. Data from recognized organizations such as WHO, World Bank, OECD, United States Environmental Protection Agency (US EPA), and United National Environmental Program (UNEP) were also included. In the assessment of the health outcome of exposure to air pollution on population study, we selected only studies that used statistics to test exposure response relationships between measured ambient PM and any health outcome of interest. We also considered studies that assessed potential risk of exposure to atmospheric PM-bound-PAHs and NPAHs.

2.3 Overview of Ambient Particulate Matter in Africa

In most African countries, PM pollution is above the annual and 24-h mean air quality guideline values recommended by the WHO (Petkova *et al.*, 2013; Naidja *et al.*, 2017; Coker and Kizito, 2018) and ambient PM has been classified among the top 10 risk factors in sub-Sahara African countries (IHME, 2013). Despite this, little data and no standards exist for the majority of African countries. Recent studies have shown that one in eight premature deaths globally can be linked to poor air quality and approximately 90% of these deaths occur in low and middle-income countries (World Health Organization, 2018a).

In this review, we selected only publications carried out in Africa with actual ambient PM measurements and that showed mean PM_{10} and/or $PM_{2.5}$ levels. Studies that are most recent and that have reported actual means of PM, sampling devices, sampling durations, and sampling site characteristics (traffic roadside, urban background, and rural sites) are shown in Figure 2.2. A summary of epidemiological studies conducted in Africa on the health effects of exposure to a mass concentration of ambient particulate matter size fractions are shown in Table 2.1.

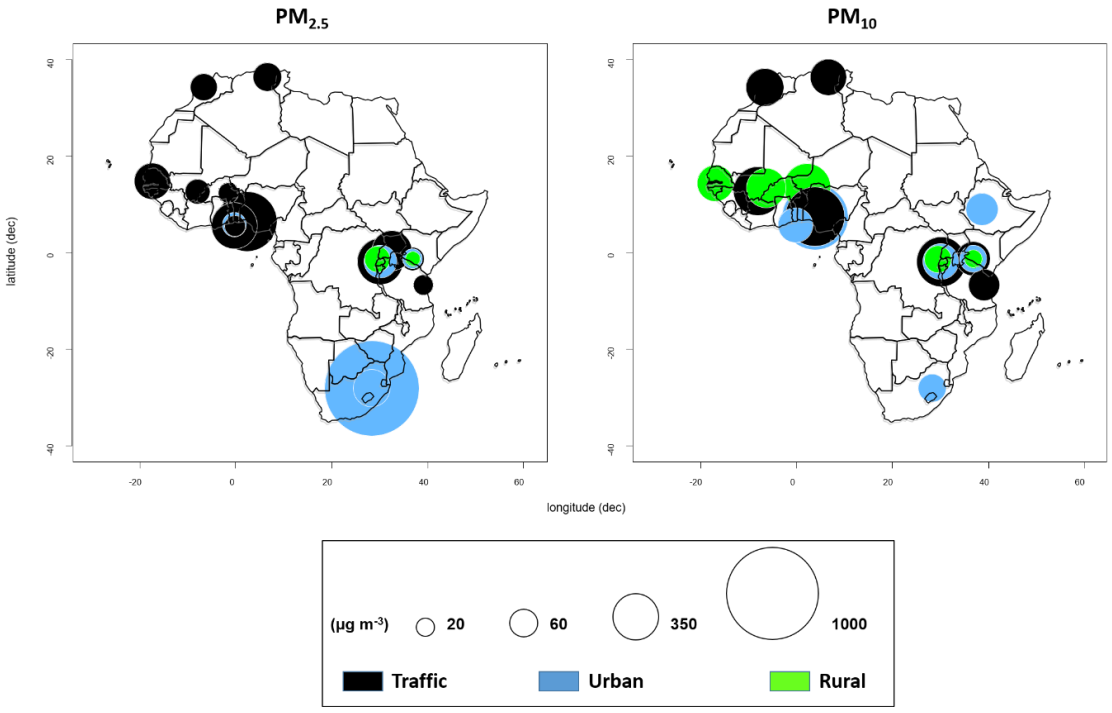

Figure 2. 2. Ambient $PM_{2.5}$ (particles less than 2.5 µm in diameter) (left), and ambient PM_{10} (particles less than 10 µm in diameter) (right), mean concentration as reported in studies from traffic (black color), urban background (blue), and rural site (green) in African countries such as Algeria (Terrouche *et al.*, 2016), Benin (Xu *et al.*, 2019), Burkina Faso (Boman *et al.*, 2009), Ethiopia (Li, 1999), Ghana (Arku *et al.*, 2008; Dionisio *et al.*, 2010), Kenya (Pope *et al.*, 2018), Mali (Garrison *et al.*, 2014), Morocco (Zghaid *et al.*, 2009), Niger (De Longueville *et al.*, 2013), Nigeria (Arku *et al.*, 2008), Rwanda (Kalisa *et al.*, 2018a), Senegal(Dieme *et al.*, 2012; De Longueville *et al.*, 2013), South Africa (Engelbrecht *et al.*, 2001; Worobiec *et al.*, 2011),Tanzania (Mkoma and Maenhaut, 2010), and Uganda (Kirenga *et al.*, 2015).

Table 2. 1. Summary of epidemiological and toxicological studies conducted in Africa on health effects of exposure to a mass concentration of ambient particulate matter size fractions. Particulate matter: ambient $PM_{2.5}$ (particles less than 2.5 μm in diameter), ambient PM_{10} (particles less than 10 μm in diameter), TSP (total suspended particles).

Study	Study Location	Type of Study	Study Population	Statistical Analysis	PM Size Fraction	Association and Health Outcome
Mentz *et al.* (2018)	Durban, South Africa	Longitudinal	N = 423 school children	Generalized estimating equation (GEE); 0–5-day lags; single lags and distributed lags	PM_{10} and $PM_{2.5}$	Exposure to PM_{10} was associated with significantly increased occurrence of respiratory symptoms among children (cough, shortness of breath, and chest tightness).
Lin *et al.* (2017)	South Africa, Ghana	Cross-sectional	N = 45,625, global aging and adult health	Logistic regression—3-level multilevel model	$PM_{2.5}$	$PM_{2.5}$ was found to be associated with overall disability and with cognition and mobility.
Makamure *et al.* (2017)	Kwazulu-Natal, South Africa	Longitudinal/questionnaire	N = 71, Children ages 7–9	Linear multivariate	PM_{10} and $PM_{2.5}$	Air pollution exposure results in increased expression of cluster of differentiation (CD14) in airway macrophages.
Ana *et al.* (2014)	Ibadan, Nigeria	Cross-sectional	N = 140 ages 15–65 years	ANOVA and Spearman-rank correlation	PM_{10}	Higher PM_{10} burden was observed to cause declining lung function.
Wichmann and Voyi. (2012)	Cape Town, South Africa	Case-crossover	N = 149,667 (RD = 13,439; CVD = 21,569; CVD = 7594)	Logistic regression	PM_{10}	PM_{10} was associated with cardiovascular disease, respiratory disease, cerebrovascular disease, and mortality.
Mustapha *et al.* (2011)	Ibadan, Nigeria	Cross-sectional	N = 1397 Schoolchildren (7–14 years)	Logistic regression	TSP, $PM_{2.5}$ and PM_{10}	Traffic pollution was associated with respiratory symptoms (wheeze, night cough, phlegm, rhinitis, and asthma in schoolchildren).
Naidoo *et al.* (2010)	Durban, South Africa	Longitudinal	N = 873 schoolchildren	Regression models	PM_{10} and $PM_{2.5}$	Schoolchildren living near industries were more likely to develop asthma and airway hyperreactivity rather than those living far away from industries.

Most of the studies were conducted in urban areas, with a high population density and a large number of potential pollution sources. Additionally, poor logistics in long-term measurements were prevalent as studies were undertaken for less than six months or for less than a 24 h period, focusing on near-roadway air pollution, using limited equipment and low flow rates, making comparison of the PM data across studies conducted in Africa difficult (Petkova *et al.*, 2013).

Limited epidemiological studies have been conducted in Africa, and available information on air quality and health has been reported by WHO, based on satellite data estimates. Four reviews on air pollution studies in Africa have been published (Petkova *et al.*, 2013; Amegah and Agyei-Mensah, 2017; Naidja *et al.*, 2017; Coker and Kizito, 2018); however, only one of these four studies addresses the health effects of air pollution in sub-Saharan countries (Coker and Kizito, 2018). Three reviews highlighted that biomass burning and road traffic are major sources of high levels of PM pollution in Africa (Petkova *et al.*, 2013; Amegah and Agyei-Mensah, 2017; Naidja *et al.*, 2017). Additionally, a study carried out in two low-income neighborhoods in Accra, Ghana indicated that combustion sources (biomass and traffic) and non-combustion sources (geological and marine) were major contributors to PM pollution (Arku *et al.*, 2008). Coker and Kizito (2018) have recently reviewed ambient air pollution and health effects in Africa and indicated that populations in sub-Saharan Africa are exposed to both acute and long-term health effects from ambient air pollution and highlighted gaps in epidemiological studies due to lack of long-term PM monitoring.

2.4 Chemical and Biological Components of Particulate Matter Worldwide

2.4.1 Chemical Components of Airborne Particulate Matter

Substantial improvements have been achieved in the chemical characterization and identification of the main PM components in developed and developing countries (Fromme *et al*., 2008; Sun *et al.*, 2015; Bootdee, *et al*., 2016). Chemical components of PM typically contribute an average of 20% to the total PM mass load (Raes *et al.*, 2000). These components are primarily emitted into the atmosphere, although some are formed in the atmosphere. Studies in the United States indicated that airborne PM contains a variety of microorganisms, some of which are pathogenic and pose severe threats to human health (Kellogg and Griffin, 2006; Polymenakou, 2012). However, the chemical composition of atmospheric PM is not distributed equally among all size ranges (Marley and Gaffney, 2006), meaning that chemical composition depends on the aerosol sources. PAHs and NPAHs are known for their harmful health effects, referring to a large group of organic compounds with two or more fused aromatic rings (Kim *et al*., 2013). In the atmosphere, PAHs (two or three rings) exist in the vapor phase, whereas multi-ringed PAHs (five

rings or more) exist in the particle phase (Abdel-Shafy and Mansour, 2015; Hayakawa, 2016a). PAHs are also capable of being transported from one region to another (intercontinental long-range transport) via air currents (Tamamura *et al.*, 2007). In addition, more than 90% of the carcinogenic PAHs appear to exist in the particulate phase of ambient air (Hayakawa, 2016a). In this section, the general overviews of PM-associated PAHs and NPAHs and airborne microorganism loadings in PM are extensively reviewed. Findings show that airborne microorganisms and organic aerosols (PAHs and NPAHs) are associated with PM and may provide reliable data for studying the response of the human body to increasing levels of air pollution.

2.4.2 Particulate Matter-Associated Polycyclic Aromatic Hydrocarbons and their Nitro-Derivatives

PAHs and NPAHs are ubiquitous environmental organic pollutants, which originate from the pyrolysis of organic matter and incomplete combustion of coal, oil, petrol, and wood (Abdel-Shafy and Mansour, 2015). NPAHs can form as secondary compounds through atmospheric reactions between PAHs and atmospheric oxidants such as ozone and nitrate radicals (Wu *et al.*, 2012). Some PAHs and NPAHs have carcinogenic and/or mutagenic properties, like benzo[a]pyrene (BaP) and 1-nitropyrene (1-NP), which are classified as Group 1 PAH and Group 2A (probably carcinogenic to humans) NPAH, respectively (International Agency for Research on Cancer, 2018). In addition, several other PAHs and NPAHs are classified in Group 2B (possibly carcinogenic to humans) (Albinet *et al.*, 2007). Given their toxicity and their wide distribution in the atmosphere, the EPA has classified 16 PAHs as priority compounds (U.S. Environmental Protection Agency, 2008).

2.4.3 Toxicity of Polycyclic Aromatic Hydrocarbons and their Nitro-Derivatives

Inhalation of PM, including $PM_{2.5}$ and PM_{10}, causes respiratory, cardiovascular, and lung diseases such as asthma, COPD, and lung cancer (Chen and Chen, 2018). In China, COPD was reported as the most common cause of human mortality, resulting from exposure to high levels of particulate air pollution at home (Li *et al.*, 2018). As PAHs and NPAHs are the major components of $PM_{2.5}$ and PM_{10}, they are thought to be responsible for these respiratory diseases (Hayakawa,

2018a). NPAHs, which exist at concentrations orders of magnitude lower than PAHs, are receiving particular attention since they possess a higher direct-acting mutagenicity and carcinogenicity than PAHs that must first undergo an enzymatic activation process (Keith and Telliard, 1979; Hayakawa *et al.,* 2018b). In 2013, research published by Pham *et al.* (2013) analyzed the mutagenicity of PMs, PAHs, and NPAHs using the Ames test with *Salmonella typhimurium* strains. PAHs such as BaP and benzo[b]fluoranthene (BbF) were found to cause indirect-acting mutagenicity of PMs produced by coal burning, wood burning and automobiles (Tang *et al.,* 2005; Hayakawa *et al.,* 2018b). Benzo[a]pyrene is the most widely studied PAH as it can be used as a marker for carcinogenic risk levels in environmental studies (Ding *et al.,* 2012). The WHO and several countries including the United States and China have recognized BaP as an epidemiological health hazard and have set protective health standards of 1 ng/m^3, 0.25 ng/m^3, and 10 ng/m^3, respectively (World Health Organization, 2000). NPAHs have also been previously observed in organic extracts of ambient PM (Hayakawa, 2018a). For example, NPAHs such as 1-nitropyrene (1-NP) and 1,3- and 1,8-dinitropyrenes (1,3-, 1,6-, and 1,8-DNPs) showed very strong direct-acting mutagenicity in emission extracts from diesel engines and wood particulates (El-Bayoumy and Hecht, 1986; Tang *et al.,* 2005; Ding *et al.,* 2012).

The latter NPAH exhibited high direct-acting mutagenic potency in the *Salmonella* bacterial mutagenicity assay, and on human lung tissue. Hayakawa (2016a) indicated that the metabolites of PAHs and NPAHs exhibited estrogenic and antiestrogenic activity in the yeast two-hybrid assay system using yeast cells expressing estrogen receptors.

2.5 Biological Components of Airborne Particulate Matter

Biological aerosols are composed of all biologically derived pathogenic or nonpathogenic matter, live or dead, and include bacteria, fungi, and viruses (Després *et al.,* 2012). The size distributions of bioaerosols vary considerably by type: pollens are typically 5–100 μm, fungal spores are 1–30 μm, and bacteria are 0.1–10 μm, while viruses are generally smaller than 0.3 μm (Després *et al.,* 2012). For example, biological aerosols represent a significant fraction of airborne PM and affect the microstructure and water uptake of aerosol particles. Bioaerosols such as bacteria, fungi, and viruses have been shown to account for a significant proportion of the mass of coarse (PM_{10}) and

fine ($PM_{2.5}$) particles. Airborne bioaerosols may be found as individual particles or agglomerates of particles (Jones and Harrison, 2004). It has been found that the dynamics of biological particles in the air are governed mainly by the particles' physical characteristics, of which size and concentration are the most important (Morawska *et al.,* 1999). Bioaerosol components, such as bacteria, fungi, and viruses, can attach to PM from varied sources including biomass, soil, and industries. Consequently, PM-associated bioaerosols can enhance their penetration into deeper parts of the lungs (D'Amato, 2002). For example, a pollen grain (>10 µm) is trapped in the nasopharyngeal tract when inhaled, whereas pollen allergens present in $PM_{2.5}$ can easily penetrate deep into lungs (Després *et al.,* 2012). As a result, the agglomeration of bioaerosols and PM can exacerbate respiratory allergies and other ailments such as pulmonary disease, cardiovascular disease, and cancer (D'Amato, 2002; Morakinyo *et al.,* 2016; Kalisa *et al.,* 2018b). The association and interaction of microorganisms and microorganism-derived allergens with airborne particulates has been documented to be part of the urban aerosphere (Woo *et al.,* 2013). Further, bioaerosols have also been identified as significant in relation to agricultural and human health (Peccia *et al.,* 2011). For example, through air dispersal during agricultural activity, many plant pathogens can travel from one region to another and cause disease outbreaks, leading to severe crop losses, famine, and mass migration (Donnison *et al.,* 2004). The transport of bioaerosols and other air pollutants in the gas phase is influenced by several factors including temperature, relative humidity, wind speed, and physical properties of the bioaerosols (D'Amato, 2002; Woo *et al.,* 2013; Kalisa *et al.,* 2018b). Davis (1987) indicated that *Peronospora tabacina* (blue mold), which is an agricultural disease that caused epidemics in United States tobacco in the late 1900s, is transmissible through the atmosphere. Airborne transmission is also one of the common ways of spreading infectious human diseases. For example, people working or living in the same environment may spread diseases such as measles, winter stomach flu, influenza, and tuberculosis (Driver *et al.,* 1994). Exposure to bioaerosols in the occupational environment is associated with a wide range of health effects with major public health impacts, including infectious diseases, acute toxic effects, allergies, and cancer (Zimmermann, 2015).

2.5.1 Particulate Matter-Associated Airborne Fungi

Fungi originate from natural sources (plants, animals, and soil) and anthropogenic activities (Jacobson, 2012). Fungal spores are a reported threat to human health (Newson *et al.*, 2000). Studies indicated that fungal spores are emitted into the atmosphere and become the most dominant biological components in airborne PM (Newson *et al.*, 2000; Jacobson, 2012). Fungi, like pollen and spores, account for large proportions of airborne PM, and fungal spores are found in the fine fractions of PM (Zhang *et al.*, 2010; Li *et al.*, 2011). A previous study, carried out in Australia, showed that PM could attach to fungal spores and airborne pollen grains and possibly change their morphology (Glikson *et al.*, 1995). For example, PM of similar size to fungal spores emitted into the atmosphere may coagulate, and their penetration into the human respiratory system may cause more serious outcomes than they would have otherwise been expected to cause alone (Després *et al.*, 2012). Studies also indicate that fungal spores and pollen contribute 4 -11% of the total mass concentration of $PM_{2.5}$ (Womiloju *et al.*, 2003). The concentration of fungal spore loadings on PM is higher in PM_{10} than in $PM_{2.5}$ air samples. This is most likely because the aerodynamic diameters of fungal spore agglomerates are between 2.5 μm and 10 μm (Deacon *et al.*, 2009). For example, Cao *et al.* (2014) found fungal spores to be the most common biological components of airborne dominant microorganism loadings in PM_{10}, being 4.5% more than in $PM_{2.5}$ (1.7%). Exposure to fungal spores loading in PM has been associated with respiratory diseases, allergies and asthma (Fröhlich-Nowoisky *et al.*, 2009). Several studies have found that *Cladosporium* spp., *Aspergillus* spp., *Penicillium* spp., and *Alternaria* spp. are the most predominant genera of fungi identified in airborne PM samples and they have been associated with symptoms of respiratory tract allergies (Boreson *et al.*, 2004; Fröhlich-Nowoisky *et al.*, 2009; Alghamdi *et al.*, 2014). Additionally, tree and grass pollens and fungal spores have been shown to exacerbate respiratory diseases such as asthma and rhinitis (Jones and Harrison, 2004; Fröhlich-Nowoisky *et al.*, 2009). The protection of sensitive populations from pathogenic fungi requires an understanding of environmental exposure to airborne fungi as a function of type and size.

2.5.2 Particulate Matter-Associated Airborne Bacteria

Airborne bacteria are one of the major components of indoor and outdoor aerosol particles (Haas *et al.,* 2013; Prussin and Marr, 2015). Airborne bacteria can be found in the air as isolated microorganisms but are more likely to be attached to other particles such as soil or leaf fragments, or found as conglomerates of a large number of bacterial cells (Després *et al.,* 2012). Some studies have shown a continuous interaction between the concentration of dust particles and microorganisms (Haas *et al.,* 2013). In an urban environment, high concentrations of airborne bacteria can have substantial effects on human health as pathogens or as triggers of asthma and seasonal allergies (Bowers *et al.,* 2011b). For example, several studies have shown that higher levels of biological components in the air, associated with PM, increased both asthma and allergic reactions (Bowers *et al.,* 2011b; Haas *et al.,* 2013). Several studies have also demonstrated that airborne bacteria are associated with small size particles (Gavett and Koren, 2001; Gou *et al.,* 2016). Nasir and Colbeck (2010) proved that up to 80% of the total viable concentration of bacteria (5036 CFU/m^3) in the atmosphere is found in particles with diameters less than 4.7 μm. Microbial allergens and pathogens were identified in PM, and their relative abundance appeared to increase as the concentration of PM pollution increased (Cao *et al.,* 2014). Cao *et al.* (2014) found that the representation of pathogens as identified within the entire bacterial community was 0.012% in $PM_{2.5}$ and 0.017% in PM_{10} samples.

2.5.3 Particulate Matter-Associated Airborne Viruses

Bioaerosols also consist of viruses responsible for various diseases that affect public health (Després *et al.,* 2012; Cao *et al.,* 2014). Due to their small size, viruses can remain airborne, come into contact with humans or animals, and potentially cause an infection. Studies have detected viruses in airborne biological contaminants, despite their small size and the difficulty associated with collection and analysis (Glikson *et al.,* 1995; Alghamdi *et al.,* 2014). Cao *et al.* (2014) employed metagenomic methods to analyze the microbial composition of Beijing's PM pollutants and show that airborne dsDNA viruses can be identified at the species level. They found that human adenovirus C (6.5%) was the dominant pathogenic airborne virus identified in the $PM_{2.5}$ and PM_{10} samples. Liang *et al.*(2014) found a significant association between ambient $PM_{2.5}$

concentrations and virus (Human influenza) levels in Beijing, which has important implications for public health and environmental actions. Exposure to airborne viruses plays an important role in microbial ecology and some infectious diseases. Studies have shown an association between viruses and bacteria that cause respiratory infections in children with asthma; additionally, Pneumococcus bacteria and the influenza virus have been shown to interact with each other (Haas *et al.*, 2013; Furuse *et al.*, 2018).

2.6 Chemical Composition of Ambient Particulate Matter in Africa

2.6.1 Atmospheric Concentrations of Polycyclic Aromatic Hydrocarbons and their Nitro-Derivatives in Africa

In Africa, urbanization and population growth have increased rapidly in recent decades. African countries account for more than a quarter of global energy consumption, with wood burning being the main energy source (World Bank, 2011; World Health Organization, 2016a). The burning of these solid fuels and biomass releases several air pollutants, gases and particulates, with two of the toxic organic compounds present in $PM_{2.5}$ and PM_{10} – PAHs and NPAHs – being of greatest environmental health concern due to their carcinogenicity and mutagenicity. The majority of studies reviewed in this study found that the mean concentrations of atmospheric $PM_{2.5}$ and PM_{10} in Africa greatly exceeded the 2006 WHO guideline values for annual and 24-h means, and these carcinogenic and mutagenic organic pollutants are a major component of PM (Figure 2.2). In the available publications on atmospheric NPAHs in Rwanda (Kalisa *et al.*, 2018a), Egypt (Nassar *et al.*, 2011), and Algeria (Ladji *et al.*, 2009) (Figure 2.3), the atmospheric concentrations of total PAHs and NPAHs show large variations between these countries. The PAH concentrations, in descending order, were Senegal, Kenya, South Africa, Mali, Uganda, Rwanda, Sierra Leone, Algeria, and Egypt. The NPAH concentrations, in descending order, were Rwanda, Algeria, and Egypt (Figure 2.3). It must be emphasized that total PAH concentrations in Senegal, Kenya, and South Africa were much higher than those in the remaining countries, suggesting that the urban atmospheres in Senegal, Kenya, and South Africa were much more polluted with PM-containing PAHs (Muendo *et al.*, 2006; Val *et al.*, 2013; Geldenhuys *et al.*, 2015).

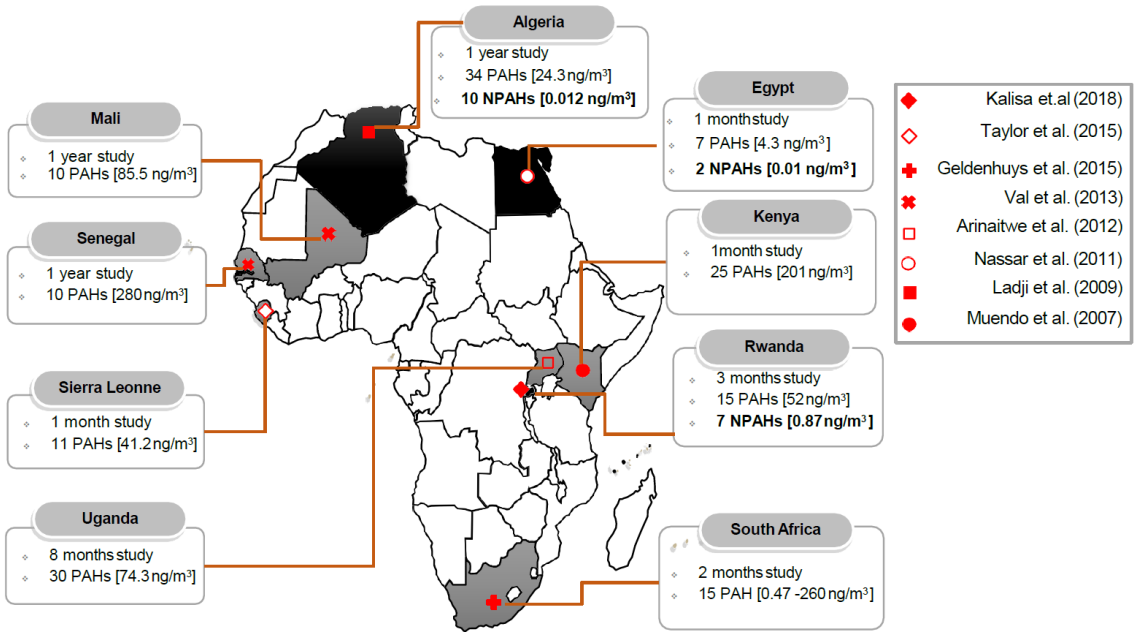

Figure 2. 3. Map of Africa showing countries where studies on polycyclic aromatic hydrocarbons (PAHs) and their nitro-derivatives (NPAHs) in ambient air were conducted. The sampling duration, concentration of PAHs and NPAHs, and number of analyzed PAHs and NPAHs species; the black color indicates PAH and NPAH studies carried out in Algeria (Ladji *et al.*, 2009), Egypt (Nassar *et al.*, 2011), and Rwanda (Kalisa *et al.*, 2018a) and the gray color indicates PAH studies carried out in Kenya (Muendo *et al*., 2006), Mali (Val *et al*., 2013),Sierra Leonne (Taylor *et al*., 2015), Senegal (Val *et al*., 2013), South Africa (Geldenhuys *et al*., 2015), and Uganda (Arinaitwe, *et al*., 2012).

2.6.2 Source and Risk Assessment of Particulate Matter-Bound Polycyclic Aromatic Hydrocarbons and their Nitro-Derivatives in Africa

NPAHs are formed from PAHs in the presence of nitrogen oxides at high temperature; this means that the corresponding PAHs increase with increasing temperature (Tang *et al.*, 2005). The combustion temperatures in wood stoves, coal stoves, and diesel engines are different (Hayakawa, 2018a). Thus, the concentration ratios of NPAHs to PAHs have been widely used worldwide and suggest that [NPAH]/[PAH] ratios are useful markers to identify the sources of PAHs and NPAHs. For example, the [NPAH]/[PAH] concentration ratios were previously determined in three different types of PM, derived from diesel engine vehicles (combustion temperature ~2700–3000 °C), coal-burning stoves (~900–1200 °C), and wood burning stoves (~500-600 °C) (Tang *et al.*, 2005). The [1-nitroperylene]/[pyrene] or [1-NP]/[Pyr] ratios of coal emissions (0.001) were much smaller than the ratio of diesel emission particles (0.36), and the [1-NP]/[Pyr] ratio was recommended as a marker for source identification of PAHs and NPAHs (Tang *et al.*, 2005).

African automobile emissions (diesel and gasoline) and biomass burning were considered major contributors of PAHs and NPAHs in urban and rural sites, respectively (Table 2.2). The [1-NP]/[Pyr] ratios in Kigali, Rwanda were 0.05 (dry season) and 0.04 (wet season) (Kalisa *et al.,* 2018a), while the values in the Greater Cairo Area, Egypt were 0.06 (winter) and 0.03 (summer) (Nassar *et al.,* 2011). The values in these two African countries were similar to those reported in East Asian cities influenced by large volumes of vehicle emissions (Hayakawa, 2018a).

High concentrations of benz(g,h,i)perylene (BPe), phenanthrene (Phe), fluoranthene (Flu), BaP, and benzo(b)fluoranthene (BbF), account for a large proportion of the total PAHs that have been commonly observed in ambient particulates in the available studies in Africa (Table 2.3). High emissions of BPe and indeno(1,2,3-cd)pyrene (IDP) have been associated with vehicle emissions (Guo *et al.,* 2003), while high emissions of Flu, BaP and BbF are associated with domestic fuel burning (Khalili *et al.,* , 1995; Ravindra *et al.,* 2008).

NPAH compounds, such as 9-nitroanthracene (9-NA) and 1-NP directly emitted from diesel engines were the most abundant NPAHs detected in African cities (Table 2.2). However, most of the sampling sites in Africa were near intersections with high traffic volumes, suggesting that these NPAHs were emitted from automobiles. Additionally, several PAH pairs, such as [fluoranthene]/([pyrene] + [fluoranthene]) or [Flu]/([Pyr] + [Flu]), [benz(a)anthracene]/([chrysene] + [benz(a)anthracene]) or [BaA]/([chrysene(Chr)] + [BaA]) and [indeno (1,2,3-cd)pyrene]/([benz(g,h,i)perylene + [indeno (1,2,3-cd)pyrene]) or [IDP]/[BPe + IDP], have been also used as markers of the source of PAHs in African countries, including Rwanda (Kalisa *et al.,* 2018a) and Kenya (Muendo *et al.,* 2006). Biomass burning and automobile emissions were the main sources of atmospheric PAHs in Kenya and Rwanda (Table 2.2). To evaluate the cancer risk of PAHs and NPAHs detected in airborne PM, the methodology developed by the US EPA was widely applied (Sexton *et al.,* 2007; Ramírez *et al.,* 2011). In African countries such as Rwanda (Table 2.3) findings from cancer risk assessment studies reported that PAHs and NPAHs present in $PM_{2.5}$ and PM_{10} were above the WHO recommended health standard (1ng/m^3) and would be classified as definite risks (Sexton *et al.,* 2007). Table 2.2 summarizes the available information on PAH and NPAH compounds analyzed in PM size

fractions, locations, sources and observed health effects, and provides the details of cited references relating to Africa.

Table 2. 2. Summary of studies conducted in Africa indicating source of PM-bound PAHs and NPAHs and associated health outcomes.

Reference	City, Country	Type of Site	PM Size	Main PAH Detected	Main NPAHs Detected	Source Identified	Association and Health Outcome
Kalisa *et al.* (2018a)	Kigali, Rwanda	Roadside/ambient air	$PM_{2.5}$ and PM_{10}	BPe, Phe, Flu, BaP, and BbF	9-NA, 2-NP+2-NFR, 6-NBaP	Wood burning and automobile emissions	The lifetime excess cancer risk exceeding the WHO guideline values and classified as definite risks.
Taylor *et al.* (2015)	Western Sierra Leone	Residence/ambient air and indoor air	$PM_{2.5}$	Phe, DBA, and BPe		Burning wood	PAH- bound $PM_{2.5}$ from biomass fuel from kitchens continue to be hazardous for people in developing countries.
Geldenhuys *et al.* (2015)	South Africa	Underground/ambient air	TSP	Pyr, Flu, and BaP		Diesel vehicle	Diesel exhaust emissions—recently confirmed as carcinogenic, which is why the health of underground workers is of concern.
Val *et al.* (2013)	Bamako, Mali	Desert area/ambient air	PM_{10}	IDP, BPe, BbF, and BaP		Traffic, biomass burning, and dust	The population of Mali—highly exposed to toxic particulate pollution that could lead to strong adverse health effects.
Dieme *et al.*(2012)	Dakar (Senegal)	Urban/ambient air	$PM_{2.5}$	BbF, BPe, IDP, and BaP		Combustion of fossil fuels	PAH and Heavy metals in $PM_{2.5}$ induced with dose-dependent toxicity, relying on inflammatory processes.
Hassan and Khoder (2012)	Dokki, Egypt	Urban/ambient air	TSP	BbF, BPe, DBA, and Chr		Unburned fossil fuels and vehicle emissions	PAHs in the particulate phase in the ambient air posing a potential health risk for the population of Egypt.
Arinaitwe *et al.*(2012)	Entebbe, Uganda	Watershed/ambient air	$PM_{2.5}$	Phe, Flu, and Pyr		Combustion of petroleum and biomass burning	Population of Uganda is likely to be exposed to toxic PAHs bound $PM_{2.5}$ from biomass burning.

Nassar *et al*. (2011)	Great Cairo, Egypt	Traffic side/ambient air	TSP	Phe, Flu, BbF, and Chr	1-NP	Gasoline engine	PAHs and NPAHs with carcinogenic and/or mutagenic health effects detected in Greater Cairo.
Ladji *et al.* (2009)	Algiers, Algeria	Suburban/ambient air	PM_{10}	Acy, Phe, and BbF	9-NA, 2-NFR	Motor vehicles	The population of Algeria is exposed to nicotine in particulates associated with PAHs.
Muendo *et al.* (2006)	Nairobi, Kenya	Traffic/ambient air	PM_{10}	Pyr, BbF, and BPe		Gasoline and diesel	Contribution of carcinogenic PAHs bound PM_{10} in Nairobi—approximately 30%.

Abbreviations of NPAH compounds: 9-nitroanthracene (9-NA), 2-nitropyrene (2-NP); 2-nitrofluoranthene (2-NFR), 1-nitroperylene (1-NP), 6-nitrochrysene, and 6-nitrobenz(a)pyrene (6-NBaP). Abbreviations of PAH compounds: Acenaphthylene (Acyl), phenanthrene (Phe), fluoranthene (Flu), pyrene (Pyr), benz(a)anthracene (BaA), chrysene (Chr), benzo(b)fluoranthene (BbF), benzo(a)pyrene (BaP), dibenz(a,h)anthracene (DBA), benz(g,h,i)perylene (BPe), and indeno (1,2,3-cd)pyrene (IDP). Particulate matter: $PM_{2.5}$ (particles less than 2.5 μm in diameter), PM_{10} (particles less than 10 μm in diameter), TSP (total Suspended particle).

2.7 Current Understanding of Bioaerosols Associated with Particulate Matter in Africa

Africa produces more than 50% of the airborne particles produced worldwide, and millions of metric tons of African desert dust is transported by natural atmospheric processes as far as the United States and Europe (Tanaka, 2012). Despite recognition of the importance of bioaerosols from African desert dust, the effects of exposure to these aerosols on humans have never been investigated. African dust contains pathogenic biological particles, which have been documented to exacerbate respiratory and proinflammatory diseases (Tanaka, 2012). Bioaerosols, their effects on human health, and their long-range transportation from Africa have been extensively studied worldwide (Kellogg and Griffin, 2006; Chin *et al.,* 2007; Griffin 2007; Deguillaume *et al.,* 2008; Prospero *et al.,* 2008; Sánchez de La Campa *et al.,* 2013; Garrison *et al.,* 2014). Investigation of the microbial content of African desert dust and related impact on humans is still in its infancy. Even though earlier work in Africa assessed bioaerosols in hospital rooms (Table 2.3), levels of typical outdoor exposure to bioaerosols are still unknown. Considering the impact of bioaerosols on human health, examining outdoor bioaerosol exposure levels in different locations and their spatial variability is important to sensitive populations. African dust can significantly increase ambient PM levels, contributing to excessive amounts of PM as set by the WHO. This is a result of a lack of valid quantitative exposure assessment methods. Characterization of bioaerosol samples is challenging and requires powerful analytical tools and knowledge of molecular biology and aerobiologic chemistry. In Africa, funding for the installation of bioaerosol sampling and air analysis is inadequate. As a result, this limits bioaerosol studies across the region, with the few that have been undertaken and completed being in collaboration with international institutions.

Early studies in Africa used cultivation approaches to assess the diversity and composition of airborne bacteria and fungi associated with PM in Egypt, Libya, and South Africa (Table 2.3). However, these studies provided a limited insight into airborne bacteria and fungi associated with PM, as only viable and culturable microorganisms can be identified through culture methods. Only one study in South Africa that investigated the transmission of *Mycobacterium tuberculosis* applied advancements in enumerating various culture-independent (high-throughput DNA

sequencing) techniques (Matuka *et al.,* 2015). The latter techniques reflect the diversity of airborne fungi and bacteria since they are very sensitive and significantly quicker than traditional methods. This process can be applied to any biological sample containing nucleic acid, as it detects viable, nonviable, culturable, and non-culturable organisms (Bowers *et al.*, 2011a; Cao *et al.,* 2014; Yan *et al.,* 2016). A few studies that have been completed in Africa on bioaerosols associated with indoor PM have found that *Bacillus* sp., *Cladosporium* sp., *Aspergillus* sp., and *Penicillium* sp. are the predominant genera of bacteria and fungi identified in airborne PM samples. These organisms have been associated with symptoms of respiratory tract allergies, asthma, and infections in patients (Table 2.3). *Bacillus* and *Staphylococcus* have been observed to dominate the bacterial aerosol community in indoor air samples in Africa (Rahoma, 2011; Osman *et al.,* 2018) and some species of bacteria including *Acinetobacter calcoaceticus* and *Corynebacterium aquaticum*, known as human pathogens, have been found in aerosols in Bamako, Mali (Val *et al.,* 2013).

Table 2. 3. Summary of the available information on the types of study, biological pollutants analyzed (either singly or in combination with PM), study population and location, observed health effects, and the details of cited references.

Study	Study Location	PM Size	Biological Components Analyzed	Enumeration Techniques	Dominant Species Identified	Association and Health Outcome
Abdel-Rahim *et al.*(2018)	Assiut, Egypt	TSP	Fungi	Culture-dependent	*Chaetomium globosum*, *Aspergillus parasiticus*, *Penicillium oxalicum*, and *Setosphaeria rostrata*	The current study suggests that improvement of antimicrobial additives of paints may be a promising approach to reduce paint biodeterioration and, subsequently, air contamination of indoor environments.
Osman et al.(2018)	Bolak, Egypt	>8 µm and <8 µm	Bacteria/Fungi	Culture-dependent	*Bacillus licheniformis*, Aspergillus, and Penicillium	Dust particles accumulated in air conditioning filters and floor surfaces and these would constitute important sources of airborne bacteria and fungi inside these hospitals.
Setlhare *et al.* (2014)	South Africa	TSP	Bacteria/Fungi	Culture-dependent	Bacillus, Kocuria, Staphylococcus, Arthrobacter, Candida, Aureobasidium, Penicillium, and Phoma	Airborne bacteria and fungi that cause disease, especially in those populations with suppressed host immunity defenses in South Africa. Fungal genera identified (e.g., Candida), cause food spoilage and fungal infections in human
Rahoma (2011)	Tobruk, Libya	0.2 µm	Bacteria/Fungi	Culture-dependent	*Bacillus thuringiensis* and *Cladosporium sp*. *Trichophyton sp*.	Inhalation of associated pathogenic viable microorganisms and chemical contaminants such as carcinogens and small particles may trigger other physiological reactions (e.g., asthma and cardiovascular events) in humans.
Kellogg *et al.* (2004)	Bamako, Mali	TSP	Bacteria/Fungi	Culture-dependent	*Acinetobacter calcoaceticus*, *Bacillus mycoides*, *Bacillus pumilus*, *Bacillus subtilis*, and *Cladosporium cladosporioides*	Opportunistic human pathogens were isolated from air samples and could cause severe respiratory diseases

2.8 Conclusions

In Africa, rapid population growth, industrialization, motorization, and urbanization have encouraged the development of dense urban centers and contributed to worsening air quality. Further to this, in African cities economic and social disparities exacerbate health inequalities. The impact of air pollution of chemical and biological origin, which varies both spatially and temporally throughout urban centers, causes further health inequities and influences vulnerable populations. Findings from currently available works have revealed the following.

- Exposure of human populations to chemical and biological aerosols is of particular concern in Africa.
- Major chemical components of PM include carcinogenic PAHs and NPAHs and the major biological components include pathogenic fungi and bacteria, although information is scarce in Africa.
- The association of chemical and biological components of PM has been linked to synergistic health effects in other continents. However, the interrelationship of these factors is complex and deserves comprehensive research in Africa.
- Chemical components of aerosols arise largely from automobiles and wood burning as the major sources of PAHs and NPAHs in Africa.
- Major knowledge gaps persist, particularly for the sub-Saharan region of Africa.

The total number of studies in Africa is extremely low and more are critically needed to better understand the contribution of both the biological and the chemical components of particulate matter to health outcomes in Africa. The limited funding and expertise in this field necessitates international and interdisciplinary collaboration.

2.9 Supplemental Information

Table S2. 1. A selection of the studies carried in African cities on ambient particulate matter size fraction. These studies show the ranges of mean PM_{10} and $PM_{2.5}$ levels and the methodology used, including information on site characteristics, sampling devices with different flowrates, sampling duration for PM and sampling period.

Reference	Study Location	Site characteristic	Sampling Device	Flowrate	Sampling duration	Sampling Period	$PM_{2.5}$ [µg/m^3]	PM_{10} [µg/m^3]
Kalisa *et al.* (2018a)	Kigali, Rwanda	Traffic	HVAS	1000 LPM	24h	Apr to Jun 2017	185	214
		Urban					81.4	98.7
	Musanze, Rwanda	Rural					45	53.7
Pope *et al*.(2018)	Nairobi, Kenya	Traffic	Low-cost (OPCs)	1m^3/h	24h	Feb to March 2018	36.6	93.7
		Urban					24.8	53
		Rural					13	19.5
Ouafo-Leumbe *et al.*(2018)	Djougou, Benin	Rural	MVPS	5 LPM	**	Nov 2005 and Oct 2009	0.7 to 47.3	1.4 to 148.3
Xu *et al*.(2019)	Cotonou, Benin	Traffic	PPS	10 LPM	12h	Jan 6 to 11 and July 5 to 10 2016	335.1	*
Djossou *et al.* (2018)	Cotonou, Benin	Traffic	MVPS	5 LPM	15min	Feb 2015 to March 2017	11 to 174	
	Abidjan, Côte d'Ivoire	Traffic					8 to 226	*
Safa and Bouacha. (2018)	Tiaret, Algeria	Traffic	CI	**	**	May to August 2016	20	37

Terrouche *et al.* (2016)	Constantine, Algeria	Traffic	LVAS	5 LPM	**	23 Dec 2011 and 8 Jan 2013	57.8	105.2
Ediagbonya *et.al* (2015)	Sapele, Nigeria	Industrial	LVAS/MVAS	5 LPM	24h	**	104 to 260.4	104.2 to 431.0
Kirenga *et al.* (2015)	Kampala, Uganda	Traffic	DUSTTRACK	**	24h	30 Jun to 27 Jul 2014	132.2	*
Bahloul *et al.* (2015)	Sfax, Tunisia	Industrial	NFAS	**	24h	Nov 23rd to Dec 16th, 2013	*	4.07 to 88.51
Ana *et al.* (2014)	Ibadan, Nigeria	Urban	**	**	6h	Jan and March 2008.	*	422
		Traffic		**				328
		Industrial		**				257
De Longueville *et al.*(2014)	Kandi, Benin	Rural	Satellite data	**	**	Feb 2003 and Dec 2007 (61 days)	*	1017
Garrison *et al.* (2014)	Mali, Bamako	Traffic	MVAS	5 LPM	24h	Sept 2012 and Jul 2013	43	210
Lowenthal *et al.* (2014)	Shobra, Egypt	Industrial	LVAS/MVAS	5 LPM	24h	Jun and Oct 2010	61.0 to 216.0	154.0 to 360.0
Val *et al.*(2013)	Bamako, Mali	Traffic	CI	30 LPM	48h	2008 to 2009	*	205.8
	Dakar, Senegal							80.7
De Longueville *et al.*(2013)	Banizoumbou, Niger	Rural	TEOM	**	**	2006 to 2007	*	187
	Cinzana, Mali	Rural						129
	M'Bour, Senegal	Rural						108

Dieme *et al.* (2012)	Faidherbe, Senegal	Traffic	HVCI	68 m^3/h	15 days	Jul 2009 and Sept 2009,	105.4	*
	Fann, Senegal	Traffic					75.1	
	Ngaparu, Senegal	Rural					16.9	
Kinney *et al.* (2012)	Nairobi, Kenya	Traffic	PFAS	4 LPM	11h	Jul 2009	98.1	*
Worobiec *et al.* (2011)	Bethlehem, South Africa	Urban	Dust-monitor	1.2 LPM	**	Jul-01	1000	*
Dionisio *et al.* (2010)	Accra, Ghana	Traffic	DustTrak	**	48h	Nov 2006 and Aug 2008	39 to 53	80 to 108
		Urban					0 to 70	57-106
Gebre, Feleke and Sahle-Demissie (2010)	Addis Ababa, Ethiopia	Urban	AP	**	24h	22 Feb to 15 Apr 2008	*	80
Mkoma *et al*. (2009)	Dar es Salaam, Tanzania	Traffic	LVAS	17 LPM	12h	Aug and Sept 2005	26	76
Odhiambo *et al*. (2010)	Nairobi, Kenya	Urban	SFU	18 LPM	8h	Feb - Apr 2003	*	239
Zghaid *et al.* (2009)	Kenitra, Morocco	Traffic	PAS	17LPM	24h	2010, 2011 and 2012 (14 months)	51.32	115.12
Boman *et al.* (2009)	Ouagadougou, Burkina Faso	Traffic	Cyclones	3LPM	24h	Nov -Dec 2007 (12 days)	27	164
Arku *et al.*(2008)	Accra, Ghana	Traffic	HCI	10 LPM	24h	30 Jun to 20 Jul 2006.	27.4	71
Laakso *et al.* (2008)	Bethlehem, South Africa	Industrial	MVAS	**	13h	July- to Dec 2002		60

	Harare, Zimbabwe						40	60
Efe and Efe. (2008)	Warri, Nigeria	Traffic	AP	**	24h	Annual 2003		126
Van Vliet and Kinney (2007)	Nairobi, Kenya	Traffic Urban	Cyclone	4LPM	12h	Feb 2016 (4 days)	414 20	*
Etyemezian *et al.* (2005)	Addis Ababa, Ethiopia	Traffic Sub -urban	MVAS	5 LPM	24h	Jan -Feb 2004	100 40	*
Engelbrecht *et al.* (2001)	Bethlehem, South Africa	Urban	ALVS	16.7 LPM	24h	June -July 1997	109	*

†**Particulate matter:** $PM_{2.5}$ (particles less than 2.5 µm in diameter), PM_{10} (particles less than 10 µm in diameter) † **Sampling device:** ALVS (Anderson Low Volume Air Sampler), MVAS (Minivol Air Samplers), LVS (Low Volume air sampler), HVCI (high volume cascade impactor), HVS (High volume sampler), PAS (Personal air Samplers), AP (Air pump), HCI (Harvard cascade impactors), OPC (Optical particle counter), PFAS (Personal filter based air Samplers), SFUAS (Stacked Filter Unit air sampler), NFAS (Nucleopore filter based air sampler), PPS (Portable personal sampler), TEOM (Tapered element oscillating microbalance) †**Flowrate:** LPM (liter per minute). * (not measured), ** (not reported).

Chapter 3 - Method Development

Part 3A - **Egide Kalisa**, Stephen Archer, Kevin Lee, Donnabella Lacap-Bugler. Evaluation of high-volume and low-volume air sampler for chemical and biological speciation of airborne particulate matter in Rwanda. Manuscript in preparation.

Part 3B - **Egide Kalisa**, Stephen Archer, Donnabella Lacap-Bugler. Effect of sampling duration and filter sample size on the measurement of $PM_{2.5}$ and PM_{10} particulate-bound PAHs and NPAH. Manuscript in preparation.

Part 3C - **Egide Kalisa**, Stephen Archer, Kevin Lee Donnabella Lacap-Bugler. Efficiency of sampling duration for DNA-based analysis of bioaerosol. Manuscript in preparation.

Chapter 3.1 Prelude

The literature review presented in the previous chapter identified the knowledge gaps in the collection and analysis of chemical and biological composition of particulate matter (PM). Chapter 3 focuses on evaluating and developing different approaches for analysing the major chemical (PAHs and NPAHs) and biological (bacterial) components of PM. The key objective of this chapter was to develop a more robust and high-resolution method of evaluating air quality and pollution. The first part of the method development was focused on developing the simultaneous PM component collection protocol (Chapter 3A). Chapter 3B determined the accuracy of calculating atmospheric concentrations of PAHs and NPAHs from different sampling durations and with differing filter size areas. Finally, the third part of the methods chapter determined the recoverable genomic DNA yield at different sampling durations of ambient air sampling and from differing filter size areas (Chapter 3C). The results obtained in this chapter were used to guide protocols for the following chapters and will be of interest to researchers investigating chemical and biological compositions of atmospheric PM.

Chapter 3A - Evaluation of high-volume and low-volume air sampler for chemical and biological speciation of airborne particulate matter in Rwanda

3A.1 Introduction

Rwanda has experienced rapid urbanization in recent years and had a population of 12 million in 2017. The small amount of air quality data available in the region indicates that the levels of ambient air pollutants, such as particulate matter (PM), are substantially higher than the World Health Organization (WHO) air quality guidelines (Kalisa *et al.*, 2018a). Crucially, the government of Rwanda currently has limited capacity to monitor and manage air pollution, and communities have no basis on which to understand the impacts of air pollution. Despite the associated health risks, air quality programs have been discontinued in recent years in Rwanda. The main problems are the severe lack of knowledge of air pollution sciences and inadequate political drive and funding to install air quality monitoring stations. This limits air quality monitoring across the region, and many countries in Africa lack air quality standards.

PM with an aerodynamic diameter <10 μm (PM_{10}) is commonly the focus of air quality monitoring in Africa and the levels are used as an indicator of air quality evaluation (Petkova *et al.*, 2013). The WHO has recognized PM_{10} as an epidemiological health hazard and has set a protective health 24-hour standard mean of 50 $\mu g/m^3$ (World Health Organization, 2006). In 2013, ambient PM was classified as a group 1 carcinogen by the International Agency on Research Cancer (IARC) (Falcon-Rodriguez *et al.*, 2016). PM contains several toxic organic compounds including polycyclic aromatic hydrocarbons (PAHs) and nitrated PAHs (NPAHs), which are well known for their carcinogenic and mutagenic properties (Durant *et al.*, 1996), and pathogenic microorganisms such as bacteria (Kalisa *et al.*, 2019a). These PM-associated chemical and biological components are major air pollutants that have been identified as causes of lung cancer, asthma, pulmonary fibrosis, oxidative stress, depressed immune response, and chronic obstructive pulmonary diseases in humans (Morakinyo *et al.*, 2016; Kalisa *et al.*, 2019a). Measurement of these PM compositions is the first step towards addressing air pollution, but monitoring is scarce to non-existent in large parts of sub-Saharan Africa.

Filtration, which involves using a sampling pump to pull the air sample through a filter cassette, is widely used for ambient air sampling. Various designs for collecting air samples are available commercially, which work on the principle of collecting a large volume of air through a filter.

High volume air samplers (HVAS) and low volume air samplers (LVAS) have been commonly used to collect samples of air particles for chemical and biological characterization (Delgado-Saborit *et al.,* 2010; Kalisa *et al.,* 2018a; Kalisa *et al.,* 2019a). The difference between an HVAS and an LVAS is the amount of air volume sampled. Studies using HVAS to collect PM onto filter have collected volumes of air of around 1440 m^3 for 24 hours' sampling (Harrison *et al.,* 1996; Hayakawa, 2018a). Other authors have used LVAS and medium-volume air samplers (MVAS) collecting < 200 m^3 (Davis and Fellin, 1987; Pandey *et al*., 2011). Several studies have used HVAS to collect PM samples for chemical characterization because of its relatively high airflow rates with low pressure drop, and high particle storage capacity. A previous study in Birmingham, UK, used LVAS and successfully determined particle-bound PAH concentrations (Delgado-Saborit *et al.,* 2010).

In this study, chemical (PAHs and NPAHs) and biological aerosol components (bacteria) associated with PM_{10} samples were collected in rural and urban sites in Rwanda using HVAS and LVAS simultaneously. This was the first study carried out in Rwanda to compare HVAS and LVAS sampler performance at two different flow rates and provide a clear comparison of the samplers' efficiency for chemical and biological speciation and determination of the abundance of PM_{10} particles. This study was a good starting point for investigating whether the LVAS, which is both cost- and time-efficient, can be used in Africa as an affordable option to characterise PM chemical and biological components and thus fill the existing research gap.

3A.2 Materials and methods

3A.2.1 Particulate matter collection

Selecting an appropriate sampling device is crucial in assessing the association of chemical and biological component loading in PM size fraction-based investigations. Factors to consider include PM size fraction, sampling duration, flow rate, sampling time, filter type and sample storage. In this study, samples were collected simultaneously using the HVAS and LVAS from the rooftop of the College of Science and Technology building, in an urban site at the University of Rwanda. Samples were also collected from a rural site located in the Northern Province of Rwanda, Musanze district, approximately 100km from the urban site (Figure 3A.1). A total of 35,

24-hour samples were collected at the urban site and 21, 24-hour samples at the rural site between April and June 2017. Each 24-hour PM_{10} sample was collected on a 126 x 166mm glass fiber filter (GFF, Whatman EPM 2000) using an HVAS (SIBATA Electric Company Limited, Japan, and HVS-RW-1000F) operating at a flow rate of 1,000 litres per minute. The LVAS (Hi-Q, CF-901/230 San Diego, CA) collected on 47 mm glass fiber filters operating at a flow rate of ~ 85 litres per minute (Figure 3A.2). Each sterilized filter was packaged in sterilized aluminium foil and was stored in a sealed bag until it was loaded into the filter cartridge. The samplers (filter holders) were sterilized with 70% ethanol before each sampling set (Gao, *et al.*, 2015). All inside surfaces of the two stages of the HVAS and LVAS samplers were maintained in a sterile condition until sampling. Gravimetrical analysis was performed as described in our previously study (Kalisa *et al.*, 2018a). In each case, the filters were analysed for both chemical and biological components; the filter was removed from the sampler, covered, inverted, and wrapped in aluminium foil inside a plastic bag and stored at −20°C in the laboratory prior to filter extraction and analysis.

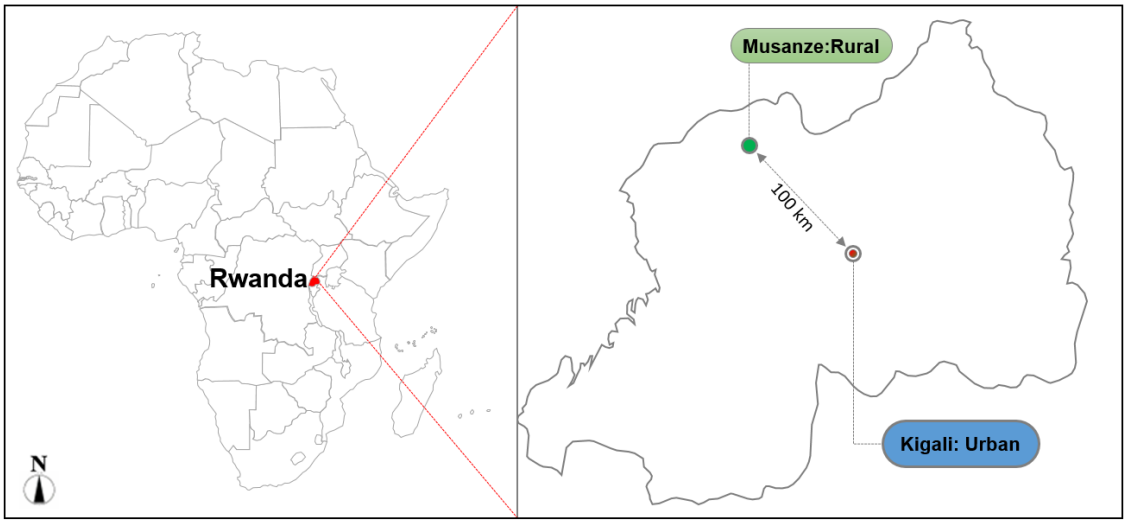

Figure 3A. 1. The sampling locations in Rwanda with reference to the African continent.

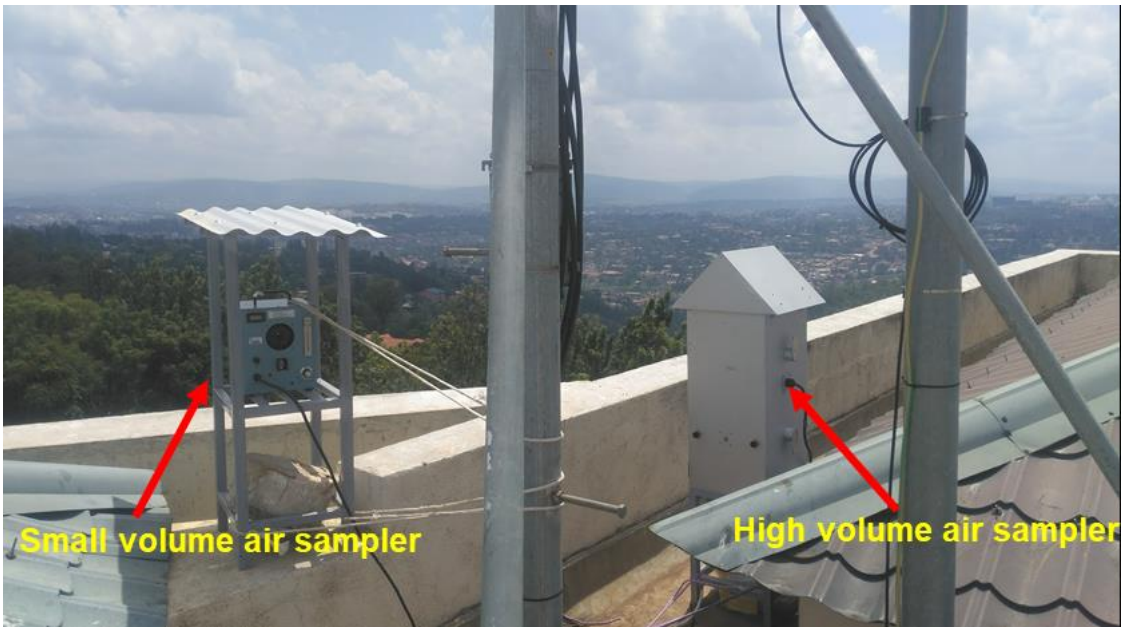

Figure 3A. 2. Simultaneous sampling using a high-volume air sampler (HVAS) and a small volume air sampler (LVAS) at the Kigali urban site, Rwanda.

3A.2.2 Meteorological conditions

Meteorological parameters such as temperature and relative humidity recorded during the sampling period were obtained from a Rwandan meteorological weather station (data available at https://meteorwanda.gov.rw/index.php?id=9).

3A.3 Analysis of PAHs and NPAHs

The filter extraction, instrumental analyses, and reagents used have been described in our previous study (Kalisa *et al.*, 2018a). Briefly, 7 samples from each sampler and from each sampling site were analysed for 15 PAHs and 6 NPAHs using two high-performance liquid chromatographic systems (HPLC-10A series, Shimadzu Inc., Kyoto, Japan), with one system equipped with a fluorescence detector and the other equipped with a chemiluminescence detector.

3A.4 Microbial analysis (bacteria)

3A.4.1 DNA extraction

The filters were cut into small pieces and were placed in nucleospin tubes (2ml) filled with ceramic beads (1.4 mm, Qiagen, Germany). To each sample 270 µL of phosphate buffer (100 mM NaH2PO4) and 270 µL of sodium dodecyl sulfate (SDS) lysis buffer (100 mM NaCl, 500 mM Tris pH 8.0, 10% SDS) were added and samples were horizontally shaken on a Vortex Genie 2 (MO BIO Laboratories Inc, Carlsbad, CA, USA) for 10 min. Samples were centrifuged at 14000 rpm for 3min and 180 µL of cetyltrimethylammonium bromide-polyvinylpyrrolidone (CTAB) extraction buffer (100 mM Tis-HCl, 1.4 M NaCl, 20 mM EDTA, 2% CTAB, 1% polyvinylpyrrolidone and 0.4% β-mercaptoethanol) was added. Samples were vortexed for 1min prior to incubation at 60°C and 300 rpm for 35 min on a rocking bed. Samples were centrifuged at 13,200 rpm for 5 min and then 350 µL of chloroform/isoamyl alcohol (24:1) was added. Samples were again vortexed for 15 s and centrifuged for 5 min at 13,200 rpm. The aqueous phase was transferred to a new Eppendorf tube then 500 µL of chloroform/isoamyl alcohol (24:1) was added. Samples were vortexed and left on a rocking bed HulaMixer (Invitrogen, Carlsbad, CA, USA) for 20 min. Samples were centrifuged for 5 min at 13,200 rpm, the aqueous phase was removed and 10 M ammonium acetate was added to the samples to achieve a final concentration of 2.5 M. The samples were vortexed and centrifuged for 5 min at 13,500 rpm. The aqueous layer was removed to a new tube and 0.5 volumes of isopropanol were added and mixed. Samples were left overnight at −20°C then centrifuged for 20 min at 14,000 rpm at 4°C. The supernatant was removed, the pellet washed with 1000 ng/µL of 70% grade ethanol and centrifuged for 1 min at

13,200 rpm. Ethanol was removed and DNA was re-suspended in 20 µL. The genomic DNA was quantified using a Qubit 2.0 Fluorometer (Invitrogen).

3A.4.2 High-throughput DNA sequencing

Amplicon libraries were constructed following the Illumina MiSeq protocol. The 16S rRNA V3–V4 region was used for the bacteria and archaea 16S rRNA gene (341F-CCTACGGGAGGCAGCAG and 907R CCGTCAATTCMTTTGAGTTT) (Archer *et al.,* 2019). The PCR thermo-cycle used was as follows: 30 cycles at 95 °C for 1 min, 55 °C for 1 min, 72 °C for 1 min, and a final extension at 72 °C for 10 min. The PCR products were purified using AMPure XP beads (Beckman-Coulter, Brea, CA, United States). The amplicons were indexed using an Illumina MiSeq protocol (Metagenomic Sequencing Library Preparation, Part # 15044223 Rev. B Illumina, San 45 Diego, CA, USA).

3A.4.3 Sequence data analysis

Bacterial Amplicon Sequence Variants (ASVs) were identified using the R package DADA2 (Callahan *et al.,* 2016). The taxonomy of the ASVs was assigned using the built-in RDP classifier in DADA2 with SILVA nr v 132 database (Quast *et al*., 2012) and sequences were clustered at 97% similarity threshold (Archer *et al.,* 2019). Diversity indices between two samplers (HVAS and LVAS) were tested using Student's *t*-test. Permutational multivariate analysis of variance (PERMANOVA) (Oksanen *et al*., 2016; Lee *et al*., 2018) was performed (Anderson *et al*., 2006) to test the effects of high flowrate and low flowrate sampler on partitioning the variance between the microbial communities. Similarities in bacterial communities between HVAS and LVAS were visualised using Principal Coordinate Analysis (PCoA).

3A.5 Results and Discussion

3A.5.1 Concentrations of PM_{10} between HVAS and LVAS

The mean 24-h concentrations of PM_{10} were higher with HVAS at urban sites (97.6 ± 43.7 µg/m^3) compared to rural sites (53.6 ± 17.13 µg/m^3) and the lowest was measured with LVAS at rural sites (45.9 ± 14.7 µg/m^3) (Figure 3A.3). The 24-h mean concentration of PM_{10} measured using

both HVAS and LVAS at the urban site exceeded the WHO's guidelines for PM_{10} (50 µg/m^3) (World Health Organization, 2006a). Higher concentrations of PM_{10} in Rwanda can be explained by the high population density and associated human activities, including the use of biomass energy sources such as wood and charcoal burning for cooking; vehicle emissions; and unpaved roads. The Wilcoxon–Mann–Whitney test showed that the mean 24-h PM_{10} concentrations were significantly higher with HVAS than LVAS at the urban site (p< 0.0001) (Duvall and Bourke, 1974; Bernstein *et al.*, 1976). However, at the rural site, the Wilcoxon–Mann–Whitney test showed no significant difference in PM_{10} concentrations measured with HVAS and LVAS (p = 0.1256). Figure 3A.4 provides a time series of the mean 24-h concentrations of PM_{10} measured at each sampling period, along with meteorological conditions such as temperature and relative humidity (RH) for both air samplers at the urban and rural sites. Meteorological parameters such temperature and RH recorded during each sampling period represented general conditions during the sampling period at both urban and rural sites (Figure 3A.4), suggesting that meteorological conditions did not significantly change during the whole sampling period; thus they are likely to have had minimal or no influence on the PM_{10} concentrations measured at both sites.

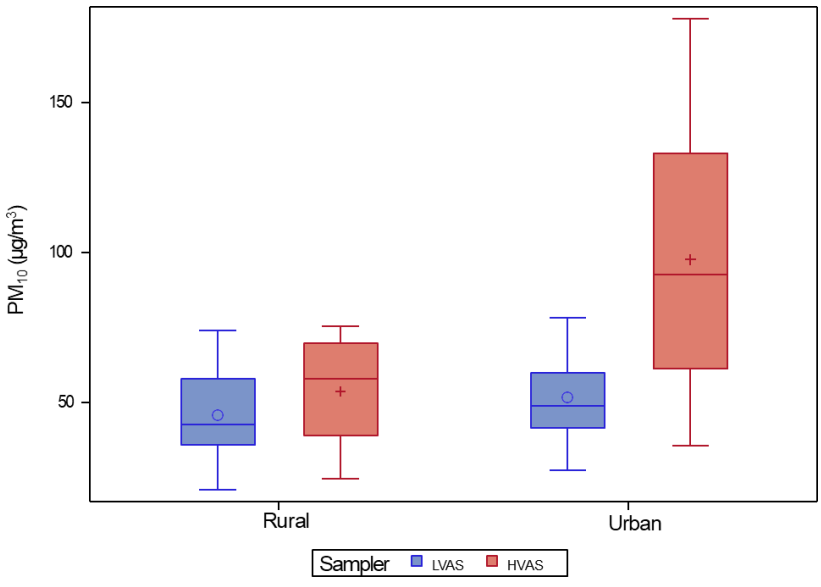

Figure 3A. 3. The mean 24-h concentrations of PM_{10} measured at urban and rural sites using a high-volume air sampler (HVAS) and a low volume air sampler (LVAS).

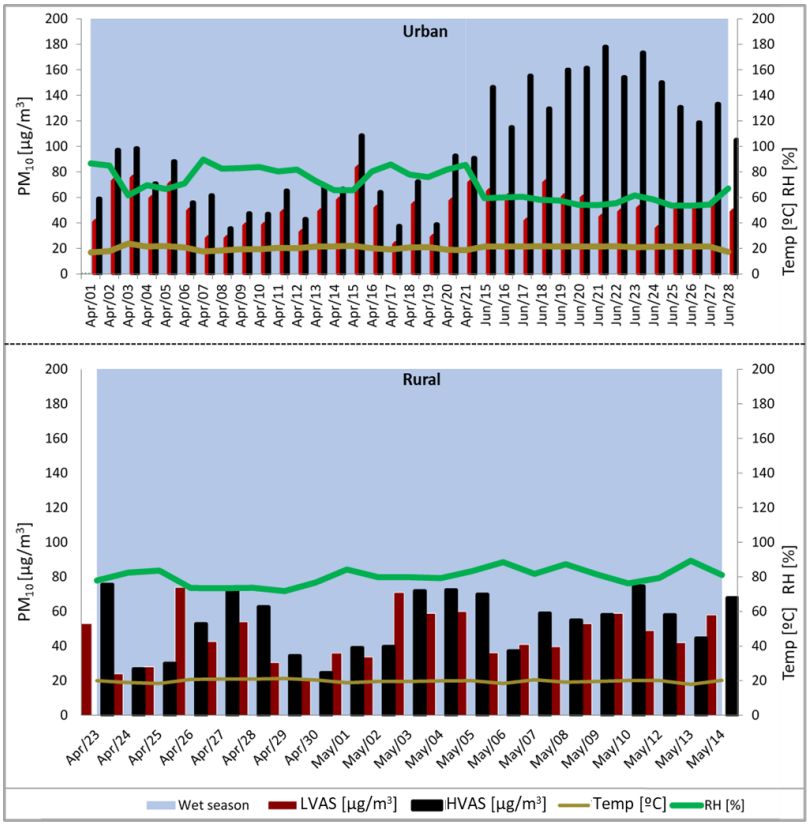

Figure 3A. 4.The mean 24-h concentrations of PM_{10} measured at the urban and rural sites using a high-volume air sampler (HVAS) and a low volume air sampler (LVAS).

3A.5.2 Atmospheric concentrations of total PAHs and NPAHs

The means of ∑15 PAHs analysed from PM_{10} were 22.90 ng/m^3 and 20.6 ng/m^3 for HVAS and LVAS respectively, while the mean of ∑5 NPAHs was 305.11 pg/m^3 with HVAS and 244.20 pg/m^3 with LVAS (Table 3A.1).

Table 3A. 1. The total PAH and NPAH concentrations (mean ± SD) from PM10, from high and low air volume air samplers at the urban site.

Analytes	HVAS (n=7)	LVAS (n=7)	*p*-value
PAH [ng/m^3]	22.90 ± 6.31	20.6 ± 11.37	0.7077
NPAH [pg/m^3]	305.11 ± 213.55	244.20 ± 171.67	0.6178

The Wilcoxon signed-rank test showed that the mean concentrations of PAHs and NPAHs from HVAS and LVAS were not statistically different (Table 3A.1). This may be due to the high degradation or volatilization of PAHs and filter face velocities between the two samplers. Despite the relatively high airflow rates and large filter area size cartridge for HVAS, the PAH and NPAH concentrations were not significantly different compared to those obtained from the LVAS with

a lower flowrate and a small filter area size. Previous studies have showed that the volatility of PAH compounds in the gas phase makes high volume (high flow rate) methods less efficient in sampling for these compounds compared to lower volume (lower flow rate) systems (Ward and Smith, 2004). Further, the volatilization of organic compounds detected in particulates is most likely dependent on a combination of temperature and filter face velocities due to flow rate, but is not a strong function of sampling time (Ward and Smith, 2004). Van Vaeck *et al.* (1984) demonstrated a loss of volatile organic compounds during high-volume particulate sampling with fiber filters, resulting in a loss of mass. Schwartz *et al.* (1981) showed that the concentrations of compounds with a lower molecular weight were more volatile and likely to show significant losses during long sampling periods. Another study showed that the measurement of organic carbon from LVAS sampling yielded higher concentrations compared to HVAS (US EPA 2001). In agreement, Delgado-Saborit *et al*. (2010) showed a successful determination of particle-bound PAH concentrations using LVAS without interference in low-volume samples.

The dominant PAHs in this study in both samplers were BPe, Flu, and BbF, accounting for 26%, 20% and 12% in HVAS and 22%, 20% and 12% in LVAS, respectively, of the total compounds detected in each sampling machine (Figure 3A.5). These compounds have been previously observed in East African and East Asian countries subject to vehicle emissions (Muendo *et al.,* 2006; Yang *et al.,* 2007; Arinaitwe *et al.,* 2012; Hayakawa, 2018a).

Higher concentrations of the predominant NPAHs in this study were found in LVAS compared to HVAS. 9-NA, 2-NP + 2-NFR were the predominant NPAHs, accounting for 60% and 35% with LVAS and 48% and 30% with HVAS of the total compounds detected at each sampler (Figure 3A.5). The LVAS was more efficient in detecting NPAHs, which exist at concentrations orders of magnitude lower than PAHs. This is due to saturation of sampled particulates on small filter area sizes compared to larger filter area sizes, which are likely to show significant losses during long sampling periods (Peltonen and Kuljukka, 1995). LVAS samples (small filter) require small solvent volumes during the extraction process compared to HVAS (large filter), which requires subsequent concentration, increasing the risk of losing analytes by evaporation in the

concentration steps, especially for NPAHs that exist at very low concentrations compared to their parent PAHs.

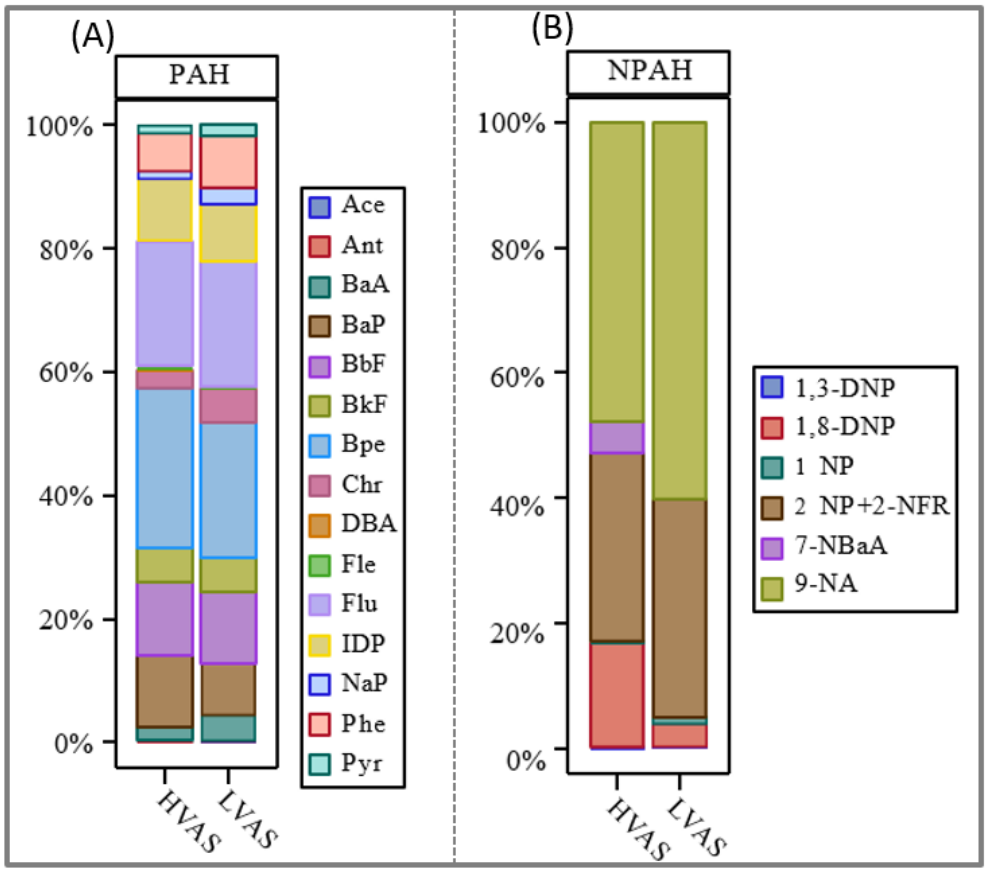

Figure 3A. 5. Percentage composition of PAHs (A) and NPAHs (B) detected in PM_{10} samples with a high-volume air sampler (HVAS) and a low volume air sampler (LVAS) at Kigali urban site, Rwanda.

3A.5.3 DNA and microbial community composition

The genomic DNA detected from PM_{10} samples with both HVAS and LVAS samplers was quantified using the Qubit Fluorometer. The results indicated that LVAS had a higher average DNA concentration (1.8 ng/µL) than HVAS (0.2 ng/µL) and that the concentration of DNA increased with increasing concentration of PM_{10} sampled with LVAS (Figure 3A.6).

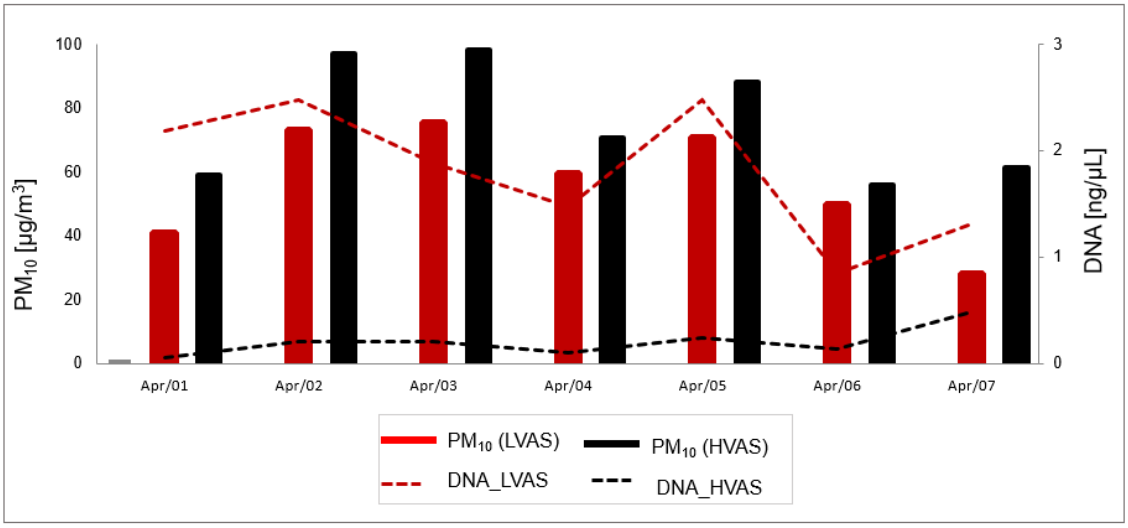

Figure 3A. 6. Variation in DNA concentrations (ng/µL) and PM_{10} concentrations (µg/m^3) detected with high volume air sampler (HVAS) and low volume air sampler (LVAS) at an urban site in Rwanda.

3A.5.4 Species richness, community diversity and microbial community composition

The bacteria species richness (as estimated by the Chao1 index) was higher LVAS than HVAS samples (Figure 3A.7). Bacteria community compositions were more in samples from HVAS compared to LVAS (as estimated by the Shannon index) (Figure 3A.7). However, the results from t-test showed that there were no statistically significant of species richness (*p*-value = 0.6146) and diversity (*p*-value = 0.7212). These results suggest that the difference in flowrate has no much effect on species richness and diversity when both samplers run simultaneously.

In this study, difference between the communities between the both samplers were observed Dominant bacterial phyla observed in both samplers were Actinobacteria, Proteobacteria, Firmicutes, Chloroflexi, Bacteroidetes, and Cyanobacteria (Figure 3A.7). Proteobacteria were more abundant in LVAS while Actinobacteria were more abundant in HVAS. In class of Proteobacteria (Figure 3A.7), Alphaproteobacteria was most abundant taxa identified in LVAS. These phyla and class detected from both samplers are commonly found in most atmospheric studies outdoor air (Woo *et al*., 2013; Bowers *et al*., 2011a; Bowers *et al*., 2013; Cao *et al*., 2014; Gao *et al*., 2017). A greater abundance of Actinobacteria, tax that usually found in soil and dust sample was most dominant in HVAS. Rwanda has dust roads and due to high flowrate and large filter size of HVAS could lead to the collection of more generated dust-associated bacteria atmosphere compare to LVAS. Data for principal coordination analysis (PCoA) was rarefied to

6499 reads and PCoA results indicated that bacterial communities exhibited distinct communities between HVAS and LVAS (PERMANOVA, $p < 0.033$) (Figure 3A.8). These results suggested that both samplers have different distinctive communities. However, HVAS communities resemble each other more than LVAS samples.

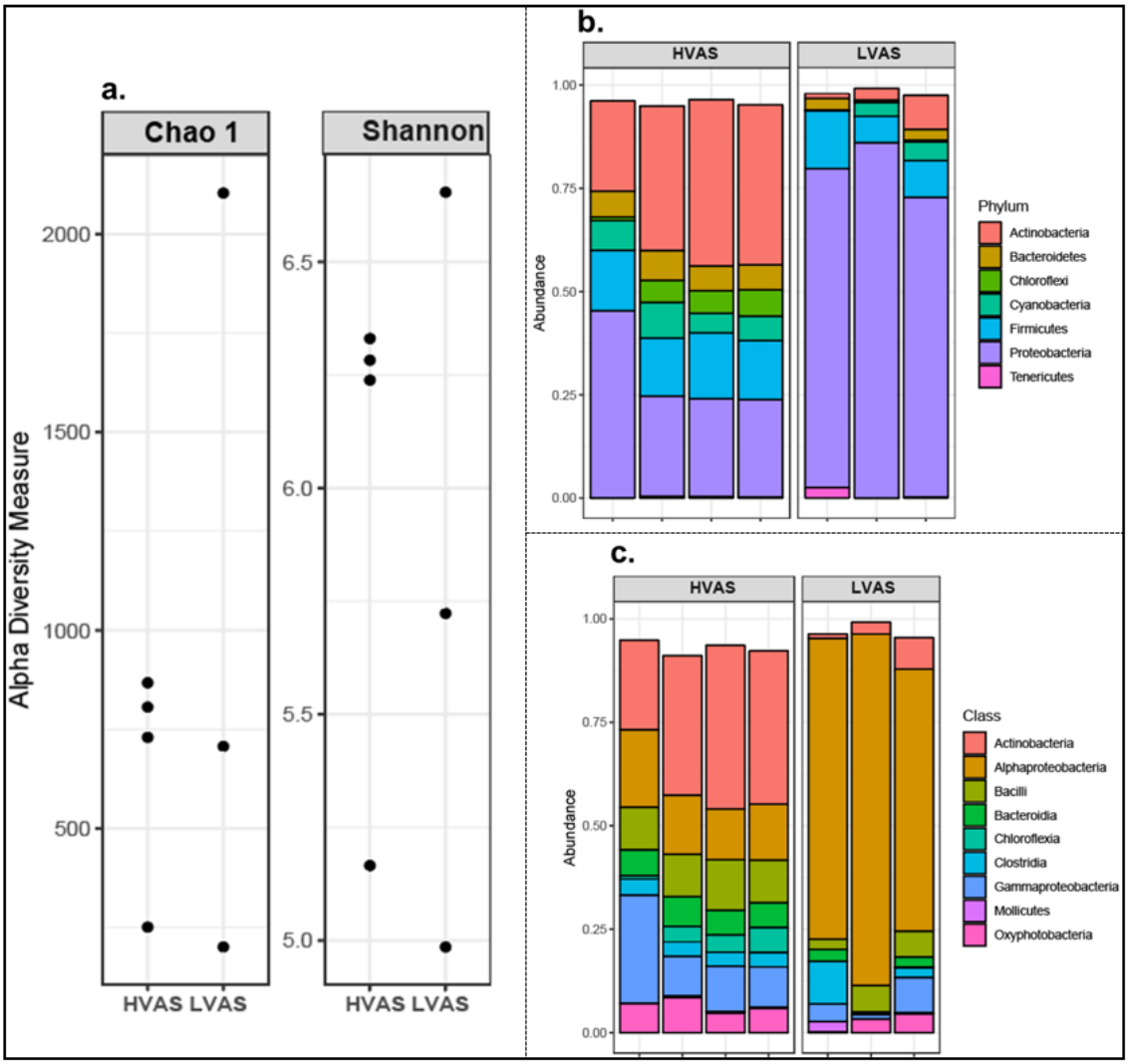

Figure 3A. 7. Characteristics of the collected PM_{10} samples and sequenced metagenomes; (a) Estimated average alpha diversity of the PM_{10} samples detected from high volume air sampler (HVAS) and low volume air sampler (LVAS); (b) Relative abundance of different bacteria species at phylum level and (c) at class level detected in PM_{10} from HVAS and LVAS.

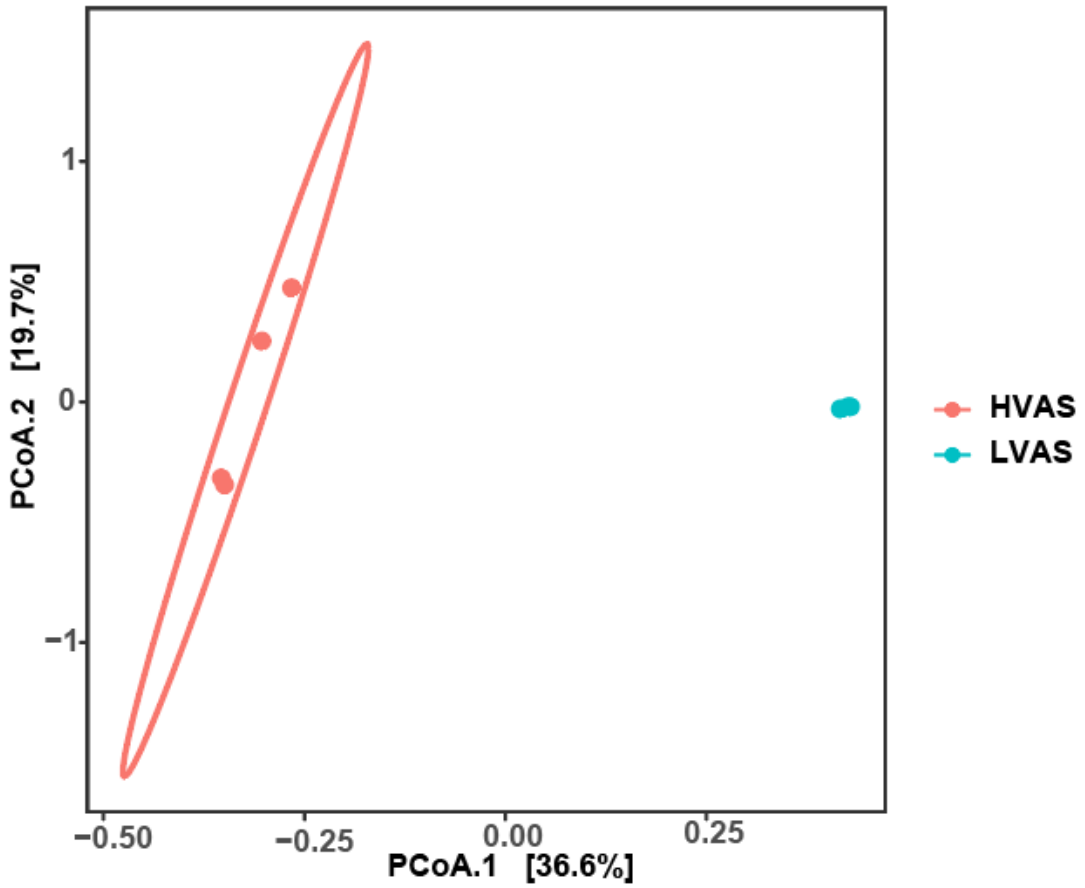

Figure 3A. 8. Principal coordination analysis (PCoA) of the Bray-Curtis dissimilarities of the bacterial relative abundance from PM_{10} samples collected from high volume air sampler (HVAS) and small volume air sampler (SVAS) at urban area in Rwanda.

3A.6 Conclusion

This study was the first investigation in Rwanda to compare HVAS and LVAS sampling systems operating in conjunction to investigate chemical (PAHs and NPAHs) and biological (bacteria) abundance and speciation associated with airborne PM_{10}. The results suggested that there was no significant difference in the concentration of PAHs and NPAHs for chemical components analysed when using the HVAS sampler at 1000 L/min as compared to the LVAS sampler at 85 L/min. However, a discrepancy was observed for biological analysis (bacteria). For example, a higher DNA yield was found in samples collected with LVAS compared to HVAS and both samplers have different distinctive bacteria communities. There was not difference in species richness and diversity between both samplers. Both samplers have successfully determined particle-bound PAH and NPAH concentrations and bacterial communities. Further study with long-term seasonal monitoring is needed to better understand the relationship between HVAS and

LVAS and the recovery of chemical and biological compositions associated with airborne particulates.

Chapter 3B - Effect of Sampling Duration and Filter Sample Size on the Measurement of $PM_{2.5}$ and PM_{10} Particulate-bound PAHs and NPAHs

3B.1 Introduction

Polycyclic aromatic hydrocarbons (PAHs) and their nitro-derivatives (NPAHs) are ubiquitous environmental organic pollutants, which are well known for their carcinogenic and mutagenic properties (Nisbet and LaGoy, 1992; Abdel-Shafy and Mansour, 2015). They are absorbed onto $PM_{2.5}$ (particulate matter with an aerodynamic diameter less than 2.5 μm), which can easily enter deep into the lungs and adversely affect the respiratory, central nervous and cardiovascular systems, and PM_{10} (particulate matter with aerodynamic diameter less than 10 μm), which is trapped in the nasopharyngeal tissues (Falcon-Rodriguez *et al.,* 2016). Substantial research has been focused on the characterization of organic aerosols, particularly Benzo(a)pyrene (BaP) as a marker for carcinogenic risk levels due to its recognized properties as an epidemiological health hazard (Abdel-Shafy and Mansour, 2015). The atmospheric standard for BaP is already set in New Zealand at 0.3 ng/m^3 as an annual guideline (Ministry for the Environment and Statistics New Zealand, 2015). However, there is little data on PAHs and NPAHs and health-related problems in New Zealand.

The characterization of PAHs and NPAHs in atmospheric particulates generally involves collecting particulate matter (PM) on a filter, followed by an extraction process (Hayakawa, 2018a; Kalisa *et al.,* 2018a, Kalisa *et al.,* 2019b). However, the accuracy of characterizing PAHs and NPAHs composition depends on the sampling procedure and extraction efficiency. Sampling duration should be long enough that the total PM collected is within the range of the analytical technique (Jutze and Foster, 1967).

There has been very little attention given to the influence of sampling duration on the measured concentrations of PAHs and NPAHs of ambient PM. Previous investigations have used different sampling durations including 23 hours (Chuesaard *et al.,* 2014), 24 hours (Hayakawa *et al.,* 1995), 48 hours (Abdallah and Atia, 2014), and 7 days (Brown and Brown, 2013) resulting in data from these studies often being difficult to compare. It has been reported that PM resistibility, sampling duration and flow rate are the most important factors affecting PM collection for organic compound analysis (Macher and Willeke, 1992); however, this has not been systematically tested. Another major knowledge gap is how much filter area is required to gain an accurate

representation of the PAH and NPAH profiles present at the time of sampling. Extraction from large filters involves the use of large volumes of toxic organic solvents and long extraction times, which is more costly and more environmentally damaging (Giergielewicz-Mozajska *et al.,* 2001). Several analyses of PAHs and NPAHs from sample filters have used different filter portion sizes for extractions, such as $5mm^2$ (Chondo *et al.,* 2013), a quarter of the total sample filter (Hayakawa *et al.,* 2014; Irei, 2016), a half of the total sample filter (Ramos De Rainho *et al.,* 2013; Xu, 2016), $10cm^2$ (Hayakawa *et al.,* 1995) and 3.14 cm^2 (Mohanraj, Solaraj and Dhanakumar, 2011) of the total sample filter. This investigation aimed to determine: (1) whether there is any relationship between the duration of ambient air sampling and the atmospheric concentrations detected of extractable PAHs and NPAHs; and (2) how much filter is necessary to gain results representative of the entire filter at different sampling durations. The study applied three sampling durations (24 hours, 5 days and 7 days) and carried out airborne PM extraction using different filter areas (half, a quarter and an eighth) cut sequentially from $PM_{2.5}$ and PM_{10} sample filters commonly used in air pollution studies (Bravo *et al.,* 2012).

3B.2 Material and methods

3B.2.1 Particulate matter collection

The sampling site was located in the Central Business District of Auckland, New Zealand, on the rooftop of a building at the Auckland University of Technology. Details of the sampling site characteristics were summarized in our previous study (Kalisa *et al.*, 2019b). The sampling period was from September to October 2016 during a period of time when temperature and relative humidity (Table 3B.1) and emission sources did not vary significantly, providing the most comparable samples practical. Each 24-hour, 5-day and 7-day sample of $PM_{2.5}$ and PM_{10} was collected using 20.3 cm × 25.4 cm glass fiber filters ($PM_{2.5}$), and 12.6 cm × 16.6 cm GFFs (PM_{10}) (GFF, 2500 QAT-UP, Pallflex Products, CT, USA). A high-volume air sampler (Sibata Scientific Technology Limited, Saitama, Japan) was used, operating at a flow rate of 1,000 litres per minute (1000L/min). All filters were sterilized, and tested blanks showed negligible organic background. The study involved the use of different $PM_{2.5}$ and PM_{10} filter size fractions for extraction (an eighth (1/8), a quarter (1/4) and a half (1/2)) for $PM_{2.5}$ filters and for PM_{10} filters. The total air volume filtered was 1,440 m^3, 7,200 m^3 and 10,080 m^3 for each sampling duration of 24 hours, 5 and 7 days, respectively.

3B.2.2 Analysis of PAHs and NPAHs

The US EPA 610 PAH mixture, which was used as a standard, contained 16 PAHs including: naphthalene (NaP), acenaphthene (Ace), acenaphthylene (Acyl), fluorene (Fle), phenanthrene (Phe), anthracene (Ant), fluoranthene (Flu), pyrene (Pyr), benz[a]anthracene (BaA), chrysene (Chr), benzo[b]fluoranthene (BbF), benzo[k]fluoranthene (BkF), benzo[a]pyrene (BaP), dibenz[a,h]anthracene (DBA), benzo[ghi]perylene (BPe), and indeno [1,2,3-cd] pyrene (IDP), and was purchased from Sigma-Aldrich (St. Louis, MO, USA). For NPAHs, our standard contained 9-nitroanthracene (9-NA), 2-nitropyrene (2-NP); 2- nitrofluoranthene (2-NFR), 1-nitropyrene (1-NP), 6-nitrochrysene (6-NC), 7- nitrobenz[a]anthracene (7-NBaA) and 6-nitrobenzo[a]pyrene (6-NBaP). These components were obtained from Chiron (Trondheim, Norway). Five deuterated internal standards for PAHs (naphthalene-d_8, acenaphthylene-d_{10}, phenanthrene-d_{10}, chrysene-d_{12}, and perylene-d_{12}) were purchased from Wako Chemical (Osaka, Japan). 2-Fluoro-7-nitrofluorene (FNF) was purchased from Sigma Aldrich (Milwaukee, WI,

U.S.A.) and was used as an internal standard for NPAH analysis. All analytes were dissolved in ethanol obtained from Kanto Chemical (Tokyo, Japan).

The extraction, standards, and instrumental analyses have been described in our previous studies (Kalisa *et al.*, 2018a, Kalisa *et al.*, 2019b). Briefly, the full-sized $PM_{2.5}$ and PM_{10} filters sampled for 24 hours, 5 days, and 7 days were divided into small pieces and placed in flasks. The volumes of solvent and water used during extraction were proportional to the area size of the extracted filter. The eighth, quarter and half size filters were each sonicated twice with respectively 40 mL, 60 mL and 80 mL volumes of benzene-ethanol (3:1, v/v). These extracts were subsequently filtered to remove filter debris. The extract was cleaned with 5% (w/v) sodium hydroxide, followed by 20% (w/v) sulfuric acid, and twice with ultrapure water using a volume proportional to the area size of the extracted filter. Dimethyl sulfoxide (DMSO) was then added, at a volume of 100 µL. The benzene was evaporated using a rotary evaporator. The remaining DMSO was dissolved in ethanol (900 µL) and the solution was filtered through a membrane filter (HLC-DISK 3, 0.45 µm, Kanto Chemical Co., Inc., Tokyo, Japan).

A 20 µL aliquot (injection volume) of the filtered solution was analysed for 14 individual PAHs (the US EPA 610 PAH mixture of 16 PAHs) except Acyl, which does not fluoresce. Nine individual NPAHs (2-NP and 2-NFR were grouped because they co-eluted) (Kalisa *et al.*, 2018a) were quantified by injecting 20 µL of the filtered solution into the HPLC system. Analysis was performed using two high-performance liquid chromatographic systems (HPLC-10A series, Shimadzu Inc., Kyoto, Japan), with PAHs being analyzed using a fluorescence detector and NPAHs analyzed using a chemiluminescence detector.

3B.3 Results and Discussion

3B.3.1 Sampling duration

The possibility of volatilization of PAHs during sampling has given rise to a number of investigations to determine the effect of sampling conditions on the collection of particulate organic matter. In this study, the concentration of $PM_{2.5}$- and PM_{10}-bound total PAHs and NPAHs decreased as sampling duration increased (Table 3B.2). The total concentration of PAHs detected in $PM_{2.5}$ and PM_{10} during 24-hour sampling (177.6 pg/m^3 and 191.3 pg/m^3, respectively) greatly exceeded the 5-day sampling (45.8 pg/m^3 and 52.8 pg/m^3) and the 7-day sampling (45.3 pg/m^3 and 57.3 pg/m^3), suggesting PAH degradation and/or volatilization from the filter as the sampling duration increased. The mean concentrations of ΣNPAHs exhibited similar trends to PAHs, with higher concentrations in the 24-hour samples for both $PM_{2.5}$ and PM_{10} (12.4 pg/m^3 and 12.8 pg/m^3) than in 7-day (9.7 pg/m^3 and 10.1 pg/m^3) and 5-day (5.1 pg/m^3 and 5.4 pg/m^3) sampling durations (Table 3B.2). Results from this study agree with Schwartz *et al.* (1981) and König *et al.* (1980) that increasing the sampling duration beyond 24 hours has the potential to decrease concentrations of PAHs and NPAHs, and thus decrease the accuracy of the results. Additionally, there was no difference in the mass of particulates sampled after 24 hours of sampling in both $PM_{2.5}$ and PM_{10} (Table 3B.2) indicating the filters were likely saturated and reached equilibrium at this point.

Table 3B. 1. Meteorological parameters [Mean ± SD] recorded from September to October 2016 and volume of air collected over 24 hours, 5 days and 7 days.

	Temperature (°C)	**RH (%)**	**Wind speed (km/h)**
24 Hours	13.8 ± 0.7	84.7 ± 2.6	9.9 ± 3.2
5 Days	15.0 ± 0.5	88.7 ± 3.7	9.6 ± 2.6
7 Days	12.8 ± 2	79.3 ± 8.8	12.95 ± 7.8

Table 3B. 2. Total PAH and NPAH mean concentrations and average mass detected in $PM_{2.5}$ and PM_{10} during 24-hour, 5-day and 7-day sampling periods.

PM size	**Parameters**	**24 Hours**	**5 Days**	**7 Days**
$PM_{2.5}$	PAH [pg/m^3]	177.6 ± 133.1	45.8 ± 34.3	45.3 ± 22.6
	NPAH [pg/m^3]	12.4 ± 8.4	5.1 ± 4.01	9.7 ± 3.5
	Mass [g]	891	953.7	959.4
PM_{10}	PAH [pg/m^3]	191.3 ± 142.3	52.8 ± 40.6	57.3 ± 31.6
	NPAH [pg/m^3]	12.8 ± 8.7	5.4 ± 4.1	10.1 ± 3.8
	Mass [g]	719.4	759.4	723.9

Diagnostic analysis of PAH to NPAH concentration ratios are used in source identification based on their combustion temperatures (Kalisa *et al.,* 2018a, Kalisa *et al*., 2019b). NPAHs are formed directly from incomplete combustion of organic materials such as diesel and gasoline. However, NPAHs can be also formed secondarily in heterogeneous reactions of PAHs and NPAHs and atmospheric oxidants such as nitrate radicals (Arey *et al.,* 1986; Liu *et al.,* 2017). In this study, the NPAH to PAH isomers composition concentration ratio was used to investigate secondary nitration during the sampling period. Supposing that emissions of PAHs and NPAHs from different sources were constant over 7 days, the comparison of the concentration ratios of PAHs to corresponding NPAHs isomers (for example [1-NP]/[Pyr], [7-NBaA]/[BaA], [6-NC]/[Chr] and [6-NBaP]/[BaP]) were investigated (Table 3B.3). The results indicated that all ratios of these compounds increased with increasing sampling duration, suggesting secondary nitration with longer sampling duration (photochemical pathways). In addition, [2-NP+2-NFR]/[1-NP] was used to examine possible secondary formation vs primary formation (Kalisa *et al*., 2018a). A [2-

NP+2-NFR]/[1-NP]value > 5 indicates strong contributions from secondary formation while a value < 5 indicates a strong contribution from primary emissions periods, suggesting that increasing sampling duration may influence the secondary formation of NPAHs.

Table 3B. 3. Diagnostic ratios of NPAH to PAH detected in $PM_{2.5}$ and PM_{10} for each sampling duration.

PM size	Duration	A	B	C	D	E
	24hours	0.14	0.06	0.10	0.00	4.97
$PM_{2.5}$	5 days	0.15	0.03	0.25	0.00	2.77
	7 days	0.18	0.56	0.32	0.01	7.49
	24hours	0.13	0.05	0.10	0.00	4.79
PM_{10}	5 days	0.14	0.02	0.22	0.00	2.74
	7 days	0.16	0.41	0.25	0.03	7.36

A: [1-NP]/[Pyr], B: [7-NBaA]/[BaA], C: [6-NC]/[Chr], D: [6-NBaP]/[BaP]), E: [2-NP+2-NFR]/[1-NP].

3B.3.2 Filter area size extraction

Previous studies have used different filter area sizes to extract PAHs and NPAHs. However, a systematic evaluation determining which size is adequate to reflect the total amount of the compounds in the filter had not been completed prior to this study. The results indicated that the concentrations of PAHs and NPAHs detected at all durations increased with increasing filter area size (one eighth <quarter < half) for both $PM_{2.5}$ and PM_{10} filters (Table 3B.4). The highest PAH concentration was detected using half of the filter area, sampled for 24 hours (Table 3B.5). Extraction of the whole filter has two main drawbacks: the use of more toxic organic solvents, long extraction times (Giergielewicz-Mozajska *et al.*, 2001) and more importantly the loss of the entire sample so that no archive can be kept for future analysis and insurance if there is a difficulty with the analysis.

The total concentrations of NPAHs exhibited similar trends to the PAHs, with higher concentrations detected in the half area size filter over 24-hour sampling, followed by 5 days and 7 days in $PM_{2.5}$ samples and in PM_{10} samples (Table 3B.4). Although the concentrations of PAHs

and NPAHs were higher in the half size filter than in the quarter or eighth filter area size, a Kruskal-Wallis test showed no significant differences in the concentrations obtained across all filter area sizes sampled over 24 hours, 5 days and 7 days (Table 3B.5), suggesting that the PAHs and NPAHs were homogeneously distributed across the entire filter. Table 3B.2 shows that there were no large differences in the mass of particulates collected over 24 hours, 5 days and 7 days. This suggests that under controlled sampling conditions, the distribution of airborne PM on the filter may be homogeneous and more likely to reach equilibrium due to the relatively long sampling duration (Collins *et al.*, 1998). A possible explanation for this phenomenon is that once equilibrium (saturation) has been reached, the airborne particulate mass on the filter stops increasing and remains constant with increasing sampling time; therefore a small filter area size can be extracted and still reflect the whole filter (Subramanian *et al.*, 2004). These results suggested that particulates sampled using HVAS are uniformly distributed over the filter. Therefore, optimizing the fraction of the filter area size generally requires at least half of the whole filter area size. A complete deposition of PAHs and NPAHs on the filter is generally assumed when any size of sample filter is extracted (Tomingas, 1979). Any filter extraction method needs to extract a representative sample, meaning that the sample reflects the diversity or composition of the total components. In this study, we found that most PAHs with lower molecular weights (2, 3 and 4 PAH rings) decreased as sampling duration increased, compared with PAHs with higher molecular weights (PAHs with 5 and 6 rings).

Table 3B. 4. Concentrations of PAHs, and NPAHs in $PM_{2.5}$ and PM_{10} detected in each filter area size and sampling duration.

Duration	**24 Hours**			**5 Days**			**7 Days**		
PAH [pg/m^3]	Eighth	Quarter	Half	Eighth	Quarter	Half	Eighth	Quarter	Half
NaP	0.5 (0.5)	0.5 (1.3)	2.3 (3)	0.3 (0.4)	0.5 (0.7)	1.2 (1.7)	0.3 (0.4)	0.1 (0.3)	0.3 (0.4)
Fle	1(1.2)	2.1(2.5)	6.1(6.8)	0.2 (0.4)	0.3 (0.4)	1.1 (1.7)	0.4 (0.5)	0.3 (0.3)	0.6 (1.0)
Phe	1.8 (1.9)	1.2 (1.6)	2.2 (2.8)	0.5 (0.5)	1.2 (1.3)	1.6 (1.8)	1(1.2)	0.4 (0.5)	2.4 (3.4)
Ant	0.6 (0.7)	1.1(1.3)	2.5 (2.9)	0.1 (0.1)	0.7 (0.8)	0.8 (0.11)	0.5 (0.6)	0.4 (0.5)	1(1.3)
Flu	1.5 (1.9)	3 (3.6)	6.6 (7.5)	0.7 (0.8)	1.5 (1.9)	3.7 (4.8)	0.7 (1)	ND (0.3)	2.4 (3.3)
Pyr	5.5 (6.5)	11.9 (13.5)	29.7 (32.6)	3 (3.3)	5.7 (6.5)	12.8 (15)	2.9 (3.5)	2.7 (3.3)	8.2 (10.2)
BaA	3.9 (3.4)	8.3 (9.2)	21.7 (23.5)	1.3 91.5)	2.6 (2.9)	5.9 (6.7)	0.9 (1.3)	1.3(1.8)	3.4(4.6)
Chr	6.1 (6.5)	12.8 (14.2)	32 (34.4)	1.9 (2.1)	3.9 (4.5)	8.7 (10.2)	1.4 (2)	2.1(2.8)	5.5 (7.6)
BbF	13.4 (13.5)	29.7 (30.6)	60.3 (64.7)	3.3 (3.5)	6.6 (7)	15.8 (16.8)	4.1(5.2)	4.3 (5.3)	8.8 (12.4)
BkF	5.3 (5.8)	11.7 (12)	23.4 (25)	1.0 (1.0)	2.1 (2.2)	5.1 (5.4)	1.5 (1.9)	1.4 (1.8)	ND (1.4)
BaP	2.3 (2.4)	8 (8.3)	21.4 (21.6)	ND (0)	0.6 (0.11)	2.3 (2.3)	1.6 (2.2)	ND (0.3)	7.8 (8.6)
DBA	1.3 (2.9)	2.6 (3.5)	5.4 (6.0)	ND (0.1)	ND (0.1)	ND (0)	0.6 (0.9)	ND (0.2)	0.2 (10
BPe	11.3 (11.9)	40.4 (41.6)	82.7 (87.1)	3 (3.0)	7.7 (9.0)	18 (21.3)	9.7 (11.3)	15.6 (17.1)	18.1(23.9)
IDP	7.1 (7.7)	14.9 (15.6)	26.7 (29.6)	1.6 (1.8)	3.3 (3.8)	6.8 (7.8)	3.8 94.5)	6.7 (7.3)	12.6 (14.6)
Σ14PAHs	**61.6 (67.6)**	**148.2 (158.8)**	**323 (347)**	**16.9 (18.5)**	**36.7 (42.2)**	**83.8 (97.7)**	**29.4 (36.5)**	**35.3 (41.8)**	**71.3 (93.7)**
				NPAH [pg/m^3]					
1,3-DNP	0.04 (0.06)	0.08 (0.11)	0.17 (0.25)	0.02 (0.04)	0.03 (0.1)	0.1(0.7)	0.03 (0.04)	0.05 (0.05)	0.07 (0.07)
1,6-DNP	1.33 (1.36)	2.74 (2.78)	5.42 (5.61)	0.66 (0.69)	0.88(0.92)	2.21(2.28)	2.47 (2.55)	2.6 (2.69)	2.56 (2.86)

1,8-DNP	0.1 (0.12)	0.16(0.18)	0.4 (0.45)	0.1(0.13)	0.16 (0.17)	0.43 (0.43)	0.05 (0.06)	0.33 (0.34)	0.35 (0.36)
1-NP	0.84 (0.87)	1.67 (1.73)	3.57 (3.7)	0.5 (0.52)	0.7 (0.75)	2.04 (2.11)	0.41(0.43)	0.72 (0.74)	1.1(1.2)
2-NP+2-NFR	1.56 (1.56)	2.74 (2.74)	5.22 (5.22)	0.42 (0.420	0.49 (0.49)	2.49 (2.68)	1.12 (1.12)	1.79 (1.87)	2.52 (2.74)
6-NBaP	0.01 (0.01)	ND (ND)	ND (0.01)	0.01(0.01)	ND (ND)	ND (0)	ND (0)	0.02 (0.02)	ND (ND)
6-NC	0.6 (0.6)	1.33 (1.33)	3.36 (3.52)	0.57 (0.57)	0.8 (0.85)	2.08 (2.08)	0.48 (0.51)	0.87 (0.92)	1.13 (1.21)
7-NBaA	0.26 (0.26)	0.5 (0.5)	0.94 (1.02)	0.06 (0.06)	0.07 (0.07)	0.05 (0.05)	0.58 (0.6)	0.96 (0.98)	1.04 (1.09)
9-NA	0.54 (0.62)	1.07 (1.1)	2.71 (2.75)	0.08 (0.09)	0.13 (0.14)	0.36 (0.37)	0.98 (1.02)	2.34 (2.37)	4.5 (4.57)
Σ9NPAHs	**5.3 (5.5)**	**10.3 (10.5)**	**21.8 (22.5)**	**2.42 (2.54)**	**3.25 (3.48)**	**9.7 (10.2)**	**6.13 (6.34)**	**9.68 (10.0)**	**13.3 (14.1)**

15PAHs = Nap+ Ace+ Fle+ Phe + Ant + Flu+ Pyr + BaA + Chr + BbF + BkF + BaP+DBA+ BPe + IDP. Σ-7NPAH = 1,8-DNP, 1.3-DNP, 9-NA + 2NP + 2NFR +1-NP +7-NBa+ 6-NBaP, Each data represents mean. ND, not detected, number in () referrer to PM_{10}

Table 3B. 5. Comparison of total PAH and NPAH concentrations detected in one half, one quarter and one eighth of total filter areas during all sampling durations (24 hours, 5 days and 7 days) using the Kruskal-Wallis test.

PM size	**Analyses**	**Half**	**Quater**	**Eighth**	***p*- value**
$PM_{2.5}$	PAH [pg/m^3]	159.3 ± 141.8	73.4 ± 64.7	35.9 ± 23.0	0.3064
	NPAH [pg/m^3]	14.9 ± 6.1	7.7 ± 3.8	4.6 ± 1.9	0.0661
PM_{10}	PAH [pg/m^3]	179.6 ± 145	80.9 ± 67.4	40.86 ± 24.8	0.2527
	NPAH [pg/m^3]	15.8 ± 6.3	7.8 ± 3.9	4.7 ± 1.98	0.059

BPe, BbF, Chr, Pyr and IDP were the most dominant PAHs found in this study (Table 3B.4), while 2-NP +2-NFR, 1,6 DNPs, 9-NA and 1-NP were the most dominant NPAHs detected in all sampling durations, with high concentration detected during 24-hour sampling (Table 3B.4). Higher losses of PAH compounds such as NaP, Fle, Flu, Chr and DBA were observed as the sampling duration increased, particularly during 7-day sampling. The NPAHs concentrations of 1-NP, 7-NBaA, 6-NC and 6-NBaP decreased with increasing sampling duration. These NPAH species are emitted mainly from combustion sources; they may be secondarily formed on the filter in the presence of NOx and corresponding PAHs. These results suggest that a shorter sampling period is more suitable to avoid the secondary formation of some NPAH compounds. Although there was a difference in the range of individual PAH and NPAH concentrations, the total PAH and NPAH profiles exhibited a relatively uniform distribution between 5-day and 7-day sampling durations compared with 24-hour sampling. PAH compounds (BPe and IDP) showed fewer losses and remained constant with long sampling duration. Losses of PAHs can be due to their reaction with other environmental pollutants during long sampling periods. For example, BaP deposited on a filter was reported to undergo chemical reactions with ozone and nitric acid, with losses as high as 85% (Peltonen and Kuljukka, 1995). Some studies noticed degradation after 2 hours' sampling (Abdallah and Atia, 2014), while other studies recorded significant losses only when samples were exposed for longer than 24 hours (e.g. loss of BaP was observed) (Brown and Brown, 2013).

3B.4 Conclusion

This study aimed to provide a thorough investigation of how sampling duration and filter area size affect the concentrations of extractable PAHs and NPAHs. The concentrations of PAHs and NPAHs decreased with increasing sampling duration. A sampling duration of 24 hours was shown to be the most effective for air pollution studies of PM when assessing PAHs and NPAHs. Losses of PAHs and NPAHs were observed as the sampling duration increased. We found that a large sampling area (at least half of the whole filter) was required for 24-hour samples for extraction of PAHs and NPAHs. The shorter sampling period is more accurate in determining PAHs with low molecular weight and NPAH concentrations in samples as there are some compounds known to volatilize or degrade easily and others are the products of secondary formation. A 24-hour sampling duration is advantageous, as it allows higher temporal resolution and shorter installations when a temporary sampling campaign is being done where equipment is not usually available, allowing greater spatial resolution for a machine. Further study, with long-term seasonal monitoring, is needed to better understand the relationship between sampling duration and the recovery of PAHs and NPAHs extracted from airborne particulates.

Chapter 3C - Efficiency of Sampling Duration for DNA-Based Analysis of Bioaerosols

3C.1 Introduction

Over the past few decades, interest in bioaerosol research has grown rapidly due to the discovery of significant impacts of bioaerosols on human health and in atmospheric events such as cloud formation, precipitation, and atmospheric chemistry (Després *et al.*, 2012; Fröhlich-Nowoisky *et al.*, 2016). In addition, studies have indicated that bioaerosols absorbed onto particulate matter (PM) may be transported over great distances (Woo *et al.*, 2013), which consequently may have indirect impacts over wider areas. The concentrations and compositions of bioaerosols in airborne particulate pollution have been studied worldwide (Kellogg *et al.*, 2004; Yamamoto *et al.*, 2012; Alghamdi *et al.*, 2014; Cao *et al.*, 2014; Dong *et al.*, 2016), but data from these studies are often difficult to compare because of differences in experimental design (Behzad *et al*., 2015). The main challenges in the characterization of bioaerosols in airborne particulates are the low density of microorganisms in the air (Behzad *et al*., 2015); the variability in airborne microbial community composition; DNA sequencing-related challenges due to low biomass (Wang *et al.*, 2015); and a lack of standardized methodologies (Luhung *et al.*, 2015) for collection of samples and extraction of their DNA. Air sampling onto filters is the most widely used technique for characterization of aerosols on ambient particles (Bein and Wexler, 2015); however, gaining sufficient biomass for downstream DNA-based analysis such as high-throughput sequencing is challenging.

As the microbial compositions of bioaerosols are highly dynamic in time and space, it is difficult to evaluate the efficacy of protocols between studies (Durand *et al.*, 2002), with few parallel sampling studies conducted to date (Dybwad *et al.*, 2012).

Due to the very low biomass concentrations in bioaerosols, several researchers have used high volume air samplers with flowrates ranging from 100 to 1000 litres per minute to collect enough biomass from air for microbiological analyses (Radosevich *et al.*, 2002). Long sampling durations are sometimes inevitable in order to collect enough biomass for airborne microbial analysis. Sampling times have been reported as 6 hours (Madsen *et al.*, 2009), 8 hours (Meadow *et al.*, 2006), 24 hours (Alghamdi *et al.*, 2014; Happo *et al.*, 2014; Yan *et al.*, 2016), 3 days (Lau *et al.*, 2006; Gou *et al.*, 2016), and sometimes even 7 days on a single filter (Zhang *et al.*, 2004; Peccia

and Hernandez, 2006; Adell *et al.,* 2012). While some studies recommend the use of long sampling durations (Fröhlich-Nowoisky *et al.*, 2016; Ferguson *et al.*, 2019), others have identified DNA losses associated with long-duration filter-based sampling (Luhung *et al.,* 2015). For high volume air filters, the most common collection method at present, the large filters required for these flow rates result in the uneven distribution of biomass across the filter, further complicating DNA extractions (Lang-Yona *et al.,* 2016). To add further complexity, most analyses of DNA from sample filters have used different-sized portions of the filter area for DNA extraction. Studies have used different sampling durations and a wide variety of filter area sizes, such as a quarter of a PM_{10} filter (Cao *et al.,* 2014), 2.2 cm^2 for a $PM_{10–2.5}$ filter (Bowers *et al.,* 2013), half of a PM_{10} filter area (Alghamdi *et al.*, 2014; Yan *et al.*, 2016) and one-eighth of the filter area (Fröhlich-Nowoisky *et al.,* 2009) to extract DNA from PM filter samples. It remains technically challenging to determine how much standard filters can be cut while still reflecting the biomass of the whole filter and providing sufficient genomic DNA yield for downstream analysis. This study aimed to undertake a thorough investigation of how sampling duration and the fraction of filter used influenced the concentration of DNA yields from bioaerosols. The purpose of this chapter was to identify which methods would be most suitable for the case studies investigated in this thesis.

3C.2 Material and methods

3C.2.1 Particulate matter collection

PM_{10} samples were collected from August to September 2016 in the Central Business District of Auckland, New Zealand. The total air volume filtered was 1,440 m^3, 7,200 m^3 and 10,080 m^3 for sampling durations of 24 hours, 5 days and 7 days, respectively. Each sample was collected on a 12.6 cm × 16.6 cm glass fiber filter (QAT-UP, Pallflex Products, CT, USA) using an HVAS operating at a flow rate of 1000L/min. Each of the filters was cut into different sized portions (half, one quarter and one eighth of the total size) and DNA was extracted using the CTAB extraction protocol. Quality control (QC) and quality assurance (QA) information on filter handling and filter storage have been described in our previous study (Kalisa *et al.*, 2018a).

3C.2.2 DNA Extraction and data analysis

DNA was extracted from PM_{10} filters for each sampling period. The filters were cut into small pieces and were placed in nucleospin tubes (2ml) filled with 0.5g ceramic beads (1.4 mm, Qiagen, Germany). Genomic DNA was extracted following a modified CTAB extraction protocol as described previously (Archer *et al.*, 2019). Genomic DNA was quantified using a Qubit 2.0 Fluorometer (Invitrogen). Comparison of DNA concentrations relating to sampling duration and filter sample size was conducted using one-way analysis of variance (ANOVA) and Student's t-test. All statistical analyses were performed using statistical analysis software (SAS, version 9.4, SAS Institute, Cary, Inc, USA).

3C.3 Results and Discussion

DNA extraction is the first step in microbial analysis of PM filter samples and plays a major role in the effectiveness of DNA-based analysis of air samples. Although new extraction kits and protocols are continuously improving the efficiency of DNA yields, most protocols and extraction kits are for soil samples and none are currently available for air samples. However, numerous studies have used high-throughput DNA sequencing to evaluate the diversity of airborne fungi and bacteria in air samples (Cao *et al.*, 2014; Yan *et al.*, 2016). DNA extraction from a large amount of filter is time-consuming and uses large volumes of reagents, making it costly; therefore, to reach the goal of more standardized analysis methods for DNA extraction, it is important to understand how the sampling duration and filter area size affect the concentration of DNA yield.

3C.3.1 Effect of sampling duration on DNA yield

Three sets of experiments were conducted to investigate the effect of sampling duration on the environmental DNA yield. The DNA concentrations of the samples are presented in ng of DNA per volume of air sampled (ng/µL). The mean concentrations of DNA were 0.16 ng/µL, 0.25 ng/µL and 0.59 ng/µL for sampling durations of 24 hours, 5 days and 7 days, respectively. An analysis of variance (ANOVA) test showed that the median DNA concentration for the 7-day sampling period was significantly higher than those for 5 days and 24 hours (Figure 3C.1) (p = 0.0433). This result confirms that long sampling durations will be sometimes required in order to collect enough biomass for adequate DNA yield and downstream analyses. These results were consistent with previous studies that analysed DNA from samples collected over 8 hours and 14 hours and found that more DNA was recovered in 14 hours compared to 8 hours (Luhung *et al.*, 2015). The latter suggested that culture-independent DNA-based analysis is not affected by long sampling duration using a filter medium.

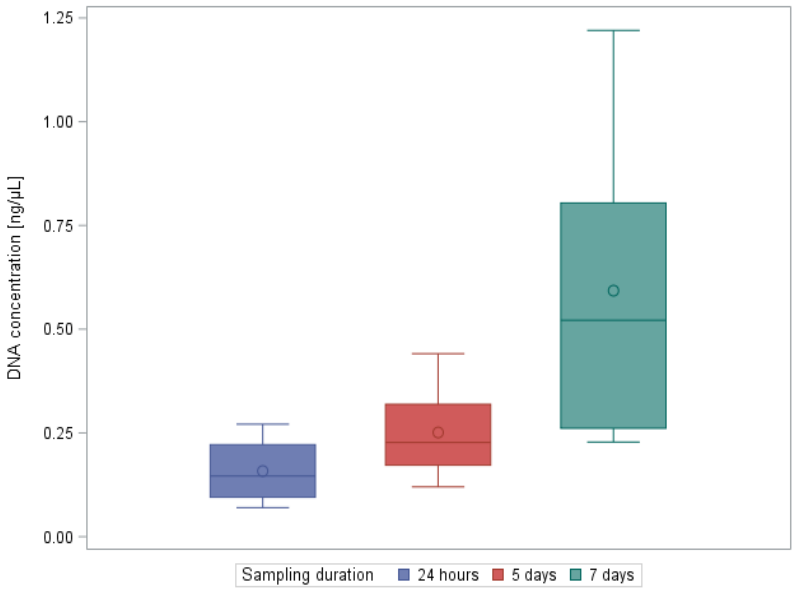

Figure 3C. 1. The average DNA concentrations from 24-hour, 5-day and 7-day sampling durations.

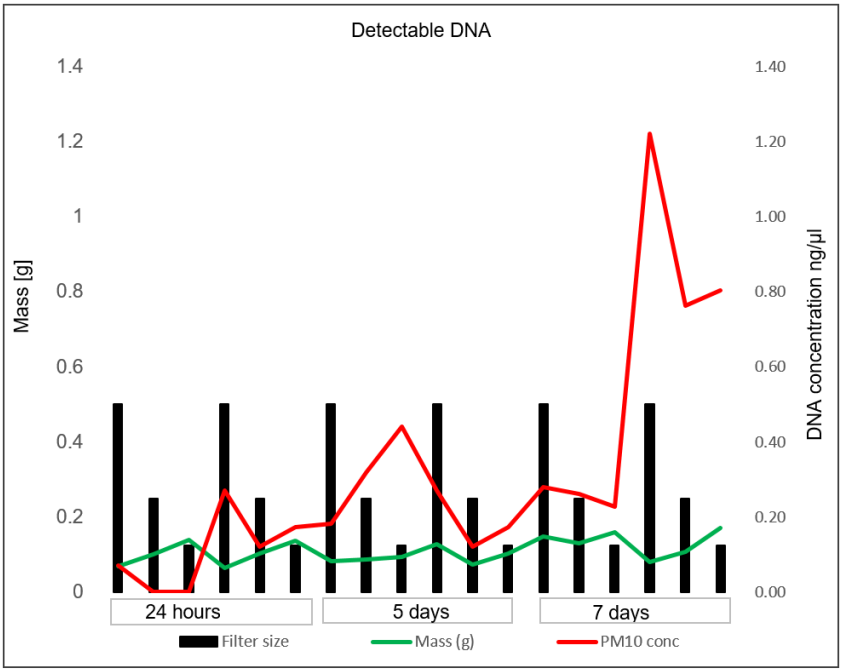

Figure 3C. 2. DNA (ng/µL) concentrations from extracted mass of PM_{10} filters collected over different sampling durations (24 hours, 5 days and 7 days) in New Zealand.

3C.3.2 Effect of filter area size on DNA yield

In this study, PM_{10} filters were divided into different sized portions: half, quarter, and one eighth of the total filter (12.6 cm × 16.6 cm). The results indicated that the concentration of DNA increased with increasing filter area size (half > quarter > eighth) for PM_{10} filters (Figure 3C.2-3). Although higher DNA concentrations were detected from the half size filter area than from a quarter and an eighth, ANOVA tests indicated that for each sample duration, there was no significant difference between a half, a quarter and an eighth of the total filter area ($p = 0.72014$). This result suggests that high volume air samplers allow collection of enough biomass and can most likely improve sampling representation and decrease variability between samples (Lin *et al.*, 1999). The results indicated that long sampling durations might allow the air to be evenly distributed across the filter (reach equilibrium or saturation) so that any filter portion size can be representative of the total filter. These findings indicated that when bioaerosol sampling is conducted over a shorter period, a larger filter portion may be needed for DNA extraction to reflect the total filter, since biomass may not be evenly distributed across filters sampled for shorter periods.

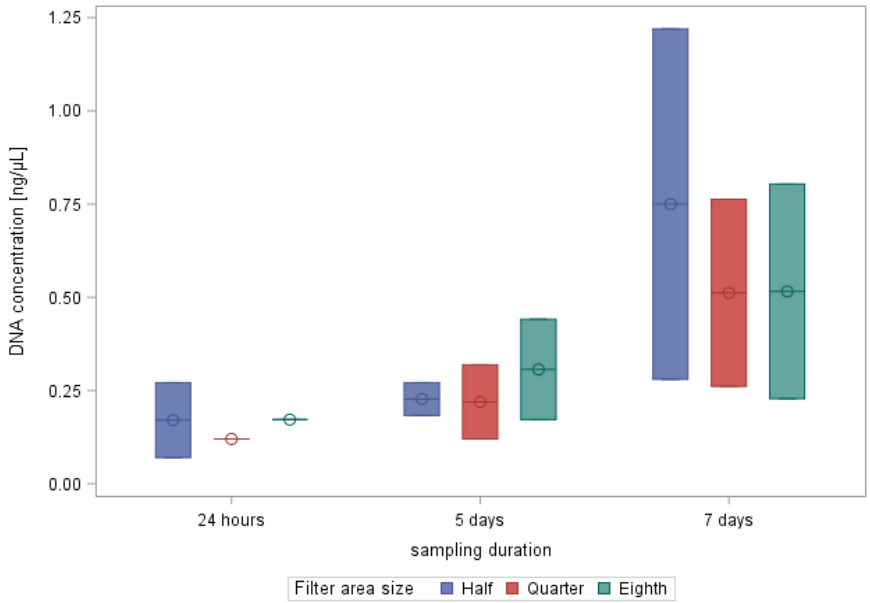

Figure 3C. 3. DNA concentration extracted from different filter size fractions: a half, a quarter and an eighth of the total filter for sampling durations of 24 hours, 5 days or 7 days.

3C.4 Conclusion

The preliminary results from this study showed that the concentrations of genomic DNA recovered in 7 days were significantly higher than 5 days and 24 hours. However, future studies should perform high-throughput sequencing to ensure there are comparable community compositions in bioaerosols across these sampling conditions. The results also indicated that at least an eighth of the filter area was required for DNA extraction in order to reflect and represent the whole filter. This study has contributed to understanding how the concentration of DNA-based bioaerosols is attenuated over time.

Chapter 4 - Pollution Characteristics and Risk Assessment of Ambient $PM_{2.5}$-Bound PAHs and NPAH in Typical Japanese and New Zealand Cities and Rural Sites

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4.1 Prelude

The methods developed for the analysis of the chemical and biological components of particulate matter in Chapter 3 were implemented in carrying out the three experimental case studies presented in this thesis. This chapter presents the findings from the chemical characterization of PM in two developed countries located in the Northern (Japan) and Southern hemispheres (New Zealand). The countries share common characteristics, which include being an archipelago, lying along the north-western and south-western margins of the Pacific respectively, and being exposed to prevailing westerly winds carrying dust particles. This study investigated the major sources of PAHs and NPAHs and determined lifetime cancer risks resulting from inhalation exposure to $PM_{2.5}$-bound PAHs and NPAHs in both countries. The study also provided information on long-range PAH and NPAH transport. Given the health effects of PAHs and NPAHs and the scarcity of information about their atmospheric levels in some areas of the world, this study serves as a foundation for further work in this area, especially in New Zealand, where there is a lack of information on carcinogenic and mutagenic pollutants like PAHs and NPAHs.

4.2 Introduction

Anthropogenic pollutants associated with fine particulate matter with an aerodynamic diameter ≤ 2.5µm ($PM_{2.5}$) pose a significant health risk to populations worldwide as they can be deposited deeper into the lungs than large particles when inhaled (Liu *et al.*, 2017; Martins and Carrilho da Graça, 2018; Kalisa *et al.*, 2019a). Although there are natural sources of $PM_{2.5}$ (Kim *et al.*, 2013), anthropogenic $PM_{2.5}$ contains several toxic organic species including polycyclic aromatic hydrocarbons (PAHs) and nitrated PAHs (NPAHs), which are well known for their carcinogenic and mutagenic properties (Abdel-Shafy and Mansour, 2015). PAHs and NPAHs are emitted into the atmosphere from anthropogenic sources such as diesel and gasoline vehicles, industrial processes, coal and wood combustion (Khalili *et al*., 1995; Ravindra *et al*., 2008; Hazarika *et al*., 2017; Hayakawa *et al.*, 2018b) and are capable of being transported from one region to another via air currents (Yang *et al.*, 2007; Tang *et al.*, 2015). NPAHs can also be formed as secondary compounds from atmospheric reactions of PAHs and atmospheric oxidants such as ozone and nitrate radicals (Hayakawa, 2016a). Further, PAHs and NPAHs are good tracers for ambient air pollution source identification and photochemical pathways (Tang *et al.*, 2005). Previous studies have indicated that more than 80% of particle-bound PAHs are associated with $PM_{2.5}$ (Hassanvand *et al.*, 2015) and that they are transported by strong winds from one region to another (Tamamura *et al.*, 2007; Yang *et al.*, 2007), over great distances (Yang *et al.*, 2009). In addition, the concentration of $PM_{2.5}$-bound PAHs and NPAHs in the atmosphere is strongly dependent on meteorological conditions, weather events and emission sources (Alam *et al.*, 2015). Therefore, PAHs and NPAHs in $PM_{2.5}$ can be useful for environmental monitoring, source identification and health assessment.

In Japanese commercial cities such as Kanazawa, the main emission sources of PAHs and NPAHs are automobiles (Hayakawa *et al.*, 2018b). However, the highest PAH concentrations detected in Kanazawa were partly associated with long-range transport PM from yellow sand storms in East Asian countries (Yang *et al.*, 2007; Hayakawa, 2016a). In the southern hemisphere, there is also evidence of long-range dust transport from the Victoria Desert, Australia, reaching New Zealand and contributing to an increase in particulate matter pollution (Marx *et al.*, 2005). However, there has been no report indicating the possible seasonal variation and sources of PAHs and NPAHs in ambient $PM_{2.5}$ in New Zealand to date. Topographical and meteorological conditions of both hemispheres could have a strong influence on spatial climatic modifications and the spatial distribution of air quality.

This is the first study that compares atmospheric PAHs and NPAHs in 'typical' city and rural areas located in different hemispheres. Therefore, the objectives of this study were: (1) to determine ambient levels of PAHs and NPAHs in $PM_{2.5}$ for Kanazawa (Japan) and Auckland (New Zealand); (2) to identify potential emission sources based on the spatial and temporal concentration variations in contrast to corresponding rural sites (Wajima in the Noto Peninsula and Tapora in the Okahukura Peninsula, respectively) and; (3) to assess the cancer risk of human exposure to PAHs and NPAHs in both countries. Given the health effects of PAHs and NPAHs and scarcity of information about their atmospheric levels in some areas of the globe; this study aimed to generate baseline reference data from which to generate models of exposure, of relevance to the science-policy interface. Results obtained from this study may provide a basis for regulatory and management standards set by the environmental authorities responsible for monitoring air quality in both hemispheres.

4.3 Material and methods

4.3.1 Study location

Japan and New Zealand provide a unique opportunity to compare air pollution in city and rural sites sharing similar characteristics. Both countries are archipelagos along the northwestern and southwestern margins of the Pacific Ocean, respectively, with lines of latitude of ~30-40 degrees (Figure 4.1). Both countries have four distinct seasons and experience prevailing westerly winds carrying dust particles (Harada and Glasby, 2000). Kanazawa City was chosen to compare with Auckland City (New Zealand's largest city) because the atmospheric concentrations of PAHs and NPAHs in Kanazawa are relatively well studied and are within the nominal range of Japanese cities (Hayakawa *et al.,* 2018b). Wajima and Tapora are both good representatives of typical rural sites away from high concentrations of vehicle emissions and industries. They are similar in terms of their distance to the city and their coastal settings. Wajima is located on the Noto Peninsula, 2.1 km south of the Sea of Japan coast and about 100 km north of Kanazawa City, while Tapora is located on the Okahukura Peninsula, 1.4 km from the coast of the Tasman Sea and about 100 km north of Auckland City. Furthermore, Wajima is in the main path of winter northwest winds originating from continental Asia and experiences long-range transport of particulate matter (Tamamura *et al.,* 2007). In Wajima, the PAH concentration due to transboundary transport from China exhibits seasonal variation (high in winter and low in summer) (Tamamura *et al.,* 2007). Thus, Wajima was used as a reference rural site to compare to Tapora, which has similar site characteristics, but where the seasonal variations in transboundary transport have never been investigated.

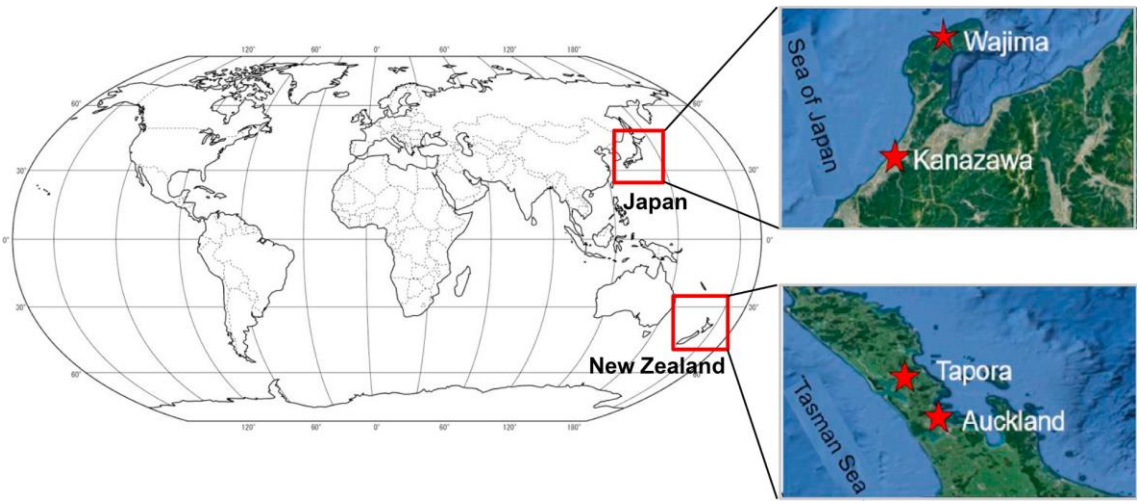

Figure 4. 1.Maps of Japan (North) and New Zealand (South); Sampling sites of Kanazawa and Wajima in Japan, and Auckland and Tapora in New Zealand are shown as solid red stars with relative positions to the Sea of Japan and the Tasman Sea.

$PM_{2.5}$ samples were collected in New Zealand and Japan from a city (Auckland and Kanazawa, respectively) and a rural site (Tapora and Wajima, respectively) (Figure 4.1). In Kanazawa (36.351N; 136.381E) and Auckland (36.854S;174.756E), the sampling locations were densely inhabited, with the air samplers located on rooftops of Kanazawa University and Auckland University of Technology buildings approximately 15~20 m above ground level (Liu *et al.*, 2017) and approximately 400 m from major roads and away from kitchen and air conditioning exhaust systems to avoid sampling biases from possible trapped pollutants in areas surrounded by dwelling and commercial buildings. At Wajima (37.23N, 136.54E) and Tapora (36.347S; 174.344E) rural sites, the sampling locations were ~15-20 km from public roadways and human activities with no significant emission sources of PAHs and NPAHs or other air pollution nearby. Sampling sites in both rural sites were located at approximately 1~3 m above ground level. $PM_{2.5}$ samples were collected during the four seasons from 2016 to 2017 in both countries. The sampling process is summarized in the Supporting Information (SI) (Table S6.1of the SI).

4.3.2 Sample collection, treatment and analytical procedures

Two 7-day $PM_{2.5}$ samples were collected at each sampling site in each season from 2016 to 2017. $PM_{2.5}$ was sampled using high-volume air samplers (Sibata Scientific Technology Limited,

Saitama, Japan) at a flow rate of 1000 L/min and fitted with a glass fiber filter (20.3 cm x 25.4 cm, 2500 QAT-UP, Pallflex Products, CT, USA).

Prior to use, all filters were pre-heated at 600°C for 5 h to lower their PAH and NPAH blank values and to remove organic contaminants. After sampling, filters were wrapped in aluminium foil, sealed inside sterilized plastic bags and shipped to the laboratory. For gravimetric mass determination of the sampled $PM_{2.5}$, the filters were equilibrated in a weighing room (daily mean temperature in the range of 20-25°C and daily mean relative humidity in the range of 30–45%) (Kalisa *et al.,* 2018a). After drying in a glass desiccator for ~48 hours in the dark, the filters were weighed, then placed in sealed plastic bags and stored at−20°C until further analysis. To ensure laboratory quality control, field blank and solvent blank experiments were conducted. Target PAHs and NPAHs were not detected in the blank samples. Recoveries of deuterated internal standards of PAHs and NPAHs were determined quantitatively based on the peak area ratios of the analytes to the deuterated internal standard and were between 75% and 104%. Detailed information about the limits of detection and limits of quantification is available in our previous studies (Hayakawa *et al.,* 2018b).

The United States Environmental Protection Agency (US EPA) 610 PAH mixture (Sigma-Aldrich, St. Louis, MO, USA) was used as a standard and included 16 PAHs: naphthalene (NaP), acenaphthene (Ace), Acenaphthylene (Acyl), fluorene (Fle), phenanthrene (Phe), anthracene (Ant), fluoranthene (Flu), pyrene (Pyr), benz(a)anthracene (BaA), chrysene (Chr), benzo(b)fluoranthene (BbF), benz(k)fluoranthene (BkF), benzo(a)pyrene (BaP), dibenz(a,h)anthracene (DBA), benz(g,h,i)perylene (BPe), and indeno(1,2,3-cd)pyrene (IDP). For NPAHs our standard contained 9-nitroanthracene (9-NA), 2-nitropyrene (2-NP); 2-nitrofluoranthene (2-NFR), 1- nitroperylene (1-NP), 6-nitrochrysene (6-NC), 7-nitrobenz(a) anthracene (7-NBaA), and 6-nitrobenz(a)pyrene (6-NBaP). These NPAHs compounds were obtained from Chiron (Trondheim, Norway). Two deuterated PAHs (perylene-d_{12} (Pyr-d_{10}) and benzo(a)pyrene-d_{12} (BaP-d_{12})) (Wako Chemical, Osaka, Japan) and an NPAH surrogate, 2-fluoro-7-nitrofluorene (FNF) (Sigma Aldrich, Milwaukee, WI, U.S.A.) were used as internal standards.

All analytes were dissolved in ethanol (Kanto Chemical, Tokyo, Japan). All other chemical reagents used in this study were of analytical-reagent grade.

Samples on $PM_{2.5}$ filters were treated as previously described by Hayakawa *et al.* (2018b). Briefly, a 49.2 cm^2 piece of the total filter was divided into 0.5 cm^2 pieces and placed in a flask. Internal standards (Pyr-d_{10}, and BaP-d_{12} for PAH and FNF for NPAH) were added. The cut filters were extracted twice by sonication using 80 mL of benzene-ethanol (3:1, v/v) and filtered. The extract was cleaned with 80 mL of 5% (w/v) sodium hydroxide, followed by 80 mL of 20% (w/v) sulfuric acid, and twice with 80 mL of ultrapure water for 15min each round. Dimethyl sulfoxide (100 µL) was added, and the solution was concentrated to 100 mL using a rotary evaporator. The residual solution was dissolved in ethanol (900 µL), and the solution was filtered through an HLC-DISK 3 with a pore size of 0.45 µm (Kanto Chemical Co., Inc., Tokyo, Japan). A 100 µL aliquot (injection volume) of the filtered solution was analyzed using two high-performance liquid chromatographic systems (HPLC-10A series, Shimadzu Inc., Kyoto, Japan), with one system equipped with a fluorescence detector and another equipped with a chemiluminescence detector to analyse PAHs and NPAHs, respectively.

This study focused on particulate PAHs, as PAHs with high carcinogenic properties have been shown to be present in the particulate phase, as opposed to the gaseous phase (Hayakawa *et al.*, 2014). For PAHs, DBA could not be quantified because of interfering peaks in several samples. Seven NPAHs were determined in which 2-NP and 2-NFR were grouped together because these two NPAHs could not be completely resolved chromatographically (Kalisa *et al.*, 2018a). Therefore, this study focused on nine PAHs (Flu, Pyr, BaA, Chr, BbF, BkF, BaP, BPe and IDP) and six NPAHs (9-NA, 2NP+2NFR, 1-NP, 6-NC, 7-NBaA and 6-NBaP). The chromatography conditions have been described previously (Hayakawa *et al.*, 2018b).

4.3.3 Data analysis

Principal component analysis (PCA) using SAS software version 9.4 (SAS Institute Inc., Cary, NC, USA) was performed to explore relationships between the PAH/NPAH data and the various sites and to provide information on their relationship to potential environmental sources (Hussain *et al.*, 2015; Liu *et al.*, 2017). Each principal component was extracted with different factor

loadings and recognized by source markers or profiles as likely pollution sources. To obtain more insight into the data characteristics, PCA was performed with individual PAH and NPAH contents as active variables (grouped together), and the sampling sites as subjects. Further data analysis was performed using ratios of various PAHs and NPAHs, which are generally characteristic of their emission sources. The Wilcoxon-Mann-Whitney test was used for pairwise comparison (urban and rural sites within each country and between countries), as implemented in SAS. PAH and NPAH quantitative data were expressed as median and interquartile range and mean and standard deviation. Three-day back trajectories were calculated using the Hybrid Single Particle Lagrangian Integrated Trajectory (HYSPLIT) model to determine the origin of air masses (Tang *et al.*, 2017).

4.3.4 Health risk assessment

Risk assessment of carcinogenic PAHs and NPAHs was conducted using the toxicity equivalency (TEQ) methodology developed by the US EPA (Nisbet and LaGoy, 1992). Health risk assessment of PAHs and NPAHs is usually estimated using BaP as a reference chemical because its toxicity is well characterized (Boström *et al.*, 2002). Therefore, the carcinogenic risk was estimated by using toxic equivalent factors (TEFs) for each PAH or NPAH compared to BaP. The concentration of each PAH and NPAH was converted to the BaP equivalent (BaPeq, carcinogenic equivalents, ng/m^3) concentration by multiplying the concentration of PAHs by the corresponding TEF (Jung *et al.*, 2010). Lifetime excess inhalation cancer risk of PAHs and NPAHs was therefore estimated by multiplying the BaPeq by the inhalation cancer unit risk factor (UR) of BaP (URBaP). The URBaP is the inhalation cancer unit risk factor of BaP (1.1×10^{-6} (ng/m^3)$^{-1}$) (Bandowe *et al.*, 2014). TEF values for PAHs and NPAHs were obtained from (Albinet *et al.*, (2008) and (OEHHA, 2011).

4.4 Results and Discussion

4.4.1 Annual concentrations of $PM_{2.5}$

The annual median (interquartile range) concentrations of $PM_{2.5}$ were 7.3 (4.8 -11.4) μg/m^3 and 5.0 (4.0 -7.5) μg/m^3 in Japan and New Zealand, respectively (Table S6.2 of the SI). Both values were under the WHO annual ambient air quality standard for $PM_{2.5}$ (10 μg/m^3) (World Health

Organization, 2006b). The Wilcoxon–Mann–Whitney test showed that the annual mean $PM_{2.5}$ concentrations were higher in city sites than in rural areas for both countries. However, this difference was not statistically significant (Table S6.2 of the SI). The low concentration of $PM_{2.5}$ measured in rural areas is likely related to low anthropogenic emissions as these sites were situated approximately 100 km away from cities and were as far as possible from roads and industrial areas.

4.4.2 Atmospheric concentration of PAHs and NPAHs in Japan

The mean of ΣPAHs in $PM_{2.5}$ in Kanazawa City was 0.53 ng/m^3, and at the Wajima rural site was 0.24 ng/m^3 (Table 4.1). The highest median concentration of PAHs was observed in Kanazawa City during autumn, accounting for 41% of the total PAHs detected at this site in all seasons (Figure 4. 2). In Wajima, the highest median concentration of total PAHs was detected in winter, accounting for 54% of the total PAHs detected at this site in all seasons (Figure 4.2,). However, the Wilcoxon-Mann-Whitney test showed that there was no statistical difference between median concentrations of PAHs in Kanazawa and Wajima (p = 0.1036) (Table 4.2). High PAH levels, despite limited local emissions, were expected at the Wajima site as it is in the main path of winter northwest winds originating from other Asian countries (Yang *et al.,* 2007; Hayakawa *et al.,* 2014) as shown by the back trajectory analysis. In Japan, most of the back trajectories of the winter samples in Wajima passed through sites of high population and industrial activity in China and Mongolia (Figure S4.1 of the SI). The highest concentration of PAHs measured at Wajima during winter was more than eight times the summer level (Table S6.2 of the SI), suggesting that PAHs at Wajima may have been strongly affected by the combustion of particulates transported from Asian countries in winter as previously shown (Yang *et al.,* 2009; Tang *et al.,* 2015). In contrast, most of the trajectories during the summer at Wajima site did not show wind trajectories from other regions, resulting in lower total PAHs.

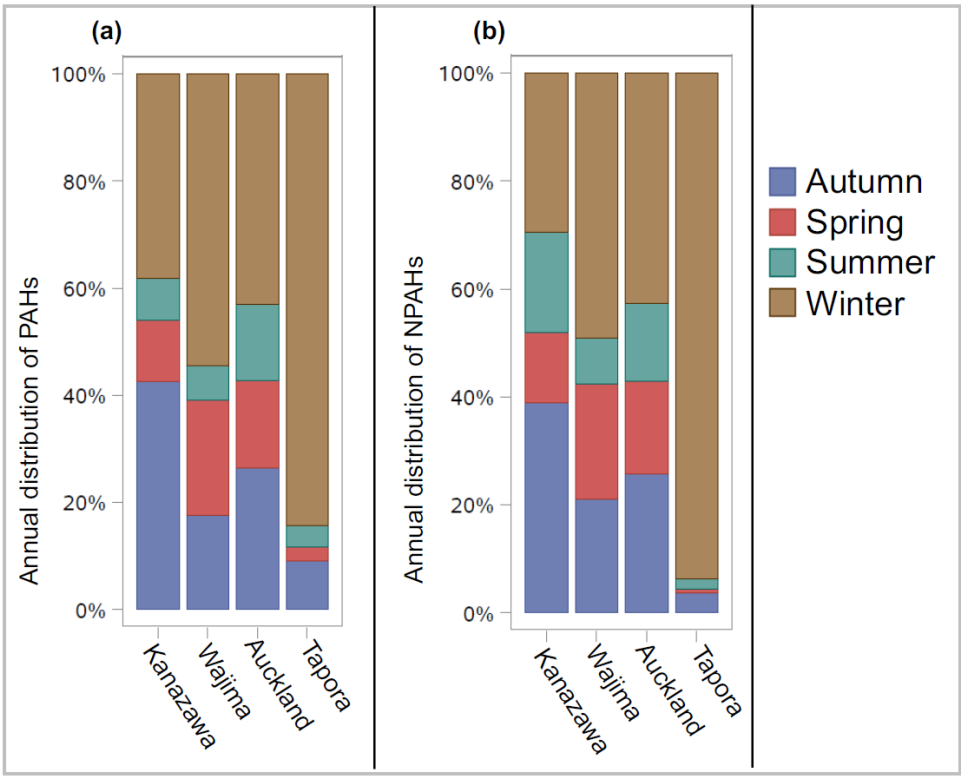

Figure 4. 2. Distributions of total (a) PAH and (b) NPAH concentrations as a percentage of total PAH and NPAH content detected in four seasons in cities (Auckland and Kanazawa) and rural sites (Tapora and Wajima).

Table 4. 1. Comparison of $PM_{2.5}$, PAH and NPAH [mean ± SD] concentrations between Japan (Kanazawa City and Wajima, rural site) and New Zealand (Auckland City and Tapora, rural site) using Wilcoxon-Mann-Whitney test.

Parameters	**Kanazawa (N=8)**	**Auckland (N=8)**	***p*-value**
PAH [ng/m^3]	0.53 ± 0.39	0.31 ± 0.19	0.4926
NPAH [pg/m^3]	33.45 ± 19.8	48.2 ± 31.7	0.1893
$PM_{2.5}$ [µg/m^3]	10.73 ± 5.1	7.38 ± 1.7	0.1883
Parameters	**Wajima (N=8)**	**Tapora (N=8)**	***p*-value**
PAH [ng/m^3]	0.24 ± 0.19	0.06 ± 0.09	0.0238
NPAH [pg/m^3]	20.39 ± 17.7	12.45 ± 19.11	0.1036
$PM_{2.5}$ [µg/m^3]	6.05 ± 2.4	4.25 ± 0.71	0.0633

The dominant PAH compounds in this study were Flu and BbF (32% and 16% in Kanazawa, and 27% and 23% in Wajima) (Figure 4.3). These compounds account for a large proportion of the total PAHs commonly observed in Japanese cities, influenced by coal burning and vehicle emissions during winter (Tang *et al.*, 2015). BaP, classified as a group 1 carcinogen in humans

(Błaszczyk *et al.,* 2017), is the most widely investigated PAH in the atmosphere because of its toxicity and it is used as a marker for carcinogenic risk in ambient air (Delgado-Saborit, Stark and Harrison, 2011). In this study, the mean concentrations of BaP detected in Kanazawa and Wajima (Table S4.2 of the SI) were lower than the WHO guideline of 1 ng/m^3 (World Health Organization, 2000). The concentrations of BaP detected in Japan were much lower in Kanazawa and Wajima than those reported in African cities (Nassar *et al.,* 2011; Kalisa *et al.,* 2018a) and Chinese cities (Tang *et al.,* 2017).

The most abundant NPAHs in Kanazawa were 2-NP+2-NFR (64%), 9-NA (16%) and 1-NP (12%) (Table S4.2 and Figure 4.3). The predominance of 2-NP+2-NFR, 9-NA and 1-NP in atmospheric particles has been previously reported in several East Asian and European cities (Tang *et al.,* 2005; Albinet *et al.,* 2008), and attributed to large emissions from biomass burning and vehicle exhausts.

Table 4. 2. Spatial variation in concentrations of PAHs and NPAHs in Japan and New Zealand.

Sites	**PAHs (ng/m^3)** median (IQR)	***p*-value**	**NPAHs (pg/m^3)** median (IQR)	***p*-value**
Kanazawa	0.45 (0.19 - 0.80)	0.1036	6.61 (4.97 - 9.26)	0.0014
Wajima	0.18 (0.12 - 0.37)		1.15 (0.53 - 1.64)	
Auckland	0.20 (0.19 - 0.43)	0.009	8.92 (6.38 - 10.29)	0.0054
Tapora	0.01 (0.01 - 0.11)		0.11 (0.05 - 3.10)	

The mean concentrations of ∑NPAH in $PM_{2.5}$ were higher in Kanazawa (33.45 pg/m^3) than in Wajima (20.39 pg/m^3) (Table 4.1). The Wilcoxon-Mann-Whitney test showed that the median concentrations of NPAHs in Kanazawa were significantly higher than in Wajima (p=0.0014) likely related to vehicular emissions of NPAHs (Keyte, Albinet and Harrison, 2016; Kalisa *et al.,* 2018b). The total concentrations of NPAHs exhibited seasonal variation (Figure 4.3 and Table S4.2 of the SI) decreasing in the following order: autumn > winter > summer > spring, for Kanazawa and winter >autumn > spring > summer, for Wajima. These findings were consistent

with previous studies carried out in Japanese cities and rural areas including Kanazawa and Wajima and showed that NPAHs were higher in winter than in summer because of highly stable air and the use of oil heating during winter (Hayakawa *et al.*, 2000; Tang *et al.*, 2002). In this study, the mean concentrations of total PAHs and NPAHs in Kanazawa and Wajima (Table 4.1) were generally lower than the mean concentrations previously reported during the summer of 2013 and winter of 2014 in four Japanese cities including Kanazawa (Hayakawa *et al.,* 2018b), suggesting that air quality in Japan may have improved in recent years. This was consistent with a recent study showing that PAHs and NPAHs have steadily decreased in Japanese cities (Sapporo, Kanazawa, Tokyo, Sagamihara and Kitakyushu) from 1997 to 2014 due to the measures taken to reduce vehicle emissions (Hayakawa, 2018a).

4.4.3 Atmospheric concentration of PAHs and NPAHs in New Zealand

This study provides the first characterization of $PM_{2.5}$-bound PAHs and NPAHs in New Zealand. The concentrations of total PAHs and NPAHs were significantly higher in Auckland than in Tapora (Table 4.1) and lower than the PAH levels previously reported in Auckland and Christchurch by Cavanagh *et al.* (2009). The lower concentrations in this study suggest that the concentration of PAHs may have decreased recently due to the measures established by the New Zealand government to decrease domestic home heating emissions from wood burning during winter (Fisher *et al.,* 2007). In agreement with previous studies, the total PAH concentrations in this study were highest in the winter (Table S4.3 of the SI and Figure 4.2) for both New Zealand sites (Khanal and Shooter, 2004; Brown *et al.,* 2005), probably due to lower temperatures and less photo-degradation during winter (Gope *et al.,* 2018).

The effect of long-range transport on PAHs concentration was not observed during all seasons in New Zealand. The trajectories from winter and summer samples in Auckland City and at the Tapora rural site showed that air parcels during the sampling period originated from the Pacific Ocean (Figure S4.2 of the SI), suggesting that there were few PAH-contaminated air masses transported from surrounding countries. Therefore, the limited number of large industries and isolation from other polluted continents explain the low concentrations of PAHs detected in New Zealand.

In New Zealand, the most dominant individual PAHs were Flu and BPe (39% and 14%) in Auckland, and Flu and BaP (34% and 14%) in Tapora (Figure 4.3). BPe and BbF are useful markers of vehicle emissions (Miguel *et al.*, 1998; Harrison and Yin, 2000; Guan *et al.*, 2017) whereas Flu and BaP are indicators of biomass burning (Khalili *et al.*,, 1995; Schauer *et al.*, 1996; Harrison and Yin, 2000). These results suggest that vehicle exhaust and biomass burning were the major sources of PAHs in New Zealand. In addition, PAHs with six rings were dominant in Auckland (Figure S4.3 of the SI). PAHs with six rings are indicators of vehicle emissions (Guo *et al.*, 2003; Phoungthong *et al.*, 2017). The annual mean of BaP (a marker for carcinogenic risk in ambient air) detected in New Zealand sites (Table S4.3 of the SI) was below the WHO (1 ng/m^3) and New Zealand (0.3 ng/m^3) annual guidelines (Ministry for the Environment and Statistics New Zealand, 2015). The annual PAH concentrations detected in $PM_{2.5}$ in New Zealand sites in this study were lower than PM_{10}-bound PAH levels previously reported in Auckland and Christchurch by Cavanagh *et al*. (2009). This previous study was carried out during the winters of 2002 and 2004 and identified domestic wood burning as the main source of PAHs.

The mean concentrations of ∑NPAH were higher in Auckland (48.2 pg/m^3) than in Tapora (12.45 pg/m^3) (Table S4.2 of the SI). The Wilcoxon-Mann-Whitney test showed that the median concentrations of NPAHs in Auckland were significantly higher than in Tapora (p=0.009). This was expected due to the proximity of the sampling site to emission sources (high traffic volumes in Auckland's main road, Queen Street). Vehicle diesel and gasoline emissions are major sources of NPAHs (Keyte, Albinet and Harrison, 2016; Kalisa, *et al.*, 2018).

The most abundant NPAHs in New Zealand site were 2-NP+2-NFR, 1-NP, 9-NA and 6-NC (Table S4.3 of the SI and Figure 4.3). The highest levels of 9-NA and 2-NP+2-NFR may have been produced by biomass burning (wood) at low temperatures in Tapora and by vehicle emissions at Auckland (Kalisa *et al.*, 2018a). It has been reported that atmospheric 9-NA can emerge from both primary emissions and secondary formation (Liu *et al.*, 2017). The highest concentration was found in both Auckland and Tapora during winter (Table S4.4 of the SI), suggesting that 9-NA concentrations were derived from automobile emissions in Auckland, while the dominant source at the Tapora rural site was secondary formation (Feilberg *et al.*, 2001). The

predominance of 2-NP+2-NFR, 9-NA and 1-NP in atmospheric particles has been previously reported in several East Asian and European cities (Tang *et al.*, 2005; Albinet *et al.*, 2008), and attributed to biomass burning and vehicle exhausts. The NPAH compound 6-NC is directly emitted from vehicles, especially diesel engines (Hayakawa *et al.*, 1995; Bamford and Baker, 2003). In this study, 6-NC (a tracer for carcinogenic NPAHs) was high in Auckland during the summer and was higher than levels obtained for other sites and seasons, suggesting that vehicle exhaust was the main source of NPAHs in Auckland.

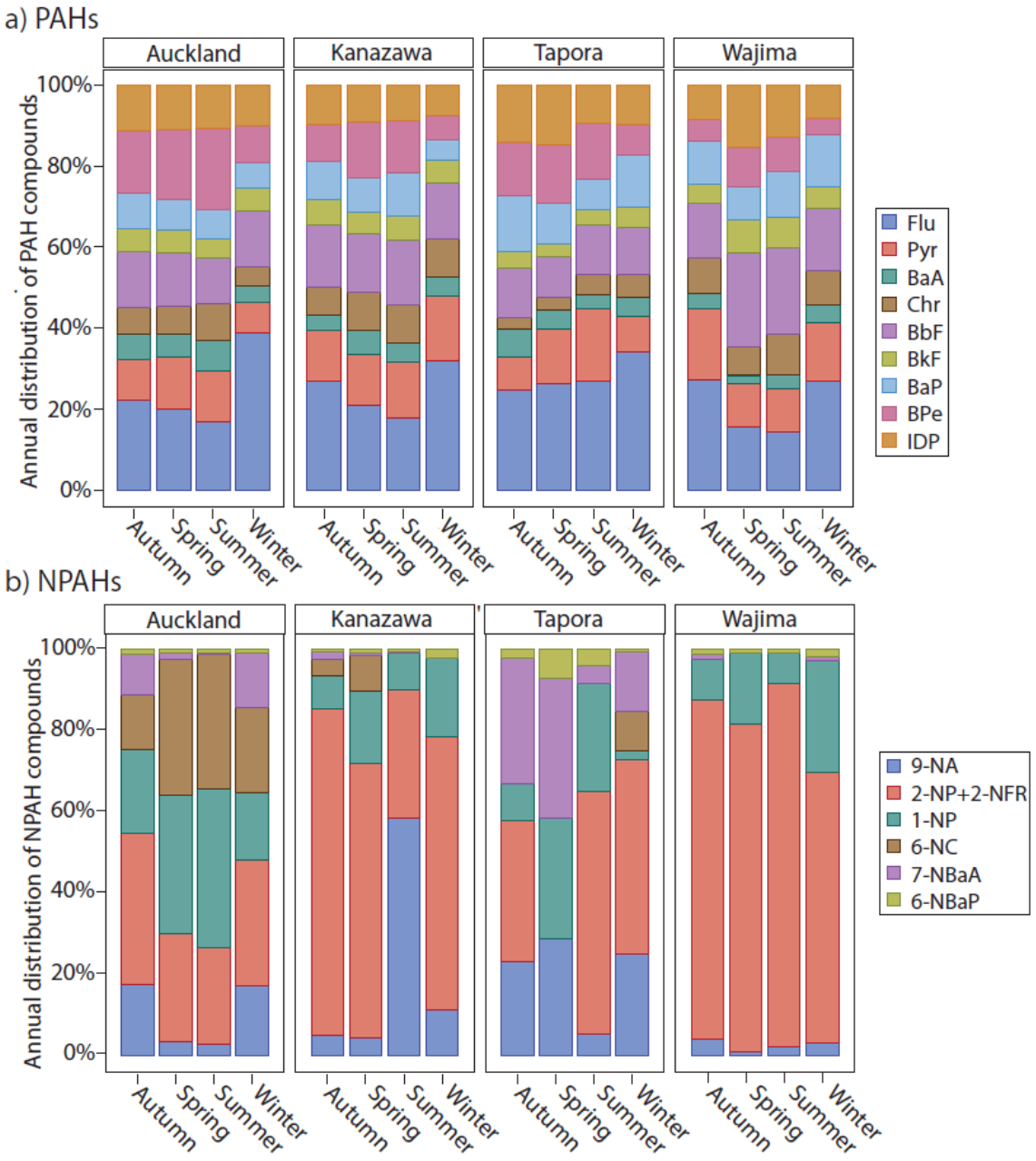

Figure 4. 3. Distributions of total PAH (a) and NPAH (b) composition detected in four seasons in cities (Auckland and Kanazawa) and rural sites (Tapora and Wajima). Abbreviation: 9-nitroanthracene (9-NA), 2-nitropyrene (2-NP); 2- nitrofluoranthene (2-NFR), 1- nitroperylene (1-NP), 6-nitrochrysene (6-NC), 7- nitrobenz(a)anthracene (7-NBaA) and 6- nitrobenz(a)pyrene (6-NBaP), fluoranthene (Flu), pyrene (Pyr), benz(a)anthracene (BaA), chrysene (Chr), benzo(b)fluoranthene (BbF), benz(k)fluoranthene (BkF), benzo(a)pyrene (BaP), benz(g,h,i)perylene (BPe), and indeno (1,2,3-cd)pyrene (IDP).

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4.4.4 Source analysis of PAHs and NPAHs using diagnostic ratio

As the PAH and NPAH isomer composition varies with combustion temperature, various ratios can be used in tracing the source of these contaminants (Hayakawa, 2018a; Kalisa, *et al.,* 2018a). The diagnostic ratios (Table 4.2) indicate that all sites in Japan and New Zealand are influenced by local emissions from different combustion sources (coal and wood fuel, vehicle emissions) and secondary formation to varying degrees. Diagnostic ratios of NPAHs to PAHs such as [1-NP]/[Pyr] in particulates are used to distinguish whether the major contributor of PAHs or NPAHs is coal combustion or automobile exhaust, since their combustion temperatures are different (Tang *et al.,* 2005). In this study, the [1-NP]/[Pyr] values were lower in winter than in summer at all sites and were comparable between cities and rural sites for both countries (Table 4.3). The ratios in summer and winter in Japan found in this study were similar to those in other Chinese and Japanese cities where coal burning predominates (Hayakawa, 2016a). The ratios in Auckland (Table 4.3) were similar to those in Seoul (0.21), Sapporo (0.14) and Tokyo (0.13); cities where diesel-engine exhaust is reported to be the major source of PAHs and NPAHs (Hayakawa, 2018). The ratios of [7-NBaA]/[BaA] for Kanazawa and Wajima (Table 4.2) during winter were in the same range as that from coal stoves (0.00063-0.0057) and similar to those previously reported in Muroran City in Japan (0.003), where iron is manufactured using coal combustion (Hayakawa *et al.*, 2016b). A [9-NA]/[1-NP] value >10 indicates substantial contributions from biomass burning while a value <10 indicates strong contributions from vehicle emissions (Chuesaard *et al.,* 2014). In this study, a contribution of [9-NA]/[1-NP] ratio >10 was observed in the Tapora rural site in New Zealand, providing further evidence for the contribution of biomass burning (domestic wood burning) during winter.

Table 4. 3. Diagnostic ratio of NPAHs to PAHs in Japan (Kanazawa and Wajima) and New Zealand (Auckland and Tapora).

	[1-NP]/[Pyr]		[9-NA]/[1-NP]		[7-NBaA/BaA]		[2-NP+2-NFR]/[1-NP]	
Sites	Summer	Winter	Summer	Winter	Summer	Winter	Summer	Winter
Kanazawa	0.02	0.01	6.44	0.58	0.003	0.0001	3.48	3.49
Auckland	0.11	0.08	0.07	1.04	0.001	0.12	13.33	2.43
Wajima	0.01	0.01	0.29	0.11	0.0001	0.001	0.61	1.88
Tapora	0.01	0.01	0.19	12.15	0.013	0.01	2.00	24.15

In addition to primary emissions, NPAHs may be found in the atmosphere as a result of secondary formation (Atkinson and Arey, 1994). The concentration ratio of [2-NFR]/[1-NP] has been used to investigate the relative contribution of NPAHs from primary formation vs secondary formation. A [2-NFR]/[1-NP] value >5 indicates strong contributions from secondary formation while a value <5 indicates a strong contribution from primary emissions (Amador-Muñoz *et al.,* 2011). In this study, 2-NP and 2-NFR could not be separated chromatographically. Therefore, [2-NP+2-NFR]/[1-NP] was used to examine possible secondary formation vs primary formation, instead of [2-NFR]/[1-NP] (Kalisa *et al.*, 2018a). A [2-NP+2-NFR]/[1-NP] ratio >5 was only observed in New Zealand sites during summer (Auckland) and winter (Tapora) (Table 4.3) while a ratio <5 was observed at all sites in Japan. These findings provided further evidence for the contribution of NPAH secondary formation in New Zealand and a strong contribution from direct emissions in Japan.

4.4.5 Source analysis of PAHs and NPAHs using PCA

To further understand the source of PAHs and NPAHs in Japan and New Zealand, PCA was used in conjunction with NPAHs to PAHs diagnostic ratio analysis (Figure 4.4a). PCA was performed on all PAH and NPAH data as one unit for both countries. The principal component axis 1 (PC1) explained 63.82% of the variance and was heavily weighted to most PAH species associated with coal combustion and domestic wood (Khalili *et al.,*1995; Ravindra *et al.,* 2008) and NPAH species (2-NP+2-NFR) from secondary formation (Kalisa *et al.,* 2018a). PC2 accounted for

19.48% of the total variance and was driven by NPAH species associated with automobile emissions (Liu *et al.*, 2017; Hayakawa, 2018a). PC1 loaded by most PAHs and PC2 loaded by most NPAHs were clustered into different two groups (Figure 4.4a), suggesting that multiple sources may contribute to atmospheric PAHs and NPAHs in New Zealand and Japan. In addition, PCA was performed on all PAHs and NPAHs with spatial clustering. The scores plot (Figure 4.4b) highlights the grouping of samples with respect to the different sampling sites. A clear separation between sites was observed in both countries; PAH and NPAH profiles detected in the two cities were closely located, while those detected in rural sites were further apart than in the cities. These findings suggest that PAHs and NPAHs in Kanazawa City in Japan and Auckland City in New Zealand may be influenced by similar sources, namely vehicular emissions. These findings were consistent with the diagnostic ratio source analysis.

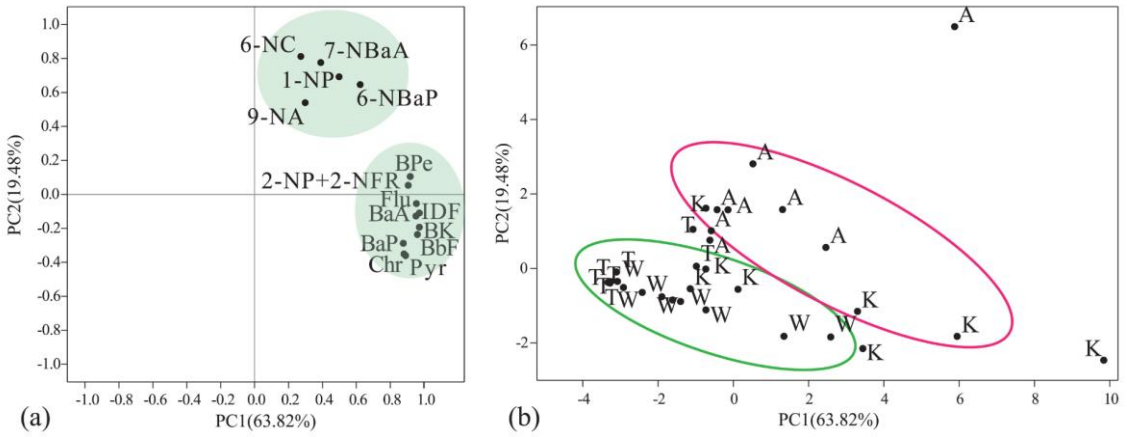

Figure 4. 4. PCA loadings indicating (a) clear grouping of PAHs and NPAHs and (b) scores plots for the first two components of all PAHs and NPAH in $PM_{2.5}$ indicating clear separation between the two cities (within the red line), Auckland (A) and Kanazawa (K) and their respective rural sites (within the green line), Tapora (T) and Wajima (W).

4.4.6 Risk Assessment

The estimated cancer risks for the Japan and New Zealand sites were calculated from the concentrations of nine PAHs and the two most carcinogenic NPAHs (1-NP and 6-NC). Total BaP equivalents (BaPeq) for the study period at all sites were much lower (Table S4.4 of the SI) compared to those reported in nine Chinese cities (Liu *et al.*, 2017), India (Masih *et al.*, 2012), urban and rural areas of Rwanda (Kalisa *et al.*, 2018a) and rural areas in southern Germany (Bari

et al., 2010). Due to the high concentration of NPAHs (1-NP and 6-NC) detected in Auckland, the total lifetime excess inhalation cancer risk calculated from PAHs and NPAHs was higher in Auckland than all the other three sampling sites (Table S4.5 of the SI). Based on these findings, the lifetime total carcinogenic risk calculated from PAHs and NPAHs in Auckland (1.32×10^{-6}) slightly exceeded the US EPA guideline values (10^{-6}) (Ramírez *et al.*, 2011), although the atmospheric concentrations of NPAHs were lower than PAHs and risks were calculated using only two NPAHs. Thus, toxicological analysis of NPAH data is urgently required to enable accurate risk assessments for atmospheric NPAH.

4.5 Conclusion

In this study, PAHs and NPAHs in $PM_{2.5}$ were characterized in Japan and New Zealand. Spatial variations of PAHs and NPAHs between urban and rural sites were predominantly due to anthropogenic combustion sources; automobiles were the main contributors of PAHs and NPAHs in both cities, whereas coal combustion and domestic wood burning were the dominant sources of PAHs and NPAHs in rural sites in both Japan and New Zealand. Long range PAH transport was shown from neighboring countries in close proximity to the Wajima site during winter. Although this preliminary study used a limited number of samples, we believe that this study can serve as the foundation for further work in this area, especially in New Zealand where there is a lack of information on PAHs and NPAHs. Further studies and long-term monitoring of air quality as a means to assess the risk of exposure to inhalation of $PM_{2.5}$-bound PAHs and NPAHs are necessary to validate these findings and evaluate mitigation strategies based on the toxicity of atmospheric PAHs and NPAHs bound to inhalable particulates. A positive matrix factorization model should be used in future studies for source apportionment of PAHs and NPAHs.

4.6 Supplemental Information

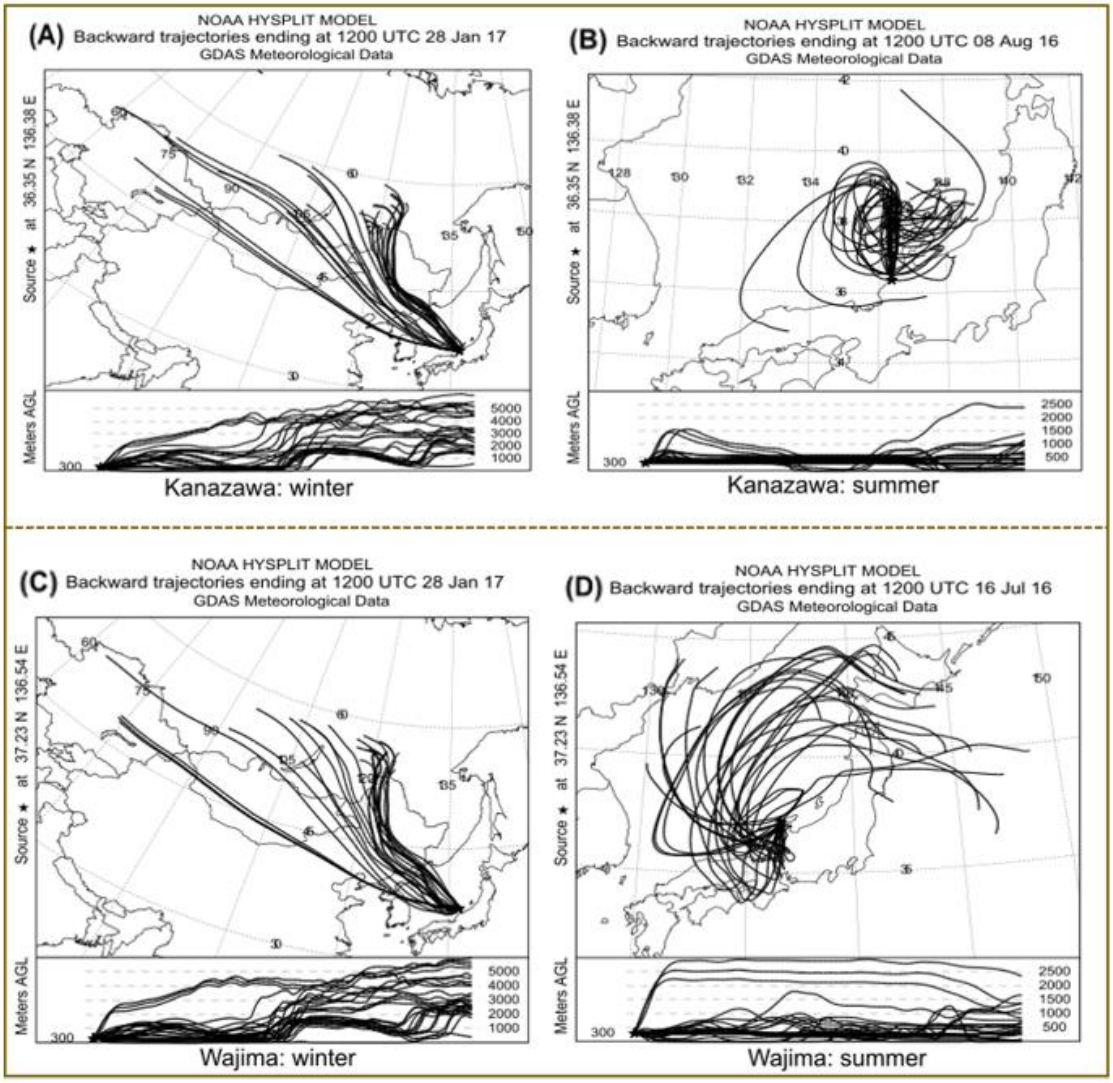

Figure S4. 1. Air mass back trajectories computed using HYSPLIT model for 72 h. Trajectories during (A) winter and (B) summer in Kanazawa City; (C) winter and (D) summer at the Wajima rural area in the Noto Peninsula in Japan. Multiple trajectories indicate different air masses originating from China and Mongolia reaching our sampling sites in Japan.

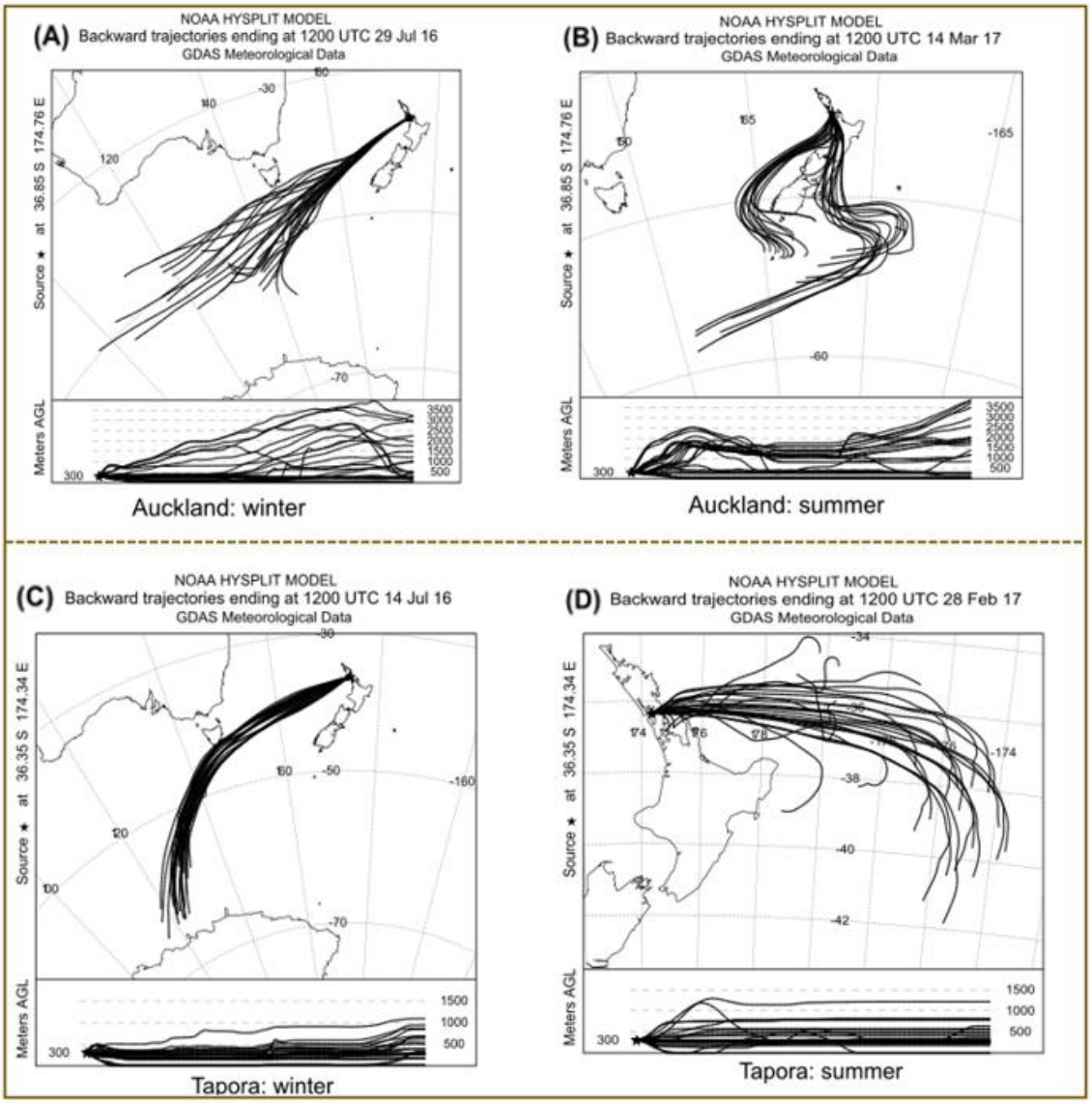

Figure S4. 2. Air mass back trajectories computed using HYSPLIT model for 72 h. Trajectories during (A) winter and (B) summer in Auckland City; (C) winter and (D) summer at Tapora rural site, Okahukura Peninsula in New Zealand. Multiple trajectories indicate different air masses originating from the Pacific Ocean reaching our sampling sites in New Zealand.

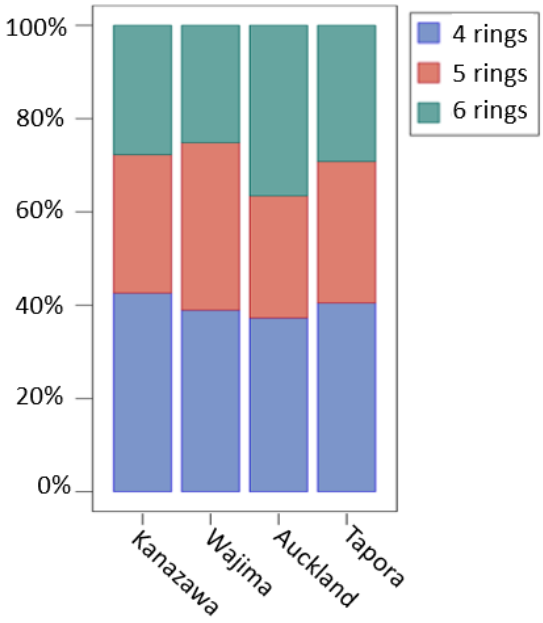

Figure S4. 3. Annual distribution of PAHs detected in $PM_{2.5}$ in Japan (Kanazawa and Wajima) and New Zealand (Auckland and Tapora). 4-ring (Flu, Pyr, BaA, Chr); 5-ring (BbF, BkF, BaP); 6-ring (IDP, BPe).

Table S4. 1. Description of sampling sites based on site type, population, area size, and temperature.

Sites	Type	Coastal setting location	Population ($\times 10^3$)	Area size (km^2)	Temperature (°C) (Summer/winter)
Kanazawa	Commercial, Urban	Sea of Japan	460	467.8	25.1/5
Auckland	Commercial, Urban	Tasman Sea	1400	1086	22.0/11
Wajima	Rural	Noto Peninsula	31	426.2	22.0/2
Tapora	Rural	Okahukura Peninsula	54	97	21.0/10

Table S4. 2. Seasonal variation in concentrations [mean ± SD] of $PM_{2.5}$, PAHs, and NPAHs in Japanese city (Kanazawa) and rural (Wajima) sites.

	Kanazawa City				**Wajima, rural**			
Season	**Autumn**	**Spring**	**Summer**	**Winter**	**Autumn**	**Spring**	**Summer**	**Winter**
$PM_{2.5}$ (μg/m^3)	7.25 ± 0.6	14.85 ± 2.4	15.25 ± 4.3	5.55 ± 8.2	4 ± 1.56	9.4 ± 0.57	6.3 ± 0.85	4.5 ± 0.14
PAH (ng/m^3)								
Flu	0.24 ± 0.10	0.05 ± 0.03	0.03 ± 0.01	0.26 ± 0.06	0.05 ± 0.01	0.03 ± 0.01	0.01 ± 0.00	0.14 ± 0.01
Pyr	0.11 ± 0.03	0.03 ± 0.01	0.02 ± 0.00	0.13 ± 0.03	0.03 ± 0.00	0.02 ± 0.01	0.01 ± 0.00	0.07 ± 0.01
BaA	0.04 ± 0.01	0.01 ± 0.00	0.01 ± 0.00	0.04 ± 0.00	0.01 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.02 ± 0.00
Chr	0.06 ± 0.01	0.02 ± 0.01	0.01 ± 0.00	0.07 ± 0.01	0.02 ± 0.00	0.01 ± 0.01	0.01 ± 0.00	0.04 ± 0.00
BbF	0.14 ± 0.07	0.03 ± 0.02	0.03 ± 0.00	0.11 ± 0.02	0.02 ± 0.00	0.05 ± 0.01	0.01 ± 0.01	0.08 ± 0.02
BkF	0.06 ± 0.03	0.01 ± 0.00	0.01 ± 0.00	0.05 ± 0.01	0.01 ± 0.01	0.02 ± 0.00	0.00 ± 0.00	0.03 ±0.01
BaP	0.08 ± 0.05	0.02 ± 0.01	0.02 ± 0.00	0.04 ± 0.01	0.02 ± 0.00	0.02 ± 0.01	0.01 ± 0.00	0.07 ± 0.01
BPe	0.08 ± 0.05	0.03 ± 0.01	0.02 ± 0.01	0.05 ± 0.01	0.01 ± 0.00	0.02 ± 0.00	0.01 ± 0.00	0.02 ± 0.00
IDP	0.09 ± 0.05	0.02 ± 0.00	0.01 ± 0.00	0.06 ± 0.01	0.01 ± 0.00	0.03 ± 0.00	0.01 ± 0.00	0.04 ± 0.01
Σ-9PAHs (ng/m^3)	**0.89 ± 0.41**	**0.24 ± 0.09**	**0.16 ± 0.03**	**0.80 ± 0.16**	**0.17 ± 0.03**	**0.21 ± 06**	**0.06 ± 0.03**	**0.53 ± 0.08**
NPAH (pg/m^3)								
9-NA	0.67 ± 0.17	0.21 ± 0.14	3.48 ± 4.71	0.75 ± 0.03	0.05 ± 0.02	0.01 ± 0.00	0.01 ± 0.01	0.09 ± 0.04
2-NP+2-NFR	11.25 ± 6.1	3.36 ± 0.20	1.88 ± 0.03	4.50 ± 1.58	1.05 ± 0.64	0.88 ± 0.62	0.40 ± 0.18	2.02 ± 0.88
1-NP	1.15 ± 0.24	0.89 ± 0.20	0.54 ± 0.10	1.29 ± 0.25	0.13 ± 0.05	0.19 ± 0.00	0.03 ± 0.02	0.83 ± 0.00
6-NC	0.57 ± 0.00	0.42 ± 0.01	ND	ND	ND	ND	ND	ND
7-NBaA	0.28 ± 0.10	0.04 ± 0.03	0.03 ± 0.01	ND	0.02 ± 0.00	ND	ND	0.03 ± 0.00
6-NBaP	0.09 ± 0.10	0.05 ± 0.01	0.04 ± 0.02	0.14 ± 1.87	0.02 ± 0.72	0.01 ± 0.00	0.00 ± 0.00	0.06 ± 0.00
Σ-6NPAH (pg/m^3)	**14.1 ± 6.66**	**4.95 ± 0.59**	**5.97 ± 4.86**	**6.68 ± 1.89**	**1.26 ± 0.72**	**1.09 ± 0.62**	**0.45 ± 0.21**	**3.03 ± 0.92**

Σ-9PAHs = Flu+Pyr + BaA + Chr + BbF + BkF + BaP+ BPe + IDP. **Σ**-6NPAH = 9-NA + 2-NP + 2-NFR +1-NP + 6-NC+ 7-NBaA+ 6-NBaP. Each data represents mean ± SD. ND = not detected. PAHs and NPAHs abbreviations are listed in the caption of Figure 4.3.

Table S4. 3. Seasonal variation in concentrations [mean ± SD] of $PM_{2.5}$, PAHs, and NPAHs in Auckland City and Tapora (rural site), New Zealand.

	Auckland City				**Tapora, rural**			
Season	**Autumn**	**Spring**	**Summer**	**Winter**	**Autumn**	**Spring**	**Summer**	**Winter**
$PM_{2.5}$ ($\mu g/m^3$)	6.5 ± 2.12	8.5 ± 2.15	6 ± 0.00	8.5 ± 0.71	4.0 ± 0.00	5.0 ± 0.00	4.5 ± 0.71	6.54 ± 0.84
PAH (ng/m^3)								
Flu	0.07 ± 0.06	0.04 ± 0.01	0.03 ± 0.00	0.21 ± 0.10	0.01 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.07 ± 0.01
Pyr	0.03 ± 0.02	0.03 ± 0.00	0.02 ± 0.00	0.04 ± 0.01	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.02 ± 0.00
BaA	0.02 ± 0.01	0.01 ± 0.00	0.01 ± 0.00	0.02 ± 0.01	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.01 ± 0.00
Chr	0.02 ± 0.01	0.01 ± 0.00	0.02 ± 0.00	0.02 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.01 ± 0.00
BbF	0.05 ± 0.01	0.03 ± 0.00	0.02 ± 0.01	0.07 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.02 ± 0.00
BkF	0.02 ± 0.03	0.01 ± 0.00	0.01 ± 0.00	0.03 ± 0.03	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.01 ± 0.00
BaP	0.03 ± 0.01	0.02 ± 0.00	0.01 ± 0.00	0.03 ± 0.01	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.03 ± 0.00
BPe	0.05 ± 0.02	0.03 ± 0.00	0.04 ± 0.00	0.05 ± 0.02	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.02 ± 0.00
IDP	0.04 ± 0.02	0.02 ± 0.00	0.02 ± 0.00	0.05 ± 0.02	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.02 ± 0.00
Σ-9PAHs (ng/m^3)	**0.33 ± 0.20**	**0.20 ± 0.02**	**0.18 ± 0.03**	**0.53 ± 0.21**	**0.02 ± 0.0**	**0.01 ± 0.00**	**0.01 ± 0.00**	**0.21 ± 0.03**
NPAH (pg/m^3)								
9-NA	1.92 ± 0.72	0.24 ± 0.06	0.18 ± 0.01	3.19 ± 2.09	0.06 ± 0.01	0.01 ± 0.01	0.00 ± 0.00	1.62 ± 0.98
2-NP+2-NFR	4.10 ± 0.32	1.98 ± 0.57	1.53 ± 0.00	5.76 ± 4.59	0.09 ± 0.00	ND	0.06 ± 0.03	3.14 ± 0.84
1-NP	2.28 ± 1.31	2.55 ± 0.12	2.52 ± 0.63	3.07 ± 0.88	0.02 ± 0.01	0.01 ± 0.01	0.03 ± 0.01	0.13 ± 0.05
6-NC	1.51 ± 0.00	2.48 ± 0.05	2.15 ± 0.36	3.84 ± 1.70	ND	ND	ND	0.64 ± 0.05
7-NBaA	1.09 ± 0.58	0.12 ± 0.07	0.01 ± 0.00	2.54 ± 2.76	0.08 ± 0.00	0.01 ± 0.01	0.00 ± 0.00	0.98 ± 0.70
6-NBaP	0.15 ± 0.13	0.08 ± 0.00	0.06 ± 0.01	0.17 ± 0.15	0.01 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.04 ± 0.00
Σ-6NPAHs (pg/m^3)	**11.5 ± 3.06**	**7.44 ± 0.88**	**6.47 ± 1.01**	**18 ± 12.17**	**0.26 ± 0.02**	**0.03 ± 0.01**	**0.10 ± 0.05**	**6.54 ± 2.63**

Σ-9PAHs = Flu+Pyr + BaA + Chr + BbF + BkF + BaP+ BPe + IDP. **Σ**-6NPAHs = 9-NA + 2-NP + 2-NFR +1-NP + 6-NC+ 7-NBaA+ 6-NBaP. Each data represents mean ± SD. ND = not detected.

Table S4. 4. Carcinogenic risk calculated from PAHs and NPAHs in Japan (Kanazawa City and Wajima, rural site) and New Zealand (Auckland City and Tapora, rural site).

		Kanazawa	Auckland	Wajima	Tapora
Analytes	**TEF**	**BaP-TEQ Levels ng/m^3**			
Flu	0.001	0	0	0	0
Pyr	0.001	0	0	0	0
BaA	0.100	0.002	0.002	0.001	0
Chr	0.010	0	0	0	0
BbF	0.100	0.008	0.004	0.004	0.001
BkF	0.100	0.003	0.002	0.001	0
BaP	1.000	0.04	0.022	0.027	0.008
BPe	0.010	0	0	0	0
IDP	0.100	0.005	0.003	0.002	0.001
6-NC	10.000	0.017	0.096	0	0.023
1-NP	0.100	0	0.001	0	0
ΣPAH toxic		0.059	0.034	0.037	0.01
ΣNPAH toxic		0.018	0.098	0	0.023
Cancer risk [PAH]		5.9×10^{-7}	3.4×10^{-7}	3.7×10^{-7}	1.0×10^{-7}
Cancer risk [NPAH]		1.8×10^{-7}	9.8×10^{-7}	0	2.3×10^{-7}
Total cancer risk [PAHs+NPAHs]		**7.7×10^{-7}**	**1.32×10^{-6}**	**3.7×10^{-7}**	**3.3×10^{-7}**

Chapter 5 - Characterization and Risk Assessment of Atmospheric $PM_{2.5}$ and PM_{10} Particulate-Bound PAHs and NPAHs in Rwanda, Central-East Africa

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Chapter 5

5.1 Prelude

Chapter 4 presents the characterization of carcinogenic PAHs and NPAHs in Japan and New Zealand aerosol. In this chapter, the same method used in chapter 4 was applied to compare developed and developing countries. Thus, carcinogenic organic compounds (PAHs and NPAHs) were characterized from ambient PM_{10} and $PM_{2.5}$ in three-land use types in a developing country, Rwanda, located in East and Central Africa (Chapter 5). The chemical signatures at each location were used to identify the likely emission sources, their temporal variations and to evaluate lifetime cancer risks resulting from inhalation exposure to PM-bound PAHs and NPAHs. This is the first study of its kind in Sub-Sahara Africa, providing important information that will allow a more comprehensive assessment of the human exposure risk to PAHs and NPAHs from various sources in urban and rural areas. Findings from this study provide clear evidence that an immediate development of emission control measures is required. Since exposure to ambient air pollution is a global problem, this research conducted in Rwanda produced findings that are relevant world-wide.

5.2 Introduction

Air pollution is the single largest environmental cause of premature mortality worldwide, with annual deaths estimated at seven million (World Health Organization, 2016a). Approximately 90% of these deaths are estimated to occur in low and middle-income countries, particularly sub-Saharan Africa (World Health Organization, 2016a). The World Health Organization (WHO) has air quality programs for most of the world's regions, some of which aim to assist countries in developing sustainable air quality policies. However, little data and no standards around air quality exist for sub-Saharan African countries such as Rwanda (World Health Organization, 2002; United Nations Environment Programme, 2006).

Rwanda is a landlocked country of 26,338 square kilometers and is the second most densely populated country in Africa, with 12 million inhabitants. Despite undergoing a rapid urbanization (DeWitt *et al.,* 2018) there is limited data on its ambient air quality and the identity of primary air pollution sources. The evaluation of particulate air pollution for a country is typically measured by the metrics of $PM_{2.5}$ (particulate matter with aerodynamic diameter less than 2.5 micrometres) that can enter the lungs and PM_{10} (particulate matter with aerodynamic diameter less than 10 micrometres) that are trapped in the nasopharyngeal tract causing adverse health impacts (World Health Organization, 2013). To date, only a single research study on household $PM_{2.5}$ has been conducted in Rwanda, and this estimated that air pollution from cooking fuel combustion was the major risk factor for the countrywide burden of disease (Rosa *et al.,* 2014). No other air quality data (aside for satellite estimates) is available for the rest of the country.

PM contains several toxic organic species including polycyclic aromatic hydrocarbons (PAHs) and nitrated PAHs (NPAHs), which are well known for their carcinogenic and mutagenic properties (Durant *et al.,* 1996). Given their toxicity and their wide distribution, the United States Environmental Protection Agency (US EPA) has classified 16 PAHs as priority compounds (Keith and Telliard, 1979). Their nitro derivatives, which exist at concentrations orders of magnitude lower than PAHs, are nonetheless potentially more dangerous since they do not undergo an initial enzymatic activation process as for PAHs (Durant *et al.,* 1996; Hayakawa *et al.,* 2018b). PAHs and NPAHs are mainly released directly from anthropogenic activities and are

products of incomplete combustion of organic materials such as coal, oil, petrol, and wood (Chang *et al.*, 2006; Ravindra *et al.*, 2008; Abdel-Shafy and Mansour, 2015; Abbas *et al.*, 2018). Although NPAHs can be formed as secondary compounds due to atmospheric reaction of PAHs and atmospheric oxidants such as ozone and nitrate radicals (Arey *et al.*, 1986), they are also suitable as tracers for the identification of ambient air pollution sources and photochemical pathways (Tang *et al.*, 2005). Data on atmospheric PAHs and NPAHs is lacking for sub-Saharan Africa. Only two publications on NPAHs exist for the rest of Africa (Egypt and Algeria) and similar emissions scenarios suggest that NPAH are also likely a previously unmeasured risk in Rwanda (Ladji *et al.*, 2009; Nassar *et al.*, 2011).

In this study, we report atmospheric $PM_{2.5}$ and PM_{10} and associated PAHs and NPAHs from three different usage areas in Rwanda during a three-month period in 2017. The objectives of this study were: (1) to investigate PAH and NPAH abundance and speciation in $PM_{2.5}$ and PM_{10}; (2) to identify their possible sources based on variation of spatial and temporal concentration; and (3) to evaluate lifetime cancer risks resulting from inhalation exposure to PM-bound PAHs and NPAHs from urban and rural areas in Rwanda. This study provides the first empirical evidence for spatio-temporal trends in $PM_{2.5}$ and PM_{10} -bound PAH and NPAH in sub-Saharan Africa and will serve as the basis for the development of standards by the Rwanda Environmental Management Authority (REMA) responsible for air quality monitoring and management.

5.3 Materials and methods

5.3.1 Sampling information

$PM_{2.5}$ and PM_{10} samples were collected daily at three locations in Rwanda (Figure 5.1). The urban background site was located away from emission sources such as industries and broadly representative of city-wide background conditions. The urban roadside site experiences high traffic volumes with frequent gridlock from mototaxis, buses, and personal vehicles. The rural area was situated approximately 100 km away from Kigali, was generally open with agricultural fields, minimal residential area, and distanced as far as possible from roads and industrial. Sampling sites were located on rooftops approximately 10~15 m above ground level. Two seasonal (wet and dry) sampling intervals were conducted at urban background and urban

roadside. Sampling at the rural site was conducted during the wet season only. Details of the sampling sites characteristics were summarized in the Supporting Information (SI) (Table S5.1).

Each sample was collected on a Whatman glass microfiber filter (GFF, Whatman EPM 2000) using high-volume samplers (SIBATA Electric Company Limited, Japan, and HVS-RW-1000F) operated at 1000L/min for a period of 24 hours (from 12:00 am to 12:00 am of the following day). A total of 88 samples ($PM_{2.5}$ and PM_{10}) were collected from the three sites in 2017 and were analyzed for PAHs and NPAHs. Quality control (QC) and quality assurance (QA) detailing all information about filter handling, filter storage and gravimetric analysis were described in Text S5.1 of the SI. Meteorological data including temperature (T), relative humidity (RH), wind speed (WS) and wind direction (WD) for both urban and rural sites were obtained from synoptic weather stations of Rwandan meteorological weather stations situated close to the sampling sites.

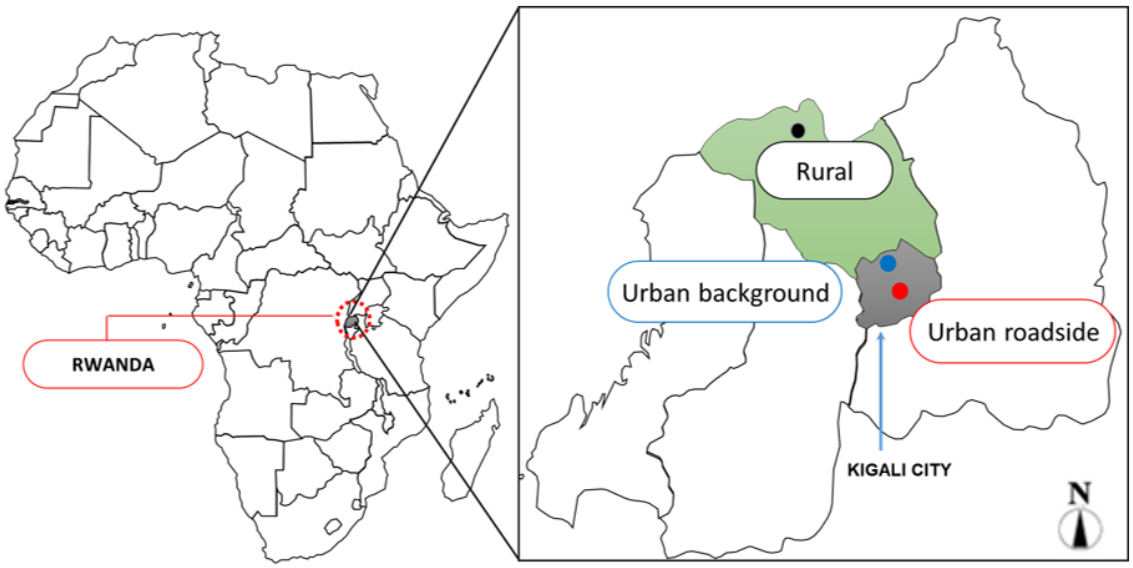

Figure 5. 1 The sampling locations in Rwanda with reference to the African continent. Figure modified from yourfreetemplates.com (full details here: https://yourfreetemplates.com/terms-of-use/).

5.3.2 Extraction and Instrumental Analyses

The extraction, instrumental analyses and standards used have been described in Hayakawa *et al.* (2018b) and are detailed in Text S5.2 of the SI. Briefly, 15 individual PAHs (US EPA 610 PAH

mixture of 16 PAHs except Acenaphthylene (Acyl), which does not fluoresce with the method used) and eight individual NPAHs (two NPAHs such as 2-nitropyrene (2-NP) and 2-nitrofluoranthene (2-NFR) were grouped together because they were not resolved completely). Therefore, 15 PAHs and 7 NPAHs were quantified in samples using two high-performance liquid chromatographic systems (HPLC-10A series, Shimadzu Inc., Kyoto, Japan), with one system equipped with a fluorescence detector and the other equipped with a chemiluminescence detector.

5.3.3 Data analysis

Principal component analysis (PCA) has been successful applied in several aerosol studies to determine the source of PAHs and NPAHs based on spatial distribution of datasets (Guo *et al.*, 2003; Liu *et al.*, 2017; Gupta *et al.*, 2018). In this study, PCA using Statistical Analysis Software (SAS, version 9.4 by SAS institute Inc. Cary.INC. USA) was performed on all PAH and NPAH data as one unit. PCA results were presented by loading and score plots. PCA was used in conjunction with NPAH to PAH diagnostic ratio approaches to further understand the PAH and NPAH concentrations in relation to their potential sources in the atmospheric environment of Rwanda. Three days' back trajectories were calculated using the Hybrid Single Particle Lagrangian Integrated Trajectory (HYSPLIT) model to determine the origin of air masses and establish source-receptor (Tang *et al.*, 2017). As PAH and NPAH data were not normally distributed, a non-parametric test with SAS was used for analysis. Therefore, the Wilcoxon-Mann-Whitney test was used to compare two groups and a Kruskal-Wallis test was used to compare more than two groups.

5.3.4 Environmental health risk assessment

To evaluate the cancer risk of PAHs and NPAHs detected in ambient air in Rwanda, we used a widely applied methodology developed by the US EPA (OEHHA, 2011; Ramírez *et al.*, 2011). In the environment, PAHs are found as mixtures and benzo(a)pyrene (BaP) is used as a PAH reference chemical due to its high carcinogenic potency and its well characterised toxicity (Abdel-Shafy and Mansour, 2015). The carcinogenic risk was, therefore, estimated by using toxic equivalent factors (TEFs) for each PAH or NPAH compared to BaP (Boström *et al.*, 2002). The concentration of each PAH or NPAH was converted to the BaP equivalent (BaPeq, carcinogenic

equivalents, ng/m^3) concentration by multiplying the concentration of PAHs with the corresponding TEF. TEF is the toxic equivalency factor of the compounds (PAHs and NPAHs). TEF values for 15 PAHs and 2 NPAHs were obtained from the literature (Collins *et al.*, 1998; Albinet *et al.*, 2008; OEHHA, 2011).

The excess cancer risk for people living in urban and rural areas in Rwanda was calculated based on EPA guidance for inhalation risk assessment following the equation below (OEHHA, 2011):

Cancer risk (CR) = URBaP × BaPeq

where URBaP is the inhalation cancer unit risk factor of BaP.

The UR BaP (unit risk) is defined as the number of people at higher risk of cancer from inhalational exposure to 1 ng/m^3 of a BaP equivalent concentration over a lifetime of 70 years. The USEPA value of UR BaP is1.1×10^{-6} (ng/m^3)$^{-1}$ and estimates the lifetime cancer risks due to PAH and/or NPAH exposures (Orakij *et al.*, 2017).

5.4 Results and Discussion

5.4.1 Concentrations of $PM_{2.5}$ and PM_{10}

The mean 24-hour concentrations of $PM_{2.5}$ and PM_{10} were higher in urban sites (urban roadside: 185.3 µg/m^3 and 214.0 µg/m^3; and urban background: 81.4 µg/m^3 and 98.7 µg/m^3), while the lowest concentrations were measured at the rural site (45.0 µg/m^3 and 53.7) for $PM_{2.5}$ and for PM_{10}, respectively. At the urban roadside, the 24-hour mean concentration of PM was more than four times the WHO's guideline for $PM_{2.5}$ and for PM_{10} (25 µg/m^3, 50 µg/m^3 respectively) (World Health Organization, 2006a) (Table S5.2 of the SI). A Kruskal Wallis test showed that $PM_{2.5}$ and PM_{10} median concentrations at the urban roadside site were significantly higher than urban background and rural sites (Table S5.3 of the SI). The Wilcoxon-Mann-Whitney test showed that the median 24-hour $PM_{2.5}$ and PM_{10} concentrations were significantly lower in the wet season than the dry season for both urban background and urban roadside sites (Table S5.4 of the SI). At the urban background site, a significant reduction of nearly 55.4% of the median concentration of PM_{10} was measured between dry (146.3 µg/m^3) and wet seasons (65.2 µg/m^3) (p<0.0001) (Table S5.5 of the SI). $PM_{2.5}$ and PM_{10} concentrations were likely elevated during the dry season in

Rwanda due to: (1) higher re-suspension of road dust from unpaved roads during the dry season; (2) the geographical features of Rwanda; (3) long-distance transport of particulates from extensive biomass burning during this season; and (4) local emissions. A detailed explanation of these four factors is provided in Text S5.3 of the SI, (Figure S5.1of the SI).

5.4.2 Atmospheric concentrations of PAHs

The mean of ∑15PAH in $PM_{2.5}$ and in PM_{10} at the urban roadside was 52.4 ng/m^3 and 105.0 ng/m^3, followed by the rural area with 31.5 ng/m^3 and 64.0 ng/m^3, and the urban background with 20.8 ng/m^3 and 23.9 ng/m^3, respectively (Table S5.2 of the SI). Kruskal Wallis tests showed that the median concentrations also followed the same trend (Table S5.3 of the SI). The highest median concentration of PAH was observed at the urban roadside and was higher in the dry season than in the values for urban roadside and urban background sites, the mean concentrations of PAHs detected in $PM_{2.5}$ and PM_{10} were significantly higher at the urban roadside site (Table S5.5 of the SI). The Kruskal Wallis test showed that the mean concentration of PAHs for the three sites during the wet season was significantly higher for the urban roadside than the urban background and rural sites (Table S5.6 of the SI), which suggests the different land uses may have an influence on the composition of these pollutants. As PAHs are known to be major components of vehicle emissions (Miguel *et al.*, 1998), the high mean values at the urban roadside site are strongly associated with the proximity of the site to a main road (KN3 Rd) with high traffic volume and frequent gridlocks.

The low concentration of PAHs measured at the urban background site is likely related to the lower road usage in an urban residential area away from major traffic or bus routes; trucks are also restricted in this area. Additionally, buildings and trees in this area tend to be lower to the ground, avoiding urban canyon effects that trap local pollution. Higher concentrations of PAHs in the rural area during the wet season are likely attributable to the use of wood burning as the primary cooking fuel by the majority of the population, as well as long-range transport of emissions from large-scale biomass burning, which is likely to be significant in this area due to wind trajectory and topography (Hersey *et al.*, 2015; DeWitt *et al.*, 2018). The rural area is mountainous and remote, surrounded by hills, and allows sampling of regional air masses long-

range transported from East Africa (DeWitt *et al.,* 2018). The lack of space in this area forced more of the population to settle at the bottom of the hills and PM-bound PAHs emitted are trapped in the inversion layer. This results in a progressive accumulation of PM-bound PAHs due to poor mixing circumstances. As was expected, the $PM_{2.5}$-bound PAH concentrations varied temporally (7.5 - 95.3 ng/m^3) between the two sites (urban roadside and urban background) and spatially (4.7 - 95.3 ng/m^3) among the three sites (Figure 5.2). The concentrations found in this study were generally lower than those previously reported in urban areas of Uganda and Kenya (Muendo *et al.,* 2006; Arinaitwe *et al.,* 2012), despite close proximity. This could be due to larger populations, greater industrialization, and the prevalence of fossil fuel power stations and diesel vehicles in those two countries compared to Rwanda (DeWitt *et al.,* 2018).

The variations in total PAHs with daily average temperature ranges at urban background (20.8 ± 3.6°C), urban roadside (21.1 ± 3.7°C) and at rural (14.8 ± 2.6°C) sites for the dates of sampling are presented in Table S5.7 of the SI. This is a very narrow range of temperature variation compared to other countries. Figure S5.2 of the SI showed that all meteorological conditions represented general conditions during all three-month sampling periods at all three sites. The wind direction during sampling showed a predominance of northerly and easterly winds at both urban sites and westerly and southerly winds at the rural site (Figure S5.3 of the SI). This suggests that local emissions were more likely to influence the PAH and NPAH levels observed in urban and rural samples. The highest concentrations of PAHs and NPAHs were recorded in June in this study and at the same time as the large-scale biomass burning changes from north-central Africa to southern Africa. Computed trajectories (Figure S5.1 of SI) showed that samples at urban sites were mainly associated with fast-moving southerly and easterly air masses.

To understand the impact of human activities on the concentration of PAHs, we investigated PAH concentrations based on work (n=60) and no-work days (combination of all public holidays, car-free days and weekends: n=30) (Table S5.8 of the SI, Figure 5.2). The Wilcoxon signed-rank test showed that the mean concentration of PAHs in $PM_{2.5}$ on workdays (37.9 ng/m^3) was significantly higher compared to non-work days (27.9 ng/m^3) (p=0.003). Car-free days, public holidays and

weekends notably reduced PM and PAHs concentrations due to a reduction in human activity (Figure 5.2 and Figure S5.4).

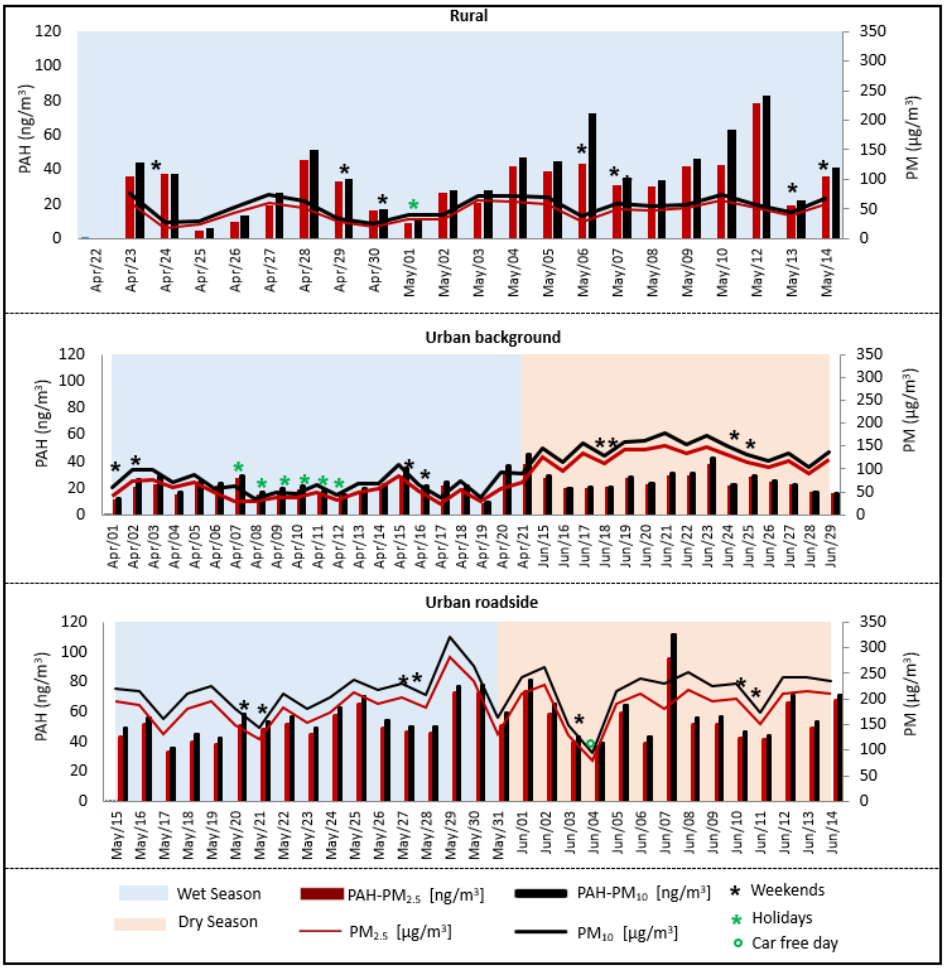

Figure 5. 2. Seasonal variation of polycyclic aromatic hydrocarbons (PAHs) detected in $PM_{2.5}$ and PM_{10} at three sites: rural, urban background, and urban roadside.

High concentrations of dominant PAH compounds in this study were fluoranthene (Flu), BaP, benzo(b)fluoranthene (BbF) and benz(g,h,i)perylene (BPe) (Figure S5.5 of the SI), which have been previously observed in East African and East Asian countries influenced by biomass burning and vehicle emissions (Arinaitwe *et al.*, 2012; Hayakawa, 2018a). BaP is classified as group 1 carcinogen for humans (Błaszczyk *et al.*, 2017). In this study, the mean concentrations of BaP detected in $PM_{2.5}$ and in PM_{10} at the urban roadside area, urban background and rural area (Table S5.1 and Table S5.9 of the SI) were higher than that recommended by WHO guidelines (1 ng/m^3) (World Health Organization, 2000).

Concentration data in this study for BaP were comparable to those observed in Kenya and in Hong Kong, where BaP was associated with domestic fuel burning (Guo *et al.*, 2003; Muendo *et al.*, 2006).

5.4.3 Atmospheric concentrations of NPAHs

The effect of vehicle emissions on NPAH concentration was evidenced during car-free days and public holidays in Kigali City. The measured NPAH mean concentration detected in PM_{10} during workdays (528.3 pg/m^3) was significantly reduced by 24.7% on no-work days (397.5 pg/m^3) (p = 0.0468) (Table S5.8 of the SI, Figure S5.4).

The mean concentrations of ∑NPAH in $PM_{2.5}$ and PM_{10} were higher at the urban roadside site (872.4 pg/m^3 and 892.6 pg/m^3) than the urban background (289.4 pg/m^3 and 310.5 pg/m^3) and rural sites (114.4 pg/m^3 and 128.5 pg/m^3) (Table S5.2 of the SI). The Kruskal Wallis test showed that the mean concentrations of NPAHs at the urban roadside (Table S5.3 of the SI) were significantly higher than the urban background and rural sites (p<0.0001). This was expected due to the proximity of the sampling site to emission sources (high traffic volumes) and vehicle emissions were reported as a major source of NPAHs (Keyte, Albinet and Harrison, 2016). The Wilcoxon-Mann-Whitney test showed that the NPAHs in $PM_{2.5}$ and in PM_{10} median concentrations were significantly lower in wet than in dry seasons for urban roadside and urban background sites (Table S5.4 of the SI). Comparing the three sites during the wet season, the Kruskal Wallis test showed that the mean concentration of NPAHs was significantly higher at the urban roadside than at urban background and rural sites (Table S5.6 of the SI). The concentrations of the total NPAH fell into a narrow range of 0.007 - 2.1 ng/m^3, a much lower variation than the total PAHs (4 - 95 ng/m^3). These observations were consistent with previous measurements of NPAHs in Egypt and in Algeria, which were typically 10-1000 times lower than the PAHs (Ladji *et al.*, 2009; Nassar *et al.*, 2011).

9-Nitroanthracene (9-NA) and 2-NP+2-NFR were the most abundant NPAH compounds identified in this study (Figure S5.5 of the SI, Table S5.9 of the SI). 9-NA can be derived from primary emissions as well as from secondary formation (Ringuet *et al.*, 2012). The highest

concentration of 9-NA was found at the urban roadside site, followed by urban background and rural sites and levels were higher in the dry season than the wet season (Table 5.1 and Table S5.9 of SI). High concentrations of 9-NA have been shown to result from direct emission from diesel vehicles, whereas its dominant source at a semirural site was atmospheric secondary formation (Feilberg *et al.,* 2001). The importance of various sources of NPAHs in the atmosphere has been investigated using correlation analyses (Feilberg *et al.,* 2001). The inter-correlations of selected NPAHs and PAHs are presented in Table S5.10 of SI for all three sampling sites and both seasons. The 9-NA concentrations in this study were significantly correlated with 2-NP+2-NFR at all sites, suggesting that the generation pathway for 9-NA was similar to that of 2-NP+2-NFR, which is most likely to occur by atmospheric formation. Interestingly, 9-NA significantly correlated with 1-nitroperylene (1-NP) at all sites, suggesting that the dominant source of 9-NA was also from direct emissions. Several reports have also suggested the generation of 1-NP from direct emission of diesel exhaust (Ratcliff *et al.,* 2010). However, the formation of 9-NA can also occur through heterogeneous reactions of nitrating agents with Ant during combustion processes (biomass burning or vehicle emissions) associated with PM rather than via gas-phase photochemical reactions in ambient air (Liu *et al.,* 2017). Thus, high levels of 9-NA at the urban roadside site were likely to be emitted directly from old diesel-powered vehicles in Rwanda. 9-NA adheres readily to particles (Liu *et al.,* 2017), similar to 2-NFR, which is also likely to occur through heterogeneous reactions of Flu. In this study, the highest concentration of Ant and Flu coincided with the highest concentration of 9-NA and 2-NFR, especially at the urban roadside site. Ant and Flu may be produced at higher levels during combustion processes and influence heterogeneous reactions that form 9-NA and 2-NFR, respectively. The highest concentration of 9-NA and 2-NP+2-NFR might have also been produced by biomass burning (wood) at low temperatures, which produced high levels of Ant and Flu at the rural site, and by vehicle emissions at the urban sites.

NPAHs such as 1,3 and 1,8-dinitropyrenes (1,3 - and 1,8-DNPs),1-NP, 7-nitrobenz(a)anthracene (7-NBaA) and 6-nitrobenzo(a)pyrene (6-NBaP) are directly emitted from vehicles, especially diesel engines (Hayakawa *et al.,* 1995; Bamford and Baker, 2003). The highest proportions of

these NPAHs were detected at the urban roadside site, suggesting that vehicle exhausts were a major contributor of NPAHs. 1-nitroperylene (1-NP), classified as group 2A carcinogenic (International Agency for Research on Cancer, 2018), was higher at the urban roadside than at urban background and rural sites. 1-NP has been identified as the principal NPAH present in diesel emissions (Ratcliff *et al.,* 2010; Hayakawa, 2018a) and in lower emissions from gasoline vehicles (Arey *et al.,* 1986). The mean concentration of 1-NP in $PM_{2.5}$ and PM_{10} at urban roadside (TableS5. 1 and Table S5.9 of the SI) was higher than previously reported in Egypt (0.7 pg/m^3) and Algeria (0.97 pg/m^3) (Ladji *et al.,* 2009; Nassar *et al.,* 2011), suggesting large contributions from diesel vehicles in the urban environments of Rwanda. The increase in the relative contribution of diesel vehicles and older diesel generators in the Rwanda traffic fleet may therefore have a stronger influence on changes in observed NPAH.

Table 5. 1. Concentrations (mean ± SD) of $PM_{2.5}$-bound PAHs and NPAHs during the dry and wet seasons at three sites in Rwanda.

		Urban background		**Urban Roadside**		**Rural**
		DRY	**WET**	**DRY**	**WET**	**WET**
		n= 15	n = 21	n = 14	n = 17	n = 21
PAH (ng/m^3)	NaP	0.08 ± 0.03	0.17 ± 0.11	0.40 ± 0.10	0.41 ± 0.13	0.45 ± 0.25
	Ace	0.02 ± 0.01	0.03 ± 0.02	0.05 ± 0.01	0.05 ± 0.02	0.08 ± 0.05
	Fle	0.13 ± 0.06	0.11 ± 0.05	0.24 ± 0.09	0.24 ± 0.17	0.14 ± 0.06
	Phe	1.85 ± 0.49	0.67 ± 0.33	3.60 ± 3.46	2.61 ± 1.17	1.80 ± 5.81
	Ant	0.03 ± 0.01	0.02 ± 0.01	0.09 ± 0.04	0.11 ± 0.03	0.02 ± 0.01
	Flu	6.08 ± 1.60	5.13 ± 2.00	9.42 ± 2.32	9.50 ± 2.34	9.58 ± 10.21
	Pyr	0.57 ± 0.19	0.22 ± 0.06	0.68 ± 0.22	0.65 ± 0.22	0.23 ± 0.21
	BaA	0.54 ± 0.16	0.27 ± 0.12	1.16 ± 0.62	1.03 ± 0.36	0.46 ± 0.38
	Chr	0.75 ± 0.20	0.37 ± 0.16	1.31 ± 0.67	1.17 ± 0.42	0.67 ± 0.61
	BbF	2.67 ± 0.70	2.17 ± 0.80	5.18 ± 1.30	5.44 ± 0.94	3.51 ± 1.61
	BkF	1.12 ± 0.31	0.99 ± 0.38	2.32 ± 0.57	2.41 ± 0.43	1.49 ± 0.67
	BaP	2.15 ± 0.66	2.25 ± 1.26	11.28 ± 4.91	7.53 ± 2.11	4.62 ± 2.38
	DBA	0.06 ± 0.06	0.05 ± 0.02	0.10 ± 0.03	0.08 ± 0.03	0.07 ± 0.03
	BPe	4.80 ± 1.31	4.98 ± 1.76	15.12 ± 3.45	14.77 ± 3.85	7.22 ± 3.11
	IDP	2.08 ± 0.53	2.07 ± 0.71	3.98 ± 1.25	4.48 ± 0.76	2.80 ± 1.13
15 PAHs (ng/m^3)		**22.95 ± 5.59**	**19.26 ± 6.79**	**54.93 ± 16.57**	**50.49 ± 11.22**	**32.53 ± 17.83**
NPAHs (pg/m^3)	1,8-DNP	9.60 ± 4.78	21.83 ± 54.79	141.23 ± 127.19	158.83 ± 152.31	7.71 ± 2.87
	1,3-DNP	0.66 ± 0.33	0.42 ± 0.31	2.17 ± 1.21	1.30 ± 0.67	0.30 ± 0.14
	9-NA	243.48 ± 122.87	93.49 ± 74.98	522.70 ± 323.98	214.48 ± 147.17	59.02 ± 34.54
	2-NP+2-NFR	154.73 ± 73.78	84.41 ± 50.14	375.44 ± 176.30	277.11 ± 108.37	85.07 ± 43.29
	1-NP	4.89 ± 2.41	2.57 ± 1.36	26.24 ± 17.06	18.18 ± 9.28	2.94 ± 1.40
	7-NBaA	59.61 ± 36.74	13.24 ± 13.18	109.34 ± 63.49	48.96 ± 37.12	ND
	6-NBaP	0.91 ± 0.48	ND	3.75 ± 2.03	2.43 ± 1.39	ND
7 NPAHs (pg/m^3)		**428.36 ± 262.24**	**190.14 ± 142.29**	**1128.79 ± 529.53**	**661.35 ± 439.70**	**129.38 ± 80.46**

15PAHs = Nap + Ace+ Fle+ Phe + Ant + Flu+ Pyr + BaA + Chr + BbF + BkF + BaP + DBA+ BPe + IDP. 7NPAH = 1,8-DNP, 1.3-DNP, 9-NA + 2-NP + 2-NFR +1-NP +7-NBaA+ 6-NBaP. Each data represents mean ± SD. ND, not detected. PAHs and NPAHs abbreviations are listed in the caption of Figure S5.5.

5.4.4 Source analysis of PAHs and NPAHs using diagnostic ratio

Different types of combustion have been shown to generate different distributions of PAHs and NPAHs isomers. In addition, the formation of NPAHs from their parent PAHs depends on increasing combustion temperature, meaning that the composition of mixed NPAHs to PAHs ratio is a useful indicator of their source (Tang *et al.*, 2005; Hayakawa *et al.*, 2018b). A diagnostic ratios analysis (Table S5.11 of the SI) indicates that all three locations are influenced by secondary formation and local emission from different combustion sources such as cooking fires, solid biomass fuel, agricultural fires and vehicle emissions, although to varying degrees. The [1-nitroperylene]/[pyrene] or [1-NP]/[Pyr] ratios of particulates from coal stoves (0.001) and automobiles (0.36) have been found to be significantly different (Tang *et al.*, 2005). In addition, [7-nitrobenz(a)anthracene]/[benz(a)anthracene] or [7-NBaA]/[BaA] ratios in coal-combustion of particulates are also much smaller than those in automobile exhaust particulates (Hayakawa *et al.*, 2016b). The [1-NP]/[Pyr] ratio values for urban roadside location during dry (0.05) and wet seasons (0.04) were higher than those at the urban background location (0.01) and rural area (0.01), suggesting that vehicular emissions were the dominant sources at the urban roadside location. These ratio values are similar to those reported in Cairo, Egypt during winter (0.06) and summer (0.03) as well as Kanazawa, Japan (0.03) and Beijing, China (0.02) where diesel-engine exhaust was found to be the major source of PAHs and NPAHs. Although the number of registered vehicles in Rwanda (80,642) is extremely low compared to other developed countries, [1-NP]/[Pyr] values in Rwanda are comparable to or even higher than western developed countries, likely due to the prevalence of older and less efficient heavy diesel vehicles, buses and moto taxis.

The higher [7-NBaA]/[BaA] ratios for urban background (0.11) and urban roadside (0.09) sites during the dry season, compared to the rural area (<0.01), suggest that vehicle emissions were the major contributors of PAHs and NPAHs in those areas as opposed to the ratios expected from coal stove usage (0.00063- 0.0057) (Huang *et al.*, 2014). The [7-NBaA]/[BaA] ratio in Kigali City was similar to that in East Asian cities (Tokyo, Sapporo, Kanazawa and Seoul), where the major

contributors of PAHs and NPAHs are automobiles (mainly diesel-engine vehicles) (Hayakawa, 2009). In this study, a contribution of 9-NA from biomass burning, designated as >10 of [9-Nitroanthracene]/[1-nitroperylene] or [9-NA]/[1-NP] ratio (Chuesaard *et al.*, 2014), was observed in all sites, with higher values at urban background and rural sites (Table S5.10 of the SI).

Further analysis of the PAH source at the three sites during the dry and wet seasons was performed using selected PAH diagnostic concentration ratios. In this study, a [fluoranthene]/([pyrene]+[fluoranthene]) or [Flu]/([Pyr]+[Flu]) ratio > 0.5 and [benz(a)anthracene]/([chrysene]+[benz(a)anthracene]) or [BaA]/([chrysene(Chr)]+[BaA]) ratio >0.35 (Table S5.10 of the SI) were consistent with the PAH source, mainly from vehicle exhaust emissions (diesel and gasoline engine) and wood burning (Schauer *et al.*, 1996; Manoli, Kouras and Samara, 2004). [Flu]/([Pyr]+[Flu]) ratios > 0.5 have been reported to suggest combustion of wood and kerosene (Tobiszewski and Namieśnik, 2012; Yunker *et al.*, 2002). This has also been reported in savanna fire particulates. Back trajectory analysis indicated that Rwanda's air quality could experience increase PM from transported savanna and forest fire emissions from nearby East African countries (Figure S5.1 of the SI). The [BaA]/([Chr]+[BaA]) ratio values of 0.38 - 0.64, >0.35 and 0.42 - 0.62 have been proposed to result from diesel engines, gasoline engines and wood burning, respectively (Schauer *et al.*, 1996; Dickhut *et al.*, 2000; Oda *et al.*, 2001). The values obtained in dry and wet seasons at the three sites suggest that vehicle exhaust emissions (diesel and gasoline engine) and wood burning were the dominant sources of PAHs in urban and rural sites, respectively.

In addition to the primary combustion sources, NPAHs may also originate from atmospheric secondary formation (Atkinson and Arey, 1994). The concentration ratio of 2-nitrofluoranthene [2-NFR]/1-nitropyrene [1-NP] or [2-NFR]/[1-NP] has been used to investigate the relative contribution of NPAHs from primary formation vs secondary formation. A [2-NFR]/[1-NP] value >5 indicates a strong contribution from secondary formation while a value <5 indicates a strong contribution from direct emissions (Amador-Muñoz *et al.*, 2011). In this study, the analytical method did not separate 2-NP and 2-NFR. Several studies have used 2-NFR combined with other NPAHs compounds (that were not separated chromatographically) over 1-NP instead of [2-

NFR]/[1-NP].(Jiang *et al.,* 2018) Since the concentration of 2-NP is much lower than that of 2-NFR in the atmosphere (Murahashi and Hayakawa 1997; Murahashi *et al.*, 1999) we used [2-NP+2-NFR]/[1-NP] instead of [2-NFR]/[1-NP] to examine possible secondary formation vs primary formation. A [2-NP+2-NFR]/[1-NP] ratio >5 was observed at all three sites (Table S5.11 of the SI) and was highest at the urban background site, followed by the rural area and urban roadside, providing further possible evidence for the contribution of NPAH secondary formation. This indicates that there may be substantial secondary background formation in areas with lower volumes of road traffic, which is consistent with a previous study that indicated [2-NFR]/[1-NP] ratios are generally higher at suburban sites relative to their proximate urban sites (Marino *et al*., 2000).

5.4.5 Source analysis of PAHs and NPAHs using PCA

To further elucidate the potential sources of PAHs and NPAHs, PCA was used in conjunction with NPAH to PAH diagnostic ratio analysis (Figure 5.3). PCA results indicated that diesel and gasoline-powered vehicles at the urban locations and wood burning at the rural location were the major sources of PAHs and NPAHs and were consistent with diagnostic ratio source analysis. Detailed results and discussion of PCA source analysis are found in Text S5.4 of the SI. In addition, PCA was performed on all PAHs and NPAHs detected in both dry and wet seasons, suggesting a seasonal effect on the concentrations, especially at the urban background with lower volumes of road traffic (Figure S5.6 of the SI).

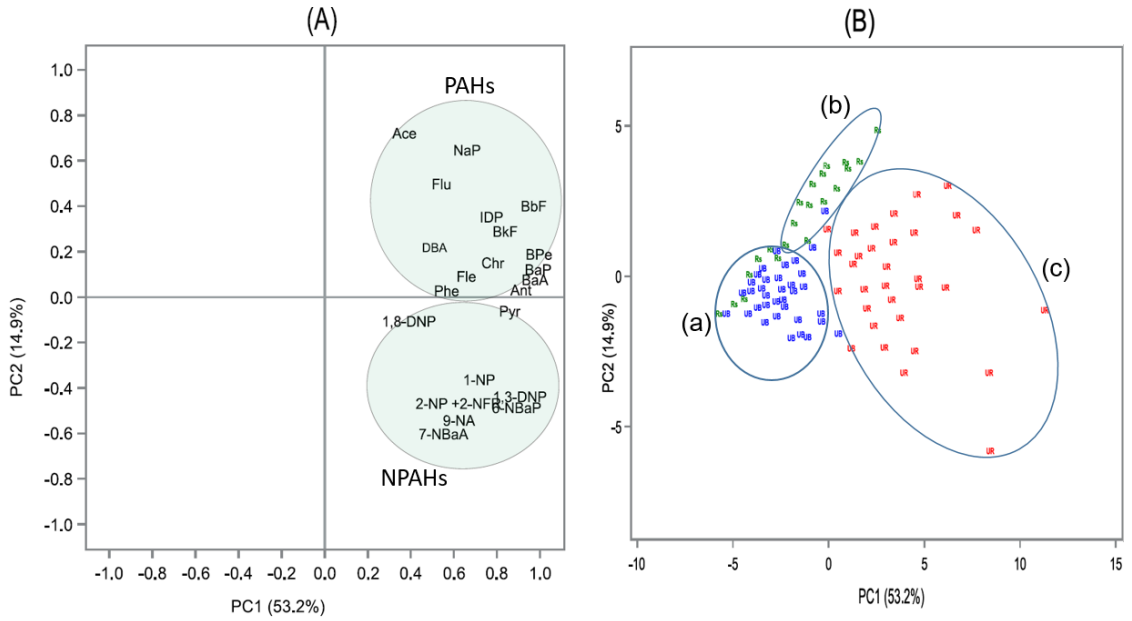

Figure 5. 3. Multivariate analysis of particulate-bound polycyclic aromatic hydrocarbons (PAHs) and nitrated PAHs (NPAHs) at three sites in Rwanda. Principal component analysis (PCA) A) loading plots and B) score plots for the first two components of all PAHs and NPAH from three sites: (a) urban background (UB = blue), (b) rural (Rs = green) and (c) urban roadside (UR = red).

5.4.6 Risk assessment

The estimated cancer risks for the three sites were calculated from the concentrations of 15 PAHs and the 2 most carcinogenic NPAHs (1-NP and 1.8-DNP). Total BaP equivalents (BaPeq) for the study period were highest at the urban roadside site, followed by the rural site and urban background (Table S5.12 of the SI). These results are comparable with other observations in Turkey (11 ng/m^3) during non-heating periods (Gaga *et al.*, 2012) but relatively higher than the values obtained in western developed countries such as the United States (0.450 ng/m^3) (Jung *et al.*, 2010) and Italy (0.79 ng/m^3) (Martellini *et al.*, 2012). There is a lower BaPeq carcinogenicity in western developed countries than that obtained in Rwanda, which can be attributed to rigorous emission standards, cleaner energy sources, more efficient industrial technology and lower populations. The atmospheric concentrations of total NPAHs were lower than those of total PAHs (Table S5.12 of the SI), and the risks were calculated using only two NPAHs. The total cancer risk calculated from 15 PAHs and 2 NPAHs was much higher at the urban roadside site than in urban background and rural sites (Table S5.12 of the SI). These values obtained at each site exceeded the USEPA guideline (10^{-6}) (Ramírez *et al.*, 2011) which suggests adverse health effects and would be classified as a definite risk (a carcinogenic risk greater than 1×10^{-4}) (Sexton

et al., 2007). Because abundant NPAHs such as 9-NA, 2-NP+2-NFR, were not determined and were excluded from the calculations due to the unavailability of TEF values, the values presented in this study are underestimated risks. Thus, toxicological analysis of NPAH data is urgently required to enable accurate risk assessments for atmospheric NPAHs. Overall, better monitoring of air quality as a means to assess the risks of human exposure to PM-bound PAHs and NPAHs would enable the development of strategies to reduce the air pollution health burden and facilitate urban planning. Future studies should use other methods of source apportionment available for larger datasets such as positive matrix factorization[1].

5.5 Supplemental Information

5.5.1 Supplementary Tables

Table S5. 1. Selected characteristics of urban and rural sites shown in Figure S5.1 and details of sample collection at each site along with the period and season.

	Kigali City		Musanze District
Sites	**Urban background**	**Urban roadside**	**Rural**
Coordinates	1.9516S, 30.0653E	1.9616S, 30.0559E	1.5364S, 29.67402E
Population	1132686		100000
Density	1,600/ km^2		690/km^2
Source of energy for cooking	Charcoal/firewood/gas	Commercial, urban	Firewood/agriculture fires
Sampling (Wet season)	01 April to 21 April 2017	15 May to 31 May 2017	23 April to 14 May 2017
Sampling (Dry season)	15 June to 30 June 2017	01 June to 14 June 2017	***

*** sampling at the rural site was conducted during wet season only.

[1] **Conclusion**: This study examined the levels, source and health risk of PAHs and NPAH in atmospheric PM for the first time in Rwanda. The mean concentrations of ΣPAHs and ΣNPAHs were higher at urban roadside than urban background and rural areas. Diesel and gasoline-powered vehicles in the urban locations and wood burning in rural location were the major sources of PAHs and NPAHs. The lifetime cancer risks resulting from inhalation exposure to PM-bound PAHs and NPAHs exceeded the WHO guidelines. This study has provided quantitative information for spatio-temporal trends in $PM_{2.5}$ and PM_{10} -bound PAH and NPAH that will serve as the basis for the development of air quality standards in Rwanda.

Table S5. 2. Spatial variation of $PM_{2.5}$, PM_{10}, PAHs and NPAHs (mean ± SD) concentration at three sites.

Analytes	Urban roadside (n=31)	Urban background (n=36)	Rural (n=21)
$PM_{2.5}$ [µg/m^3]	185.3 ± 39.7	81.4 ± 40.9	45.0 ± 15.7
PAH in $PM_{2.5}$ [ng/m^3]	52.4 ± 13.8	20.8 ± 6.5	31.5 ± 16.4
NPAH in $PM_{2.5}$ [pg/m^3]	872.4 ± 529.7	289.4 ± 230.8	114.5 ± 72.2
PM_{10} [µg/m^3]	214.0 ± 43.1	98.7 ± 43.6	53.7 ± 17.1
PAH in PM_{10} [ng/m^3]	105.0 ± 27.6	23.9 ± 7.73	64.0 ± 33.9
NPAH in PM_{10} [pg/m^3]	892.6 ± 537.5	310.5 ± 236.9	128.5 ± 66.3

Table S5. 3. Comparison of $PM_{2.5}$, PM_{10}, PAHs and NPAHs median (Q1-Q3) concentration using Kruskal Wallis test.

Analytes	Urban roadside (n=31)	Urban background (n=36)	Rural (n=21)	*p*-value
$PM_{2.5}$ [µg/m^3]	190.7(153.5-211.6)	72.6(48.9-119.7)	50.6(32.7-58.9)	< 0.0001
PAH in $PM_{2.5}$ [ng/m^3]	50.5(42.0-59.2)	19.9(16.1-25.8)	33.4(19.2-41.6)	< 0.0001
NPAH in $PM_{2.5}$ [pg/m^3]	789.5(411.3-1125)	195.5(133.8-441.1)	103.9(66.4-159.5)	< 0.0001
PM_{10} [µg/m^3]	221.2(179.8-240.8)	94.8(62.7-134.7)	58.0(39.1-69.9)	< 0.0001
PAH in PM_{10} [ng/m^3]	101.1(83.9.0-118.4)	22.7(19.5-28.6)	66.7(38.4-83.1)	< 0.0001
NPAH in PM_{10} [pg/m^3]	808.0(425.7-1142)	213.0(145.5-493.7)	124.2(76.0-181.2)	< 0.0001

Table S5. 4. Comparison of $PM_{2.5}$, PM_{10}, PAHs and NPAHs median concentration between dry and wet season at each site using Wilcoxon-Mann-Whitney tests.

	Analytes	**Wet season Median (IQR)**	**Dry season Median (IQR)**	***p*-value**
Rural	$PM_{2.5}$ [µg/m^3]	50.6 (31.7-58.9)	-	n/a
	PAH in $PM_{2.5}$ [ng/m^3]	33.4 (19.2-41.6)	-	n/a
	NPAH in $PM_{2.5}$ [pg/m^3]	103.9 (66.4-159.5)	-	n/a
Urban background	$PM_{2.5}$ [µg/m^3]	49.9 (38.2-59.6)	126.3 (112.7-141.0)	0.2354
	PAH in $PM_{2.5}$ [ng/m^3]	18.7 (14.8-21.8)	21.6 (18.9-26.8)	0.5430
	NPAH in $PM_{2.5}$ [pg/m^3]	151.5 (129.8-198.3)	404.5 (251.7-626.4)	0.0155
Urban roadside	$PM_{2.5}$ [µg/m^3]	184.4 (153.5-194.8)	205.8 (181.1-211.7)	<0.0001
	PAH in $PM_{2.5}$ [ng/m^3]	48.9 (44.5-51.4)	51.4 (41.5-66.1)	0.0759
	NPAH in $PM_{2.5}$ [pg/m^3]	466.5 (346.5-1034)	902.4 (688.3-1396)	0.0052
Rural	PM_{10} [µg/m^3]	58.0 (39.1-69.9)	-	n/a
	PAH in PM_{10} [ng/m^3]	66.7 (38.4-83.1)	-	n/a
	NPAH in PM_{10} [pg/m^3]	124.2 (75.9-181.2)	-	n/a
Urban background	PM_{10} [µg/m^3]	65.2 (47.4-88.1)	146.3 (129.5-160.0)	<0.0001
	PAH in PM_{10} [ng/m^3]	21.8 (17.5-25.8)	23.6 (20.0-28.8)	0.3585
	NPAH in PM_{10} [pg/m^3]	162.5 (126.5-225.6)	413.5 (260.1-642.1)	0.0047
Urban roadside	PM_{10} [µg/m^3]	211.6 (179.8-225.4)	232.4 (216.0-241.8)	0.1577
	PAH in PM_{10} [ng/m^3]	97.2 (88.9-102.8)	102.8 (83.0-132.4)	0.7382
	NPAH in PM_{10} [pg/m^3]	482.1 (362.2-1053.3)	921.4 (708.6-1416.9)	0.0208

Table S5. 5. Comparison of PAHs and NPAHs detected in $PM_{2.5}$ and in PM_{10} (mean ± SD) concentration between urban background and urban roadside sites during the dry season.

Analytes	**Urban roadside (n=15)**	**Urban background (n=14)**	***p*-value**
PAH in $PM_{2.5}$ [ng/m^3]	54.9 ± 16.6	22.9 ± 5.6	<.0001
NPAH in $PM_{2.5}$ [pg/m^3]	1128.8 ± 529.5	428.3 ± 262.2	0.0001
PAH in PM_{10} [ng/m^3]	109.8 ± 33.1	25.1 ± 6.8	<.0001
NPAH in PM_{10} [pg/m^3]	1155.1 ± 540.6	445.3 ± 266.9	0.0001

Table S5. 6. Comparison of PAH and NPAH concentrations detected in $PM_{2.5}$ and in PM_{10} (mean ± SD) for three sites during the wet season using the Kruskal Wallis test.

Analyte	**Urban roadside (n=17)**	**Urban background (n=21)**	**Rural (n=21)**	***p*-value**
PAH in $PM_{2.5}$ [ng/m^3]	50.5 ± 11.2	19.3 ± 6.8	31.5 ± 16.4	< 0.0001
NPAH in $PM_{2.5}$ [pg/m^3]	661.3 ± 439.1	190.1 ± 142.3	114.2 ± 72.2	0.0005
PAH in PM_{10} [ng/m^3]	101.0 ± 22.4	23.1 ± 8.4	64.0 ± 33.9	< 0.0001
NPAH in PM_{10} [pg/m^3]	676.6 ± 440.9	209.5 ± 151.2	145.6 ± 86.3	0.0006

Table S5. 7. Meteorological parameters during sampling period.

Meteorological parameters	**April (Wet)**	**May (Wet)**	**June (Dry)**
Temperature (min-max), °C	9.2-29.1	10.7-29.1	14.4-30.1
Temperature (mean ± SD), °C	18.7 ± 4.2	18.1 ± 4.3	21.5 ± 3.8
Wind speed (min-max), m/s	0 - 33.2	0 -19.4	0-14.2
Wind speed (mean ± SD), m/s	2.1±1.9	1.8 ± 1.2	2.3 ± 1.4
Relative humidity (min-max), %	42.3-100	34.8-100	32.2-95.3
Relative humidity (mean ± SD), %	79.5 ± 14.7	77.9 ± 16.9	59.5 ± 15.5
Meteorological parameters	**Urban background**	**Urban roadside**	**Rural**
Temperature (min-max), °C	14.2 - 29.4	14.3 - 30.1	9.2 - 21.6
Temperature (mean ± SD), °C	20.8 ± 3.6	21.1 ± 3.7	14.8 ± 2.6
Wind speed (min-max), m/s	0 - 33.2	0 -19.4	0 -5.6
Wind speed (mean ± SD), m/s	2.4 ± 1.9	2.2 ± 1.4	1.3 ± 0.6
Relative humidity (min-max), %	32.2 - 99.5	33.0 - 96.3	55.9-100
Relative humidity (mean ± SD), %	69.0 ± 18.1	65.8 ± 16.3	86.9 ± 11.6

Table S5. 8. Comparison of mean concentrations of total $PM_{2.5}$, PM_{10}, PAHs and NPAHs during workdays and non-work days.

	Workdays	**No -work days**	
Analyte	**[mean ± SD]**	**[mean ± SD]**	***p* -Value**
	n =60	n=30	
$PM_{2.5}$ [µg/m^3]	122.9 ± 70.3	83.0 ± 56.2	0.0005
PM_{10} [µg/m^3]	143.6 ± 78.8	99.5 ± 62.1	0.0009
PAH in $PM_{2.5}$ [ng/m^3]	37.9 ± 20	27.9 ± 12.7	0.003
PAH in PM_{10} [ng/m^3]	68.7 ± 46.0	49.2 ± 31.9	0.0039
NPAH in $PM_{2.5}$ [pg/m^3]	497.3 ± 500.7	380.3 ± 406.1	0.0674
NPAH in PM_{10} [pg/m^3]	528.3 ± 511.6	397.5 ± 407.7	0.0468

Workdays (normal days: n=60) and Non-work days (combination of all public holidays, car-free day and weekends: n=30)

Table S5. 9. Concentrations (mean ± SD) of PM_{10} -bound PAHs and NPAHs during the dry and wet seasons at the three sites in Rwanda.

		Urban background		**Urban Roadside**		**Rural**
		DRY	WET	DRY	WET	WET
		n = 15	n= 21	n = 14	n = 17	n = 23
PAH (ng/m^3)	NaP	0.24 ± 0.04	0.29 ± 0.13	0.60 ± 0.11	0.49 ± 0.11	0.61 ± 0.31
	Ace	0.02 ± 0.01	0.03 ± 0.02	0.05 ± 0.01	0.05 ± 0.02	0.09 ± 0.05
	Fle	0.14 ± 0.06	0.13 ± 0.05	0.27 ± 0.09	0.26 ± 0.18	0.18 ± 0.08
	Phe	2.2 ± 0.65	1.03 ± 0.53	4.79 ± 4.96	3.45 ± 1.59	2.30 ± 5.90
	Ant	0.03 ± 0.01	0.03 ± 0.02	0.11 ± 0.05	0.13 ± 0.03	0.02 ± 0.01
	Flu	6.43 ± 1.82	5.57 ± 2.41	10.26 ± 2.54	10.32 ± 2.45	10.55 ± 10.27
	Pyr	0.61 ± 0.21	0.27 ± 0.08	0.81 ± 0.31	0.75 ± 0.23	0.28 ± 0.21
	BaA	0.62 ± 0.21	0.44 ± 0.23	1.53 ± 0.80	1.37 ± 0.41	0.62 ± 0.40
	Chr	0.90 ± 0.29	0.57 ± 0.30	1.73 ± 0.83	1.56 ± 0.48	0.90 ± 0.63
	BbF	2.93 ± 0.85	2.70 ± 1.08	5.80 ± 1.56	6.10 ± 1.07	6.06 ± 1.30
	BkF	1.24 ± 0.38	1.21 ± 0.50	2.60 ± 0.64	2.70 ± 0.46	1.77 ± 0.78
	BaP	2.25 ± 0.71	2.57 ± 1.45	11.75 ± 5.20	8.08 ± 2.09	5.32 ± 2.67
	DBA	0.07 ± 0.06	0.05 ± 0.02	0.10 ± 0.03	0.09 ± 0.04	0.07 ± 0.03
	BPe	5.19 ± 1.52	5.84 ± 2.16	16.01 ± 3.63	15.75 ± 3.87	8.19 ± 3.45
	IDP	2.23 ± 0.62	2.39 ± 0.87	4.31 ± 1.29	4.84 ± 0.77	3.15 ± 1.25
Total PAHs (ng/m^3)		**25.14 ± 6.75**	**23.09 ± 8.41**	**60.72 ± 19.47**	**55.94 ± 11.49**	**37.55 ± 19.53**
	1,8-DNP	10.39 ± 4.83	25.18 ± 54.05	142.84 ± 128.98	160.01 ± 152.18	10.44 ± 3.41
	1,3-DNP	0.66 ± 0.33	0.41 ± 0.32	2.21 ± 1.21	1.34 ± 0.66	0.32 ± 0.15
	9-NA	253.70 ± 135.28	100.11 ± 78.68	531.90 ± 327.51	218.95 ± 148.39	62.93 ± 37.83
	2-NP+2-NFR	141.35 ± 90.22	73.59 ± 56.64	362.71 ± 205.05	236.61 ± 146.68	69.21 ± 55.14
	1-NP	4.70 ± 2.94	2.35 ± 1.71	18.21 ± 15.53	17.18 ± 10.48	3.04 ± 1.41
	7-NBaA	59.61 ± 36.74	9.81 ± 12.39	109.34 ± 63.49	48.96 ± 37.12	0.88 ± 0.00
	6-NBaP	0.91 ± 0.48	0.06 ± 0.04	3.75 ± 2.03	2.44 ± 1.39	ND
Total NPAHs (pg/m^3)		**445.31 ± 266.95**	**207.76 ± 147.61**	**11550.07 ± 540.60**	**676.56 ± 440.90**	**145.67 ± 86.32**

15PAHs = Nap + Ace+ Fle+ Phe + Ant + Flu+ Pyr + BaA + Chr + BbF + BkF + BaP + DBA+ BPe + IDP. 7NPAH = 1,8-DNP, 1.3-DNP, 9-NA + 2-NP + 2-NFR +1-NP +7-NBaA+ 6-NBaP. Each data represents mean ± SD. ND, not detected. PAHs and NPAHs abbreviations are listed in the caption of Figure S5.5.

Table S5. 10. Inter-correlation coefficients of PAHs and NPAHs at the three sampling locations; urban background, urban roadside and rural sites in Rwanda.

Rural	NaP	Ace	Ant	Flu	Pyr	9-NA	2-NP+2-NFR	1-NP
NaP	1	0.92[b]	0.47[c]	0.52[a]	0.39	0.43	0.70[a]	0.66[a]
Ace		1	0.55[a]	0.39	0.28	0.48[c]	0.66[a]	0.64[a]
Ant			1	-0.2	-0.14	0.27	0.61[a]	0.39
Flu				1	0.94[b]	0.05	0.14	0.31
Pyr					1	-0.1	0.11	0.25
9-NA						1	0.87[b]	0.77[b]
2-NP+2-NFR								0.92[b]
1-NP								1
Urban background	NaP	Ace	Ant	Flu	Pyr	9-NA	2-NP+2-NFR	1-NP
NaP	1	0.62[b]	0.18	0.09	-0.33	-0.1	0.06	0.14
Ace		1	0.22	0.44[a]	-0.27	-0.17	-0.04	0.14
Ant			1	0.46[c]	0.56[b]	0.50[a]	0.63[b]	0.53[a]
Flu				1	0.44[c]	0.19	0.37[c]	0.55[b]
Pyr					1	0.64[b]	0.64[b]	0.56[b]
9-NA						1	0.93[b]	0.87[b]
2-NP+2-NFR							1	0.97[b]
1-NP								1
Urban roadside	NaP	Ace	Ant	Flu	Pyr	9-NA	2-NP+2-NFR	1-NP
NaP	1	0.58[b]	0.48[c]	0.29	0.45[a]	0.13	0.27	0.18
Ace		1	0.26	-0.01	0.09	0.14	0.53[c]	0.40[c]
Ant			1	0.68[b]	0.74[b]	0.1	0.19	0.1
Flu				1	0.83[b]	0.17	0.13	0.09
Pyr					1	0.46[a]	0.39[c]	0.23
9-NA						1	0.85[b]	0.76[b]
2-NP+2-NFR							1	0.95[b]
1-NP								1

(a) $p<0.01$, (b) $p<0.001$, (c) $p<0.05$. PAHs and NPAHs abbreviations are listed in the caption of Figure S5.

Table S5. 11. Diagnostic ratios between PAHs and NPAHs in $PM_{2.5}$ in different seasons at urban background, urban roadside and rural sites in Rwanda.

	Urban Background		**Urban Roadside**		**Rural**
Isomer ratios	Dry	Wet	Dry	Wet	Wet
[1-NP]/[Pyr]	0.01	0.01	0.05	0.04	0.01
[9-NA]/[1-NP]	49.82	36.43	19.92	11.8	19.24
[BaA]/([Chr]+[BaA])	0.42	0.43	0.48	0.47	0.42
[Flu]/([Pyr]+[Flu])	0.91	0.93	0.93	0.94	0.98
[7-BaA]/[BaA]	0.11	0.05	0.09	0.05	<0.01
[2-NP+2-NFR]/[1-NP]	31.66	32.89	14.31	15.24	28.9

PAHs and NPAHs abbreviations are listed in the caption of Figure S5.5

Table S5. 12. Carcinogenic risk calculated from PAHs and NPAHs.

		Rural	**Urban background**		**Urban roadside**	
		Wet	Dry	Wet	Dry	Wet
Analyte	TEF	BaP-TEQ Levels ng/m^3				
NaP	0.001	0.000	0.000	0.000	0.000	0.000
Ace	0.001	0.00	0.000	0.000	0.000	0.000
Fle	0.001	0.000	0.000	0.000	0.000	0.000
Phe	0.001	0.002	0.002	0.001	0.004	0.003
Ant	0.01	0.000	0.000	0.000	0.001	0.001
Flu	0.001	0.01	0.006	0.005	0.009	0.01
Pyr	0.001	0.00	0.001	0.000	0.001	0.001
BaA	0.1	0.046	0.054	0.027	0.116	0.103
Chr	0.01	0.007	0.008	0.004	0.013	0.012
BbF	0.1	0.351	0.268	0.218	0.519	0.545
BkF	0.1	0.149	0.112	0.099	0.232	0.241
BaP	1	4.62	2.146	2.25	11.276	7.531
DBA	5	0.331	0.326	0.239	0.494	0.395
BPe	0.01	0.072	0.048	0.05	0.151	0.148
IDP	0.1	0.28	0.208	0.207	0.398	0.448
1.8-DNP	0.1	0.001	0.001	0.002	0.014	0.016
1-NP	0.1	0.000	0.000	0.000	0.003	0.002
Σ15-PAH toxic		**5.868**	**3.18**	**3.099**	**13.215**	**9.437**
Σ2-NPAH toxic		0.001	0.001	0.002	0.017	0.018
Cancer risk Σ15-PAH		5.1×10^{-4}	3.0×10^{-4}	3.0×10^{-4}	1.15×10^{-3}	8.2×10^{-4}
Cancer risk Σ2-NPAH		9.1×10^{-8}	1.3×10^{-7}	2.1×10^{-7}	1.5×10^{-6}	1.5×10^{-6}
Total cancer risk		**5.1×10^{-4}**	**3.0×10^{-4}**	**3.0×10^{-4}**	**1.2×10^{-3}**	**8.2×10^{-4}**

5.5.2 Supplemental texts

5.5.2.1 Quality control (QC) and quality assurance (QA)

All filters were pre-heated for 4 hours to remove organic contaminants prior to use. After sampling, filters were wrapped in aluminum foils, inside a sterilized plastic bag sealed in polyethylene and shipped to the laboratory. For gravimetric mass determination of the sampled $PM_{2.5}$ or PM_{10}, the filters were equilibrated in controlled weighing room (daily mean temperature in the range of 20 - 25°C and controlled within ± 2°C, daily-mean relative humidity in the range of 30 - 45% and controlled within ± 5%). After being dried in a glass desiccator for at least 48 hours in the dark, the filters were weighed and kept in sealed plastic bags and stored at -20°C until further analysis. To assure laboratory quality control, field blank and solvent blank experiments were conducted (target PAHs and NPAHs were not detected in all blank samples). The recoveries of the deuterated internal standards of PAHs and NPAHs were determined quantitatively based on the peak area ratios of the analytes to the deuterated internal standard and were between 60% and 102%. As part of the QC and QA protocol the limit of detection (LOD) and limit of quantification (LOQ) has been established previously. Detailed information can be found in Hayakawa *et al.* (2018b).

5.5.2.2 Extraction and analytical procedures

The US EPA 610 PAH mixture (Sigma-Aldrich, St. Louis, MO, USA) was used as a standard and included 16 PAHs: naphthalene (NaP), acenaphthene (Ace), Acenaphthylene (Acyl), fluorene (Fle), phenanthrene (Phe), anthracene (Ant), fluoranthene (Flu), pyrene (Pyr), benz(a)anthracene (BaA), chrysene (Chr), benzo(b)fluoranthene (BbF), benz(k)fluoranthene (BkF), benzo(a)pyrene (BaP), dibenz(a,h)anthracene (DBA), benz(g,h,i)perylene (BPe), and indeno(1,2,3-cd)pyrene (IDP). For NPAHs our standard contained 9-nitroanthracene (9-NA), 2-nitropyrene (2-NP); 2-nitrofluoranthene (2-NFR),1-nitroperylene (1-NP), 6-nitrochrysene (6-NC), 7-nitrobenz(a)anthracene (7-NBaA) and 6- nitrobenz(a)pyrene (6-NBaP). These NPAH compounds were obtained from Chiron (Trondheim, Norway). All analytes were dissolved in ethanol (Kanto Chemical, Tokyo, Japan). All other chemical reagents used in this study were of analytical reagent grade.

Samples on $PM_{2.5}$ and PM_{10} filters were treated following the method of Hayakawa *et al.* (2018b). Briefly, a 49.2 cm^2 piece of the total filter was divided into 0.5 cm^2 sections and placed in a flask. Five deuterated PAHs (naphthalene-d_8, acenaphthylene-d_{10}, phenanthrene-d_{10}, chrysene-d_{12}, and perylene-d_{12}) and a NPAH surrogate (2-fluoro-7-nitrofluorene) were added as internal standards. During all treatment processes, samples were protected from light to avoid photochemical degradation of PAHs and NPAHs (Kamiya *et al.*, 2017). The small pieces of the filter were sonically extracted twice with 80 mL of benzene-ethanol (3:1, v/v) and filtered. The extract was cleaned with 80 mL of 5% (w/v) sodium hydroxide, followed by 80 mL of 20% (w/v) sulfuric acid, and twice with 80 mL of ultrapure water. Dimethyl sulfoxide (100 µL) was added and benzene was evaporated using a rotary evaporator. The residual solution was dissolved in ethanol (900 µL) and the solution was filtered through a membrane filter (HLC-DISK 3, 0.45 µm, Kanto Chemical Co., Inc., Tokyo, Japan). A 100 µL aliquot (injection volume) of the filtered solution was analysed using two high-performance liquid chromatographic systems (HPLC-10A series, Shimadzu Inc., Kyoto, Japan), with one system equipped with a fluorescence detector and the other with a chemiluminescence detector to analyse PAHs and NPAHs, respectively. To assure laboratory quality control, the concentrations of target PAHs and NPAHs compounds in the negative controls were all under the detection limit of the instrument and therefore the results were not adjusted for background level. HPLC systems for the determination of PAHs and NPAHs in atmospheric particulates (PM) have been described previously (Tang *et al.*, 2005).

5.5.2.3 Discussion of reasons for high concentrations of $PM_{2.5}$ and PM_{10} during the dry season in Rwanda

5.5.2.3.1 Re-suspended road dust from unpaved roads

A recent study indicated that vehicles moving on unpaved roads during the hot season generate a significantly greater amount of PM emission than paved roads (Penkała, Ogrodnik and Rogula-Kozłowska, 2018) Of the total 14,000 km of road in Rwanda, 40% are paved, and in the capital city of Kigali, only 18% of roads are paved (African Development Bank Group, 2013). The effects of unpaved roads have been shown in the 24-hour mean $PM_{2.5}$ concentrations in neighboring

Uganda, where high proportions of unpaved roads resulted in higher particulate levels (161 μg/m^3) than those with paved roads (68.5 μg/m^3) (Kirenga *et al.*, 2015).

5.5.2.3.2 The geographical features of Rwanda

Rwanda is a land-locked country surrounded by high mountains, with calm winds, high air pressure and low levels of precipitation. Countries with wet and dry seasons and that are surrounded by mountains typically experience a pronounced increase in PM levels during the dry season due to vertical temperature inversion (Petkova *et al.,* 2013). In a basin architecture, PM emitted is trapped in the inversion layer (a layer with a positive temperature gradient) (Largeron and Staquet, 2016). This results in a progressive accumulation of PM due to poor mixing and can reach harmful levels within hours. Findings in similar geographical and seasonal conditions were reported in Chiang Mai, Thailand (Chuesaard *et al.,* 2014).

5.5.2.3.3 Long-distance transport of particulates from extensive biomass burning

It is important to note that Rwanda's prevailing wind direction changes from northerly to southerly during the dry season at the same time as the large-scale biomass burning area changes from north central Africa to southern Africa (Archibald *et al.,* 2010; Strydom and Savage, 2016). The results suggest that the atmospheric concentration of PM in Rwanda could be strongly affected by biomass combustion particulates transported from Eastern and South Africa during the dry season. During the dry season, savannah fires and other combustion sources are significant contributors of PM. Back trajectory analysis indicates increased local PM corresponds to activities in neighboring countries. Samples with the highest and lowest levels of total $PM_{2.5}$ from each sampling site were used to compute three-day reference backward trajectories at 1000, 500 and 100 m above ground level. Samples with high levels of PM were associated with the highest-altitude westerly and northwesterly air masses (about 3500 m altitude) that originated from neighboring East African countries such Uganda, Tanzania and Kenya, as well as South Africa (Figure S5.1A of the SI). These East African countries likely have high concentrations of PM from biomass burning (Van Vliet and Kinney, 2007; Mkoma *et al.,* 2009; Kinney *et al.,* 2012).

Samples with low levels of PM (Figure S5.1B of the SI) were associated with slow-moving local air masses travelling at lower altitude (500 m) within Rwanda. These findings are in strong agreement with a recent study carried out at the Rwanda Climate Observatory situated at Mt. Mugogo (2590 m above sea level), indicating that during Rwanda's dry seasons, the country experiences pollution transported from the southern biomass burning seasons in Africa from June to August (DeWitt *et al.*, 2018).

5.5.2.3.4 Local emissions

The high population density and biomass energy sources used, such as wood and charcoal burning for cooking, and practicing the slash-and-burn method of agriculture, all contribute to increased PM concentrations (Drigo *et al.*, 2013).

5.5.3 Supplemental Figures

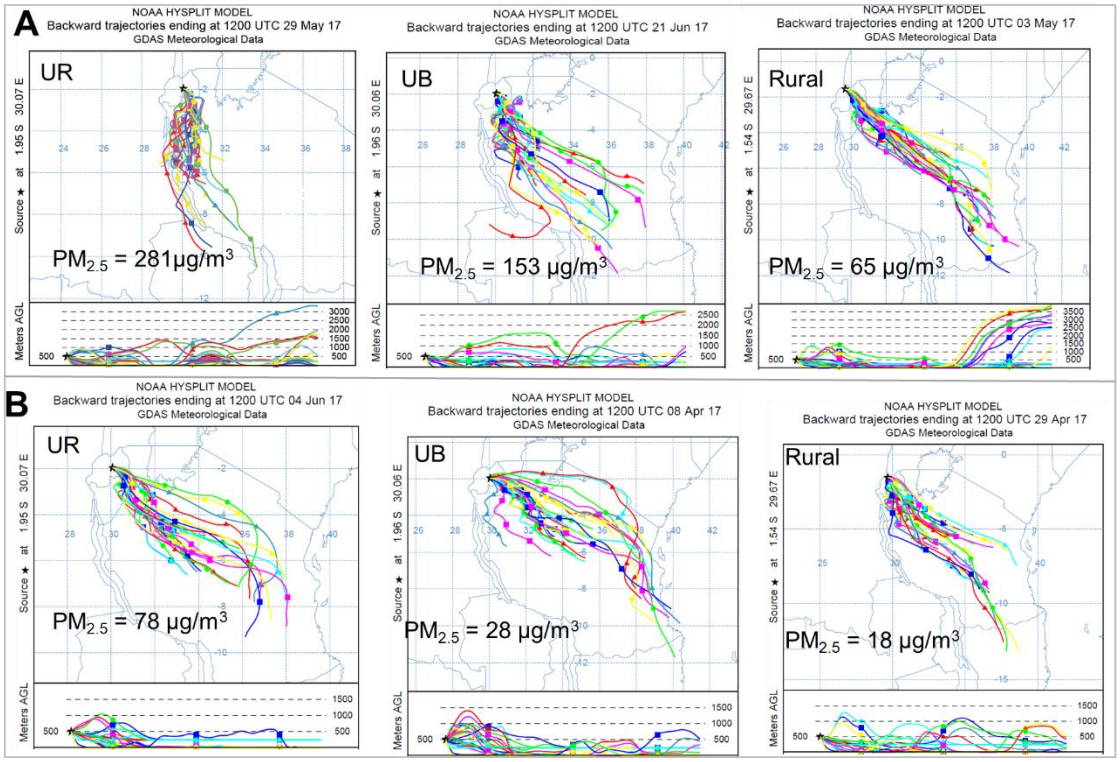

Figure S5. 1. Backward air trajectories for selected samples with (A) high levels of $PM_{2.5}$ and (B) lowest levels $PM_{2.5}$ at each sampling site: urban roadside (UR), urban background (UB) and rural area computed using the Hybrid Single Particle Lagrangian Integrated Trajectory (HYSPLIT).

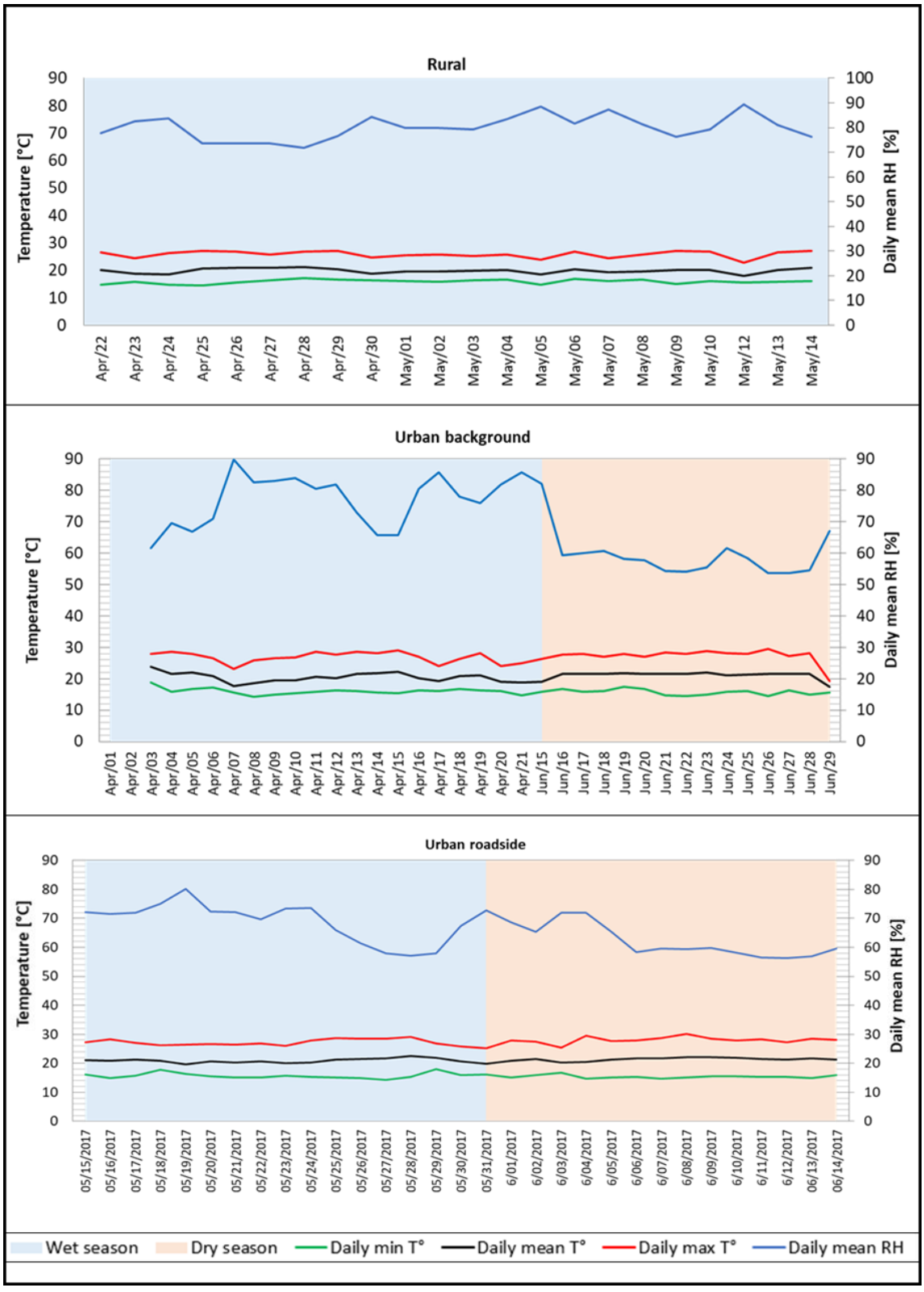

Figure S5. 2. Temporal variation of meteorological conditions during sampling period (April, May, June) at three sites in Rwanda with daily relative humidity and daily temperature.

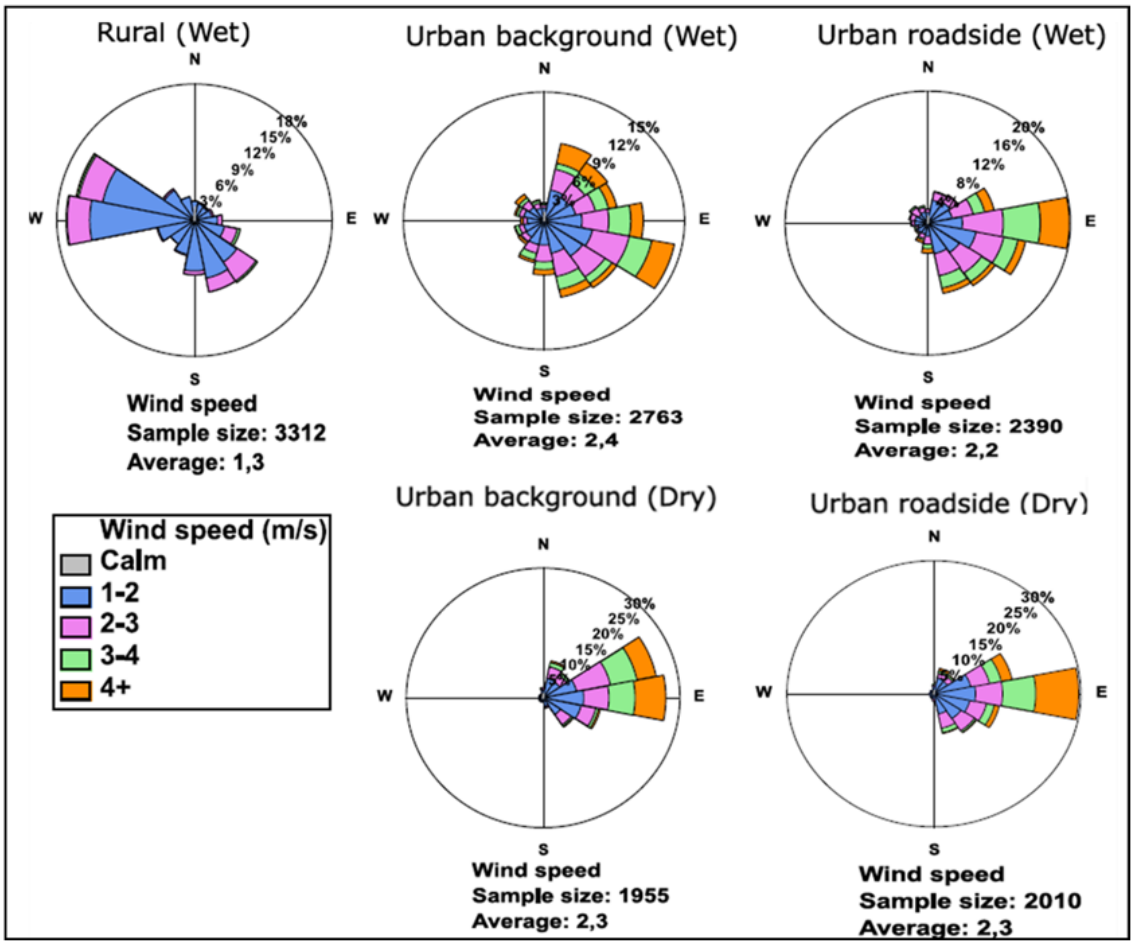

Figure S5. 3. Wind rose plot showing the wind speed and direction during the sampling dates at the rural site (wet season), urban background site (wet and dry seasons) and urban roadside site (wet and dry seasons).

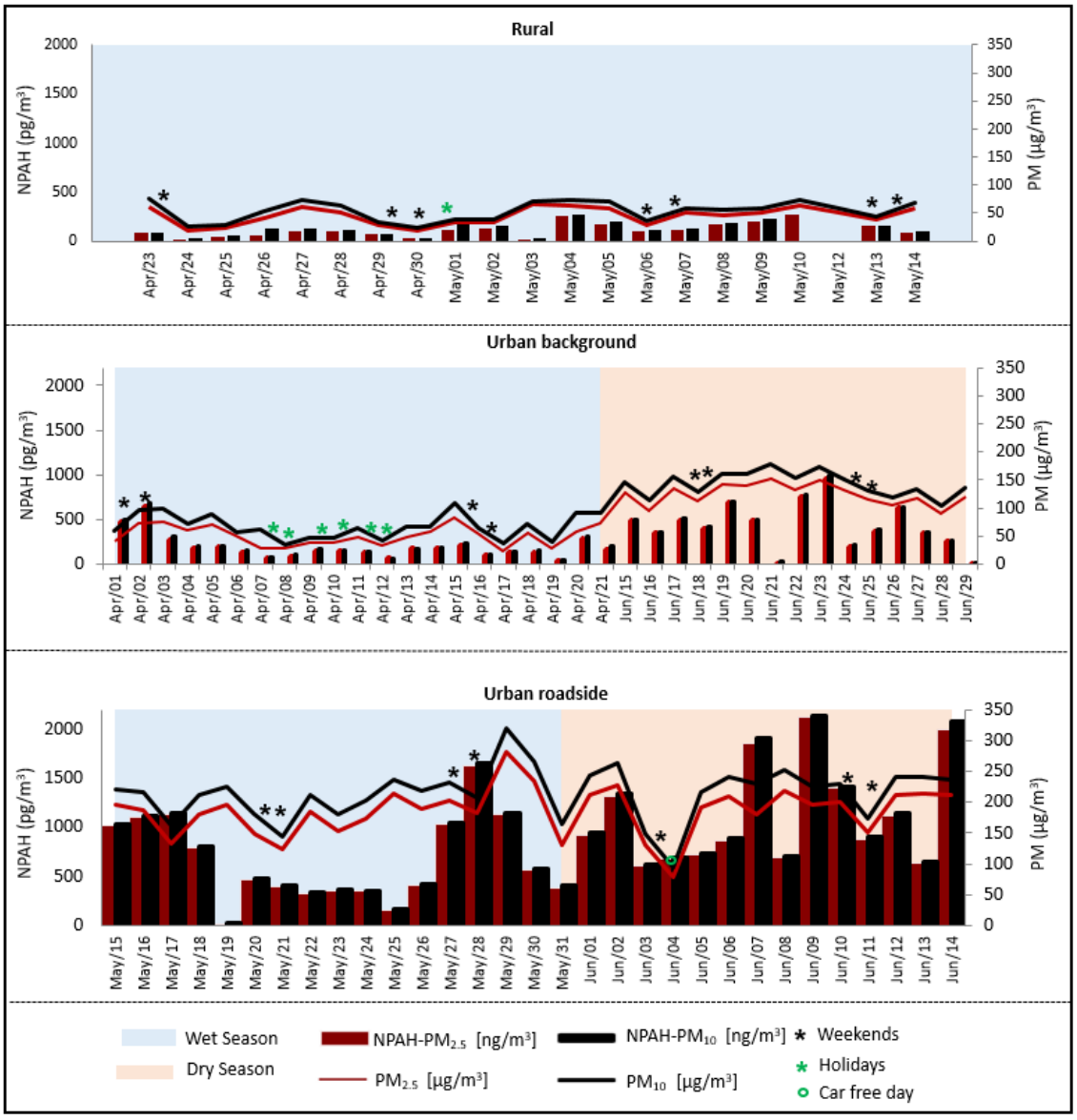

Figure S5. 4. Seasonal variation of nitrated polycyclic aromatic hydrocarbons (NPAHs) detected in ambient $PM_{2.5}$ and PM_{10} in Rwanda; rural area, urban background, and urban roadside sites.

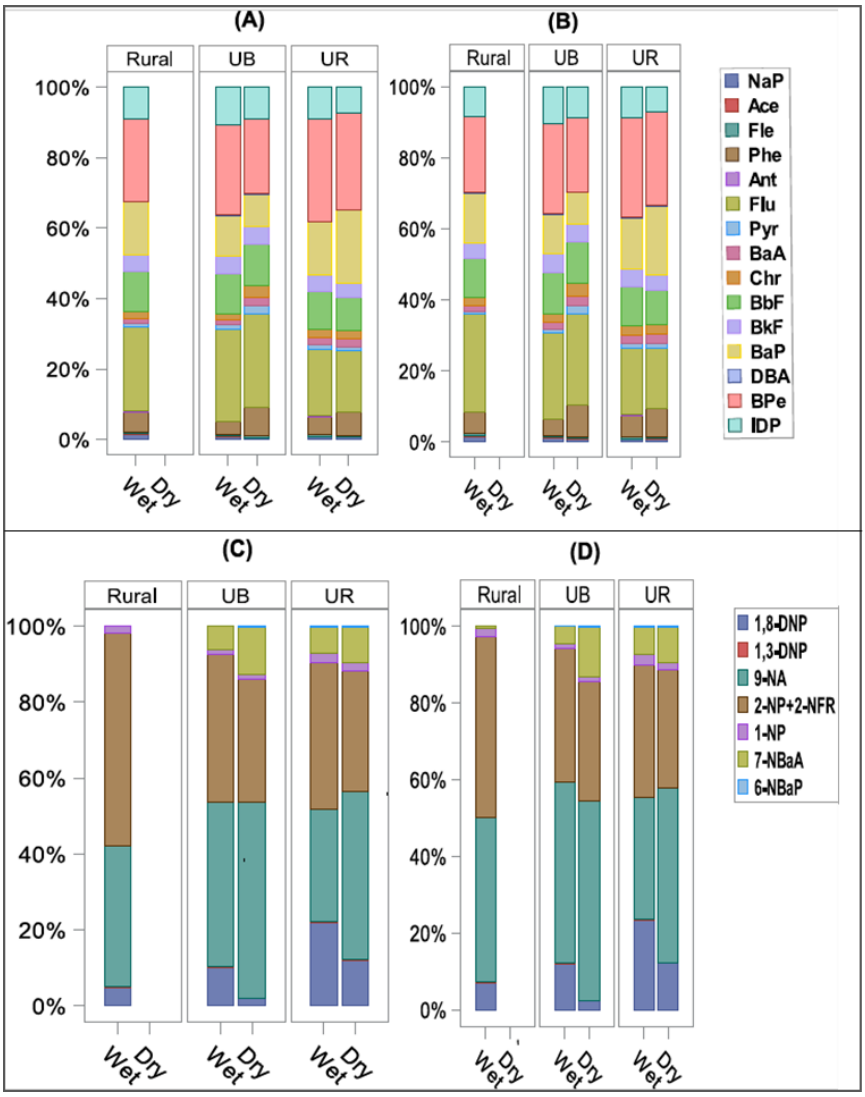

Figure S5. 5. Compositions of individual polycyclic aromatic hydrocarbons (PAHs) and nitrated PAHs (NPAHs) indicating seasonal variation of each compounds detected in $PM_{2.5}$ (A and C) and PM_{10} (B and D) in rural, urban background (UB), and urban roadside (UR) sites in Rwanda. PAH and NPAH abbreviations: (NaP), acenaphthene (Ace), fluorene (Fle), phenanthrene (Phe), anthracene (Ant), fluoranthene (Flu), pyrene (Pyr), benz(a)anthracene (BaA), chrysene (Chr), benzo(b)fluoranthene (BbF), benzo(k)fluoranthene (BkF), benzo(a)pyrene (BaP), dibenz[a,h]anthracene (DBA), benz(g,h,i)perylene (BPe), and indeno(1,2,3-cd)pyrene (IDP), 9-Nitroanthracene (9-NA), 2-nitropyrene (2-NP); 2- nitrofluoranthene (2- NFR), 1,3 and 1,8-dinitropyrenes (1,3 - and 1,8- DNPs),1-nitroperylene (1-NP), 7-nitrobenz(a)anthracene (7-NBaA) and 6-nitrobenzo(a)pyrene (6-NBaP).

5.5.4. Detailed discussion of PCA source analysis

The results shown in Figure 5.3 reveal that two principal component axes (PC1 and PC2 hereafter) explained 68.1% of the data variance. PC1 explained 53.2% of the variance and had a high positive loading for NPAHs (except Pyr). PC1 was highly loaded with NPAH species associated with diesel and gasoline-powered vehicles[26] and species from secondary formation (2-NP+2-NFR and 9-NA). 9-NA has two origins: primary emissions and secondary formation. PC2 accounted for 14.9% of the total variance, and was driven by Ace, NaP and Flu, (marker of biomass burning) (Schauer *et al.*, 1996; Ravindra, Sokhi and Van Grieken, 2008) and was highly predominant in the rural area. The rural area is generally open with agricultural fields and local emissions from agriculture fires and solid biomass fuel burning, suggesting that Ace, NaP and Flu were delivered from wood burning. In particular, low-temperature wood combustion is used for residential heating and cooking in rural areas of Rwanda during dry and wet seasons. Thus, PC2 was considered a source representing domestic fuel wood burning. PC1, loaded by most NPAHs and PC2, loaded by most PAHs were clustered into different groups (Figure 5.3A), suggesting that multiple sources may contribute to the atmospheric PAHs and NPAHs in Rwanda. During all sampling periods, PAHs and NPAHs mainly originated from solid fuel combustion (wood), diesel and gasoline vehicle primary emissions and secondary generation. These findings were consistent with diagnostic ratio source analysis.

In addition, PCA was performed for all PAHs and NPAHs with spatial clustering. The score plot (Figure 5.3A) showed that there was very good grouping of samples with respect to the different sampling sites. The score plot samples of urban background and rural areas did not show a clear separation, suggesting that the composition of PAHs and NPAHs at both sites may have originated from similar sources. However, samples from the urban roadside site were found to be well separated from the other two sites (Figure 5.3B), suggesting that their composition may have originated from different sources.

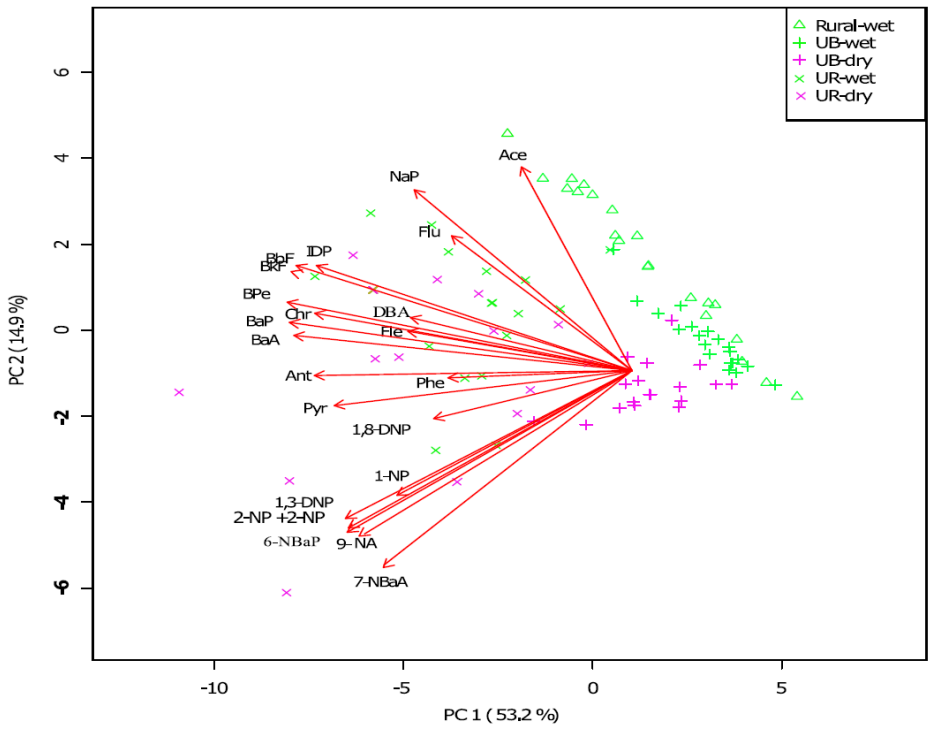

Figure S5. 6. Principal component analysis (PCA) scores and biplot (score plots combined with loading plots) coded by season (green = wet, pink = dry) and by site (rural site (Δ), urban background (UB) (+) and urban roadside (UR) (x). The PCA biplot of the PAHs and NPAHs data showing the loading of each variable (arrows) and the scores of each compound coded by seasons. The length of the arrows approximates the variance of the variables, whereas the angels between them approximate their correlations. Individual PAH and NPAH compounds close together correspond to observations that have similar scores on the PCA components in each season and site.

Chapter 6 - Simultaneous chemical and microbial characterization of atmospheric PM_{10} and $PM_{2.5}$ Particulate from three land-use types in Rwanda, Central-East Africa

Modified content of this chapter was submitted for publication as

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6.1 Prelude

PM_{10} and $PM_{2.5}$-bound PAH and NPAH, their sources, and health risk assessment in Rwanda were presented in the previous chapter. In this chapter the spatial and temporal variation in the microbial community structure (bacteria and fungi) of the same PM samples are characterized using 16S rRNA and ITS gene amplicon sequencing. The relationship of the microbial community with the PAHs and NPAHs concentrations is also discussed. This is the first study to present a multi-domain assessment of bio-aerosols from three land-use types in Africa using high-throughput DNA analyses. The putative pathogenic and allergenic signals from the data sets are also identified. The data presented here are useful for epidemiological studies to develop public health policies regarding the monitoring and management of particulate air pollution and the protection of public health, and to create a baseline dataset for further studies.

6.2 Introduction

Aerosolized particulate matter (PM) is a complex mixture of solid particles and liquid droplets that has been recognized as the greatest environmental cause of premature human mortality worldwide, particularly in Sub-Saharan Africa (World Health Organization, 2016a; Bauer *et al*., 2019). $PM_{2.5}$ and PM_{10} are commonly the focus of air quality monitoring as indicators of a countries air quality of (Petkova *et al.,* 2013). Their toxicity arises largely from their chemical and biological components (Woo *et al.,* 2013; Jalava *et al.,* 2015), which have substantial effects on the atmospheric environment and human health (Kalisa *et al.,* 2019a). Major chemical components of PM include carcinogenic polycyclic aromatic hydrocarbons (PAHs) and nitrated PAHs (NPAHs) (Hayakawa, 2018; Kalisa *et al.,* 2018a, Kalisa *et al.,* 2019a) and major biological components include fungi, bacteria, and viruses (Kalisa *et al.,* 2019a). Despite increasing recognition that biological particles may represent a significant portion of the PM in the atmosphere (Jaenicke, 2005), most studies of atmospheric aerosols typically focus on the chemical components (Yoo *et al.*, 2017). Studies indicated that health responses may be enhanced when chemical and biological constituents of PM are understood in combination (Adhikari *et al.*, 2006; Rosselli *et al.,* 2015; Morakinyo *et al.,* 2016). These components vary substantially by time, location, season and climate, which results in spatio-temporal variation in composition, concentration and toxicity (Harrison and Yin, 2000; Brodie *et al.,* 2007; Maki *et al.,* 2015; Gou *et al.,* 2016). Airborne microorganisms can be transported through the atmosphere from one region to another (Maki *et al.,* 2019). Bacteria are likely to represent a significant portion of the $PM_{2.5}$ aerosol fraction due to their small size, while fungi are the most dominant biological components in larger airborne particles (PM_{10}) (Zhang *et al.,* 2010; Li *et al.,* 2011). Cao *et al.* (2014) indicated that increased PM pollution resulted in increased bioaerosol concentration and allergenic materials in China. Although airborne microorganisms have been found to be closely associated with PM, few studies have characterized both the chemical and biological composition of PM (Sánchez de La Campa *et al.*, 2013; Gandolfi *et al.,* 2015; Jalava *et al.,* 2015; Yan *et al.*, 2018) and none of these studies have investigated microbial association with PAHs and NPAHs.

Organic compounds including PAHs are also known to be degraded by bacterial communities in soil (Parajuli *et al*., 2017). For example, Uyttebroek *et al*. (2006) and García-Díaz *et al.* (2013) have reported that species of Actinobacteria contain efficient degraders of PAH in soil.

Sub-Saharan Africa is a major source of dust storms, which transport material to other continents, including America and Europe (Schuerger *et al.*, 2018). Several studies have characterized the microbial components of Saharan dust and indicated that African dust contains viable microbes, including bacteria and fungi (Griffin *et al.*, 2001; Prospero *et al.*, 2005; Sánchez de La Campa *et al.*, 2013). In recent years, the relatively high species richness of PM-associated airborne microorganisms has been revealed by high-throughput DNA sequencing (Hospodsky *et al.*, 2012; Woo *et al.*, 2013; Cao *et al.*, 2014; Yan *et al.*, 2016). This approach more accurately reflects the diversity of airborne fungi and bacteria since it is more sensitive (as well as significantly quicker) than culture-based techniques.

This is the first study in Africa that demonstrates the spatial and temporal variation of bacteria and fungi in aerosol size fractions using high-throughput amplicon sequencing, and their relationship with PM chemical composition. Specifically, we amplified and sequenced marker genes to characterize both bacterial and fungal community structures and correlated this with PAH and NPAH concentrations in PM_{10} and $PM_{2.5}$ from rural, urban background and urban roadside locations in Rwanda. This unique dataset allowed us to address the following objectives: (1) To describe the total microbial community structure (bacteria and fungi) and their variability with regard to airborne PM size, season and land-use type; (2) To determine the relationships between microbial communities (bacteria and fungi) and chemical components (PAHs and NPAHs) associated with PM using multivariate analysis, and (3) To identify the effect of meteorological factors (temperature and relative humidity) on chemical and biological aerosol compositions. The data presented here will be useful in epidemiological studies to develop public health policies regarding the monitoring and management of particulate air pollution and the protection of public health.

6.3 Materials and Methods

6.3.1 Particulate matter collection

PM_{10} and $PM_{2.5}$ samples were collected (24-hour period per sample) on a Whatman glass microfiber filter (20.3 cm x 25.4 cm, GFF, Whatman EPM 2000) using high-volume samplers (SIBATA Electric Company Limited, Japan, and HVS-RW-1000F), which were equipped with $PM_{2.5}$ and PM_{10} fractionating inlets at an average flow rate of 1000 L/min at three locations in Rwanda, over three months from April to June 2017. Two sites were chosen to represent the Kigali City area, with different vehicle traffic intensities (Figure 6.1). The urban background site (1.9616S, 30.0640E) was located away from emission sources, such as industries, and was broadly representative of city-wide background conditions. The urban roadside site (1.9519S, 30.0739E) was located adjacent to a major road and experienced high private vehicles, buses and mototaxis traffic volumes. The rural area (1.5367S, 29.68253E), was situated approximately100 km away from Kigali City and was generally open, with agricultural fields and minimal residential areas, roads and industrial infrastructures. Quality control (QC) and quality assurance (QA) detailing all information about filter handling, filter storage, and gravimetric analysis were described in our previous study (Kalisa *et al.,* 2018a). After gravimetric analysis, each filter PM sample was cut into two equal parts; one was analyzed for chemical components (PAHs and NPAHs) while the other was analyzed for biological components (bacteria and fungi). Figure S6.1 of the Supplemental Information (SI) describes the filter sample analysis flow chart for chemical and biological component sampling, filter extraction and analysis.

Wet and dry season sampling intervals were conducted at urban background and urban roadside sites. Sampling at the rural site was conducted during the wet season only due to time constraints. Meteorological data including temperature (T), relative humidity (RH), wind speed (WS), and wind direction (WD) for both urban and rural sites were obtained from synoptic weather stations at Rwandan meteorological weather stations situated close to the sampling sites. The summary of all meteorological conditions from each site and concentrations (mean ± SD) of collected $PM_{2.5}$ and PM_{10} by site in Rwanda is presented in Table S6.1 of SI

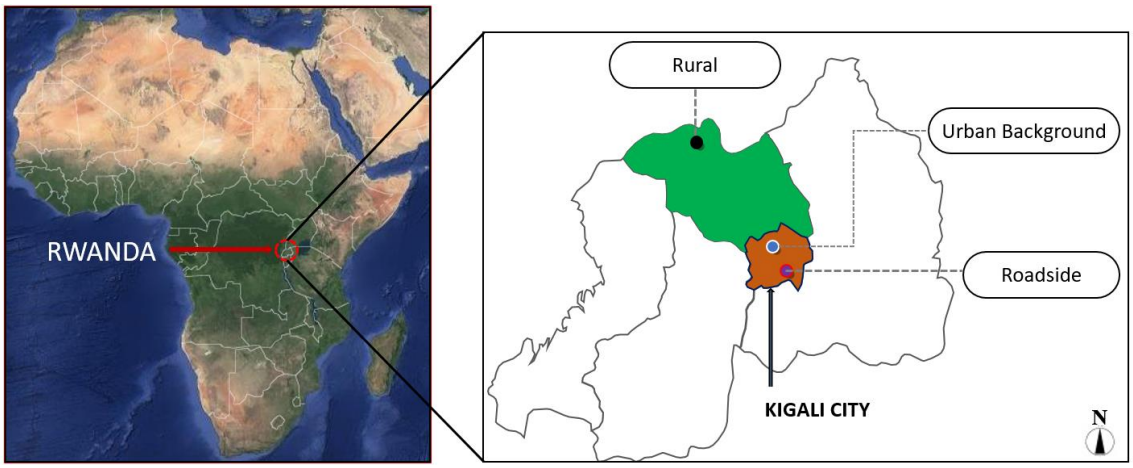

Figure 6. 1. Sampling locations in Rwanda with reference to the African continent. Figure modified from Google map and yourfreetemplates.com (full details here: https://yourfreetemplates.com/terms-of-use/).

6.3.2 DNA extraction and high-throughput DNA sequencing

DNA was extracted from the PM_{10} and $PM_{2.5}$ filters for each sampling period. Each filter was cut into small pieces and placed in a Nucleospin bead tube (2ml, Machinery–Nagel, Germany) filled with ceramic beads (1.4 mm, Qiagen, Germany). Genomic DNA was extracted following a modified CTAB extraction protocol as described previously (Archer *et al.,* 2019). The genomic DNA was quantified using a Qubit 2.0 Fluorometer (Invitrogen). Amplicon libraries were constructed following the Illumina MiSeq protocol (Metagenomic Sequencing Library Preparation, Part # 15044223 Rev. B Illumina, San 45 Diego, CA, USA). The 16S rRNA V3–V4 region was used for bacteria and archaea and the Internal Transcribed Spacer region ITS1 forward and ITS2 reverse was used for fungi (Bellemain *et al.,* 2010; Archer *et al.,* 2019). The PCR thermo-cycle used was as follows: initial denaturation of 5 min or 3 min (ITS); 30 cycles at 95 °C for 1 min, 55 °C for 1 min (16S rDNA) or 51°C for 1 min (ITS), 72 °C for 1 min, and a final extension at 72 °C for 10 min. The PCR products were purified using AMPure XP beads (Beckman-Coulter, Brea, CA, United States). The amplicons were indexed using a Nextera XT index kit (Illumina).

6.3.3 Sequence data analysis

16S rRNA Amplicon Sequence Variants (ASVs) were identified using the R package DADA2 (Callahan *et al.,* 2016). The taxonomy of the ASVs was assigned using the built-in RDP classifier

in DADA2 with SILVA nr v132 database (Quast *et al.*, 2012). USEARCH version 9.0.2132 (Edgar, 2010) was used to process the ITS amplicon sequence data. Fungal Operational Taxonomic Units (OTUs) based on internal transcribed spacer (ITS) sequences were clustered at the 97% similarity threshold. The total reads were 2,004,520 and 3,330,401 for 16S and ITS, respectively. Alpha diversity and species richness were estimated using Shannon and Chao1 indices (Archer *et al.*, 2019). Diversity indices were tested using Student's *t*-test and one-way analysis of variance (ANOVA). Permutational multivariate analysis of variance (PERMANOVA) (Oksanen *et al.*, 2016; Lee *et al.*, 2018) on weighted UniFrac distance matrix was performed using the R package vegan v.2.5.4 (Anderson *et al.*, 2006) to test the effects of sampling site, location, PM size fraction ($PM_{2.5}$ and PM_{10}) and season on partitioning the variance between the microbial communities. Multivariate analysis was applied to determine the relationship between chemical and biological aerosol concentration and meteorological factors. Spatial and temporal similarities in bacterial and fungal communities were visualised using Principal Coordinate Analysis (PCoA). The Mantel test, run with 999 random permutations, was conducted to correlate the community distance matrices for the two particle sizes derived from high-throughput sequencing. The correlations between environmental variables (meteorological data, particulate mass concentration), airborne bacterial abundances and communities on chemical compositions of PM_{10} (PAHs and NPAHs) were investigated using Spearman′s rank correlation coefficients, redundancy analyses (RDA) and variation partitioning (VPA). RDA and VPA were conducted as previously described by Borcard, Legendre and Drapeau (1992) and Legendre (2008). Briefly, the set of chemical compounds (PAHs and NPAHs) were regressed on the significant sets of the bacterial community and the subset of environmental variables in two separate RDAs. For each RDA, the significance of the global model was tested, and a suitable subset of explanative variables was selected by the forward selection method developed by Blanchet *et al*. (2008). Two final RDAs were then performed with the significant subsets of the variables selected (Blanchet, Legendre and Borcard, 2008) and included in a VPA. The significance of each variable in the chemical composition was evaluated using the permutation test. VPA was performed to further determine the relative importance of the environmental factors and bacterial communities on

chemical composition. All statistical analyses were performed using R version 3.5.3 and statistical analysis software (SAS, version 9.4 by SAS institute Inc. Cary. Inc. USA). The sequencing reads were deposited in EMBL-EBI European Nucleotide Archive (ENA) under study accession number PRJEB33617.

6.3.4 Chemical composition analysis

The approach used for chemical characterization of atmospheric particle samples is reported in our previous paper (Kalisa *et al*., 2018a). Briefly, 16 PAH mixture (Sigma-Aldrich, St. Louis, MO, USA), and 8 NPAHs (Chiron, Trodheim, Norway) were used as standards (Table S6.2 of the SI). All analytes were dissolved in ethanol (Kanto Chemical, Tokyo, Japan). One portion from (49.2 cm^2) the half of each of the PM_{10} and $PM_{2.5}$ sample filters was cut into pieces and placed in a flask. Five deuterated PAHs (naphthalene-d_8, acenaphthylene-d_{10}, phenanthrene-d_{10}, chrysene-d_{12}, and perylene-d_{12}) and a NPAH surrogate (2-fluoro-7-nitrofluorene) were added as internal standards. The PM_{10} and $PM_{2.5}$ filter samples were sonically extracted with benzene-ethanol and the extract was cleaned with sodium hydroxide, sulfuric acid, and ultrapure water. Detailed extraction steps were previously reported in Hayakawa (2018a), Kalisa *et al*. (2018a), and Kalisa *et al*. (2019b). 100 µL of the extract were analyzed using two high-performance liquid chromatographic systems (HPLC-10A series, Shimadzu Inc., Kyoto, Japan), with one system equipped with a fluorescence detector for PAHs analysis and the other equipped with a chemiluminescence detector for NPAH analysis.

6.4 Results and discussion

6.4.1 Species richness and community diversity

A total of 176 filter samples (88 PM_{10} and 88 $PM_{2.5}$) collected from April to June 2017 were processed for chemical and biological analyses. A total of 2,004,520 16S rRNA ASVs and 3,330,401 ITS OTUs sequence reads were obtained after quality filtering. Based on taxonomic classification as described in the materials and methods section, 30,894 16S rRNA ASVs and 9,962 ITS OTUs sequences were determined (97% sequence similarity threshold for OTUs). For bacteria, PM_{10} samples, bacterial species richness exhibited variations for each land-use type (Table 6.1) increasing in the following order: rural > urban background > urban roadside (Table

6.1). For $PM_{2.5}$ samples collected during the wet season exhibited the highest species richness at the urban background site, followed by the urban roadside, then the rural site (Table 6.1). Rwandan air samples had a higher Shannon diversity index score in both PM sizes than those observed in Chinese cities such as Changsha (Runlan *et al.*, 2019), Beijing (Yan *et al.*, 2018) and Hong Kong (Woo *et al.*, 2013), which could be due to the differences in meteorological factors, population, topography and urbanization *(*Bowers *et al.*, 2011a*;* Woo *et al.*, 2013; Maki *et al.*, 2015; Yan *et al.*, 2018).

The fungal species richness (as estimated by the Chao1 index) was higher in rural sites than urban background and urban roadside sites for the PM_{10} samples. For $PM_{2.5}$ samples exhibited the highest fungal species richness at the urban roadside than urban background site (Table 6.1). The results also showed a higher species richness among the PM_{10} samples taken during the wet season compared to the dry season at the urban sites (*t-test*, $p = 0.026$). However, no significance difference was observed for species richness between dry and wet season for $PM_{2.5}$ samples (*t-test*, $p = 0.1109$).

A comparison of the species richness and diversity based on working days (normal days n = 60) versus non-working days (combination of all public holidays, car-free day, and weekends: n = 30) showed no significant difference (*t*-test, for $PM_{2.5}$, $p = 0.1594$, for PM_{10}, $p = 0.1649$) for bacteria and (*t*-test, $p= 0.6396$, $p= 0.3737$) for fungi, indicating that PM air pollution levels do not significantly affect species richness and community diversity of bacteria and fungi. In agreement with previous studies that found no significant differences in species richness and community diversity between days with different air pollution levels (Woo *et al.*, 2013; Yan *et al.*, 2018).The decreasing diversity with human influence may be due to environmental homogenisation in the lower atmosphere (Dueker *et al.*, 2018).

Table 6. 1. Average diversity index, PAH and NPAH concentrations from PM_{10} and $PM_{2.5}$ from different land-use types: urban roadside, urban background and rural sites.

			Bacteria		Fungi	
PM	SITE	Season	Chao 1	Shannon	Chao 1	Shannon
PM_{10}	Rural	Wet	477.3	5.47	2654.31	4.91
	UB	Dry	271.07	5.18	67	2.8
		Wet	577.35	5.74	2345.78	4.51
	UR	Dry	523.84	5.66	651.62	4.28
		Wet	454.47	5.5	1628.34	4.85
$PM_{2.5}$	Rural	Wet	567.42	5.54	ND	ND
	UB	Dry	1706.89	6.67	ND	ND
		Wet	2363.23	6.24	1347.88	3.78
	UR	Dry	3022.6	6.6	2010.54	3.73
		Wet	498.49	5.35	941.29	3.51

ND: not detected in sample

6.4.2 Community variation with land-use type, season and PM size fraction

Bacterial communities exhibited distinct communities by site in PM_{10} and in $PM_{2.5}$ samples (PERMANOVA, $p < 0.001$) (Figure 6.2 a, b) while fungal communities were significantly distinct between sites for PM_{10} (PERMANOVA, $p < 0.001$) but not for $PM_{2.5}$ (PERMANOVA, $p < 0.071$) (Figure 6.2). PCoA analysis of the fungal communities also revealed spatial differences (three land-use types) in PM_{10} samples (Figure 6.2 c, d). These findings were consistent with previous work showing that urban and rural sites in the United States may often harbor distinct bacterial and fungal communities, due in part to shifts in the relative contribution of bacterial source environments across distinct land-use types (Bowers *et al.*, 2011a; Yamamoto *et al.*, 2012). However, in Hong Kong no significant differences were observed in samples from different sites along the urbanization gradient for either bacterial or eukaryotic community (Woo *et al.*, 2013).

PM_{10} samples collected from the urban background and urban roadside sites showed clear clustering of samples collected during the dry and wet seasons for bacteria (PERMANOVA, $p = 0.003$) but this was not observed for $PM_{2.5}$ (PERMANOVA, $p = 0.137$) (Figure S6.2 of the SI). For fungal communities, clustering was not evident for the $PM_{2.5}$ and PM_{10} samples as there was an overlap of samples from the two-land use-types during dry and wet seasons (PERMANOVA, $p = 0.096$, $p = 0.121$, respectively).

The dissimilarities of airborne bacterial communities (from all sites and seasons) for the two particle sizes were compared via a Mantel test. The overall results showed that there was no significant difference in community dissimilarity based on the particle size (R = 0.16, p = 0.056).

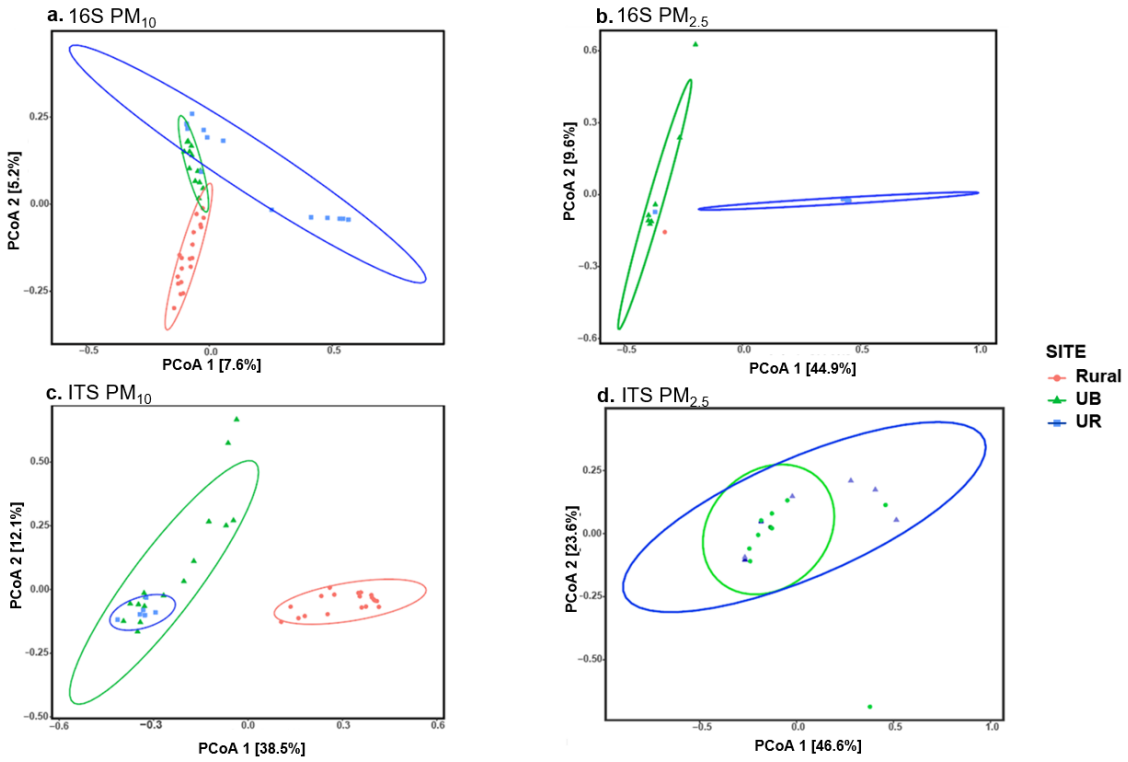

Figure 6. 2. Principal coordinates analysis (PCoA) of the Bray-Curtis dissimilarities of the bacterial relative abundance from PM_{10} samples (a) and $PM_{2.5}$ samples (b) and the fungal relative abundance from PM_{10} samples (c) and $PM_{2.5}$ samples (d) from three land use-types: Rural site, urban background (UB) and urban roadside (UR).

6.4.3 Microbial community composition

6.4.3.1 Bacteria

Dominant bacterial phyla observed in this study included Proteobacteria, Actinobacteria, Firmicutes, Chloroflexi, Bacteroidetes, and Cyanobacteria, which is consistent with previous studies (Figure 6.3) (Lee *et al.*, 2010; Bowers *et al.*, 2011a; Bowers *et al.*, 2013; Cao *et al.*, 2014; Gao *et al.*, 2017). The bacterial composition of Rwandan bio-aerosols revealed clear trends for land-use type and PM size. For example, the most abundant taxa identified from the rural site samples were Proteobacteria and Firmicutes (34% and 21%, respectively in PM_{10} and 77.6% and 14.6%, respectively in $PM_{2.5}$). The urban background site had the highest numbers of Proteobacteria and Actinobacteria (62% and 19%, respectively in $PM_{2.5}$ and 42.5 % and 26.5%,

respectively in PM_{10}). Dust and soil communities support a greater abundance of Actinobacteria (Lacap-Bugler *et al*., 2017), which would explain the high relative abundance of Actinobacteria during the dry season in urban locations found in this study. Kigali City is densely populated and soil-dwelling bacteria such as Actinobacteria can be easily released into the atmosphere due to anthropogenic activities (Runlan *et al.,* 2019). In Hong Kong, Cyanobacteria were the most abundant phyla due to the city's coastal location associated with sea winds, which transport Cyanobacteria into the city (Woo *et al.,* 2013). This suggests that variation in airborne bacteria can be affected by geographic characteristics and meteorological factors such as wind directions. Firmicutes (spore-forming microorganisms) (Maki *et al.,* 2019) were observed in high abundance during the wet season in rural site compared to urban background and urban roadside sites. The dominance of Firmicutes has been observed in winter bioaerosol communities in the United States and Italy (Brodie *et al.,* 2007; Franzetti *et al.,* 2011). This is consistent with the wet season in rural areas of Rwanda, where the average temperature goes down to 9ºC. In this study, Proteobacteria dominated the smaller fraction ($PM_{2.5}$) while Firmicutes were dominant in the larger fraction size (PM_{10}) (Figure 6.3). This is consistent with a previous study in China where the ability of Firmicutes to form aggregates was thought to contribute to their association with large particle fractions (Gou *et al.,* 2016). The authors also reported that Proteobacteria preferentially dominated smaller particle fractions rather than PM_{10}. This was also supported by Cao *et al.* (2014), who found a higher abundance of Proteobacteria in $PM_{2.5}$ than PM_{10} in China.

In this study, the classification of the more abundant genera of bacteria in the different PM sizes and land-use types (Figure S6. 3 of the SI) suggested that soil- and plant-associated bacterial genera were abundant at rural sites while dust-associated bacteria were abundant in urban sites. This was expected as the rural sampling site was generally open, with agricultural fields, and located a long way from roads, suggesting that soil, biomass energy sources such as wood and plant leaves, and the practice of slash-and-burn agriculture may be the main sources of airborne bacteria in this location. Other studies have also shown high relative abundance of bacterial in and around agricultural areas (Bowers *et al.,* 2011a). Anthropogenic activities in the urban environments can also be sources of airborne microbes (Brodie *et al.,* 2007; Bowers *et al.,* 2011b).

Many roads in Rwanda are unpaved; in addition, urban roads tend to have more human activities, including both pedestrian and motor vehicle traffic, than roads in rural sites. These disturbances can generate airborne PM, which in turn can act as a supportive material for airborne microorganisms. This may explain why the dust-associated bacteria were most abundant in urban sites.

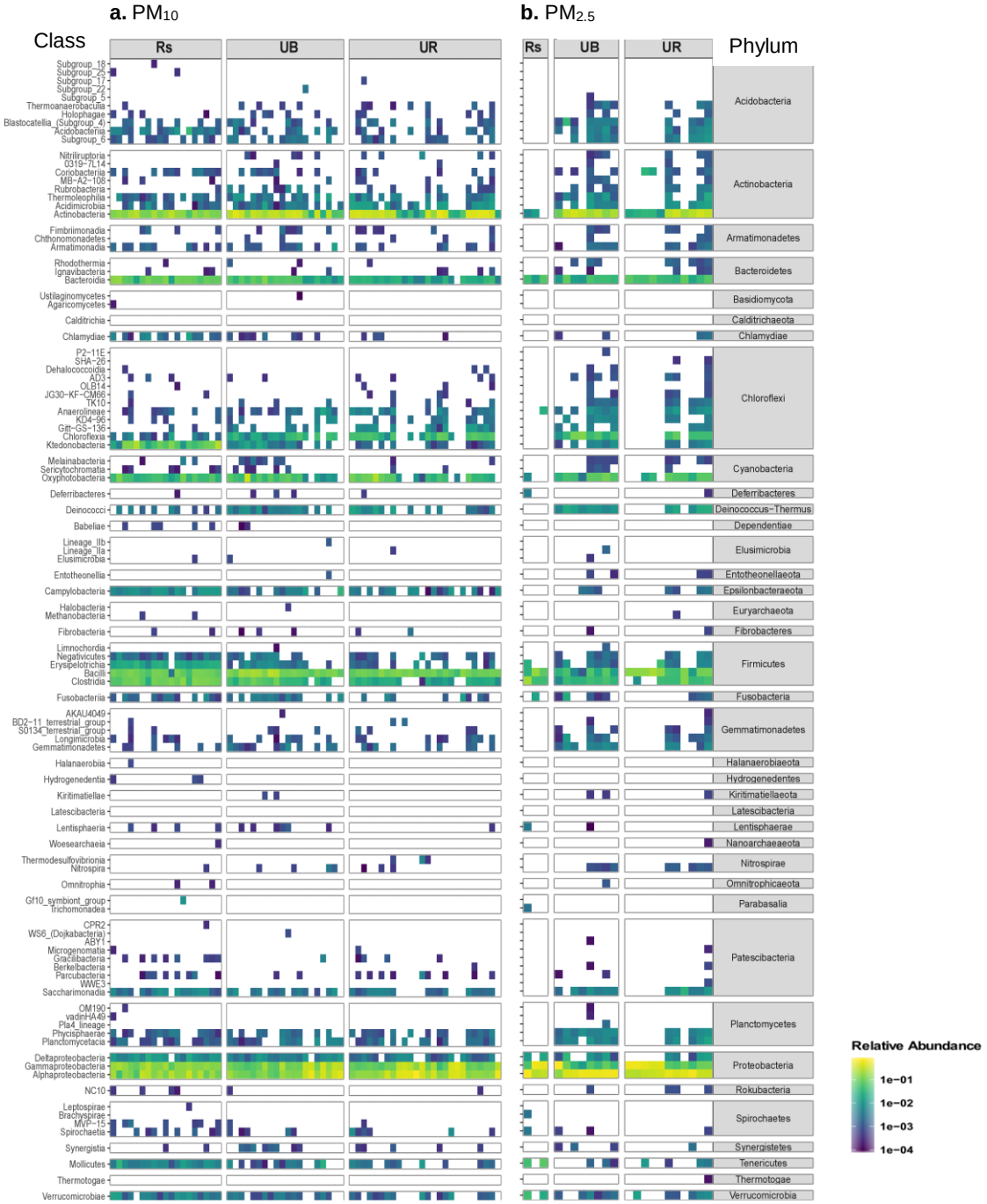

Figure 6. 3. Heatmap diagram showing distribution of ASVs-16S relative abundance at phylum and class levels detected from three land-use types in PM_{10} (a) and $PM_{2.5}$ (b) samples from rural (Rs), urban background (UB) and urban roadside (UR) sites.

6.4.3.2 Fungi

Dominant fungal phyla observed in this study included Ascomycota and Basidiomycota, which are consistent with previous bioaerosol studies (Fröhlich-Nowoisky *et al.,* 2009; Yamamoto *et al.,* 2012; Yan *et al.,* 2016). There was spatial variation in fungal communities identified in the PM_{10} size fraction. This was also observed in the $PM_{2.5}$ size fraction comparing urban background and urban roadside sites. Ascomycota were dominant in $PM_{2.5}$ in urban locations (urban roadside: 81.7%; urban background: 67%) whereas Basidiomycota were more dominant in PM_{10} samples especially in rural sites, representing 71% of the total OTUs from each site (Figure 6.4). The remaining OTUs identified were associated with unclassified/unidentified fungi. The dominant Ascomycota and Basidiomycota associated with PM in Rwanda are similar to fungi found in the United States (Yamamoto *et al.,* 2012; Bowers *et al.,* 2013; Dannemiller *et al.,* 2014; Womack *et al.,* 2015). The greater dominance of Ascomycota in $PM_{2.5}$ than in PM_{10} at urban sites could be due to Ascomycota having unicellular forms that can be aerosolized, in contrast to Basidiomycota, which are too large to be easily aerosolized (Womack *et al.,* 2015). Fröhlich-Nowoisky *et al.* (2009) collected atmospheric particulates by filtration and demonstrated that Basidiomycetes occur frequently together with coarse particles due to their larger spores (5–10 μm) compared to Ascomycota, which are found in connection with fine particles (2–5 μm). In contrast, Yamamoto *et al.* (2012) found the opposite trend. Dothideomycetes and Sordariomycetes were more dominant fungal classes found in this study (Figure 6.4). Consistent with previous atmospheric studies conducted in Germany (Fröhlich-Nowoisky *et al.,* 2009), Seoul, Korea (Shin *et al.,* 2015; Abd Aziz *et al.,* 2018) and the United States (Yamamoto *et al.,* 2012). Dothideomycetes and Sordariomycetes were the two most abundant fungal classes of airborne Ascomycota found in urban locations in $PM_{2.5}$ samples (Figure 6.3). Agaricomycetes and Tremellomycetes were the most abundant classes of Basidiomycota found in PM_{10} and the abundance increased over time during the wet season. Agaricomycetes have been previously detected in air samples as the most abundant class in the Basidiomycota (Fröhlich-Nowoisky *et al.,* 2009; Yamamoto *et al.,* 2012; Woo *et al.,* 2013). Agaricomycetes are mushroom-forming fungi (Hibbett *et al.,* 2002) commonly

found in tropical soil (Wardle and Lindahl, 2014) and on leaf surfaces (Kembel and Mueller, 2014).

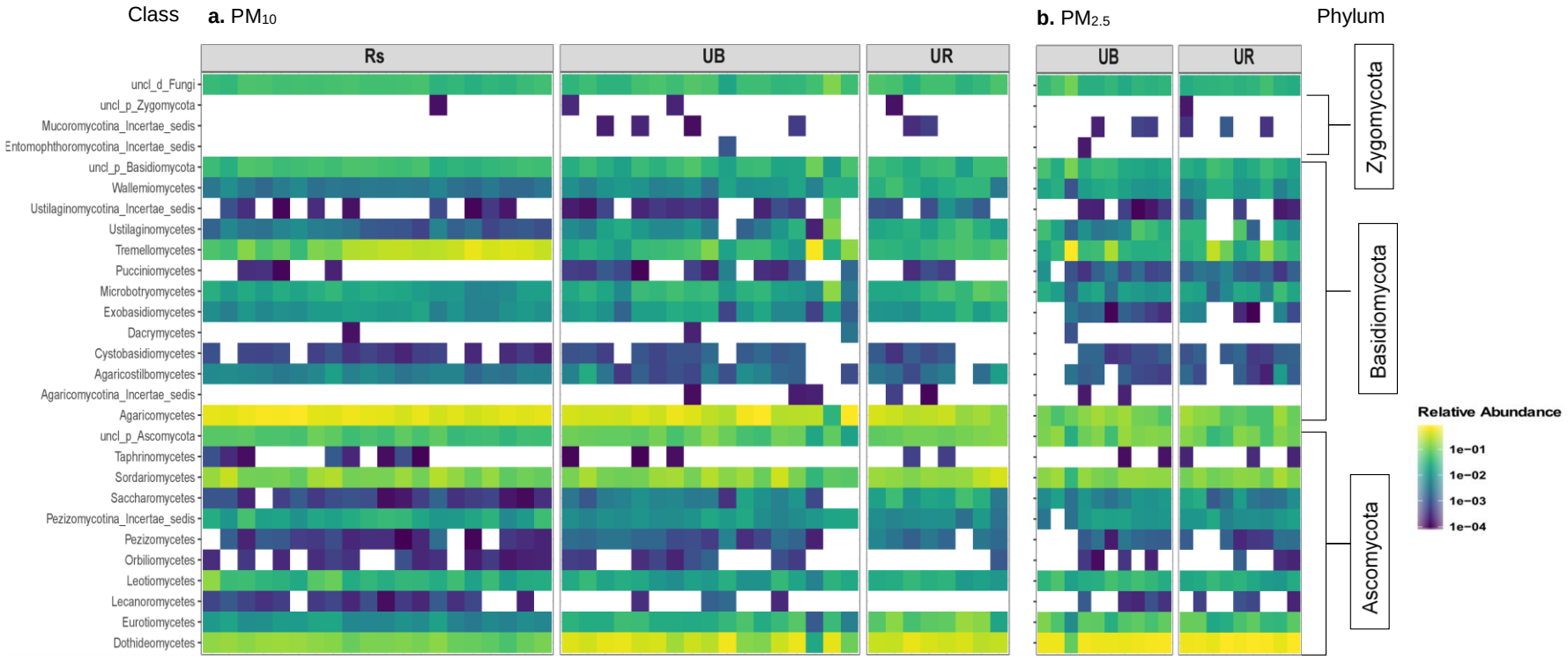

Figure 6. 4. Heatmap diagram showing distribution of OTUs -ITS relative abundance at phylum and class level from three land-use types detected in PM_{10} (a) and $PM_{2.5}$ (b) samples from the rural (Rs), urban background (UB) and urban roadside (UR) sites.

6.5 Potential bacterial and fungal pathogens

We identified a range of bacterial and fungal genera and species associated with health risks based on previous studies on airborne allergenic or pathogenic bacterial and fungal species (Figure S6.3-S6.4 of the SI, Table S6.3-S6.4 of the SI). At the genus level, these potentially pathogenic genera include *Pseudomonas, Acinetobacter, Delftia, Staphylococcus, Sphingomonas* and *Clostridium* (Figure S6.3 of the SI) (Woo *et al.,* 2013). The taxa identified in this data set were consistent with those found other recent air surveys of ambient PM pollution in China (Cao *et al.,* 2014; Gou *et al.,* 2016; Gao *et al.,* 2017; Yan *et al.,* 2018), in African dust (Kellogg and Griffin, 2006; Polymenakou *et al.,* 2008) and in Europe (Genitsaris *et al.,* 2017). The diverse genera *Pseudomonas* and *Acinetobacter* contained potential human pathogens linked to respiratory tract diseases such as lung infections, including pneumonia (Noto *et al.,* 2015) nosocomial infections (Xu *et al.,* 2010), and bacteraemia (Wong *et al.,* 2017). Human-associated *Delftia* and *Sphingomonas* were detected at high levels in $PM_{2.5}$ at urban sites (Figure S6.3 of the SI), which means they are more likely to penetrate deeper into the human lung, potentially triggering serious diseases (Brook *et al.,* 2004). We also found *Pseudomonas*, a genus that includes pathogenic species such as *Acinetobacter johnsonii* and *Pseudomonas monteilii*. Their relative abundances are presented in the Table S6.3 of the SI. *Acinetobacter johnsonii* is a human pathogen isolated in the African atmosphere that has been linked to several diseases, including pneumonia, meningitis, and bacteraemia (Polymenakou *et al.,* 2008). *Pseudomonas monteilii* is an important opportunistic and nosocomial pathogen capable of affecting respiratory metabolism (Shariff and Beri, 2017). Various clinically allergenic fungi (Figure S6.4 of the SI) were also detected from the samples (Yamamoto *et al*., 2012). *Alternaria* were abundant allergenic genera of fungi found in $PM_{2.5}$ at urban sites; this genus contains species that are common human allergens and can trigger asthmatic attacks (Denning *et al.,* 2006; Simon-Nobbe *et al.,* 2008). Fungal pathogenic species such as *Aspergillus fumigatus* and *Blumeria graminis* (Table S6.4 of the SI) were also detected. *Aspergillus fumigatus* is a major fungal allergen known to cause invasive infections and it can colonize the bronchial tubes of the lung, causing allergic bronchopulmonary aspergillosis (Greenberger, 2002; Nierman *et al.,* 2005; Shin *et al.,* 2015). Some plant pathogen species such

as *Blumeria graminis* (Fröhlich-Nowoisky *et al.*, 2009) were also found in this study and their relative abundance was high in the PM_{10} samples compared to $PM_{2.5}$.

6.6 Correlation between microbial community and chemical components

Aerosolized PM is a complex mixture of chemical and biological components. While previous studies suggest high concentrations of PM and its adsorbed chemical components might inhibit or promote the growth of some airborne microorganisms (Cao *et al.*, 2014; Kalisa *et al.*, 2019a), the relationships between the chemical and microbial compositions associated with PM remains poorly understood. Spearman correlation coefficients were calculated to describe the relationships between the environmental factors ($PM_{2.5}$, PM_{10}, PAHs, NPAHs, Temp and RH) and bacterial diversity and richness (Table S6.5-S6 of the SI).

A Spearman correlation test showed that PM_{10} and $PM_{2.5}$ bacterial components (genus level) were significantly correlated with total PAHs and NPAHs while no significant correlation was observed between PM and microbial (bacterial and fungal) diversity and richness. This result was consistent with a study carried out in Saudi Arabia, which found no significant correlations between the concentrations of microorganisms and PM mass concentrations (Alghamdi *et al.*, 2014). Numerous genera including *Acinetobacter, Pseudomonas, Methylbacterium* and *Micrococcus* have been found to degrade PAHs especially those with lower molecular weight (2 to 4 rings) as their sole carbon source (Ghosal *et al.*, 2016). Interestingly, in this study *Acinetobacter, Pseudomonas, Methylbacterium* and *Micrococcus* were significantly negatively correlated with PAH compounds (phenanthrene (Phe), anthracene (Ant) and pyrene (Pyr)) and NPAH compound such as 9-nitroanthracene (9-NA), 1-nitroperylene (1-NP), 7-nitrobenz(a)anthracene (7-NBaA), and 6-nitrobenz(a)pyrene (6-NBaP) (Table S6.4 of the SI), suggesting that the negative correlation observed in this study might be explained by the degradation of PAH and NPAH species by bacteria in the atmosphere.

In the present study, RH and temperature were also significantly correlated with *Acinetobacter, Pseudomonas, Methylbacterium* and *Micrococcus* (Table S6.7 of the SI).

To further understand the correlation between the chemical and biological compositions of PM, redundancy analysis (RDA) was performed based on chemical species (PAHs and NPAHs) and the most dominant bacteria at genus level for $PM_{10,}$ ($PM_{2.5}$ data did not yield significant results). The RDA results showed that 29.46% of the chemical species variation could be predicted by bacteria (genus level), particularly the relative abundance of *Methylbacterium* (R^2adj = 14.69 %, p = 0.001), *Delftia* (R^2_{adj} = 10.05 %, p = 0.001) and *Ornithinimicrobium* (R^2_{adj} = 4.72 %, p = 0.006). There was a significant correlation with chemical compounds of PAHs and NPAHs displayed by segregation of the samples according to land-use types (R^2_{adj} = 29.43 %, p = 0.001). It is likely that these spatial clusterings (Figure 6.5) are driven by high levels of the bacteria *Delftia* and *Ornithinimicrobium* in urban roadside sites and a high relative abundance of *Methylbacterium* in the rural site (Figure 6. 5). Furthermore, the results of a linear model based on environmental factors and PAH and NPAH species showed that 46.85 % (R^2_{adj}, p = 0.001) of the variation of the chemical species could be predicted by concentration of PM_{10} (R^2_{adj} = 35.90 %, p = 0.001), NPAH (R^2_{adj} = 5.92 %, p = 0.002) and RH (R^2_{adj} = 2.42 %, p = 0.007) (Figure 6.5). Urban roadside in PM_{10} samples were characterized by high concentrations of the chemical species, particularly 7-NBaA, 9-NA, 1,8- DNP, 2-NP +2-NFR and 1-NP. Conversely, the concentrations of these chemical species and PM_{10} decreased gradually in the urban background and rural samples, whereas the RH increased significantly in those samples.

The variation partitioning analysis (VPA) showed that the environmental factors and bacterial variables together explained 44.95% (R^2_{adj}, p = 0.001) of the total variation in PAH and NPAH compounds. Indeed, most of the variation explained by the bacterial community could not be disentangled from the variation explained by the environmental variables (R^2 of the interaction = 28.8 %, p = 0.001) and 55% of the variation of the PAHs and NPAHs remained unexplained (Figure 6.5). Further investigations are needed to identify the unexplained factors causing the variation of PAH and NPAH compounds in the atmosphere.

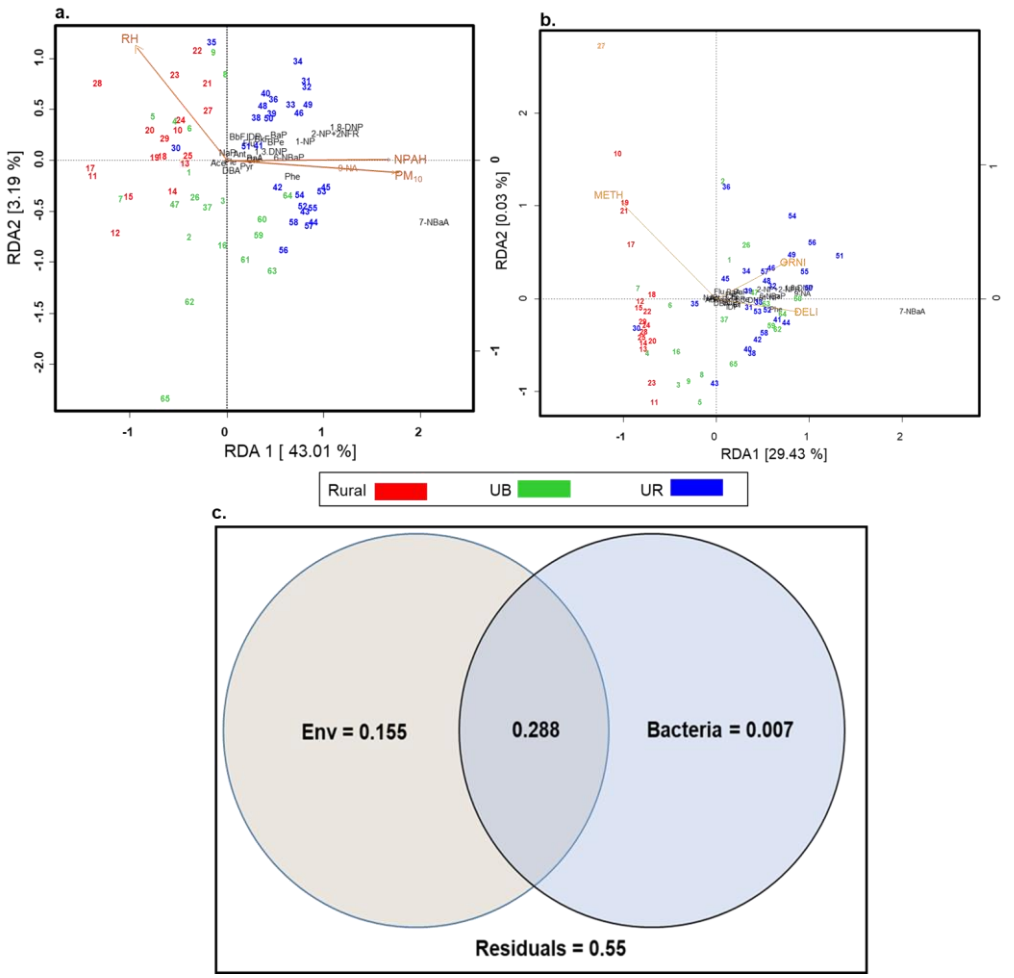

Figure 6. 5. Redundancy analysis (RDA) and variation partitioning analysis (VPA) analysis of the contribution of bacterial communities (genus level) and the environmental variables to the variation in chemical species (PAHs and NPAHs) detected in PM_{10} samples at land-use types in Rwanda: Rural, urban background (UB) and urban roadside (UR) sites. (a) RDA figure showing the variation of the chemical species can be predicted by selected environmental variables (PM_{10}, NPAHs and relative humidity); (b) RDA figure showing that the chemical species variation can be predicted by selected groups of bacteria such as *Methylobacterium* (METH), *Delftia* (DELI) and *Ornithinimicrobium* (ORNI) in three land-use type; (c) VPA analysis showing the percentage of the total variation of chemical species that explained of both environmental factors and bacterial communities. Each diagram represents the PAH and NPAH variation partitioned into the relative effects of each factor or combination of factors. Residuals indicate the fraction of the variation of PAHs and NPAHs compounds that remains unexplained. PAHs and NPAHs abbreviations are listed in the Table S6.2 of the SI.

6.7 Conclusion

This study was the first of its kind to demonstrate the spatio-temporal variation in bacterial and fungal communities in PM_{10} and $PM_{2.5}$ in Africa using high-throughput DNA analyses and to demonstrate the relationship between microbial community structure and chemical composition of PM size (PAHs and NPAHs). The results showed that the diversity and composition of the airborne bacterial and fungal communities varied by site, PM size fraction, and season. The results indicated that PM air pollution levels do not affect species richness and community diversity of bacteria and fungi in PM_{10} and $PM_{2.5.}$ Correlation between chemical and microbial community composition was observed in this study and 44.9% of the total variation of the chemical composition was explained by environmental factors and the bacterial community structure. Further study is required to investigate the role of chemical and biological components of PM size fractions in disease causation. Our findings will be useful in epidemiological studies to develop public health policies regarding the monitoring and management of particulate air pollution and the protection of public health. Data provided in this study will be important basis for future studies of the chemical and biological compositions of aerosols.

6.8 Supplemental Information

6.8.1 Supplemental Tables

Table S6. 1. Meteorological parameters (mean ± SD) and concentrations (mean ± SD) of collected $PM_{2.5}$ and PM_{10} at urban background (UB), urban roadside (UR) and rural sites in Rwanda.

PM	SITE	Season	N	PM [µg/m^3]	PAH [ng/m^3]	NPAH [pg/m^3]	Temp (°C)	RH (%)
PM_{10}	Rural	Wet	23	53.6 ± 17.1	37.5 ± 19.5	145 ± 86.3	14.8 ± 2.6	86.9 ± 11.6
	UB	Dry	15	143.0 ± 21.4	25.13 ± 6.7	445 ± 266	21.29 ± 1.1	57.04 ± 4.55
		Wet	21	67.0 ± 21.8	23.07 ± 8.0	207 ± 147	19.2 ± 4.6	77.6 ± 8.1
	UR	Dry	14	216.7 ± 46.3	60.7 ± 19.4	1155.0 ± 540	21.45 ± 0.6	61.9 ± 5.6
		Wet	17	211 ± 41.6	55.9 ± 11.4	676 ± 440.9	20.8 ± 0.78	69.9 ±6.7
$PM_{2.5}$	Rural	Wet	23	45.0 ± 15.6	32.53 ± 17.8	129.3 ± 80.4	14.8 ± 2.6	86.9 ± 11.6
	UB	Dry	15	124.3 ± 18.5	22.9 ± 5.5	428 .4 ± 262	21.29 ± 1.1	57.04 ± 4.55
		Wet	21	50.7 ± 16	19.25 ± 6.7	190.1 ± 142.2	19.2 ± 4.6	77.6 ± 8.1
	UR	Dry	14	188.0 ± 41.3	50.0 ± 16.6	1128.7 ±529.3	21.45 ± 0.6	61.9 ± 5.6
		Wet	17	183.0 ± 39.3	50.5 ± 11.2	439.0 ± 67	20.8 ± 0.78	69.9 ± 6.7

Table S6. 2. Name and abbreviation of EPA 16 PAH mixture and 8NPAHs analyzed in this study.

PAHs	NPAHs
Naphthalene (NaP)	9-nitroanthracene (9-NA)
Acenaphthene (Ace)	2-nitropyrene (2-NP)
Acenaphthylene (Acyl)	2-nitrofluoranthene (2-NFR)
Fluorene (Fle)	1-nitroperylene (1-NP)
Phenanthrene (Phe)	6-nitrochrysene (6-NC)
Anthracene (Ant)	7-nitrobenz(a)anthracene (7-NBaA)
Fluoranthene (Flu)	6- nitrobenz(a)pyrene (6-NBaP)
Pyrene (Pyr)	1,3 and 1,8-dinitropyrenes (1,3 - and 1,8- DNPs)
Benz(a)anthracene (BaA)	
Chrysene (Chr)	
Benzo(b)fluoranthene (BbF)	
Benz(k)fluoranthene (BkF)	
Benzo(a)pyrene (BaP)	
Dibenz(a,h)anthracene (DBA)	
Benz(g,h,i)perylene (BPe),	
Indeno(1,2,3-cd)pyrene (IDP)	

Table S6. 3. Description of pathogenic airborne bacteria detected from air sample in Rwanda and their relative abundance in PM_{10} and $PM_{2.5}$.

Phylum	Species	Description	PM_{10}	$PM_{2.5}$
Proteobacteria	*Methylobacterium adhaesivum*	Opportunistic pathogens found in immunocompromised patients (Truant *et al.,* 1998)	4.52E-04	0.00E+00
Proteobacteria	*Stenotrophomonas maltophilia*	An emerging opportunistic human pathogen involved in infections in patients with cystic fibrosis (Lira *et al.,* 2012).	2.87E-04	0.00E+00
Firmicutes	*Turicibacter sanguinis*	Cause acute appendicitis inflammation in Human (Lira *et al.,* 2012).	2.33E-04	8.74E-06
Epsilonbacteraeota	*Arcobacter cryaerophilus*	Have been associated with diarrhoea and bacteraemia in Human (Figueras *et al.,* 2014).	1.89E-04	0.00E+00
Proteobacteria	*Acinetobacter guillouiae*	Healthcare-associated infections and are restricted mainly to catheter-related bloodstream infections associated with nosocomial infection (Singh *et al.,* 2013).	1.06E-04	0.00E+00
Proteobacteria	*Acinetobacter johnsonii*	Human pathogens that have been linked to several diseases such as pneumonia, meningitis, and bacteremia (Polymenakou *et al.,* 2008).	8.18E-05	0.00E+00
Proteobacteria	*Enterobacter cloacae*	Human pathogen that increased obesity (Okhravi *et al.,* 1998).	2.95E-05	7.01E-06
Proteobacteria	*Acinetobacter schindleri*	Opportunistic pathogens in hospitalized patients (Okhravi *et al.,* 1998)	4.83E-05	0.00E+00
Proteobacteria	*Vibrio alginolyticus*	Human pathogen. It causes otitis and wound infection (Citil *et al.,* 2015)	4.02E-05	0.00E+00
Proteobacteria	*Pseudomonas syringae*	Plant pathogens (Xin, Kvitko and He, 2018)	3.76E-05	0.00E+00
Bacteroidetes	*Porphyromonas endodontalis*	Chronic periodontitis associated with periapical lesions (Bedran *et al.,* 2012)	3.22E-05	0.00E+00
Proteobacteria	*Photobacterium damselae*	Marine bacterium that causes infections and fatal disease in a wide range of marine animals and in humans (Rivas *et al.,* 2011).	1.07E-05	0.00E+00
Proteobacteria	*Pseudomonas monteilii*	The species is capable of respiratory metabolism. Important opportunistic and nosocomial pathogens (Elomari *et al.,* 1997).	8.05E-06	0.00E+00

Table S6. 4. Description of the pathogenic and allergic fungi detected in air samples in Rwanda and their relative abundance in PM_{10} and $PM_{2.5}$.

Phylum	*Species*	Description	PM_{10}	$PM_{2.5}$
Ascomycota	*Aspergillus clavatus*	*A. clavatus* is allergenic, causing the occupational hypersensitivity pneumonitis known as malt-worker's lung (Vermani *et al.*, 2010).	2.25E-06	5.79E-06
Ascomycota	*Aspergillus flavus*	*A. flavus* is allergenic and is a known pathogen of plants, humans and animals and can colonize the bronchial tubes of the lung (Abdalla, 1988).	0.001016	0.003181
Ascomycota	*Aspergillus fumigatus*	*A. fumigatus* is a major fungal allergen known to cause invasive infections and can cause allergic bronchopulmonary aspergillosis (Greenberger, 2002).	0.000324	0.000343
Ascomycota	*Aspergillus japonicus*	Causes positive reactions in the skin prick test (Adhikari *et al.*, 2004).	7.32E-06	2.25E-05
Ascomycota	*Aspergillus tamarii*	Pathogenic fungus, with potential as a dual-purpose microbial control organism, against both insect pests and plant pathogens (Vermani *et al.*, 2010).	0.002084	0.014327
Ascomycota	*Beauveria bassiana*	Causes white muscardine disease and infection in a patient with acute Lymphoblastic Leukaemia (Herrero *et al.*, 2012).	4.11E-05	1.86E-05
Ascomycota	*Blumeria graminis*	Fungus that causes powdery mildew on grasses, including cereals (Simon-Nobbe *et al*., 2008).	1.69E-06	9.64E-06
Ascomycota	*Candida boidinii*	*C. tropicalis* is a clinically relevant species and may be an etiological agent of candidemia, specifically in Latin American countries and Asia (Simon-Nobbe et al., 2008).	1.69E-06	2.57E-06
Ascomycota	*Candida tropicalis*	Causes a wide range of diseases such as oropharyngeal candidiasis, angular cheilitis, balanoposthitis, oral thrush and vulvovaginal candidiasis (Zuza-Alves, Silva-Rocha and Chaves, 2017).	9.18E-05	0.000283
Ascomycota	*Penicillium brevicompactum*	*Penicillium brevicompactum* as probable new antigens in a cool and dry climate (Dannemiller *et al.*, 2012).	0.000384	0.000225
Ascomycota	*Penicillium citrinum*	A *Penicillium* species that causes mortality in mosquitos (Dannemiller *et al.*, 2012).	0.000726	0.000777
Ascomycota	*Alternaria dauci*	Plant pathogen that cause diseases (Alternaria leaf speck) on lettuce and celery grown infields (Simon-Nobbe *et al*., 2008).	0.000435	0.00603

Table S6. 5. Spearman correlation of environmental variables, chemical and biological components of aerosols (bacteria) detected in $PM_{2.5.}$

	$PM_{2.5}$	PAHs	NPAHs	Temp	RH	CHAO1	Shannon
$PM_{2.5}$	1	0.58**	0.68**	0.50*	-0.61**	0.04	0.09
PAHs		1	0.57**	0.10	-0.16	-0.01	0.10
NPAHs			1	0.46*	-0.63**	-0.05	0.07
Temp				1	-0.89**	-0.17	-0.10
RH					1	-0.04	-0.17
CHAO1						1	0.84**
Shannon							1

** Correlation is significant at the <0.01 level
* Correlation is significant at the <0.05 level

Table S6. 6. Spearman Correlation of environmental variables, chemicals and biological components (bacteria) in aerosols detected in $PM_{10.}$

	PM_{10}	PAHs	NPAHs	Temp	RH	CHAO1	Shannon
PM_{10}	1	0.64**	0.72**	0.48**	-0.65**	-0.02	0.03
PAHs		1.00	0.54**	-0.002	-0.15	0.02	0.00
NPAHs			1.00	0.46**	-0.55**	0.01	0.04
Temp				1	-0.86**	-0.03	0.02
RH					1.00	0.09	0.03
CHAO1						1	0.93**
Shannon							1

** Correlation is significant at the <0.01 level
* Correlation is significant at the <0.05 level

Table S6. 7. Correlation of bacterial communities with known degraders of PAH and NPAH species.

	PM_{10}	Temp	RH	Phe	Ant	Pyr	1,3 DNP	9-NA	1-NP	7-NBaA	6-NBaP
Acinetobacter	-0.57**	-0.44**	50**		-0.46**	-0.39**	-0.34**	-0.30*		-0.35**	-0.32**
Methylbacterium	-0.46**	-0.31*	47**	-0.29*	-0.42**	-0.27*	-0.35**	-0.38**	-0.28*	-0.42**	-0.35**
Micrococcus	-0.34**	-0.31*	0.47**	-0.30*	-0.30*	-0.36**		-0.31*	0-.29*	-0.38**	-0.25*
Pseudomonas	-0.33**		0.23*			-0.30*	-0.25*	-0.25*	-0.30*	-0.30*	-0.31*

** Correlation is significant at the <0.01 level
* Correlation is significant at the <0.05 level

6.8.2 Supplemental Figures

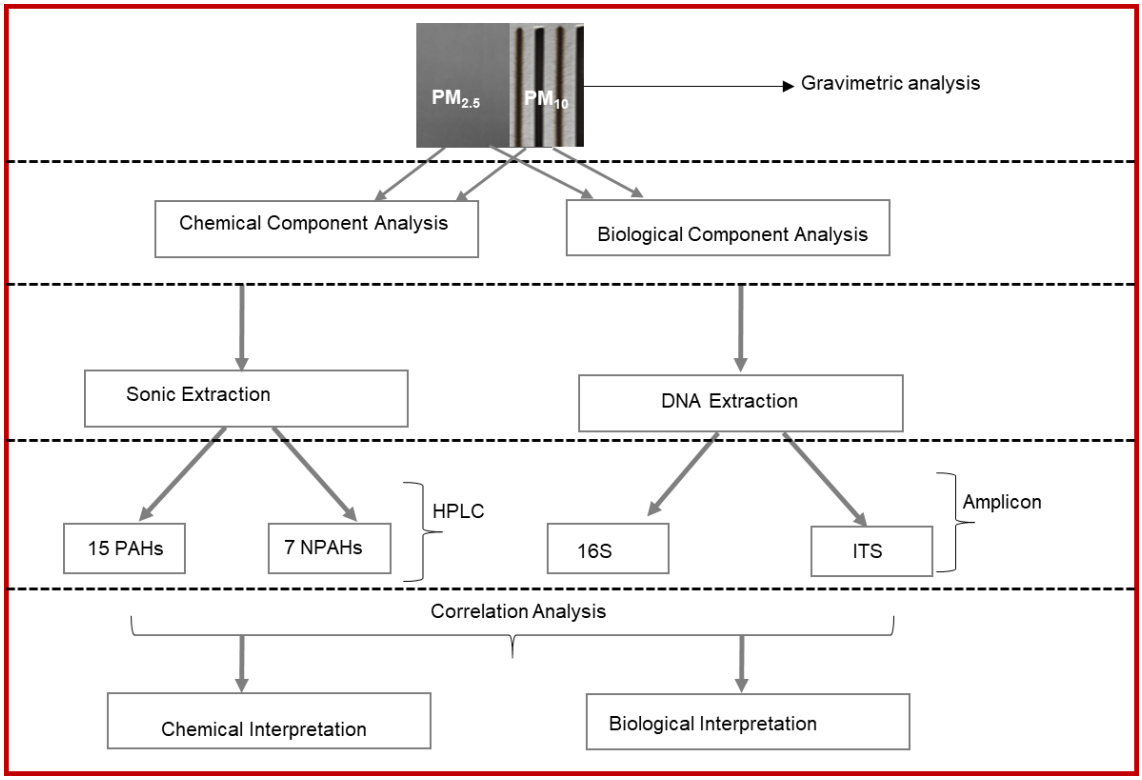

Figure S6. 1. Filter sample analysis flow chart. Filter after air sampling and gravimetric analysis, preparation of the filter for downstream analyses and analysis of the sample components and chemical and biological data analysis and interpretation.

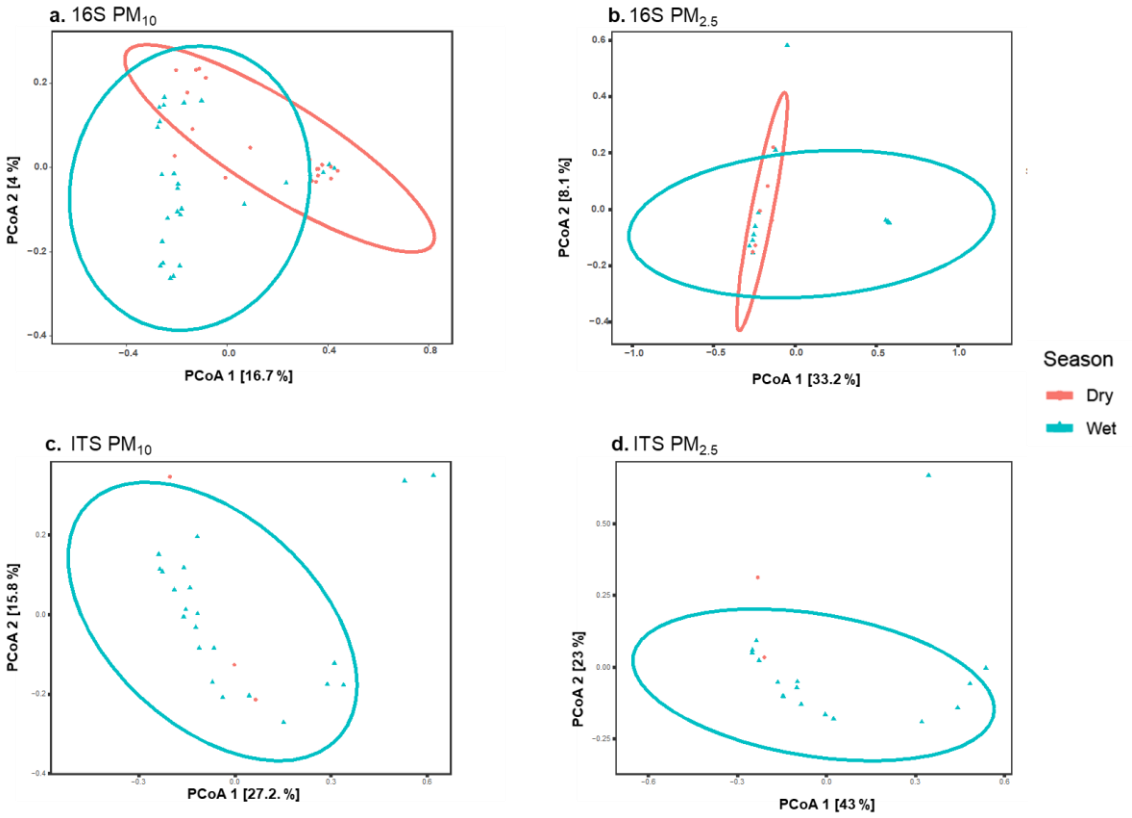

Figure S6. 2. Principal coordinates analysis (PCoA) of the Bray-Curtis dissimilarities of bacterial communities (relative abundances) from PM_{10} samples (a) and $PM_{2.5}$ samples (b) and the fungal communities from PM_{10} samples (c) and $PM_{2.5}$ samples (d) from dry and wet seasons in Rwanda.

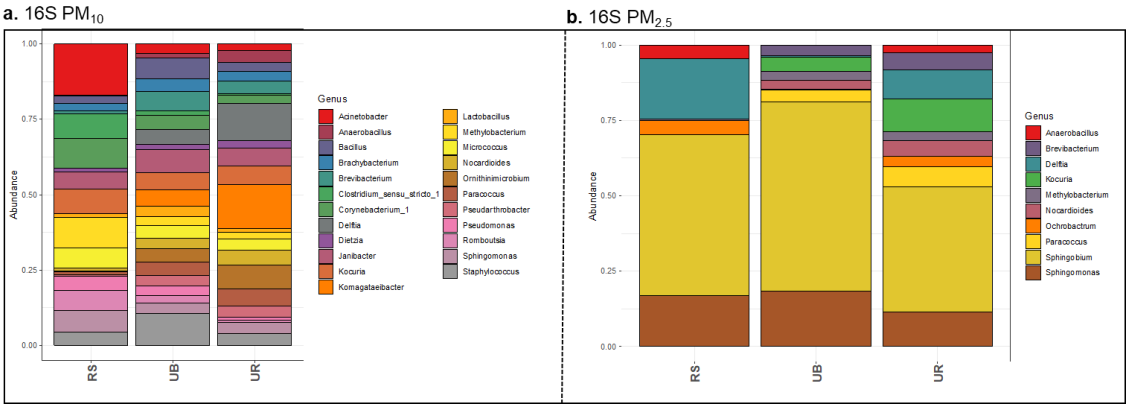

Figure S6. 3. Heatmap of relative abundances of the most abundant bacterial genera for PM_{10} samples (a) and $PM_{2.5}$ (b) from three land-use types: rural (Rs), urban background (UB) and urban roadside (UR) site.

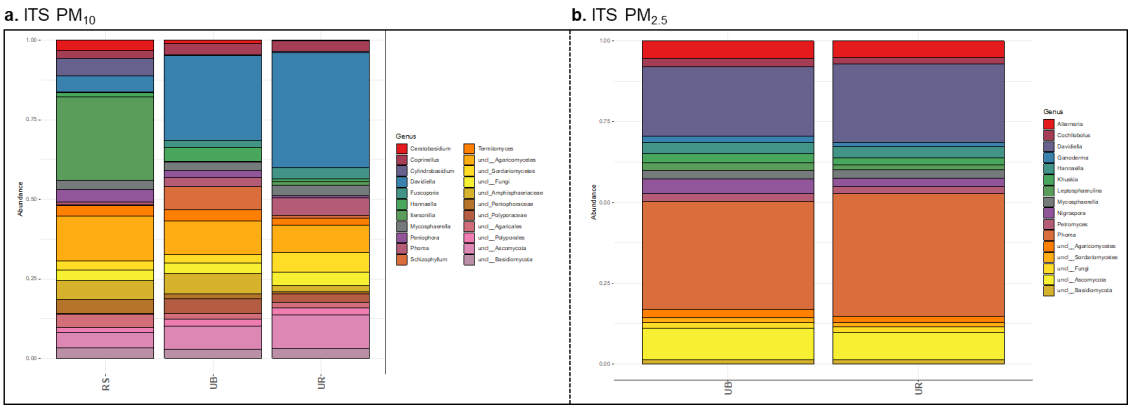

Figure S6. 4. Heatmap of relative abundances of the most abundant fungal genera for PM_{10} samples (a) and $PM_{2.5}$ (b) from three land-use types: rural (Rs), urban background (UB) and urban roadside (UR) site.

Chapter 7- General Discussion and Conclusions

7.1 Discussion of key findings

Given the health effects of the chemical and biological components of PM and the scarcity of information about atmospheric levels of PM in some parts of the world, monitoring of these components is necessary to evaluate mitigation strategies. The overall aim of this thesis was to characterize the chemical and biological components of PM in Japan, New Zealand, and Rwanda. Due to time constraints and funding limitations the chemical composition (PAHs and NPAHs) of PM was investigated in urban and rural environments in Japan, New Zealand, and Rwanda, while the biological composition (bacteria and fungi) of PM was only investigated in urban and rural environments in Rwanda.

The aim of this thesis was to bridge the current knowledge gap regarding the chemical and biological composition of aerosols **(Chapter 2)**, develop techniques and experimental designs to analyse the chemical and biological components of aerosols **(Chapter 3 parts A, B and C),** and characterize and determine the risk assessment of the chemical and biological components of PM in Japan, New Zealand, and Rwanda **(Chapters 4-6).**

7.2 Method Development

A key objective of this project was to improve the established methods for evaluating air quality and gain a more thorough understanding of the biological and chemical components of PM. Three sub-chapters were dedicated to evaluating method improvements that were subsequently used in the experimental case studies. The first part of the method development (Chapter 3 Part A) aimed to develop protocols for the simultaneous collection of the major chemical and biological components of PM. Part B aimed to determine whether any relationship existed between ambient air sampling duration and the atmospheric concentrations of extractable PAHs and NPAHs from different filter size areas. Part C aimed to determine whether any relationship existed between the ambient air sampling duration and extractable DNA yields from bioaerosols from differing filter size areas.

7.2.1 Chapter 3 Part A - Evaluation of high-volume and low-volume air samplers for chemical and biological components of airborne particulate matter in Rwanda.

Currently there is no standard protocol for collecting and analysing the chemical and biological components of PM simultaneously**.** High volume air samplers (HVAS) are widely used to collect

air samples onto filters for various chemical and biological component characterizations. However, using HVAS is costly, which limits this type of study in Africa. The purpose of this study was two-fold: to compare a low volume air sampler (LVAS), which is significantly more economical, with HVAS to characterize chemical and biological components in Rwanda, and to identify which methods would be most suitable for the case studies investigated in this thesis. Secondly, the aim was to provide a protocol to enable aerosol science researchers to simultaneously assess the chemical and biological composition of aerosol pollutants. In the first stages of developing an appropriate experimental method, HVAS and LVAS were deployed simultaneously in urban and rural locations over a 3-month period in Rwanda to collect particulate matter (PM) with aerodynamic diameters of ≤10 μm (PM_{10}). Seven PM_{10} samples from each air sampler were then analysed to test the comparability of the characterised chemical components (PAHs and NPAHs) and biological components (bacteria). The chemical composition and microbial community diversity of the PM_{10} samples were characterized using high performance liquid chromatography (HPLC) and high-throughput DNA sequencing, respectively. The results demonstrated that both chemical and biological composition could be performed off-line (in parallel) on the same sample. Chemical composition analysis revealed no significant difference in the concentrations of PAHs and NPAHs between the two samplers. Microbial analysis revealed that there was no great difference in bacteria communities' composition between both samplers. Results suggest that the difference in flow rate of the samplers would not have a significant effect on the chemical and biological composition analyses of PM when both samplers run for up to 24 hours. Therefore, the evaluated LVAS sampler was as effective as the standard HVAS and both systems could be considered in the chemical and biological characterization of aerosols. As the HVAS is costly, which may prevent study of this kind in Africa, LVAS is an option.

7.2.2 Chapter 3 Part B - Effect of sampling duration and filter sample size on the measurement of $PM_{2.5}$ and PM_{10} particulate-bound PAHs and NPAHs

Sampling duration and PM filter extraction are important considerations in the efficiency of aerosol collection for analysis of organic compounds. Early investigations of PAH and NPAH analysis in ambient PM collected on filters used different sampling durations and different filter area sizes. This raised the question of to what extent are the PAH and NPAH profiles obtained

from various sampling durations and reduced filter area sizes representative of the actual PAH and NPAH concentration present at the time of sampling. This study undertook a thorough investigation of how the following factors influenced the experimental outcomes: (i) the effect of sampling duration on the concentration of atmospheric PAHs and NPAHs; and (ii) the effect of filter area size of $PM_{2.5}$ and PM_{10} filters on PAH and NPAH recoverability. The study applied three sampling durations (24 hours, 5 days and 7 days) and carried out airborne PM extraction using different filter area sizes (an eighth, a quarter and a half), cut sequentially from $PM_{2.5}$ and PM_{10} sample filters. Ambient $PM_{2.5}$ and PM_{10} samples were collected in Auckland, New Zealand using HVAS. Fourteen PAHs and nine NPAHs were extracted from each filter area size and analyzed using HPLC with fluorescence and chemiluminescence detectors, respectively. The concentrations of total PAHs and total NPAHs per cubic metre of air decreased as the sampling time increased; i.e. 24 hours > 5 days > 7 days. The results also showed no significant difference in PAH and NPAH concentrations extracted from one half, one quarter or one eighth of the original filters. The amount of particulates collected during 24-hour sampling was sufficient for the analysis of PAHs and NPAHs but required a larger sampling area (at least a half of the whole filter) for extraction to reflect and represent the whole filter.

There was no difference in the mass of particulates sampled after 24 hours of sampling for both $PM_{2.5}$ and $PM_{10,}$ indicating the filters were likely saturated and had reached equilibrium; thus, increasing the sampling time would not have much effect on the amount of PM collected. Diagnostic ratio analysis of NPAHs to PAHs showed an increase with increasing sampling duration, suggesting that long sampling times may cause losses and secondary reactions of the PAH and NPAH compounds on the filter. These results suggest that shorter sampling durations are preferable, to avoid secondary reactions (degradation and formation) of compounds on the filter and to increase the temporal resolution of the study. This study contributed to the understanding of how the atmospheric concentrations of PAHs and NPAHs attenuate with increasing sampling duration and demonstrated the effect of filter area size on the accuracy of the analyses. Further study, with simultaneous long-term seasonal monitoring is required to validate

these findings and help reach the goal of a more standardized analysis method for aerosol composition.

7.2.3 Chapter 3 Part C - Efficiency of sampling duration for DNA-based analysis of bioaerosol

Biological aerosols have very low biomass concentrations in air, compared to water and soil. To date there is no universal standard for DNA extraction of air samples, which often leaves researchers with limited options for downstream analyses, especially when culture-independent methods are required. To overcome the technological and methodological limitations of previous approaches to aerosol sampling using impactor collection, which are known to introduce significant sampling biases in airborne microbial sampling, in this study an HVAS was used to collect enough biomass for DNA extraction. The impacts of three important factors that can influence the performance of culture-independent DNA-based analysis of air samples were investigated in this thesis as a preliminary study. These factors included the effect of sampling duration on the DNA yield and the effect of filter area size on DNA recoverability. PM_{10} samples were collected in the Central Business District of Auckland, New Zealand. The total air volume filtered was 1,440 m^3, 7,200 m^3 and 10,080 m^3 for sampling durations of 24 hours, 5 days and 7 days, respectively. Each of the filters was cut into different sized portions (half, one quarter and one eighth of the total) and DNA was extracted using the cetyl trimethyl ammonium bromide (CTAB) extraction protocol and quantified using a Qubit 2.0 Fluorometer. ANOVA tests showed that the concentrations of the genomic DNA (gDNA) recovered in 7 days were significantly higher than 5 days and 24 hours ($p = 0.0433$) and the results showed no significant difference in gDNA concentrations extracted from half, quarter and one eighth of the original filter size samples ($p = 0.72014$). Due to the low concentrations of bioaerosols, collecting enough biomass for the desired analysis methods is an issue, and at least an eighth of the total filter area size was required for DNA extraction to reflect and represent the whole filter. This study contributed to the understanding that the accuracy of studies of DNA-based bioaerosols depends on sampling time and extraction methods. Further studies should perform high-throughput sequencing to validate these findings on the community diversity of bioaerosols.

7.3 Chapter 4 - Pollution characteristics and risk assessment of ambient $PM_{2.5}$-bound PAHs and NPAHs in typical Japanese and New Zealand cities and rural sites

This was the first study to compare the atmospheric behavior of PAHs and NPAHs in comparable developed countries located in different hemispheres (Chapter 4) (Kalisa *et al*., 2019 b). Japan was used for comparison with New Zealand because the two countries share similar characteristics: both are part of archipelagos, situated along the northwestern and southwestern margins of the Pacific respectively, and subject to prevailing westerly winds carrying dust particles. Nine PAHs and six NPAHs were measured in $PM_{2.5}$ samples collected using HVAS over one year (2016-2017) spanning winter, summer, spring and autumn in two cities: Kanazawa (Japan) and Auckland (New Zealand) and their respective rural background sites: Wajima on the Noto Peninsula (Japan) and Tapora on the Okahukura Peninsula (New Zealand). PAHs and NPAHs were analysed using HPLC with fluorescence detection and chemiluminescence detection, respectively. Results showed that the mean concentrations of ΣPAHs and ΣNPAHs followed similar distribution profiles in both countries, with higher concentrations in the urban than the rural sites (Kalisa *et al*., 2019 b). The mean of ΣPAHs in Kanazawa City was 0.53 ng/m^3 and represented the highest total concentration compared to the other three sites. Conversely, the highest ΣNPAHs were measured in Auckland City (48.2 pg/m^3). Diagnostic ratios and principal component analysis revealed that automobiles were the main source of PAHs and NPAHs in both cities, whereas coal combustion and domestic wood burning were the dominant sources of PAHs and NPAHs in rural sites in both Japan and New Zealand. Back trajectory analyses showed that high levels of PAHs detected at Wajima were the result of long-range transport from China and Mongolia in winter, which was not observed in New Zealand, probably because of its isolation from other continents (Kalisa *et al.*, 2019 b).

The findings from this study were consistent with several previous studies that measured PAHs and NPAHs at Japanese sites (Hayakawa *et al.*, 2018b). This study can serve as the foundation for further work in this area, especially in New Zealand, where there is a lack of information on PAHs and NPAHs. Although the levels of $PM_{2.5}$ were generally below the WHO regulatory limits in New Zealand and Japan, their chemical compositions, particularly with regard to PAHs and

NPAHs, may still cause problems in vulnerable populations such as children and elderly; thus, continuous monitoring of these compounds and development of emission control measures is needed.

7.4 Chapter 5 - Chemical and microbiological characterization of atmospheric $PM_{2.5}$ and PM_{10} particulate across three land-use types in Rwanda, Central-East Africa

In the first study of its kind in Africa, this thesis undertook a field study to characterize the chemical and biological composition of PM in urban background, urban roadside and rural sites in Rwanda. The scale and complexity of the dataset meant that it was separated into an initial chemical analysis (Chapter 5) (Kalisa *et al*., 2018a) and a biological analysis with links to the chemistry results (Chapter 6) (Kalisa *et al*: Manuscript submitted and under review).

Rwanda is a landlocked country located in the Sub-Saharan region, with rapid urbanization and the highest population density in Africa. Information on air quality is scarce to non-existent for the whole country. Available data on PM are satellite estimates and there is no ground-based monitoring data for PM or its chemical and biological composition in urban or rural areas of Rwanda, or in Africa in general (Kalisa *et al*., 2019a). The Rwandan Environmental Management Authority (REMA), responsible for air quality compliance and the formulation of policies for environmental protection, has recently launched a law for effective control of air pollution emissions, which was published in 2016. However, controlling these emissions alone will not achieve effective improvement of air quality. Integrated control of all sources of air pollution is needed, via a robust regulatory framework based on rigorous scientific evidence. Such a framework is not currently possible due to lack of knowledge of the sources, causes and impacts of air pollution. In this study, PM with aerodynamic diameters of $\leq$2.5 μm and $\leq$10 μm ($PM_{2.5}$ and PM_{10}, respectively) was collected using HVASs over a three-month period (from 1 April to 30 June 2017) in different land-use areas, and during the wet and dry seasons in Rwanda. Half of each PM filter sample was analysed for chemical composition (PAHs and NPAHs) using two high-performance liquid chromatographic systems, with one system equipped with a fluorescence detector and the other equipped with a chemiluminescence detector. This study involved designing experiments, deploying equipment, and overcoming logistical difficulties associated

with reliable data collection in Rwanda. The data showed that ambient $PM_{2.5}$ and PM_{10} levels were higher in the dry season than in the wet season and greatly exceeded WHO guidelines. The analyses also showed higher concentrations of PAH and NPAH compounds at the urban roadside site than at the urban background and rural sites. Multivariate statistical analysis tools and models (HYSPLIT) were used to explore correlations within the data and to determine the origins of air masses. In parallel, a methodology developed by the US EPA was applied to evaluate the cancer risk of PAHs and NPAHs detected in Rwanda's ambient air. It was found that poor outdoor air quality in rural areas of Rwanda is due to cooking with wood or other vegetation products, widespread seasonal burning and long-range transport from neighboring countries in close proximity. Significant production of particles from diesel and gasoline-powered vehicles, due to a heavy reliance on the importation of used vehicles with poor or degraded emissions control, was a major source of poor air quality in urban locations (Kalisa *et al.,* 2018a). In Rwanda, more than 70 % of on-road vehicles were manufactured before 2005 (Duhuze, 2018), supporting the findings of this study that air pollution in Kigali was significantly higher at roadside locations than at urban background locations (Kalisa *et al.,* 2018a).

Health risk assessments demonstrated that lifetime cancer risks resulting from inhalation of PM-bound PAHs and NPAHs exceeded WHO safe limits. The study showed the impact of human activities on air quality by analysing air pollution during work days and non-workdays, finding a more than 30% drop in air pollution during the country's car-free days and holiday seasons (Kalisa *et al.,* 2018a). Based on the 2015 report of the Global Burden of Disease (GBD) study (Cohen *et al.,* 2017) and according to the WHO global urban ambient air pollution database , the 2016 $PM_{2.5}$ concentration for urban Rwanda was estimated at 40.7 $\mu g/m^3$ (World Health Organization, 2016b); this study averaged 81.4 $\mu g/m^3$ (urban background), which is 100% higher. In addition, the Health Effects Institute (HEI) reported that the population-weighted annual average $PM_{2.5}$ in Rwanda was 43 $\mu g/m^3$ in 2017 (Health Effects Institute, 2019). The present study showed that the mean concentration of $PM_{2.5}$ was significantly higher at urban roadside (185.3 $\mu g/m^3$) than urban background (81.4 $\mu g/m^3$) and rural sites (45.0 $\mu g/m^3$). These results suggest that levels of ambient $PM_{2.5}$ in urban Rwanda and population weighted $PM_{2.5}$ averages for all of Rwanda as reported by

the WHO and HEI are likely to be underestimated. This study provided clear evidence that immediate development of emission control measures is required, and quantitative information on the sources of PM would allow a more comprehensive assessment of the risk of human exposure to PM from various sources in Rwanda. Exposure to PM is a regional problem and this study, conducted in Rwanda, can be used to shine a light on the issue and produce findings relevant to other countries in Sub-Saharan Africa.

7.5 Chapter 6- Simultaneous chemical and microbial characterization of atmospheric PM_{10} and $PM_{2.5}$ particulate from three land-use types in Rwanda, Central-East Africa

This study described the total microbial community structure (bacteria and fungi) and its variability with regard to airborne PM size, season and sampling site. This was the first study in the African continent to demonstrate the variation in spatial and temporal characteristics of bacteria and fungi in aerosol size fractions using high-throughput sequencing. In addition, available studies on the chemical and biological composition of PM, performed in developed countries suggest that there may be an association between the chemical and biological components in PM size fractions. However, the interrelationship of these factors has never been investigated in Africa and very little is known in other areas worldwide (Kalisa *et al*., 2019a). In this study the chemical composition (PAHs and NPAHs) and the microbial community structure (16S rRNA gene and ITS region) were characterized, and the relationships were investigated using multivariate statistics. Microbial analysis revealed that the diversity and composition of the airborne bacterial and fungal communities varied by site, PM size fraction, and season. The majority of the biological material originated from soil, dust and plants. Redundancy analysis (RDA) identified that PAH and NPAH species were significantly negatively correlated with microbial communities known to be highly efficient PAH degraders in the soil. Variation partitioning analysis (VPA) indicated that environmental factors and the bacterial community structure (genus level) together explained 44.95% of the total variation in chemical composition. This interaction of chemical and biological aerosols may constitute a health concern since it may cause changes in composition and structure (Du *et al.*, 2018). To our knowledge, this is the first study to demonstrate the correlation between PAH/NPAH compounds and microorganisms in the

atmosphere, suggesting the potential for discovering highly efficient PAH and NPAH degraders, which may enhance further research into the behavior of PAH/NPAH-degrading microorganisms in the atmosphere. This data may be used as a reference for future urban and rural planning efforts to reduce pollution and the spread of airborne microbial allergens and pathogens associated with PM size fractions.

7.6 Data Synthesis

This is the first study to undertake a simultaneous chemical and biological characterization of particulate matter ($PM_{2.5}$ and PM_{10}) in developed countries (Japan and New Zealand) and a developing country (Rwanda). The chemical composition (PAHs and NPAHs) of PM was investigated in urban and rural environments in Japan, New Zealand, and Rwanda, while the biological composition (bacteria and fungi) of PM was only investigated in urban and rural environments in Rwanda. This investigation provided the most comprehensive characterization of PAHs and NPAHs, as well as airborne microorganism associated with particulates matter of the size fraction of health concern. Airborne $PM_{2.5}$ and PM_{10} was collected in urban and rural areas of Japan, New Zealand and Rwanda. The focus was on the USEPA priority-listed PAHs and NPAHs, and microorganisms (bacteria and fungi) loading on the $PM_{2.5}$ and PM_{10} size fractions.

There is a significant experimental complexity in simultaneously assessing the chemical and biological components of PM, which is usually related to the difficulty in measuring concentrations of biological and chemical components of PM together, due to differences in flow rates, which often results in a time difference between samplers. For example, some authors have attempted to investigate the association of biological and chemical compounds by performing characterizations of the collected particles in parallel (one sampler designed for chemical components analyses in conjunction with another sampler for biological component analyses) (Adhikari *et al.,* 2006). However, this approach must be carefully managed to avoid potential experimental bias since the collection of these compounds changes significantly with time, flowrates and space. Additionally, the concentrations of the biological and chemical components of ambient aerosols change with time and space, so any variations in time and space introduced between the two measurements may affect the outcome. Since the rate of change of these two

concentrations may not be the same, more reliable results could be achieved if time series of the concentrations of biological and chemical components could be compared. In this thesis methods were developed to overcome these challenges. The chemical and biological components were analysed from the same filter using a single air sampler with uniform flow rate, and sampling duration for every sampling site. Thus, the investigation of the association of chemical and biological components of aerosols did not experience any experimental bias.

The High-Volume Air Sampler (HVAS) was used to collect samples in the case studies because it is equipped with an inlet impactor fitted with a $PM_{2.5}$ and PM_{10} size-selection filter. This allowed accurate chemical and biological analyses to be conducted over short sampling intervals, making it possible to identify and measure potentially toxic chemical and biological components that can penetrate the lungs. For the Rwandan study, a 24-hour sampling period was selected as the most suitable sampling duration for analysis of PAHs/NPAHs and airborne microorganisms in consideration of the PM concentration at the sampling site.

Sampling duration and filter extraction are important considerations in the efficiency of aerosol collection for analysis of organic compounds and airborne microorganisms. Early investigations of chemical and biological aerosol analysis in ambient PM collected on filters used different sampling durations and different filter area sizes. This raised the question of to what extent are the chemical and biological aerosol profiles obtained from various sampling durations and reduced filter area sizes representative of the actual concentration present at the time of sampling. Extraction of the whole filter has two main drawbacks: the use of larger volumes of toxic solvents and long extraction times, and more importantly the loss of the entire sample so that no archive can be kept for future analysis and insurance if there is a difficulty with the analysis. This study undertook a thorough investigation of how the sampling duration and filter area size affected the concentration of atmospheric PAHs and NPAHs and DNA concentration. The study applied three sampling durations (24 hours, 5 days and 7 days) and carried out airborne PM extraction using different filter area sizes (an eighth, a quarter and a half), cut sequentially from PM sample filters collected in New Zealand. The preliminary study showed that 24-hour sampling was the most efficient for analysis of PAHs and NPAHs compared to 5 and 7 days, while more gDNA was

obtained with a longer sampling duration (7 days). This study compared PAHs and NPAHs between New Zealand and Japan; and the 7-day sampling period was previously found to be most suitable for analysis of these compounds in Japan (Tang *et al.*, 2002; Yang *et al.*, 2007). Therefore, the longer sampling duration of 7 days was selected to be used in collecting PM samples in New Zealand in order to allow direct comparisons and analysis for both chemical and biological components of PM. There was no significant difference in filter area size analysis, thus any filter area size used was a representative of the total filter. The methods developed in this thesis described the sampling and analytical procedures in detail and the results obtained will be of particular interest to researchers investigating chemical and biological compositions of atmospheric PM.

The experimental chapters investigated the concentrations of PAHs and NPAHs, determined major sources of PAHs and NPAHs, and determined lifetime cancer risks resulting from inhalation exposure to PM-bound PAHs and NPAHs in Japan, New Zealand and Rwanda. The results obtained from these countries provided two unique opportunities to characterize the air pollution in 'typical' developing and developed cities. Globally, emissions from motorized vehicles are a growing concern as road traffic increases. Automobile emissions are the leading cause of air pollution in developed countries, including Japan and New Zealand, but in developing countries like Rwanda, they often decrepit state of many vehicles and poor regulatory oversight means petrol or diesel-driven engines can be just as bad in their outputs, especially of PM bound PAHs and NPAHs that have carcinogenic and mutagenic health impacts. Exposure to potential carcinogen was vastly higher in Kigali than in Kanazawa or Auckland (Kalisa *et al*., 2018a, Kalisa *et al.*, 2019b), although the number of registered vehicles in Rwanda is much lower. The impact of vehicle emissions in Rwanda is higher because the heavy diesel vehicles, buses, and moto-taxis are mostly older and imported as second-hand vehicles. Additionally, Japan and New Zealand have more stringent emission standards and monitoring, as well as programmes that encourage the implementation of low-emission public transport. The findings of this thesis suggest that the establishment of clear air quality policies and mitigation measures, along with a strong environmental health programme, could guide developing countries like Rwanda in reducing air

pollution and its associated health impacts. As a result, the current study could have a positive impact on both the scientific community and policymakers because of its differentiation of pollution sources and the observation of elevated concentrations of pollutants compared with corresponding studies performed in more developed countries, and may assist in the development of air quality measures and policies. Since exposure to carcinogenic and mutagenic PAHs and NPAHs in ambient air pollution is a global problem, this research conducted in Japan, New Zealand, and Rwanda generated findings that are relevant world-wide.

The $PM_{2.5}$ and PM_{10} sample filters collected in Rwanda were analyzed for PAH and NPAH and for microbial community structure (bacterial and fungal rRNA gene sequences). This is the first study to present a multi-domain assessment of bio-aerosols from three land-use types in Africa using high-throughput DNA analysis. The data presented here are useful for epidemiological studies to develop public health policies regarding the monitoring and management of particulate air pollution and the protection of public health, and to create a baseline dataset for further studies. While most in the scientific community and those involved in epidemiological studies are of the opinion that the total PM is an important driver of resultant health effects, there is still some degree of uncertainty regarding the major components considered to be most harmful, and the importance of each, or the synergistic effects of PM components. This study provides useful data for epidemiological studies and on the nature of chemical and biological aerosols composition loading on airborne particulate samples, which can be used for setting up an effective exposure limit for PM compositions.

7.7 Strengths and limitations of the study

7.7.1 Strengths

The aim of this thesis was to characterize the chemical and biological composition of PM in Japan, New Zealand and Rwanda. This work added a significant new dimension to the understanding of

the chemical and biological composition of the particle size fractions of the greatest health concern in typical developed and developing countries.

Given the relevance of the different species of air pollutants in relation to human health, this work closed the gap in research previously carried out on the African continent. In this thesis, epidemiological and toxicological studies conducted in African cities were reviewed for the first time to summarize the association between the biological and chemical components of PM and their associated health outcomes. The detailed information on levels, source and health risk assessment of exposure to carcinogenic of PAHs and NPAHs in atmospheric particulate were provided. This information will serve as a basis on which decision and policy makers can establish environmental management programmes to protect the public from exposure to carcinogenic organic pollutants and pathogenic microorganisms in Africa. This study may also encourage researchers to continue to fill the data gap in air pollution research in this neglected yet rapidly developing and highly vulnerable region of the world.

New methods were developed, and this provided different approaches for analysing the major PM bound chemicals, such as PAHs and NPAHs, and airborne microorganisms associated with PM that overcome the limitations of current methods. This approach can be used in both developed and developing countries with high efficiency and accuracy. This study confirmed that low-cost monitors, LVAS, can measure both the chemical and biological components of particulate air pollution which can be an alternative monitoring device in Rwanda. This is an affordable, and accurate method to monitor air quality in many areas of the country that currently lack air pollution data. The study reported the concentrations and sources, and the cancer risk of human exposure to ambient $PM_{2.5}$ and PM_{10}-bound PAHs and NPAHs in typical Japanese, New Zealand and Rwandan cities and rural sites. It showed that multivariate receptor models such as principal component analysis (PCA) and diagnostic ratios of NPAH to PAH concentrations can be successfully applied to infer the sources of PAHs and NPAHs in both developed and developing countries. This study also demonstrated that the HYPLIT back trajectory model can be used to investigate long range transport of PAHs and NPAHs in both maritime and landlocked areas. This is the first study to present a multi-domain assessment of bio-aerosols in three land-use types in

Africa using high-throughput DNA analysis. This work provides a significant novel dimension to the understanding of bioaerosols in a sub-Saharan country (Rwanda) influenced by wood burning and identified putative pathogenic and allergic microorganisms in air. This study showed how land-use types, PM size, and season affect bacterial and fungal communities. The data presented here are useful for epidemiological studies to enable the development of public health policies on the monitoring and management of particulate air pollution and the protection of public health, and to create a baseline dataset for further studies. This was the first study in Africa to investigate the relationship between the chemical and microbial components of PM. The data obtained will be useful in epidemiological studies and in setting up effective PM exposure limits, rather than using the existing measure of total mass. It will also be useful in designing effective PM control strategies. The study demonstrated that diesel vehicles were major source of air pollution in Kigali City, and that car-free days significantly reduced air pollution in Rwanda. As a result of these findings, the Rwandan government has introduced car-free days for other cities which initially were only held in Kigali City. This study established that LVASs can play an important role in the continued monitoring of air quality in Rwanda to investigate the effect of the government's new policies on air pollution control. The Rwandan government and the WHO had previously relied on satellite data for air quality estimations when assessing the impact of ambient air pollution on health. However, the findings from this thesis suggest that the satellite estimated data for ambient PM in urban Rwanda are probably underestimates and provide clear evidence that long-term ground monitoring of air quality is required.

7.7.2 Limitations

This research generated a comprehensive chemical and biological dataset ever collected in Africa in order to address the knowledge gap with regards to PM air pollution. Due to the logistical complexity of sample collection and the extensive variety of analytical techniques, the period of study was limited and lacks the long-term monitoring component necessary for extensive regulatory review. However, information provided in this thesis can be used as a baseline for further long-term analysis. This study attempted to gain the best temporal and spatial resolution possible with the equipment and time that was practically available. It would have been more

meaningful to simultaneously collect samples from all sites in the three case studies to ensure there was no bias in the data. Due to time constraints and funding limitations, biological components were not analyzed for Japan and New Zealand, but samples were stored for future analyses.

7.8 Future Directions

The research presented in this thesis suggests a range of future work to improve the understanding of atmospheric aerosol composition in developed and developing countries.

This study used the back trajectory Hybrid Single Particle Lagrangian Integrated Trajectory (HYSPLIT) model to determine the origin of air masses and establish source receptors (Kalisa *et al.*, 2018a,Kalisa *et al.*, 2019b). Further investigation with more advanced instrumentation such as high-resolution time-of-flight aerosol mass spectrometry (HR-ToF-AMS) should be conducted in these countries to quantify the contributions of various local and regional sources to air pollution. This data may help to initiate international action, along with local and national efforts to cut air pollution and reduce its effects on health (World Health Organization, 2006b).

In this study, source identification of PAHs and NPAHs was investigated using diagnostic ratio analysis of NPAH to PAH concentrations and principal component analysis (PCA), which showed that the use of diesel and gasoline-powered vehicles in urban locations and wood burning in rural locations were the major sources of PAHs and NPAHs. However, future studies should also use positive matrix factorization (PMF) models to apportion the sources of PAHs and NPAHs across urban and rural areas of Japan, Rwanda and New Zealand and determine the contributions of primary emissions and secondary formation.

So far, the negative influences of air pollution in Rwanda and Sub-Sahara Africa in general have received relatively little attention from a public health viewpoint. Future work will involve long-term monitoring in most areas of Rwanda and working with the public to provide information and raise awareness of the issues of poor air quality (Nahayo *et al.*, 2019). This long-term monitoring, with increased community awareness, will have the potential to generate sustainable frameworks

to encourage economic growth without jeopardising public health, and create more liveable environments.

In Rwanda, more than 85% of biomass fuel is wood and charcoal, used for cooking and heating in both urban and rural areas (MININFRA, 2018). Recent studies published in the Lancet indicate that sub-Saharan African countries have the highest per capita levels of premature death and disease due to indoor pollution (Forouzanfar *et al.,* 2016; Landrigan *et al.,* 2018). This is of particular concern in Rwanda, where more than 8000 deaths recorded in 2002 were attributable to indoor air pollution, with more than 90% of those deaths occurring in children under 5 years of age (Gatskie and Karch, 2010). However, there is a severe lack of information on indoor air pollution in Rwanda. Therefore, future studies should use modern techniques and monitoring, emissions modelling, questionnaires and health assessments to evaluate the interdependence of indoor and outdoor air pollution. This will enable effective mapping of air pollution in both urban and rural locations of Rwanda and will identify current knowledge gaps and data needs. The relationships between indoor and outdoor air pollution will inform epidemiological studies that currently rely on estimates of ambient outdoor air pollution to estimate total exposure in populations.

Using HVASs can be costly and may limit studies in Africa. This thesis has confirmed that low-cost monitors can be successfully used to measure air pollution in Rwanda and provide an affordable, reliable network to monitor air quality in many areas of the country. Therefore, future studies can develop the capacity and expertise of Rwanda stakeholders to undertake air pollution monitoring campaigns using low-cost sensors and also quantify the effects of meteorological conditions on air quality, which have been previously found to be correlated (Kalisa *et al.,* 2018b). This will enable the gathering of large amounts of data in typically hard-to-access locations such as homes and workplaces.

To promote social welfare, the Rwandan government has recently implemented “car-free” Sundays, on which major roads are blocked off, there are no business activities, and people take part in mass exercise sessions on car-free streets. This study demonstrated for the first time how

a car-free day policy, along with other non-working days, can significantly reduce air pollution in Kigali City (Kalisa *et al.*, 2018a). Very recently, the car-free day policy was extended to most cities of Rwanda and expanded to the first and third Sundays of each month. In addition, in 2017 Rwanda implemented a new policy to curb the use of older imported cars and increase taxes on personal vehicles (RRA, 2019). As a result, vehicle importation dropped by more than 20% during the first 6 months of 2017 (Ngabonziza, 2017). Extensive monitoring is required to quantify the effects of these policies on air quality in Rwanda. Monitoring networks for bioaerosols should be established to enable the mitigation of respiratory diseases during severe air pollution episodes.

Airborne microorganisms have been found to live in tight association with PM, but few studies have characterized biological compositions in developed countries such as Japan and New Zealand. Therefore, future studies should use a combination of both culture-independent and culture-dependent techniques for better characterisation of biological aerosols in air samples; this could distinguish between viable and non-viable organisms present in PM.

This thesis has demonstrated the relationship between the chemical (PAH and NPAH) and biological components (bacteria and fungi) of PM. However, the synergistic health effects of this association are still far from being understood and deserve some comprehensive research. Further study is required to investigate the roles of the chemical and biological components of PM size fractions in disease causation. Considering the impacts of bioaerosols on human health, examining both indoor and outdoor bioaerosol exposure levels in different locations, and their spatial variability, is important for vulnerable populations living in these areas.

7.9 Conclusion

This study investigated, for the first time, the chemical and biological compositions of particulate matter in Japan, New Zealand, and Rwanda, and developed a novel technique for simultaneously assessing chemical components (PAHs and NPAHs) and biological components (bacteria and fungi) associated with particulate matter. The effect of long-range PAH and NPAH transport from neighboring countries was confirmed, suggesting that air pollution is a global problem and requires international collaboration to determine mitigation measures. The levels of PM in Japan and New Zealand were below the WHO limits, but the chemical compositions of PAHs and

NPAHs could still pose problems to vulnerable populations. For instance, because of the high concentration of NPAHs detected in Auckland City, the lifetime total cancer risk calculated from PAHs and NPAHs exceeded the guidelines used by the US Environmental Protection Agency. In developing countries like Rwanda, this study provided the first characterization of PAHs and NPAHs and demonstrated that PM concentrations and lifetime cancer risks resulting from inhalation exposure to PM-bound PAHs and NPAHs exceed the World Health Organization safe limits. This study highlighted the effect of anthropogenic activities on air pollution in Rwanda. Automobile emissions were found to be the main source of PAHs and NPAHs in all three countries. However, because of the preponderance of second-hand vehicles more than 10 years old in Rwanda, the lack of transport infrastructure and vehicle emission control, and the lack of air quality monitoring and standards, the levels of these carcinogenic compounds and the resulting health risk assessments are significantly higher than in Japan or New Zealand, although they have far more vehicles and industries compared to Rwanda. The data obtained from this investigation have a positive impact in the scientific community as a result of the differentiation of pollution sources and the elevated concentrations of pollutants in a developing country compared with corresponding studies performed in more developed countries. This was the first study to characterize bioaerosols in Africa using next generation sequencing to find the proportions of pathogenic and opportunistic bacteria and fungi. The diversity and composition of the airborne bacterial and fungal communities varied by site, PM size fraction, and season. A range of pathogenic and allergic bacteria and fungi were detected in in PM size fraction of health concern. Significant correlations between chemical and biological compositions of particulate matter were observed in this study, providing useful data for epidemiological studies and in setting up effective exposure limits for PM composition, rather than using the existing measure of total mass. It will also be useful in designing effective PM control strategies. Effect of meteorological conditions (temperature and RH) on chemical and microbial components of PM was observed in this thesis. The findings of this research are informative to public health in Japan, New Zealand and Africa in terms of understanding how human-modified environments impact public health. Exposure to toxic PAHs and NPAHs as well as pathogenic microorganisms associated with particulate matter

air pollution is a global problem and research conducted in Japan, New Zealand and Rwanda has produced findings that may be relevant world-wide.

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Appendices: Screenshot of published papers in peer-reviewed journals

Appendix 1: Chemical and biological aerosol in Africa (a review).

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Review

Chemical and Biological Components of Urban Aerosols in Africa: Current Status and Knowledge Gaps

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Abstract: Aerosolized particulate matter (PM) is a complex mixture that has been recognized as the greatest cause of premature human mortality in low- and middle-income countries. Its toxicity arises largely from its chemical and biological components. These include polycyclic aromatic hydrocarbons (PAHs) and their nitro-derivatives (NPAHs) as well as microorganisms. In Africa, fossil fuel combustion and biomass burning in urban settings are the major sources of human exposure to PM, yet data on the role of aerosols in disease association in Africa remains scarce. This review is the first to examine studies conducted in Africa on both PAHs/NPAHs and airborne microorganisms associated with PM. These studies demonstrate that PM exposure in Africa exceeds World Health Organization (WHO) safety limits and carcinogenic PAHs/NPAHs and pathogenic microorganisms are the major components of PM aerosols. The health impacts of PAHs/NPAHs and airborne microbial loadings in PM are reviewed. This will be important for future epidemiological evaluations and may contribute to the development of effective management strategies to improve ambient air quality in the African continent.

Keywords: polycyclic aromatic hydrocarbons; nitrated polycyclic aromatic hydrocarbons; microorganisms; particulate matter; carcinogenic

Appendix 2: PAHs and NPAHs in Japan and New Zealand

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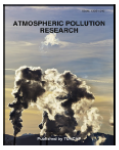

Pollution characteristics and risk assessment of ambient $PM_{2.5}$-bound PAHs and NPAHs in typical Japanese and New Zealand cities and rural sites

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ABSTRACT

Nine polycyclic aromatic hydrocarbons (PAHs) and six nitro-PAHs (NPAHs) were measured in $PM_{2.5}$ samples collected over one year (2016–2017) in two cities: Kanazawa (Japan) and Auckland (New Zealand) and their respective rural background sites: Wajima in Noto Peninsula (Japan) and Tapora in Okahukura Peninsula (New Zealand). The mean concentrations of ΣPAHs and ΣNPAHs followed similar distribution profiles in both countries, with higher concentrations in the urban than rural sites. The mean of ΣPAHs in Kanazawa City was 0.53 ng/m^3 and represented the highest total concentration compared to the other three sites. Conversely, the highest ΣNPAHs concentrations were measured in Auckland City (48.2 pg/m^3). Diagnostic ratios and principal component analysis revealed that automobiles were the main sources of PAHs and NPAHs in both cities, whereas coal combustion and domestic wood burning were the dominant sources of PAHs and NPAHs in rural sites in Japan and New Zealand. Back trajectory analyses showed that high levels of PAHs detected at Wajima were the result of long-range transport from China and Mongolia in winter, which was not observed at the other sites. Considering the adverse health effects of $PM_{2.5}$, further studies and continuous monitoring of atmospheric PAHs and NPAHs are necessary to evaluate mitigation strategies in both hemispheres.

Appendix 3: PAHs and NPAHs in Rwanda

Environmental Science & Technology

Article

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Characterization and Risk Assessment of Atmospheric $PM_{2.5}$ and PM_{10} Particulate-Bound PAHs and NPAHs in Rwanda, Central-East Africa

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Supporting Information

ABSTRACT: Exposure to airborne particulates is estimated as the largest cause of premature human mortality worldwide and is of particular concern in sub-Saharan Africa where emissions are high and data are lacking. Particulate matter (PM) contains several toxic organic species including polycyclic aromatic hydrocarbons (PAHs) and nitrated PAHs (NPAHs). This study provides the first characterization and source identification for PM_{10}- and $PM_{2.5}$-bound PAHs and NPAHs in sub-Saharan Africa during a three-month period that spanned dry and wet seasons at three locations in Rwanda. The 24-h mean $PM_{2.5}$ and PM_{10} concentrations were significantly higher in the dry than the wet season. PAH and NPAH concentrations at the urban roadside site were significantly higher than the urban background and rural site. Source identification using diagnostic ratio analysis and principal component analysis (PCA) revealed diesel and gasoline-powered vehicles at the urban location and wood burning at the rural location as the major sources of PAHs and NPAHs. Our analysis demonstrates that PM concentrations and lifetime cancer risks resulting from inhalation exposure to PM-bound PAHs and NPAHs exceed World Health Organization safe limits. This study provides clear evidence that an immediate development of emission control measures is required.