

**A PROSPECTIVE COHORT STUDY ON AMBIENT AIR
POLLUTION, AIRBORNE POLLEN (AND FUNGAL SPORES)
AND RESPIRATORY MORBIDITIES INCLUDING
CHILDHOOD ASTHMA IN ADOLESCENTS FROM THE
WESTERN CAPE PROVINCE**

By

Toyib Adedamola Olaniyan

Thesis presented for the degree of

Doctor of Philosophy

In the School of Public Health and Family Medicine

University of Cape Town

October 2018

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Supervisor: Professor Mohamed Aqiel Dalvie
Co-supervisor: Professor Mohamed Fareed Jeebhay
Co-supervisor: Professor Martin Rössli

This thesis is presented in fulfilment of the requirements for the degree of Doctor of Philosophy (PhD) in the School of Public Health and Family Medicine, Faculty of Health Sciences, University of Cape Town. The work on which this thesis is based is original research and has not, in whole or in part, been submitted for another degree at this or any other university. The contents of this thesis are entirely the work of the candidate, or in the case of multi-authored published papers, constitutes work for which the candidate was the lead author. The contribution of the candidate to included multi-authored papers is further delineated in the preface to the thesis and in the introduction to each included paper as appropriate.

Signed by candidate

Toyib Adedamola Olaniyan

October 2018

ABSTRACT

Background: The epidemiological studies investigating environmental risk factors associated with asthma among children living in informal settlements are scant as are studies on the independent and co-pollutant effect of short- and long-term exposures to ambient air pollutants as well as fungal spores on asthma-associated outcomes. This study systematically investigated these factors among schoolchildren residing in informal settlements in the Western Cape province of South Africa.

Methods: A cohort study of grade-4 schoolchildren (n=590) recruited from six primary schools in four informal settlements was conducted over 12 months. In addition, a panel study, investigated the children for 2 consecutive school weeks in both summer and winter. Spirometry and fractional-exhaled nitric oxide (FeNO) measurements were conducted during the school day, while the International Study on Asthma and Allergy in Children (ISAAC) standardised questionnaire was administered to the parent or guardian at the child's home at baseline and follow-up. The presence of atopy was determined based on a positive Phadiatop test on sera. In the cohort study, annual NO₂ and PM_{2.5} levels were computed for each child's address using a land-use regression model. Daily PM₁₀ levels obtained from a stationary monitor near two of the study areas were used for the panel study. Airborne pollen and fungal spore measurements were obtained directly from a stationary monitor placed in each study area.

Results: The prevalence of doctor-diagnosed asthma was 3.4% and only half of them were on asthma treatment. The prevalence of wheezing in the past 12 months (12.9%), airway obstruction (17.6%) and airway inflammation (10.2%) was much higher. The presence of damp conditions, visible mould growth, passive smoking as well as paraffin-use for cooking and heating were significant indoor risk factors for asthma. The estimated annual average NO₂ level of 16.6 µg/m³ was below the WHO annual exposure standards, however more than a third of children were exposed to annual PM_{2.5} levels above the 10 µg/m³ WHO standard and the allergic symptom threshold level of 100 spores/m³ for *Alternaria* spores. In the panel study, daily exposure of schoolchildren to *Alternaria* and *Cladosporium* spores independently decreased FEV₁ (-27.56 ml, 95% CI: -50.60 to -4.51 ml per 10 spores/m³ increase in *Alternaria*; and -86.19ml, 95% CI: -131.69 to -40.70 ml per 50 spores/m³ increase in *Cladosporium* respectively) from lag day-0 to lag day-5, especially in the winter monitoring period. In the cohort study, an interquartile range increase of 14.2 µg/m³ in annual NO₂ was associated with an risk of new onset ocular-nasal symptoms (adjusted odds ratio – aOR: 1.63, 95% CI: 1.01 – 2.60), wheezing (aOR: 3.57, 95% CI: 1.18 – 10.92), more than two or more asthma symptom score (aOR: 1.71, 95% CI: 1.02 – 2.86), and airway inflammation defined as FeNO > 35ppb (aOR: 3.10, 95% CI: 1.10 – 8.71), independent of PM_{2.5} exposures. In addition, an interquartile increase of 83.1 spores/m³ in 24-hour annual *Alternaria* spore levels was associated with an increased risk of airway inflammation incidence and having a ≥ 10% increase in FeNO at follow-up both in the single-pollutant model and two-pollutant model.

Conclusion: This study demonstrated a large proportion of undiagnosed and untreated asthma in schoolchildren living in informal settlements, with both indoor and outdoor mould exposures playing an important role in addition to ambient chemical pollutants. The incidence of new onset asthma symptoms and airway inflammation associated with NO₂ at levels below the WHO Air Quality Standards raises the issue of the adequacy of these standards in protecting respiratory health. Raised long-term levels of airborne *Alternaria* spores contributing to increased airway inflammation is likely to form the basis for the increased risk of acute symptoms and airway effects observed in association with exposure peaks.

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PREFACE

This thesis includes published papers, as per general provision 6.7 in the General Rules for the Degree of Doctor of Philosophy (PhD) of the University of Cape Town, and with the approval in 2018 of the University Doctoral Degrees Board. The following three papers are formally included as part of the thesis:

Olaniyan, T. A., Dalvie, M. A., & Jeebhay, M. F. (2015). Ambient air pollution and childhood asthma: A review of South African epidemiological studies. *Current Allergy and Clinical Immunology*, 28(2), 122–127.

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The contribution of the candidate to each paper is outlined in the introduction to each paper. The candidate was the lead author for each paper and drafted all versions of the manuscripts. The candidate was responsible for circulating the manuscripts to co-authors, reviewing co-author comments and suggestions before integrating them into the manuscript as appropriate. All co-authors critically reviewed and approved the submitted manuscripts prior to submission.

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CHAPTER 1

INTRODUCTION

1.1 Introduction

Childhood asthma is the most common chronic disease in children globally and ranks in the top 20 contributors of global disability-adjusted life years (DALY) in all children; and among the top 10 causes of DALY amongst 5 – 14 year olds (1). The global mortality rates of childhood asthma is approximately 0.7 per 100 000 (2). Asthma impacts significantly on the quality of life of a child as it can interfere with physical exercise, results in school absenteeism as well as underperformance at school due to sleep interruption. It is estimated that two-thirds of asthmatic children suffer from a noticeable disability and approximately ten million school days are missed yearly (3). Instances of severe asthma in children leading to frequent school absences might consequently affect the child's education and probably career choice later in life. South Africa currently ranks 25th globally with regard to asthma prevalence and has the fifth highest number of deaths due to asthma amongst children (4). However a high prevalence of undiagnosed and untreated childhood asthma has been reported previously in Cape Town especially among individuals from low socio-economic backgrounds (5). It was reported that 47% of asthmatic children were unrecognized and only 3% of these children were on daily treatment (5). Townend *et al.*, have also reported that a high prevalence of airway obstruction is significantly associated with poverty in several countries (6).

The rapid growth and urbanization in developing countries and the reports from the World Health Organization on the attributable effects of ambient air pollution (7,8), has fuelled public interest, government regulatory agencies and the urgency for studies to better understand the role of air pollutants especially in vulnerable populations such as children in developing countries from low socio-economic backgrounds living with underlying risk factors. Among other reasons, the increasing outdoor air pollution may also contribute to the increase in asthma prevalence in developing countries (5). Ambient air pollution is a major environmental health concern globally affecting the population in both highly industrialised and developing countries. The key criteria ambient air pollutants (PM_{2.5}, PM₁₀, CO, NO₂, SO₂ and O₃) identified by the WHO are usually composed of background concentrations (7). These pollutants greatly differs to traffic-related pollutants in their near-road concentrations and sources (9). While sources of ambient air pollution include mobile sources (such as; cars, trucks, buses, trains, and planes), stationary sources (such as; power plants, industrial facilities and factories), and natural sources (such as; volcanoes, wild fires, and wind-blown dust), traffic-related pollutants such as NO₂, NO_x, black carbon and ultrafine particles, are mainly from tail-pipe emissions of vehicles (10). Hence, this thesis focuses on ambient air pollution of background concentrations with reference to traffic-air pollutants were appropriate.

An estimated 23% of all deaths and 24% of the global burden of disease can be attributed to environmental factors, with ambient air pollution (especially particulate matter) estimated to be responsible for 3.2 million deaths per year (3.1% of global total DALYs) (7). In 2016, the WHO estimated that only about one in every

ten people breathe clean air, according to the WHO Air Quality Guidelines (7). The WHO further reported 3.7 million premature deaths in both urban and rural areas to be attributed to air pollution, which is mainly due to small particulate matter of 10 micrometre or less in diameter (PM₁₀). Low-and middle-income countries (LMIC), where air pollution emissions from power-plants, traffic, open waste burning and other combustion sources are very common, account for about 88% of these deaths (7). The WHO estimated that a 15% reduction in ambient air pollution-related deaths can be expected from reducing ambient particulate matter from 70 to 20µg/m³ (7). Respiratory diseases are the leading cause of death from ambient air pollution, responsible for over half of the deaths reported (7). Of special concern is the effect of ambient air pollution on children as their immune systems and lungs are not fully developed with the onset of exposures in early-life (8,9). Furthermore, children tend to spend more time outdoors in parks and play grounds, and likely to breathe a greater amount of air pollutant per body weight compared to adults similarly exposed.

The evidence for the extent to which air pollution affects children's respiratory health is inconclusive suggesting much need for further investigation (14,15). Modest increased risk of asthma incidence (16–20) and prevalence (21–32) have been reported, with inconsistency in demonstrating the causal relationship between new-onset asthma and ambient air pollution exposure in children (16,17,19,33–40). However, more consistent evidence in the role of ambient air pollution exposure particularly traffic-related pollutants in reducing lung function in children (especially in infants) have been reported (16,26,41–48). There is also growing evidence of the association of short-term exposure (42,49–53) to ambient air pollution with increased FeNO levels, but inconclusive evidence of long-term air pollution effects of background origin (52) and traffic-related air pollutants such as NO₂ and NO_x (54,55) on airway inflammation.

Furthermore, although environmental and lifestyle factors appear to drive the increasing susceptibility to developing allergic diseases such as asthma, the increased susceptibility in response to pollen allergen in population exposed to high levels of pollution remain elusive. With increasing climate fluctuations, it is important to account for other co-exposures when exploring air pollution epidemiology. Pollen is a major air contaminant that have been implicated in adverse respiratory health outcomes. At the individual level, little is known about the combined effect of allergens and air pollutants on human respiratory health since this association is difficult to analyse in uncontrolled settings. The current knowledge on the pathogenesis of allergies and asthma due to the combined exposure to biological agents (such as pollen or fungal pollen) and air pollutants have been primarily based on animal or *in vitro* laboratory studies (8). However, most epidemiological studies of biological pollutants have been focused on its effect on asthma exacerbation (56), reported respiratory symptoms (57), and allergic sensitization (58), while limited studies have focused on its effect on objective asthma indicators such as lung function and airway inflammation. Several

epidemiological studies exploring the short-term effects of ambient air pollution and fungal spores on the respiratory health of children have mostly been conducted using time-series data with the outcome of interest usually obtained at the population level from hospital records for asthma visit, over-the counter asthma medication or emergency visits of asthma attacks (58–63). This outcome measure limit the exploration of the effects at the individual level. Because ambient air pollutants and fungal spores vary on a daily basis with meteorological factors, it is reasonable to postulate the possibility of confounding or effect modification in studies exploring the short- or long-term effects of either ambient air pollutants or outdoor fungal spores on respiratory health. The relatively few studies to have explored this combined association between ambient air pollutants and fungal spores was on acute changes in lung function estimated at same day or 1-day lag period nor was evidence of interactive effect between air pollutants and biological pollutants on lung function presented (64–66).

The main research questions addressed in this thesis was to determine the short- and long-term effects of air pollutants and fungal spores on asthma-associated outcomes in schoolchildren from low-income settings in sub-Saharan Africa. The study was conducted due to the fact that there are difficulties in extrapolating reported effects of air pollutants and biological pollutants to children from informal settlements in South African population as majority of these studies were mainly from Western countries, as opposed to developing countries with the greatest disease burden due to air pollution. In addition, it is also difficult to compare these effects estimates from various studies due to large differences among studies with regard to study design, air pollution exposure assessment, health outcomes explored, confounders adjusted-for, and statistical analysis method. Thirdly, although the effect of the co-exposure of air pollutants and allergens have been demonstrated in experimental studies, epidemiological studies of this association at the individual level using objectively assessed respiratory health measures in children are very scarce with no studies in the Southern hemisphere or South Africa (67). It is envisaged that this thesis will generate crucial data that is lacking in the international literature on air pollution, biological pollutants and asthma in low-income settings of sub-Saharan Africa, where patterns of co-exposure, co-morbidities and susceptibility may largely differ from those in Western countries, given the multifactorial nature of respiratory diseases especially asthma.

1.2. Thesis aim

The aim of this thesis was to investigate the short- and long-term effects of ambient air pollution and fungal spores on asthma-associated outcomes among schoolchildren from four informal settlements in the Western Cape Province of South Africa.

1.3. Objectives of the thesis

1. To determine the prevalence of asthma-like outcomes (such as respiratory symptoms, presence of airway obstruction on spirometry, and airway inflammation) and their association with indoor exposures prevalent in these four informal settlements.
2. To characterise ambient air pollutants estimated at both the participant's home address and stationary air quality monitor, including fungal spores, and their respective association with meteorological parameters
3. To examine the effect of daily variations in particulate matter and fungal spores levels on lung function during winter and summer in a panel study of participating schoolchildren in these settlements.
4. To examine the independents and co-effect of air pollutants and fungal spores on the onset of asthma-associated outcomes among participating schoolchildren in these settlements.

1.4. Structure of the thesis

Chapter 2 provides a comprehensive literature review from epidemiological evidence of the effect of ambient air pollution and the co-effect with airborne pollen or fungal spores on the respiratory health of children. It includes two published literature review, one focusing mainly on local South African studies (15) and the other explored evidence from the international literature (14). Prior to submission, an updated literature review on the current state of evidence was also included. The rationale of this chapter was to identify gaps in the literature, provide context for the thesis and research questions being explored.

Chapter 3 provides a publication which details the description of the methods employed in this thesis (68). This includes a detailed description of the study population and design, the various exposure assessment considered, and the outcome measures explored, including their definitions.

Chapter 4 gives an overview of the methods used to assess exposures of participating schoolchildren to ambient air pollutants, airborne pollen and fungal spores during the cohort and panel study period. Secondly, the chapter presents the descriptive results of the daily concentrations of PM₁₀, SO₂ and O₃ from stationary air monitor closest to Khayelitsha and Marconi-Beam during the panel study analyses in chapter 6 and the estimated annual levels of PM_{2.5} and NO₂ from LUR at the participants' residence, used in the cohort study analysis in chapter 7. This chapter further presents the characteristics of airborne pollen and fungal spores measured in the four study areas throughout the panel and cohort study periods, and lastly describes the correlation between air pollutants, pollen, fungal spores, and climatic factors.

Chapter 5 presents the prevalence of asthma-related outcomes in these four informal settlements. It also describes the association of these asthma-like outcomes with important indoor household characteristics

that are prevalent in these settlements that will be taken into consideration in the analysis in chapter 6 and chapter 7.

Chapter 6 provides rare evidence lacking in demonstrating the independent and co-pollutant effects of daily variation in particulate matter and fungal spores on children's lung function. This is the fourth study that have explored these effects on lung function at the individual level, but the first to estimate longer lagged (of up to 5-days) short-term effects of PM₁₀ and fungal spores on daily repeated objectively assessed lung functions of unselected school children (selection not based on asthma or atopic status) at the individual level.

Chapter 7 provides the first epidemiological study to demonstrate the independent and co-pollutant effects of long-term exposures to air pollutants and outdoor fungal spores on the development of asthma-associated outcomes, in schoolchildren living in informal settlements.

Chapter 8 presents a summary of the most important findings of this thesis, its strengths and limitations, including recommendations for public health policies and future research directions.

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CHAPTER 2

Literature review

2.1 Ambient air pollution and childhood asthma: A review of South African Epidemiological Studies

Paper overview

This paper documents evidence from local studies in South Africa with regard to the effects of ambient air pollutants on childhood asthma.

Contribution to the thesis

The article critically appraised the evidence from epidemiological studies of air pollution conducted in South Africa to identify gaps in the literature onto which the present thesis is built on. The need for a longitudinal study in South Africa with objective standardised estimation of air quality and asthma outcomes was established in this paper.

Role of candidate

The candidate did the literature search, wrote the manuscript, incorporated input from supervisors who reviewed the manuscript, and the candidate submitted the final manuscript for publication.

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AMBIENT AIR POLLUTION AND CHILDHOOD ASTHMA: A REVIEW OF SOUTH AFRICAN EPIDEMIOLOGICAL STUDIES

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ABSTRACT

Childhood asthma is the most common chronic disease in children globally and ranks in the top 20 contributors of global disability-adjusted life years (DALY) in all children, and among the top 10 causes of DALY amongst 5-14 year olds. The growing increase in the prevalence of childhood asthma symptoms has been correlated with the increase in outdoor air pollution in developing countries. An estimated 23% of all deaths and 24% of the global burden of disease can be attributed to environmental factors, with ambient air pollution (especially particulate matter) responsible for 3.2 million deaths per year (3.1% of global total DALYs). This review focuses on epidemiological evidence from South African studies to interrogate the evidence for the link between ambient air pollution and childhood asthma. The review suggests a positive association between ambient air pollution and the risk of childhood asthma. However, the strength of this association is compromised by methodological issues related to the paucity of use of standardised instruments for assessing asthma outcomes and the lack of more detailed exposure characterisation approaches, which need to be addressed in studies contemplating further investigation into this area of research.

BACKGROUND

Childhood asthma is the most common chronic disease in children globally and ranks in the top 20 contributors of global disability-adjusted life years (DALY) in all children; and among the top 10 causes of DALY amongst 5-14 year olds.¹ The global mortality rates of childhood asthma is approximately 0.7 per 100 000.² Findings from the International Study of Asthma and Allergies in Childhood (ISAAC) showed that approximately 13% of adolescents between the ages of 13-14 years have symptoms of asthma. Previous epidemiological studies have reported five or more episodes of wheezing in one-third of asthmatic children in the first 12 months of having asthma.^{3,4} There is a significant global variation between countries, in the prevalence of asthma symptoms (characterised by wheezing in the past 12 months), of up to a 13-fold difference. Although symptoms of asthma are more apparent in many high-income countries (HICs), some countries in the low- and middle-income range also have a high prevalence of asthma symptoms. Africa has the highest prevalence of approximately 51% of severe asthma symptoms among children with current wheeze.² In Cape Town, asthma was prevalent in 34.4% and 17% of urban and rural children,

respectively, aged 10 to 14 years.⁵ A similar distribution has been observed in children aged 8-17 years living in Kenya's capital Nairobi, with a prevalence of 22.9% compared to 13.2% in rural Kenya.⁶

AMBIENT AIR POLLUTION

Ambient air pollution is a major environmental health issue globally affecting the population in highly industrialised and developing countries. An estimated 23% of all deaths and 24% of the global burden of disease can be attributed to environmental factors, with ambient air pollution (especially particulate matter) responsible for 3.2 million deaths per year (3.1% of global total DALYs).^{7,8} The WHO reported 3.7 million premature deaths in both urban and rural areas caused by air pollution, which was mainly due to small particulate matter of 10 microns or less in diameter (PM₁₀). It is estimated that a 15% reduction in ambient air pollution-related death can be expected from reducing ambient particulate matter from 70 to 20 µg/m³. Respiratory disease is the leading cause of deaths from ambient air pollution, responsible for over half of the deaths reported.⁹ Recent studies also show long-term exposure to nitrogen dioxide (NO₂) to exacerbate bronchitis symptoms



Figure 1: Traffic-related pollutant sources



Figure 2: Industrial-related pollutant sources

in asthmatic children¹⁰⁻¹² NO₂ is also linked to reduced lung function growth as reported in North America and other European cities.^{7,11,13} Furthermore, studies have suggested that air pollution, particularly traffic-related, contribute to the development of asthma, atopy and infant mortality (Figure 1).¹⁴ Other studies have also demonstrated that industrial air pollutants, such as emissions from refineries, are also a major source of air pollution and associated with asthma (Figure 2).¹¹ Epidemiological studies have shown changes in respiratory symptoms and pulmonary function in individuals with asthma following exposure to sulphur dioxide (SO₂) for durations as short as 10 minutes.^{12,15-17} High levels of ozone (O₃) have also been reported to have harmful effects including breathing problems, triggering asthma and reduced lung function.^{18,19} Table I outlines the different ambient air quality standards for the four criteria pollutants and demonstrates that the South African standards are not as stringent as WHO standards for particulate matter, sulphur dioxide and ozone.

METHODOLOGY

Selection criteria included peer-reviewed articles of primary research in the form of epidemiological studies, investigating the association between ambient air pollution and childhood asthma, conducted in South Africa since 2004. The rationale for inclusion of post-2004 articles was to have more recent evidence to corroborate that of a previously published review in 2004.²² Subject-specific

databases (such as Medline through PubMed; CINAHL; Highwire; and the Cochrane library) were searched laterally using keywords combined by Boolean operators such as 'AND/OR' commands. Keywords were identified using the PICO (Population-Intervention-Comparison-Outcome) acronym and Medical Subject Headings (MeSH terms). The first stage of literature search was a sensitive (broad) search strategy with the query; 'Children AND Air pollution AND Asthma AND South Africa'. This was followed by a more specific search including all elements of the selection criteria and different MeSH terms to narrow-down the search with the query; '(Child* OR Infants OR Adolescent) AND (Ambient air pollution OR Particulate Matter OR Sulphur dioxide OR Carbon monoxide OR Nitrogen dioxide) AND (Asthma OR wheezing) AND South Africa) AND "last 10 years"[PDat] AND Humans[Mesh]'. Truncations (*) were used to identify all possible endings of keyword such as 'Child*', whose base does not change when used differently (e.g. Child, Children, Children's). Reference-list searching was done to identify one additional article.

CRITICAL APPRAISAL OF RESULTS

A sum of four studies was included in this detailed review, with three of these using a cross-sectional study design and the other also including a longitudinal study component. A descriptive summary of the findings from these studies is presented in Table II.

TABLE I: INTERNATIONAL AND NATIONAL AMBIENT AIR QUALITY GUIDELINES

POLLUTANT	AVERAGING PERIOD	WHO AMBIENT AIR QUALITY GUIDELINES (µg/m ³) ²⁰	SOUTH AFRICAN NATIONAL AMBIENT AIR QUALITY STANDARDS (µg/m ³) ²¹
Particulate Matter (PM ₁₀)	1-year	20	40
	24-hour	50	75
Particulate Matter (PM _{2.5})	1-year	10	-
	24-hour	25	-
Sulphur dioxide (SO ₂)	24-hour	20	125
	10-minutes	500	500
Nitrogen dioxide (NO ₂)	1-year	40	40
	1-hour	200	200
Ozone (O ₃)	8-hour daily maximum	100	120

TABLE II: DESCRIPTIVE SUMMARY OF SOUTH AFRICAN STUDIES OF AMBIENT AIR POLLUTION AND CHILDHOOD ASTHMA

AUTHORS	MALULEKE AND WORKU (2009)	WHITE ET AL. (2009)	SHIRINDE ET AL. (2014)	NAIDOO ET AL. (2006)
AIM OF STUDY	Focus on identifying key predictors of asthma in school children.	Assessment of asthma symptom prevalence in priority area of Cape Town (characterised by petrochemical refinery environs) compared to other places in Cape Town.	Association of wheeze with reported outdoor air pollution in a priority area (Ekurhuleni Metropolitan Municipality).	Association of respiratory outcomes in exposed children to ambient air pollution.
STUDY DESIGN	Cross-sectional	Cross-sectional	Cross-sectional	Cross-sectional and longitudinal study design (Panel Study)
STUDY POPULATION	Random sample of 742 school children between 13-14 years in Polokwane, Limpopo Province.	2361 school aged children between 11 to 14 years from 17 schools in defined areas (Cape Town).	3764 school aged children between 13 and 14 years from 16 selected schools in Tembisa and Kempton in Ekurhuleni Metropolitan Municipality.	422 school aged children in grade 3 to 6 from 4 schools in South Durban (exposed) and 3 schools in North Durban (unexposed).
EXPOSURE MEASUREMENT	Monthly particulate matter (PM) data from monitoring stations recorded between 2002 and 2005 to quantify pollution levels; questions on smoke in the environment was used as the exposure index in the analysis.	Distance from the refinery and meteorological estimates exposure index (wind speed, wind direction and proportion blown yearly) linked to each child's residential address using the refinery as the putative point source.	Reported frequency of trucks passing near the home on weekdays.	Assessment of pollutants (SO ₂ , PM, NO _x and CO) from monitoring stations near selected schools.
POLLUTANT SOURCES	Silicon smelting, automobile emission, industrial activities, smoke and biomass fuels.	Petrochemical refinery	Traffic related pollutants	Industrial activities mainly related to petrochemical refineries and gasoline/diesel powered vehicles.
OUTCOME MEASUREMENT	Parental-reporting of the prevalence of cough in the child.	Children ISAAC written and video questionnaires to assess asthma symptoms.	Children ISAAC written questionnaire.	Standardised questionnaire, spirometry, serial peak flow and skin prick test.
RESULTS	Presence of smoke in the environment was strongly associated with asthma (OR; 2.39 CI: 1.34-4.98).	Positive associations between meteorologically estimated exposure (MEE) with having to take an inhaler to school (OR; 1.22 CI; 1.06-1.40), frequent wheezing at rest (OR; 1.27 CI; 1.05-1.54) and recent waking with wheezing (OR; 1.33 CI; 1.06-1.66). No associations were observed when simple distance from the refinery was used as a marker for exposure.	The frequency of trucks passing near homes during weekdays increased the likelihood of ever-wheeze (OR; 1.32 CI; 1.01-1.73), current wheeze (OR; 1.61 CI; 1.15-2.24) and current severe wheeze (OR; 2.22 CI; 1.28-3.77).	School children from South Durban (industrial pollutant area) were more likely to have persistent asthma (OR; 1.82 CI; 1.05-3.14) and marked airway hyper responsiveness on methacholine challenge (OR; 2.55 CI; 1.03-6.28) compared to school children in North Durban.
POTENTIAL BIAS	Potential bias: • Selection bias (exclusion bias); • Information bias (misclassification bias).	Potential bias: • Selection bias; • Information bias (recall and reporting bias).	• Potential bias: • Misclassification bias; • Information bias (recall and reporting bias).	Potential bias: Selection bias; Short follow-up (restricted to short term effect inference).
CONCLUSION	Environmental air pollution was an important risk factor for asthma in children.	An increased prevalence of asthma among children living around the refinery area and related to refinery emissions.	Children living in one of the air pollution priority areas have an increased risk of wheezing due to exposure to air pollution sources.	Children in South Durban, when compared to those residing in North Durban, have an increased risk of persistent asthma and airway hyper responsiveness due to ambient air pollution exposures.

The study by Maluleke and Worku (2009) used a cross-sectional study design to identify key predictors of asthma in children living in Polokwane province.²³ The use of a cross-sectional study design limited detailed exploration of the temporal relationship between environmental pollutant exposures and childhood asthma. Furthermore, the exposure assessments used in this study were constrained by the lack

of information on the proximity of the monitoring stations to the selected schools. Although the authors controlled for some covariates (socio-economic status, environmental tobacco smoke, diet and pet ownership), atopy and indoor air pollution was not controlled. Atopy and indoor air pollution may have skewed the findings positively, as it has also been shown to be independently associated with asthma

symptoms in children.^{24,25} The results of the study demonstrated that children in a smoke environment were twice as likely to develop asthma, compared to unexposed children (OR; 2.39 95% CI; 1.34-4.98). The reliability of the result was constrained by the subjective assessment of asthma as reported by parental-reported cough among children, without any other objective asthma endpoints reported.

The study by White et al. (2009) also used a cross-sectional study design to determine if the prevalence of asthma in school children was higher in the Milnerton area, where a petrochemical refinery is located, compared to other areas in Cape Town.²⁶ Furthermore, due to the lack of objective quantitative measurements of criteria air pollutants, a specific pollutant effect could not be measured. Although the authors controlled for parity, passive smoking, family history of asthma and atopic diseases, distance from major road, there was no information on smoking status and pollen data exposures in the control group. Thus, no inference about the role of both the smoking status and pollen exposures as potential confounders could be made. The overall results of the study demonstrated an eight fold increased likelihood of asthma symptoms (OR; 8.92) in the priority area compared to other areas in Cape Town. However, the wide confidence interval of 4.79 to 16.63 limited the precision of the result. Furthermore, the multivariate analysis demonstrated a positive association between meteorologically estimated exposures (MEE) but not simple distance from the refinery and the following endpoints: having to take an inhaler to school (OR; 1.22 CI; 1.06-1.40); frequent wheezing at rest (OR; 1.27 CI; 1.05-1.54); and recent waking with wheezing (OR; 1.33 CI; 1.06-1.66).

The limitation of generalisation across different socio-economic class categories was somewhat addressed in the study by Shirinde et al. (2014), which explored the association of wheeze with air pollution among children in 16 schools from two urban areas (Tembisa and Kempton Park) of different socio-economic status.²⁷ Although the study had an appreciably large sample size of 3764, the use of cross-sectional study design limited the exploration of the temporal association between air pollution and asthma. Furthermore, a crude subjective exposure measure of exposure was used based on questions on the frequency of trucks passing by the homes of children on weekdays. This may have introduced a form of misclassification bias since children spend most of their time in school during weekdays and may, therefore, have limited insight on exposures closer to their home. There were also no questions on other sources of air pollution such as distance of industries from residential areas, nor any objective quantitative data on environmental pollution, such as data from monitoring stations to quantify specific pollutants. Although the authors took into account the presence of confounders such as residential area, sex, mother's education level, residential history, domestic fuel exposure, diet, active and passive smoking, both in the study design and analytical approaches, there is a possibility of residual confounding due

to atopic disease and unmeasured socio-economic status factors such as occupation, income levels, parity, etc. The overall findings of the study suggested that the frequency of trucks passing near homes in weekdays increased the likelihood of ever-wheeze (OR; 1.32 CI; 1.01-1.73), current wheeze (OR; 1.61 CI; 1.15-2.24) and current severe wheeze (OR; 2.22 CI; 1.28-3.77) in these children.

The only study with a longitudinal study component was reported by Naidoo et al. (2006), which investigated asthma in a representative group of school children exposed to ambient air pollution in South Durban and compared them with school children in a non-exposed area of North Durban.²⁸ The use of a panel study design increased the power of the analysis and the ability to examine the effects of short-term fluctuations in air pollutant exposure on acute respiratory changes across the four seasons. Both selected communities had a similar socio-economic profile and the schools were randomly selected in each community area. However, recruitment of students in each area was done using a dual pronged strategy, which was characterised by inclusion of a randomly selected group of students as well as a group selected on the basis of known or probable persistent asthma. Monitoring stations located close to schools were used to assess exposure to ambient air pollution in both the exposed and non-exposed groups. Measurement bias was also reduced by random selection of schools having less than 15% of its students from surrounding communities away from the monitoring stations. Similarly, the use of an objective marker of asthma outcome (bronchial hyper responsiveness) together with a standardised validated questionnaire reduced the risk of measurement bias. The results of the study demonstrated that children from the exposed South Durban area were more likely to have persistent asthma (OR; 1.82 CI; 1.05-3.14) and marked airway hyperactivity following the methacholine challenge testing (OR; 2.55 CI; 1.03-6.28), compared to children in North Durban. Furthermore, results from the serial daily peak expiratory flow, over three consecutive weeks across the four phases (season), showed intraday variability (a marker of worsening of asthma) of mean (std. dev.) PEF [16.3 (12.4) vs 15.7 (11.9)] and mean FEV1% [18.0 (12.1) vs 17.0 (12.0)] to be marginally greater, on average, among children in South Durban, compared to those in North Durban. However, inference is limited to the short term effect of air pollution measured in the four seasons.

DISCUSSION

Most of the studies reviewed had methodological limitations constraining their ability of demonstrating the strength of association between ambient air pollution and childhood asthma. Three of the four studies reviewed used only a cross-sectional study design that limited the inference of a temporal relationship between air pollution on the development or presence of childhood asthma. This is important since it is possible that families, with symptomatic children at an early age, may move to homes in lower exposed areas. However, the study that used a longitudinal study compo-

ment had a short follow-up period which limited the ability to quantitatively assess the level of exposure and other covariates with the initial onset or progression of asthma. Other limitations in studies included the lack of detailed and systematic exposure assessment of criteria pollutants. This limited the ability to explore dose-response relationships and time-activity patterns in a meaningful manner. Furthermore, comparisons with other countries or regions in the world were, therefore, not possible. The use of a standardised quantitative measurement of criteria pollutants from monitoring stations located near the resident or school of the study participants could have limited exposure misclassification to the extent that it did exist.

Similarly, there were variations among studies reviewed regarding the assessment of asthma outcomes in exposed children. Asthma outcome was assessed mainly by subjective, indirect approaches using questionnaires in three of the four studies. This had the potential to introduce a form of information bias when administering questionnaires, due to possible recall and general awareness of the association between asthma with air pollution. The use of simple standardised objective measurements using peak expiratory flow measurements, spirometry and/or exhaled nitric oxide (FeNO) could have reduced such bias.

There was also evidence of selection bias operating in three of the four studies. The lack of detailed information in the inclusion and exclusion of study participants, together with a lack of randomisation of children, irrespective of known asthma-status across all socio-economic groups, limited the internal and external validity of the results. Furthermore, some studies dealt inadequately with confounding factors that were not reported on, such as socio-economic factors, smoking status, atopy and indoor air pollutants (domestic fuel combustion). These factors may have skewed the risk estimation since they are also known to be associated with childhood asthma.^{24,25,29} Had the data been collected for these confounders, this could have been appropriately controlled for, during statistical analysis using either stratification or multivariate analysis.

The limitations in methodology, in these studies, have also been reported by Wichmann and Voyi in their review on the effect of air pollution on ill health using evidence from South African studies.²² Their review focused on both indoor and outdoor air pollution and the health outcomes reported on all measures of ill-health, including respiratory health associated with air pollution exposure. Similar methodological issues were reported that limited the reliability and validity of the results. Future studies of the association between air pollution and childhood asthma need to address these issues to increase the strength of the evidence and render comparison with findings reported from other parts of the world, possible.

CONCLUSION

The review suggests a positive association between the risk of childhood asthma and ambient air pollution. However, the strength of this association is compromised by methodological issues that need to be addressed, in future studies, contemplating further investigations into this area of research.

It is recommended that a longitudinal study with a reasonably long follow-up period (more than 3 years) be conducted, using the following:

1. Objective standardised quantitative measurements of criteria pollutants (such as those obtained from pollutant-specific monitoring stations, supplemented by more detailed exposure characterisation studies related to both indoor and outdoor pollutant sources, using more advanced approaches in exposure modelling techniques, such as land use regression models, in addition to more traditional dispersion modelling techniques).
2. Asthma outcomes, using simple instruments according to standardised procedures (such as those obtained from serial peak expiratory flow measurements, spirometry or exhaled nitric oxide levels).

This will increase the reliability and validity of the measurements, to better quantify the risk and take into account other possible covariates that may potentially bias or confound these associations.

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2.2 Air Pollution, pollen and childhood asthma, is there a link?

Paper overview

This paper documents evidence from the international literature with regard to the combined effects of both ambient air pollutants and biological pollutants such as pollen and fungal spores on childhood asthma.

Contribution to the thesis

The article critically appraised the evidence from epidemiological studies of the combined effect of air pollutants and airborne pollen or fungi to identify gaps in the literature onto which the present thesis is built on. This paper highlights possible mechanisms involved in the interrelationship between air pollutants and biological allergen with asthma, and summarises the evidence on the independent and/or co-effect of multiple environmental exposures on childhood asthma. The need for future studies to explore this combined effect at the individual level was identified.

Role of candidate

The candidate did the literature search, wrote the manuscript, incorporated input from supervisors who reviewed the manuscript, and the candidate submitted the final manuscript for publication.

Publication status

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AIR POLLUTION, POLLENS AND CHILDHOOD ASTHMA – IS THERE A LINK?

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ABSTRACT

While the interplay between ambient air pollutants and their role in the development of asthma continues to remain a subject of detailed study, a link appears to be postulated between ambient air pollutants and airborne pollens in the increasing prevalence of allergic airway disease. With an increasing focus on climate change and the effects of global warming, pollen allergy has become the focus of recent studies looking at the interrelationship between ambient air pollutants and pollen exposures in individuals with allergic respiratory disease. At the population level, little is known about the potential synergistic effects between pollen allergens and air pollutants since this type of association poses challenges in uncontrolled real-life settings. The current knowledge of the pathogenesis of allergies and asthma due to the combined exposure to biological agents such as pollens and air pollutants has been based primarily on animal or *in vitro* studies. This article highlights possible mechanisms involved in the interrelationship between air pollutants and pollen allergens with asthma, and summarises the evidence on the independent and/or co-effect of multiple environmental exposures (ambient air pollutants and pollens) on childhood asthma.

INTRODUCTION

Childhood asthma is the most common chronic disease in children globally and ranks among the top 20 contributors to global disability-adjusted life years (DALY) in all children; it also ranks among the top ten causes of DALY in 5–14-year-olds.¹ The growing increase in the prevalence of childhood asthma symptoms has been correlated with the global increase in outdoor air pollution.² Ambient air pollution is a major environmental health concern globally that affects populations in highly industrialised and developing countries. As recently documented by the World Health Organisation (WHO), 98% of cities in low- and middle-income (LMI) countries with more than 100 000 inhabitants do not meet WHO air-quality guidelines, whereas in high-income countries the percentage decreases to 56%.³ According to recent global burden of disease indicators, the estimated ambient air pollution (especially particulate matter) was responsible for 5.5 million deaths per year in 2013 and 141.5 million DALY (14% of global total DALY); in terms of DALY, it is the fifth leading global risk factor.⁴ The WHO has reported 3.7 million premature deaths in both urban and rural areas

caused by air pollution, which is due mainly to small particulate matter of 10 microns or less in diameter (PM₁₀). LMI countries, in which air-pollution emissions from power plants, traffic, open waste burning and other combustion sources are very common, account for approximately 88% of these deaths.⁵

While the relationship between ambient air pollutants and their role in the development of asthma continues to remain an area of detailed study, with our increasing understanding of the implications of climate change and global warming, a link has been postulated between ambient air pollutants and airborne pollens in the increasing prevalence of allergic airway diseases, including the exacerbation of asthma and the role of air pollutants in modifying the effect of pollen exposures. Pollen allergy has been used more recently to study the interrelationship between ambient air pollutants and respiratory allergy, including asthma in sensitised individuals.⁶

The aim of this article is to identify the mechanisms involved in the interrelationship between air pollutants



Figure 1: Power plants' pollution and pollen

and pollen allergens with asthma and to summarise the evidence for the independent and/or co-effect of environmental exposures (ambient air pollutants and pollens) on childhood asthma.

AMBIENT AIR POLLUTANTS

Ambient air pollutants commonly arise from primary and secondary sources. The primary pollutants are emitted directly from sources that include gaseous pollutants – for example, sulphur dioxide (SO₂) and mono-nitrogen oxides (NO_x) as well as particulate matter (PM) (e.g. soot). The secondary pollutants arise from primary pollutants in the atmosphere that combine with UV-rays from sunlight and/or moisture (e.g. ozone (O₃), secondary particles such as sulphates). The air pollutants referred to in this article are the four key primary sources – PM, SO₂, nitrogen dioxide (NO₂) and O₃ – identified by the WHO 2005 Air Quality Guidelines (AQG), reviewed previously.¹

ALLERGENIC POLLEN AND POLLEN ALLERGY

Although airborne pollens comprise a small proportion of the atmosphere, they are important causative agents of allergic respiratory disease in sensitised individuals. Allergic respiratory disease has become a public health problem due to the increased prevalence of airborne allergenic pollen causing clinical disease and resulting in escalating costs to patients and society at large.⁷ Globally, the common non-animal-related allergens that are common sensitisers are pollens from trees, weeds, grass and fungal spores. Important grass-pollen allergens are *Cynodon dactylon* (Bermuda grass) and *Lolium perenne* (Rye grass). These tropical grasses are commonly found along latitudes between 30°N and 31.4° + 7.5°S and have their optimal growth at temperatures between 24°C and 37°C. Bermuda grass has been linked to allergic conjunctivitis, asthma, seasonal allergic rhinitis exacerbation and asthma.⁸ Grass-pollen allergy is also a common respiratory allergy (median prevalence of 16.9%) in most European countries and also in South Africa.⁹ Approximately 80% of South Africans react to *Eragrostis* (lovegrass) and Buffalo grass pollens.¹⁰ Besides grass, birch (mostly in east-central Europe) and ragweed (predominantly in northern countries) are among the major pollens responsible for causing rhinitis and



Figure 2: Traffic-related pollution and pollen

asthma. Cypress and *Parietaria* (wall pellitory) also play a major role in southern countries.

INTERACTION BETWEEN AIR POLLUTANTS AND POLLEN IN ASTHMA AETIOLOGY

Exposure to single pollutants has been the focus of many studies on respiratory and allergic risk factors. However, multiple exposures to several pollutants occur in 'real life'. There are always complex mixtures of pollutants from various sources in the environment, which often contribute jointly to synergistic or additive toxic effects.^{11,12} Owing to the intrinsic electrostatic properties and porous surfaces of ambient respirable PM, they readily adhere to airborne allergens released from pollens. PM interacts with aero-allergens via modulation of the allergenicity of airborne allergens, in this way promoting airway sensitisation.^{13,14} *Phleum pratense* pollen releases more allergen-containing granules when treated with various concentrations of O₃ and NO₂ under experimental conditions compared to exposure to air only (see Figure 3).¹⁵ The bio-availability of airborne pollen allergens has also been identified to increase owing to the effect of traffic-related pollutants.¹⁵ Air pollutants may increase acute responses to allergens in various ways:

- increasing epithelial permeability;
- 'priming' allergen-induced responses caused by airway inflammation, in this way enhancing the recruitment and activation of inflammatory cells;
- increasing airway oxidative stress, and
- increasing the release of neuropeptide.⁷

The access of inhaled allergens to the immune system may be facilitated by impaired mucociliary clearance and airway mucosal damage, which enhances atopic sensitisation risk and symptoms exacerbation in sensitised individuals.^{16,17}

MECHANISMS BY WHICH AIR POLLUTANTS CAUSE ASTHMA

The development of asthma has been better understood owing to the availability of techniques for investigating gene polymorphisms and variants associated with the increased risk of asthma. There are four groups of genes attributed to conferring susceptibility to the development of

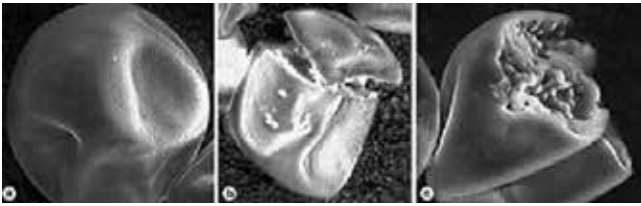


Figure 3: (a) Intact pollen; (b) damaged pollen following the treatment of 50 ppm of NO₂; (c) damaged pollen following treatment with 0.7 ppm of O₃, showing visible pollen cytoplasmic granules from the broken grain (Reproduced with permission)¹⁵

asthma. They are those controlling:

- airway repair and remodelling, including airway development;
- immune system responses;
- bronchial hyperresponsiveness, and
- the endogenous production of anti-oxidants in the airways (see Figure 4).

Various mechanisms enable either different gene types on their own or those interacting with environmental exposures (pollutants and allergens) through gene-environment interactions to cause asthma. These mechanisms include oxidative stress and damage, airway wall remodelling, immunological effects and inflammatory pathways, and the enhancement of respiratory sensitisation to allergens.¹⁸ It is postulated that air pollution increases the likelihood of sensitisation to allergens by:

- acting as a carrier for allergens to the lower respiratory tract and parts of the lungs that would otherwise be difficult for allergens to reach;
- increasing the permeability of the epithelium and thereby increasing the surface area of immunological cells responding to allergens;
- interacting with antigenic protein and in this way increasing its potency, and
- air pollutants (especially PM), acting as adjuvants, serving as a depot and in this way preventing antigens from dispersing.

During this process, PM stimulates the division of Type-2-helper T cells or activates antigen-presenting cells.¹⁹

EXPOSURE-RESPONSE STUDIES BETWEEN AMBIENT AIR POLLUTANTS AND EXACERBATION, PREVALENCE AND INCIDENCE OF CHILDHOOD ASTHMA

In the literature, the evidence for the association between ambient air pollution exposure and asthma prevalence or asthma induction in children is inconclusive.^{20,21} A systematic review by Anderson et al²² of 21 studies on the prevalence of wheeze symptoms or asthma diagnosis and levels of air pollution in five or more communities demonstrated that only 11% of studies showed a positive, statistically significant association between the pollution-outcome estimates. Epidemiological evidence from a pooled analysis of five European birth-cohort studies

– GINI and LISA (both Germany, divided into north and south areas), MAAS (England, United Kingdom), PIAMA (the Netherlands), and BAMSE (Sweden)²⁰ – showed no association with an increased prevalence of childhood asthma, while some suggested effects on only a sub-population of age-groups²³ and girls.²⁴ The results of a systematic review and a meta-analysis by Bowatte et al²¹ of pooled five birth-cohort studies (PIAMA, Oslo, BAMSE, Vancouver and BCO^{25–29}) showed a modest association between the incidence of asthma and longitudinal childhood exposure to NO₂: OR 1.09; 95% CI 0.96 to 1.23 per 10 µg/m³ increase in pollutant. However, the association was highly variable between all the studies pooled together, with a heterogeneity of 75.5%. A recent study evaluating the association with traffic-related pollution found no association between incidence of asthma with the measured pollutants at baseline and follow-up.³⁰ However, there is evidence from epidemiological studies that exposure to ambient air pollutants in early childhood plays an important role in the exacerbation of asthma and other respiratory symptoms, with a greater effect being observed in asthmatic children.^{31–36}

EXPOSURE-RESPONSE STUDIES BETWEEN AIR POLLUTANTS AND AIRWAY INFLAMMATION AND LUNG FUNCTION DEFICITS IN CHILDHOOD ASTHMA AIRWAY INFLAMMATION

There is growing evidence of an association between ambient air pollution and increased exhaled nitric-oxide (FeNO) levels, a subclinical marker of airway inflammation in children. In the Southern California Children's Health Study (SCCHS), higher FeNO levels were associated with both background and traffic-related pollutants. Among children aged seven to 11 years, cumulative lagged averages of daily O₃ (over 1–23 days), PM₁₀ (over 1–7 days), and PM_{2.5} (over 1–8 days) were associated with 14.3% (p<0.01), 9.3% (p<0.05), and 17.4% (p<0.001) higher FeNO levels across an IQR of 15.42 ppb of O₃, 12.97 µg/m³ of PM₁₀, and 7.5 µg/m³ of PM_{2.5}.³⁷ Similarly, longitudinal analysis of chronic exposures to PM_{2.5} and NO₂ (scaled to the IQR of 2.4 µg/m³ and 1.8 ppb, respectively) showed that PM_{2.5} and NO₂ exposures were associated with 4.94 ppb (p = 0.005) and 2.29 ppb (p = 0.02) increase in FeNO, respectively.³⁷

LUNG FUNCTION DEFICITS

There is more consistent evidence for the role of ambient air pollution exposure, particularly traffic-related pollutants, in reducing lung function, especially in young children. A large proportion of epidemiological studies^{38–43} found a consistent association between ambient air pollution exposure and reduced lung function in children, with three studies showing mixed results^{23,44,45} Some studies demonstrated significant associations with only traffic-related pollutants such as NO₂, PM_{2.5} and PM₁₀^{39,42,44,45} in younger children^{23,42} non-asthmatics³⁸ and boys.³⁹ Findings from the SCCHS were consistent in demonstrating a significant positive association with lung-function deficits.⁴⁶

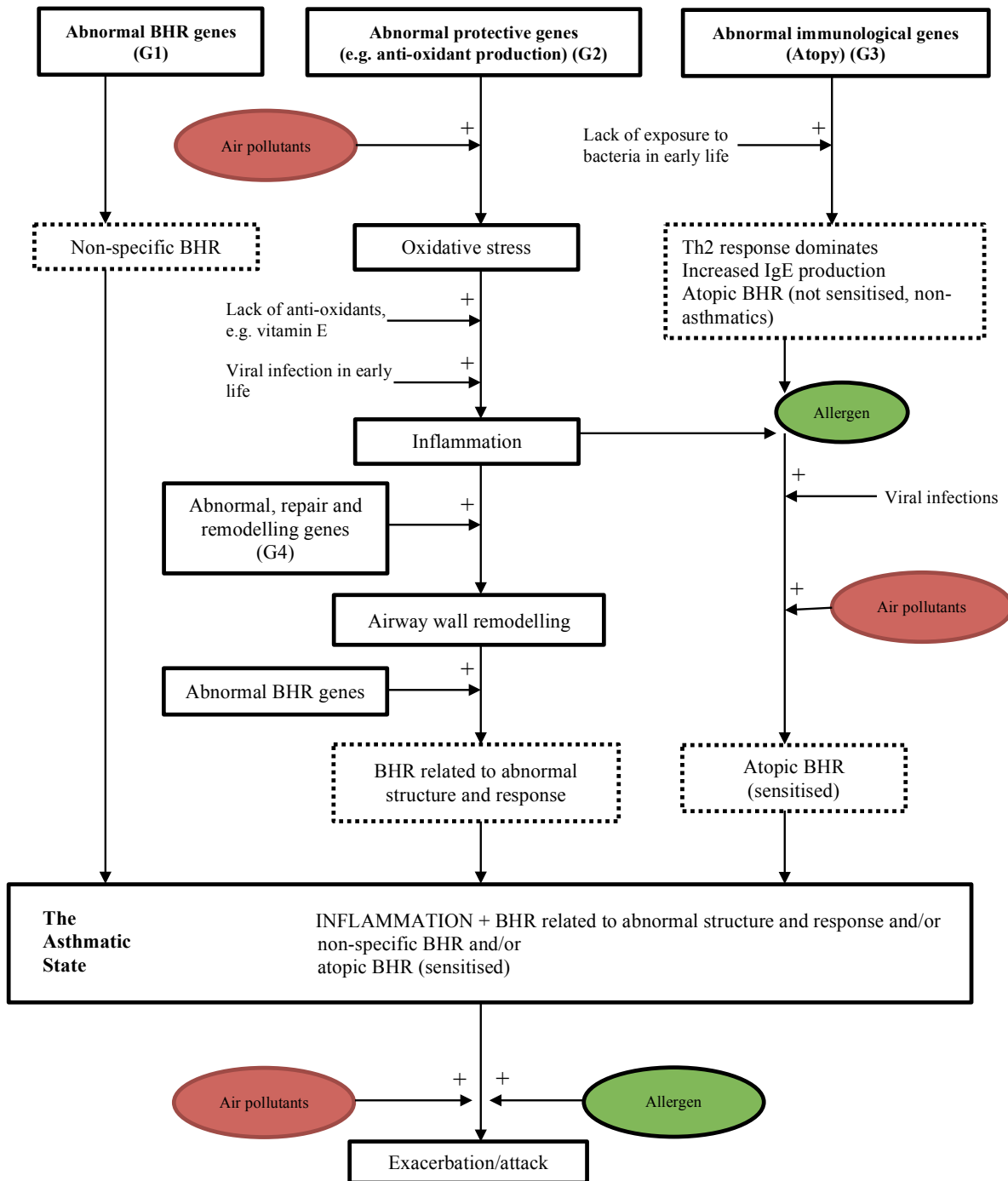


Figure 4: Hypothetical network of causation of asthma in relation to ambient air pollution exposure. BHR, bronchial hyperresponsiveness; IgE, immunoglobulin E; Th2, Type 2 helper cells (adapted and reproduced with permission)18

For example, exposures to NO₂, PM_{2.5}, elemental carbon (EC), and acid vapour were significantly associated with reduced FEV₁ in children followed from age ten to 18 years. Similarly, the proportion of low FEV₁ (defined as the per cent FEV₁ below 80%) was four times greater in children from communities with the highest level of PM_{2.5} compared to those with the lowest level (7.9% vs 1.6%, p = 0.002).⁴⁶

EXPOSURE-RESPONSE STUDIES BETWEEN POLLEN EXPOSURE AND THE EXACERBATION, PREVALENCE AND INCIDENCE OF CHILDHOOD ASTHMA

The association between pollens and the onset of allergic disease and exacerbations has also been reported in various studies (Table I);^{47,48} however, inconsistencies

were evident in the reported associations in these studies. Positive correlations between cedar-pollen counts and asthma in six- to seven-year-old children have been reported in the Japanese ISAAC study.⁴⁷ In contrast, no association between increased prevalence of asthma and airborne pollen was found in a Hungarian study.⁴⁹ In a large international epidemiological study,⁵⁰ grass-pollen allergy was associated with seasonal asthma exacerbations, with elevated risks for sensitised participants in early summer in southern Europe (OR March/April = 2.60, 95% CI: 1.70–3.97; OR May/June = 4.43, 95% CI: 2.34–8.39). In northern Europe, a similar association was observed for birch-sensitised individuals (OR May/June = 2.94, 95% CI: 1.92–4.50; OR July/August = 2.01, 95% CI: 1.38–2.94). A significant association was also observed between grass pollens and hospital visits for asthma attacks in a French study,⁵¹ with an interquartile range increase of 17.6 grains/m³ of the Poaceae grass family for a 54% increased risk of asthma attacks. However, three of the reviewed studies showed no association between pollen exposure and asthma exacerbation in children (see Table I).^{52–54} With regard to asthma incidence, a nine-year German longitudinal study⁴⁸ found a significant association between new onset of asthma with previous sensitisation to grass pollen (RR 1.79, 95% CI: 1.01–3.19). In an Australian birth cohort study,⁵⁵ cumulative exposure to pollen between four and six months was associated with asthma among 620 children aged six to seven years (OR 1.4 95% CI: 1.1–1.7). Similarly, in a birth-cohort study of 514 children followed from birth to 24 months of age, children exposed to pollen and spores in the first three months of life were reported to be at increased risk of early wheezing, independent of other seasonal factors, lower respiratory infections and ambient air pollutants.⁵⁶

EXPOSURE-RESPONSE STUDIES BETWEEN CO-EXPOSURE TO AIR POLLUTANTS AND POLLEN AND ASTHMA

At the population level, little is known about the possible synergistic effect between allergens and air pollutants since this association is difficult to analyse in uncontrolled settings. The current knowledge of the pathogenesis of allergies and asthma due to combined exposure to biological agents (e.g. pollen, mites) and air pollutants has been based primarily on animal or in vitro laboratory studies.² Several clinical laboratory studies have demonstrated that, following prior exposure to ambient air pollutants, there was a greater effect of allergen challenge on asthma.^{57,58} In 2015, Brandt et al demonstrated a significantly higher house-dust mite (HDM)-specific memory T cells in the lungs of mice exposed to both HDM and diesel exhaust particles (DEPs) compared to mice exposed to only saline, DEPs or HDM alone.⁵⁷ The authors' hypothesised that early-life exposures to DEP may increase the risk of allergic asthma development in children, should exposure to DEPs potentiate recall responses to allergen. The same authors further tested the hypothesis by assessing the effect of

early-life exposure to DEP in children sensitised and non-sensitised to HDM. Their result showed a two-fold higher prevalence of asthma at seven years in sensitised children exposed to DEP at birth, compared to non-sensitised children.⁵⁷ However, the clinical relevance of such modification in the wider population is unclear, because inconsistent results were found in other epidemiological studies.

A number of the reviewed articles demonstrated a positive association between increased pollen exposures and asthma exacerbation (see Table I).^{33,59,56,60–62} However, the effects persisted after controlling for several air pollutants and meteorological factors, indicative of non-modifiable effects of air pollution and seasonality. Similarly, in a Spanish study of 3 939 participants, an increase of 10 µg/m³ in SO₂ and NO₂ was associated with a higher risk of asthma emergency room visits (RR 5.2 95% CI: 0.5–10.1 for SO₂ and RR 2.6 95% CI: 0.3–5.0 for NO₂), while the risk level remained the same with the combined effect of plant species such as Urticaceae and Poaceae (RR 5.7 95% CI: 0.9–10.6 for SO₂ and RR 2.7 95% CI: 0.4–5.1 for NO₂).⁶³

In conclusion, although the synergistic effect of the combined exposure to air pollutants and allergens has been demonstrated in experimental studies, studies of this association at the population level remain inconclusive, indicating the need for more research to explore this further.

DECLARATION OF CONFLICT OF INTEREST

The authors declare no conflict of interest.

This article has been peer reviewed.

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TABLE I: STUDIES OF THE ASSOCIATION BETWEEN AMBIENT POLLEN AND/OR AIR POLLUTANTS AND CHILDHOOD ASTHMA

AUTHOR(S)	AIM	STUDY DESIGN	STUDY POPULATION	EXPOSURE MEASUREMENTS	OUTCOME MEASUREMENTS	RESULTS	CONCLUSION
Gleason et al, 2014 ³³	Studied transient impact of ozone, PM _{2.5} and pollen on emergency department visit for childhood asthma	Time-stratified case-crossover design	Children aged 3–17 years residing in New Jersey, United States between 2004 and 2007	Daily concentrations of pollen. Daily averages of PM _{2.5} and daily eight-hour average of O ₃ from fixed-monitoring station, and meteorological data from New Jersey State Climatologist	Daily number of emergency visits for childhood asthma	Multipollutant model showed positive associations between daily emergency department visits and same-day O ₃ (RR _{IOR} = 1.08, 95% CI 1.06–1.10), three-day tree pollen average (RR _{IOR} = 1.19, 95% CI 1.17–1.20), three-day grass pollen average (RR _{IOR} = 1.08, 95% CI 1.09–1.11) respectively	Ozone, grass and weed pollen are independent risk factors for asthma exacerbation
Tosca et al, 2014 ⁶⁴	Investigated relationship between asthma exacerbation, pollen, air pollution and meteorological factors	Time-series ecological study design	Children residing in Genoa, Italy between 2002 and 2011	Daily concentrations of pollen (<i>Betulaceae</i> , <i>Utricaceae</i> , <i>Gramineae</i> , <i>Oleaceae</i>), outdoor air pollutants, and meteorological variables	Number of emergency calls for asthma exacerbation	Number of emergency calls associated with pollens in spring (r = 0.498), SO ₂ (r = 0.622), NO ₂ = 0.58, NO (r = 0.699), rainfall (r = 0.0818), and wind speed (r = 0.727)	Environmental factors may induce exacerbation of asthma
Yoshida et al, 2013 ⁴⁷	Evaluated association between pollen exposure and prevalence of allergic diseases	Ecological analysis	Children aged between six and seven years and those aged between 13 and 14 years residing in Japan	Daily pollen data on Japanese cedar and Japanese cypress	Allergic diseases assessed using International Study of Asthma and Allergy in childhood (ISAAC) questionnaire	Cedar but not cypress pollen counts positively associated with prevalence of asthma in six- to seven-year-old children (r = 0.49, p = 0.003) but not in other age group.	Evidence of ecological associations between pollen counts and prevalence of asthma
DellaValle et al, 2012 ⁵⁹	Investigated relationship between ambient pollen concentration and severity of asthma symptoms among asthmatic children	Cross-sectional study	430 asthmatic children aged 4–12 years in Connecticut, MA and New York	Mixed-effect models used to estimate daily exposures to tree, grass, weed, and total pollen	Daily symptoms of rescue medication use, night symptoms, wheeze, persistent cough, chest tightness, shortness of breath, and night symptoms	23% increased likelihood of any respiratory symptoms (95% CI; 1.01–1.50) and 11% increased likelihood in rescue medication use (95% CI; 1.02–1.21), comparing the highest quintile of weed pollen to the lowest, while adjusting-for O ₃ , PM _{2.5} , maximum temperature, antibiotic use, and season	Daily asthmatic symptoms are associated with pollen exposure
Harley et al, 2009 ⁵⁶	Assessed risk of early childhood wheezing following fungal and pollen exposure in first months of life	Birth-cohort study	514 children enrolled before birth and followed for 24 months	Ambient aeroallergens measured throughout study period	Early wheezing obtained from medical records	Positive association between seasonal patterns (spore season) and odds of early wheezing (adjusted OR = 3.1, 95% CI; 1.3–7.4) controlling for PM _{2.5} and lower respiratory infections	Early wheezing in children exposed to pollen and spores in first three months of life is independent of air pollutant exposure and lower respiratory infections

AUTHOR(S)	AIM	STUDY DESIGN	STUDY POPULATION	EXPOSURE MEASUREMENTS	OUTCOME MEASUREMENTS	RESULTS	CONCLUSION
Heguy et al, 2008 ⁶⁰	Explored short-term effects of grass and weed pollen exposure on childhood asthma (re) admission to emergency department	Time-series analysis	Children aged 0–9 years living in Montreal, Canada between 1994 and 2004	Daily concentrations of pollen. Bi-hourly measurements of CO, SO ₂ , NO ₂ , and O ₃ from fixed-monitoring stations, and meteorological data from Meteorological Service of Canada	Daily number of emergency visits for childhood asthma	Positive association between emergency department visit and grass pollen concentration three days following exposure (mean per cent change = 1.73%; 95% CI: 0.24–3.25%) after adjusting for air pollutants and meteorological variables	Increasing risk of emergency department visit with increasing grass pollen concentrations
Endre et al, 2007 ⁴⁹	Measured prevalence of childhood asthma in relation to air pollution and total pollen count	Time-series ecological study design	Children under care of paediatricians between 1995 and 2003 in Budapest, Hungary	Online measurements of CO, NO ₂ , and SO ₂ at eight points in Budapest, O ₃ measurements at two stations, while pollen and fungal counts were calculated in Budapest	Doctors reported childhood asthma from response to survey questionnaire	Significant increase in prevalence of reported asthma from 1.88% in 1995 to 2.68% in 2003 (p < 0.0001). No increase in levels of air pollutants and pollens across studied years	Increase of 50% in prevalence of asthma in study period, independent of air pollution and pollen exposures
Atkinson et al, 2006 ⁶¹	Investigated short-term effects of daily counts of fungal spores on asthma exacerbation	Time-series ecological study design	Children aged 0–14 years residing in London between 1992 and 1993	Daily concentrations of fungal spores, pollen, outdoor air pollutants, and meteorological variables	Daily counts of visit for childhood asthma to family doctors, emergency department, and hospital admissions	Changes in fungal spore concentrations from lower to upper quartiles showed increase risk of emergency department visit (RR 1.06 95% CI: 0.94–1.18) and hospital admissions (RR 1.07 95% CI: 0.97–1.19). Observed effect was independent of air pollutants	Exacerbation of asthma may be induced by increased fungal spore concentrations independent of other environmental factors such as air pollution
Dales et al, 2000 ⁵²	Explored association between emergency department visit for asthma and daily concentrations of both pollen and fungal spores	Time-series ecological study design	Children reporting to children's hospital between 1993 and 1997	Daily concentrations of fungal spores, pollen, outdoor air pollutants (SO ₂ , NO ₂ , O ₃), and meteorological variables (barometric pressure, relative humidity, temperature)	Emergency department visit for asthma reporting at the children's hospital	Fungal spores, but not pollen, associated with percentage increase in number of asthma visits to emergency department (8.8%, p < 0.05)	Fungal spores implicated in exacerbation of asthma in children
Newson et al, 2000 ⁵³	Explored association between fungal spore counts and asthma admission	Time-series ecological study design	Children and adults residing in Trent, England between 1987 and 1994	Daily counts of 25 spore taxa measured in Derby	Hospital admission for asthma including spore counts on six days of asthma epidemics	No association found between total spore count and hospital admission, except for total spore count above the 90th percentile showing associations with four of the six epidemic days (OR = 9.92, 95% CI: 1.41–109.84)	Although no specific taxon was implicated, there is some evidence that high rates of asthma admissions tend to occur on days with high total mould spore counts

AUTHOR(S)	AIM	STUDY DESIGN	STUDY POPULATION	EXPOSURE MEASUREMENTS	OUTCOME MEASUREMENTS	RESULTS	CONCLUSION
Garty et al, 1998 ⁵⁴	Examined association between emergency department asthma visit and air pollution, weather, and airborne allergens	Prospective cohort study	Asthmatic children aged 1–18 years reporting at Children's ER between January and December, 1993	Daily concentrations of airborne allergens, air pollutants, and meteorological variables	Number of emergency room visits for asthma	Positive correlation between number of emergency room visits and SO ₂ and NO _x , high barometric pressure and negative associations with O ₃ and temperature, but no association with airborne pollens and spores	High levels of NO _x and SO ₂ associated with emergency room visits for asthma but not airborne pollen
Rosas et al, 1998 ⁶²	Assessed the relationship between environmental factors such as aeroallergens, air pollution and weather and emergency department admissions for asthma	Time-series ecological study design	Children under 15 years of age residing in Mexico City, Mexico	Daily concentrations of airborne allergens, air pollutants, and meteorological variables	Number of emergency room admissions for asthma	Both grass and fungal spores associated with asthma admissions in both wet and dry seasons, adjusting for air pollution	Positive association between pollen and asthma exacerbation in children

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2.3 Updated epidemiological evidence of the effects of ambient air pollution and airborne pollen (including fungal spores) as a co-exposure on respiratory health of children

This summarises the epidemiological evidence of the effect of ambient air pollution and the co-effect with airborne pollen or fungal spores on the respiratory health of children from local (South Africa) and international studies. The rationale is to provide an update on the current state of evidence from epidemiological studies in order to identify gaps in the literature.

2.3.1 Search Strategy

In February, 2018, an update search was done searching PubMed/Medline, ScienceDirect, Embase and Highwire for studies using the search terms “Air pollution” AND “asthma” AND “child*”. This was followed by a more specific search using the search terms (Ambient air pollution OR outdoor air pollution OR Pollen) AND (asthma or lung function or respiratory or allergic disease or airway inflammation) AND (children or paediatrics or child*). The search began by identifying previously reviewed articles on the topic to avoid repetition, then identifying primary research articles not included in the identified reviews. For the purpose of rigour, identified reviewed articles were critically assessed for completeness. Selection criteria for the primary research articles included peer-reviewed primary research articles aimed at exploring the association between ambient air pollution (and co-effect of pollen or fungal spores) on various aspect of children’s respiratory health such as asthma symptoms, asthma exacerbation, lung function, and airway inflammation from 2010 till date using epidemiological study design. Reference-list searching was done to identify additional article.

2.3.2 Descriptive Summary of evidence

For the purpose of this review, primary research articles reviewed would be referred to as ‘independently reviewed articles’ excluding already published reviews included. The independently reviewed articles included both international studies and only one local study done in South Africa met the inclusion criteria. A total of 4 reviewed articles (1–4) were identified around the topic area with an additional 63 primary research articles meeting the specific selection criteria. Thirty-one primary articles explored the effects of ambient air pollutants on respiratory symptoms, 20 focused on effects on lung function, while 9 articles explored the effects on airway inflammation. In addition, 8 articles explore the effects of either pollen or fungal spores on asthma-like outcomes in children. Amongst the articles that explored the effects of air pollution on respiratory symptoms, 13 used a cross-sectional study design, 6 longitudinal/cohort study, 6 panel study, 3 case cross over, and 3 time-series studies. Similarly, most of the studies focusing on lung function were cross-sectional in design (12 studies), with others using panel study design (3 studies) and

longitudinal/birth cohort study design (5 studies). Six of the studies exploring the effects on airway inflammation used a cross-section study design, while 2 used panel design and only one birth cohort study. Of the 8 articles that explored the effects of either pollen or fungal spores on childhood asthma, 6 used a time-series study design with only 2 longitudinal study.

Tables 2.3.1 to table 2.3.4 summarises the outcomes of these primary research articles.

Table 2.3.1: Epidemiological studies of ambient air pollutants effect on children's respiratory symptoms

Author(s), year	Study design	Study population	Exposure(s)	Outcome(s)	Main findings
Hasunuma et al., 2018 (5)	Panel study	138 school children from 2 schools, including an additional 71 asthmatic children from medical institutions in Fukuoka, Western Japan	Daily hourly concentration of photochemical oxidants (O _x), NO ₂ and PM _{2.5} from stationary monitor	Measurements of PEF and recording of nasal symptoms, cough and medication-use in diary obtained twice daily for 3 months from April 1 to June 30, 2013	Lag of up to 5-days were considered. There was an increased risk of coughing at Lag-0 amongst children without asthma (OR: 1.34, 95% CI: 1.11 - 1.60), children not using long-term medication (OR: 1.52, 95% CI: 1.12 - 2.07), and children using long-term medication (OR: 1.06, 95% CI: 0.93 - 1.20) per 10 ppb increase in oxidant.
Mentz et al., 2018 (6)	Panel study	423 school children from four communities in South and North Durban, South Africa	Measurements of NO, NO ₂ , SO ₂ , CO, O ₃ , PM _{2.5} and PM ₁₀ from multiple monitoring site around the city and those set-up at the respective schools for a 6-day cycle during the study period	Daily record of respiratory symptoms	Increased risk of cough at various lag (lag 0 to 5) for the measured pollutants, with greater effects in South as compared to North Durban. An increased risk of cough at lag-0 for an IQR increase in PM ₁₀ (OR: 1.07, 95% CI: 1.02 – 1.13), NO ₂ (OR: 1.14, 95% CI: 1.06 – 1.22), NO (OR: 1.09, 95% CI: 1.02 – 1.16), and SO ₂ (OR: 1.14, 95% CI: 1.06 – 1.22)
Gouveia et al, 2018 (7)	Time-series	Children respiratory mortality from four large urban Latin-America cities: Rio de Janeiro, Sao Paulo, Mexico City and Santiago	Daily measurements of PM ₁₀ and O ₃ obtained from stationary monitors	Mortality due to respiratory diseases in children	Percentage increase risk of deaths (0.47%, 95% CI: 0.09% - 0.85%) due to respiratory diseases in children (1-5 years old) from PM ₁₀
Keet et al., 2017 (8)	Time-series	Data on 7,810,025 children aged 5 -20 years enrolled in Medicaid in the US from 2009 - 2010	Two-average of PM _{2.5} and PM ₁₀ predicted at the zip-code area level from monitoring data and geographic characteristics	Asthma prevalence and morbidity, adjusting-for age, sex, urban city at the area level, education,	Although PM _{2.5} effect was greater than PM ₁₀ , an increase of 1 µg/m ³ in PM ₁₀ was associated with increased asthma prevalence (RR: 1.006, 95% CI: 1.001 - 1.011), ER visits (RR: 1.017, 95%

poverty and confounding due unmeasured spatial data CI: 1.001 - 1.033), and hospital admissions (RR: 1.023, 95% CI: 1.003 - 1.042)

Fan et al., 2017 (9)	Cross-sectional	2134 school children from 10 schools in Taiyuan, China	School based measurement of PM ₁₀	Respiratory symptoms obtained from questionnaires	An increase of 10 µg/m ³ in PM ₁₀ was associated with daytime attacks of breathlessness (OR: 1.07) and nocturnal cough (OR: 1.13)
Samoli et al., 2017 (10)	Panel study	186 children aged 10 years followed for 5 weeks during the 2013-2014 academic year in Athens and Thessaloniki, Greece	Daily O ₃ estimated from weekly personal and fixed school site measurements	Daily record of respiratory symptoms, absenteeism and PEF	There was an increased risk of any symptom (OR: 1.19, 95% CI: 0.98 - 1.44), attributed mainly to stuffy nose (OR: 1.23, 95% CI: 1.00 - 1.51) following a 10 µg/m ³ increase in O ₃ . However, there was no effect of O ₃ exposure on absenteeism and PEF.
Shan et al., 2016 (11)	Time-series	Paediatric hospital visit for wheeze in children under 3 years from January to December 2014 in Shanghai, China	Daily measurements of PM _{2.5} , PM ₁₀ , NO ₂ , CO, and SO ₂ , including meteorological data from fixed monitoring sites	Daily hospital visit for wheeze	There was a percent increase of nearly 20% in hospital visits per IRQ increase in PM _{2.5}
Yang et al., 2016 (12)	Birth Cohort study	3,701 children from the PIAMA birth cohort study in the Netherlands	LUR used to model OP (assessed by spin resonance OP(ESR) and dithiothreitol assay OP(DTT)) , PM _{2.5} , and NO ₂	Repeated questionnaire until age 14 years, allergic sensitization measurement, lung function, FeNO at age 12 years	For an IQR increase in OP (DTT) exposure, there was an association with asthma incidence (OR: 1.10 95%CI: 1.01 – 1.20); asthma symptoms prevalence (OR: 1.08 95%CI: 1.02 – 1.16); and rhinitis (OR: 1.15 95%CI: 1.05 – 1.26), while FEV ₁ and FVC had a negative association which was sensitive to NO ₂ adjustment. However, there was no association of respiratory health with PM _{2.5} and OP(ESR).
Hasunuma et al., 2016 (13)	Nested case-control	A baseline survey on 63,266 1.5 year-old children and follow-up at 3 years of age on 43,343 preschool children in Japan	NO _x and elemental carbon (EC) at residential address measured at baseline including time-activity	Asthmatic cases were matched with controls on a 1 to 4 ratio based on age, gender and area.	There was no association between the incidence of asthma at baseline and follow-up with measured pollutants. However, there was an association of persistence of asthma symptoms with NO _x (OR: 6.02 95%CI: 1.51 – 23.92) when

			pattern at follow-up measured by dispersion model.		comparing the upper 5 th and the lower 25 th centiles of NO _x
Molter et al., 2015 (14)	Birth Cohort study	10,377 children aged 4-5 year and 8-10 years participating in five European birth cohorts: GINI and LISA (both Germany, divided into north and south areas), MAAS (England, UK), PIAMA (the Netherlands), and BAMSE (Sweden).	Land-use regression model for NO ₂ , NO _x , PM ₁₀ , and PM _{2.5} , at each child's residential address.	ISAAC questionnaire used to collect information on asthma and current wheeze	The result of the analysis showed no significant association between prevalence of asthma amongst 8-10 years with 10µg/m ³ increase in NO ₂ (OR: 1.10 95%CI: 0.81 – 1.49); 5µg/m ³ increase in PM _{2.5} (OR: 1.23 95%CI: 0.78 – 1.95); and 10µg/m ³ increase in PM ₁₀ (OR: 0.88 95%CI: 0.63 – 1.24).
Ranzi et al., 2015 (15)	Panel study	69 asthmatic children aged 6-7 years residing in PO Valley in Italy	A combination of geographic (residential address) and environmental measurements (annual means of NO ₂ , SO ₂ , and PM ₁₀) which incorporated industrial and traffic sources. Each participants were assigned into 3 areas based on these combination.	Parental reporting of respiratory symptoms. PEF measured twice daily for 8-weeks	There was a small association of PM ₁₀ exposure with phlegm (OR: 1.05 95%CI: 1.00 – 1.10) and cough (OR: 1.03 95%CI: 0.99 – 1.08), with an increase odds of 18% in the most polluted area (OR: 1.18 95%CI: 1.02 – 1.37)
Rovira et al, 2014 (16)	Cross-sectional	2672 children aged 6 -7 years and 2524 adolescent aged 13 - 14 years in Tarragona, Spain.	Residence near 2 petrochemical sites living in the city with medium vehicular traffic compared to those with low vehicular traffic with no industry	ISAAC questionnaire for prevalence of asthma and respiratory symptoms. Lung function measurements in subsample of 959 adolescent in the 4 areas	Similar crude prevalence across the study areas. Following adjustment, children and adolescent residing in the exposed area had a significant higher prevalence of nocturnal cough in the preceding year (PR= 1.29; 95%CI, 1.05 – 1.57) and respiratory hospitalization (PR= 1.49; 95% CI, 1.06 – 2.09). There was no reduced lung function values among the adolescents residing in the exposed area

Molter et al., 2014 (17)	Birth Cohort study	1185 babies followed through from birth to 11 years of age within a population-based birth cohort study in the UK	Micro-environmental exposure model from birth to age 11 to assess monthly PM ₁₀ and NO ₂ exposure of each child.	Parental-reported prevalence of current wheeze and asthma at ages 3, 5, 8 and 11 years	There was an increase prevalence of asthma from age 3, 15.2% to 23.3% at age 5, while prevalence of current wheeze decreased from ages 3 to 8 (23.7 to 18%). However, there was no significant association between the mean monthly exposures to PM ₁₀ and NO ₂ and either prevalence of asthma and current wheeze.
Wendt et al., 2014 (18)	Case-crossover	18,289 incident asthma cases were identified from Children enrolled on the Medicaid in Harris County Texas between 2005-2007	Ambient air pollutant (O ₃ , NO and PM _{2.5}) concentrations are obtained from the monitoring stations between 2005 and 2007. Meteorological and aeroallergen data are also collected from the monitoring stations. Primary pollutant from traffic and industrial related sources. The average concentration of pollutant measured are as follows: 8-h max O ₃ , 37.87ppb; 1-h max NO ₂ , 39.26ppb; 24-h PM _{2.5} , 14.97µg/m ³	Asthma cases were identified from the Medicaid Analytic Extract enrolment and claim files.	New asthma onset in the warm season (May-October) was significantly associated with each 10ppb increase in O ₃ (OR=1.07 95%CI 1.02-1.08), NO ₂ (OR=1.07 95%CI 1.03-1.11) and PM _{2.5} (OR=1.12 95%CI 1.03-1.22). The strongest association was observed when a 6-day cumulative average period was used as the exposure metric.
Nishimura et al., 2013 (19)	Cross-sectional	3343 Latino and 977 African American with and without asthma in five urban regions in the US and Puerto Rico.	Data from local ambient air monitoring stations to estimate annual mean exposures to O ₃ , NO ₂ , SO ₂ , PM _{2.5} and PM ₁₀ .	Self-reported doctor-diagnosed asthma.	The odds of physician-diagnosed asthma increased by 17% for a 5ppb increase in mean NO ₂ during the first-year of life (OR; 1.17 95%CI: 1.04-1.31). However, there was no reported significant association for the other pollutants with childhood asthma.

Ripabelli, 2013 (20)	Cross-sectional	1004 exposed children from the industrial area compared to 920 from lower industrial area of Termoli, Molise region, Italy	Residence within industrial area compared to lower industrial area	Modified ISAAC questionnaire to identify confirmed and probable asthma cases in 89 children and adolescent. Analysis of paediatricians' databases on drug prescription for symptoms control and treatment	24.7% of the 89 participants administered with the ISAAC questionnaire had asthma among which 7.9% confirmed and 16.8% probable asthma cases with majority (77.3%) from the exposed area. However, there was no clusters found when all the asthma cases were georeferenced based on residences. A higher prevalence of 13.7% of diagnosed asthma cases was found within the exposed group when analysing drug prescription.
Stoner et al., 2013 (21)	Longitudinal study	6950 representative sample of children born in 2001 and followed through kindergarten-age in the US	Air toxic exposure were represented by 'total' National Air Toxics Assessment (NATA) respiratory hazard index, respiratory index from on-road mobile sources or diesel PM	Parental-reported asthma symptoms and emergency room visits	An overall 17.7% of 5.5 year-olds reported doctor-diagnosed asthma and 6.8% reported being hospitalized for an asthma attack. However, there was no association with greater asthma prevalence or hospitalization with air toxic exposures ($P > 0.05$). The adjusted logistic regression models also showed that there was no difference in the likelihood of asthma in children exposed to the highest respiratory hazard index as compared to those from the lowest respiratory hazard index.
Mino & Ceballos, 2013 (22)	Cross-sectional	305 randomly selected school children aged 6-14 years in Santa Marta, Columbia	fmrOMNI low volume samplers to measure PM ₁₀ concentrations.	ISAAC questionnaire to identify asthma and allergy symptoms. Lung function assessed via spirometry	The prevalence of respiratory symptoms was 39.3% with an increased risk (OR = 2.19, P=0.0015) of upper respiratory tract symptoms for living in exposed areas.
Kim et al., 2013 (23)	Cross-sectional	4545 elementary school children from 7 schools with diverse air pollution levels in four Korean cities	Classification of schools into 1. No-traffic or other pollutants area, 2. Traffic-related areas, and 3. Traffic-related with other pollutant such as industrial and filling station sources. The three areas were evaluated based	ISAAC questionnaire to identify asthma and allergy symptoms	There was a significant risk of asthma and allergy symptoms and treatment in children in the school group with traffic-related pollutants and school group with complex pollutants compared to school groups with no traffic-related pollutions (OR: 2.12 95%CI: 1.41 – 3.19 and OR: 1.59 95%CI: 1.06 – 2.37 respectively)

on levels of black carbon, PM₁₀, SO₂, NO₂ and O₃.

Altug, 2013 (24)	Panel study	1880 school children aged 9 – 13 years from 16 public schools from suburban, urban and urban-traffic regions in Eskisehir, Turkey.	Passive samplers for air measurements of O ₃ , NO ₂ , and SO ₂ , both in summer and winter.	ISAAC questionnaire to identify asthma and allergy symptoms. Lung function assessed via spirometry both in summer and winter	A significant association was more observed only in Girls with altered lung function (only the summer test) living in suburban and urban regions as compared with urban-traffic region (OR: 1.49 95%CI: 1.04 - 2.14 and OR: 1.69 95%CI: 1.06 – 2.71 respectively). An increase of 10µg/m ³ of O ₃ was significantly associated with impaired lung function only in Girls for the summer test (OR: 1.11 95%CI: 1.03 – 1.19). However, no association was found in boys and for the winter test. Similarly, no association was found between measured air pollutants and respiratory symptoms.
Jung <i>et al.</i>, 2012 (25)	Birth cohort study	405 children aged between 5-6 years old in New York City	Two-week integrated residential monitoring of PM _{2.5} and black carbon between October and May, 2011. Repeated residential monitoring after 6 months on only 262 participants	Repeated ISAAC questionnaire to identify new wheeze during a 3years follow-up and compared to a reference group with reported never-wheezing, remitted wheeze or persistent wheeze. Specific IgE to cockroach, cat, mouse and dust was done at ages 5 and 7 years	For an IQR increase, a significant association was found between PM _{2.5} and new wheeze (adjusted OR: 1.51 95%CI: 1.05 – 2.16) but not for black carbon. In the sub-group of participants with repeated measurement after 6 months, a positive association was found with new wheeze development. However, there was no association of measured pollutants with IgE levels.

Sarnat et al., 2012 (26)	Panel study	58 asthmatic children (aged 6 to 12 years) from two schools in Ciudad Juarez, Mexico and El Paso, Texas, USA.	Pollutant measurements using indoor and outdoor 48-hr size-fractionated PM, 96-hr nitrogen dioxide and 48-hr black carbon, were collected over a period of 16 weeks at each school. Primary pollutant from traffic-related sources. Average ambient concentrations of pollutant measured are as follows: 48-h PM ₁₀ , 35.0µg/m ³ ; 48-h PM _{2.5} , 9.6µg/m ³ ; 96-h NO ₂ , 14.0ppb; 48-h O ₃ , 30.0ppb.	Respiratory symptom surveys completed by parent/guardian and exhaled nitric oxide	There was a small (2.4% change in eNO per 4.9 µg/m ³ increase in ambient PM _{2.5}) but consistent association between eNO and the different pollutant metrics, with an estimated increase of 1% to 3% in eNO per interquartile range increase in pollutant concentration. Only ambient 48-h PM _{2.5} showed a significant association (P<0.001).
Portnov et al., 2012 (27)	Cross-sectional	Analysis on medical records of 3922 school children living in Haifa Metro in Northern Israel.	GIS estimates on ambient air pollutant such as PM ₁₀ and SO ₂	Hospitalization for childhood asthma	The results shows childhood asthma to be significantly associated with areas with high levels of PM ₁₀ compared to the lowest level (OR: 2.58, 95%CI: 1.52 – 4.41), but no association was found with SO ₂
Hwang et al., 2012 (28)	Cross-sectional	1,819 school children residing in a metropolitan area (Seongbuk) and a semirural area (Andong) in Korea	Exposure proxy was at the area level	Self-reported and physician-diagnosed asthma	There was no significant difference in the prevalence of self-reported asthma across the two areas (12.8% vs. 13.6%). However, the prevalence of physician-diagnosed asthma was higher in the semirural area (15.0%) as compared to the metropolitan area (6.8%).
Kaszniak-Kocot et al., 2011 (29)	Cross-sectional	1,130 children aged 13 – 15 years residing in Upper Silesia, Poland	Distance from heavy traffic road	ISAAC questionnaire to identify asthma and allergy symptoms	The prevalence of bronchial asthma, doctor-diagnosed asthma and current wheezing in the past 12 months was 4.5%, 8.7% and 12.6% respectively. Children residing near a heavy traffic road including damp-dwellings and heated coal-fired furnaces were nearly twice more likely to have asthma.

Kim et al., 2011 (30)	Longitudinal study	1,743 school children followed-up for 2 years residing in metropolitan and industrial areas of Korea	GIS estimates on the 5-year mean concentration of ozone	ISAAC questionnaire to identify asthma and allergy symptoms	The result shows an association of 12-months prevalence of wheeze per 5ppb increase in ozone exposure (OR: 1.372, 95%CI: 1.016 – 1.852). Furthermore there was a significant association between newly developed sensitization to outdoor allergen and ozone (P-trend= 0.007).
Clark et al., 2010 (31)	Nested case-control	37,401 children from South-western British Columbia, Canada were assessed from outpatient and hospitalization records for asthma incidence identification up to 34-years of age. Controls were matched on age and sex on a 1:5 ratio from the eligible cohort.	Ambient air pollution data from the gestational period and first year of life using data from monitoring station and LUR for CO, PM _{2.5} , PM ₁₀ , NO ₂ , SO ₂ , O ₃ , BC, wood smoke. Data on point sources and proximity was also collected	Asthma diagnosis from outpatient and hospitalization records	Statistical significant increase risk of asthma diagnosis with early life exposure to BC, CO, NO, NO ₂ , PM ₁₀ and point source proximity. Highest risks was associated with traffic-related pollutants; a 10µg/m ³ increase in NO (OR: 1.08 95%CI: 1.04 – 1.12); a 10µg/m ³ increase in NO ₂ (OR: 1.12 95%CI: 1.07 – 1.17); and a 100µg/m ³ increase in CO (OR: 1.10 95%CI: 1.06 – 1.13).
Akinbami, et al., 2010 (32)	Cross-sectional	34,073 eligible children aged 3-17 years from the 2001-2004 National Health Interview Survey residing in metropolitan areas in the US.	12-month mean levels of SO ₂ , NO ₂ , O ₃ and PM from monitoring stations.	Questions on current asthma and asthma attack	Compared to children in the lowest quartile of O ₃ levels, children in the highest quartile are more at risk for current asthma (OR: 1.56 95%CI: 1.15 – 2.10) and recent asthma attack (OR: 1.38 95%CI: 0.99 – 1.91). However, no association was found for SO ₂ and NO ₂ levels, and only to a less degree for PM.
Penard-Mornand et al., 2010 (33)	Cross-sectional	6,683 school children aged 9-11 years from 108 schools in six French communities.	Dispersion model used to estimate the 3-years averages for PM ₁₀ , SO ₂ , NO _x , NO ₂ , CO, and benzene.	Reported asthma and allergies using the ISAAC questionnaire, exercise-induced asthma, and skin prick test	4,907 children residing at their current address for the last 3 years had a significant association of asthma (lifetime, past year & exercise-induced) with SO ₂ , PM ₁₀ , NO _x , CO, and benzene. The association persisted among 2,213 residing at their present address since birth, for lifetime asthma with PM ₁₀ (OR: 1.4, 95% CI: 1.0 – 2.0) and an IQR increase in benzene (OR: 1.3, 95% CI: 1.0 – 1.9).

Cara et al., 2010 (34)	Cross-sectional	Surveyed 297 exposed children (Calarasi) vs. 237 non-exposed children (Roseti) aged 6-7 years in Romania	An industrial area near iron and steel factory in Calarasi as the exposed area and a non-industrial area in Roseti	ISAAC short questionnaire to identify asthma and allergy symptoms. FEV ₁ and PEF	Children from the exposed area were 7-times more likely to respond positively to at least one of the ISAAC questions as compared to those from the non-exposed area (OR: 7.2 95%CI: 3.6 – 14.3), and remained significant after adjusting for covariates. There was also a strong association between asthma symptoms at school age and early life wheezing ((OR: 9.0 95%CI: 3.4 – 23.5)
Pan et al., 2010 (35)	Cross-sectional	11,860 children aged 3 – 12 years residing in six heavy industrial province in Northern China	Daily measurements of Total suspended particle (TSP), SO ₂ and NO ₂ were obtained from the monitoring stations	Respiratory symptoms questions from the Epidemiologic Standardization project Questionnaire of American Thoracic Society (ATS-DLD-78-A), including additional questions on household characteristics, breastfeeding, respiratory illness history and familial history.	For every IQR increase in TSP, SO ₂ and NO ₂ , there was a significant increase in the prevalence of persistent cough (21-28%), current asthma (39-56%), and persistence phlegm (21-30%), with stronger associations with TSP than SO ₂ and NO ₂ .

Table 2.3.2: Epidemiological studies of the effects of ambient air pollutants on children's lung function

Author(s), year	Study design	Study population	Exposure(s)	Outcome(s)	Main findings
Tsui et al., 2017 (36)	Cross-sectional study	481 boys and 535 girls non-asthmatic children aged 6 - 15 years in 44 schools in Taiwan	Estimate of PM ₁₀ , O ₃ , SO ₂ , NO ₂ , and CO using kriging method based on monitoring data	Spirometry indices such as FEV ₁ , FVC and maximal midexpiratory flow	A 10 µg/m ³ increase in PM ₁₀ was associated with percent deficit in FEV ₁ (-2.00%, 95% CI: -2.96% to -0.75%), and maximal midexpiratory flow (-2.28%, 95% CI: -4.04% to -0.51%)
Fan et al., 2017 (9)	Cross-sectional study	Total of 695 third-or fourth grade school children (382 from polluted area and 313 from clean area categorised according to the EPA of Chonqing, China, between 2010 and 2015)	Categorization into clean and polluted area based on the EPA website between 2010 and 2015	Spirometry indices such as FEV ₁ and FVC	A significantly higher FVC and FEV ₁ amongst school children in the clean area compared to the polluted area (<i>p</i> < 0.05)
Neisi et al., 2017 (37)	Cross-sectional study	105 elementary school children in Ahvaz, Iran	Measured levels of PM _{2.5} and PM ₁₀ during normal and dusty days close to the school	Spirometry indices such as FEV ₁ and FVC, including FeNO	Significant difference in mean values of FVC during dusty and normal days (<i>p</i> < 0.05). A significant higher FeNO levels in the dusty area (20.3 ppb) compared to the clean area (14.23 ppb)
Zhang et al., 2017 (38)	Longitudinal study	233 school children from three schools with four lung function measurements between December 2013 and May 2015 in Shanghai, China	Daily air quality data on PM _{2.5} , PM ₁₀ , SO ₂ , and NO ₂ obtained from stationary monitors near the schools, including meteorological data	Spirometry indices for small airways such as MEF25%, MEF50%, MEF75% and FEF _{25%-75%} .	Significant adjusted effects of PM _{2.5} , PM ₁₀ and SO ₂ on all small airway indices (<i>p</i> < 0.05)
Samoli et al., 2017 (10)	Panel study	186 children aged 10 years followed for 5 weeks during the 2013-2014 academic year in Athens and Thessaloniki, Greece	Daily O ₃ estimated from weekly personal and fixed school site measurements	Daily record of respiratory symptoms, absenteeism and PEF	There was an increased risk of any symptom (OR: 1.19, 95% CI: 0.98 - 1.44), attributed mainly to stuffy nose (OR: 1.23, 95% CI: 1.00 - 1.51) following a 10 µg/m ³ increase in O ₃ .

However, there was no effect of O₃ exposure on absenteeism and PEF.

Jung et al., 2016 (39)	Cross-sectional study	Children aged 9 - 14 years, including adults (15 - 64 years) and the elderly (65 years and older) in an industrial complex of Gwangyang Bay in South Korea	Spatial interpolation of daily O ₃ levels	Spirometric indices such as FEV ₁ and FVC	Significant negative effect of O ₃ on FEV ₁ and FVC, especially on lag-0 and lag-2 in children
Wong et al., 2016 (40)	Cross-sectional study	2,833 children aged 6 - 18 years from the Canadian Health Measures Survey, 2007 - 2011	Weighted average of NO _x and PM _{2.5} within 25 km of respondent's residence estimated from the National Pollutant Release Inventory data	Spirometric indices such as FEV ₁ , FVC, FEV ₁ /FVC	All except PM _{2.5} showed negative effect on FEV ₁ and FEV ₁ /FVC only in males. Null effect in females for PM _{2.5} , while no effect was found between NO _x and any of the lung function indices
Yang et al., 2016 (12)	Birth Cohort study	3,701 children from the PIAMA birth cohort study in the Netherlands	LUR used to model OP (assessed by spin resonance OP(ESR) and dithiothreitol assay OP(DTT)) , PM _{2.5} , and NO ₂	Repeated questionnaire until age 14 years, allergic sensitization measurement, lung function, FeNO at age 12 years	For an IQR increase in OP (DTT) exposure, there was an association with asthma incidence (OR: 1.10 95%CI: 1.01 – 1.20); asthma symptoms prevalence (OR: 1.08 95%CI: 1.02 – 1.16); and rhinitis (OR: 1.15 95%CI: 1.05 – 1.26), while FEV ₁ and FVC had a negative association which was sensitive to NO ₂ adjustment. However, there was no association of respiratory health with PM _{2.5} and OP (ESR).
Ierodiakonou et al., 2016 (41)	Longitudinal study	1,003 asthmatic children aged 5-12 years participating in a 4-year clinical study in 8 North	Daily measurement of O ₃ , CO, NO ₂ , and SO ₂ from monitoring stations linked to postal code of each child	Spirometry obtained from pre- and post-bronchodilator at each baseline and follow-up (2 months after baseline and	Both 4-months moving average of CO and O ₃ were negatively associated with FEV ₁ /FVC ratio (P < 0.05). Likewise, increase 4-months average of NO ₂ is associated with reduced post-bronchodilator FEV ₁ and %predicted FVC. In addition long term exposure to an

		American cities (US & Canada)		every 4 months subsequently	IQR increase in SO ₂ was associated to reduce PC ₂₀ (-6% 95% CI: -11%, -1.5%)
Neophytou <i>et al.</i>, 2016 (42)	Cross-sectional	1,968 (1,449 Latinos and 519 African Americans) asthmatic children participating in the SAGE and GALA II study from five regions in US and Puerto Rico	Daily ambient air concentrations of O ₃ , NO ₂ , SO ₂ , PM _{2.5} and PM ₁₀ data from monitoring stations and the residential history of each participant	Lung functions accessed through spirometry	There was a 7.7% decrease in FEV ₁ (95%CI: -11.8%, -3.5%) for a 5 µg/m ³ increase in lifetime PM _{2.5} exposure in the overall sample. Although percent African ancestry is a significant predictor of lung function, the global genetic ancestry did not significantly modify the associations.
Amadeo <i>et al.</i>, 2015 (43)	Cross-sectional	1,436 children (8-13 years) randomly selected from 27 elementary schools distributed over 91 classrooms in Guadeloupe (French West Indies)	Medium-term exposure to close-proximity pollution and background air pollution data are derived from air monitoring stations for a period of 2 consecutive weeks prior to clinical examination of children. Primary pollutant from traffic and industrial related sources. The average background concentrations of measured pollutant are as follows: O ₃ , 54.1µg/m ³ ; NO ₂ , 14.8µg/m ³ ; SO ₂ , 4.7µg/m ³ ; PM ₁₀ , 23.9µg/m ³	Baseline peak expiratory flow (PEF) before running and variation in PEF after running was used to assess exercise-induced asthma. ISAAC questionnaires	There was a 16% prevalence of asthma in the sampled children. A PEF decrease (β=-0.32; 95%CI -0.61- -0.03) was associated with a 1µg/m ³ increase in medium-term exposure to ambient O ₃ with the association stronger in asthmatics than non-asthmatics. A significant association was found between PM ₁₀ and PEF decrease, but there was no change or reduction in PEF with NO ₂ , SO ₂ and PM ₁₀ .

Barone-Adesi et al., 2015 (44)	Cross-sectional study and Meta-Analysis	4, 884 children aged 9-10 years from the Child Heart and Health Study in England (CHASE) including 13 articles included in the meta-analysis.	Dispersion modelling of NO ₂ , NO, NO _x and PM	Spirometry to obtain lung function	There was no association between all the pollutant except O ₃ with either FEV ₁ and FVC in CHASE. However, in the meta-analysis, a 10µg/m ³ increase in NO ₂ was associated with a deficit of 8ml in FEV ₁ (95% CI: -14ml, -1ml), thus translating to a 7% increase in the prevalence of abnormal lung function in children.
Chen et al., 2015 (45)	Cross-sectional	1,494 non-asthmatic school children aged 6-15 years from 24 districts in Taiwan.	Daily measurements of PM ₁₀ , PM _{2.5} , SO ₂ , O ₃ , CO and NO ₂ were obtained from the monitoring stations	Pulmonary function test from spirometry	There was deficit (changes) of -103ml and -142 ml in FVC; -86ml and -131ml in FEV ₁ ; and -102ml and -188ml in MMEF for an IQR increase in PM _{2.5} and O ₃ in the last 2-months respectively. In a subgroup of children aged 6-10 years, PM _{2.5} was associated with decrease FEV ₁ /FVC.
Rovira et al, 2014 (16)	Cross-sectional	2672 children aged 6 -7 years and 2524 adolescent aged 13 -14 years in Tarragona, Spain.	Residence near 2 petrochemical sites living in the city with medium vehicular traffic compared to those with low vehicular traffic with no industry	ISAAC questionnaire for prevalence of asthma and respiratory symptoms. Lung function measurements in subsample of 959 adolescent in the 4 areas	Similar crude prevalence across the study areas. Following adjustment, children and adolescent residing in the exposed area had a significant higher prevalence of nocturnal cough in the preceding year (PR= 1.29; 95%CI, 1.05 – 1.57) and respiratory hospitalization (PR= 1.49; 95% CI, 1.06 – 2.09). There was no reduced lung function values among the adolescents residing in the exposed area
Mino & Ceballos, 2013 (22)	Cross-sectional	305 randomly selected school children aged 6-14 years in the city of Santa Marta, Columbia	fmrOMNI low volume samplers to measure PM ₁₀ concentrations.	ISAAC questionnaire to identify asthma and allergy symptoms. Lung function assessed via spirometry	The prevalence of respiratory symptoms was 39.3% with an increased risk (OR = 2.19, P=0.0015) of upper respiratory tract symptoms for living in exposed areas.

Molter <i>et al.</i>, 2013 (46)	Birth cohort study	1185 children followed from birth to the age of 11 years in Manchester, England	Micro-environmental exposure model from birth to age 11 to assess monthly PM ₁₀ and NO ₂ exposure of each child.	Spirometry (specific airway resistance and FEV ₁ following pre- and post-bronchodilator) performed during clinic visits at ages 3, 5, 8, and 11 years	There was a significant less growth in percent predicted FEV ₁ over time with life-time exposure to PM ₁₀ and NO ₂ both before (-0.83%, 95% CI: -1.39, -0.28 for a 1-unit increase in NO ₂ and 1.37%, 95% CI: -2.52, -0.23 for a 1-unit increase in PM ₁₀) and after bronchodilator (-1.20%; 95% CI: -1.97, -0.43 and -3.59%; 95% CI: -5.36, -1.83, respectively). However, there was no association of lifetime exposure with specific airway resistance over time. Furthermore, there was no significant association in the cross-sectional analyses of detailed exposure estimates for the summer and winter prior to 11 years of age and lung function at 11 years.
Altug <i>et al.</i>, 2013 (24)	Panel Study	1880 school children aged 9 – 13 years from 16 public schools from suburban, urban and urban-traffic regions in Eskisehir, Turkey.	Passive samplers for air measurements of O ₃ , NO ₂ , and SO ₂ , both in summer and winter.	ISAAC questionnaire to identify asthma and allergy symptoms. Lung function assessed via spirometry both in summer and winter	A significant association was more observed only in Girls with altered lung function (only the summer test) living in suburban and urban regions as compared with urban-traffic region (OR: 1.49 95%CI: 1.04 - 2.14 and OR: 1.69 95%CI: 1.06 – 2.71 respectively). An increase of 10µg/m ³ of O ₃ was significantly associated with impaired lung function only in Girls for the summer test (OR: 1.11 95%CI: 1.03 – 1.19). However, no association was found in boys and for the winter test. Similarly, no association was found between measured air pollutants and respiratory symptoms.
Jacobson <i>et al.</i>, 2012 (47)	Panel study	309 school children aged 6 – 15 years from the same school in the subequatorial Amazon region of Brazil.	Daily measurements of PM _{2.5} considering the 24-hour, 12-hour, and 5-hour averages near the school.	Daily measurement of peak expiratory flow rate except on weekends from August to December, 2006.	There was a reduction in the PEF for a 10µg/m ³ increase in PM _{2.5} which varied averagely between 0.26l/min (95%CI: -0.49, -0.04) and 0.38l/min (95%CI: -0.71, -0.04). In the non-asthmatic group, the effect ranged from 0.38l/min (95%CI: -0.63, -0.13) to 0.53l/min (95%CI: -0.90, -0.16). However,

there was no significant effect among asthmatics.

Lee <i>et al.</i>, 2011 (48)	Cross-sectional	3,957 Grade-7 school children from 14 Taiwanese communities	Air pollution data on PM _{2.5} , PM ₁₀ , SO ₂ , NO _x , NO ₂ , and CO from air monitoring stations for 2005 – 2007.	Lung function test using spirometry	Traffic-related pollutants such as CO, NO _x , and NO ₂ were more associated with chronic adverse effects on FVC and FEV ₁ , and sub-chronic adverse effects specifically on MMEF and PEF only among boys. There was no effect of other pollutants on either boys or girls.
He <i>et al.</i>, 2010 (49)	Longitudinal study	1,983 children from three district (categorized as high-medium- & low-polluted areas) in Guangzhou China followed for 6-months.	Daily measurements of PM ₁₀ , SO ₂ and NO ₂ were obtained from the monitoring stations	Pulmonary function test from Spirometry. Parental-reported questionnaire (ATS-DLD-78-C) on respiratory symptoms	Following adjustment for covariates, with the low-polluted district (LPD) as the reference, there was significant deficits in the annual growth rates in boys, for FEF-25, -0.153 l/s, p = 0.004 in high-polluted district (HPD) and -0.136 l/s, p = 0.008 in medium-polluted district (MPD) respectively, and for FEF 25-75, -0.167 l/s, p = 0.021 in HPD and -0.176 l/s, p = 0.013 in MPD respectively. While a deficit of -0.123 l/s, p = 0.043 for FEF-25 in girls in the HPD.

Table 2.3.3: Epidemiological studies of the effects of ambient air pollutants on airway inflammation in children

Author(s), year	Study design	Study population	Exposure(s)	Outcome(s)	Main findings
Neisi et al., 2017 (37)	Cross-sectional study	105 elementary school children in Ahvaz, Iran	Measured levels of PM _{2.5} and PM ₁₀ during normal and dusty days near the school	Spirometry indices such as FEV ₁ and FVC, including FeNO	Significant difference in mean values of FVC during dusty and normal days ($p < 0.05$). A significant higher FeNO levels in the dusty area (20.3 ppb) compared to the clean area (14.23 ppb)
Carlsen et al., 2016 (50)	Panel study	95 healthy elementary school children aged 11 years from northern Sweden sampled between 11 April to 6 June, 2011	Daily estimate of PM _{2.5} , NO ₂ , NO _x , NO and O ₃ , including birch pollen during the study period near the school	FeNO	An IQR increase of PM ₁₀ was associated with an increase in FeNO between 6.9ppb (95% CI: 0.0 - 14.0) and 7.3ppb (95% CI: 0.14 - 14.9) in the multi-pollutant model.
Liu et a., 2014 (51)	Birth-cohort study	1985 (192 asthmatics and 1793 non-asthmatics) children aged 10 years from the GINIplus and LISApplus studies in Germany	Short-term estimates of 24h NO ₂ and PM ₁₀ . Long term estimated of annual concentrations of NO ₂ , PM ₁₀ , PM _{2.5}	FeNO	24h NO ₂ increases eNO by percentage change of 18.30% (95% CI: 11.63% - 25.37%) in the single pollutant model and by 14.62% (95% CI: 6.71% - 23.11%), while 24h PM ₁₀ was only significant in the single-pollutant model (9.59%, 95% CI: 4.80% - 14.61%). However, there was no significant association between long term air pollution and eNO.
Altug, 2014 (52)	Cross-sectional	605 school children aged 9 – 13 years from Eskisehir, Turkey.	Passive samplers for air measurements of weekly O ₃ , NO ₂ , and SO ₂ , estimated in the school playgrounds during the study period	ISAAC questionnaire to identify asthma and allergy symptoms. FeNO measurements including lung function assessed via spirometry	A 10 µg/m ³ increase in O ₃ increases the risk of having-cold (OR: 1.21) and runny nose on test-day (OR: 1.28) in the adjusted model ($p < 0.05$). None of the pollutants had a significant effect on FeNO. However, amongst children with upper respiratory tract complains, weekly

average of O₃ was negatively associated with PEF.

Sarnat et al., 2012 (26)	Panel Study	58 asthmatic children (aged 6 to 12 years) from two schools in Ciudad Juarez, Mexico and El Paso, Texas, USA.	Pollutant measurements using indoor and outdoor 48-hr size-fractionated PM, 96-hr nitrogen dioxide and 48-hr black carbon, were collected over a period of 16 weeks at each school. Primary pollutant from traffic-related sources. Average ambient concentrations of pollutant measured are as follows: 48-h PM ₁₀ , 35.0µg/m ³ ; 48-h PM _{2.5} , 9.6µg/m ³ ; 96-h NO ₂ , 14.0ppb; 48-h O ₃ , 30.0ppb.	Respiratory symptom surveys completed by parent/guardian and exhaled nitric oxide	There was a small (2.4% change in eNO per 4.9 µg/m ³ increase in ambient PM _{2.5}) but consistent association between eNO and the different pollutant metrics, with an estimated increase of 1% to 3% in eNO per interquartile range increase in pollutant concentration. Only ambient 48-h PM _{2.5} showed a significant association (P<0.001).
Eckel et al., 2011 (53)	Cross-sectional	2,143 school children aged 7 - 11 years from the Southern California Health Study	Traffic-related pollutant estimated from: distance to road, total street length within circular buffers, density of traffic within buffers, annual averages of NO _x from dispersion model, predicted annual averages of NO, NO ₂ and NO _x from intercommunity based-model	FeNO	Total street length within 50m, 100m, and 200m buffer were associated with higher FeNO percentage difference [(46.7%, 95% CI: 14.3 - 88.4), (12.4%, 95% CI: -8.8 - 38.4), (4.1%, 95% CI: -14.6 - 26.8)] respectively, with stronger associations at small buffers. There was no significant association of other TRAP metrics with FeNO

Graveland et al., 2011 (54)	Cross-sectional	812 school children from nine Dutch schools, all within 400m of a highway	Daily measurements of PM ₁₀ obtained from stationary monitor, while long-term exposure assessed from traffic-related characteristics such as traffic density and distance to highway	FeNO	Across the range of PM ₁₀ exposure of 44 µg/m ³ , there was a significant increase in FeNO (geometric mean ratio 2.24, 95% CI: 1.37 - 3.65). There was no significant effect of TRAP with FeNO.
Berhane et al., 2011 (55)	Cross-sectional	2,240 school children aged from 13 communities in the Southern California Health Study	Daily 24-hour average of PM _{2.5} , PM ₁₀ and 8-hour average of O ₃ measured from central stationary monitor during a period of up to 30 days prior FeNO test	FeNO	An IQR increase of 7.5 µg/m ³ in PM _{2.5} (lagged over 1 -8 days), increase of 12.97 µg/m ³ in PM ₁₀ (lagged over 1 - 7 days) and an increase of 15.42 ppb in O ₃ (lagged over 1 - 23 days) were significantly associated with percentage increase in FeNO of 17.42% (<i>p</i> < 0.01), 9.25% (<i>p</i> < 0.05), and (<i>p</i> < 0.01) respectively. These effects were all higher in warm season
Flamant-Hulin et al., 2010 (56)	Cross-sectional	104 children (34 asthmatics and 70 non-asthmatics) in France	Measurements of NO ₂ and PM _{2.5} from active and passive samplers in the school	FeNO	High exposures to PM _{2.5} and NO ₂ associated with higher FeNO in both groups of children, with stronger association found for atopic non-asthmatic children

Table 2.3.4: Epidemiological studies of the effects of airborne pollen and fungal spores on children's respiratory health

Author(s), year	Study design	Study population	Exposure(s)	Outcome(s)	Main findings
Tham et al., 2017 (57)	Time-stratified case-crossover	Hospitalization of asthma in Australian children over 5 years	Daily measurements of 20 fungi taxa, grass pollen, air pollutants and meteorological variables from central monitoring sites	Children hospitalised for asthma	Modest association between some fungal taxa such as <i>Coprinus</i> (Lag-0, Lag-1, OR: 1.03, 95% CI: 1.01 - 1.06), <i>Chaetomium</i> (Lag-2, OR: 1.08, 95% CI: 1.00 - 1.20), and <i>Periconia</i> (Lag-0: OR: 1.03, 95% CI: 1.00 - 1.07) with non-modifying effect of grass pollen and air pollutants
Bono et al., 2016 (58)	Time-series	Daily number of respiratory-related emergency admissions for children in Turin, Italy	Daily concentrations of background NO ₂ , PM _{2.5} and O ₃ , including total aeroallergens from <i>Betula</i> , <i>Ambrosia</i> , <i>Urticaceae</i> , <i>Gramineae</i> , <i>Corylaeae</i> , and <i>Cupressaceae</i> from central monitoring sites	Children hospitalised for respiratory reasons	Increase in ER admissions by 0.7% (95% CI: 0.1% - 1.2%) following a one-day increase of 10 grains/m ³ in aeroallergen, and by 1.3% (95% CI: 0.3% - 2.2%) after a five-day increase of 10 µg/m ³ in NO ₂ .
Ito et al., 2015 (59)	Time-series	Daily sales of over-the-counter allergy medication and ER visit for asthma in New York, US between 2002 and 2012	Daily measurements of nine spring tree pollen genera (maple, elm, poplar, ash, beech, birch, oak, hickory, and sycamore), air pollutants and meteorological variables	Number of over-the-counter sales and ER admissions	A cumulative rate ratio of 1.9 (95% CI: 1.7 - 2.1) and 1.7 (95% CI: 1.5 - 1.9) increase in medication sales and ER visits per 0-to-98th percentile increase in ash
Chen et al., 2014 (60)	Longitudinal study	100 school children studied between October 2007 and June 2008, and two additional measurements in	Daily concentrations of fungal spores measured weekly to correspond to lung function	5 to 10 repeated lung function measurements during the study period over 10 months. A total of	Only <i>Cladosporium</i> showed a one-day lagged adjusted significant negative association with FVC and FEV ₁ . Above an identified threshold of 1514 spores/m ³ , each doubling of

		June, and November 2009 in New Taipei City, Taiwan	measurements, including air pollutant (SO ₂ , NO ₂ , CO, O ₃ , PM _{2.5} , and PM ₁₀ from stationary monitor	824 measurements available for analysis	<i>Cladosporium</i> at lag-1 was significantly associated with a deficit of 0.231 (95% CI: -0.35 to -0.11) in FEV ₁ , and a deficit of 0.251 in FVC (95% CI: -0.37 to -0.13)
Chen et al., 2011 (61)	Longitudinal study	100 school children studied between October 2007 and June 2008 in New Taipei City, Taiwan	Daily concentrations of fungal spores measured weekly to correspond to lung function measurements, including air pollutant (SO ₂ , NO ₂ , CO, O ₃ , PM _{2.5} , and PM ₁₀ from stationary monitor	5 to 10 repeated lung function measurements during the study period over 8 months. A total of 682 measurements available for analysis	Unchanged effect of fungal spores in the presence of PM _{2.5} on FEV ₁ and FVC. An IQR increase of 1.3 log spores/m ³ was associated with a deficit of 0.101 (95% CI: -0.17 to -0.02) in FEV ₁ , and a deficit of 0.121 in FVC (95% CI: -0.21 to -0.03) in the single pollutant model. This effect remained unchanged in the two-pollutant model with PM _{2.5} [FEV ₁ : -0.111 (95%CI: -0.19 to -0.03), and FVC: -0.141 (95%CI: -0.23 to -0.05)]
Jariwala et al., 2011 (62)	Time-series	Paediatric patient admission for asthma in New York, US during 1999	Daily concentrations of pollen and air pollutants such as NO ₂ , SO ₂ and O ₃ from central monitoring sites	Daily number of ER admission for asthma	High correlation between peak spring season indicative of high tree pollen and ER admission ($r = 0.90$, $p = 0.03$), and strong positive correlation between asthma ER admissions in winter and levels of SO ₂ and NO ₂
Raphoz et al., 2010 (63)	Time-series	ER asthma visits among children aged - to 9 years in Montreal, Canada, between 1994 and 2004	Daily measurements of 20 fungal spores, air pollutants and meteorological variables from central monitoring sites	Daily number of ER admission for asthma	Increase in first ER admissions at all lag days (up to Lag-6) except the concurrent day, following exposures to <i>Basidiomycete</i> , with effects observed from lag-3 and diminished at lag-6 for <i>Deuteromycetes</i> and <i>Cladosporium</i> . However, except for <i>Basidiomycetes</i> , there is little evidence of readmissions.
Huynh et al., 2010 (64)	Time-series	Asthma attacks consultation at general practitioners office in the Greater Paris Area between 2003 and 2007	Daily counts of common pollen such as <i>Betula</i> , <i>Poaceae</i> , <i>Cupressa</i> , and <i>Fraxinus</i> , including air pollutants concentrations	Weekly data of asthma attack visits to GP from the French GP Sentinel Network	An increase relative risk of 1.54 (95% CI: 1.33 - 1.79) following an IQR increase of 17.6 grains/m ³ in <i>Poaceae</i> during the pollen season, independent of air pollutants and influenza.

from central monitoring
sites

2.3.3. Main findings of exposure-response studies for ambient air pollution and airborne pollen/fungal spores on childhood respiratory health including asthma

2.3.3.1 Ambient air pollution associations with respiratory symptoms including asthma prevalence and incidence

The role of air pollution in the induction of asthma in children remains unclear, however recent studies have begun to somewhat show consistent but not conclusive associations. In the previous literature review section [chapter 2.2 (65)], the results of a systematic review and a meta-analysis done by Bowatte *et al.*, (66) on a pool of five birth cohort studies [PIAMA, Oslo, BAMSE, Vancouver and BCO (31,67–70)], showed a modest association between the incidence of asthma and longitudinal childhood exposure to NO₂ (OR 1.09; 95% CI 0.96 to 1.23 per 10 µg/m³ increase). However, the association was highly variable between the studies pooled together, with a heterogeneity of 75.5%. Nevertheless, there was an increasing association between asthma incidence and exposure to NO₂ in early childhood with increasing age until 6 years. Similarly, the heterogeneity ranges from 0% to 62.6% in the pool of studies across the years. There was however no pattern of association after 6 years between asthma and long-term exposure to NO₂.

Furthermore, with regard to the influence of PM on the incidence of asthma and wheeze, four birth cohort studies [PIAMA, BAMSE, Vancouver and BCO (31,68–70)] showed an increased incidence of asthma in children longitudinally exposed to PM_{2.5} (OR 1.14 95% CI 1.00 - 1.30 per 2µg/m³). Nonetheless, there is substantial heterogeneity across the studies ($I^2 = 77.1\%$). The age-specific meta-analysis showed an increasing trend of risk of asthma from ages 3 to 12 years, with childhood exposure to PM_{2.5}. Similarly, the heterogeneity across the studies for the age-specific meta-analysis ranged from 0% to 52.3%. Furthermore, when proximity to road was used as a proxy to determine the increased risk of developing asthma from long-term air pollution exposure, only 2 (GINI & LISA) of the 6 cohort studies [GINI & LISA, GINI plus & LISA plus, BCO, Oslo, CCCEH and CCAAPS (31,67,71–74)] reported a significant association at ages of 2 and 6 years. With regard to the development of wheeze from air pollution, using proximity to road as a proxy, only 2 [CCAAPS and CCCEH (72,73)] from the 4 cohort study reported a significant association at ages 1 and 5 years.

Findings from the Southern California Children's Health Study (SCCHS) for the cohort recruited between 1993 to 2004, reported a higher risk of new-onset asthma with an IQR of 6.2 ppb NO₂ (adjusted hazard ratio (HR): 1.29, 95%CI: 1.07 – 1.56) (1). Similarly, regional O₃ levels were associated with asthma incidence with the association modified by exercise in the same cohort. However, in the subsequent cohort recruited between 2002 and 2016, there was no association of regional NO₂ (adjusted HR: 1.37 95%CI: 0.69 – 2.71) and O₃ (adjusted HR: 1.01 95%CI: 0.49 – 2.11) levels with asthma incidence (1). Similar

inconsistencies were found in the reviewed primary articles with mixed results in the 5 articles (12,13,18,19,25) focusing on causal relationship between ambient air pollutants and incidence of asthma. For example, a nested case-control study which followed-up 43,343 preschool children in Japan for over a year, found no association between the incidence of asthma at baseline and follow-up with measured pollutants. However, there was an association of persistence of asthma symptoms with NO_x (OR: 6.02 95%CI: 1.51 – 23.92) when comparing the upper 5th and the lower 25th centiles of NO_x (13).

Although some of the findings showed an increased risk of asthma incidence, the size of the risk appears to be modest with borderline confidence intervals. These results add to the growing inconsistency in demonstrating the causal relationship between new-onset asthma and long-term exposure to ambient air pollution in children. It also shows contradictory evidence of the role of traffic-related pollutants in inducing new-onset of asthma in children.

With regard to the effect of ambient air pollutants on asthma prevalence, the previous literature review section [chapter 2.2 (65)] reported the risk of air pollution in only 11% of the 21 studies from a systematic review done by Anderson *et al.*, (3) in 5 or more communities. The 13 studies which showed positive associations were pooled together in a quantitative meta-analysis, with air pollution analysed as a continuous variable. Only one odds ratio and lifetime prevalence of asthma from any one of the studies were included in the analysis to minimise bias, selected *a priori*. No association was found between levels of air pollution at the community and asthma prevalence [NO₂ – OR; 1.00 (95% CI, 0.95-1.06); PM₁₀ - OR; 1.00 (95% CI, 0.94-1.07); O₃ - OR; 1.01 (95% CI, 0.96-1.07); SO₂ - OR; 1.03 (95% CI, 0.97-1.09). There was no evidence of publication bias in the estimates, as revealed by the funnel plots and statistical tests (3).

In another systematic review by Bowatte *et al.*, (66) on a pool of 10 studies (67,69,75–82) from 6 birth cohort studies (GINI & LISA, PIAMA, BAMSE, Oslo and COPSAC and INMA), only 2 of the cohort studies reported significant associations of an increased risk of wheeze following NO₂ exposures at ages 1 to 6 years (69,80). Similarly, in the same review, 8 studies (69,70,75–77,79,80,82) from 4 cohort studies (GINI & LISA, PIAMA, BAMSE and COPSAC) reported long-term exposure to PM and wheeze. The association was reported as prevalence in 5 of the studies, with only 1 cohort study (PIAMA) that showed a significant increase in the risk of wheeze related to exposure to PM in children at ages 1 to 8 years (69).

Findings from the Southern California Children's Health Study (SCCHS) for the cohort recruited between 1993 and 2004, reported a significant association of higher local NO₂ levels with higher asthma prevalence (adjusted OR; 1.83 (95% CI, 1.04-3.22) for an IQR increase of 5.7ppb in NO₂) (1). Significant effects of ambient air pollutant on asthma prevalence was also found in 11 of the primary articles reviewed in this section (5,6,8–10,15,16,22,23,29–31,33–35). For example, the only South African study found an increased

risk of cough at lag-0 for an IQR increase in PM₁₀ (OR: 1.07, 95% CI: 1.02 – 1.13), NO₂ (OR: 1.14, 95% CI: 1.06 – 1.22), NO (OR: 1.09, 95% CI: 1.02 – 1.16), and SO₂ (OR: 1.14, 95% CI: 1.06 – 1.22) (6). However, there were inconsistencies in the findings from the primary articles reviewed on the effect of higher prevalence of asthma from ambient air pollution exposure. There is evidence of no effect of various ambient air pollutants on the prevalence of asthma from 6 of the reviewed articles (10,20,21,24,28,32). For example; there was no cluster of asthma cases based on geo-referenced residences between 1004 exposed children from an industrial area and 920 children from a lower industrial area in a large Italian study (20). Similarly, there was no association between measured NO₂, SO₂, and O₃ with respiratory symptoms amongst 1800 school children in Eskisehir, Turkey. These null findings were similar to those reported in the previous review [chapter 2.2 (65)] from a pool of 5 European birth cohort studies [GINI and LISA (both Germany, divided into north and south areas), MAAS (England, UK), PIAMA (the Netherlands), and BAMSE (Sweden) (14)] which showed no association with increased prevalence of childhood asthma, with some suggested effects on only sub-population of age-groups (17).

The results of the meta-analyses (3,66) and primary articles reviewed appear contradictory as one would expect an increasing prevalence, if higher concentration of ambient air pollutants is associated with increased asthma incidence, because an important determinant of prevalence is incidence. Exposure misclassification in the between-community prevalence studies, which are mostly based on air-quality assessment at community-level, might have reduced the power to detect a real effect in these studies. Hence, pooling together the findings from the meta-analyses and the five large European birth-cohort studies, it is inconclusive to show an association of an increasing prevalence of childhood asthma with ambient air pollution exposure either in the short- or long-term.

2.3.3.2. Ambient air pollution associations with lung function

Another chronic effect of ambient air pollution exposure is the deficit in growth of lung function in children. The majority (9,12,36–39,41,42,45,46,49) of the 20 primarily reviewed articles focusing on the effect on lung function found consistent association of ambient air pollution exposure with reduced lung function in children, while seven showed mixed or null effect of air pollution on lung function deficit (10,16,24,40,43,44,46–48). For example; in the Child Heart and Health Study in England (CHASE) study, there was no association between all the pollutants except O₃ with either FEV₁ or FVC (44). Similarly, in a large birth cohort study of 1185 children followed from birth to age of 11 years, there was no association of lifetime exposure with specific airway resistance over time (46). Some studies found effect only in sub-groups. In the Canadian Health Measure Survey conducted between 2007 and 2011, null effects were reported in females following exposure to PM_{2.5} (40). Similar sub-group effect was reported in the previous review in chapter 2.2 (65). Some studies showed significant association with only traffic-related pollutants

such as NO₂, PM_{2.5} and PM₁₀ (44,45,48,83), and in subgroups such as younger children (45,46), non-asthmatics (47) and boys only (48). However, findings from the Southern California Children's Health Study (SCCHS) were reported in chapter 2.2. (65) to be consistent in demonstrating a significant association of deficit in lung function in children following ambient air pollution exposure (1).

Thus, put together, there is more consistent evidence of the role of ambient air pollution exposure, particularly traffic-related pollutants in reducing lung function in children, as compared to the inconclusive evidence about its role in new-onset asthma.

2.3.3.3. Ambient air pollution associations with airway inflammation

Airway inflammation has been documented as a plausible mechanism underlying the effects of ambient air pollutants on exacerbation of asthma (84). The use of FeNO has been developed and validated over the years as a non-invasive marker of airway inflammation (85). In chapter 2.2. (65), the effects of ambient air pollutants on airway inflammation assessed by FeNO measurements in the Southern California Children's Health Study was reported. However, mixed results were found in this updated literature review. While 4 of the 9 articles found convincing effects of air pollution on increased FeNO levels (37,50,55,56), others found significant effect for only short term air pollution exposure (51), within only total street length in small buffers such as 50m (53), null effects of TRAP (53,54) and null effects of long-term air pollution exposure (51).

Hence, there is a growing evidence of the association of short-term exposure to ambient air pollution with increased FeNO levels, which thus provides a subclinical marker of airway inflammation in children. However, future research is needed to elucidate the role of FeNO in asthma incidence following long-term exposure to ambient air pollution.

2.3.3.4. Exposure-response studies of pollen or fungal spores exposure on asthma

The effects of pollen and fungal spores on asthma prevalence and incidence were reported in chapter 2.2 (65) independent of the effect of air pollution at the population level. However, most of the articles (57–59,62–64) reviewed in this updated review were mainly done at the population level using time-series data. This study design limits the inference of the effects of pollen and fungal spores below the aggregated population level as the outcomes of interest were mainly obtained from records of hospital admissions for asthma and sales number of asthma medication. However, only three studies explored the effect of fungal spores at the individual level. Above an identified threshold of 1514 spores/m³, each doubling of *Cladosporium* at lag day-1 was significantly associated with a deficit of -0.23L (95% CI: -0.35 to -0.11) in FEV₁, and a deficit of -0.25L in FVC (95% CI: -0.37 to -0.13) independent of particulate air pollutant in a longitudinal study of 100 school children studied between 2007 and 2009 in New Taipei City in Taiwan

(60). The authors of this study argued the effect of *Cladosporium* on lung function to be more potent than PM_{2.5} due to the effect observed at a threshold in the thousands range (that is 1514 spores/m³). The same authors previously reported an unchanged effect of fungal spores in the presence of PM_{2.5} on FEV₁ and FVC in the same cohort of school children. An IQR increase of 1.3 log spores/m³ was associated with a deficit of -0.10L (95% CI: -0.17 to -0.02) in FEV₁, and a deficit of -0.12L in FVC (95% CI: -0.21 to -0.03) in the single pollutant model. This effect remained unchanged in the two-pollutant model with PM_{2.5} [FEV₁: -0.11L (95%CI: -0.19 to -0.03), and FVC: -0.14L (95%CI: -0.23 to -0.05)] (61). Although not included in the reviewed articles in this section due to the publication period criteria, the only individual-based study prior to the Taiwanese study was conducted in Pennsylvania, US during the summer of 1991 amongst 108 children. The American authors found a deficit of -1.0 l/m (95% CI: -1.9 l/min to -0.2l/min) in morning PEF per 10,000 spore/m³ in *Cladosporium* independent of air pollutant (86). *Cladosporium* has the potential of reaching and depositing at distal regions of the respiratory tracts due to its small particle size of aerodynamic diameter between 1.75 to 1.87 µm in the atmosphere. Thus, more epidemiological studies are needed in other regions of the world to explore the effects of fungal spores at the individual level on children's respiratory health especially lung function. It is also important to assess the combined effect of fungal spores with air pollutant, as air pollutants have been reported in the previous section in chapter 2.2 to increase the allergenicity of pollen in experimental studies (65).

2.3.4. Research gaps and needs

The in-depth evaluation of evidence from birth cohort studies with frequent follow-ups is one of the main strength of the systematic review done by Bowatte *et al.*, (66). Trends across different age strata were able to be demonstrated in the age-stratified meta-analysis. However, there was substantial heterogeneity across the studies reviewed, which may be due to variants definitions in outcomes (asthma, wheeze, bronchial hyperresponsiveness and allergic sensitization) and exposure (proximity to roads, monitoring stations, and Land-Use Regression and dispersion models).

Asthma outcome was assessed mainly by subjective indirect approaches using standardized questionnaires in 24 of the reviewed studies (with 8 of those studies combining questionnaire with other outcome measures). This had the potential to introduce a form of information bias when administering questionnaires due to possible recall and general awareness of the association between asthma and air pollution. Thirteen studies assessed asthma using daily count of ED visits or hospital admissions for asthma. This measure of outcome has the potential of introducing health seeking behaviour bias which thus limits generalization. Furthermore, in most of the reviewed articles, there was no data on daily counts of upper respiratory disease and respiratory infection visits as these are common illnesses amongst infants and children. The lack of

control for daily count of upper respiratory disease visits and respiratory infections might have confounded the estimation of asthma. Consequently, the use of simple standardised objective measurements using peak expiratory flow measurements, spirometry and/or exhaled nitric oxide (FENO) could have reduced such bias. Objective lung function measurement was only done in 16 of the reviewed articles, with FeNO being done in 9 studies exploring the effect of ambient air pollutants on airway inflammation (table 2.3.3).

Similarly, the lack of detailed and systematic exposure assessment of criteria pollutants at the individual level was evident across the studies. The extrapolation of group data for exposure levels at the area-level or from monitoring station to individuals might have introduced a form of ecological bias in the estimation of exposure as seen in over 30 of the independently reviewed studies. It is also difficult to estimate how well a population-weighted spatial average of measurements from different monitoring stations approximates the ambient concentration across the entire study areas. This measurement error limited the ability to explore dose-response relationships and time-activity patterns in a meaningful manner and also impacts the statistical power to detect effects. Perhaps the use of more novel exposure measures such as LUR, GIS-based indicator of pollution, micro-environmental exposure model, and dispersion model might reduce the exposure measurement error by accounting for spatial and temporal variations. However, only 3 of the reviewed studies employed the use of LUR (12,14,31); 5 studies used GIS estimates (15,27,29,30,53); 4 studies used dispersion model (13,33,44,53); and only 2 studies used micro-environmental exposure model (17,46).

Most of the studies reviewed independently also had methodological limitations constraining their ability to demonstrate the strength of association between either ambient air pollution, airborne pollen or fungal spores with childhood asthma (especially long-term association). Three of the studies reviewed used a case-crossover study design which limited inference to only short-term effects of air pollution (13,18,31). Similarly, it was difficult to infer long-term associations, as the 5 panel studies reviewed (6,10,15,26,47,50) were limited to assess acute exposure effects. Most of the studies reviewed independently used only a cross-sectional study design that limited the inference of a temporal relationship between air pollution on the development or presence of childhood asthma. This is important since it is possible that families with symptomatic children at an early age may move to homes in lower exposed areas. Perhaps the use of longitudinal study design including birth-cohort studies with long follow-up would quantitatively assess the level of exposure and other covariates with the initial onset or progression of asthma. However, only 5 studies reviewed used a longitudinal study design with the lowest follow-up of 6-months (49), and longest follow-up of four years (41). Six of the reviewed articles was a birth cohort study which helped to assess the effect across different age-groups (12,14,17,25,46,51).

Furthermore, evidence of selection bias was previously reported in chapter 2.1 (87) in 3 of the 4 South African studies (88–90). The lack of detailed information in the inclusion and exclusion of study participants, together with a lack of random sampling of children irrespective of known asthma-status across all socio-economic groups, limited the internal and external validity of the results. In addition, in this updated literature review, some studies dealt inadequately with confounding factors that were not reported-on such as socio-economic factors, smoking status, pollen or fungal exposure, upper respiratory tract diseases, respiratory infections, atopy, and indoor air pollutants (domestic fuel combustion). The heterogeneity of the study findings from the systematic reviews and meta-analyses (3,66) might also be related to unmeasured confounders. Environmental factors such as fungal spores, pollen and meteorological conditions might drive asthma and allergies through their complex interactions, thus modifying or confounding the effects of ambient air pollutants exposures. Similarly, socio-economic factors (including malnutrition and crime) which often lead to living in more polluted areas may also increase allergic diseases and asthma risk and thus confound the effect of ambient air pollutants. These factors may have skewed the risk estimation since they are also known to be associated with childhood asthma (91–93). Had the data been collected for these confounders, this could have been appropriately controlled-for during statistical analysis.

2.3.5. Conclusion

Overall, there is evidence suggestive of the negative effects of ambient air pollutants on children respiratory health, however mixed results were presented in this review on the effects of ambient air pollution on children's lung function, airway inflammation, prevalence and incidence of asthma. This review also identifies the lack of evidence at the individual level with regard to the role of biological pollutants such as pollen and fungal spores on the respiratory health of children. Climate change is hypothesized to affect the distribution of pollen by altering the start-date and length of pollen season, thus increasing the exposure periods to concentrations of airborne pollen and fungal spores. There is also a need for future studies to explore the lag effect including the combined effects of ambient air pollutants with biological pollutants on children's respiratory health, as increased allergenicity of pollen has been hypothesized in the presence of chemical pollutants in experimental studies, especially in developing countries with very few studies and the greatest burden of disease due to air pollution.

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CHAPTER 3

METHODS

3.1 A prospective cohort study on ambient air pollution and respiratory morbidities including childhood asthma in adolescents from Western Cape Province: Study Protocol

Paper overview

This paper documents the methodological procedures employed in this thesis

Contribution to the thesis

The article gives the detailed description of the study population and design, the various exposure assessments considered, and the outcome measures explored, including their definitions.

Role of candidate

The candidate participated in all aspect of the study, drafted the manuscript, incorporated input from supervisors and collaborators who reviewed the manuscript, and the candidate submitted the final manuscript for publication.

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STUDY PROTOCOL

Open Access



A prospective cohort study on ambient air pollution and respiratory morbidities including childhood asthma in adolescents from the western Cape Province: study protocol

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Abstract

Background: There is evidence from existing literature that ambient air pollutant exposure in early childhood likely plays an important role in asthma exacerbation and other respiratory symptoms, with greater effect among asthmatic children. However, there is inconclusive evidence on the role of ambient air pollutant exposures in relation to increasing asthma prevalence as well as asthma induction in children. At the population level, little is known about the potential synergistic effects between pollen allergens and air pollutants since this type of association poses challenges in uncontrolled real life settings. In particular, data from sub-Saharan Africa is scarce and virtually absent among populations residing in informal residential settlements.

Methods/design: A prospective cohort study of 600 school children residing in four informal settlement areas with varying potential ambient air pollutant exposure levels in the Western Cape in South Africa is carried-out. The study has two follow-up periods of at least six-months apart including an embedded panel study in summer and winter. The exposure assessment component models temporal and spatial variability of air quality in the four study areas over the study duration using land-use regression modelling (LUR). Additionally, daily pollen levels (mould spores, tree, grass and weed pollen) in the study areas are recorded. In the panel study asthma symptoms and serial peak flow measurements is recorded three times daily to determine short-term serial airway changes in relation to varying ambient air quality and pollen over 10-days during winter and summer. The health outcome component of the cohort study include; the presence of asthma using a standardised ISAAC questionnaire, spirometry, fractional exhaled nitric-oxide (FeNO) and the presence of atopy (Phadiatop).

(Continued on next page)

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Discussion: This research applies state of the art exposure assessment approaches to characterize the effects of ambient air pollutants on childhood respiratory health, with a specific focus on asthma and markers of airway inflammation (FeNO) in South African informal settlement areas by considering also pollen counts and meteorological factors. The study will generate crucial data on air pollution and asthma in low income settings in sub-Saharan Africa that is lacking in the international literature.

Keywords: Ambient air pollution, Environmental pollution, Outdoor air pollution, Traffic-related pollutants, Pollens, Childhood asthma, Respiratory morbidities, Allergic diseases, Lung function, Airway inflammation,

Background

Childhood asthma is the most common chronic disease in children globally and ranks in the top 20 contributors of global disability-adjusted life years (DALY) in all children; and among the top 10 causes of DALY amongst 5–14 year olds [1]. The global mortality rates of childhood asthma is approximately 0.7 per 100,000 [2]. Asthma impacts significantly on the quality of life of a child as it can interfere with physical exercise, results in school absenteeism as well as underperformance at school due to sleep interruption. It is estimated that two-thirds of asthmatic children suffer from a noticeable disability and approximately ten million school days are missed yearly [3]. Instances of severe asthma in children leading to frequent school absences might consequently affect the child's education and probably career choice later in life. Furthermore, asthma in children creates a burden on the family since caregivers often take time 'off-work' to cater for sick children, hence a major cause of parental work absenteeism. Limitation of social life due to lack of sleep has been found in 50% of parents with asthmatic children and negligence of other siblings as more time is devoted to the asthmatic child [4].

Among other reasons, the increasing outdoor air pollution may also contribute to the increase in asthma prevalence in developing countries [5]. Ambient air pollution is a major environmental health concern globally affecting the population in both highly industrialised and developing countries. An estimated 23% of all deaths and 24% of the global burden of disease can be attributed to environmental factors, with ambient air pollution (especially particulate matter) estimated to be responsible for 3.2 million deaths per year (3.1% of global total DALYs) [5, 6]. In 2016, the WHO estimated that only about one in every ten people breathe clean air, according to the WHO Air Quality Guidelines [7]. The WHO further reported 3.7 million premature deaths in both urban and rural areas to be attributed to air pollution, which is mainly due to small particulate matter of 10 μm or less in diameter (PM_{10}). Low-and middle-income countries (LMIC), where air pollution emissions from power-plants, traffic, open waste burning and other combustion sources are very common,

account for about 88% of these deaths [7]. The WHO estimated that a 15% reduction in ambient air pollution-related deaths can be expected from reducing ambient particulate matter from 70 to 20 $\mu\text{g}/\text{m}^3$ [7]. Respiratory diseases are the leading cause of death from ambient air pollution, responsible for over half of the deaths reported [7]. Of special concern is the effect of ambient air pollution on children as their immune systems and lungs are not fully developed with the onset of exposures in early-life [8, 9]. Furthermore, children tend to spend more time outdoors in parks and play grounds, and likely to breathe a greater amount of air pollutant per body weight compared to adults similarly exposed. Recent studies have suggested that the development of asthma, atopy and infant mortality is associated with air pollution, particularly traffic-related pollution [10]. The evidence for the extent to which air pollution affects children's respiratory health is inconclusive suggesting much need for further investigation.

Various studies have demonstrated that exposure to ambient air pollutants in early childhood plays an important role in the exacerbation of asthma and other respiratory symptoms with greater effect amongst asthmatic children [11–16]. Furthermore, studies have demonstrated the association between ambient air pollution and lung function deficits and more recently on increased FeNO in children [17–24]. It has been postulated that continuous exposure to air pollutants, particularly outdoors where children spend most of their time, may exacerbate the impact of early childhood exposures that lead to asthma and other respiratory symptoms expression. However, it should be noted that the bulk of these evidence comes from regions outside sub-Saharan Africa. Furthermore, there is inconclusive evidence on the role of ambient air pollutant exposures in relation with increasing asthma prevalence as well as asthma induction in children [25, 26].

Although environmental and lifestyle factors appear to drive the increasing susceptibility to developing allergic diseases such as asthma, the increased susceptibility in response to pollen allergen in population exposed to high levels of pollution remain elusive. While the pathogenesis of asthma and allergies have demonstrated a link

between combined exposure to air pollutants and pollens in in-vitro and animal studies, little is known about their synergistic effect at the population level [27]. A growing body of evidence has demonstrated a link between enhanced risk of atopic sensitization and exacerbation of symptoms due to the interaction between air pollutants and airborne allergens [27–29]. Nevertheless, more research is needed to clarify the mechanism by which air pollutants and pollens induce airway changes in exposed individuals. Some recent evidence have demonstrated that pollen allergen can also induce non-atopic reactions such as irritation and inflammation in addition to allergic responses such as immunoglobulin production [27]. However, very few epidemiological studies have taken into account separating allergic and non-allergic asthma and underlying mechanisms due to the common inflammatory component present in both mechanisms. To better evaluate the relative contribution of the role air pollution in allergic and non-allergic asthma, more research is needed since air pollution is considered to be a major risk factor for non-allergic respiratory diseases.

Most studies of the association between air pollution and childhood asthma have been performed in industrialised settings. Given the multifactorial nature of these diseases, the need for research is particularly eminent in LMIC where patterns of co-exposure, co-morbidities and susceptibility may largely differ from those in Western countries. The WHO also recommended in the 2016 report on the effect of ambient air pollution [7] that more epidemiological studies are needed to estimate the long-term health effect of air pollution especially in LMIC plagued with unacceptable levels of air pollution. Further studies in developing countries are needed so as to render comparison between South African and other developing countries to findings reported from other parts of the world, thereby contributing to the evidence on the associations between air pollution and asthma in children.

Methods/design

Aim & objectives

The aim of this prospective cohort study is to investigate the association between ambient air pollution and co-exposure to pollen with respiratory morbidities focusing on asthma among children in the Western Cape, South Africa.

The objectives are:

1. To determine the prevalence of asthma (defined by asthma symptom score, presence of airway obstruction on spirometry, and elevated FeNO) in primary school children residing mainly in informal settlement areas of the Western Cape with diverse exposure to air pollutants and airborne pollen.
2. To characterize baseline exposures, temporal trends and spatial distribution of ambient air pollutants and relevant airborne pollen and their association with meteorological parameters on their dispersion.
3. To examine the effect of air pollutants and pollen levels on asthma outcomes controlling for potential confounders and exploring the potential for effect modification:
 - a) To examine the effect of daily variations in ambient air pollutants and airborne pollen levels across seasons on asthma outcomes (defined by respiratory symptoms and changes in serial peak flow and FEV₁ measurements) in a panel study of participating children in the Western Cape.
 - b) To examine the exposure-outcome relationships (at baseline and at the 12 month follow-up assessment) between exposure to ambient air pollutants and airborne pollen with asthma outcomes (defined by asthma symptom score, presence of airway obstruction on spirometry, and elevated FeNO).
 - c) To explore longitudinal 12 month change in ambient air pollutants and airborne pollen on changes in asthma symptom score, lung function (FEV₁ and FEV₁/FVC ratio), and FeNO in this cohort.
4. To examine whether increased baseline FeNO predicts new onset asthma (defined by increasing asthma symptom score or presence of new asthma symptoms).

Study population and design

The study areas includes three areas identified in a needs analysis conducted by the Western Cape Department of Environmental Affairs and Development Planning (DEA&DP) in 2013. The prioritised areas include an urban industrialised area in Cape Town (Milnerton/Milnerton Ridge including Marconi-Beam, Phoenix and Joe Slovo), a peri-urban area outside Cape Town with a large informal sector (Khayelitsha) and a rural area (Oudtshoorn) more than 400 km outside Cape Town. Additionally, an area (Masiphumulele, Noordhoek) with negligible ambient air pollutants exposure and community of similar socio-economic status as the prioritised areas is identified. These four study areas are selected to maximize contrasts in exposure levels to the different ambient air pollutants.

The study comprises of a baseline study and a 12-months follow-up study of the cohort in which all examinations including a child and caregiver questionnaire, lung function testing in the form of spirometry, exhaled nitric-oxide and allergy testing will be conducted. Additionally, a panel study is embedded investigating seasonal relationships between short-term variation in ambient air pollution and lung function measured through daily peak flow measurements in children during a 2-week period in summer and winter. This ensures a design whereby the exposures, potential confounding factors and health outcomes are monitored seasonally and repeatedly in a prospective manner.

Sampling

In total 600 participants are selected from children attending primary schools located in the three prioritised and one less-exposed areas. A list of all schools in the study area is obtained from the Department of Education in the Western Cape Province. One or two schools from each area located nearest to the City of Cape Town metro air quality monitoring stations are selected. One hundred and fifty Grade-4 students in each study area are targeted. Grade-4 students are selected for the study since they will not leave primary school to enter senior school during the study period. The average age of Grade-4 students is 10 years; as such they are old enough to adhere to instructions during the various tests and reasonably confident to answer simple questions regarding their symptoms. This age group also provides a key phase in the onset of childhood asthma which often is preceded by atopic dermatitis and allergic rhinitis (with reference to the 'atopic march'). Although, there are gender differences in asthma reported in the literature, all eligible Grade-4 students are included in the study and gender will be adjusted-for, stratified and assessed for interaction in the analyses.

After meeting the school principal, obtaining permission from the schools board, and obtaining the Grade-4 class lists and addresses, the houses of the school children are visited by trained field staff to obtain the caregivers (parent or guardian) consent. Random sampling is used to select 75 students from the list of consented children when the number of consented children exceeds 75 in each school.

All Grade-4 students attending selected schools qualify for inclusion in the study. Baseline data is collected between February 2015 and August 2015, while the follow-up data is obtained from May 2016 to September 2016. However, spirometry is rescheduled for participants with a positive response obtained from a pre-test questionnaire to any of the following items: If the child had a recent operation (in last 12 months); If the child feels like vomiting or has any pain; If the child is being treated for

Tuberculosis; If the child had flu, sinusitis or lung infection in the last 3 weeks. However, children with positive response to 'having epilepsy' are excluded from doing spirometry.

Questionnaire

Trained interviewers administered questionnaires to child participants and their caregivers in their spoken language in the cohort study. The use of mobile technology is implemented in the administration and capture of questionnaires. For interviews of child participants, the questionnaire contains items on demographic factors and respiratory health incorporating the standardized and validated International Study of Asthma and Allergies in Childhood (ISAAC) questionnaire [30]. For interview of caregiver, the questionnaire contains items on:

- i) Demographic factors (age, gender, education) of child
- ii) Birth history (Mother's age at child's birth, birthweight, maternal smoking during pregnancy)
- iii) Diet (child's nutritional habit)
- iv) Health impairment (presence of other illness and vaccination profile)
- v) Respiratory conditions and allergy (presence of respiratory symptoms such as cough, wheeze, shortness of breath and chest tightness, including other allergic conditions such as eczema, itch/rash, food allergy, pet allergy)
- vi) School attendance (school absenteeism to assess the impact of respiratory illnesses such as asthma)
- vii) Asthma severity (doctor-diagnosed asthma and other allergic diseases, presence of current wheeze)
- viii) Health services utilization (regular hospital visits)
- ix) Asthma medication
- x) Exposure to indoor pollution and allergens (such as heating and cooking fuels, dust mites, cockroaches, rats, molds and pets)
- xi) Household characteristics and hazards
- xii) Respiratory health of the child based on the ISAAC questionnaire (assessing the presence of rhinoconjunctivitis) [30]
- xiii) Child residential history to assess the duration of exposure in the sampling study area

The questionnaires are translated and back-translated. The questionnaires is administered at all phases of the study.

Pulmonary function assessment

Pulmonary function assessments for the cohort study are conducted during school visits, comprising spirometry conducted according to the American Thoracic Society (ATS) guidelines [31] and fractional-exhaled nitric

oxide measurement (FeNO). Spirometry and FeNO is conducted at baseline and in the follow-up period. Additionally, two intensive phases (once during peak winter and another during peak summer) of serial peak flow measurements are conducted bihourly per day during the school time over a two week period for all school child participants (panel study). During this period, school children are asked to complete a bihourly symptom activity log recording symptoms, odour, location and activity. The serial peak-flow measurement is conducted simultaneously at all schools.

Spirometry

An experienced technologist conducts spirometry using a flow-volume spirometer (EasyOne 2001–3 Spirometer). Spirometers are calibrated at least twice a day using a three-litre syringe. The temperature and humidity are monitored on a daily basis. The technologist is blinded to the exposure status of participants. Spirometry is performed in a sitting position with nose clips. Each child participant performs a maximum of up to eight trials to produce three acceptable curves. Test reproducibility is used as a guide to whether further attempts will be necessary. Reproducibility criteria is based on the two best tracings for both FEV₁ and FVC varying by no more than 150 ml or 5%, whichever is greater. However, failure to meet reproducibility criteria does not result in exclusion of the spirogram results from the statistical analysis. Poor reproducibility may also be an independent marker of airway dysfunction [32]. The lung function indices of primary interest include forced vital capacity (FVC) and forced expiratory volume in one-second (FEV₁). The best FEV₁ and FVC is used regardless of whether they belong to the same tracing. Lung volumes obtained by spirometry are adjusted for body temperature and pressure according to the temperature and atmospheric pressure measured on a continuous basis throughout the day. Special instructions are given to participants to ensure that tested individuals do not take any anti-asthmatic inhalers (12 h before) or oral asthma medications (48 h before) prior to the test. Participants are administered an inhaled bronchodilator and have spirometry repeated after a minimum of 10-min waiting period. Special precautionary measures include having readily available oxygen and B₂-adrenergic agents for nebulization. Additionally, emergency medical personnel are on-site or within quick access at all times during the tests. All participating children are assessed during school hours.

Fractional exhaled nitric oxide (FeNO)

Fractional-exhaled nitric oxide (FeNO) measurement is a recognized non-invasive method for assessing allergic airway inflammation [33]. The Fractional-exhaled nitric

oxide (FeNO) testing is conducted by a trained nurse. A hand-held portable nitric oxide sampling device (NIOX MINO® Airway Inflammation Monitor; Aerocrine AB, Solna, Sweden) is used. The child is advised to sit comfortably and breathe normally for about five minutes to acclimatize, and thereafter instructed to inhale close to total lung capacity (TLC). This is followed by an immediate exhalation at a constant flow rate of 50 ml/s for at least four seconds. Two technically adequate measurements are performed (at least 30-s interval) in line with the American Thoracic Society/European Respiratory Society recommendations [34]. The average value of the two measurements is used in the analysis. Special instructions are provided to participants to ensure that tested individual avoids strenuous exercise, eat nor drink (at least an hour) prior to the test. The passive smoking history and date of last medication of bronchodilator is recorded. It is important to note that spirometric manoeuvres may reduce exhaled NO levels; hence FeNO measurements are done before spirometry. Furthermore, children with upper and lower respiratory tract infection have their measurements deferred until recovery or had their measurement taken with infection state recorded. The participants' height and weight is measured and this information is used to compute the body mass index (BMI). Ambient NO and temperature are also recorded.

Panel study: Collection of bihourly symptoms log and peak flow data

In the panel study, each child is asked to perform serial peak flow measurements bihourly (just before school starts, during the 1st break and 2 h thereafter) and complete an activity symptoms log in between serial peak flow measurements on each school day over each of the 2-week sampling phases (summer and winter phases). The activities recorded in the symptoms log include symptoms, odour detection and information about location during the previous 2 h. Three consecutive manoeuvres of peak expiratory flows are performed by each child immediately prior to completion of the bihourly logs using an individual Microlife Peak flow meter (Microlife PF100). Each child is trained to use the device before fieldwork starts and reminded daily during both phases by trained research assistants. The Microlife PF100 meets the ATS standards, and it is easy to use on children due to its user-friendly interface. The device measures both Peak Expiratory Flow (PEF) and FEV₁. This procedure is largely dependent on the child's cooperation, efforts and ability to inhale maximally. To obtain a maximum value for the PEF with a rapid upstroke, the children are encouraged to blow vigorously as they physically can. To avoid measurement errors, the children are advised to blow out quickly, and avoid flexing the neck, as this might lead to the PEF dropping by

10% due to visco-elastic properties of the trachea [35]. Testing are conducted simultaneously at all schools according to the American Thoracic Society/European Respiratory Society (ATS/ERS) guidelines. Trained research assistants are present at each school at all times to oversee the measurements.

Assessment of allergic status

Allergy tests: Phadiatop levels

Samples of blood (9 ml) are drawn from each participant using a Becton Dickinson Vacutainer SST tube (with gel medium and clot activator) by the nurse. Each sample is labelled and stored in a cooler box at 4-degrees Celsius before being transported to the laboratory for spinning down. The blood is allowed to clot for 1–2 h at room temperature (20–24 degrees Celsius) and then centrifuged at 1350 rpm for 10-min at room temperature. The serum is then transferred to another tube and stored at –20-degrees Celsius in a freezer. These serum samples are then transferred to the main freezers where they are stored at –80-degrees Celsius. The samples are then couriered to the SANAS accredited (ISO 15189:2007), National Institute for Occupational Health (NIOH) Immunology laboratory for immunological analysis. Presence of sensitization to common aeroallergens (house dust mites, grass pollens, cat, dog, and cockroach) are determined by the Phadiatop® test (Phadia AB, Uppsala, Sweden). The analysis is done blinded to further details of the participant. The Phadiatop test is conducted in the baseline study as well as in the follow-up period. A positive result would be indicative of the presence of atopy.

Exposure characterisation

Ambient air pollutants

Exposure to ambient air pollution is estimated from a) geographic information system-based indicators b) air monitoring data from the stationary monitors in each of the study areas, c) available dispersion and chemical modelling, d) fine-scale land-use regression modelling based on two air pollution measurement campaigns in winter and summer in each study area at a total of 130 homes of study participants.

a) *Geographic information system (GIS)-based measures:*

GIS-based indicators are extracted as an exposure proxy for point and mobile sources of air pollution such as traffic-related pollutant (NO₂ and PM_{2.5}). The distance of each participants' address to point sources, local and major roads, highways is computed using ArcGIS software. In addition, several buffers (50 m, 150 m and 200 m) are created around each participant's address, and the total roadway length is calculated using ArcGIS. It should be noted that roadway length within each buffer can

also be weighted-by the average traffic frequency and vehicular types plying each roadway (depending on data availability). Additionally, other GIS data may also be used such as land use/classification, street network, building and population density which would also be calculated for different buffer sizes.

- b) **Fixed-site air monitoring data:** Ambient air sulphur dioxide (SO₂), nitrogen dioxide (NO₂), Particulate Matter of 2.5 μm in diameter (PM_{2.5}), Particulate matter of 10 μm in diameter (PM₁₀), and ozone (O₃) measurements from the City of Cape Town's and DEA&DP stationary air quality monitoring stations in each study area are obtained throughout the study period. The sampled air quality data are stored in the City of Cape Town's Data Acquisition System (DAS). The storing, pre-validation and transmission of data on the Air Quality Monitoring Network is the responsibility of the Western Cape Department of Environmental Affairs and Development Planning. Most pollutants are measured at seconds-to-minutes resolution using the US Environmental Protection Agency (EPA) approved methods and the values are expressed as one-hour averages.
- c) **Dispersion and chemical modelling:** Dispersion and chemical modelling is used to estimate air pollution levels over specific time periods across the different study areas. Input data for these models are emissions from major sources such as industry, household or domestic fuel-use obtained from household surveys, traffic estimated from the number and types of vehicles in the areas, biomass fuel burning and meteorological variables (such as wind speed, wind direction, temperature, humidity, and precipitation). High resolution distribution of primary pollutants concentrations is provided by the dispersion modelling, while concentrations of secondary pollutants such as ozone and particulates are derived from the chemical modelling. CALPUFF system is used to create the dispersion modelling at a resolution of 300 m over the study areas [36]. This system captures complex terrain and diurnal wind regimes such as land-sea breezes more effectively. The model is based on the principle that meteorological factors influences the direction and dispersion of pollutants from their point sources, and thus residential exposure depends on their location upwind versus downwind of the point sources. On the other hand, chemical modelling is used to model pollutant transformation in addition to secondary formation of aerosols which may impact on the concentrations of PM_{2.5} in the Province. A comprehensive Air Quality Model with Extensions (CAMx) is used to model

chemical pollutants due to its suitability for the integrated assessment of particulate and gaseous air pollution over many temporal scales. The data from the model is used to estimate the average exposures at each participant's residence in the short-, medium-, and long-term through the study period (daily, weekly, monthly and annually). These model output are however highly dependent on the quality of their input emission data.

- d) **Land-use regression (LUR) modelling:** To refine the spatial resolution of exposures at the individual level, Land Use Regression (LUR) models are used to estimate the spatial distribution of air pollutant concentrations across each study area and for all relevant locations. This is based on ambient air pollutant measurements in all four study areas using passive diffusion samplers to measure NO₂, SO₂ and O₃ and active pump-based 37 mm filter samples (PM_{2.5}, PM_{2.5} absorbance). In addition, real-time continuous air pollutant measurements (NO₂, O₃, CO, PM₁₀, PM_{2.5}, and PM_{1.0}) is recorded at the schools and reference site per study area. In three study areas, 40 houses per study area including participating schools and a background reference site are selected to represent the full range of exposure levels within the study area. However, for the Noordhoek study area, a total of 20 houses is selected due to its small geographical size. Measurements in each selected house are conducted for a week, over a 4-week sampling period per area, while measurements at schools and the reference site are done weekly throughout the entire 4-week sampling period. GIS-based model predictors are derived from ArcGIS software described above. Temporal adjustment within each and across both seasonal measurement campaigns (summer and winter) are done by using data from schools, reference sites and from the City of Cape Town's and DEA&DP air quality monitoring stations including meteorological data from the South African Weather Service (SAWS). Additionally, questionnaire responses on ambient and indoor air pollution (such as heating and cooking fuels), including dust mites, pets, moulds and use of pesticides at home are considered in the exposure assessment.

Pollen measurements

Tree pollen, grass pollen, weed pollen and mould spores are measured in all the four study areas from July 2015 to March 2017, using the Burkard 7-day recording volumetric Spore Trap which allows for continuous spore trapping. There are varying concentrations of pollen and spore at heights above ground level, thus the trap is placed above the ground level (ideally a flat rooftop) at a

minimum height of 1.5 m to avoid dust contamination. Pollen and fungal spores are measured at each study area for one-calendar year in order to properly assess the seasonal peaks of the local aeroallergens. The average daily concentration is expressed as particles/m³ using the concentration conversion below;

$$\frac{N \times \text{total area of strip (48mm} \times \text{14mm)}}{\text{Area of stip analysed (field diameter} \times \text{length of traverse} \times \text{no of traverses} \times \text{14.4)}}$$

Where N = the pollen or fungal spore concentration per m⁻³.

14.4 = volume of air sampled per 24 h period, at 10⁻¹ l/min.

Field diameter = width of microscope field.

Meteorological factors

Meteorological factors such as rainfall, sunshine, temperature, barometric pressure, humidity, wind speed and wind direction are obtained (minimum, maximum and daily averages) from the South African Weather Service (SAWS). These factors are considered in the LUR models as well as in the statistical analyses of the associations between ambient air pollutants and respiratory health outcomes in children. These factors have been found to influence the spatial and temporal distribution, deposition and formation (in the case of secondary pollutant such as ozone), of pollutants including the altered spatial and temporal distribution of allergens (pollens, moulds and house dust mite) [27, 37]. Meteorological factors have also been shown to influence the exacerbation of allergic respiratory conditions including asthma especially in vulnerable groups such as children [27].

Statistical analysis

Sample size calculation

The sample size required for the study was calculated using the following formula below;

$$\frac{N \approx 4P(1-P)(Z_{\alpha/2} + Z_{\beta})}{(RD)^2}$$

Where N = sample size, Z_{α/2} is the alpha risk, and Z_β is the power

$$\frac{P = P_0 + P_1}{2}$$

P₀, the prevalence of asthma is urban areas of Cape Town estimated at 34.4% [38].

P₁, the prevalence of asthma amongst exposed school children, 48.8% found in a study conducted in Durban, South Africa [39].

Therefore $\frac{P=0.344+0.488=0.416}{2}$

RD is the risk difference P₁ – P₀ = 0.488–0.344 = 0.144.

Using an alpha risk of 0.05 and a power of 80% ($\beta = 0.20$).

$$\text{Thus, } \frac{N \approx 4 \cdot (0.416) \cdot (1 - 0.416) \cdot (1.96 + 0.842)}{(0.144)^2} = 368$$

The approximate sample size calculated for the study is therefore 368.

Outcome variables

Doctor-diagnosed asthma

A positive response by the parent or guardian to the question in the questionnaire, 'if the child participant was previously diagnosed by a doctor to have asthma' and treated as a dichotomous variable (Yes, No).

Asthma symptoms scores

The operational definition of asthma based on asthma symptoms will be a sum of positive answers to eight main asthma symptoms and bronchial hyper-responsiveness questions from the Child Caregiver question, which generates a continuous asthma score ranging from 0 to 8 [40]. Continuous asthma scores will be produced from the following questions below;

1. How many episodes of wheezing or whistling has the child had in the past 12 months?
2. In the past 12 months, how many times has the child had wheezing that made it hard for him or her to breathe or catch his or her breath?
3. In the past 12 months, how many times has the child complained that his or her chest felt tight or heavy?
4. Does the child get short of breath walking with other children of his/her own age on level ground?
5. In the past 12 months, how many times has the child coughed with exercise or running or playing hard?
6. In the past 12 months, how many times has the child's sleep been disturbed due to wheezing, coughing, chest tightness or shortness of breath?
7. Has a doctor or nurse ever told you that the child has asthma?
8. Has the child ever taken any medication for asthma?

Airway obstruction

Airway obstruction will be defined as a reduced FEV₁ (less than 80% of the predicted value- using the multi-ethnic reference values for spirometry for the 3–95 year age range [41]) and a reduced FEV₁/FVC ratio (less than 0.8) recorded as a dichotomous variable (Yes, No).

Airway inflammation

Airway inflammation will be assessed using the fractional exhaled nitric oxide (FeNO) levels, which is a marker of eosinophilic airway inflammation. Elevated levels of FeNO have been implicated in the detection of

subclinical airway inflammation, even in the absence of lung function impairment and absence of asthma symptoms [42]. Children with FeNO levels less than 15 ppb will be considered normal; those with levels between 15 and 35 ppb will be categorized as 'elevated', while children with FeNO levels above 35 ppb will be categorized as having 'high' levels [42].

Atopy

Atopy will be defined as individuals with serum Immunoglobulins E (IgE) antibodies above 0.35kUA/l on the Phadiatop test. Although this measurement does not provide information on the specific allergens the individual is sensitized-to, it does give an indication of the presence of an allergic sensitization to common inhalant allergens. Serum IgE levels are also linked to airway hyper-responsiveness in the presence or absence of asthma symptoms [38].

Variability in PEF and FEV₁

Increased intraday variability and lower nadir values (minimum best or daily lowest valid values) are markers of worsening of asthma. Thus, the average within-day variability in PEF and FEV₁ over the 10-days in each sampling season (summer and winter) will be calculated for each participant including the average minimum best values of PEF and FEV₁ over the 10-days.

Rapid decliners in FEV₁

This will be used as another marker of airway obstruction and defined as children whose FEV₁ reduces by 30 ml/year (the upper limit of physiological decline) and/or children with deficit in lung function growth. The deviation from the normal lung function growth curve will also be explored using the multi-ethnic reference values for spirometry for the 3–95 year age range [41].

New-onset asthma

A new asthma case will be defined as a child with no prior parental report of a physician diagnosis of asthma in the baseline survey whose parent or guardian reports a doctor diagnosis of asthma in the follow-up year. Additionally, participants defined as rapid decliners in FEV₁ on follow-up and/or participants with appreciable changes in the asthma symptom score will also be classified as new asthma cases.

Statistical analysis protocol

Analysis protocol for objectives 1

Descriptive analyses is used to examine the characteristics of the study population, to characterize the asthma symptom score (ASS), the distribution of the various pulmonary function tests (PFT), and FeNO measurements during year-1. The ASS is based on the sum of

positive answers to eight main asthma symptoms and bronchial hyper-responsiveness questions, while airway obstruction and airway inflammation is based on definitions previously described. Univariate linear, logistic, Poisson or ordered logistic regression analyses will be used to examine the association between variables, including demographic characteristics, personal characteristics, and household characteristics with respiratory outcomes including allergic status, ASS, spirometric indices and FeNO.

Analysis protocol for objectives 2

The mean annual levels and seasonal variations, annual and seasonal exceedances of South African Ambient Air Quality Standards and annual maximum levels in NO₂, SO₂, PM, and O₃ in each area will be determined from continuous data from ambient air quality monitoring stations during the study period and also dispersion modelling data. Annual and seasonal averages of NO₂, SO₂ and PM, will be determined from average 24-h levels, while for O₃, an eight-hour daytime average (from 10 a.m. to 6 p.m.) and of the one-hour maximal levels will be computed. Missing data (e.g. from technical failures) in ambient air quality monitoring data will be imputed based on regression models using measurement data from other close monitoring sites and meteorological variables. Furthermore, spatial variation in pollutants in each area will be determined using LUR as described above. The LUR models constructed for the time period of the measurement campaigns will be extrapolated to other time windows including long-term averages by using data measurements from the stationary air quality monitoring stations within each area as well as meteorological data.

The total airborne pollen measured in all four study areas will be described as a continuous variable and later categorize as follows; Tree pollen in count/m³ ranging from 0 to 15 'low', 16–90 'moderate', 91–1500 'high' and above 1500 'very high'; Grass pollen in count/m³ ranging from 0 to 5 'low', 6–20 'moderate', 21–200 'high' and above 200 'very high'; Weed pollen in count/m³ ranging from 0 to 10 'low', 10–50 'moderate', 51–500 'high' and above 500 'very high'; and mould spores in count/m³ ranging from 0 to 900 'low', 900–2500 'moderate', 2500–25,000 'high' and above 25,000 'very high'. Time-series analysis will be used to investigate the seasonality of each airborne pollen count while their correlation between meteorological variables such as temperature, relative humidity, rainfall and wind speed and direction two to 3 days before measured levels will also be explored.

Analysis protocol for objectives 3

i) Protocol for objectives 3a.

Exposures: In the panel study, the hourly and daily averages of each pollutants (24-h averages for PM, SO₂ and NO₂, and 8-h average for O₃) and airborne pollens will be determined for the same day as well as for a lag period of 7-days.

Outcomes: Daily nadir (minimum best) and intraday variability of peak expiratory flow (PEF) and FEV₁ will be used as markers for the worsening of asthma.

Covariates: The potential confounders determined *apriori* include: age, gender, race/ethnicity, asthma medication use, respiratory allergy history, atopic status, BMI, hour and day (of the week) of PEF, season, and temperature. Other covariates with *P-value* < 0.20 from the bivariate analysis will also be included in the multivariate model.

Analytical method: A general additive multiple linear regression model (GAM) will be fitted for predicting the percent change in within day PEF and FEV₁, and nadir PEF and FEV₁ for an interquartile range (IQR) increase in particular pollutant and airborne pollen while taking into consideration possible confounders and effect modifiers including penalized splines for seasonal and meteorological variations. The IQR scaling will enable the percentages to be directly relevant to the exposure experienced by the participants and will also make the percentages for different pollutants directly comparable to each other. To account for within-subject correlation, Generalized Estimating Equation (GEE) regression model will also be used as it focuses on estimating the average response through the fixed-effect or random-effect, as opposed to a regression parameters that predicts the effect of changing one or more covariates on a given participant.

ii) Protocol for objectives 3b.

Exposures: The hourly and daily averages of each pollutants (24-h averages for PM, SO₂ and NO₂, and 8-h average for O₃) and airborne pollens will be used to construct a lag of up to 30 days. The annual averages of air pollutants from the LUR will also be considered.

Outcomes: Asthma symptom score (ASS) will be on an ordinal scale ranging from 0 to 8 as defined above. Airway obstruction will be defined as a dichotomous variable with reduced FEV₁ (less than 80% of the predicted value) and a reduced FEV₁/FVC ratio (less than 0.8). Airway inflammation will be assessed using the log-transformed FeNO levels on a continuous scale and also as a polychotomous (ordered) variable as categorized above.

Covariates: The potential confounders include; age, gender, race/ethnicity, asthma medication use, respiratory allergy history, atopic status, BMI, and day (of the week) of test date, season, and temperature. Other covariates with *P-value* < 0.20 from the bivariate analysis will also be included in the multivariate model.

Analytical method: Multiple linear regression will be used to model the associations between IQR range increase (or a $10 \mu\text{g}/\text{m}^3$ increase where applicable) in pollutants (air pollutants and airborne pollen) and continuous outcome of interest while taking into consideration possible confounders and effect modifiers. Multiple logistic regression will be used in the case of a binary outcome while multiple ordinal regression will be used for ordinal outcomes such as ASS. In addition, to examine the co-dependency of the various pollutants, the correlation structure will be used to guide the selection of a two-pollutant model to avoid potential multicollinearity. Sensitivity analysis on the lag structure will also be done using different exposure windows up to 60 days prior to examinations, whenever appropriate, to evaluate the temporality of possible effects. For the purpose of this analysis, long-term effects of pollution levels will be investigated as potential confounders or effect modifiers of the short-term effects in models that will include random intercepts.

iii) **Protocol for objectives 3c.**

Exposure: The change in annual averages of 24-h period for NO_2 , SO_2 and PM, and 8-h daytime average for O_3 , including airborne pollen levels across the cohort study period.

Outcomes: Changes in ASS at follow-up, changes in levels of FeNO (ΔFeNO) or percentage predicted FEV_1 ($\Delta\%\text{FEV}_1$) and FEV_1/FVC ratio ($\Delta\text{FEV}_1/\text{FVC}$) between the two study periods.

Covariates: The potential confounders include; age, gender, race/ethnicity, asthma medication use, respiratory allergy history, atopic status, BMI, hour and day (of the week) of test, season, and temperature. Adjustment for short-term effects of air pollution using appropriate lag structure at each study period together with potential confounders and effect modifiers taken into consideration, will help assess the independent effects of long-term air pollution. Time independent covariates (ΔZ) such as race/ethnicity effects will be assessed together with time-elapsing between the two child-specific yearly test dates (ΔAge), while time-varying covariates (ΔW) effects will be assessed considering all possible transitions or changes over time for categorical and continuous covariates respectively. The most appropriate linear distributed lag models will be chosen from all possible types of lag-based models for short-term effects of air pollution, and the model selection will be based on the Akaike Information Criterion (AIC). The confounding effect of ambient temperature will be tested using the lag structure selected for the short-term air pollutant effects. Potential effect modifiers such as gender, asthma status, respiratory allergy, baseline outcome status (obstruction status, FeNO levels) and season will be tested in the final model.

Analytical method: Two strategies will be employed in these analyses. The first is a cohort analysis starting with a disease-free cohort to explore new-onset of asthma, while the second will be a change-analysis to investigate the changes in the respiratory outcomes over time. To explore the association between the asthma score and pollutant concentrations both defined at follow-up, in a subpopulation reporting neither symptoms nor asthma at baseline, a negative binomial regression model (with a log link) will be used to account for extra-Poisson variation due to the distribution of the asthma score, with a majority of zeros. The result will be expressed as ratios of the mean asthma scores (RMS). The pollutants effect will be scaled for an increase of $10 \mu\text{g}/\text{m}^3$ higher concentration. The subpopulation of interest will be considered a sample being in all likelihood free of asthma at baseline. Thus, new onset of symptoms (a reflection of asthma incidence) might be interpreted following the occurrence of symptoms at follow-up. To account for the participant reporting only one of the symptoms at follow-up, further analysis will be performed by comparing those with none or only one symptoms (coded as participants free of symptoms) at follow-up with those with at least two symptoms. This will also be followed-up by considering those reporting none, one or two symptoms as participants free of symptoms, comparing them with participants reporting at least three symptoms.

Multiple linear regression will be used to assess the relationship between changes in levels of FeNO (ΔFeNO) or percentage predicted FEV_1 ($\Delta\%\text{FEV}_1$) and FEV_1/FVC ratio ($\Delta\text{FEV}_1/\text{FVC}$) between the two study periods and the corresponding changes in 'long term' and 'short term' air pollution and airborne pollen levels. A 12-month period prior to the day of tests will be used to assess the effects of changes in long term pollution levels, while adjusting for short term levels based on lags of up to 60 days prior to the day of tests. The model will assume a general form provided below;

$$\begin{aligned} & \Delta\text{FeNO} (\Delta\%\text{FEV}_1 \text{ or } \Delta\text{FEV}_1/\text{FVC}) \\ & = \beta_0\Delta\text{Age} + \beta_1\Delta\text{AP}^{\text{LT}} + \beta_2\Delta\text{AP}^{\text{ST}} \\ & + \beta_3\Delta\text{P}^{\text{LT}} + \beta_4\Delta\text{P}^{\text{ST}} + \beta_5\Delta\text{Temp}^{\text{ST}} \\ & + \gamma^T Z \times \Delta\text{Age} + \gamma^T \Delta W + \varepsilon \end{aligned}$$

where ΔAge , $\Delta\text{AP}^{\text{LT}}$, $\Delta\text{AP}^{\text{ST}}$, $\Delta\text{P}^{\text{LT}}$, $\Delta\text{P}^{\text{ST}}$, and $\Delta\text{Temp}^{\text{ST}}$ denotes the time elapsed between the two tests, changes in long-term air pollution levels, short-term pollution, changes in long-term pollen levels, short-term pollen levels, and temperature levels, respectively. The change-on-change model will allow for exploring the determinants of change in FeNO or $\%\text{FEV}_1$ or FEV_1/FVC rather than determinants of level of the actual FeNO or $\%\text{FEV}_1$ or FEV_1/FVC . In an attempt to explore the change in obstructive pattern

defined by reduced FEV₁ less than 80% of the predicted value and a reduced FEV₁/FVC ratio less than 0.8, a binary variable will be created with participant changing from non-obstructive airways to obstructive airways coded as 1; those without change in obstructive airways pattern also coded as 1; those with changes from obstructive airways to non-obstructive airways coded as 0; and those without change in non-obstructive airways pattern also coded as 0. Thus, a multiple logistic regression will be used to model the association between change in obstructive patterns and change in exposure taking into consideration confounding and effect modifiers.

Analysis protocol for objectives 4

Predictor: Baseline FeNO levels.

Outcome: Crude incidence rates for new-onset asthma (defined by increasing ASS or presence of new asthma symptom) will be calculated by dividing the number of cases by the total person-years at risk. Follow-up is considered complete at the time of reported diagnosis for children who developed new-onset asthma.

Covariates: The potential confounders include; age, gender, race/ethnicity, asthma medication use, respiratory allergy history, atopic status, BMI, season, temperature including average ambient air pollution and airborne pollen levels on the day of the FeNO measurement.

Analytical approach: To investigate the relationship between baseline FeNO with new-onset asthma, incident rates will be calculated while exploring series of multivariate modelling approaches to adjust for potential confounders and heterogeneity of effects within subgroups of children. A cohort analyses starting with a disease-free cohort will be fitted to investigate the association between FeNO and new-onset asthma adjusting for possible confounders including lifetime history of wheezing. Fitted models with appropriate interaction terms with statistical significance tested by partial likelihood ratio test will be used to assess heterogeneity of associations among subgroups. Thereafter, stratified analyses will be performed in the presence of such significant interaction. Splines and piecewise cubic polynomials joined smoothly at a number of breakpoints (knots) will be used to explore the nonlinearity nature of FeNO effects.

Discussion

This research intends to explore state of the art approaches in the characterization of exposure to ambient air pollutant to investigate its effects on children's respiratory health (with a specific focus on asthma) in South African informal settlement areas, a heavily under researched setting. The research employs the application of more novel outcomes and exposure measures such as biological markers of airway inflammation (such as FeNO) in addition to traditional measures (spirometry,

peak expiratory flow, asthma symptom score, and allergic sensitization to common inhalants) as well as ambient air pollution estimates derived from LUR modelling and GIS-based indicators in addition to dispersion modelling and air quality monitoring stations data. Additionally, the study measures pollen levels (mould spores, tree, grass and weed pollen) in the study areas supplemented with meteorological factors (such as wind direction, wind speed, temperature, humidity, pressure and sunshine) that may impact on pollutant levels and pollen counts. This study attempts to investigate the synergistic effect of air pollutant and pollen exposures on both new-onset childhood asthma and asthma exacerbations at the population level. Furthermore, this study promises to provide additional data for the Western Cape Province of South Africa to further explore the association between ambient air pollution and respiratory morbidity (childhood asthma) in South Africa. The study will generate crucial data on air pollution and asthma in low income settings in sub-Saharan Africa that is lacking in the international literature.

A demonstrable positive association between ambient air pollution and asthma in children in the Western Cape would support the importance and urgency of improving ambient air quality in the Province and particularly in the study areas. This would contribute towards highlighting the need for ongoing ambient air quality monitoring and strategies for the continued reduction of air pollutants from likely sources such as traffic, passive smoking, industries, and domestic fuels. There is potential to use these information towards increasing and promoting asthma awareness and asthma education in communities with a high prevalence of asthma including the study communities. It is believed that such public health promotion initiatives would assist in reducing associated asthma morbidity. Education programmes will emphasize the need for household environmental management including indoor dust control, ownership of certain pets, pest and bio-aerosol control, prevention of smoking indoors and actively discouraging adults from smoking particularly around children or in the vicinity of their rest, recreational or study areas. These measures will, in conjunction with ambient air quality management, result in an improved quality of life of asthmatics, particularly those with persistent asthma including those who are susceptible. Since air pollutants have been shown to influence the allergenic content in plants by influencing pollen production and allergenic protein in pollen grains, abatement of air pollution will mitigate effects of climate change, allergen exposures, air quality and health status of vulnerable populations.

Furthermore, the findings from this project will also help the Department of Environmental Affairs and Development Planning (DEA&DP) in the Western Cape, South Africa to develop regional and spatial plans that consider these

findings (with regard to location of industries and major roads close to residential areas), and review environmental exposure standards. This will contribute towards highlighting the importance of ongoing monitoring of air pollution pollen exposures near urban areas and near schools, with results communicated to the public in a concise and understandable means. This would contribute towards the protection of public health during periods of high levels of ambient air pollution whereby authorities can use this information as part of their decision-making processes.

Abbreviations

AIC: Akaike information criterion; ATS: American thoracic society; BMI: Body mass index; CAMx: Comprehensive air quality model; DALY: Disability-adjusted life years; DAS: Data acquisition system; DEA&DP: Department of Environmental Affairs and Development Planning; ERS: European respiratory society; FeNO: Fractional-exhaled nitric oxide; FEV₁: Forced-expiratory volume in one second; FVC: Forced vital capacity; GAM: Generalized additive models; GEE: Generalized estimating equation; IgE: Immunoglobulin E; IQR: Interquartile range; ISAAC: International study of asthma and allergy in childhood; LMI: Low and middle income countries; LUR: Land-use regression; NIOH: National institute of occupational health; NO₂: Nitrogen dioxide; O₃: Ozone; OR: Odds ratio; PEF: Peak expiratory flow; PM_{1.0}: Particulate matter with diameter of 1.0 microns; PM₁₀: Particulate matter with diameter of 10 microns; PM_{2.5}: Particulate matter with diameter of 2.5 microns; RD: Risk difference; RMS: Ratio of mean score; SAWS: South Africa Weather Services; SO₂: Sulphur dioxide; UCT: University of Cape Town

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Availability of data and materials

The data that support the findings of this study are available from the corresponding author upon reasonable request and with permission of the Western Cape Department of Environmental Affairs and Development Planning, South Africa in a form which ensures privacy of study participants.

Authors' contributions

TO participated and MA and MJ supervised all aspect of the study and drafted the manuscript. MR, RB, MT, MD and KH had a key role in the design and development of the air pollution exposure measurement of the study. DB played a key role in the design and measurement of pollen across the study areas while RN, BP, JL and NK provided technical input to the study design. All authors read and approved the final manuscript.

Authors' information

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Ethics approval and consent to participate

The study is being done in accordance with the Declaration of Helsinki of the 25th world Medical Assembly [43]. The study proposal was approved by the University of Cape Town's Research Ethics Committee (HREC REF: 697/2014). Permission to include school children in the study is obtained from the Western Cape Department of Education. During recruitment, permission is requested from the principles of selected schools, school governing bodies and thereafter from the parents of children from participating schools to include their children in the study after conducting information meetings at the schools to inform them of the study, the time commitments and testing procedures.

The houses of school children are visited by trained field staff to explain the study and answer questions from parents/guardians, obtain the caregivers (parent or guardian) consent. Informed consent from care-givers and assent from child participants is obtained in person before testing. The participants are asked if they consent/assent to participate in the study before testing. The consent form explains the purpose of the proposed research, describes the testing and risks and discomforts of the testing, the expected benefits of the research, explains how confidentiality will be preserved and how the consent will be documented, informs that there will be no costs for testing and lists the details of persons to be contacted about any aspect of the research. It also serves to assure the participant should they decline to perform any or all aspects of the program that they will not be penalised in any way. All information and data is held with strict confidentiality and stored on the premises of the University of Cape Town. All files are locked in filing cabinets. To protect confidentiality, the consent forms and contact information sheets are not stored with confidential study information but kept in a separate locked file cabinet. A copy of the final report of the study will be made available to the school that child participants attend. Additionally, an information sheet on the risks of air pollution and how to manage these risks will be distributed to school staff and caregivers of participating students. A seminar on the results of the study and managing of the risks of air pollution will be held at the participating schools after completion of the study.

Consent for publication

The consent to publish the result of the findings was obtained in writing from the parent or caregiver during the consent procedure. It was stated clearly in the consent form that only summary results will be presented in reports and publications. Individual results will be provided to the parents, participants and their physicians if authorized to do so in writing.

Competing interests

The authors declare that they have no competing interest.

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CHAPTER 4

Characterization of exposures to ambient air pollutants and airborne pollen of schoolchildren residing in four informal settlements in Western Cape, South Africa

4.1 Introduction

Various exposure metrics have been used to assign exposure levels to ambient air pollution and pollen in epidemiological studies investigating the health effect of air pollution and/or pollen. Reported exposure measures from questionnaires have been used in identifying exposures to outdoor air pollutants such as frequency of heavy trucks around the home, and mode of transportation to work or school; and exposures to indoor pollutants such as use of paraffin for cooking and heating, presence of pets and smoker in the home. Participants in epidemiological studies of air pollution have commonly been assigned average data from routinely measured air quality from fixed monitoring station closest to their homes in assessing short-term exposure (1–3). This assignment of exposure from the routinely measured concentration is relatively cheaper as compared to other exposure assessment methods, and provides temporal consistency when data is available. However, it is limited by its ability to characterise intra-urban contrasts related to mobile local sources such as traffic emissions, because fixed monitoring air quality stations are often spatially less dense. To overcome some of the limitations of assigning one single average to a cluster of participants within a geographical space with varying pollutant levels, interpolation methods such as kriging and inverse distance weighting has been widely used (4,5). These interpolation methods use data from multiple fixed monitoring stations and assign more weight to monitoring stations closest to the participant's address. However, interpolation is more useful in estimating pollutants with relatively small local source such as PM_{2.5} and in more rural areas, because the assumption of a smooth spatial change in concentration is somewhat weak in urban areas with local sources such as major roads resulting in sharp changes in concentrations levels.

Geographic-information system (GIS) parameters such as the total street length around certain buffer radii and the distance to a major road have also been adopted commonly (6–8) to assess air pollutant exposure. This method has been widely used as a proxy for traffic-related pollutants mainly estimated from traffic-volumes. However, it is also limited by the inability to identify traffic-types (that is; vehicular types and exhaust released) or distinguish between traffic-related pollutant (e.g. NO₂ and NO_x) and background pollutant such as PM_{2.5}, which differs widely from its near-road side concentrations (9).

Knowledge of major pollutant sources with within a geographical area allows the use of dispersion and chemical transport modelling (DCTMs) to determine emissions of ambient air pollutants (10–13). This method is also often limited by the spatial scale they cover. An extension to the use of chemical transport modelling is the use of satellite monitoring of aerosol optical density (AOD), to estimate surface concentration of pollutants such as PM_{2.5} and NO₂ (14–17). However, there is less reliability for assessing surface concentrations of ozone, due to its concentration in the stratosphere. The satellite method is also limited by its spatial resolution (usually about 10 x 10 km for NO₂ and PM_{2.5}), sparse information on particle size distribution and chemical composition, temporal and spatial variation in assessing surface

concentrations and clouds interference. However, the advantage of incorporating the satellite method is its availability globally as compared to surface-monitoring that are only available in urban areas of some countries, and its usefulness in providing background ambient air pollutants (18).

Briggs and colleagues mapped out traffic-related pollutants such as NO₂ in Huddersfield and Amsterdam using a regression-based approach in a GIS environments in 1997 (19), after which a number of epidemiological studies have attempted to quantify individual long-term air pollution exposures at various geographical locations using land-use regression (LUR) methods (20–27). Few studies have been done in Africa using these approaches (28). In comparison to the aforementioned air pollutant measurement methods, the LUR takes into account the spatial nature of air pollutants within the geographical boundaries being modelled or estimated, with the possibility of including a temporal component. The land-use regression models uses empirical models by combining monitored air pollution data from limited number of locations with land-use parameters obtained from GIS (which accounts for the spatial variation). Background air quality data from fixed monitoring sites are then used to account for temporal variations in the models. In the absence of uncertainty about emission factors or physical-chemical transformation processes, LUR models are by far the method of choice. However, LUR models are limited in its characterisation of atmospheric formation processes, and it is less transferrable to other areas due to its empirical nature. In the absence of a wide spatial coverage (that is; lack of information on other spatial clusters such as transportation mode, schools or offices), the use of LUR in estimating the total individual exposure, might be affected by the spatial extent of the model. Although the methodology to account for time-activity patterns such as the use of fine-scale spatial maps, GPS or mobile phones is available, it is often difficult to apply in large population-based epidemiological studies. Hybrid models incorporating multiple assessment methods in one framework have been developed in studies, in recognition of the limitation of any single method (18). For example, de Hoogh *et al.*, used data from satellite and chemical transport model as predictors in LUR, using supervised stepwise selection methods to estimate annual PM_{2.5} and NO₂ in Western Europe (29).

With regard to the identification and quantification of airborne pollen and fungal spores, epidemiological studies are limited by the lack of standardised criteria (30). Questionnaire data on water damages or leaks, dampness, presence of visible mould growth, and mould odour have been used to indicate fungal exposure in indoor spaces (31–33). Determination of atmospheric bio-aerosol composition have been done by quantitation methods such as direct or culture-based volumetric air sampling methods (30,34,35). The Burkard spore trap is the most commonly used for ambient sampling of pollen and fungal spores. Many investigators have highlighted the absence of certain fungi (such as *Stachybotrys chartarium*) in the air, but found to be abundant on surfaces. Such cases leads to false-negative findings if the sampling was aimed at

detecting the presence of the specific organism in any reservoir (36). In the absence of culturable spores, false-negative results are common if the sampling was designed to estimate the presence of airborne fungus (36). It is argued that the use of culture-based methods for estimating fungal spores limits the number and types of fungi identified, as compared to direct spore counting. For example, in a study measuring *Alternaria* in low-income inner-cities of the US, *Alternaria* antigens were observed even in the absence of culturable *Alternaria* (37). Nonetheless, although direct spore counting enables the detection of a wide variety of fungal spores' taxa, it lacks the specificity offered by methods such as culture or quantitative polymerase chain reaction (PCR) (35).

Newer techniques such as immunoassays, polymerase chain reaction (PCR), and genomic sequencing are also available for detecting fungal and pollen levels(34). The detection of specific allergens is achievable by immuno-detection. This method, particularly for fungi, have been successful due to its allergen production variability (based on the strain, substrate and age) and the presence of large number of potential allergens per sample (36). Although antibodies for a variety of fungal allergens are commercially available, this method is yet to be widely used. The identification of specific fungal taxa in samples have been made possible by the development of DNA probes. The comparison of fungal isolates from different sources is an important advantage of the PCR method. PCR techniques have also been used to identify the airborne nature of non-culturable clinically relevant fungus such as *Pneumocystis carinii* (38). Here in Cape Town, an important fungus *Emergomyces africanus* which causes AIDS-related mycosis in South Africa, was recently detected for the first time in air samples from modified Burkard spore trap by the use of quantitative PCR assay (39). However, quantitative PCR is often limited by chemical inhibition such as airborne particulate matter (40) and the unavailability of pan-generic fungal primers, as most of the current primers are species-specific (41).

Ambient air sampling has been the most common method used in virtually all studies assessing the prevalence of pollen and fungal spores. However, in some situations, personal exposure measures prove very useful. For instance, quantification of the actual number of pollen grains entering the nasal airways (42). This particular study highlights the variation in exposure amongst individuals due to differences in activities and location. Exposures during specific activities are better captured with personal exposure measurements. For example, lawn cutters were shown to be exposed to higher levels of both fungal spores and pollen as compared to the general population (43). This finding is consistent with previous literature that have documented exposures to high-level of fungal spores during farming activities (44,45).

This chapter gives an overview of the methods used to assess exposures of the participating schoolchildren to ambient air pollutants, airborne pollen and fungal spores during the cohort and panel study period. Secondly, the chapter presents the descriptive results of the daily concentrations of PM₁₀, SO₂ and O₃ from

stationary air monitor closest to Khayelitsha and Marconi-Beam used in the panel study analyses in chapter 6. It also presents the estimated annual levels of PM_{2.5} and NO₂ from LUR models, at the participants' residence, used in the cohort study analysis in chapter 7. This chapter further presents the characteristics of airborne pollen and fungal spores measured in the four study areas throughout the panel and cohort study periods, and lastly describe the correlation between air pollutants, pollen, fungal spores, and climatic factors.

4.2 Methods

4.2.1 Ambient air pollutant measurements

In the panel study, which was conducted between the 15th and 26th of February, 2016 in summer and from the 25th of July to the 5th of August 2016 in winter, air quality data from a stationary monitor was used to estimate the short-term exposures of the study participants to air pollutants over this period. For the cohort study, LUR models were used to estimate the individual exposure to various air pollutants in the study areas over the one-year follow-up period between 17th of February 2015 to 16th of September 2016. In addition to the adopted ambient air pollution exposure assessment used in this thesis, GIS-based exposure measures and dispersion modelling were speculated to be used in the methodology section in Chapter 3 (46), however only stationary monitoring and LUR were finally used due to study feasibility and logistics. Details of the various exposure assessments have been previously published and reported in chapter 3 (46). A brief description is therefore given hereunder.

4.2.1.1 Stationary air quality monitoring

In the panel study, the data from the stationary monitors of the City of Cape Town and the Western Cape Department of Environmental Affairs and Development Planning located in the four study areas, were incomplete for 2015 and 2016 with missing data during the year. Thus, this data could not be used for exposure assessment in the panel and cohort studies. Complete data was available from an air quality stationary monitor located at the Cape Town International airport located approximately 16km and 19km from Khayelitsha and Marconi-Beam respectively. Daily hourly concentrations of PM₁₀, SO₂ and O₃ were obtained from this stationary monitor to estimate the daily variations in ambient air pollutants. Estimation of daily air pollutants levels for the panel data was therefore limited to only Khayelitsha and Marconi-Beam. For PM₁₀ and SO₂, 24-hour daily averages were computed from the hourly data, while O₃ was calculated using the 8-hour daily maximum average.

4.2.1.2 Land-use Regression

The annual average concentration of PM_{2.5} and NO₂ was estimated at each participant's address by land-use regression (LUR) models developed specifically for this study (47). In brief, the air pollution monitoring

campaigns were performed during 2015-2016 in each study area. Weekly measurements of PM_{2.5} and NO₂ were performed in both winter and summer at 140 sites (40 sites each in three study areas, except 20 sites in Masiphumulele) within a period of 1 year. These measurements in each area were temporally adjusted using available routinely monitored air quality from only two monitoring sites (airport and George monitoring site), to obtain the seasonal (winter/summer) and annual averages. The stationary air monitor data used for Masiphumulele and Oudtshoorn were collected about 45km from these main study areas, due to incomplete or lack of air quality data for the respective study area. Predictors of exposure, obtained or collected on-site, such as household density, nearby traffic (e.g. major roads, bus stops, and train stations), waste burning sites, and land-use derived from geographic information system (GIS) were used to evaluate the spatial variation in the annual average concentrations. To maximize the adjusted explained variance, regression models were developed using a supervised stepwise approach, and the models were validated using leave-one-out-cross-validation (LOOCV). The NO₂ model explained 62% of the annual variance, while PM_{2.5} had a lower explanatory power of 36% (47). The LUR model was used to estimate annual average concentration of PM_{2.5} and NO₂ for each participant's address. The average concentration of PM_{2.5} and NO₂ were scaled to compute an interquartile range increase for each pollutant for the current analysis.

4.2.2 Pollen and Fungal spores Measurements

Airborne fungal spores and pollen from grass, trees, and weeds were measured in all four study areas between July 2015 and February 2017, with each site having a measurement period over a 52-week-calendar year to cover all possible seasonal peaks. The sampling was done using the Burkard 7-day recording volumetric spore trap, located on a flat rooftop of a building close to the school having a minimum height of 1.5m to avoid dust contamination. Microscopic analysis of pollen was conducted by a trained counter, and the resulting daily pollen and fungal spores' counts were used to estimate the average annual concentrations in particles per cubic meter of air for each study area. The daily concentration of each pollen and fungal taxa were extracted for corresponding days of the panel study, and annual averages were computed from weekly means for the cohort study. Figure 4.1 shows the four study areas with the land cover and vegetation where the sampling units were placed. Nama Karoo biomass is the predominant vegetation found in Oudtshoorn, while Fynbos vegetation dominates the three other study areas.

4.2.3 Meteorological data

Hourly climatic data were obtained from the South African Weather Services (SAWS) for each of the study areas for both 2015 and 2016. The daily means were computed from the hourly observations, and thereafter used to calculate the annual averages. The climatic data of interest were maximum temperature, average temperature, humidity and pressure.

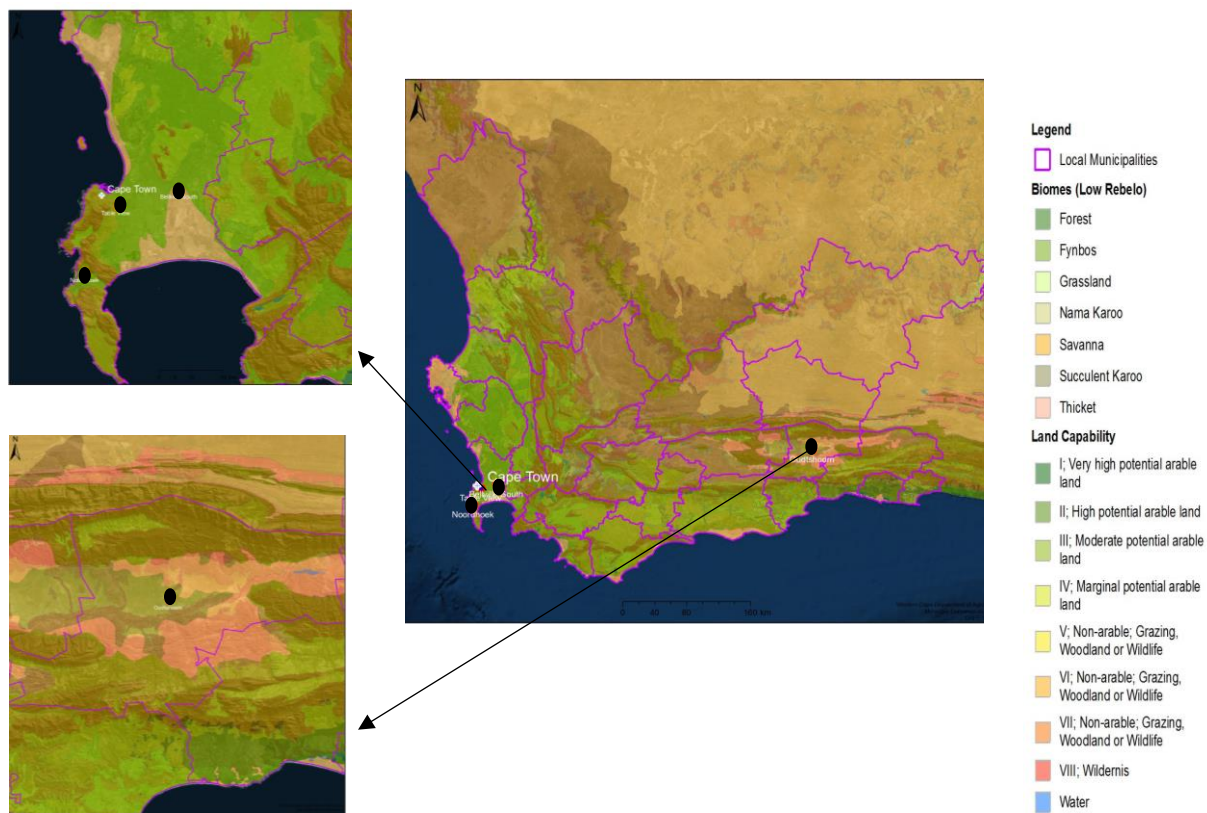


Figure 4.1: Spatial map of land cover and vegetation showing the location of the pollen sampling sites in the four study areas

4.3 Results

4.3.1 Ambient air pollutants levels in the panel study

A summary of the criteria pollutants measured at the stationary air quality monitor at Cape Town International Airport during the panel study is presented in Table 4.1. The maximum averages for PM₁₀, SO₂ and O₃ were 44.42 µg/m³, 17.46 µg/m³ and 67.5 µg/m³ respectively, and were all recorded during the summer campaign of the panel study. Furthermore, all pollutants were below the WHO air quality reference guidelines (24-hour PM₁₀ – 50 µg/m³; 24-hour SO₂ – 20 µg/m³; 8-hour daily maximum O₃ – 100 µg/m³) (48) and South African National Ambient Air Quality Standards (SAAQS) (24-hour PM₁₀ – 75 µg/m³; 24-hour SO₂ – 125 µg/m³; 8-hour daily maximum O₃ – 120 µg/m³) (49) during the panel study. There was also no significant difference between the average summer and winter concentrations of measured pollutants (PM₁₀: 26.53 µg/m³ vs. 22.30 µg/m³, $p > 0.05$; SO₂: 8.71 µg/m³ vs. 9.40 µg/m³, $p > 0.05$; O₃: 42.05 µg/m³ vs. 44.99 µg/m³, $p > 0.05$).

4.3.2 Ambient air pollution pollutants levels in the cohort study

The annual averages for PM_{2.5} and NO₂ estimated using LUR in the four study areas are presented in Figure 4.2A and 4.2B respectively. Air pollutants estimates were only available for a total of 553 participants' addresses of the 590 school children recruited in the study. The missing 37 estimates were due to incorrect residential addresses, which were difficult to geocode. The LUR estimates range from 0.75 µg/m³ to 62.89 µg/m³ for PM_{2.5} and 0.13 µg/m³ to 39.90 µg/m³ for NO₂. Schoolchildren residing in Khayelitsha had the highest annual mean for PM_{2.5} of 11.89 µg/m³ (higher than the WHO air quality reference guideline of 10 µg/m³) and 24.22 µg/m³ for NO₂. The air pollutant levels in Marconi-Beam followed closely, with an annual PM_{2.5} average of 10.90 µg/m³ and 23.22 µg/m³ for NO₂. Exposures to annual PM_{2.5} above the WHO air quality reference guidelines of 10 µg/m³, were found in 108 of the 135 participants (80%) sampled from Khayelitsha and in 86 of 136 children (63.24%) from Marconi-Beam (Table 4.2). Overall, nearly half of the children in this study were exposed to levels of PM_{2.5} above the WHO air quality guideline (46.84%). A maximum annual mean of 62.89 µg/m³ for PM_{2.5} was recorded in a participant's home in Oudtshoorn. None of the children were exposed to annual levels of NO₂ above the WHO air quality reference guideline of 40 µg/m³ (48).

Across all the four study areas, the winter averages of PM_{2.5} were significantly higher than the summer means (13.42 µg/m³ vs. 8.11 µg/m³, $p < 0.001$). More specifically, the average winter means for PM_{2.5} in Khayelitsha of 14.95 µg/m³ was significantly higher than a summer average of 9.73 µg/m³ ($p < 0.001$). Besides Oudtshoorn with a higher average summer mean of NO₂ than winter, all other areas showed a higher winter average compared to summer. For example, the winter mean NO₂ levels in Khayelitsha was significantly higher than the summer average (30.30 µg/m³ vs. 18.80 µg/m³, $p < 0.001$) (appendix Figures S4.1 A – D).

Table 4.1: Daily levels of criteria pollutants measured from the stationary air quality monitor located at Cape Town International airport, during the sampling phases of the panel study

	Summer (8 Feb - 26 Feb 2016)	Winter (18 July - 5 Aug 2016)	Combined
Particulate matter - PM ₁₀ (µg/m ³)			
mean ± SD	26.53 ± 10.87	22.30 ± 5.67	24.02 ± 8.30
median (IQR)	27.96 (17.83 - 33.79)	22.08 (17.67 - 27.54)	22.17 (17.71 - 28.38)
min-max	11.75 - 44.42	14.67 - 32.04	11.75 - 44.42
Sulphur dioxide - SO ₂ (µg/m ³)			
mean ± SD	8.71 ± 3.79	9.40 ± 4.46	9.12 ± 4.15
median (IQR)	7.79 (6.17 - 9.83)	8.75 (5.67 - 14.25)	8.27 (5.88 - 12.08)
min-max	3.86 - 17.46	3.21 - 17.42	3.21 - 17.46
Ozone - O ₃ (µg/m ³) *			
mean ± SD	42.05 ± 11.50	44.99 ± 8.59	43.57 ± 10.04
median (IQR)	41.80 (32.38 - 49.00)	44.31 (38.43 - 53.13)	42.38 (37.38 - 50.50)
min-max	27.50 - 67.50	26.13 - 57.63	26.13 - 67.50

* Ozone was calculated using the 8-hour daily maximum average

All other pollutants were computed on a 24-hour daily average

SD: standard deviation, IQR: interquartile range from the 25th to the 75th percentile

Stationary air quality monitor only relevant for Khayelitsha and Marconi-Beam area

Table 4.2: Air pollutant levels above the World Health Organization air quality guidelines and the allergic symptom thresholds for fungal spores

Pollutants	Khayelitsha	Marconi-Beam	Masiphumulele	Oudtshoorn	All areas
Air pollutants ^x					
PM _{2.5} (µg/m ³)	108/135 (80.0)	86/136 (63.24)	40/116 (34.48)	25/166 (15.06)	259/553 (46.84)
NO ₂ (µg/m ³)	0/135 (0.0)	0/136 (0.0)	0/116 (0.0)	0/166 (0.0)	0/553 (0.0)
Fungal spores ^y					
<i>Alternaria</i> (spores/m ³)	27/52 (51.9)	34/52 (65.4)	5/52 (9.6)	10/52 (19.2)	76/208 (36.5)
<i>Cladosporium</i> (spores/m ³)	0/52 (0.0)	1/52 (1.9)	0/52 (0.0)	7/52 (13.5)	8/208 (3.9)

values expressed as n/N (%); where n is the number of participants (for air pollutants) and number of weeks (for fungal spores), and N is the total number of participants (for air pollutants) and total number of weeks (for fungal spores)

^x The total number of participants with annual estimates from the land-use regression

^y The total number of weeks sampled in a 52-calendar pollen measurements

WHO air quality guidelines for annual PM_{2.5} is 10 µg/m³ and annual NO₂ is 40 µg/m³

Threshold for allergic health effects for *Alternaria* is 100 spores/m³ while that of *Cladosporium* is 3000 spores/m³

Figure 4.2A

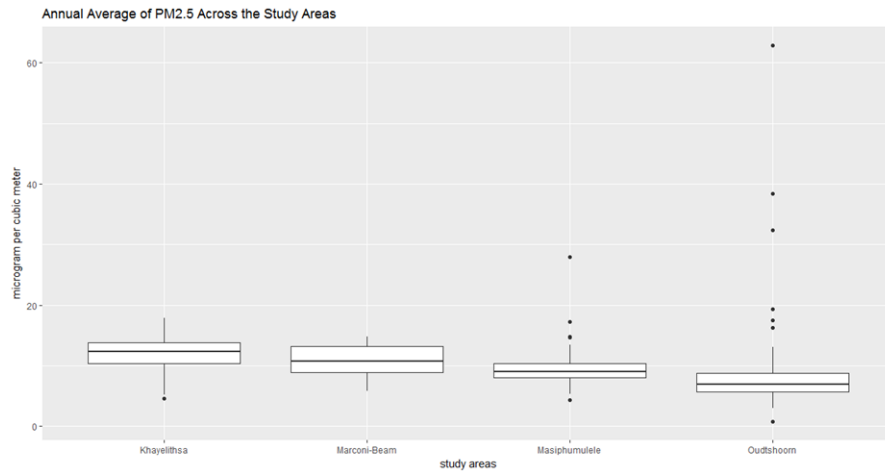


Figure 4.2B

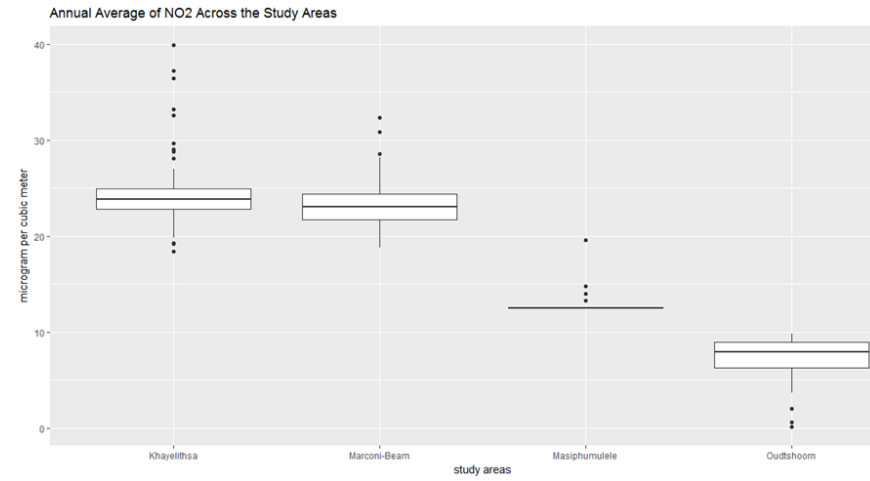


Figure 4.2A and 4.2B: The annual averages of PM_{2.5} and NO₂ estimated from land-use regression in all four study areas

4.3.3 Levels of airborne pollen and fungal spores in the 4 study areas

Three categories of airborne pollen namely; grass, trees, and weeds, as well as fungal spores were measured in this study. Various sub-classifications within these broad categories were measured. The sub-classifications included; 24 fungal spores (*Alternaria*, *Ascospores*, *Cladosporium*, *Aspergillus*, *Basidiospores*, *Bispora*, *Chaetomium*, *Curvularia*, *Epicoccum* amongst others), 4 grass (*Cyperaceae*, *Restinaceae*, *Typhaceae*, and *Poaceae* – commonly known as grass), 17 trees (*Betula* – commonly known as Birch, *Cupressaceae* – commonly known as Cypress, *Oleacea*, commonly known as Olive, *Myrataceae* – commonly known as Eucalyptus, and *Ulmus* – commonly known as Stinkwood amongst others), and 31 weed (*Amaryllidaceae*, *Asteraceae*, *Boraginaceae*, *Bruniaceae*, *Myricaceae* – commonly known as Waxberry, *Liliaceae* to list a few). The full list of the specific-genera for the measured pollen and fungal spores is presented in Appendix 4.2 (Table S4.1).

One should be aware that the meteorological seasons in the Southern hemisphere differ from those in the Northern hemisphere. In the South, the spring season starts 1st of September and ends 30th of November; the summer starts 1st of December and ends 28th of February (29th February in a Leap Year); fall (autumn) starts 1st of March and ends 31st of May; while winter starts on the 1st of June and ends 31st of August.

Figures 4.3A to 4.3D present the weekly total concentrations of fungal spores and the three broad categories of pollen (grass, trees, and weed) measured over the 52 week measurement period in the 4 study areas. The highest peak for total fungal spores (34,823.52 spores/m³) in the 4 areas was recorded in week 30 (mid-July, 2016) in Masiphumulele, while week 31 (4th week in July), week 36 (1st week in September), and week 45 (last week of October) had the peak concentration of total fungal spores as high as 32,923.44 spores/m³ in Oudtshoorn. However, the total fungal spores, on average, were relatively lower in Khayelitsha and Marconi-Beam throughout the year (Figure 4.3A).

Besides Oudtshoorn, which had a peak in week 37 (2nd week in September), the total grass grains peaked in week 41 (1st week in October) in the other three study areas, with the highest value of 197.28 grains/m³ recorded in Marconi-Beam. This peak period represents the grass pollen season in South Africa, which usually occurs at the beginning of spring. However, the levels of total grass grains were generally very low at all other times of the year outside the short peak period (Figure 4.3B).

The total tree counts were relatively low (almost non-detectable) in all the study areas, with the exception of multiple spikes in Oudtshoorn in week 26 (last week in June), week 34 (mid-August, 2016), week 37 (1st week of September), and week 41 (1st of October). The highest spike of 832.32 counts/m³ was recorded in Oudtshoorn in mid-August, 2016 (Figure 4.3C). *Cupressaceae* (Cypress) was the main tree pollen responsible for the spikes, with as high as 796 counts/m³ reported in mid-August in Oudtshoorn.

The total weed counts in all four study areas peaked at week 10 (1st week of March) with the highest concentrations of 577.44 counts/m³ recorded in Masiphumulele (Figure 4.3D). These peaks across all the four areas mark the beginning of autumn, following an extensive three months of summer which is suitable for its production. *Myricaceae* (Waxberry) was the main weed responsible for the peaks observed in all of the four study areas. A highest level ever of Waxberry (364 counts/m³) was reported in Khayelitsha, Cape Town in the first week of March. However, very low levels of weed were measured at all other times of the year in all four study areas.

Due to the relatively low levels of tree, grass and weed pollen recorded in the four study areas, exposure, predominantly to fungal spores (*Alternaria*, *Ascospores* and *Cladosporium*) were considered further in both the panel and cohort study based on their abundance as observed in the sampling and their known clinical significance in causing allergic symptoms.

4.3.3.1 Levels of fungal spores during the panel study

Figures 4.4A to 4.4C show the daily concentrations of the three fungal spores considered in the panel study. The panel study was conducted from the 15th - 26th of February, 2016 in summer, and from the 25th of July – 5th August, 2016 in winter.

Overall, the levels of *Alternaria* were higher in the summer than in the winter phase of the panel study in all the 4 areas, although the highest concentration (216 spores/m³) for *Alternaria* was recorded in Marconi-Beam during the winter phase. This level was twice the threshold (100 spores/m³) to elicit allergic symptoms for *Alternaria* (50). In addition, there were two days recorded above this threshold in the summer phase, with the highest concentration of 167.04 spores/m³ reported in Marconi-Beam (Figure 4.4A).

With the exception of Oudtshoorn, the daily concentrations of *Cladosporium* were also generally higher in summer as compared to the winter phase of the panel study. However, in Oudtshoorn, the allergic symptom threshold (3000 spores/m³) for *Cladosporium* was exceeded twice during the winter phase, with the highest concentration of 22,334.4 spores/m³ (Figure 4.4C) recorded. These overall patterns (that is; high levels of *Alternaria* and *Cladosporium* in summer) observed in the panel study is largely expected to be due to the xerophilic nature of *Alternaria* and *Cladosporium*.

In contrast to the patterns observed during the winter and summer phase for *Alternaria* and *Cladosporium*, the daily levels for *Ascospores* were relatively higher during the winter compared to the summer phase in all the four study areas (Figure 4.4B). This is consistent with the hydrophilic nature of *Ascospores*, as they tend to peak during cold humid conditions. However, the spike observed in Oudtshoorn in summer was preceded by heavy rainfall days, thus creating a suitable humid environment for *Ascospores* sporulation.

4.3.3.2 Levels of fungal spores during the cohort study

Figures 4.5A to 4.5C show the weekly average concentrations of the three fungal spores considered in the cohort study. These measurements were recorded from July 2015 to February 2017, enabling the computation of a minimum of 52-weeks of continuous measurement in each study area.

Marconi-Beam generally had the highest average level of *Alternaria* over the 52-week calendar period in the 4 study areas, although the highest concentration of 519.12 spores/m³ was recorded in Khayelitsha in week 42 (mid-October, 2016). This level is five times the allergic symptoms threshold for *Alternaria* (100 spores/m³) (Figure 4.5A). Overall, 36.5% of the weeks in a 52-week calendar period had levels above the allergic symptoms threshold for *Alternaria* in all the four study areas. Thirty-four of the 52-weeks (65.4%) in Marconi-Beam had levels above the threshold for *Alternaria*, followed closely by Khayelitsha with over half of the year above the threshold (Table 4.2). In all four study areas, levels of *Alternaria* were generally higher than the threshold all year round, except during the cold weeks, as expected (due to xerophilic nature of *Alternaria*) from the 1st week in July (week 27) to the 1st week in September (week 36) (Figure 4.5A).

On the other hand, overall, Oudtshoorn had the highest level of *Ascospores* and *Cladosporium* with a maximum concentration of 23,378.4 spores/m³ and 29,591.28 spores/m³ respectively. These concentrations were recorded at week 36 (1st week in September) for *Ascospores* (Figure 4.5B) and week 45 (1st week in November) for *Cladosporium* (Figure 4.5C). Seven of the 52 weeks (13.5%) had levels above the allergic symptoms threshold for *Cladosporium* of 3000 spores/m³ in Oudtshoorn. Three of these seven days were recorded during the cold months (week 30 – 32, that is; 4th week in July to 2nd week in August), while the other four spikes were recorded during the expected warmer months [week 4 (4th week in January), week 5 (1st week in February), week 13 (4th week of March), and week 45 (2nd week in November)]. The other study areas were generally below this threshold, except for a single week (week 21 – 4th week in May) in Marconi-Beam with levels above the allergenic threshold of *Cladosporium* (Table 4.2).

Figure 4.3A

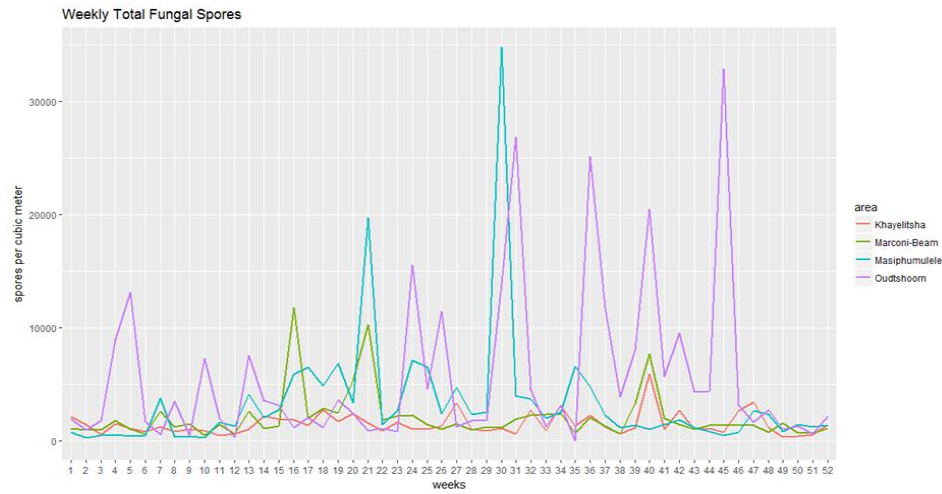


Figure 4.3B

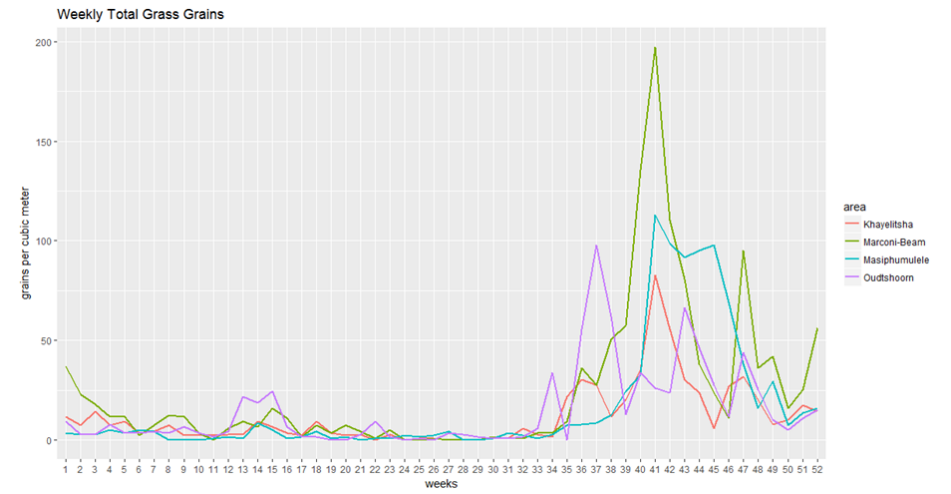


Figure 4.3C

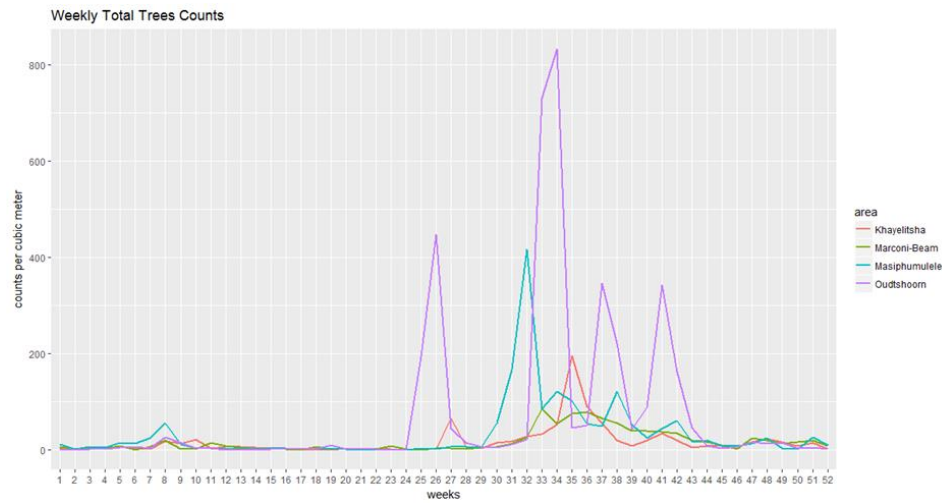


Figure 4.3D

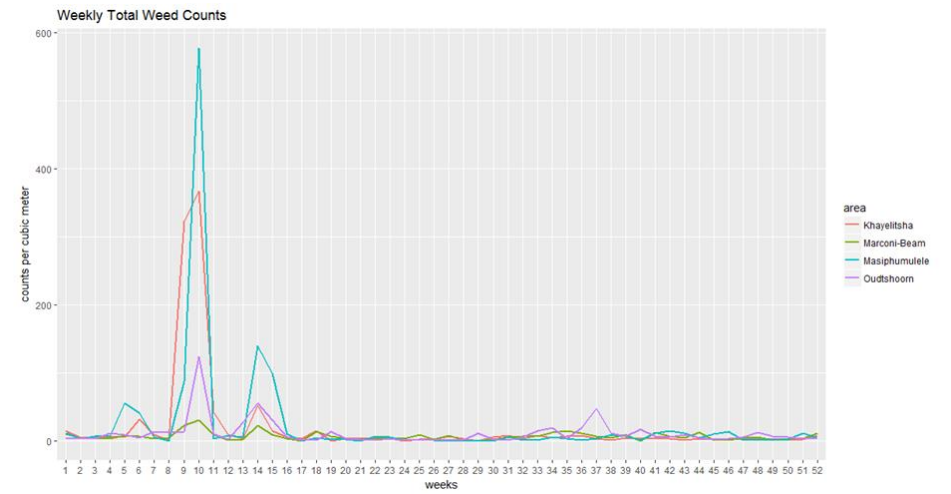


Figure 4.3A – 4.3D: Weekly total concentration of fungal spores (4.3A), grass (4.3B), trees (4.3C) and weed (4.3D) measured in the four study areas during a 52-week calendar period

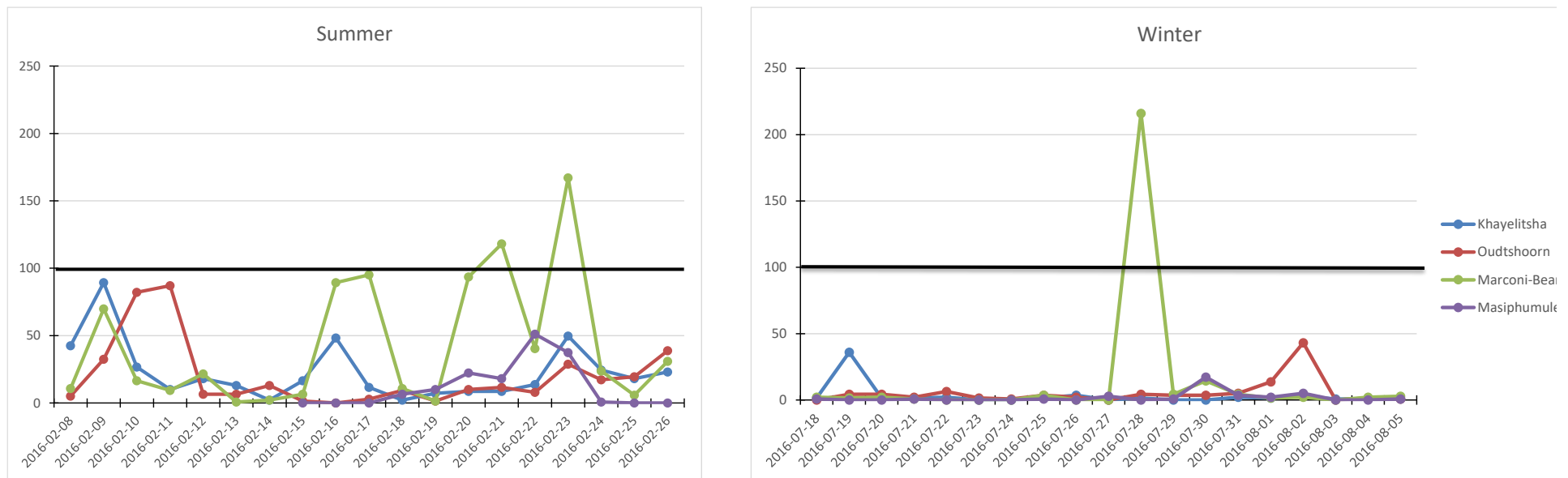


Figure 4.4A: Daily concentration of *Alternaria* (spores/m³) measured in the summer and winter phases across the four study areas over a 2 week period of the panel study in 2016 (black line represents the allergic symptoms threshold of 100 spores/m³ - Kasprzyk *et al.*, 2015)

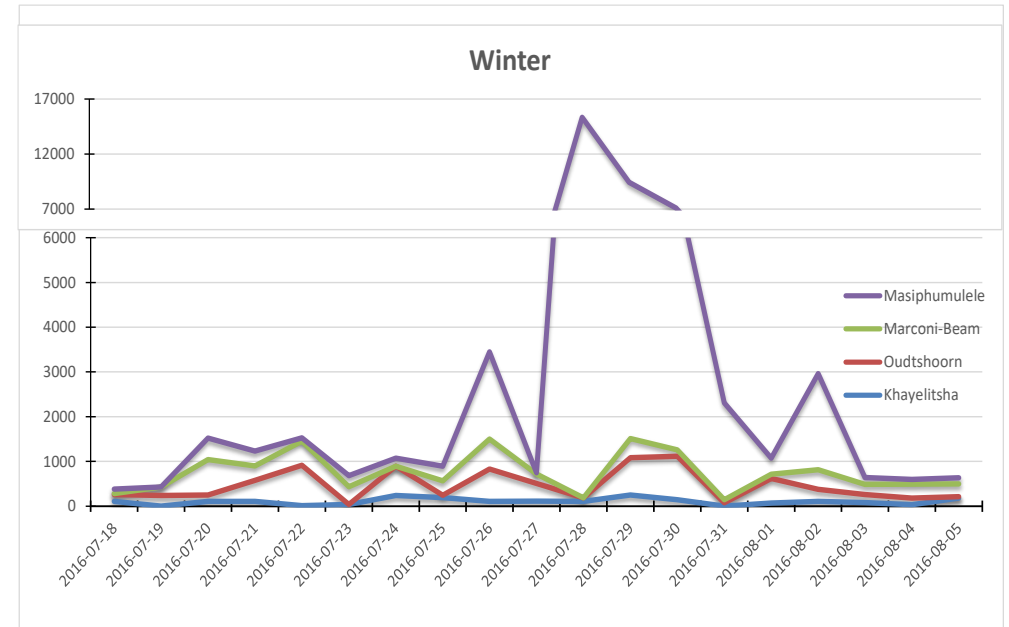
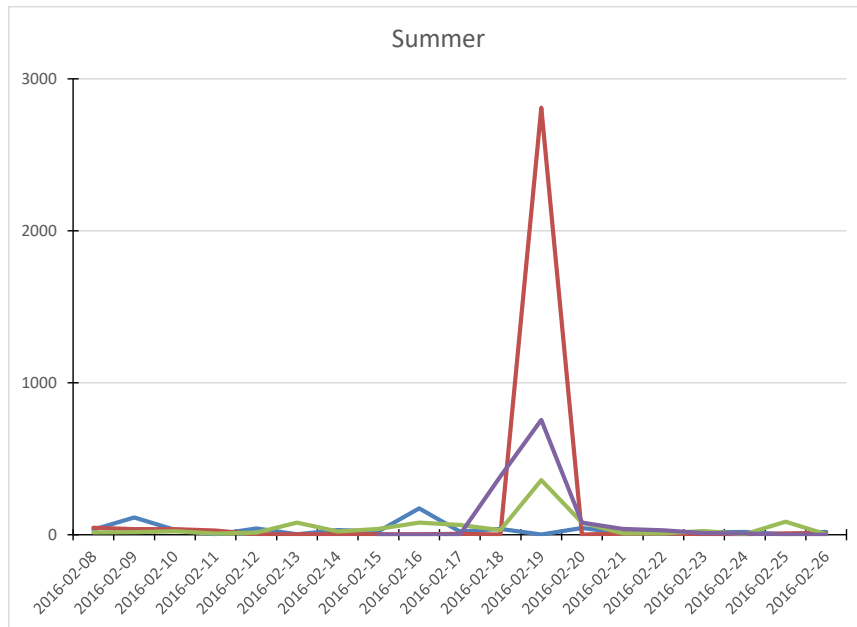


Figure 4.4B: Daily concentration of *Ascospores* (spores/m³) measured in the summer and winter phases across the four study areas over a 2 week period of the panel study in 2016

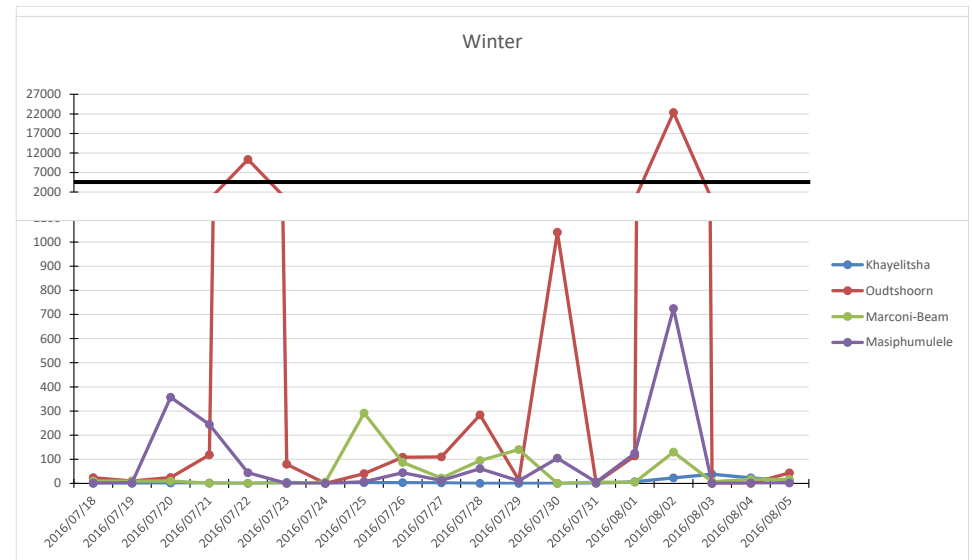
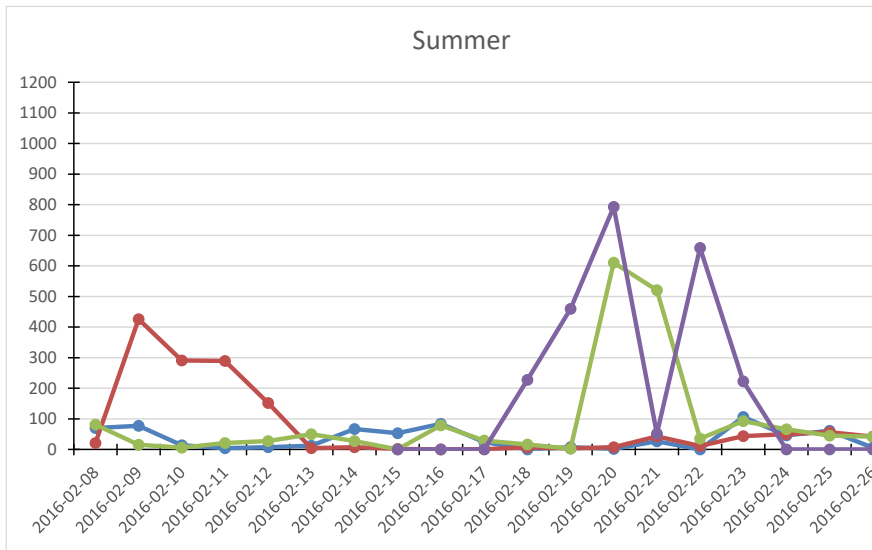


Figure 4.4C: Daily concentration of *Cladosporium* (spores/m³) measured in the summer and winter phases across the four study areas over a 2 week period of the panel study in 2016 (black line represents the allergic symptoms threshold of 3000 spores/m³ - Kasprzyk *et al.*, 2015)

Figure 4.5A

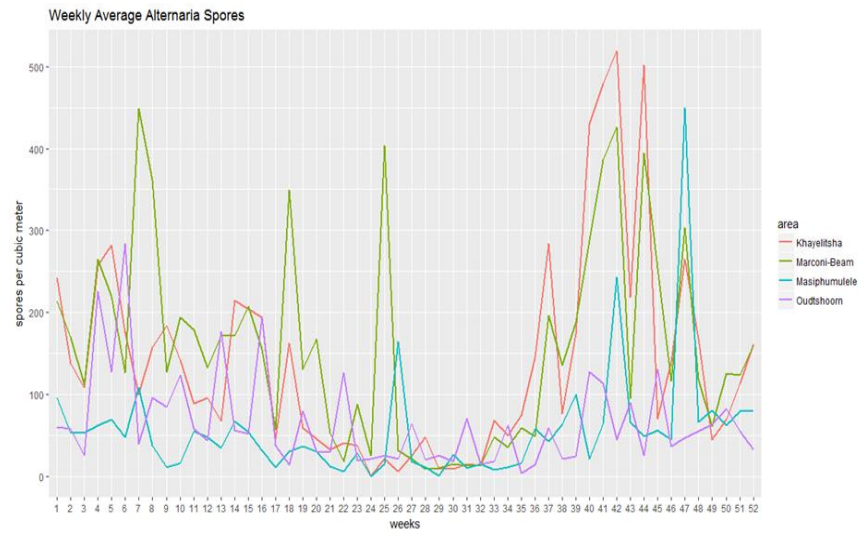


Figure 4.5B

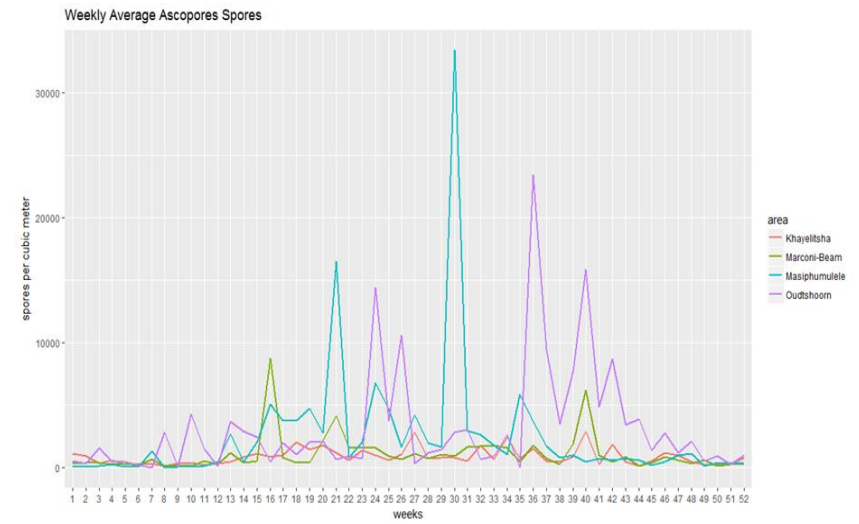


Figure 4.5C

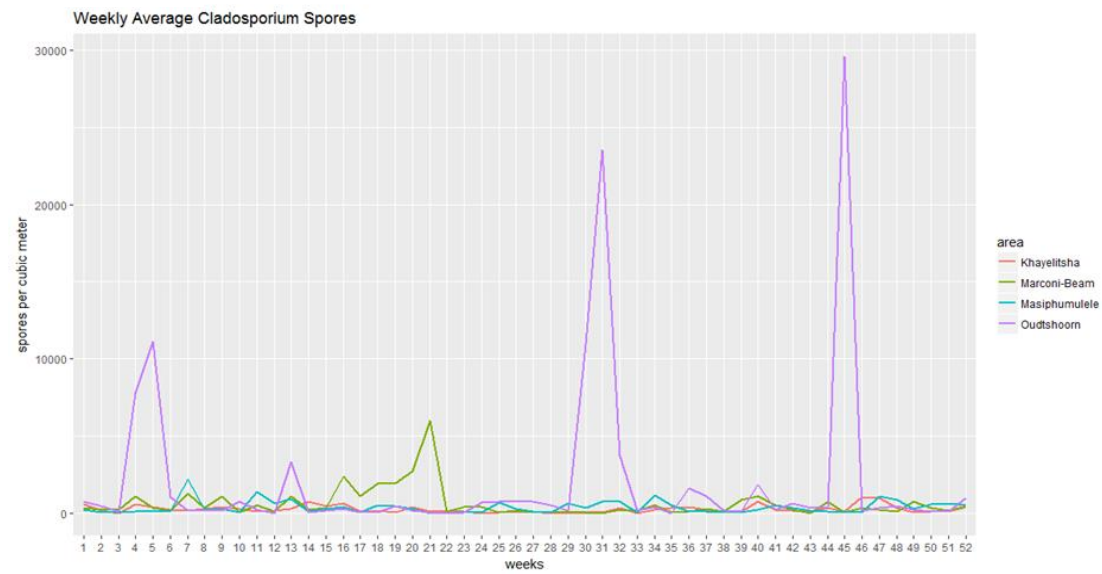


Figure 4.5A – 4.5C: Weekly average concentration of *Alternaria* (4.5A), *Ascospores* (4.5B), and *Cladosporium* (4.5C) measured in the four study areas during a 52-week calendar period

4.4 Correlation between ambient air pollutants, fungal spores and climatic factors

Figure 4.6A to 4.6D shows the summary statistics of the meteorological factors measured in the four study areas. Oudtshoorn had the highest average monthly mean temperature, with a maximum monthly mean temperature of 25.9°C and maximum temperature of 34.4°C (Figure 4.6A), while Masiphumulele was on average, the coolest area with an average monthly mean temperatures below 20°C throughout the year (Figure 4.6B). These differences in temperature can be explained by the climatic and geographical location of Oudtshoorn, which is typically semi-arid and located inland compared to the other three areas that are more coastal and with a Mediterranean climate. Marconi-Beam was the most humid amongst all the study areas, with humidity ranging from 73% to 84% throughout the year (Figure 4.6C). The time series graph show an inverse relationship between humidity and temperature (correlation coefficient, $r = -0.58$, $p < 0.05$ Table 4.3), with the winter months (June, July and August) having the highest humidity levels of all the study areas. However, the pressure (range: 975.5 hPa – 983.4 hPa) in Oudtshoorn was relatively lower throughout the year compared to the other three areas (Figure 4.6D).

There was low correlation between the measured criteria pollutants during the cohort period in the 4 areas ($PM_{2.5}$ vs. NO_2 , $r = 0.33$, $p > 0.05$). $PM_{2.5}$ had a strong positive correlation with *Alternaria* ($r = 0.89$, $p < 0.01$) and a strong negative correlation with *Ascospores* ($r = -0.96$, $p < 0.01$) and *Cladosporium* ($r = -0.76$, $p < 0.01$) (Table 4.3). Interestingly, between the fungal spores, there was a strong negative correlation between *Ascospores* and *Alternaria* ($r = -0.93$, $p < 0.01$), and a strong positive correlation between *Ascospores* and *Cladosporium* ($r = 0.79$, $p < 0.01$). There was also a strong negative correlation between humidity with $PM_{2.5}$ ($r = -0.81$, $p < 0.01$), and *Alternaria* ($r = -0.86$, $p < 0.01$), including a strong positive correlation with *Ascospores* ($r = 0.81$, $p < 0.01$) (Table 4.3). *Alternaria* also showed a positive correlation with maximum temperature ($r = 0.68$, $p < 0.05$) and a negative correlation with pressure ($r = 0.75$, $p < 0.05$).

Figure 4.6A

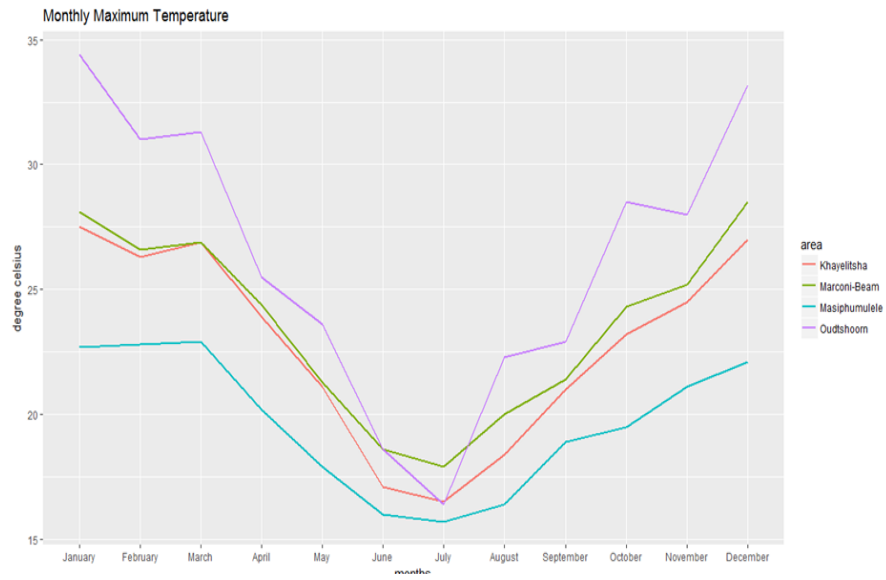


Figure 4.6B

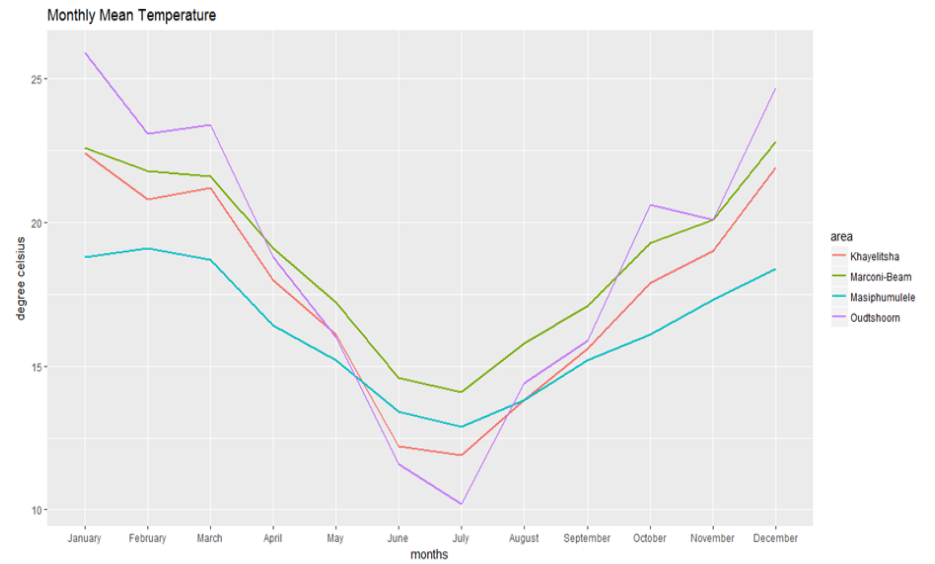


Figure 4.6C

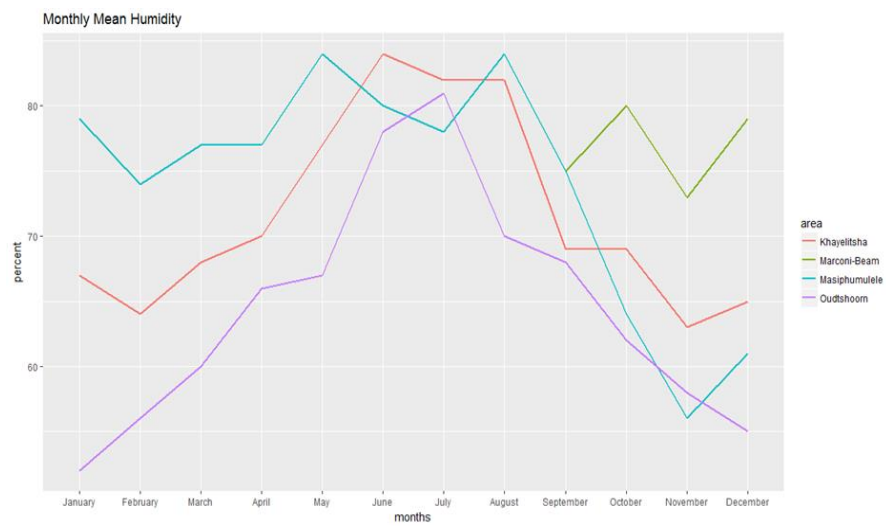


Figure 4.6D

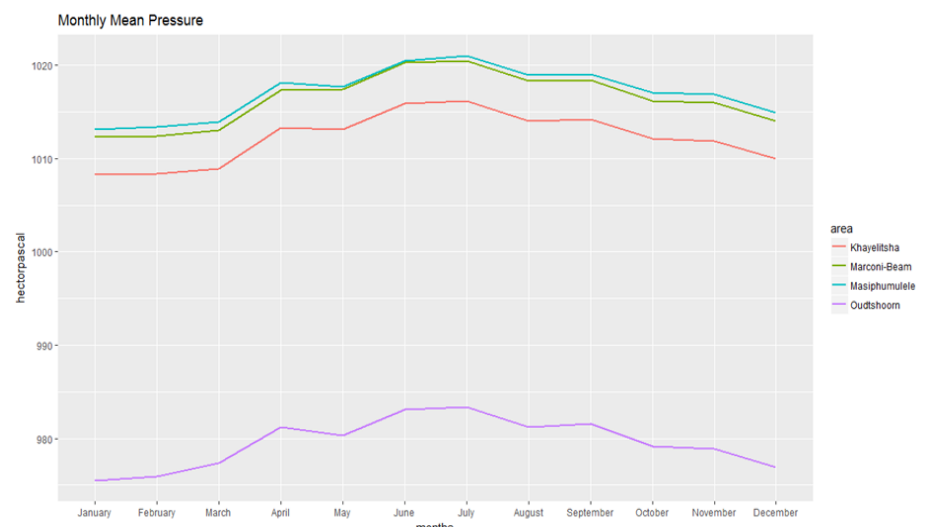


Figure 4.6A – 4.6D showing monthly average maximum temperature (4.6A), mean temperature (4.6B), mean humidity (4.6C), and mean pressure (4.5C) across the four study areas during the cohort sampling period in 2015

Table 4.3: Spearman's correlation among air pollutants, airborne pollen and climatic variables using annual averages

	PM _{2.5}	NO ₂	<i>Alternaria</i>	<i>Ascospores</i>	<i>Cladosporium</i>	Maximum temperature	Temperature	Humidity	Pressure
PM _{2.5}	1.0000	0.3271	0.8905	-0.9596	-0.7616	0.3567	-0.0686	-0.8143	-0.5903
NO ₂		1.0000	0.2845	-0.3338	-0.2938	0.0600	-0.0829	-0.2501	-0.1476
<i>Alternaria</i>			1.0000	-0.9285	-0.5343	0.6801	0.2949	-0.8582	-0.7543
<i>Ascospores</i>				1.0000	0.7924	-0.3598	0.0718	0.8128	0.5644
<i>Cladosporium</i>					1.0000	0.2071	0.6454	0.6356	0.2626
Maximum temperature						1.0000	0.8712	-0.5765	-0.8070
Temperature							1.0000	-0.1025	-0.4458
Humidity								1.0000	0.9102
Pressure									1.0000

Bold text represents correlations of 70% and more
TSL: Total street length

4.5 Discussion

4.5.1 Ambient air pollution exposure characteristics in the 4 study areas

The maximum averages for PM₁₀, SO₂ and O₃ were all recorded during the summer campaign of the panel study. These higher levels in summer, especially for ozone is largely due to photochemical reaction of NO₂ with ultra-violet rays from sunlight, which occurs predominantly during warmer months (51). The daily averages of pollutants measured in this study were much less than those reported in a recent local study in Durban, South Africa. For example, the Durban study recorded a 24-hour PM₁₀ mean and maximum concentration of 51.8 µg/m³ and 182.8 µg/m³ respectively in participating schools in Southern Durban (52) compared to our mean and maximum concentrations of 24.02 µg/m³ and 44.42 µg/m³ respectively. Furthermore, in this current study, all pollutants were below the WHO air quality reference guidelines (24-hour PM₁₀ – 50 µg/m³; 24-hour SO₂ – 20 µg/m³; 8-hour daily maximum O₃ – 100 µg/m³) (48) and South African National Ambient Air Quality Standards (SAAQS) (24-hour PM₁₀ – 75 µg/m³; 24-hour SO₂ – 125 µg/m³; 8-hour daily maximum O₃ – 120 µg/m³) (49) during the panel study.

In the cohort study, school children residing in Khayelitsha had the highest annual mean for PM_{2.5} of 11.89 µg/m³ (a level higher than the WHO air quality reference guideline of 10 µg/m³) with 80% of these children above this threshold (48). Marconi-Beam exposures followed closely, with an annual PM_{2.5} average of 10.90 µg/m³ with 63.24% of these children above the WHO PM_{2.5} annual reference threshold (48). Furthermore, nearly half of the children in this study were exposed to levels of PM_{2.5} above the WHO air quality guideline (46.84%). The annual estimates from the land-use regression range from 0.75 µg/m³ to 62.89 µg/m³ for PM_{2.5} and 0.13 µg/m³ to 39.90 µg/m³ for NO₂. Large fractions of spatial variance in pollutants within small geographical boundaries have been reported in previous studies such as those from the European Study of Cohorts for Air Pollution Effects (ESCAPE) projects, who used a similar standardised LUR approach (24). However, the annual levels of PM_{2.5} estimated in this current study were relatively higher than those of the network of studies in the ESCAPE projects. For instance, in our current study, the maximum estimated levels of PM_{2.5} across the four study areas ranged from 14.80 µg/m³ in Marconi-Beam to 62.89 µg/m³ in Oudtshoorn, as compared to a concentration of 10.80 µg/m³ in the Manchester Asthma and Allergy Study (MAAS) in the UK (53) to 21.40 µg/m³ in the GINI/LISA North (54) project in Germany. On the contrary, maximum concentration of NO₂ measured in three of the ESCAPE studies were much higher than levels reported in this current study. For example, maximum levels of annual NO₂ ranging from 52.1 µg/m³ in PIAMA study in the Netherlands (55) to 59.8 µg/m³ in GINI/LISA North study in Germany (54) compared to 39.9 µg/m³ estimated in our current study. None of the children in this current study were exposed to levels of NO₂ above the WHO air quality reference guideline of an annual level of 40 µg/m³ (48).

Across all the four study areas, estimated levels of PM_{2.5} and NO₂ were on average higher during winter as compared to summer. Similar higher annual mean NO₂ levels during the cold period (May – August) have been reported in a previous study here in Cape Town (30.7 µg/m³ vs. 22.2 µg/m³) measured between 2001 and 2006 from three stationary monitors around the city (56). The higher levels of pollutants in winter can be partly explained by the heating patterns during winter. More than half of the homes in this current cohort used paraffin as the major source of fuel for cooking and heating across the four communities (data shown in Table 5.1 in chapter 5). A common practice in these communities during winter is the burning of tyres outside the home where family members tend to sit around to keep warm. Another possible explanation for the lower levels of NO₂ might be the closure of an oil refinery, a major source of NO₂ in Marconi-Beam, during the LUR summer campaign measurements. It should also be noted that the average wind speed in Cape Town is higher during the summer months (monthly average of 6 m/s), as compared to those recorded in the winter months (monthly average of 3 m/s). Thus, the higher wind speed in summer months disperses air pollutants causing lower measured levels, as compared to pollutant stagnation in winter months, resulting in higher measured levels.

In this study, an inverse correlation between average annual temperatures, PM_{2.5} and NO₂ was observed. Of importance is the correlation between the different pollutants measured. We reported a low correlation between the measured criteria pollutants (PM_{2.5} vs. NO₂, $r = 0.33$, $p > 0.05$) while a strong positive correlation was observed between PM_{2.5} and *Alternaria* ($r = 0.89$, $p < 0.01$). A strong negative correlation was also observed between PM_{2.5} and *Ascospores* ($r = -0.96$, $p < 0.01$) and *Cladosporium* ($r = -0.76$, $p < 0.01$). Such strong correlations were taken into account when building multi-pollutant models in investigating the effects of both air pollutants and fungal spores on the respiratory health of children reported in the subsequent chapters in this thesis.

4.5.2 Airborne pollen and fungal spores levels in the 4 study areas

Due to the relatively low levels of tree, grass and weed pollen recorded in the four study areas, exposure, predominantly to fungal spores (*Alternaria*, *Ascospores* and *Cladosporium*) were considered further in both the panel and cohort study based on their abundance as observed in the sampling and their known clinical significance in causing allergic symptoms. The highest peak for total fungal spores (34,823.52 spores/m³) in the 4 areas was recorded in Masiphumulele during the winter month of July. Although not applicable to all the study areas, fungal spores have been reported in various sites across the world, to be present all year round. Fungal spores are ubiquitous in nature, and thus are present in all part of the world both in the warm season with low humidity and in cold season with high humidity. Xerophilic spores such as *Alternaria* and *Cladosporium* are predominantly common during the warm season, while hydrophilic spores such as *Ascospores* are commonly found during the colder months (30).

The occurrence of ambient fungal spores throughout the year reported in this study is consistent with findings from other parts of the world. For example, a large study comparing spores level in 12 central and eastern European countries found spores present all through the year (50). In this current study for instance, although the highest concentration of 216 spores/m³ for *Alternaria* was recorded in Marconi-Beam during the winter phase of the panel study, on average, the levels of *Alternaria* were higher in the summer than in the winter phase in all the 4 areas. However, the spikes recorded in winter can be partly explained by the drought currently experienced in the Western Cape Province of South Africa with dry warmer winter days. The level recorded in Marconi-Beam was twice the threshold of 100 spores/m³ to elicit allergic symptoms for *Alternaria* (50) with an addition of two days recorded above this threshold in the summer phase in Marconi-Beam. In the cohort study, 34 of the 52-weeks (65.4%) in Marconi-Beam had levels above the threshold for *Alternaria*.

Except during the cold weeks, as expected (due to xerophilic nature of *Alternaria*), levels of *Alternaria* were generally higher than the threshold all year round, in all four study areas. Various studies have shown that the concentration of spores in the air determines the intensity of allergic reaction for participants exposed to *Alternaria*. A threshold of 100 spores/m³ has generally been used commonly as the threshold level required to elicit the first allergic reaction, with severe inhalant allergic symptoms observed at a level as high as 300 spores/m³ (50). However, lower threshold levels of 50 spores/m³ have also been used in other studies (57,58). In this study, the peak *Ascospores* concentration was recorded during the winter months that had the highest humidity levels, while *Alternaria* concentration peaks were recorded during months with relatively lower humidity and high temperature. Similar patterns were observed in a five year time-series study comparing the distribution of fungal spores in Worcester, England (57). In this current study, we reported a strong negative correlation between *Ascospores* and *Alternaria* ($r = -0.93, p < 0.01$). Such inverse relationship between *Alternaria* and *Ascospores*, could be explained by their respective association with humidity (59). In this study, there was a strong negative correlation between humidity and *Alternaria* ($r = -0.86, p < 0.01$), and a strong positive correlation with *Ascospores* ($r = 0.81, p < 0.01$). These relationships between the fungal spores were also considered to avoid multicollinearity when building multi-pollutant models.

In the panel study, the daily concentrations of *Cladosporium* were generally higher in summer as compared to the winter phase, except in Oudtshoorn where the allergic symptom threshold of 3000 spores/m³ for *Cladosporium* was exceeded twice during the winter phase in Oudtshoorn (50). It should be noted that Oudtshoorn has drier winters with relatively lower humidity as compared to the other three areas due to its semi-arid climatic nature, coupled with the extended drought currently experienced in the Western Cape. Across the 52-week measurement period in the cohort study, Oudtshoorn had the highest level of

Cladosporium with a maximum concentration of 29,591.28 spores/m³ recorded at the beginning of summer. Seven of the 52 weeks (13.5%) had levels above the allergic symptoms threshold for *Cladosporium* of 3000 spores/m³ in Oudtshoorn. However, the proportion of weeks per annum above the threshold of *Cladosporium* is similar to that measured in a five year study comparing fungal spores' distribution in Worcester, England. In the latter study, the proportions of *Cladosporium* levels measured above the threshold was 11.1% (that is; 40 of 360 days) in year 2007 and 2008, and 22,2% (80 of 360 days) in year 2006 and 2010 (57).

4.5.3 Strength and limitations of the exposure assessments

Very few epidemiological studies of air pollution conducted in Africa have used advanced exposure assessment methods such as land-use regression, making the quantification of the health effect of air pollution difficult to compare with those from the developed countries in which these methods were employed (28). This study is therefore bolstered by the use of LUR to estimate the annual exposures to criteria pollutants such as PM_{2.5} and NO₂ in an under-resourced and under-research informal communities in the Western Cape Province of South Africa. The use of the LUR takes into account both the spatial and temporal nature of air pollutants within the geographical boundaries being modelled or estimated. However, the use of monitoring stations outside the study area of Masiphumulele and Oudtshoorn, in adjusting-for temporal trends in the LUR model could have affected the predictive power of the model. Routinely monitored air quality within these areas was either incomplete or not available. The assessment of air pollutant exposure mostly at the children's home, using LUR annual estimates fails to incorporate space (school) and time activity patterns as compared to personal monitoring (which is especially challenging in children). The measurement of airborne pollen and fungi were obtained from a single monitoring site in each area, thereby introducing possible area-level bias due to lack of spatial variation and exposure misclassification, as time-activity patterns were not available. It is unclear to what extent these factors effect exposure assessment in this study but the participating school children in each area are probably exposed to similar levels of biological pollutants due to compulsory school time table and lack of air conditioning facilities in the class rooms of participating school children.

4.6 Conclusion

This chapter has provided information on the levels of ambient air pollutants, airborne pollen, and fungal spores in informal housing settings of participating school children using standardised and robust measurement protocols. Amongst all the biological pollutants measured, fungal spores of *Alternaria*, *Ascospores* and *Cladosporium* were the most prevalent in the study areas. Although estimated NO₂ levels are generally below the WHO ambient air quality reference guidelines, over a third of school children in

this study were exposed to PM_{2.5} levels above the WHO guidelines and allergic symptoms threshold levels for *Alternaria*. With the effects of aeroallergens present in fungal spores hypothesized to be exaggerated in the presence of air pollutants, future chapters will investigate the independent and combined effect of air pollutants and fungal spores on the respiratory health of school children in these communities, taking into consideration meteorological factors that have been identified to potentially confound such relationships.

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CHAPTER 5

Asthma-related outcomes associated with indoor air pollutants among schoolchildren from four informal settlements in two municipalities in the Western Cape Province of South Africa

Abstract

The health impact of indoor air pollution in informal settlement households has not been extensively studied in South Africa. This cross-sectional study investigated the association between asthma and common indoor exposures among schoolchildren from four informal settlements located in two municipalities in the Western Cape Province. A total of 590 children, aged 9 – 11 years, were recruited. The International Study of Asthma and Allergies in Children (ISAAC) questionnaire, was administered to caregivers. Pulmonary function assessment included spirometry and fractional-exhaled nitric oxide (FeNO). Phadiatop test for atopy was done. The prevalence of doctor-diagnosed asthma was 3.4% (n=20) among whom only 50% were on treatment. The prevalence of current wheeze was 12.9% and 17.6% had airway obstruction ($FEV_1 < \text{lower limit of normal}$) while 10.2% had airway inflammation ($FeNO > 35 \text{ ppb}$). In adjusted logistic regression models, dampness, visible mould growth, paraffin-use for cooking, and passive smoking were associated with a two to three-fold increased risk in upper and lower airway outcomes. The strongest association was that of visible mould growth with rhinitis (adjusted odds-ratio – aOR 3.37, 95% CI: 1.69 – 6.71). Thus, there is a need for improved diagnosis of childhood asthma and indoor air quality in informal settlement households.

5.1. Introduction

South Africa currently ranks 25th globally with regard to asthma prevalence and has the fifth highest number of deaths due to asthma amongst children.¹ However a high prevalence of undiagnosed and untreated childhood asthma has been reported previously in Cape Town especially among individuals from low socio-economic backgrounds.² It was reported that 47% of asthmatic children were unrecognized and only 3% of these children were on daily treatments.² Townend *et al.*, have also reported a high prevalence of airway obstruction, to be significantly associated with poverty in several countries.³ At the international level, the importance of adequate, accessible and sustainable housing in the cities by 2030 is one of the targets of the recent Sustainable Development Goal on sustainable cities.⁴ While at the national level, housing the poor is an integral part of developmental policies. For instance, according to human settlements vision 2030 of South Africa and within the last 20 years, 2.8million housing units have been delivered by the government.⁵ However, according to the 2011 census, 13% of all households in South Africa still dwell in shacks and informal dwellings. In 2014, despite the effort of the South African Department of Housing to resettle people living in informal settlements, it was reported that one in four South Africans still lives in informal dwellings, with a potential increase to one in three by 2050.⁶ This number is increasing daily in Western Cape Province, with the movement of people from other provinces or rural areas into urban settings. The housing structure and sanitation including the quality of life remains a big worrying factor in these rapidly growing settlements.⁵

Asthma exacerbation and episodes occur mostly at home, especially during the weekends indicating the indoor home environment to be an important contributor to asthma-related outcomes.⁷ It has been shown that asthma symptoms get worse at certain times of the year, while severe storms or sudden weather change can trigger asthma symptoms.⁸ The weather and climate of the Western Cape is dominated by cold wet winters and hot dry summers, and can lead to increased asthma symptoms amongst vulnerable groups, such as young children and the elderly. The World Health Organization estimates that, household air pollution from biomass fuel, together with second-hand tobacco smoke and household-related risk such as mould are responsible for over 50% of respiratory morbidities in children under five years in low- and middle-income countries.⁹ Indoor allergen and chemical exposures are the most important household risk factors associated with asthma and allergy severity.¹⁰ However, there is inconsistent evidence of the effects of allergen exposure from pets on childhood asthma as found in a systematic review by Chen *et al.*¹¹

A recent review by Patelarou *et al.*, suggested insufficient evidence for quantitative dose-response relationships between asthma outcomes and indoor air chemical and bio-aerosol exposures, except for endotoxin and mould exposure indicators for which there was good evidence for childhood wheeze and asthma respectively.¹² However, this review focused primarily on industrialised developed countries, which

appear to have wide contrasts in the composition of household indoor air pollutants compared to developing countries. While the composition of some of these pollutants may have similarities to more established households in South Africa, bio-aerosols and indoor chemical air pollutant concentrations in informal households are likely to vary substantially in composition due to household materials, cleaning habits, ventilation and different patterns of fuel-use for cooking and heating.

The aim of the study in this chapter was to determine the prevalence of childhood asthma-related outcomes and to investigate their association with indoor air quality exposures, through a cross-sectional study undertaken in four informal settlements located in two municipalities of the Western Cape province of South Africa.

5.2. Methods

5.2.1. Study Population

This analysis forms part of a baseline study of a larger cohort investigation of the effects of both outdoor air pollutants and airborne fungal spores on childhood asthma in schoolchildren from these communities. This study also forms part of a broader study funded by the Western Cape Department of Environmental Affairs and Development Planning (Study Tender: Conduct Comprehensive Human Health Risk Assessment (HRA) Studies within identified areas across the Western Cape, Ref: EADP7/2013). A detailed study protocol has been published elsewhere.¹³ In summary, 590 fourth grade schoolchildren were recruited from six primary schools located in four informal settlements from two municipalities in the Western Cape Province of South Africa. Three of these communities (Khayelitsha, Marconi-Beam, and Oudtshoorn) were selected based on a previous ‘needs analysis’ aimed at undertaking a human health risk assessment (HHRA) of susceptible population groups that are affected by air pollution in the province.¹⁴ An additional area (Masiphumulele), with relatively low air pollution levels was included to reflect contrasting air quality. However, all four informal settlements have similar and comparable socio-demographic profiles. There is some spatial variability in that the three study areas (Khayelitsha, Marconi-Beam, and Masiphumulele) are located about 20km from Cape Town metropolis, having a distinct coastal climate, whereas the fourth area (Oudtshoorn) located inland in the Karoo region about 500km from Cape Town, has a semi-arid climate. A list of primary schools in the selected communities were obtained, and information letters about the study was sent to school principals, followed by a detailed information session in the respective schools. The specific selection of grade-4 pupils was to include participants that are old enough to perform acceptable lung function test manoeuvres. Lung function pre-test questionnaire was administered to consenting children. An average total of 150 schoolchildren (in accordance with the sample-size calculation¹³) were recruited from each of the four communities, and selection was based on passing the pre-test lung function questionnaire. Qualifying criteria include children without 1) any recent operation in the last 12 months, 2)

any pain or nausea, 3) history of epilepsy. In scenarios where the number of qualifying pupils were more than 150 per area, random numbers were used for random selection.

Approval was granted by the University of Cape Town's Human Health Research Ethics Committee (HREC REF: 697/2014) and permission to include schoolchildren was obtained from the Western Cape Department of Education. Consent was obtained from each participating child's parent or guardian prior to questionnaire administration (appendix S5.1 – caregiver informed consent form), including an additional one from the child (appendix S5.2 – child assent form) prior to performance of health tests. All information was kept confidential and anonymity was maintained through unique participant identifiers used throughout the study.

5.2.2. Data Collection

The baseline fieldwork was conducted during February and September 2015. More specifically, the fieldwork was completed for an average of two weeks in each study area; Khayelitsha in February, Oudtshoorn in March and April, Marconi-Beam in July, and Masiphumulele in September of 2015. These differences in data collection period was accounted for in the analyses. A total of 898 grade-4 schoolchildren were eligible to participate from the six schools, from which 642 (response rate of 71.5%) parents or guardian consented to their child or ward participating in the study. Following the lung function pre-test questionnaire, and random selection, completed data from 590 participants were finally obtained and analysed.

The validated standardised International Study of Asthma and Allergies in Children (ISAAC) questionnaire was administered to parent or caregivers of participating children to obtain; information on demographic characteristics including birth history and various household characteristics such as leaks, dampness, visible mould growth, fuel-type for cooking and heating and cigarette smoke exposures, as well as detailed information on their medical history and respiratory symptoms (appendix S5.3 – caregiver questionnaire). Pulmonary function assessments comprising spirometry and fractional-exhaled nitric oxide (FeNO) measurements according to the American Thoracic Society (ATS) guidelines¹⁵ were conducted during school time. Atopy status was determined using the Phadiatop test on serum collected from each child.

Data collected from the ISAAC questionnaire for the outcomes of interest included: parental or caregiver report of doctor diagnosis of asthma, current wheezing in the past 12 months, presence of ocular-nasal symptoms in the past 12 months, and rhinitis defined as the presence of either itchy eyes or running nose or sneezing in the absence of cold or flu in the last 12 months. Asthma symptom score (0 – 8) was generated from a sum of 8 positive responses to asthma-like symptoms obtained from the ISAAC questionnaire, and a high score was defined as two or more symptoms.^{16,17} The objective outcomes obtained from pulmonary

function testing included the forced-expiratory volume in one second (FEV₁), forced vital capacity (FVC), FEV₁/FVC, and the forced mid-expiratory flow (FEF₂₅₋₇₅), including their respective cut-off values below the lower limit of normal according to the Global Lung Initiative (GLI) equations.¹⁸ The presence of significant airway inflammation was assessed through high FeNO levels defined as being >35ppb.¹⁹ Atopy was defined as a positive Phadiatop test using a cut-off of ≥ 0.35 PAU/L.²⁰

5.2.3. Statistical analyses

All data analyses were done using statistical package STATA version 14.2. The prevalence of asthma-related outcomes and proportions of risk factors including household characteristics were computed by dividing the number of positive response to a certain question (or case definition for pulmonary function) by the total number of answers obtained for this specific question (or children available for that particular test). Hence, the sample size varies slightly for each question or tests. Univariable logistic regression was used to assess the association of each host characteristics identified *a priori* with the respective outcome of interest. Saturated multiple logistic regression models were used to explore the association of individual indoor exposures with the various asthma-associated outcomes. These models were adjusted for individual host characteristics such as age, gender, body mass index, prenatal maternal smoking, birth weight, atopy, and study area. Study areas was included to capture any area-specific variation (such as season of the year during which lung function was done) or characteristics not captured in the model and the other variables were selected *a priori* from the literature. The consistency in the variables adjusted for in the various models (that is; saturated model) was to enable the comparisons of the risk estimated from each indoor exposure model. An additional model building approach (sensitivity analysis) was employed to test the reliability of the above model strategy. This involved a stepwise selection of covariates, and testing the contribution of each variable using a likelihood ratio and the Akaike Information Criteria (AIC). Important predictor variables indicative of indoor household exposures explored include: dampness, presence of visible mould growth, pets in the home, smokers in the home, and the use of paraffin for cooking and heating. Each of these indoor exposures were assessed for their respective association with the individual asthma-associated outcome of interest.

5.3. Results

The median age of children in the study was 10 years, with an approximately equal proportion of male and female children (49.5:50.5). The majority of children (96.3%) lived in the same house since birth (data not shown). Children residing in Oudtshoorn had the lowest prevalence of atopy (28.2%), but the highest prevalence of exposure to prenatal maternal smoking (23.2 %) compared to those in the other three areas. On the other hand, children residing in Masiphumulele had the highest prevalence of visible mould growth (15.6%) and dampness (13.9%) in their homes (Table 5.1). The most common fuel type for cooking and

heating was electricity and paraffin, with minimal (below 5% reported) or no use of other fuel types such as wood, animal dung, and gas (data not shown). However, children residing in Marconi-Beam reported the lowest use of electricity (42.7%) and the highest use of paraffin (74.7%) compared to the other three areas.

The prevalence of respiratory symptoms and asthma-related outcomes including pulmonary function results are presented in Tables 5.2 and 5.3 respectively. While 12.9% of the responding parents or caregivers reported their child or ward to have current wheeze, only 3.4% reported doctor-diagnosed asthma and only 1.7% were on asthma medication (that is, only 50% of those reported to be diagnosed with asthma). A relatively higher proportion, 10.2% and 17.6% were classified as having clinically significant airway inflammation ($\text{FeNO} > 35\text{ppb}$) and airway obstruction ($\text{FEV}_1 < \text{LLN}$) respectively. Female children had relatively smaller lung volumes than their male counterparts. Furthermore, they also demonstrated a significant higher prevalence (23.7% vs. 14.4%, $p < 0.01$) of small airway obstruction ($\text{FEF}_{25-75} < \text{LLN}$) but male children were more likely to have current asthma symptoms including severe asthma (Table 5.3 and appendix Table S5.4).

Age, sex, and atopic status were strongly associated with asthma and related outcomes (table 5.4). Being a female child increased the likelihood (aOR: 1.84, 95%CI: 1.18 – 2.89) of having small airway obstruction ($\text{FEF}_{25-75} < \text{LLN}$), while older age increased the odds of having airway inflammation (aOR: 1.39, 95%CI: 1.05 – 1.85). Similarly, atopic children were four-times more likely (aOR: 4.44, 95%CI: 2.08 – 9.51) to have an asthma symptom score (ASS) ≥ 2 and as expected more likely of having high FeNO ($\text{FeNO} > 35\text{ppb}$). Conversely, reported prenatal smoking was associated with a decreased odds of having a high FeNO (aOR: 0.23, 95%CI: 0.07 – 0.74).

In the multiple regression models to assess the effect of indoor household exposures, dampness and visible mould growth significantly increased asthma-related outcomes (Table 5.5). The presence of dampness in the home was associated with a two-fold increased odds of current wheeze (aOR: 2.60, 95%CI: 1.18 – 5.71). A similar trend was observed between rhinitis and household dampness (aOR: 3.00 95%CI: 1.47 – 6.13) as well as visible mould growth (aOR: 3.37 95%CI: 1.69 – 6.71).

Paraffin use for cooking/heating and passive smoking were also significant risk factors for asthma outcomes. Paraffin-use was associated with a two-fold increased likelihood of having significant airway inflammation (aOR: 2.31, 95%CI: 1.05 – 5.06) and an increased risk of rhinitis (aOR: 1.69 95%CI: 1.05 – 2.70). Furthermore, having a smoker in the home significantly increased the odds of current wheeze (aOR: 1.79, 95%CI: 1.02 – 3.15) (Table 5.5). The results of the sensitivity analysis using the stepwise approach were relatively similar to the saturated model and are presented in appendix Table S5.5.

Table 5.1: Demographic characteristics and reported indoor exposures by caregivers of schoolchildren residing in four informal settlements in the Western Cape Province

Characteristics	Khayelitsha	Oudtshoorn	Marconi-Beam	Masiphumulele	All areas
<i>Range of Ns for the variables presented</i>	<i>(n1 = 162, n2 = 147)</i>	<i>(n1 = 168, n2 = 163)</i>	<i>(n1 = 150, n2 = 140)</i>	<i>(n1 = 110, n2 = 111)</i>	<i>(n1 = 590, n2 = 561)</i>
Demographics, n1 (%)					
Age (years)	10 (9 – 10)	10 (9 – 10)	10 (10 – 11)	10 (10 – 11)	10 (9 – 10)
Gender (Male)	70 (45.8)	78 (45.9)	76 (50.7)	68 (58.1)	292 (49.5)
Weight (kg)	30.1 (27 – 35.8)	27.3 (24.8 – 31.2)	33 (29 – 37.6)	33.6 (29.1 – 40)	30.4 (26.8 – 35.9)
Height (cm)	133 (127 – 137)	132 (128 – 137)	137 (133 – 142)	137 (132 – 142)	135 (130 – 139)
Body mass index (Kg/m ³)	17.2 (15.8 – 19.7)	15.7 (14.7 – 17.2)	17.3 (15.9 – 19.3)	17.7 (16.2 – 19.7)	16.9 (15.5 – 17.2)
Atopy (positive Phadiatop), n2 (%)	61 (41.5)	46 (28.2)	61 (43.6)	47 (42.3)	215 (38.3)
Birth history, n1 (%)					
Prenatal maternal smoking	35 (21.6)	39 (23.2)	21 (14.0)	13 (11.8)	108 (18.3)
Birth weight (kg)	3.2 (2.9 – 4.0)	3 (2.6 – 3.2)	3.1 (3.0 – 3.6)	3.6 (3.1 – 3.9)	3.2 (2.9 – 3.6)
Reported indoor exposures, n1 (%)	n = 130	n = 161	n = 135	n = 110	n = 536
Visible mould growth	11 (6.8)	19 (11.4)	5 (3.3)	17 (15.6)	52 (8.8)
Dampness (leaks)	8 (4.9)	12 (7.2)	7 (4.7)	15 (13.9)	42 (7.2)
Pet ownerships	47 (29.0)	43 (25.8)	15 (10.0)	12 (11.1)	117 (20.0)
Fuel for cooking/heating					
Electricity	129 (79.6)	106 (63.5)	64 (42.7)	54 (50.0)	353 (60.1)
Paraffin	63 (38.9)	85 (50.9)	112 (74.7)	67 (62.0)	327 (55.7)
Smoker in the home	49 (30.3)	55 (32.9)	26 (17.3)	34 (31.2)	164 (27.9)

Continuous variables, median (interquartile range); categorical variables, number (%)

Table 5.2: Respiratory symptoms and related disease outcomes of schoolchildren residing in four informal settlements in the Western Cape Province

<i>Range of Ns for the variables presented</i>	Khayelitsha (n1=162, n2=135)	Oudtshoorn (n1=168, n2=161)	Marconi-Beam (n1=150, n2=135)	Masiphumulele (n1=110, n2=110)	All areas (n1=590, n2=541)
Past medical history, n1 (%)					
Doctor-diagnosed asthma *	5 (3.1)	7 (4.2)	4 (2.7)	4 (3.6)	20 (3.4)
Doctor-diagnosed eczema	4 (2.5)	8 (4.8)	12 (8.0)	7 (6.4)	31 (5.3)
Doctor-diagnosed hay fever	2 (1.2)	12 (7.2)	15 (10.0)	7 (6.4)	36 (6.1)
Upper airway symptoms, n1 (%)					
Ocular-nasal symptoms	48 (29.6)	45 (26.8)	29 (19.3)	29 (26.4)	151 (25.6)
Rhinitis	12 (7.4)	26 (15.6)	41 (27.3)	39 (36.1)	118 (20.1)
Asthma-like symptoms, n1 (%)					
Shortness of breath walking with other children on level ground *	4 (2.5)	12 (7.1)	4 (2.7)	5 (4.6)	25 (4.2)
Symptoms history in the past 12 months, n1 (%)					
Current wheeze *	12 (7.4)	21 (12.5)	17 (11.3)	26 (23.6)	76 (12.9)
Wheezing during physical activities *	3 (1.9)	5 (3.0)	2 (1.4)	2 (1.8)	12 (2.1)
Cough during physical activities *	3 (1.9)	4 (2.4)	2 (1.4)	1 (0.9)	10 (1.7)
Chest tightness *	5 (3.1)	7 (4.2)	3 (2.0)	4 (3.6)	19 (3.2)
Sleep disturbances due to wheeze, chest tightness or shortness of breath *	5 (3.1)	6 (3.6)	3 (2.0)	4 (3.6)	18 (3.1)
Use of asthma medication *	5 (3.1)	3 (1.8)	1 (0.7)	1 (0.9)	10 (1.7)
Asthma symptom score, n2 (%)					
Score 0	114 (84.4)	149 (92.6)	116 (85.9)	83 (75.5)	462 (85.4)
Score 1	16 (11.9)	4 (2.5)	5 (3.7)	16 (14.6)	41 (7.6)
Score ≥ 2	5 (3.7)	8 (5.0)	14 (10.4)	11 (10.0)	38 (7.0)

Categorical variables, number (%)

* Asthma symptom score ranging from 0 -8 derived from a sum of positive answers to eight main asthma symptoms and bronchial hyperresponsiveness questions from the ISAAC questionnaire

Table 5.3: Pulmonary function and airway inflammatory indices of schoolchildren residing in four informal settlements in the Western Cape Province stratified by gender

Variables	Male	Female	p-value	Overall
<i>Range of Ns across the variables presented</i>	(n1=257, n2=292)	(n1=261, n2=297)		(n1=518, n2=589)
Spirometric indices, (n1)				
FEV ₁ (litres)	1.63 ± 0.29	1.52 ± 0.28	0.000	1.57 ± 0.29
FVC (litres)	1.91 ± 0.34	1.75 ± 0.30	0.000	1.83 ± 0.33
PEF (l/s)	3.72 ± 0.81	3.63 ± 0.85	0.218	3.68 ± 0.83
FEF ₂₅₋₇₅ (l/s)	1.99 ± 0.61	1.91 ± 0.64	0.102	1.95 ± 0.63
FEV ₁ < 80% Predicted	36 (14.0)	48 (18.4)	0.176	84 (16.2)
FEV ₁ < LLN	42 (16.3)	49 (18.8)	0.467	91 (17.6)
FVC < 80% Predicted	23 (9.0)	26 (10.0)	0.694	49 (9.5)
FVC < LLN	36 (14.0)	28 (10.7)	0.257	64 (12.4)
FEV ₁ /FVC < 0.80	43 (16.7)	34 (13.0)	0.236	77 (14.9)
FEV ₁ /FVC < LLN	29 (11.3)	41 (15.7)	0.141	70 (13.5)
FEF ₂₅₋₇₅ < 80 Predicted	77 (30.0)	110 (42.0)	0.004	187 (36.0)
FEF ₂₅₋₇₅ < LLN	37 (14.4)	62 (23.7)	0.007	99 (19.1)
Airway inflammation (FeNO), (n2)				
Normal (< 15ppb)	172 (58.9)	188 (63.3)	0.274	360 (61.1)
Elevated (15 - 35 ppb)	92 (31.5)	77 (25.9)	0.134	169 (28.7)
High (>35 ppb)	28 (9.6)	32 (10.8)	0.634	60 (10.2)

Continuous variables, mean ± SD; categorical variables, number (%)

FEV₁: forced expiratory volume in 1 sec; FVC: forced vital capacity; PEF: peak expiratory flow; FEF₂₅₋₇₅: forced mid expiratory flow

Predicted values computed using the Global Lung Initiative (GLI) reference equation

Bold text denotes statistical significance at p < 0.05

LLN: lower limit of normal below the 5th percentile

Table 5.4: Association between host factors and asthma-related outcomes among schoolchildren using unadjusted logistic regression models

Outcomes	Age (years)*	Gender (male)	Body mass index*	Prenatal maternal smoking	Birth weight (kg)*	Atopy
	OR (95% CI)					
Doctor-diagnosed asthma	1.01 (0.61 – 1.66)	1.80 (0.70 – 4.63)	0.86 (0.70 – 1.05)	0.78 (0.22 – 2.72)	0.93 (0.44 – 1.98)	1.84 (0.74 – 4.62)
Asthma symptom score ≥ 2	1.25 (0.89 – 1.76)	1.00 (0.52 – 1.93)	1.06 (0.97 – 1.15)	0.70 (0.26 – 1.83)	0.82 (0.48 – 1.43)	4.44 (2.08 – 9.51)
Current wheezing	1.18 (0.91 – 1.53)	1.54 (0.94 – 2.52)	1.02 (0.95 – 1.09)	1.01 (0.54 – 1.88)	1.42 (0.93 – 2.17)	1.34 (0.81 – 2.23)
Ocular-nasal symptoms	0.87 (0.70 – 1.07)	1.10 (0.75 – 1.59)	1.03 (0.97 – 1.08)	0.80 (0.49 – 1.31)	0.85 (0.62 – 1.16)	1.46 (0.99 – 2.16)
Rhinitis	1.27 (1.02 – 1.57)	1.09 (0.73 – 1.64)	1.02 (0.97 – 1.08)	0.59 (0.33 – 1.06)	2.20 (1.51 – 3.20)	1.02 (0.67 – 1.55)
FEV ₁ < LLN	1.19 (0.94 – 1.51)	0.85 (0.54 – 1.33)	0.93 (0.87 – 1.01)	1.09 (0.61 – 1.95)	1.19 (0.80 – 1.76)	0.97 (0.60 – 1.57)
FVC < LLN	1.19 (0.90 – 1.56)	1.36 (0.80 – 2.30)	0.87 (0.78 – 0.96)	0.94 (0.47 – 1.87)	1.21 (0.77 – 1.90)	0.66 (0.36 – 1.18)
FEV ₁ /FVC < 0.80	1.05 (0.81 – 1.36)	1.34 (0.82 – 2.18)	1.01 (0.94 – 1.08)	1.01 (0.54 – 1.90)	1.12 (0.74 – 1.69)	1.60 (0.97 – 2.63)
FEV ₁ /FVC < LLN	0.97 (0.74 – 1.28)	0.68 (0.41 – 1.14)	1.05 (0.98 – 1.12)	1.16 (0.62 – 2.19)	1.25 (0.81 – 1.94)	1.31 (0.77 – 2.22)
FEF ₂₅₋₇₅ < LLN	1.09 (0.86 – 1.38)	0.54 (0.35 – 0.85)	1.01 (0.95 – 1.07)	0.85 (0.47 – 1.54)	1.26 (0.86 – 1.85)	1.08 (0.69 – 1.71)
FeNO > 35 ppb	1.39 (1.05 – 1.85)	0.94 (0.55 – 1.62)	1.07 (1.00 – 1.45)	0.23 (0.07 – 0.74)	1.38 (0.85 – 2.23)	34.55 (10.62 – 112.40)

OR: Odds ratio; each OR is a separate unadjusted regression model

*association expressed per 1 unit increase, while the binary variables have the reference as NO (except in gender which have female as the referent group)

Bold text denotes statistical significance at $p < 0.05$

FEV₁: Forced expiratory volume in 1 sec; FVC: Forced vital capacity; PEF: peak expiratory flow; FEF₂₅₋₇₅: Forced mid expiratory flow

FeNO: Fractional exhaled nitric oxide

LLN: lower limit of normal below the 5th percentile

Table 5.5: Associations between indoor household exposures and asthma-related outcomes among schoolchildren using saturated adjusted logistic regression models

Outcomes	Dampness (leaks)	Visible mould	Pets in the home	Paraffin use	Smokers in the home
	OR (95% CI)				
Doctor-diagnosed asthma ^a	NE	1.83 (0.38 – 8.89)	1.26 (0.37 – 4.24)	1.50 (0.52 – 4.36)	0.32 (0.07 – 1.47)
Asthma symptom score ≥ 2 ^a	1.99 (0.66 – 6.03)	0.49 (0.11 – 2.27)	1.90 (0.76 – 4.71)	0.73 (0.34 – 1.57)	0.63 (0.24 – 1.63)
Current wheeze ^a	2.60 (1.18 – 5.71)	1.05 (0.43 – 2.55)	0.88 (0.42 – 1.81)	1.52 (0.87 – 2.67)	1.79 (1.02 – 3.15)
Ocular-nasal symptoms ^a	1.03 (0.49 – 2.19)	1.85 (0.97 – 3.54)	1.04 (0.63 – 1.73)	1.10 (0.73 – 1.67)	0.60 (0.38 – 0.97)
Rhinitis ^a	3.00 (1.47 – 6.13)	3.37 (1.69 – 6.71)	0.97 (0.52 – 1.80)	1.69 (1.05 – 2.70)	1.15 (0.69 – 1.91)
FEV ₁ < LLN ^b	0.39 (0.11 – 1.31)	0.69 (0.28 – 1.72)	1.41 (0.78 – 2.57)	0.96 (0.58 – 1.59)	1.00 (0.58 – 1.74)
FVC < LLN ^b	0.42 (0.10 – 1.80)	1.51 (0.63 – 3.65)	1.13 (0.55 – 2.31)	1.10 (0.61 – 1.97)	0.71 (0.36 – 1.42)
FEV ₁ /FVC < 0.80 ^b	1.03 (0.40 – 2.63)	0.67 (0.25 – 1.79)	1.52 (0.81 – 2.84)	1.46 (0.85 – 2.51)	0.71 (0.38 – 1.30)
FEV ₁ /FVC < LLN ^b	0.98 (0.36 – 2.69)	0.78 (0.29 – 2.11)	1.63 (0.85 – 3.11)	1.55 (0.87 – 2.75)	0.84 (0.45 – 1.57)
FEF ₂₅₋₇₅ < LLN ^b	1.14 (0.49 – 2.65)	0.75 (0.32 – 1.76)	1.07 (0.58 – 1.95)	1.15 (0.71 – 1.86)	0.70 (0.40 – 1.23)
FeNO > 35 ppb ^a	0.93 (0.29 – 2.93)	0.42 (0.08 – 2.09)	0.81 (0.29 – 2.24)	2.31 (1.05 – 5.06)	0.52 (0.21 – 1.29)

OR: Odds ratio; each OR is a separate adjusted logistic regression model

^a controlling-for age, gender, body mass index, prenatal maternal smoking, birthweight, atopy and study area, all fitted in a saturated model

^b controlling-for only prenatal maternal smoking, birthweight, atopy and study area, since these variables take into account age, gender and height in computing their respective cut-off

Bold text denotes statistical significance at $p < 0.05$

FeNO: Fractional exhaled nitric oxide FEV₁: Forced expiratory volume in 1 sec; FVC: Forced vital capacity; PEF: peak expiratory flow; FEF₂₅₋₇₅: Forced mid expiratory flow

LLN: lower limit of normal below the 5th percentile

5.4. Discussion

The reported prevalence of 3.4% doctor-diagnosed asthma in this current study falls at the lower end of the prevalence range found in previous studies using similar validated ISAAC questionnaires. However, the prevalence of doctor-diagnosed asthma reported in this study is within the range of those reported in the global paediatric asthma prevalence surveys on African children with reported proportions from 2.5% in Ethiopia and Burkina Faso to almost 10% in Swaziland.²¹ The prevalence of doctor-diagnosed asthma from recent cross-sectional studies from other low-middle-income countries were as high as 7.5% in Iran²², 6% in India²³, 4.8% in China¹⁰, 3% in Senegal²⁴, 2.5% in Mongolia²⁵, to 2% in Georgia.²⁶

Moreover, significant differences in asthma prevalence have been noted between urban and rural children of similar age group.²¹ For example, in this current study, the proportion of children residing near an urban area (Marconi-Beam) with two or more asthma symptom score (ASS ≥ 2) is twice as high as compared to those residing in a rural area of Oudtshoorn (10.4% vs. 5.0%). This difference could be due to the presence of a petrochemical refinery in close proximity to Marconi-Beam.²⁷ Similarly, in a previous study in Cape Town, South Africa, a lower asthma symptoms occurrence was reported in rural children (17%) as compared to urban children (34.4%).²⁸ The prevalence of childhood asthma also varied from 1.4% to 3.5% in rural areas and 1.5% to 4.1% in urban areas of Eastern European countries such as Poland, Ukraine, and Belarus.²⁹

However, the reported prevalence of current wheeze (12.8%) in this study was lower than ranges reported elsewhere in South Africa; 18.2% in Tembisa and Kempton Park of Gauteng Province³⁰, 20.3% in Cape Town of Western Cape Province, 27.6% in Polokwane of Limpopo Province and 24.5% in Durban of KwaZulu Natal Province.³¹ It should be noted that, these other South African studies were conducted in highly industrial areas, therefore the prevalence of current wheeze is expected to be higher than the current study.

In comparison to other large studies from the southern hemisphere, a higher prevalence of current wheeze (32.6%) was reported in the ISAAC phase III study in Wellington, New Zealand amongst children aged 13-14 years in the written questionnaire.³² Although a large variation in asthma symptoms has been reported in global studies using the ISAAC questionnaire³³, this study found the prevalence of reported asthma symptoms (current wheeze, 12.8%) to be similar to the more objective measures generally used (airway obstruction identified on spirometry, 17.6% and airway inflammation identified by high FeNO, 10.2%) in this study. This represents a strength of this study using a constellation of study tools to measure asthma morbidity.

An important finding in this study was the high proportion of under-diagnosed and untreated asthma in the schoolchildren residing in informal settlements that were studied. While the prevalence of doctor-diagnosed asthma was surprisingly low (3.4%) and only 50% among them on asthma treatment, a much higher prevalence of wheezing in the past 12 months (12.9%), airway obstruction (17.6%) and airway inflammation (10.2%) was found. The under-recognition and under-treatment of asthma among schoolchildren in Cape Town residing in low income communities in South Africa has been reported over two decades ago.² More recently, a Nigerian study of low-income communities also found a high proportion of under-diagnosed childhood asthma with 2.2% reporting doctor-diagnosed asthma in relation to 24.4% having possible asthma (asthma-related symptoms).³⁴ The findings of these studies support the conclusions of a recent review that there is considerable under-diagnosis and under-treatment of asthma in low-income countries and that this is responsible for the misreporting of the global burden of asthma published by the World Health Organization in 2014^{35,36} and that from the ISAAC Phase III study.³² The high proportions of undiagnosed and untreated asthma in low-income communities are indicative of poor health care utilization uptake as well as poor identification of asthma symptoms compounded by limited access to asthma controller medication.^{2,34,35}

This study also found that the use of paraffin for cooking and heating significantly increased the risk of having airway inflammation (FeNO > 35 ppb), and was significantly associated with rhinitis. Paraffin, together with electricity is the most common source of energy in informal settlement communities in South Africa, as well as in many other low-and middle-income countries, among communities of low socio-economic status.³⁷ The level, duration and time pattern of exposure in microenvironments, determines the extent of personal exposure to pollutants from sources such as paraffin, since children spend considerable proportion of their time indoors.³⁸ This is consistent with other studies that estimated indoor air pollution from biomass fuel-use including paraffin. A recent study of Nigerian rural schoolchildren reported an increased risk of severe asthma symptoms (OR: 2.37, 95% CI: 1.16 – 4.84) with biomass fuel-use.³⁹ Here in South Africa, schoolchildren aged 13 – 14 years residing in homes using biomass fuel for cooking in Polokwane, had an increased risk of asthma (OR: 1.50, 95% CI: 1.09 – 2.10).⁴⁰ Another study of 1074 primary schoolchildren in Serbia found that children with greater exposure to by-products of combustion from biomass-fuel reported a higher prevalence of respiratory symptoms.⁴¹

However, there is currently insufficient evidence of the effect of quantitatively measured chemical indoor air pollutants on asthma outcomes with even fewer studies using objective measures of indoor air pollutant characterisation to better understand these risks. For example, Van Strien *et al.*, found that days of wheezing increased with indoor NO₂ levels in homes (RR: 2.20 95% CI: 1.40 – 3.40)⁴², while Tavernier *et al.*,⁴³ and Raaschou-Nielsen *et al.*,⁴⁴ were unable to demonstrate an association between high levels of

indoor NO₂ and the risk of asthma and wheezing, respectively. However, it is difficult to determine the contribution of outdoor air pollution (including allergen and chemical pollutants) to the indoor environment or the degree of exposure a child is exposed to outside the home. There is therefore a need for studies that further characterise indoor and outdoor air pollutant exposures, in order to objectively evaluate their effect on asthma outcomes in children.

Other notable indoor exposures found to be significantly associated with asthma-like outcomes were dampness and visible mould growth. This study found schoolchildren from low-income informal settlements residing in houses with damp conditions and visible mould growths to have an increased risk of rhinitis and wheeze. Previous studies that found dampness and mould as a major source of indoor allergens associated with increased risk of asthma development and exacerbation in children were mostly conducted in Europe and North America, which thus have varying aeroallergen composition as compared to those in the southern hemisphere. However, the findings of this study are consistent with those reported in previous studies. In a meta-analysis of 31 studies, visible mould was significantly associated with an increased risk of rhinitis (OR: 1.82, 95%CI: 1.56 – 2.12).⁴⁵ Elsewhere, a 53% increased odds of wheeze from household dampness and mould was found independent of allergy status in a meta-analysis of 17 international studies.⁴⁶ A larger meta-analysis of more than 200,000 children from 61 studies also found a 68% and 49% increase odds of wheeze and asthma respectively in children residing in houses with visible mould.⁴⁷ The result of our study supports the evidence of the role of microbial causal agents and justifies the prevention of indoor dampness and mould, which in turn will reduce symptoms such as rhinitis. The relationship between dampness and mould for the development of asthma was evident in a meta-analysis of 16 studies, which found a significant association of dampness (effect estimates – EE: 1.33, 95%CI: 1.12 – 1.56) and visible mould (EE: 1.29, 95%CI: 1.04 – 1.60).⁴⁸

In this study, an investigation of host-related risk factors demonstrated a relatively higher prevalence (23.2%) of prenatal maternal smoking among schoolchildren in Oudtshoorn compared to the other study areas. This is consistent with that reported in women from the rural Western Cape.⁴⁹ However, the Oudtshoorn children had a relatively lower prevalence of atopy (28.2%) compared to the other study areas (range: 41.5% - 43.6%). The lower prevalence of atopy in these children may partly be due to the more rural in-land geographical location of Oudtshoorn compared to the coastal location of the other study areas with different aeroallergen exposures. These findings are also consistent with the lower prevalence of airway inflammation (FeNO > 35ppb), in Oudtshoorn children (2.4%) compared to the other areas (range: 8.5% - 16%). Atopy is a known risk factor associated with airway inflammation,²⁰ which was evident in this study as atopic children were significantly more likely to have high FeNO (> 35 ppb).

Gender differences in asthma-related outcomes have been well documented in the literature.^{50,51} In this study, female children had a higher prevalence of both large ($FEV_1 < LLN$) and small airway obstruction ($FEF_{25-75} < LLN$), while male children were more likely to have asthma symptoms and more severe asthma (defined by asthma medication-use and sleep disturbances due to wheezing, chest tightness, or shortness of breath). The latter findings are consistent with other studies in the literature, which report that the prevalence and risk of asthma to be higher in male children before puberty and higher in female children post puberty.⁵¹

Nonetheless, there are few limitations to the study that require some consideration. Although the majority of the children in this study reported to have lived in the same house since birth, the cross-sectional nature of the analyses may have limited the inference of causality by the risk factors identified. Furthermore, the possibility of recall bias in reporting respiratory symptoms in the last 12 months may have led to possible outcome misclassification. However, this bias was reduced as objective measures such as spirometry and FeNO corroborated the subjective information obtained from the questionnaires. Furthermore, there might have been reporting bias due to social desirability in reporting known unhealthy behaviours such as smoking habits including maternal smoking. The challenges of performing spirometry in children are well known.⁵² However, quality control was achieved with ongoing training of the field staff and post spirometry quality control checks were done by an experienced lung function technologist to ensure that all spirograms used in the data analysis conformed to the American Thoracic Society (ATS) standards.^{15,53} A possible limitation in this study was also the subjective measure of indoor exposures of potential allergens and chemical pollutants from cooking fuels, which was not corroborated with objective environmental measurements. However, our findings are similar to other studies that have used similar subjective assessments of indoor pollutants.^{24,34,54-56}

5.5. Conclusion

The study in this chapter found that there is likely a high proportion of under-diagnosed and untreated asthma amongst schoolchildren from informal settlements areas in the Western Cape Province of South Africa, as extrapolated from the cross-sectional study undertaken. The study also provided circumstantial evidence that schoolchildren from low-income informal settlements residing in houses with damp conditions and visible mould growth had an increased risk of rhinitis and wheeze. The study further provided circumstantial evidence that the use of paraffin for cooking and heating was associated with an increased risk of rhinitis and airway inflammation. Seasonal differences in the association between air pollution and childhood respiratory health including asthma is further explored in other research outputs of this study.

In the absence of providing new housing, recommendations include interventions to improve housing structure or redesign of new housing contemplated for these communities such as more effective ventilation

to reduce dampness and mould growths. Such interventions would also lead to a reduction in the concentration of chemical pollutants from the use of paraffin for cooking and heating in such microenvironments. Additionally, interventions such as indoor dust control, avoidance of indoor tobacco smoking, and improved ambient air quality management will contribute further towards reducing asthma morbidity and mortality in this vulnerable population. Awareness of asthma symptoms especially wheeze, which is the most common symptom, should be improved and education to seek medical assistance could be done in schools and community health centres. It further highlights the need to make asthma control medication accessible in health centres serving these communities, which has the potential to reduce asthma severity and the need for hospitalization.

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CHAPTER 6

Short term seasonal effects of airborne fungal spores and particulate matter on lung function in a panel study of schoolchildren

Abstract

Background: Few epidemiological studies have investigated the combined effect of short-term fungal spores and particulate matter on lung function in children.

Methods: A panel study was conducted testing for two consecutive school weeks each in summer and winter among 313 schoolchildren from two informal settlements in the Western Cape Province of South Africa. Outcomes included forced expiratory volume in one second (FEV₁) and peak expiratory flow (PEF) measured three times daily and concurrently at all schools. Daily PM₁₀ levels were obtained from a stationary monitor near the two study areas (Khayelitsha and Marconi-Beam), while daily fungal spore levels were measured using spore traps in each study area throughout the year. The effects of PM₁₀ and two most commonly encountered fungi, *Alternaria* and *Cladosporium* spores, on lung function were analysed for lag periods up to five-days, adjusting-for confounding variables and examining for effect modification.

Results: Daily *Alternaria* and *Cladosporium* spores significantly decreased FEV₁ and PEF, especially in winter. The strongest adverse effect was on FEV₁ (mean deficit: -369.34 ml, 95% CI: -650 to -87.77 ml) resulting from a cumulative increase of 50 spores/m³ in *Cladosporium* from lag day-0 through -5. There was also a significant higher deficit in FEV₁ following an increase of 10 spores/m³ in *Alternaria* at high median PM₁₀ levels, suggestive of potential effect modification by PM₁₀.

Conclusion: The study provides evidence that increased daily exposure to ambient fungal spores, *Alternaria* and *Cladosporium* results in lung function deficits especially in winter. These effects can be lagged and are modified by ambient PM₁₀ exposure. Increased sporulation of fungi such as *Alternaria* and *Cladosporium* due to higher winter temperatures and drought as experienced in the Western Cape province of South Africa, is likely to increase the risk of asthma and have an adverse effect on lung function.

6.1. Introduction

Several epidemiological studies investigating the short-term effects of ambient particulate matter and fungal spores on the respiratory health of children have been reported. However, most of these studies used time-series data with the outcome of interest obtained mainly at the population level using hospital records for asthma visits, over-the counter asthma medication or emergency visits for asthma attacks (1–6). These outcome measures limit the exploration of the respiratory health effects at the individual level. Furthermore, exposures to pollen or fungal spores in these studies were obtained mostly from single or few pollen monitoring stations averaged per area. Since ambient particulate matter and fungal spores vary daily with meteorological factors, exploration of the short-term effects of either ambient particulate matter or outdoor fungal spores on respiratory health need to take into consideration these meteorological factors.

Very few studies have investigated the association between fungal spores and lung function, taking into consideration short-term particulate matter. A Taiwanese study of elementary and middle-school children (n=100), using repeated lung function measurements over 8-months (between October 2007 and June 2008 excluding January 2008) found that an IQR increase of 1.3 log spores/m³ was associated with a deficit of 0.11 L (95% CI: -0.19 to -0.03 L) in FEV₁, and a deficit of 0.14 L (95% CI: -0.23 to -0.05 L) in FVC independent of PM_{2.5}, with the latter having no effect (7). The authors expanded their investigation to assess the effect of specific fungi taxa on lung function, by considering two additional monthly visits in June and November 2009. The study found that *Cladosporium* spores at lag day1, above an identified threshold of 1514 spores/m³, was associated with reduced FEV₁ of -0.23 L (95% CI: -0.35 to -0.11 L) as well as FVC of -0.25 L (95% CI: -0.37 to -0.13 L) independent of 24-hour ambient air pollutant exposures such as CO, O₃, SO₂, NO₂, PM_{2.5} and PM_{10-2.5} (8). Another study of American children (n=108) reported a deficit of 1.03 l/min in morning PEF (95% CI: -1.9 to -2.0 l/min) associated with a 10,000 spores/m³ increase in *Cladosporium* spore levels, adjusting-for both meteorological variables and 24-hour particulate matter (9). However, none of these studies reported any interactive effects between ambient fungal spores and air pollutants on lung function, nor reported effects across longer lag-days. Furthermore, these studies purposefully included selected schoolchildren based on their asthmatic or allergic status, to enable the detection of acute effects on vulnerable populations.

The overall findings of the review of the literature in chapter 2 (10) indicate that there is little evidence from epidemiological studies of the combined effects of air pollutants and aeroallergen from fungal spores on individual level respiratory health outcomes using objective measures such as lung function among otherwise healthy schoolchildren. There is also no evidence on the time lag of these effects, nor any effect modification between air pollutants and fungal spores, nor the confounding effect of time-varying factors such as meteorological variables on these associations. Furthermore, there has been no previous study

investigating the association between fungal spores and lung function conducted in sub-Saharan Africa. This study in this chapter therefore sought to examine the effect of daily variations in ambient fungal spore levels and particulate matter of aerodynamic diameter of $10\mu\text{g}/\text{m}^3$ (PM_{10}) on lung function during winter and summer in a panel study of otherwise healthy schoolchildren in the Western Cape province of South Africa. Panel studies are regularly being used to investigate short-term and cumulative causal-effect relationships between health outcomes and environmental exposures (11–13). Panel studies provide valuable insights complementary to cross-sectional studies, which cannot capture the temporal variation of exposures and short-term outcomes.

6.2. Methods

6.2.1. Study Population and Design

The panel study was conducted between 15th February to 26th February 2016 for the summer sampling period, and 25th July to 5th August 2016 for the winter sampling period. The participants in this study were part of the larger cohort study investigating the effects of outdoor air pollutants, airborne pollen, and fungal spores on childhood asthma in schoolchildren residing in four informal settlements in the Western Cape Province of South Africa. A detailed description of the study population, sampling and measurements has been published elsewhere in chapter 3 (14). In summary, a total of 553 of the 590 grade-4 school children recruited from six primary schools residing in four informal settlements (Khayelitsha, Marconi-Beam, Masiphumulele, and Oudtshoorn) participated in the panel study. The current analysis is however restricted to only two study areas [Khayelitsha and Marconi-Beam (337 schoolchildren)] with complete PM_{10} levels, to enable the construction of a multi-pollutant model with fungal spores. The study areas have similar and comparable socio-demographic profiles, with Khayelitsha and Marconi-Beam being an urban settlement within 20 km radius of Cape Town metropolis having a coastal climate.

6.2.2. Data Collection

6.2.2.1. PM_{10} and Fungal Spore Data

Daily hourly concentrations of PM_{10} , air quality data were available from an air quality stationary monitor positioned at the Cape Town International airport (operated by the Airports Company South Africa) located approximately 16km and 19km from Khayelitsha and Marconi-Beam respectively. Ambient fungal spores (such as *Alternaria* and *Cladosporium*) were measured with Burkard 7-day recording volumetric spore traps, located in each of the study areas close to the schools.

6.2.2.2. Lung Function Assessments

Each child was required to perform serial peak flow measurements bihourly (just before school started, during the first break and two hours thereafter) on each school day over each of the 2-week

sampling phases (summer and winter phases) at the same time in all the study areas. The total schooldays during which lung function measurements were obtained in summer was 10, while that in winter was nine, due to a national public holiday on the 3rd of August 2016. In total, observations (number of school days X number of participants) of 3370 and 30337 were expected in summer and winter respectively. Three consecutive manoeuvres of PEF and FEV₁ were performed by each child using an individual Microlife Peak flow meter (Microlife PF100) during each session. The best effort per session was stored by the device, with a total of three sessions per child per day being collected. These measurements were used to compute the daily average PEF and FEV₁.

6.2.3. Statistical analyses

Exposure metrics: The daily average, based on 24 hourly averages of PM₁₀ and airborne fungal spores (*Alternaria* and *Cladosporium*) were determined for the same day as well as for a lag period of 5-days. The airborne fungal spore measurements selected in statistical analysis were those known to be allergenic and found to be abundant in the study areas during the sampling period. The levels of PM₁₀ and the airborne fungal spores determined are represented per scale for every interquartile range (IQR) increase in these measurements. These scales enabled the percentages to be directly relevant to the exposure experienced by the participants and made the percentages for different pollutants directly comparable to each other.

Outcomes: These included daily averages of peak expiratory flow (PEF) and forced expiratory volume in the first second (FEV₁).

Covariates: The potential covariates determined *a priori* included time invariant variables obtained from questionnaires during the home visits of the cohort study such as childbirth weight and maternal smoking status; those obtained during lung function tests conducted during the overall cohort study such as age, age-square, gender, height, height-square, atopy status (Phadiatop test), study area to account for any unmeasured characteristics at the area level, and time varying meteorological variables (temperature, humidity, pressure and wind speed) obtained from each study area.

Analytical procedure: Before running the regression models, imputations (assignments) for missing air quality data (less than 5% data over the panel study period) was done using the ‘Multivariate Imputation by Chained Equation (MICE)’ package in the R statistical package (15). The missing values were imputed with plausible data values drawn from a distribution specific for each missing data point. The number of imputed datasets was specified as 5 (m=5) and predictive mean matching ‘pmm’ was used as the imputation method. The exposure-response regression models used were built using the ‘distributed lag linear and non-linear models (dlm)’ package in the R statistical software (16). A cross-basis matrix accounting for multiple measurements by participants was built for particulate matter (PM₁₀), *Alternaria*, *Cladosporium*

and meteorological parameters such as temperature, humidity, pressure and wind speed, and thereafter included in a regression function together with other covariates. The effect of PM₁₀, *Alternaria* and *Cladosporium* spore levels was assumed to be linear (fun="lin"), while the meteorological factors were modelled through a natural cubic spline with 4 degrees of freedom. The lagged effects of PM₁₀, *Alternaria* and *Cladosporium* were specified up to 5 days lag, with a 2nd degree polynomial function. The final model was run for summer and winter separately, and thus included the cross-basis matrices, adjusting for other covariates, including a smoothing function of 'day of testing' with 4 degrees of freedom to correct for daily trend. The lag-response relationship for PM₁₀, *Alternaria* and *Cladosporium* was specified for values used in the estimation, approximating every interquartile range increase in the respective pollutants (20µg/m³ for PM₁₀, 10 spores/m³ for *Alternaria* and 50 spores/m³ for *Cladosporium*). In single-pollutant models, adjustments were made for time-varying covariates such as meteorological variables and time-invariant variables. The multi-pollutant models estimating the independent effect comprised PM₁₀, *Alternaria* and *Cladosporium* spore levels, adjusting for the effect of one against the other. In addition, random-effect models were used to estimate the average effects across time and between participants, while accounting for the multiple measurements contributed by each participants. The assumption of the model was satisfied by confirming the analysis of the residuals. Stratified analysis across median concentration of PM₁₀ (22.25 µg/m³ in summer and 22.08 µg/m³ in winter) was employed to assess the potential for effect modification by PM₁₀ on both *Alternaria* and *Cladosporium* on lung function.

6.3 Results

The measured concentrations of both PM₁₀ and airborne fungal spores, and meteorological data are presented in Table 6.1 and Figure 6.1. The levels of PM₁₀ levels were relatively similar during the summer and winter phases of the panel study (Table 6.1). Overall, xerophilic fungal spore levels of *Alternaria* and *Cladosporium* were higher during the summer phase (Table 6.1). In Marconi Beam, there were 3 days (2 in summer and 1 in winter) on which the allergic health effect threshold of ≥ 100 spores/m³ for *Alternaria* (17) (Figure 6.1) were exceeded (Figure 1). A correlation matrix of the day-to-day concentrations or levels of the pollutants and climatic variables are presented in appendix Table S6.1. There was a moderate positive correlation between PM₁₀ and temperature (Spearman's correlation, $r = 0.41$, $p < 0.05$), and a moderate negative association was found between PM₁₀ and humidity ($r = -0.49$, $p < 0.05$). The host characteristics and the measured lung function indices are also presented in Table 6.1. The lung function parameters were, on average, similar in both summer and winter (Table 6.1). A total of 2518 and 2162 lung function measurements were utilised for the analyses from 276 and 258 participants in summer and winter respectively. The missing lung function measurements were the result of participants being absent from school on the specific test days.

6.3.1. Effect of Particulate Matter (PM₁₀) on Lung Function

There was no significant negative effect of a 20 $\mu\text{g}/\text{m}^3$ increase in PM₁₀ on daily mean PEF and FEV₁ in summer or winter (Figure 6.2A, Table S6.2 and Table S6.3), except for a deficit of 41.82 l/min in PEF (95% CI: -77.44 to -6.20 l/min) in winter on lag day-5 in the multi-pollutant model (Figure 6.2A). Against a priori hypothesis, PM₁₀ showed positive correlation with daily mean PEF on lag day-2 and day-3 in winter in the multi-pollutant model (Figure 6.2A) and with daily mean FEV₁ from an overall cumulative lag of up to 5-days from lag day-0 in winter in both the single- and multi-pollutant models (Table S6.3).

6.3.2. Effect of *Alternaria* on Lung Function

A significant deficit in FEV₁ during the winter phase was observed with lag (from approximately 13.04 ml in lag day-0 to 6.59ml in lag day-3) to null following an increase of 10 spores/m³ in *Alternaria* in the single-pollutant model (Table S6.3). In the multi-pollutant model, an increase of 10 spores/m³ in 5-day lagged *Alternaria* was significantly associated with a deficit of 9.43 ml in FEV₁ (95% CI: -17.14 to -1.71 ml) in winter (Figure 6.3B). There was also an overall cumulative deficit of 39.77ml in FEV₁ (95% CI: -58.95 to -20.59 ml) in the single-pollutant model in winter from lag day-0 through to lag day-5 (Table S6.3). This effect was substantially reduced (30.7% drop) in the multi-pollutant model with a deficit of 27.56ml in FEV₁ (95% CI: -50.60 to -4.51 ml). A similar substantial reduction in the effect of *Alternaria* on FEV₁ was also evident in the random-effect model examining the average effect across time and between participants (Table 6.2). There was evidence of potential effect modification by PM₁₀ on the effect of *Alternaria* on FEV₁. The size of the deficit in FEV₁ was significantly higher at high median levels of PM₁₀ as compared to low median levels in both summer and winter (Table 6.3).

6.3.3. Effect of *Cladosporium* on Lung Function

In the multi-pollutant model, there was an increasing deficit in daily mean PEF from lag day-3 through lag day-5 with an increase of 50 spores/m³ in *Cladosporium* in winter (Figure 6.2C) and an overall cumulative deficit of 173.44 l/min (95% CI: -276.83 to -70.05 l/min) (Table S6.2). This effect was not observed in the single-pollutant model in winter. There was also a significant deficit in PEF in summer from lag day-1 through lag day-5 with an overall cumulative deficit of 29.54 l/min (95% CI: -48.94 to -10.14 l/min) in the single-pollutant model (Table S6.2). However, the average effect from the random-effect models were much smaller. An average deficit of 0.68 l/min and 1.35 l/min in PEF was observed in summer in the single and multi-pollutant model respectively per 50 spores/m³ increase in *Cladosporium* (Table 6.2). With regard to FEV₁, an increase of 50 spores/m³ in *Cladosporium* was significantly associated with an overall cumulative deficit of 370 ml (95% CI: -590.09 to -150.87 ml) in summer and 134.23 ml (95% CI: -171.04 to -97.42 ml) in winter in the single-pollutant model. These overall cumulative effects were substantially confounded (35.8% drop) in the multi-pollutant model in winter (-86.19 ml, 95% CI: -131.69 to -40.70 ml)

but not in summer (-369.34 ml, 95% CI: -650.91 to -87.77 ml) (Table S6.3). As with PEF, the average deficit in FEV₁ over time between participants from the random-effect models was much lower (Table 6.2). There was no evidence of effect modification by PM₁₀ on the effect of *Cladosporium* on the lung function indices on interest.

Table 6.1: Levels of PM₁₀, airborne fungal spores, meteorological factors, host characteristics and lung function indices measured daily during the sampling phases

Parameter	Summer (15 Feb - 26 Feb 2016)	Winter (25 July - 5 Aug 2016)	Combined
Exposures			
PM ₁₀ (µg/m ³)	22.3 (17.8 - 33.8)	22.1 (17.7 - 27.5)	22.1 (17.7 - 28.5)
<i>Alternaria</i> (spores/m ³)	16.6 (8.6 - 42.5)	1.4 (0.0 - 2.9)	4.3 (1.4 - 23.0)
<i>Cladosporium</i> (spores/m ³)	27.4 (7.9 - 66.9)	5.1 (0.7 - 22.3)	12.2 (2.2 - 50.4)
Meteorological factors			
Temperature (C)	21.7 (20.9 - 23.4)	14.2 (12.9 - 15.9)	18.5 (14.2 - 21.7)
Humidity (%)	68.0 (60.0 - 70.0)	77.0 (72.0 - 83.0)	70.0 (64.0 - 77.0)
Pressure (hPa)	1010.1 (1007.5 - 1011.8)	1016.9 (1013.9 - 1021.1)	1012.7 (1009.9 - 1016.9)
Wind Speed (m/s)	4.4 (2.4 - 5.4)	3.2 (2.0 - 4.3)	3.70 (2.3 - 4.9)
Host characteristics			
Age (years)	-	-	10.0 (9.0 - 10.0)
Gender (Male)*	-	-	149 (50.5)
Weight (kg)	-	-	31.2 (27.9 - 36.3)
Height (cm)	-	-	135 (130.0 - 140.0)
Body mass index (Kg/m ³)	-	-	17.2 (15.8 - 19.4)
Atopy (positive Phadiatop)*	-	-	131 (42.1)
Prenatal maternal smoking*	-	-	49 (15.8)
Birth weight (kg)	-	-	3.2 (3.0 - 3.8)
Lung function indices			
Average PEF (l/min)	229.92 ± 64.53	240.18 ± 70.23	234.66 ± 67.41
Average FEV ₁ (litres)	1.55 ± 0.38	1.57 ± 0.41	1.56 ± 0.39

Exposures and climatic variables are presented as 24-hour daily average

Exposures, meteorological factors and continuous host characteristics are presented as median (interquartile range)

* Categorical variable presented as number (proportion)



Figure 6.1: Concentration of *Alternaria* (spores/m³) – top panel, and *Cladosporium* (spores/m³) – bottom panel, in summer and winter across the two study areas over a 2-week period in 2016 (black line represents the allergenic threshold of 100 spores/m³ - Kasprzyk et al., 2015)

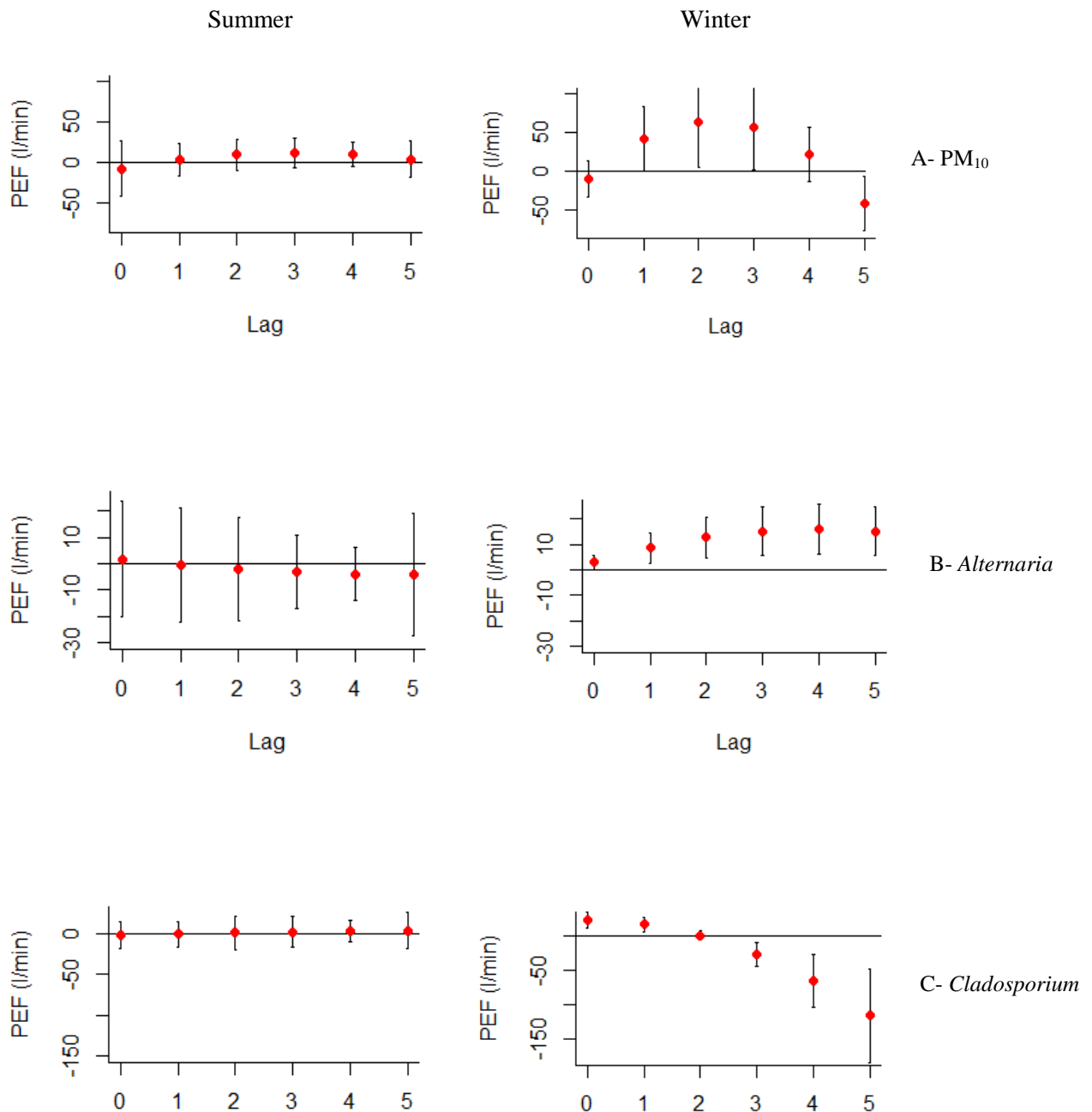


Figure 6.2A, 6.2B, 6.2C: Each panel represents the adjusted multi-pollutant lag-response relationship on peak expiratory flow (PEF) across summer and winter for an interquartile range increase in pollutants (A- 20 $\mu\text{g}/\text{m}^3$ for PM₁₀; B- 10 spores/ m^3 for *Alternaria*; C- 50 spores/ m^3 for *Cladosporium*).

Multi-pollutant model includes PM₁₀, *Alternaria* and *Cladosporium*, adjusting for time-varying confounders such as humidity, temperature, pressure and wind speed at lag 0 and other covariates such as age, age-square, gender, height, height-square, childbirth weight, maternal smoking status, atopy, study area including a smoothing function (natural cubic spline) of 'day of test' with 4 degrees of freedom

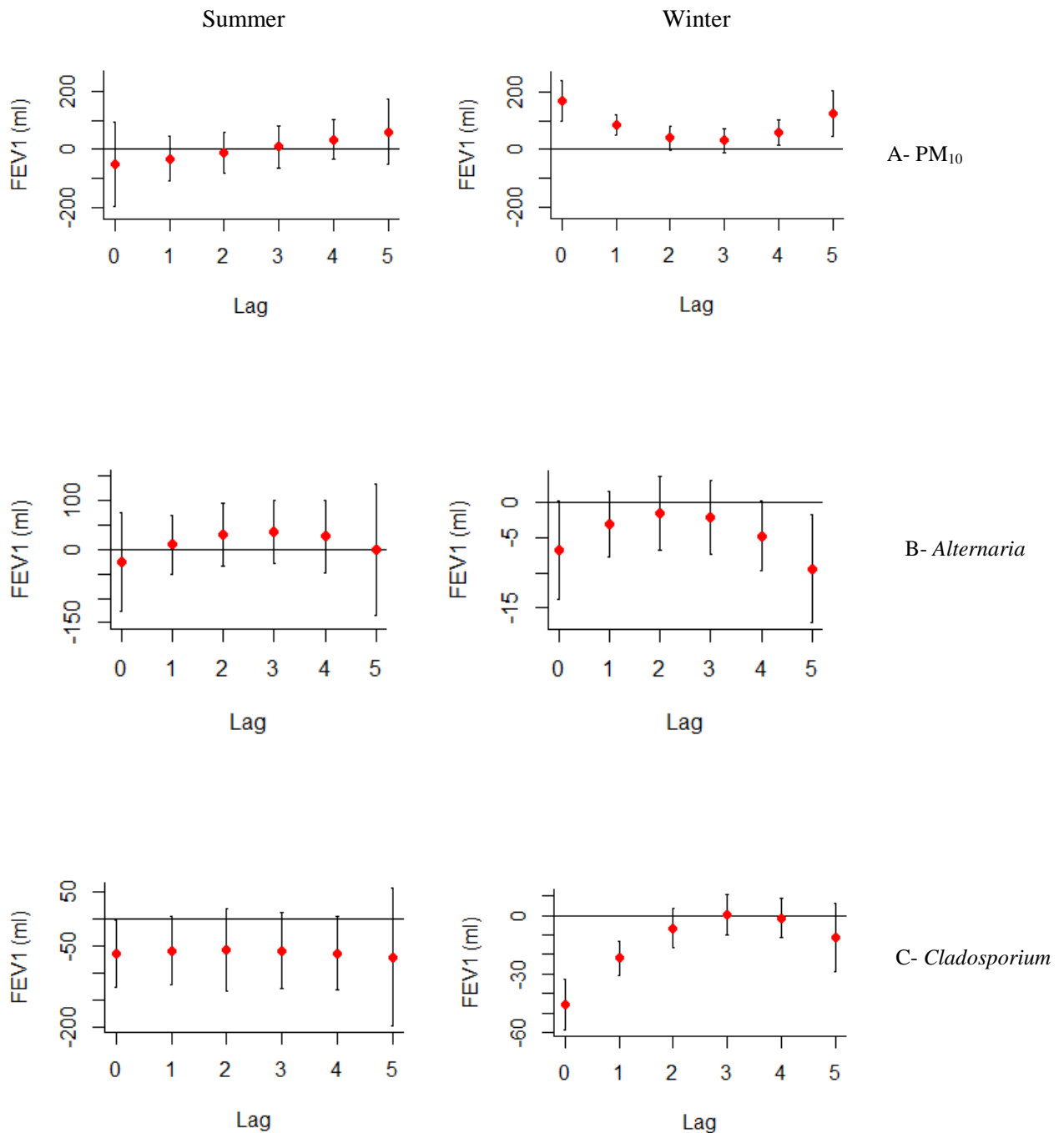


Figure 6.3A, 6.3B, 6.3C: Each panel represents the adjusted multi-pollutant lag-response relationship on forced expiratory volume in 1 second (FEV₁) across summer and winter for an interquartile range increase in pollutants (A- 20 $\mu\text{g}/\text{m}^3$ for PM₁₀; B- 10 spores/ m^3 for *Alternaria*; C- 50 spores/ m^3 for *Cladosporium*).

Multi-pollutant model includes PM₁₀, *Alternaria* and *Cladosporium*, adjusting for time-varying confounders such as humidity, temperature, pressure and wind speed at lag 0 and other covariates such as age, age-square, gender, height, height-square, childbirth weight, maternal smoking status, atopy, study area including a smoothing function (natural cubic spline) of 'day of test' with 4 degrees of freedom

Table 6.2: Average effects per interquartile range increase in PM10, *Alternaria* and *Cladosporium* on PEF and FEV₁ from single and multi-pollutant random-effect models

		Effects estimated as beta coefficients (95% Confidence interval)			
		PEF (l/min)		FEV ₁ (ml)	
	Pollutants	Summer	Winter	Summer	Winter
Single pollutant	PM ₁₀	4.97 (-0.68 to 10.62)	-0.07 (-7.22 to 7.08)	278.07 (250.14 to 305.99)	62.61 (13.70 to 111.52)
Multi-pollutants		9.49 (3.20 to 15.78)	-3.44 (-10.91 to 4.02)	253.32 (224.90 to 281.74)	67.71 (21.67 to 113.74)
Single pollutant	<i>Alternaria</i>	0.14 (-0.24 to 0.52)	-0.09 (-0.36 to 0.19)	-25.97 (-28.58 to -23.37)	-5.62 (-8.33 to -2.90)
Multi-pollutants		1.00 (0.54 to 1.46)	-0.40 (-0.80 to 0.01)	-16.50 (-19.76 to -13.23)	-2.11 (-4.63 to 0.41)
Single pollutant	<i>Cladosporium</i>	-0.68 (-1.16 to -0.20)	0.40 (-0.01 to 0.80)	-7.06 (-7.81 to -6.31)	-8.86 (-10.79 to -6.93)
Multi-pollutants		-1.35 (-1.93 to -0.77)	0.75 (-0.19 to 1.32)	-3.65 (-4.63 to -2.66)	-8.66 (-10.56 to -6.77)

Each estimates represents the average effects across time and between participants from random-effect models for an interquartile range increase in pollutants (20 µg/m³ for PM₁₀; 10 counts/m³ for *Alternaria*; 50 counts/m³ for *Cladosporium*)

PM₁₀: Particulate matter of diameter 10 micrograms

Peak expiratory flow (PEF)

Forced expiratory volume in one second (FEV₁)

Single pollutant model includes only either PM₁₀, *Alternaria* or *Cladosporium*, adjusting for time-varying confounders such as humidity, temperature, pressure and wind speed and individual host characteristics such as age, age-square, gender, height, height-square, childbirth weight, maternal smoking status, atopy, and study area.

Multipollutant model includes PM₁₀, *Alternaria* and *Cladosporium*, adjusting for time-varying confounders such as humidity, temperature, pressure and wind speed and individual host characteristics such as age, age-square, gender, height, height-square, childbirth weight, maternal smoking status, atopy, and study area

All models were also controlled for heteroskedasticity

Bold text indicates significance at $p < 0.05$

Table 6.3: Stratified analysis showing the average effects per interquartile range increase in *Alternaria* and *Cladosporium* on PEF and FEV₁ from single pollutant random-effect models

		Effects estimated as beta coefficients (95% Confidence interval)			
		Summer		Winter	
		High PM ₁₀	Low PM ₁₀	High PM ₁₀	Low PM ₁₀
PEF (l/min)	<i>Alternaria</i>	-1.94 (-5.58 to 1.68)	-0.04 (0.38 to 0.30)	7.93 (5.05 to 10.81)	-0.03 (-0.51 to 0.45)
	<i>Cladosporium</i>	-0.53 (-1.75 to 0.70)	0.42 (-0.09 to 0.94)	0.24 (-1.37 to 1.86)	-0.63 (-1.19 to -0.07)
FEV ₁ (ml)	<i>Alternaria</i>	-36.11 (-49.13 to -23.10)	-6.15 (-9.12 to -3.18)	-34.58 (-43.07 to -26.08)	-17.38 (-20.35 to -14.41)
	<i>Cladosporium</i>	-12.70 (-15.90 to -9.50)	-13.94 (-16.26 to -11.61)	13.94 (5.29 to 22.59)	-4.75 (-5.61 to -3.89)

PEF: Peak expiratory flow

FEV₁: Forced expiratory volume in one sec

PM₁₀: Particulate matter of diameter 10 micrograms

Single pollutant model includes only either *Alternaria* or *Cladosporium*, adjusting for time-varying confounders such as humidity, temperature, pressure and wind speed and individual host characteristics such as age, age-square, gender, height, height-square, childbirth weight, maternal smoking status, atopy, and study area.

Effects estimate stratified across median summer (22.25 µg/m³) and winter (22.08 µg/m³) levels of PM₁₀

Bold text indicates significance at $p < 0.05$

6.4 Discussion

To the best of our knowledge, this is the first study to estimate longer lag (of up to 5-days) short-term effects (independent and combined effect) of PM₁₀ and fungal spores on daily repeated lung function of schoolchildren (selection not based on asthma or atopic status) at the individual level. That the study was conducted in a developing country, is important since these countries globally have the greatest burden of lung disease due to air pollution (18). Most of the previous epidemiological studies that have investigated the short-term respiratory health effects of fungal spores and air pollutants used time-series analyses with air quality and pollen data often from single or few stationary monitors, and associated these exposure metrics with number of asthma hospital admissions or reported asthma symptoms (5,6,19–21). Only three epidemiological studies (7–9) could be identified that explored short-term effects of outdoor fungal spores on lung function in schoolchildren, with none of the studies reporting effects beyond 1-day lag nor were any interactive effects between ambient air pollutants and fungal spores on reduced lung function reported.

The most important finding in the study was the consistent statistically significant independent effect of fungal spores on lung function. A significant cumulative effect of *Cladosporium* on deficit in FEV₁ from lag day-0 to lag day-5 was found in the multi-pollutant model in both summer and winter. These deficits in FEV₁ remained significant over time and between participants across the two seasons in the random-effect models. Furthermore, a cumulative reduction in FEV₁ was also found during winter in the multi-pollutant model following an increase of 10 spores/m³ in *Alternaria* (-27.56 ml, 95% CI: -50.60 to -4.51 ml) from lag day-0 to lag day-5.

Another notable finding in this study was the lagged (i.e. delayed) associations of PM₁₀, *Alternaria* and *Cladosporium* exposure with lung function, which indicates that these exposures trigger sustained reactions in the airways. These lagged lung function effects could decline, remain the same or increase over time. For instance, a significant declining deficit in FEV₁ (from approximately 13.04 ml on lag day-0 to 6.59ml on lag day-3) in winter was shown to approach the null following an increase of 10 spores/m³ in *Alternaria* in the single-pollutant model (Table S6.3). On the other hand, there was an increasing deficit in PEF following an increase of 50 spores/m³ in *Cladosporium* from lag day-2 through to lag day-5 (-3.99 l/min to -6.16 l/min) in summer in the single-pollutant model, and lag day-3 through to lag day-5 (-27.74 l/min to -116.49 l/min) in winter in the multi-pollutant model. This highlights the importance of epidemiological studies to explore the effect of variation in environmental factors on respiratory health outcomes in considering these lag-response relationships.

The largest independent deficit in FEV₁ was observed on the same day (lag day-0) in both summer (-63.19 ml, 95% CI: -125.67 to -0.71 ml) and winter (-45.84ml, 95% CI: - 58.9 to - 32.77 ml) following an increase

of 50 spores/m³ in *Cladosporium*, compared to the other pollutants. However, the effect in winter at lag day-0 showed a gradual decline approaching null at lag day-3, and then followed a steady increase deficit from lag day-4 to lag day-5. A Taiwanese study of schoolchildren (n=100) followed monthly for 10 months reported significant deficits in FEV₁ (-0.23 L, 95% CI: -0.35 to -0.11 L) per doubling increase of 1514 spores/m³ of *Cladosporium* only on lag day-1 but no association on lag day-0, -2, and -3 (8). The deficit in FEV₁ on lag-day-1 observed in this current study during winter was approximately three times as high (-0.66 L, 95% CI: -0.91 to -0.39 L) when exposure to *Cladosporium* was scaled to a similar level as in the Taiwanese study (per 1514 spores/m³ increase in *Cladosporium*). A possible explanation for the stronger effect observed in the current study may have been the larger number of observations available for analyses (2518 in summer and 2162 in winter) compared to the Taiwanese study (824 observations). The larger number of observations in the current study may have contributed to the detection of more subtle exposure-response relationships when compared to the Taiwanese study.

The biological mechanisms by which fungal spores adversely affect children's lung function is not fully understood. The inhalable spore size (aerodynamic diameter of approximately 1.87 μm) (22) and the allergen component of *Cladosporium* and pro-inflammatory agents (such as 1, 3-β-D glucans and mycotoxins) may have contributed to the effects on lung-function observed in this study. Migration of eosinophils and neutrophils inducing both Th1 and Th2 cytokine types have been reported when human epithelial cells are exposed to *Cladosporium* extracts during in vitro culture (23,24). Chronic respiratory effects including allergic inflammation in the lungs on administration of fungal spores have also been found in animal studies (25). The lung function effects observed in this study may therefore have resulted from inflammatory responses to unique biological properties (26,27) and *Cladosporium* antigen activity (28). However, specific sensitization to *Alternaria* and *Cladosporium* was not tested in the children who participated in this study and could not be explored further.

An interesting finding in the study was the increased effect of *Cladosporium* when controlling-for PM₁₀ and *Alternaria* in the multi-pollutant model, accounting for an additional cumulative deficit of 158.55l/min in PEF per increase of 50 spores/m³ *Cladosporium* exposure in winter following a cumulative effect of up to 5-day lag, when compared to the single pollutant model. There was also evidence of the modifying effects by PM₁₀ on the adverse effect on lung function due to fungal spore exposures. In both summer and winter, increased levels of *Alternaria* was associated with a significantly higher deficit in FEV₁ at higher median concentrations of PM₁₀. A possible explanation for the co-effect of PM₁₀ and fungal spores may be due to the increased duration of allergens being airborne, the increased allergic effect of pollen or spores in the presence of particulate matter, which may promote airway sensitization (29,30), and/or the increased exposure to allergenic constituents given that ambient particulate matter also function as carriers of

allergenic components. Allergens released from pollen and spores adhere to these particles, due to porous surfaces and intrinsic electrostatic properties. Under experimental conditions, *Phleum pratense* pollen has been shown to release more allergen-containing granules on contact with air pollutants, compared to exposure to air only (31). Traffic-related pollutants have also been documented to increase the bioavailability of airborne pollen allergens (31). The acute response to allergens may be increased by air pollutants in various ways either by increasing airway oxidative stress, enhancing the recruitment and activation of inflammatory cells following airway inflammation, increasing epithelial permeability, as well as increased release of neuropeptides (32).

This study has also demonstrated that time-varying covariates such as meteorological factors, and more broadly seasonality, have an impact on the association between both biological pollutants (such as ambient fungal spores) and air pollutants on the respiratory health of children. There were seasonal differences in the levels of fungal spores, with a study area (Marconi-Beam) having three days with levels above the allergic threshold for *Alternaria* spores. Consistent increased deficit in PEF was only observed in the multi-pollutant model in winter on lag day-3 to lag day-5 and for an overall cumulative increase up to lag day-5 from lag day-0 for *Cladosporium* (-173.44 l/min, 95% CI: -276.83 to -70.05 l/min). None of these effects were observed in summer. When the effect size was rescaled to a level of that used in an American study of 108 school children, the deficit in PEF observed in the current study (-0.87 l/min, 95% CI: -1.38 to -0.35 l/min per 10,000 spores/m³ increase in *Cladosporium* up to lag day-5) in winter was comparable to a deficit of 1.03 l/min (95% CI: -1.86 to -0.20 l/min per 10,000 spores/m³ increase in *Cladosporium*) observed in the morning, independent of raised air pollutants (9). However, this latter study was on selected group of children known to have reported chronic respiratory symptoms, resulting in the detection of slightly larger effects observed for *Cladosporium* in this group.

There were some unexpected results that showed wintertime PM₁₀ to be associated with significantly higher lung function. It is difficult to explain these findings with plausible biological mechanisms given the associations between PM₁₀ and lung function reported in previous panel studies and experiments. Results may be spurious associations, explained by some other unmeasured factors correlated with PM₁₀ during the winter but not in summer. The rather limited range of PM₁₀ concentrations during the study periods may increase the risk for spurious findings. The most important limitation of this study was that estimation of exposures to PM₁₀ levels were based on a stationary air quality monitor not located in the two study areas (16km from Khayelitsha and 19km from Marconi-Beam). The data from this monitor was used because the air pollution data from the routine stationary monitors located in the study areas were inadequate for the study periods of interest. Nevertheless, the more complete daily data from the airport monitor did provide background exposure data that was partially useful for exposure estimation in this panel study. Due to the

lack of adequate data from the monitors in all four study areas, two study areas had to be excluded from the analysis, even though the health data were collected from all four study areas. However, since exposure assessment for fungal spores was based on measurements from a pollen and spore monitoring station in each of the four study areas, a certain degree of spatial variability and exposure gradient across the study areas was present to study the fungal spore effects.

The use of stationary monitor in assessing exposures often introduces exposure misclassification since time-activity patterns were not available. Children's exposure to fungal spores may be modified by the amount of time spent outdoors, indoors or when exercising. However, due to the compulsory school timetable, it was assumed that the amount of time spent on various activities were similar among the schoolchildren. It was also assumed that all schoolchildren were directly exposed to ambient pollutants at school since there were no air conditioners in the classrooms of the participating schools. It is therefore unlikely that the observed effects of fungal spores on lung function in this study were overestimated since differential exposure misclassification would only exist if the proportion of time spent outdoors or indoors was related to the fungal spore level.

6.5 Conclusion

The current study in this chapter has provided evidence that exposure of schoolchildren to *Alternaria* and *Cladosporium* spores negatively impacted on lung function parameters. There was also evidence suggestive of the effect modification by PM₁₀ on the effect of *Alternaria* on reduced lung function. The effects of ambient fungal spores on lung function was found to be stronger in winter and lagged beyond the actual day of exposure. Should current climate predictions hold, longer sporulation of xerophilic fungi such as *Alternaria* and *Cladosporium* due to higher temperatures and drought conditions, as experienced in the Western Cape province of South Africa, is likely to increase the risk of asthma due to longer exposure duration even at low levels of exposure to these spores. The increasing levels of air pollutants associated with climate change may also lead to greater allergenic potency of fungal spores as was demonstrated in the modifying effect between PM₁₀ and fungal spores on lung function indices. It is therefore important that the public should be provided with real-time information and alerts on air quality and allergy indices, and that such information be communicated in a comprehensible manner based on the socio-demographic background of target populations. Future studies incorporating specific allergen sensitization tests with more rigorous monitoring and quantification of daily fungal spore exposures, and air pollutant levels to account for spatiotemporal variations would enhance further investigations of their association on adverse respiratory health effects in children.

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CHAPTER 7

The effects of ambient air pollution and airborne fungus on the respiratory health of school children residing in informal settlements: a prospective cohort study

Abstract

Background: No previous epidemiological study has investigated the combined effect of long-term ambient air pollutants of NO₂ and PM_{2.5} with *Alternaria* spores exposure on asthma outcomes among schoolchildren in Africa. The aim of this study was to investigate the independent and co-pollutant effect of long-term exposures to ambient air pollutants and fungal spores on the development of asthma-associated outcomes in a cohort of schoolchildren.

Methods: A total of 590 grade-4 schoolchildren residing in four informal settlements were followed-up over 12 months. Spirometry and fractional exhaled nitric-oxide (FeNO) measurements were conducted on each child in addition to the International Study on Asthma and Allergy in Children (ISAAC) questionnaire that was administered to the caregiver at baseline and follow-up. Annual NO₂ and PM_{2.5} levels were estimated for each child's home using land-use regression modelling, while *Alternaria* spore measurements were obtained directly from a stationary monitor placed in each study area. Single- and two-pollutant models were constructed to assess the independent and co-pollutant effects of both air pollutants (NO₂ and PM_{2.5}) and *Alternaria* spores on incident asthma-associated outcomes adjusting-for host characteristics and indoor exposures.

Results: A total of 522 schoolchildren had complete follow-up after 12 months. There was no differential loss to follow-up, since participants' characteristics were similar among children lost to follow-up. The average annual concentration of PM_{2.5} and NO₂ were 10.01 µg/m³ and 16.62 µg/m³ respectively, across the four study areas. Fifty-two percent of participants were exposed to allergenic levels of *Alternaria* above the threshold of 100 spores/m³. In the two-pollutant-adjusted models, an interquartile range (IQR) increase of 14.2 µg/m³ in NO₂ was associated with an increased risk of new onset ocular-nasal symptoms (adjusted odds ratio – aOR: 1.63, 95% CI: 1.01 – 2.60), wheezing (aOR: 3.57, 95% CI: 1.18 – 10.92), more than two or more asthma symptom score (aOR: 1.71, 95% CI: 1.02 – 2.86), and airway inflammation defined as FeNO > 35ppb (aOR: 3.10, 95% CI: 1.10 – 8.71), independent of PM_{2.5} exposures. However, an interquartile increase of 83.1 spores/m³ in 24-hour annual *Alternaria* spore levels was associated with an increased risk of airway inflammation incidence and having a ≥ 10% increase in FeNO at follow-up both in the single-pollutant model and two-pollutant model.

Conclusion: This study found a significant association of ambient *Alternaria* spores and NO₂ (below local and international guidelines), independent of PM_{2.5} exposure, on 12-months incidence of asthma-associated outcomes.

7.1. Introduction

In the literature review reported in chapter 2, several studies have demonstrated a modest risk in asthma incidence (1–5) and prevalence (6,7,16,17,8–15) following exposure of children to various ambient and traffic-related air pollutants. An increased risk of 9% (OR: 1.09 95% CI: 0.96 - 1.23) per 10 $\mu\text{g}/\text{m}^3$ increase in NO_2 was associated with childhood asthma incidence in a pool of five birth cohort studies [PIAMA, Oslo, BAMSE, Vancouver and BCO (15,18–21)], as did $\text{PM}_{2.5}$ by 14% (OR 1.14 95% CI 1.00 - 1.30 per $2\mu\text{g}/\text{m}^3$) in a pool of four birth cohort studies [PIAMA, BAMSE, Vancouver and BCO (15,19–21)]. Furthermore, the evidence for the deleterious effect of air pollution on children’s lung function is also increasing, with a reported deficit in lung function (mainly forced expiratory volume in one second - FEV_1) between 0.5% and 3% following long-term exposures to traffic-related pollutants such as NO_2 (1,9,22–29). There is also growing evidence of the positive association between increased short-term exposure (23,30–34) to ambient air pollutants such as particulate matter with increased FeNO.

However, the evidence of long-term health effects associated with O_3 , SO_2 and $\text{PM}_{2.5}$ is inconclusive (33), as is the relationship between traffic-related air pollutants such as NO_2 (35,36) on airway inflammation. Most of the reported epidemiological studies investigating the effects of air pollution on lung function have focussed on total airway resistance and large airway obstruction as opposed to small airway obstruction and airway inflammation, which may be an important site for early asthma pathology. Furthermore, since most of these studies were conducted in industrialised countries, it is difficult to extrapolate these effects to children residing in informal settlements of developing countries experiencing a greater disease burden due to air pollution (37). In addition, methodological differences in study design, air pollution exposure assessment, health outcomes explored, confounders adjusted-for, and statistical analytical techniques contribute to the challenges in making comparisons across studies.

With increasing climate fluctuations, which may influence local flora, it is important to account for co-exposure to airborne biological pollutants such as pollen and fungal spores in the presence of ambient air pollutants. There is increasing focus on the effect of ambient fungal spores on asthma due to its known allergenic properties (38–40). Previous epidemiological studies investigating the effect of pollen and fungal spores have focused primarily on their short-term effects. The outcomes reported were mainly respiratory symptoms (41) and allergic sensitization (42), with few studies focussing on lung function and airway inflammatory effects. Most of these studies (43–48) were conducted at the population level using time-series pollen or fungi data limiting the inference of health effects associated with pollen and fungal spores at the individual level, and the outcomes of interest were mainly obtained from records of asthma hospital admissions and asthma medication sales.

Very few epidemiological studies in children have explored the combined effect of fungal spores and air pollutants on asthma outcomes at an individual level. A longitudinal study of schoolchildren (n=100) followed for 8 months (October 2007- June 2008) in Taiwan, demonstrated an unchanged independent negative effect of fungal spores on FEV₁ (-0.11 L, 95% CI: -0.19 to -0.03) and FVC (-0.14 L, 95% CI: -0.23 to -0.05) at lag day-1. In this study an IQR increase of 9.9µg/m³ in PM_{2.5}, adjusting for fungal spores, was associated with a deficit of 0.07 L (95% CI: -0.17 to 0.03) in FEV₁ and -0.10 L (95% CI: -0.18 to -0.03) in FVC (49). A follow-up study of the same cohort of school children found a doubling increase in *Cladosporium* above an identified threshold of 1514 spores/m³ at lag day-1 to be significantly associated with a deficit of 0.23 L (95% CI: -0.35 to -0.11) in FEV₁ and a deficit of 0.25 L in FVC (95% CI: -0.37 to -0.13), independent of the presence of particulate air pollutants (50). Another study among children in the US (n=108) in the summer (1991) found a deficit of 1.0 L/m in morning PEF per 10,000 spore/m³ in *Cladosporium* independent of air pollutants (95% CI: -1.9 L/min to -0.2L/min), but no association between particulate matter and PEF after adjusting for fungal spores (51). Furthermore, in chapter 6 of this thesis, a short-term effect of *Alternaria* and *Cladosporium* on FEV₁ deficit, independent of PM₁₀ exposure has also been found.

To our knowledge, none of the previous studies reported in the literature investigated the effect of long-term exposures to ambient fungal spores in the presence of chemical air pollutants on lung function. In our current study, there is reason to suggest possible effects on respiratory outcomes due to long-term exposure to fungal spores in this cohort of schoolchildren, due to the presence of fungal spores, especially *Alternaria*, all year round in all four study areas investigated. In chapter 4, the presence of *Alternaria* reaching its allergic symptom threshold of 100 spores/m³ (52) was present in nearly half of the 52-week measurements in all four study areas (table 4.2 and figure 4.5A), probably in part due to the extended drought being experienced in the Western Cape Province, which thus favours the prolonged sporulation of xerophilic fungi such as *Alternaria*. Furthermore, the effects of indoor mould presence on asthma symptoms and airway inflammation reported in chapter 5 as well as the persistent effect of *Cladosporium* on acute lung function deficit in chapter 6, suggested consideration of the long-term effects on asthma and lung function. A review of the literature as outlined in chapter 2.2 (53), found that increased allergenicity of grass pollens in the presence of traffic-related airborne pollutants has been reported in experimental studies (54), pointing to the possibility of interactive effects between fungal spores and airborne pollutants.

This current chapter investigated the effect of long-term exposures to ambient air pollutants, NO₂ and PM_{2.5}, including *Alternaria* spores on the onset of asthma-related symptoms, lung function and airway inflammation among schoolchildren residing in four informal settlements in the Western Cape Province of South Africa.

7.2. Methods

7.2.1. Study population

A detailed description of the study population has been previously published (55). In brief, 590 fourth grade schoolchildren were recruited from six primary schools located in four informal settlements in the Western Cape Province of South Africa. Three of these communities (Khayelitsha, Marconi-Beam and Oudtshoorn) were selected based on a previous needs analysis aimed at undertaking a human health risk assessment (HHRA) of susceptible population groups that are affected by air pollution in the province (56). An additional area (Masiphumulele) with relatively low air pollution levels was included to reflect contrasting air quality. However, all four informal settlements have similar and comparable socio-demographic profiles. There is some spatial variability in that the three study areas (Khayelitsha, Marconi-Beam, and Masiphumulele) are located about 20km from Cape Town metropolis, having a distinct coastal climate, whereas the fourth area (Oudtshoorn) located inland in the Karoo region about 500km from Cape Town, has a semi-arid climate. A list of primary schools in the selected communities were obtained, and information letters about the study was sent to school principals, followed by a detailed information session in the respective schools. The specific selection of grade-4 pupils was to include participants that were old enough to perform acceptable lung function test manoeuvres. Lung function pre-test questionnaire was administered to consenting children. A total of 150 schoolchildren (in accordance with the sample-size calculation (55)) were recruited from each of the 4 communities, and selection was based on passing the pre-test lung function questionnaire. Qualifying criteria include children without 1) any recent operation in the last 12 months, 2) any pain or nausea, 3) history of epilepsy. In scenarios where the number of qualifying pupils were more than 150 per area, random numbers were used for random selection. The baseline testing was conducted in the four areas between February and September of 2015, while the 12 months follow-up was completed in September 2016.

7.2.2. Air Pollution data

The annual average concentration of PM_{2.5} and NO₂ was estimated at each participant's address by land-use regression (LUR) models developed specifically for this study (57). In brief, the air pollution monitoring campaigns were performed during 2015-2016 in each study area. Weekly measurements of PM_{2.5} and NO₂ were performed in both winter and summer at 140 sites (40 sites each in three study areas, except 20 sites in Masiphumulele) within a period of 1 year. These measurements in each area were temporally adjusted using available routinely monitored air quality from only two monitoring sites (airport and George monitoring site), to obtain the seasonal (winter/summer) and annual averages. The stationary air monitor data used for Masiphumulele and Oudtshoorn were collected about 45km from these main study areas, due to incomplete or lack of air quality data for the respective study area. Predictors of exposure, obtained or

collected on-site, such as household density, nearby traffic (e.g. major roads, bus stops, and train stations), waste burning sites, and land-use derived from geographic information system (GIS) were used to evaluate the spatial variation in the annual average concentrations. To maximize the adjusted explained variance, regression models were developed using a supervised stepwise approach, and the models were validated using leave-one-out-cross-validation (LOOCV). The NO₂ model explained 62% of the annual variance, while PM_{2.5} had a lower explanatory power of 36% (57). The LUR model was used to estimate annual average concentration of PM_{2.5} and NO₂ for each participant's address. The average concentration of PM_{2.5} and NO₂ were scaled to compute an interquartile range increase for each pollutant for the current analysis.

7.2.3. *Alternaria* data

Alternaria spores level were measured in all four study areas between July 2015 and March 2017, with each site having measurements for an entire one-calendar year to cover all possible seasonal peaks. The measurements were conducted using the Burkard 7-day recording volumetric spore trap (one trap per area), located on a flat rooftop having a minimum height of 1.5m to avoid dust contamination. Trained counters conducted microscopic analysis of the spores. The resulting daily spores were used to estimate the average daily annual concentrations in particles per cubic meter of air for each study area. *Alternaria* spores was selected for long-term analysis based on its clinical significance and relative abundance to other airborne pollen and spores in the study areas. More specifically, long-term exposure to *Alternaria* was assessed using the 24-hour annual levels since these spores were present throughout the year as reported in chapter 4. The spores were scaled to compute an interquartile range increase to make the effect estimates for different exposure parameters more comparable. Further details on *Alternaria* including other pollen and fungal spores measurements is presented in chapter 3 (55).

7.2.4. *Health outcome and covariate data*

The ISAAC standardised questionnaire was administered to the parent or guardian of each child at baseline and follow-up by trained interviewers. The questionnaire obtained information on previous information of rhinitis, doctor-diagnosed asthma, ocular-nasal symptoms, wheezing and other respiratory symptoms; birth history including the child's birth weight and maternal smoking during pregnancy; and household characteristics such as use of cooking fuel, presence of visible mould growth, dampness, presence of furry pets and smoker in the home. Age, gender, height and weight were obtained at the time the lung function test was conducted at the child's school. An asthma symptom score was computed from a sum of 8 asthma-associated outcomes, using a cut-off score of two or more symptoms (58,59). The incidence of newly reported symptoms at follow-up was calculated as the proportion of new symptoms reported by the participants who were symptom-free at baseline.

Spirometry was performed at baseline and follow-up according to the American Thoracic Society (ATS) and European Respiratory Society (ERS) guidelines (60). The main lung function parameters of interest were FEV₁, FVC, FEV₁/FVC and FEF₂₅₋₇₅. The Global Lung Function Initiative (GLI) equation was used to derive cut-off for normal lung function according to the age, gender, height and ethnicity of the child (61). The lower limit of normal (LLN) was used as the cut-off, with large airway obstruction defined as FEV₁ < LLN and small airway obstruction defined as FEF₂₅₋₇₅ < LLN respectively. In addition, the ratio of FEV₁/FVC < 0.8 was also used as an index of airway obstruction (60). The incidence of new airway obstruction at follow-up was computed as the proportion of subjects with new airway obstruction among participants whose lung function was normal using the cut-off of the LLN at baseline. In addition, participants were defined as ‘substantial decliners’ if they had a fall of more than 30 ml in FEV₁ after the 12 months of follow-up.

Airway inflammation using a non-invasive method, fractional-exhaled nitric oxide (FeNO), was also measured. A FeNO concentration of more than 15 ppb was considered ‘elevated’ and a level greater than 35 ppb was regarded as ‘high’, suggestive of the presence of airway inflammation (62). Longitudinally, a 10% increase from baseline FeNO measured at 12 months follow-up was considered to be a clinically significant increase in FeNO (63). Further details of the outcome measurements have also been reported in chapter 3 and previously published (55).

7.2.5. Data analysis

Only participants with complete follow-up data and free of disease for the corresponding outcome were included in the analysis. Multiple logistic regression analysis was used to assess the association between annual air pollutants and fungal spores with the incidence of respiratory outcomes of interest in the single-pollutant models, considering adjusting-for sex, age, height, weight, atopy, maternal smoking, child birthweight, dampness (leaks), visible mould growth, presence of pets and smoker in child’s home, cooking with paraffin, and study area. Correlation between these covariates were assessed with the ‘variance inflation factor’. Covariates such as age, height, weight and childbirth weight were later dropped from the models due to multi-collinearity. Multiple logistic regression was also used to assess the association between pollutants (that is; NO₂ and PM_{2.5}, and PM_{2.5} and *Alternaria*) and the incidence of abnormal lung function measures and airway inflammation (FeNO > 35 ppb), increase in FeNO (≥ 10% from baseline), airway obstruction and decline in FEV₁ > 30ml/year. To assess the independent effects, the two-pollutant models (that is; NO₂ and PM_{2.5}, and PM_{2.5} and *Alternaria*) further adjusted-for one another, in addition to the covariates in the single-pollutant model above. The covariates adjusted-for in the models for airway obstruction patterns (FEV₁ < LLN, FVC < LLN and FEF₂₅₋₇₅ < LLN) excluded age, gender and height since these variables were already accounted-for in the computation of their respective lower limit of normal lung

function values. NO₂ was not included in the model exploring the co-effect of air pollutants with *Alternaria* due to multi-collinearity with *Alternaria* assessed through the variance inflation factor (VIF). In the current analyses, binary outcomes in the logistic regression were estimated as odds ratio with their respective 95% confidence interval, for a given interquartile range (IQR) increase in exposure (4.95 µg/m³ for PM_{2.5}, 14.22 µg/m³ for NO₂ and 83.1 spores/m³ for *Alternaria*). All statistical analyses were done using STATA 14.2 and a two-side α-level ≤ 5% was considered statistically significant.

7.3. Results

7.3.1. Characteristics of the study population

The study population at baseline consisted of 590 schoolchildren among whom 522 had complete follow-up data after 12 months. There was no evidence of differential loss to follow-up since the demographic data and other characteristics did not differ between the children with and without complete follow-up (appendix Table S7.1). Characteristics of the study population, reported respiratory symptoms and lung function indices at baseline and follow-up for participants with complete follow-up data are presented in Table 7.1. There was a significant difference in reported rhinitis (20.4% vs. 3.6%), ocular-nasal symptoms (24.6% vs. 31.7%), asthma-symptom score ≥ 2 (7.3% vs. 18.5%), pet ownership (20.2% vs. 7.6%) and presence of smoker in the child's home (27.8% vs. 18.1%) between the baseline and follow-up phases. As to be expected the average lung volumes were significantly greater at follow-up. However, there was a significantly greater proportion of children with airway obstruction at follow-up [FEV₁ < LLN (22.7% vs. 17.5%); FEV₁/FVC < 0.8 (22.7% vs. 14.7%); FEF₂₅₋₇₅ < LLN (24.4% vs. 18.5%)]. The proportion of subjects with new onset large airway obstruction (FEV₁ < LLN) was 15.5%, and slightly higher (16.4%) for small airway obstruction (FEF₂₅₋₇₅ < LLN) (Table 7.3).

7.3.2. Air pollutants and fungal spores exposure

Annual PM_{2.5} levels at the children's current home addresses estimated by LUR showed that 46.8% of the participants were exposed to levels above the WHO reference air quality guidelines (64) (PM_{2.5} > 10 µg/m³) (Table 7.2), whereas none of the children were exposed to annual NO₂ above the WHO reference air quality guideline (> 40 µg/m³). Khayelitsha had the highest average annual PM_{2.5} level of 11.9 µg/m³ and NO₂ level of 24.2 µg/m³, while Oudtshoorn had the lowest average annual levels of 8.1 µg/m³ and 7.6 µg/m³ for PM_{2.5} and NO₂ respectively (appendix Table S7.2). The annual NO₂ and PM_{2.5} concentrations and climatic factors for each study area are presented in the appendix (Table S7.2). The estimated annual average concentration of both PM_{2.5} and NO₂ were similar at the child's residence and at the school (home vs. school annual mean PM_{2.5}: 10.0 µg/m³ vs. 9.7 µg/m³, and NO₂: 16.6 µg/m³ vs. 19.5 µg/m³) (data not shown). A

total of 52.2% of school children were exposed to greater than the allergic symptom threshold (52) for *Alternaria* (> 100 spores/m³) (52) (Table 7.2). Among the airborne biological pollutants investigated, *Alternaria* fungal spores are the most dominant in the study areas. The exposure response analyses were therefore limited to *Alternaria*.

7.3.3. Effects of PM_{2.5} on incidence of reported respiratory symptoms, airway obstruction, lung function decline and airway inflammation

New onset adverse respiratory outcomes including rhinitis, doctor-diagnosed asthma, wheezing and asthma symptom score ≥ 2 were not associated with PM_{2.5} in the two-pollutant which adjusted for NO₂ and other covariates (Table 7.4A). The incidence of ocular-nasal symptoms was however significantly associated with an IQR increase of 4.95 $\mu\text{g}/\text{m}^3$ in annual PM_{2.5} (adjusted odds ratio – aOR: 1.48, 95% CI: 1.08 – 2.02) in the single-pollutant model and in the two-pollutant model that adjusted for *Alternaria* (aOR: 1.46, 95% CI: 1.04 – 2.05) (Table 7.4B). There was no association of PM_{2.5} with any of the lung function parameters and airway inflammation (Table 7.5A and 7.5B).

7.3.4. Effects of NO₂ on incidence of reported respiratory symptoms, airway obstruction, airway inflammation and lung function decline

An IQR increase of 14.22 $\mu\text{g}/\text{m}^3$ in annual NO₂ was independently associated with the incidence of ocular-nasal symptoms (aOR: 1.63, 95% CI: 1.01 – 2.60), asthma symptom score > 2 (aOR: 1.71, 95% CI: 1.02 – 2.86) and wheezing (aOR: 3.67, 95% CI: 1.15 – 11.70) in the two-pollutant model, which adjusted for annual PM_{2.5} and other covariates (Table 7.4A). There was no significant effect of NO₂ on newly reported doctor-diagnosed asthma, and rhinitis at follow-up. In the two-pollutant model, an IQR increase of 14.22 $\mu\text{g}/\text{m}^3$ in annual NO₂ was significantly associated with a three-fold (aOR: 3.10, 95% CI: 1.10 – 8.71) increased risk of new onset airway inflammation (FeNO > 35 ppb) (Table 7.5A). Although none reached statistical significance, an IQR increase of 14.22 $\mu\text{g}/\text{m}^3$ in annual NO₂ was positively associated with incidence of large (FEV₁ $<$ LLN) and small (FEF₂₅₋₇₅ $<$ LLN) airway obstruction (Table 7.5A).

7.3.5. Effects of Alternaria on incidence of reported respiratory symptoms, airway obstruction, airway inflammation and lung function decline

No significant associations were observed between the incidence of rhinitis, doctor-diagnosed asthma, ocular-nasal symptoms, wheezing or asthma symptom score ≥ 2 with an interquartile increase in annual 24-hour *Alternaria* spores levels in the single-pollutant and two-pollutant model (Table 7.4B). However, an interquartile increase of 83.1 spores/m³ in 24-hour annual *Alternaria* spore levels was associated with an increased risk of having a $\geq 10\%$ increase in FeNO at follow-up, while adjusting-for baseline FeNO (> 20

ppb) both in the single-pollutant model (aOR: 1.65, 95% CI: 1.15 – 2.37) and two-pollutant model (aOR: 1.82, 95% CI: 1.23 – 2.70). Similarly, an interquartile increase of 83.1 spores/m³ in 24-hour annual *Alternaria* spore levels was associated with an increased risk of airway inflammation (FeNO > 35ppb) at follow-up, both in the single-pollutant model (aOR: 3.52, 95% CI: 1.31 – 9.47) and two-pollutant model that adjusted-for PM_{2.5} (aOR: 3.70, 95% CI: 1.30 – 10.57) (Table 7.5B). There was no association between *Alternaria* and the incidence of both large and small airway obstruction.

Table 7.1: Baseline and follow-up (12 months) demographic, respiratory symptom and lung function characteristics of schoolchildren residing in the four selected informal settlements of the Western Cape

	Number	Baseline	Follow-up
<i>Host characteristics</i>			
Age, years	522	9.93 ± 0.92	10.93 ± 0.92
Gender, females	522	264 (50.6)	264 (50.6)
Atopy, positive Phadiatop	561	215 (38.3)	-
Weight, kg	522	32.29 ± 7.97	37.71 ± 9.89
Height, cm	522	134.41 ± 7.82	141.04 ± 7.46
<i>Birth history</i>			
Low birth weight (< 2.5 kg)	527	41 (7.8)	-
Maternal smoking	527	98 (18.6)	-
<i>Reported indoor exposure</i>			
Visible mould growth	525	46 (8.8)	31 (5.9)
Dampness (leaks)	525	38 (7.2)	33 (6.3)
Pet ownerships	525	106 (20.2)	40 (7.6)
Paraffin-use	525	293 (55.8)	270 (51.4)
Smoker in the home	525	146 (27.8)	95 (18.1)
<i>Reported respiratory outcomes</i>			
Rhinitis	525	107 (20.4)	19 (3.6)
Doctor-diagnosed asthma	476	16 (3.4)	10 (2.1)
Ocular-nasal symptoms	537	132 (24.6)	170 (31.7)
Wheezing **	475	53 (11.2)	61 (12.8)
Asthma-symptom score ≥ 2 ***	491	36 (7.3)	91 (18.5)
<i>Lung function indices</i>			
FEV ₁ , litres	463	1.57 ± 0.29	1.76 ± 0.35
FVC, litres	463	1.83 ± 0.33	2.10 ± 0.39
FEF ₂₅₋₇₅ , litres/sec	463	1.95 ± 0.62	2.08 ± 0.73
FEV ₁ < LLN	463	81 (17.5)	105 (22.7)
FVC < LLN	463	40 (8.6)	49 (10.6)
FEV ₁ /FVC < 0.8	463	68 (14.7)	105 (22.7)
FEF ₂₅₋₇₅ < LLN	463	86 (18.5)	113 (24.4)
<i>Airway inflammatory marker</i>			
FeNO, ppb *	520	12.5 (9.0 – 18.6)	12 (8.5 – 20)
FeNO > 15 ppb	520	199 (38.3)	214 (41.2)
FeNO > 35 ppb	520	49 (9.4)	67 (12.9)

Analysis restricted to only participants with complete follow-up as indicated in the 'number'

Continuous variable expressed as mean ± SD; Categorical variable expressed as n (%)

* Skewed data represented as median (interquartile range)

** Presence of wheeze in the past 12 months

*** Asthma symptoms score computed from 8 asthma associated symptom questions

Bold text represents significant difference between baseline and follow-up at p < 0.05

FEV₁: forced expiratory volume in 1 sec; FVC: forced vital capacity; FEF₂₅₋₇₅: forced expiratory flow between the 25th and 75th percentile of FVC

FeNO: Fractional exhaled nitric oxide

LLN: lower limit of normal below the 5th percentile

Table 7.2: Distribution of land-use regression estimated annual average air pollutants levels, *Alternaria* and meteorological variables across the four selected study areas

Variables	Mean	SD	Max.	Min.	Median	IQR	% above WHO guidelines
<i>Air pollutants</i>							
PM _{2.5} (µg/m ³)	10.01	4.4	62.9	0	9.4	5.0	46.8
NO ₂ (µg/m ³)	16.62	7.6	39.9	0	14.0	14.2	0
<i>Alternaria</i> (spores/m ³)	107.3	43.9	158.3	56.3	140.1	83.1	52.2
<i>Meteorological factors</i>							
Maximum temperature (°C)	23.3	2.2	26.3	19.7	23.6	2.2	
Temperature (°C)	17.9	0.9	18.8	16.3	18.7	1.3	
Humidity (%)	72.2	5.2	78.3	64.4	71.7	10.3	
Pressure (hPa)	1006.2	15.5	1017.0	979.6	1012.2	20.5	

Max: Maximum; Min: Minimum; IQR: interquartile range

The allergic symptom threshold for *Alternaria* is 100 spores/m³

Table 7.3: Incidence of reported symptoms, degree of airway obstruction, airway inflammation and substantial decline in lung volume over 12 months of schoolchildren residing in the four selected informal settlements of the Western Cape

Outcomes	Number	Incidence
Rhinitis	418	17 (4.1)
Doctor-diagnosed asthma	509	11 (2.2)
Ocular-nasal symptoms	405	126 (31.1)
Wheezing *	456	23 (5.0)
Asthma-symptom score ≥ 2 **	455	81 (17.8)
FEV ₁ < LLN	382	59 (15.5)
FVC < LLN	408	38 (9.3)
FEV ₁ /FVC < 0.8	395	65 (16.5)
FEF ₂₅₋₇₅ < LLN	378	62 (16.4)
Δ FEV ₁ > 30ml/year ***	463	55 (11.9)
Airway inflammation (FeNO > 35ppb)	520	28 (5.9)
Increase in FeNO from baseline ($\geq 10\%$) ***	520	227 (43.7)

Incidence calculated as proportion of cases among case-free participants at baseline (number) except in ' Δ FEV₁ > 30ml/year' and longitudinal increase in FeNO

* presence of wheeze in the past 12 months

** Asthma symptoms score computed from 8 asthma associated symptom questions

FEV₁: forced expiratory volume in 1 sec; FVC: forced vital capacity; FEF₂₅₋₇₅: forced expiratory flow between the 25th and 75th percentile of FVC

LLN: lower limit of normal below the 5th percentile

Δ FEV₁ > 30ml defined as substantial decline (i.e. a fall in FEV₁ by over 30ml at 12 months follow-up)

*** the number is the total number of participants with complete follow-up with regard to ' Δ FEV₁ > 30ml and FeNO respectively'

FeNO: Fractional exhaled nitric oxide

Table 7.4A: Single- and two-pollutants association between air pollutants with incidence of reported respiratory symptoms at follow-up in adjusted models

	Annual PM _{2.5} (µg/m ³)		Annual NO ₂ (µg/m ³)	
	Single-pollutant	Two-pollutants	Single-pollutant	Two-pollutants
Rhinitis (n = 418)	1.20 (0.76 – 1.89)	1.11 (0.59 – 2.10)	2.22 (0.80 - 6.18)	2.12 (0.74 – 6.12)
Doctor-diagnosed asthma (n = 509)	0.86 (0.32 – 2.28)	0.49 (0.14 – 1.72)	2.48 (0.70 - 8.81)	3.60 (0.90 – 14.38)
Ocular-nasal symptoms (n = 405)	1.48 (1.08 – 2.02)	1.29 (0.93 – 1.79)	1.86 (1.21 - 2.89)	1.63 (1.01 – 2.60)
Wheezing (n = 456)	1.09 (0.71 – 1.66)	0.89 (0.42 – 1.92)	3.41 (1.18 - 9.83)	3.57 (1.18 – 10.92)
Asthma-symptom score ≥ 2 * (n = 455)	1.07 (0.84 - 1.37)	0.99 (0.74 – 1.32)	1.70 (1.04 - 2.78)	1.71 (1.02 – 2.86)

Effect estimated as odds ratio (OR) adjusted for sex, atopy, maternal smoking, dampness (leaks), visible mould growth, pets, cooking with paraffin, smoker in child's home, and study area in the single-pollutant models

In addition to the above adjusted covariates, two-pollutants model for PM_{2.5} & NO₂ was adjusted for each other

Effects estimated per interquartile range increase in annual PM_{2.5} of 4.95 µg/m³; and annual NO₂ of 14.22 µg/m³

Bold text denotes statistical significant at $p < 0.05$

* Asthma symptoms score computed from positive responses to 8 asthma associated outcomes doctor diagnosed asthma, asthma medication-use, wheeze and breathless, chest-tightness, shortness of breath at rest, woken up by shortness of attack, shortness of breath after exercise and ever asthma

Table 7.4B: Single- and two-pollutants association between PM_{2.5} and *Alternaria* with incidence of reported respiratory symptoms at follow-up in adjusted models

	Annual PM _{2.5} (µg/m ³)		<i>Alternaria</i> (spores/m ³)	
	Single-pollutant	Two-pollutants	Single-pollutant	Two-pollutants
Rhinitis (n = 418)	1.20 (0.76 – 1.89)	1.20 (0.35 – 2.83)	1.04 (0.38 – 2.81)	0.99 (0.35 – 2.83)
Doctor-diagnosed asthma (n = 509)	0.86 (0.32 – 2.28)	0.69 (0.22 – 2.21)	1.37 (0.38 – 4.86)	1.83 (0.44 – 7.61)
Ocular-nasal symptoms (n = 405)	1.48 (1.08 – 2.02)	1.46 (1.04 – 2.05)	1.14 (0.74 – 1.75)	1.03 (0.64 – 1.67)
Wheezing (n = 456)	1.09 (0.71 – 1.66)	1.02 (0.60 – 1.75)	1.82 (0.70 – 4.73)	1.86 (0.66 – 5.22)
Asthma-symptom score ≥ 2 * (n = 455)	1.07 (0.84 – 1.37)	1.05 (0.81 – 1.35)	1.25 (0.76 – 2.06)	1.22 (0.72 – 2.07)

Effect estimated as odds ratio (OR) adjusted for sex, atopy, maternal smoking, dampness (leaks), visible mould growth, pets, cooking with paraffin, smoker in child's home, and study area in the single-pollutant models

In addition to the above-adjusted covariates, two-pollutants model for PM_{2.5} & *Alternaria* was adjusted for each other. NO₂ was not included in the model due to multi-collinearity with *Alternaria* assessed through the variance inflation factor (VIF)

Effects estimated per interquartile range increase in annual PM_{2.5} of 4.95 µg/m³; and 24-hr annual *Alternaria* of 83.1 spores/m³

Bold text denotes statistical significant at $p < 0.05$

* Asthma symptoms score computed from positive responses to 8 asthma associated outcomes doctor diagnosed asthma, asthma medication-use, wheeze and breathless, chest-tightness, shortness of breath at rest, woken up by shortness of attack, shortness of breath after exercise and ever asthma

Table 7.5A: Single- and two-pollutants association between air pollutants with incidence of airway obstruction, lung function decline, and airway inflammation at follow-up in adjusted models

	Annual PM _{2.5} (µg/m ³)		Annual NO ₂ (µg/m ³)	
	Single-pollutant	Two-pollutants	Single-pollutant	Two-pollutants
FEV ₁ < LLN * (n = 382)	1.21 (0.82 – 1.79)	1.10 (0.70 – 1.72)	1.49 (0.85 – 2.60)	1.41 (0.76 – 2.61)
FVC < LLN * (n = 382)	0.96 (0.61 – 1.52)	0.91 (0.54 – 1.54)	1.13 (0.77 – 3.22)	1.20 (0.59 – 2.42)
FEV ₁ /FVC < 0.8 (n = 408)	0.99 (0.67 – 1.44)	0.86 (0.54 – 1.37)	1.49 (0.86 – 2.59)	1.62 (0.88 – 3.01)
FEF ₂₅₋₇₅ < LLN * (n = 378)	1.16 (0.81 – 1.66)	1.01 (0.93 – 1.10)	1.78 (0.99 – 3.16)	1.78 (0.94 – 3.28)
Δ FEV ₁ > 30ml/year (n = 463)	1.07 (0.73 – 1.58)	0.95 (0.59 – 1.53)	1.52 (0.84 – 2.76)	1.57 (0.82 – 3.01)
FENO > 35 ppb (n = 520)	1.12 (0.77 – 1.64)	0.97 (0.52 – 1.80)	3.05 (1.15 – 8.07)	3.10 (1.10 – 8.71)
≥10% increase in FeNO ** (n = 520)	0.97 (0.78 – 1.20)	0.92 (0.73 – 1.16)	1.26 (0.87 – 1.81)	1.31 (0.89 – 1.93)

Effect estimated as odds ratio (OR) adjusted for sex, atopy, maternal smoking, dampness (leaks), visible mould growth, pets, cooking with paraffin, smoker in child's home, and study area in the single-pollutant models

In addition to the above adjusted covariates, two-pollutants model for PM_{2.5} & NO₂ was adjusted-for each other

FEV₁: forced expiratory volume in 1 sec; FVC: forced vital capacity; FEF₂₅₋₇₅: forced expiratory flow between the 25th and 75th percentile of FVC

* effect estimates were not adjusted-for age, gender and height as this has already been accounted-for in the calculation of the lower limits of normal (LLN)

** significant increase in baseline FeNO ≥ 10% at follow-up, adjusted effects included adjustment for baseline FeNO > 20 ppb

Effects estimated per interquartile range increase in annual PM_{2.5} of 4.95 µg/m³; and annual NO₂ of 14.22 µg/m³

Table 7.5B: Single- and two-pollutants association between PM_{2.5} and *Alternaria* with incidence of airway obstruction, lung function decline, and airway inflammation at follow-up in adjusted models

	Annual PM _{2.5} (µg/m ³)		<i>Alternaria</i> (spores/m ³)	
	Single-pollutant	Two-pollutants	Single-pollutant	Two-pollutants
FEV ₁ < LLN* (n = 382)	1.21 (0.82 – 1.79)	1.13 (0.74 – 1.75)	1.50 (0.87 – 2.60)	1.30 (0.71 – 2.40)
FVC < LLN* (n = 382)	0.96 (0.61 – 1.52)	0.92 (0.55 – 1.53)	1.00 (0.51 – 1.95)	1.20 (0.57 – 2.48)
FEV ₁ /FVC < 0.8 (n = 408)	0.99 (0.67 – 1.44)	0.89 (0.57 – 1.38)	1.36 (0.79 – 2.32)	1.52 (0.84 – 2.77)
FEF ₂₅₋₇₅ < LLN* (n = 378)	1.16 (0.81 – 1.66)	1.08 (0.73 – 1.61)	1.46 (0.84 – 2.53)	1.42 (0.78 – 2.58)
Δ FEV ₁ > 30ml/year (n = 463)	1.07 (0.73 – 1.58)	1.01 (0.66 – 1.56)	1.26 (0.71 – 2.22)	1.29 (0.70 – 2.39)
FeNO > 35 ppb (n = 520)	1.12 (0.77 – 1.64)	0.96 (0.51 – 1.80)	3.52 (1.31 – 9.47)	3.70 (1.30 – 10.57)
≥10% increase in FeNO** (n = 520)	0.97 (0.78 – 1.20)	0.87 (0.68 – 1.12)	1.65 (1.15 – 2.37)	1.82 (1.23 – 2.70)

Effect estimated as odds ratio (OR) adjusted for sex, atopy, maternal smoking, dampness (leaks), visible mould growth, pets, cooking with paraffin, smoker in child's home, and study area in the single-pollutant models

In addition to the above adjusted covariates, two-pollutants model for PM_{2.5} & NO₂ was adjusted-for each other. NO₂ was not included in the model due to multi-collinearity with *Alternaria* assessed through the variance inflation factor (VIF)

FEV₁: forced expiratory volume in 1 sec; FVC: forced vital capacity; FEF₂₅₋₇₅: forced expiratory flow between the 25th and 75th percentile of FVC

* effect estimates were not adjusted-for age, gender and height as this has already been accounted-for in the calculation of the lower limits of normal (LLN)

** significant increase in baseline FeNO ≥ 10% at follow-up, adjusted effects included adjustment for baseline FeNO > 20 ppb

Effects estimated per interquartile range increase in annual PM_{2.5} of 4.95 µg/m³; and 24-hr annual *Alternaria* of 83.1 spores/m³

7.4. Discussion

The strongest and most consistent adverse respiratory health effect by a pollutant found in the study was that of NO₂ on the incidence of various asthma-related outcome measures over a 12-months follow-up period. Besides ocular-nasal symptoms, the effect of NO₂ on the 12-months incidence of wheeze, two or more asthma symptom score and airway inflammation was lower in the single-pollutant model than that observed with PM_{2.5} in the two-pollutant model, suggesting an increased independent effects of NO₂.

An important finding in this study, was the significant association of incidence airway inflammation defined by FeNO > 35 ppb with increase annual NO₂ and 24-hour annual *Alternaria* exposure, independent of co-exposure to PM_{2.5}. The association with new onset airway inflammation among children with normal baseline FeNO, supports the hypothesis that airway inflammation is a marker of early asthma pathology amongst healthy children. Although previous epidemiological studies have well defined the association of FeNO following acute exposure to ambient air pollutants (23,30,32,65,66), the effect of long-term air pollution on FeNO is still a growing field. Results from the Southern California Children Health Study found significant association of both NO₂ and PM_{2.5} on longitudinal changes in FeNO in children after 12 months (67). An increase of 2.29 ppb (95% CI: 0.36 – 4.21) in FeNO was associated with an IQR increase of 1.8 ppb in NO₂ over 12 months independent of asthma and allergy status. In this current study, only healthy children (that is; those with normal FeNO < 35 ppb) were considered in the follow-up analysis, hence once could hypothesize that that increasing levels of NO₂ and *Alternaria* may further increase asthma risk amongst non-asthmatic children with normal baseline FeNO levels. This hypothesis however needs to be tested in future studies.

Of importance is the independent effect of long-term exposure to *Alternaria* spores on longitudinal increase in FeNO (10% increase from baseline) at follow-up, after adjusting-for participants with baseline elevated FeNO > 20 ppb, and long-term PM_{2.5} exposure, suggesting a possible allergenic inflammatory role for fungal spores. To the best of our knowledge, no other studies have explored such long-term effects of *Alternaria* spores on airway inflammation in children. Fungal spores have been postulated in animal studies to induce cytological changes in macrophages and inflammatory responses such as the production of nitric oxide, interleukin-6, interleukin-10, alpha-interferon, and interleukin-1 β (68,69). Hence, the effect of *Alternaria* spores reported in this chapter may possibly be due to lung inflammatory responses towards specific structural components or metabolic products of these microbes.

Fungi are ubiquitous microorganisms, which can be found both indoors and outdoors. The association between exposure to fungal spores assessed through the presence of mould and dampness in indoor environments and the development of asthma in children has been reported in previous studies and also supported by the findings reported in chapter 5. The systematic review and meta-analysis conducted by

Quansah *et al*, reported the increased risk of developing asthma following exposures to visible mould (OR: 1.29, 95% CI: 1.04 – 1.60) and dampness (OR: 1.33, 95% CI: 1.12 – 1.56) (70). In this current analysis, the independent effects of indoor visible mould and dampness (as well as other covariates) were adjusted-for in both the single- and two-pollutant models. It is therefore unlikely that the effect of *Alternaria* observed was due to indoor fungal spores' exposure.

The findings from the single- and two-pollutant models with regard to the effects of long-term exposure to NO₂ and PM_{2.5} on new onset small airway obstruction stresses the importance of this site in the pathology associated with these exposures. In this current study, an IQR increase of 14.22 µg/m³ in annual NO₂ marginally increased the risk of new onset small airway obstruction (FEF₂₅₋₇₅ < LLN) by 78% (aOR 1.78, 95% CI: 0.94 – 3.28). The effect of long-term NO₂ exposure on small airway obstruction found in the current study is important as an early warning for asthma, since changes in large airway assessed through FEV₁ measured on routine spirometry often occur when the asthma is more advanced. This is of particular importance in the four communities investigated in this study, since substantial under-recognition and under-treatment of asthma was previously reported in this cohort in chapter 5 (71). This finding of small airway dysfunction (FEF₂₅₋₇₅ < LLN), which is sub-clinical may also explain the underreporting of asthma symptoms reported previously in this cohort in chapter 5 (71).

The effect of long-term NO₂ exposure on the incidence of reported wheezing (aOR: 3.67, 95% CI: 1.15 – 11.70) is consistent with the findings of some studies. In the Southern California Children's Health Study (SCCHS), an increased risk of new-onset asthma was associated with an IQR increase of 6.2 ppb in NO₂ [adjusted hazard ratio (HR): 1.29, 95% CI: 1.07 – 1.56] (72) in a cohort recruited between 1993 and 2004, when annual NO₂ was approximately 40 µg/m³. However, this effect was not observed in cohorts recruited between 2002 and 2016, which were exposed to reduced levels of annual NO₂ (< 10 µg/m³) (73). Despite the average annual level of 16.6 µg/m³ in NO₂ from all the four study areas being substantially lower than that of the 1993-2004 California cohort, this current study found significant effect association with airway inflammation and new onset respiratory symptoms, which may indicate that respiratory effects could occur at levels lower than the WHO air quality reference annual value of 40 µg/m³ (64). These findings add to the growing body of evidence and points to the need to possibly review the reference guideline level.

This study is strengthened by the longitudinal analysis of the effect of long-term exposure to NO₂, PM_{2.5} and *Alternaria* on the development of new asthma-related outcomes in a rarely investigated children population residing in informal settlements, with little lost to follow-up. The analysis was also restricted to otherwise 'healthy children' at baseline to specific outcome being investigated, which enabled the evaluation of new onset of asthma-like outcomes adjusting for host and indoor characteristics previously found to be risk factors in this cohort (71).

Despite the positive findings observed in this study, there are some limitations worthy of mentioning. There was limited association of PM_{2.5} with most of the asthma-related outcomes investigated in this current study. This may be due to both the low spatial variability (R² of 29%) in the annual PM_{2.5} model used to estimate exposure, and the small spatial contrast of PM_{2.5} estimates (IQR of 4.95 µg/m³) across cohort. While it is acknowledged that the air pollutant exposure estimates at the home level, using LUR annual estimates in this study, could have been improved by incorporating space (school) and time activity patterns through personal monitoring, this would have been costly and challenging to implement especially in a study of children. Furthermore, airborne *Alternaria* exposure was obtained from a single monitoring site in each area without information on time-activity patterns of the children, thereby introducing possible exposure misclassification due to a lack of spatial variation. However, it was assumed that the participating schoolchildren in each area were exposed to similar levels of air pollutants and spores due to having to follow a standard compulsory school timetable (thus similar time-activity patterns would be present in all schools) and the absence of air conditioning facilities in the classrooms of all participating schoolchildren. Perhaps a significant limitation in the current study was the short follow-up period of 12 months, which may also have precluded the investigation of certain asthma-associated outcomes such as significant loss in lung function. The current analyses of the effects of air pollutants and fungal spores focused primarily on early subclinical airway obstructive patterns and airway inflammation rather than lung function growth. It is envisaged that this cohort of children would be followed-up for another 5 years beyond the scope of the current study.

7.5. Conclusion

Despite low home-outdoor annual mean concentrations of NO₂ – all compliant with WHO guideline values, this study found independent association of NO₂ with new-onset of asthma-associated outcomes. This included reported ocular-nasal symptoms, two or more asthma symptom score, wheezing and airway inflammation among schoolchildren residing in four informal settlements in the Western Cape. There was also evidence of an independent effect of *Alternaria* spores on new onset of airway inflammation and FeNO increase at follow-up in this cohort of schoolchildren. This is the first epidemiological study to demonstrate the independent and co-effect of long-term exposures to NO₂ and *Alternaria* spores on the development of asthma-associated outcomes in an under-researched vulnerable population in sub-Saharan Africa. Although annual levels of NO₂ estimated, in the four study areas were generally below the WHO and South African Ambient Air Quality Standards (SAAQS), the presence of an independent effect below these guidelines, underscores not only the need to adopt these standards in all countries, but calls indeed for a revision of current standards to protect vulnerable populations. In the light of the limitations of the current air quality regulations and the lengthy time required to revise current regulations, it is important to reduce personal exposures. This could be done by implementing strategies such as reducing road commuting times of schoolchildren, building parks and schools away from high-traffic roads, and introducing public warnings for high pollution days. Reducing air pollutant

levels may mitigate the long-term effects of climate change, which has been postulated to affect the timing and length of mould season for species such as *Alternaria*, as being currently experienced in the Western Cape Province due to the current drought. Future studies are needed to replicate these findings employing longer follow-up periods in such under-researched population.

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CHAPTER 8

Summary of study findings, conclusions and recommendations

8.1 Summary of study findings

This thesis has generated important data that is sparsely reported in the international literature on the characterisation of exposures to air pollutants and fungal spores in sub-Saharan Africa (chapter 4), the prevalence and indoor risk factors for childhood asthma associated outcomes (chapter 5), and the short- and long-term effects of air pollutants and fungal spores on childhood asthma and associated outcomes in children from low-income settings in sub-Saharan Africa (chapter 6 and 7). The patterns of co-exposure, co-morbidities and susceptibility may largely differ from those in industrialised countries, given the multifactorial nature of respiratory disease including asthma. In addition to the detailed discussion outlined in the individual chapters, the main key findings in this thesis are presented below.

8.1.1 Ambient levels of chemical and biological air pollutants measured in the study

Alternaria, *Ascospores* and *Cladosporium* were the most prevalent fungal spores measured in the four study areas both in the panel and cohort study (Chapter 4). The highest peak level for total fungal spores (34,823.52 spores/m³) was recorded in Masiphumulele during the winter month of July. Levels of *Alternaria* spores were generally higher than the allergic symptom threshold throughout the year in all four study areas, except during the cold weeks of the monitoring period. During the panel study, mean levels of *Alternaria* and *Cladosporium* spores were higher in summer, while *Ascospores* were higher in winter. Over the 52 week measurement period that covered the period during which the cohort subjects were evaluated, Marconi-Beam generally had the highest average level of *Alternaria* spore levels among the 4 study areas with 34 of the 52-weeks (65.4%) recording levels above the allergic symptom threshold for *Alternaria* (1).

During the panel study, ambient air pollutants readings were obtained from the Cape Town International Airport stationary air quality monitor, which was restricted in estimating exposures only for participants residing in Khayelitsha and Marconi-Beam. The maximum daily average levels of PM₁₀, SO₂ and O₃ were 44.42 µg/m³, 17.46 µg/m³ and 67.5 µg/m³ respectively, were all recorded during summer. During the panel study, all pollutants were below the WHO air quality reference guidelines (24-hour PM₁₀ – 50 µg/m³; 24-hour SO₂ – 20 µg/m³; 8-hour daily maximum O₃ – 100 µg/m³) (2) and the South African National Ambient Air Quality Standards (SAAQS) (24-hour PM₁₀ – 75 µg/m³; 24-hour SO₂ – 125 µg/m³; 8-hour daily maximum O₃ – 120 µg/m³) (3).

The annual estimates computed from the land-use regression models ranged from 0.75 µg/m³ to 62.89 µg/m³ for PM_{2.5} and 0.13 µg/m³ to 39.90 µg/m³ for NO₂. However, school children residing in Khayelitsha had the highest annual mean PM_{2.5} exposures of 11.89 µg/m³ (a level higher than the WHO air quality reference guideline of 10 µg/m³) with 80% of these children exposed above this threshold (2). Although estimated NO₂ levels are generally below the WHO ambient air quality reference guidelines, over a third of school

children were exposed to PM_{2.5} levels above the WHO guidelines and allergic symptom threshold levels for *Alternaria* spores over this study period

There was a low correlation between the measured criteria pollutants during the cohort period in the 4 areas (PM_{2.5} vs. NO₂, $r = 0.33$, $p > 0.05$). However, PM_{2.5} had a strong positive correlation with *Alternaria* spores ($r = 0.89$, $p < 0.01$) and a strong negative correlation with *Ascospores* ($r = -0.96$, $p < 0.01$) and *Cladosporium* ($r = -0.76$, $p < 0.01$). Interestingly, between the fungal spores, there was a strong negative correlation between *Ascospores* and *Alternaria* ($r = -0.93$, $p < 0.01$), and a strong positive correlation between *Ascospores* and *Cladosporium* ($r = 0.79$, $p < 0.01$). Furthermore, there was a strong negative correlation between humidity with PM_{2.5} ($r = -0.81$, $p < 0.01$), and *Alternaria* ($r = -0.86$, $p < 0.01$), including a strong positive correlation with *Ascospores* ($r = 0.81$, $p < 0.01$). With regard to meteorological factors, *Alternaria* spore levels also showed a positive correlation with maximum temperature ($r = 0.68$, $p < 0.05$) and a negative correlation with pressure ($r = 0.75$, $p < 0.05$).

It was hypothesised that the effects of allergens present in fungal spores would demonstrate an exaggerated asthmatic response in the presence of air pollutants, hence chapters 6 and 7 of this thesis investigated the independent effect of air pollutants and fungal spores on the respiratory health of schoolchildren in these communities, taking into consideration meteorological factors that were identified to potentially confound such relationships.

8.1.2 Asthma-associated outcomes and indoor air exposure risk factors

In this study, a high proportion of under-diagnosed and untreated asthma was reported in participating schoolchildren from informal settlements areas in the Western Cape province of South Africa in chapter 5. While the prevalence of doctor-diagnosed asthma was surprisingly low (3.4%) and only 50% among them on asthma treatment, a much higher prevalence of wheezing in the past 12 months (12.9%), airway obstruction (17.6%) and airway inflammation (10.2%) was found. The study also provided evidence that school children from low-income informal settlements residing in houses with damp conditions and visible mould growth had an increased risk of ocular-nasal symptoms, rhinitis and wheeze. Previous studies reporting dampness and mould as a major source of indoor allergens associated with increased risk of new-onset asthma and exacerbation in children have been mostly conducted in Europe and North America, with different aeroallergen exposures compared to inhabitants in the southern hemisphere.

The study further provided evidence that the use of paraffin for cooking and heating was associated with an increased risk of rhinitis, airway inflammation and airway obstruction. Paraffin, together with electricity is the most common source of energy in informal settlement communities in South Africa as well as among

communities of low socio-economic status in many other low-and middle-income countries (4). The level, duration and time pattern of exposures in micro-environments, are likely to play a key role in determining the extent of personal exposure to pollutants from sources such as paraffin, since children spend a considerable proportion of their time indoors especially in winter.

8.1.3 Acute effects of particulate matter and fungal spores exposure on asthma-associated outcomes (panel study)

The panel study (described in Chapter 6) provided important evidence, which in general has been very sparse, demonstrating the co-pollutant effects of daily variation in particulate matter and fungal spores on children's lung function. This is the fourth study to have explored these association on lung function at the individual level (that is; non-aggregated level data), but the first to estimate longer lag (of up to 5-days) short-term effects (independent and co-pollutant effects) of PM₁₀ and fungal spores in otherwise healthy schoolchildren (selection not based on asthma or atopic status). Exposure of schoolchildren to *Alternaria* and *Cladosporium* spores negatively impacted on lung function since they independently decreased FEV₁ (-27.56 ml, 95% CI: -50.60 to -4.51 ml per 10 spores/m³ increase in *Alternaria* spores and -86.19ml, 95% CI: -131.69 to -40.70 ml per 50 spores/m³ increase in *Cladosporium* spores respectively from lag day-0 to lag day-5).

The panel study also provided further evidence suggestive of the effect modification by PM₁₀ on the effect of *Alternaria* on reduced lung function. The findings from the study presented in chapter 6 suggested that the effects of ambient fungal spores were more apparent in winter and furthermore demonstrated delayed effects beyond the actual day of exposure. Should current climate predictions hold, longer sporulation periods of xerophilic fungi such as *Alternaria* and *Cladosporium* due to higher temperatures and drought, such as those experienced currently in the Western Cape province of South Africa, is likely to increase the risks associated with longer exposure duration, even at relatively lower levels. The increasing levels of ambient air pollutants associated with climate change may also lead to greater allergenic potency of fungal spores in modifying the risk as demonstrated in the modifying effect between PM₁₀ and fungal spores on lung function indices reported in chapter 6.

8.1.4 Long-term effects of ambient air pollutants and fungal spores exposure on asthma associated outcomes

To our knowledge, chapter 7 reports the first epidemiological study demonstrating the independent and co-pollutant association of long-term exposures to ambient air pollutants and fungal spores on the development of asthma-associated outcomes in a cohort of schoolchildren living in informal settlements. This study used a longitudinal follow-up design to investigate the effect of a 12-month duration of exposure to both ambient

air pollutants and outdoor fungal spores on the incidence of asthma-associated outcomes in schoolchildren. The strongest and most consistent adverse respiratory health effect by a pollutant found in the study was that of NO₂ on the incidence of various asthma-related outcome measures over a 12-months follow-up period. Besides ocular-nasal symptoms, the effect of NO₂ on the 12-months incidence of wheeze, two or more asthma symptom score and airway inflammation was lower in the single-pollutant model than that observed with PM_{2.5} in the two-pollutant model, suggesting an increased independent effects of NO₂.

An important finding in this study, was the significant association of incidence airway inflammation defined by FeNO > 35 ppb with increase annual NO₂ and 24-hour annual *Alternaria* exposure, independent of co-exposure to PM_{2.5}. The association with new onset airway inflammation among children with normal baseline FeNO, supports the hypothesis that airway inflammation is a marker of early asthma pathology amongst healthy children.

The study findings presented in chapter 7 also provided evidence of the independent effect of long-term exposure to *Alternaria* spores on longitudinal increase in FeNO (10% increase from baseline) at follow-up, after adjusting-for participants with baseline elevated FeNO > 20 ppb, and long-term PM_{2.5} exposure, suggesting a possible allergenic inflammatory role for fungal spores. To the best of our knowledge, no other studies have explored such long-term effects of *Alternaria* spores on airway inflammation in children.

8.2 Strengths and limitations of panel and cohort studies

The panel and cohort study described in this thesis had a few notable strengths leading to novel findings highlighted below;

- The panel study represented the first attempt to estimate the longer lag (of up to 5-days) short-term effects (independent and co-pollutant) of both PM₁₀ and fungal spores on repeated objective measures of lung function among schoolchildren at the individual level – this population is closer to the context in other developing countries having the greatest burden of respiratory disease due to air pollution.
- The cohort study in this thesis is the first to evaluate the long-term effects of exposure to both air pollutants and fungal spores on new-onset of asthma and associated outcomes.
- The independent effects of both air pollutants and fungal spores in a multi-pollutant setting were explored for the first time, and provided some evidence of ‘effect modification’ between air pollutants and fungal spores at an individual level.
- This thesis utilized the recently developed and increasingly used LUR to quantify exposure to long-term ambient air pollutants, a methodology lacking in previous air pollution studies in Africa.

There are some limitations in the studies that are outlined below;

- Air pollutant exposure estimates at the home level, using LUR annual estimates could have been improved through the incorporation of space (school) and time activity patterns by personal monitoring if spatial information on these activities were available and collected.
- The use of data collected from monitoring stations outside the study area of Masiphumulele and Oudtshoorn to adjust-for temporal trends in the LUR model could have possibly affected the predictive power of the LUR model.
- The low spatial variability (R^2 of 29%) explained by the annual $PM_{2.5}$ model in the LUR estimates previously reported may have precluded the observation of possible effects of $PM_{2.5}$.
- Airborne fungal spore exposures were obtained from a single monitoring site in each area without information on time-activity patterns of the children, possibly introducing exposure misclassification due to a lack of spatial variation.
- Specific test for sensitization to *Alternaria* and *Cladosporium* was not done in this study to further determine the role of allergic sensitization in the observed effects.
- Other known biological pollutants such as weed, tree and grass pollen were not investigated further due to relatively low concentrations during the study period.
- The short follow-up period of 12 months for the cohort study may have precluded the investigation of certain asthma-associated outcomes such as clinically significant changes in lung function.

8.3 Conclusion

This study confirmed a high proportion of under-diagnosed and untreated asthma among schoolchildren living in informal settlements and demonstrated an increased risk of upper and lower airway symptoms, airway inflammation and airway obstruction in those residing in houses with damp conditions, visible mould growth and paraffin-use for cooking and heating in the home. Among the outdoor biological pollutants measured, fungal spores of *Alternaria* and *Cladosporium* were the most prevalent in the study areas. Although estimated NO_2 levels were generally below the WHO ambient air quality reference guidelines, over a third of schoolchildren were exposed to $PM_{2.5}$ levels above the limits proposed by the WHO guidelines and allergic symptom threshold levels for *Alternaria*. This cohort study demonstrated the independent and co-pollutant effect of long-term exposure to ambient chemical air pollutants and fungal spores on the occurrence of new-onset asthma associated outcomes, in an under investigated vulnerable population in sub-Saharan Africa. The independent effects of chronic exposure to NO_2 annual median levels of $14.0 \mu\text{g}/\text{m}^3$ on new-onset of asthma-associated outcomes among schoolchildren were also demonstrated. There was also evidence of an independent effect of long-term exposure to *Alternaria* spores on an increase

in overall allergic airway inflammation at follow-up in this cohort of schoolchildren. The panel study also provided evidence that delayed effects beyond the actual day of short-term exposure to *Alternaria* and *Cladosporium* was independently associated with a deficit in FEV₁, especially during winter. Furthermore, there was evidence suggestive of the effect modification by PM₁₀ on the effect of *Alternaria* on reduced lung function.

8.4 Recommendations

The following policy recommendations can be drawn from the findings of the thesis;

1. In light of the increased risk of asthma-associated outcomes due to poor indoor air quality such as mould, cooking fumes and second-hand tobacco smoke observed in these communities, an improved housing structure or redesign of new housing contemplated for these communities is recommended to have more effective ventilation to reduce dampness and mould growth. It is envisaged that such interventions would also lead to a reduction in the concentration of chemical pollutants from the use of paraffin for cooking and heating in such micro-environments. Consideration should also be given to providing alternatives to domestic fuel combustion activities.
2. Educational programmes in public spaces such as community centres tied to the goals of the National Asthma Education Programme (NAEP) is recommended to emphasize the need for household environmental management including indoor dust control, pest and bio-aerosol control, prevention of smoking indoors and actively discouraging adults from smoking particularly around children or near their rest, recreational or study areas. It is envisaged that such interventions will contribute further towards reducing asthma morbidity and mortality in this vulnerable population.
3. With the high prevalence of under-recognition and under-treatment of asthma in these communities, awareness of asthma symptoms should be improved and education to seek medical assistance could be provided by schools and community health centres. The study findings further highlight the need for asthma prevention and control medication to be made accessible in local community health centres serving these communities. This has the potential to reduce asthma severity and the need for hospitalization of children.
4. The increased risk associated with longer exposure duration to *Alternaria* spores is likely to increase should current climate conditions persist in the Western Cape province of South Africa. Furthermore, reducing air pollutant levels may also contribute towards mitigating the long-term effects of climate change, which has been postulated to affect the timing and length of the mould season for species such as *Alternaria*.
5. The potential modifying effect between PM₁₀ and fungal spores on children's lung function indices highlights the increased risk of allergic health effects of fungal spore resulting from increasing

levels of air pollutants associated with climate change. It is therefore important that the public should be provided with real-time information and alerts on air quality and allergy indices and that such information be communicated in an appropriate and comprehensible manner to different target populations.

6. Although annual levels of NO₂ estimated in the four study areas were generally below the WHO and South African Ambient Air Quality Standards (SAAQS), the presence of an independent effect below these exposure guidelines, suggests the need to review the current standards so as to protect vulnerable populations. In the light of the limitations of the current air quality regulations and the lengthy time required to revise current regulations, it is important to reduce personal exposures in the interim. This could be done by implementing administrative strategies of exposure reduction such as reducing road commuting times of schoolchildren, building parks and schools away from high-traffic roads and introducing public warnings for high fungi/pollen and pollution days.
7. Local government in the Western Cape needs to expand and enhance air quality measurements for effective monitoring of criteria pollutants within the province. Real-time air quality measurements are proposed to inform vulnerable populations such as children and their caregivers on current levels of air pollution in their areas in an accessible communication strategy. This would contribute towards the protection of public health during periods of high levels of ambient air pollution and strengthen exposure assessment data required for epidemiological studies.

The following recommendations are made for future studies;

1. With regard to the long-term effect of both air pollutants and fungal spores on new-onset asthma associated outcomes, future studies are needed to replicate these novel findings by employing longer follow-up periods (including the follow-up of this current cohort of children) and increasingly robust exposure assessments.
2. Regarding the limitations previously described, future studies should incorporate more rigorous quantification of daily fungal spore measurements and especially airborne chemical pollutants levels. Detailed accounting for spatiotemporal variation would also be beneficial to further investigate the effect of daily variations in both chemical air pollutants and fungal spores on new onset asthma, asthma exacerbations and loss of lung function in children.

References

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Appendices

Appendix 4.1

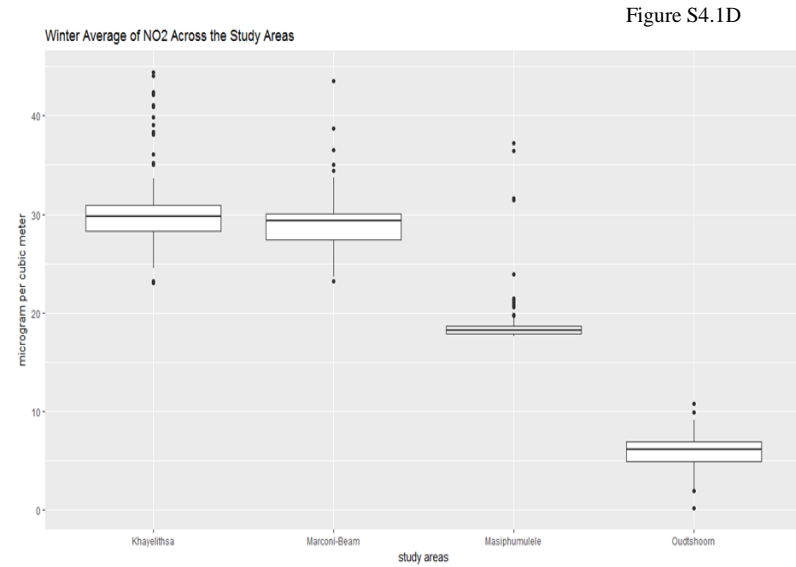
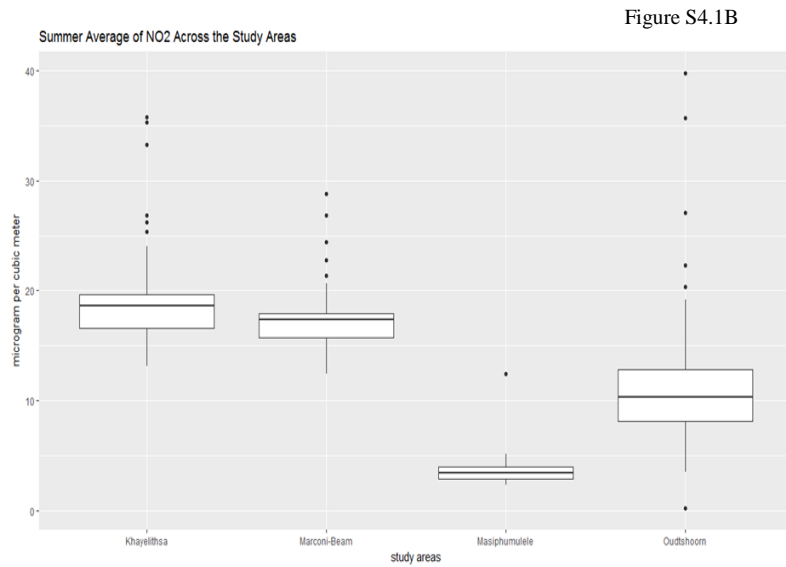
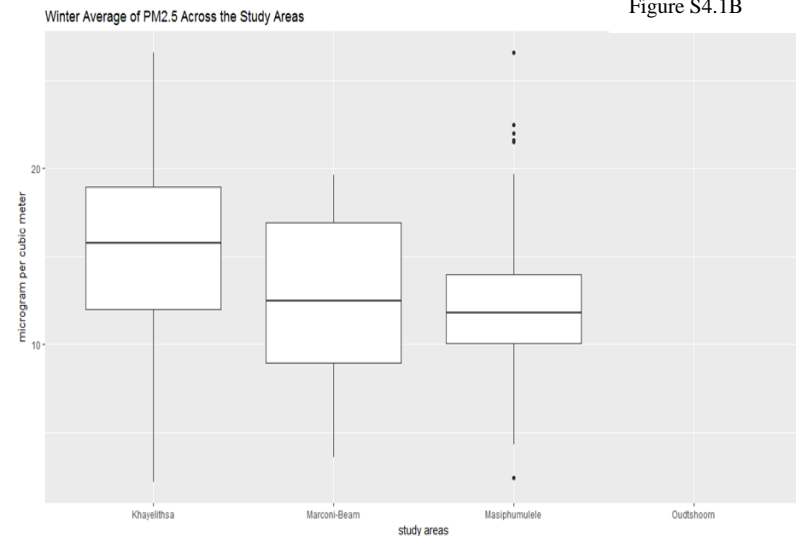
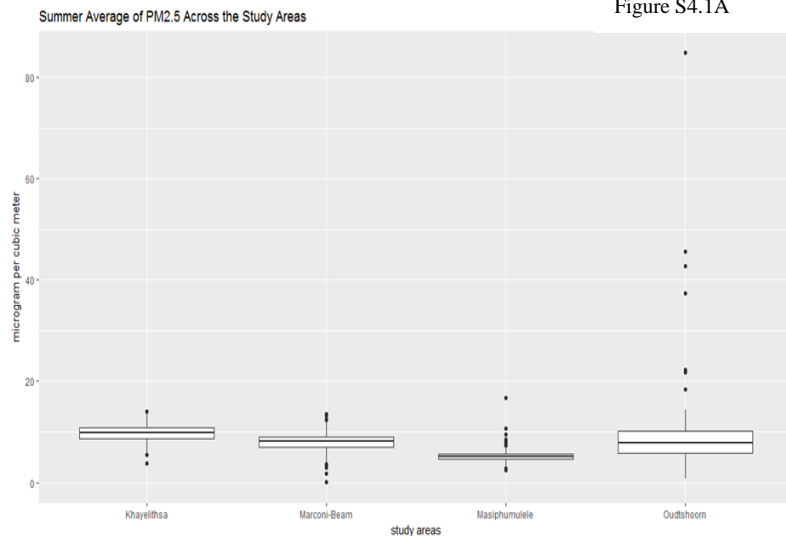


Figure S4.1A to S4.1D: The seasonal averages of PM_{2.5} (figure S4.1A- summer and figure S4.1B- winter) and NO₂ (figure S4.1C- summer and figure S4.1D- winter) estimated from land-use regression in all four study areas

Appendix 4.2

Table S4.1: Specific-genera of measured pollen and fungal spores in the four study areas

Category	Classification	Common name
Fungal Spores	<i>Alternaria</i>	
Fungal Spores	<i>Ascospores</i>	
Fungal Spores	<i>Aspergillus / Penicillium</i>	
Fungal Spores	<i>Basidiospores</i>	
Fungal Spores	<i>Bispora</i>	
Fungal Spores	<i>Chaetomium</i>	
Fungal Spores	<i>Cladosporium</i>	
Fungal Spores	<i>Curvularia</i>	
Fungal Spores	<i>Epicoccum</i>	
Fungal Spores	<i>Nigrospora</i>	
Fungal Spores	<i>Fusarium</i>	
Fungal Spores	<i>Helminthosporium</i>	
Fungal Spores	<i>Leptosphaeria</i>	
Fungal Spores	<i>Periconia</i>	
Fungal Spores	<i>Pithomyces</i>	
Fungal Spores	<i>Pleospora</i>	
Fungal Spores	<i>Puccinia</i>	
Fungal Spores	<i>Rusts</i>	
Fungal Spores	<i>Smuts</i>	
Fungal Spores	<i>Spegazzinia</i>	
Fungal Spores	<i>Stemphyllium</i>	
Fungal Spores	<i>Tetraploa</i>	
Fungal Spores	<i>Torula</i>	
Fungal Spores	<i>Mildew</i>	
Grass	<i>Cyperaceae</i>	Sedge
Grass	<i>Poaceae</i>	Grass
Grass	<i>Restionaceae</i>	Reed
Grass	<i>Typhaceae</i>	Bullrush
Trees	<i>Acacia</i>	Acacia
Trees	<i>Betula</i>	Birch
Trees	<i>Cupressaceae</i>	Cypress
Trees	<i>Ginko</i>	Maidenhair
Trees	<i>Hippocastinaceae</i>	Chestnut
Trees	<i>Moraceae</i>	Mulberry
Trees	<i>Myrtaceae</i>	Eucalyptus
Trees	<i>Oleaceae</i>	Olive
Trees	<i>Pinus / Podocarpus</i>	Pine
Trees	<i>Platanus</i>	Plane

Trees	<i>Populus</i>	Poplar
Trees	<i>Prosopis</i>	Mesquite
Trees	<i>Rhus</i>	Kareeboom
Trees	<i>Quercus</i>	Oak
Trees	<i>Salix</i>	Willow
Trees	<i>Ulmus</i>	Stinkwood
Trees	<i>Casuarina</i>	Pine
Weeds	<i>Amaryllidaceae</i>	Lily
Weeds	<i>Asteraceae</i>	Daisy
Weeds	<i>Boraginaceae</i>	Shrub
Weeds	<i>Bruniaceae</i>	Fynbos sp
Weeds	<i>Caryophyllaceae</i>	Weed
Weeds	<i>Chenopodiaceae</i>	Goosefoot
Weeds	<i>Ericaceae</i>	Fynbos sp
Weeds	<i>Euphorbiaceae</i>	Fynbos sp
Weeds	<i>Iridaceae</i>	Iris
Weeds	<i>Juncus</i>	Rush
Weeds	<i>Liliaceae</i>	Lily
Weeds	<i>Loranthaceae</i>	Mistletoe
Weeds	<i>Malvaceae</i>	Weed
Weeds	<i>Myricaceae</i>	Waxberry
Weeds	<i>Oenotheracea</i>	e.primrose
Weeds	<i>Oxalidaceae</i>	Sorrel
Weeds	<i>Plantago</i>	plantain
Weeds	<i>Polygonaceae</i>	Weed
Weeds	<i>Polypodiaceae</i>	fern
Weeds	<i>Proteaceae</i>	Fynbos sp
Weeds	<i>Ranunculaceae</i>	Weed
Weeds	<i>Rosaceae</i>	Weed
Weeds	<i>Rumex</i>	Sorrel
Weeds	<i>Rutaceae</i>	Weed
Weeds	<i>Solanaceae</i>	Weed
Weeds	<i>Taraxacum</i>	Dandelion
Weeds	<i>Thymelaceae</i>	Weed
Weeds	<i>Tiliaceae</i>	Weed
Weeds	<i>Umbelliferae</i>	Weed
Weeds	<i>Urticus</i>	nettle
Weeds	<i>Parietaria</i>	Weed

Appendix S5.1: Caregiver Informed Consent Form

Consent to participate in a survey investigating asthma in schoolchildren due to exposures to ambient air pollution and other environmental pollutants in the Western Cape

1. **Title of research project**

An Epidemiological Cohort of school children investigating Asthma Outcomes Following Exposure to Ambient Air Pollution and Other Environmental Pollutions

2. **Name of researchers**

Mohamed Aqiel Dalvie (BSc, Honours, MSc, PhD)

Mohamed Jeebhay (MbChB, MMED, PhD)

Rajen Naidoo (MbChB, MMED, PhD)

Toyib Adedamola Olaniyan (BSc Hons., MSc)

Purpose of the research project

The Department of Environmental Health and Development Planning, is conducting this survey investigating the effect of air pollution and other environmental pollutants on asthma in school children. This study is necessary and important because air pollution in the Western Cape is significant and could result in health effects especially in children. In order to effectively study the health effects from air pollution, it is important to follow participants for a long time. This study is therefore very important as no previous long term study following children for three years has been done before in the Western Cape. We would like to interview you about your child's health and conduct testing on your child during this year (2015), with a follow-up into 2016. The study will benefit residents in the Western Cape exposed to air pollution and other environmental pollutants.

3. **Description of the research project**

If you agree for you and your child to participate, we will interview you once during 2015 and 2016 while your child will be undergoing two sets of tests at school during these years. The testing in the study includes:

Your interview: A member of our study team will interview you at your home for about 15 minutes once during 2015 and 2016. You will be asked questions about your child's breathing problems, your child's medication and use of health services, your current and previous employment history; smoking habit (if any); home and environment.

Set 1 testing procedure of your child at school during one day each in 2015 and 2016:

- a) **Complete questionnaires:** A member of our study team will interview your child in privacy at the school for about 5 minutes. Your child will be asked about any breathing or chest problems.

Breathing tests

- Your child will be asked to blow several times into a machine which measures how well your child's lungs are working. This test will last for about 30 minutes.

- Your child will also be asked to blow two times into a NIOX MINO machine, which measures nitric oxide produced by the airways. This machine is used to detect if a person has allergic airway inflammation which is present in asthma or rhinitis. This test will last for about 30 minutes.

- b) **Urine test:** We will collect a urine sample (in privacy) in a plastic container from your child. The sample will be analysed for pesticides.
- d) **Blood test:** A nurse will draw 9 ml blood from a vein on your child's arm. The blood will be analysed to test your child's allergy status.

Set 2 testing procedure of your child at school during summer 2016 and winter 2016 for two weeks

- a) **Breathing test:** Your child will be asked to blow 3 times a day into a handheld device every day for 2 weeks each in summer and winter to measure how well your child's lungs are working. The disruption of school activities will be minimal as measurements will be taken in the morning before school starts, during the first break and then once during classes. Your child will be trained to use the device and a researcher will be present at the school to assist. This test will last for about 10 minutes each.
- b) **Symptoms log:** On each day before the 2nd and 3rd breathing test, your child will be asked to complete a brief questionnaire on asthma symptoms and your child's activities during the previous 2 hours. This questionnaire will last for about 5 minutes each.

4. Risks and discomforts of the research

- a) **From the blood tests.** A single needle stick will be felt when the blood is taken. Sometimes a small bruise may occur from the needle stick, but this is minor and will heal quickly. The total amount of blood taken is quite small and the body will quickly replace it. Blood samples will be used only to measure allergy and will be destroyed at the end of the study.
- b) **From the questionnaires and urine tests.**
There are minimal risks associated with completing the questionnaire and from the urine test. The only risk is a loss of confidentiality about personal information but the data will be seen only by study personnel. All reports will present aggregate data in which individuals will not be identifiable.

- c) **From breathing tests:** There is a small chance that the initial breathing tests could cause your child to become light-headed or faint. Having your child complete the test in a seated position under the observation of trained personnel greatly reduces the chance of your child having such a problem. Your child will be given medicine (salbutamol) to breathe in that works to open your child's lungs. Although very rare, this medication can briefly cause a fast heartbeat, tremor, nervousness or chest pain. A nurse knowledgeable in the treatment of such problems will be immediately available.

6. **Expected benefits to you and to others**

You will be provided with a detailed clinical report, results and interpretation of your child's lung function test, and referral to your family practitioner or local clinic for further management if any problems are found through testing.

The results of the study would help you and others know the risks associated with various occupational and environmental pollutions for adults and children. This would further allow you to manage and/or reduce your risk. A copy of the final report of the study will be made available at the school that your child attends. Additionally, an information sheet on the risks of air pollution and how to manage these risks will be distributed to school staff and caregivers of students. A seminar on the results of the study and the managing of the risks of air pollution will be held at the school after the completion of the study.

The results obtained from this questionnaire at large would help the government of the Western Cape know the degree to which health is affected by environmental pollution. This would further help in further planning in reducing environmental exposures in residential areas.

7. **Costs to you resulting from participation in the study**

The study is offered at no cost to you. In the event a problem is discovered and you wish to be seen by a doctor for it, we can recommend to you who to see. However, the study cannot pay for these additional medical visits or treatments.

The University of Cape Town (UCT) has insurance cover for the event that research-related injury or harm results from your child's participation in the study. The insurer will pay all reasonable medical expenses in accordance with the South African Good Clinical Practice Guidelines (DoH 2006), based on the Association of the British Pharmaceutical Industry Guidelines (ABPI) in the event of an injury or side effect resulting directly from your participation in the study. You will not be required to prove fault on the part of the University.

The University **will not be liable** for any loss, injuries and/or harm that your child may sustain where the loss is caused by:

- The use of unauthorised medicine or substances during the study.
- Any injury that results from your child not following the protocol requirements or the instructions that the study nurse may give.

- Any injury that arises from inadequate action or lack of action to deal adequately with a side effect or reaction to the study medication.
- An injury that results from negligence on your child's part

By agreeing to participate in this study, you do not give up your right to claim compensation for injury where you can prove negligence, in separate litigation. In particular, your right to pursue such a claim in a South African court in terms of South African law must be ensured. Note, however, that you will usually be requested to accept that payment made by the University under the SA GCP guideline 4.11 is in full settlement of the claim relating to the medical expenses.

An injury is considered study-related if, and to the extent that, it is caused by study activities. You must notify the study nurse immediately of any side effects and/or injuries during the study, whether they are research-related or other related complications.

UCT reserves the right not to provide compensation if, and to the extent that, your child's injury came about because your child chose not to follow the instructions that your child were given while taking part in the study. Your right in law to claim compensation for injury where you prove negligence is not affected.

8. Confidentiality of information collected

Your and your child's name will not appear in any reports on this study. The records from the questionnaires will be kept completely confidential and will be seen only by members of the study team.

9. Documentation of the consent

One copy of this signed document will be kept together with our research records for this study. A copy of the information sheet about the study will be given to you to keep.

10. Contact person.

You may contact one of the following persons for answers to further questions about the research, your rights, or any injury you may feel is related to the study.

Name of person: MA Dalvie (The principal investigator) telephone 021 4066610

Name of person: Lamees Emjedi (Ethics administrator) telephone 021 4066492

11. Voluntary nature of participation

You and your child's participation in this project is voluntary. Subsequent to your consent, you and/or your child may refuse to participate in or withdraw from the study at any time without penalty or loss of benefits to which you may otherwise be entitled.

9. Consent of the participant

I have read the information given above, or it has been read to me. I understand the meaning of this information. By signing this form, I hereby consent for me and my child to participate in the study. I also understand that I am free to withdraw myself and my child from the study at any time without penalty.

Printed name of caregiver

Signature, Mark, or Thumb Print

Interviewer's name (Print)

Signature

Witness (Print) (If caregiver is illiterate)

Signature

DATE: _____

DATE: _____

Appendix S5.2 Child Assent Form

Child Assent Form

Introduction

Hi [child's name]! My name is _____ and I would now like to talk to you about your health. Before I begin, I want to assure you that we have your parent's or guardian's permission to approach you. You now have the right to refuse to participate, after I explain to you what we want to do.

1. **Title of research project**

An Epidemiological Cohort study of Children investigating Asthma Outcomes following exposure to Ambient Air Pollution and Other Environmental Pollutions

2. **Purpose of the research**

The Department of Environmental Health and Development Planning, is doing this study to find out if air pollution and other environmental pollutants cause asthma in school children in the Western Cape. We would like to do tests on you this year (2015) and the following year (2016).

3. **Description of the research project**

If you agree to participate, you will be asked to do the following set of tests on one day each in 2015 and 2016:

a) A short interview in 2015 and 2016.

I want you to know that the answers you give me to the questions I ask about your health will be private and we won't share your answers with other kids or with your parents. Only project members of this study will see the answers and they will use these answers to help you improve your health. There are no right or wrong answers to these questions I will ask you. We want to know how you feel. Also, if you do not want to answer one particular question or if you want to stop at any time and not answer any more questions, you can do that by telling me you don't want to continue. Nothing will happen to you if you decide not to answer these questions. But your participation is important and will help us understand health problems in children and this will help other children who might have it in the future.

b) Breathing tests in 2015 and 2016.

You will be asked to blow several times into a machine which measures how well your lungs are working. This test helps us find out if you may have a breathing problem like asthma.

- c) **Urine sample in 2015 and 2016:** We will collect a urine sample from you to test for chemicals.

- d) **Blood sample in 2015 and 2016:** A nurse will draw a small blood sample from you to test if you have allergies.

You will also be asked to do the following set of tests later in 2015:

- e) **A daily log:** You will be asked to log your activities and any breathing problems each day for a period of 2 weeks once during the winter months and repeated in the summer months,

- f) **Further test about your breathing:** We will ask you to blow into a hand held device several times a day during the same periods of 2 weeks during the summer and again during the winter.

4. Risks and discomforts of the research project

- a) **From the blood tests.** You will feel one prick from the needle when the nurse takes your blood. Sometimes a small bruise may occur from the needle stick, but this will heal quickly. The amount of blood taken is quite small and the body will quickly replace it. Blood samples will be used only to measure allergy and will be destroyed at the end of the study.

- b) **From the questionnaires and urine tests.**
There are little risks from the short interview and from the urine test. The only risk is that somebody might come to know about your private information but only project members of this study will see your information and they will use the information to help you improve your health. Your name will not be written in the reports of this study.

- c) **From breathing tests:** There is a small chance that you can become light-headed or faint at the start of the breathing tests. But you will sit down when doing these breathing tests and there will be trained project members to oversee you so that there is less chance of this happening. We will give you a medicine called salbutamol to breathe in that works to open your lungs. There is only a very small chance that this medicine can cause a brief fast heartbeat, cause you to tremble, be nervous or give you a pain in the chest. A nurse that knows how to treat such problems will be immediately available to help if this happens.

5. Expected benefits to you and to others

You parents will be given a detailed report of your tests and they will be asked to take you to your doctor or clinic if we find any problems with your health.

The results of the study would help you and other people know what the risks are from air and other environmental pollutants. This will help you and your parents to manage and/or reduce your risk. A copy of the final report of the study will be given to your school. Additionally, an information sheet on the risks of air pollution and how to manage these risks will be given to your school and parents. A presentation of the results of the study and how to manage the risks of air pollution will be made at the school after the completion of the study.

The results from this study will also help the government of the Western Cape know if air and environmental pollution affects the health of people. This will help them to reduce air and environmental pollution in homes.

6. Confidentiality of information collected

Your name will not appear in any reports on this study. The records of questionnaires, breathing tests, blood samples, urine samples and logs, will be kept completely confidential and will be seen only by study team.

7. Contact person.

You may contact one of the following persons for answers to further questions about the research, your rights, or any injury you may feel is related to the study.

Name of person: Prof MA Dalvie (The principal investigator) telephone 021 4066610

Name of person: Prof. Marc Blockman (Chairperson of the Faculty of Health Sciences Human Research Ethics Committee at the University of Cape Town) telephone 021 4066496

You may contact Prof. Blockman if you have any questions or concerns about your rights or welfare as research participants.

8. Assent for your participation

The information above has been read to me. I understand the meaning of this information, Dr./Mr./Ms. _____ has offered to answer any questions concerning the study. By signing this form, I agree to participate in the study. I also understand that I am free to withdraw from the study at any time without penalty.

Printed name of child

Signature, Mark, or Thumb Print

Interviewer's name (Print)

Signature

Witness (Print)

Signature

DATE: _____

DATE: _____

Appendix S5.3 Caregiver questionnaire (extracted from the online version)

Question name	Question text
Child_Study_Number	What is the child's study number?
Respondent_Request	Is it okay for me to start?
Respondent_start_question	Do you have any question for me before I start?
Realtionship_to_Child	What is your relationship to the child?
Responsible_Person	Are you the person most responsible for care of the child or most familiar with any health problems (s)he has?
Realtion_to_child	How are you related to the child?
Child_Birthplace	In what country was the child born?
Biological_Mother_Age	How old was the biological mother of the child when the child was born? (Years)
Mother_smoking_status	Did the child's biological mother smoke at any time while she was pregnant with the child?
Newborn_Care	Did the child receive any new-born care in an intensive care unit, premature nursery or any other type of special care facility?
Child_days_ICU	How many days did the child receive any new-born care in an intensive care unit, premature nursery or any other type of special care facility?
Child_Birth_Weight	What was the child birth weight?
Child_Breakfast	How often does the child eat breakfast?
Child_Eating_Habits	During the past 12 months, has the child changed eating habits to try to lose weight?
Change_Of_Eating_Habits	During the past 12 months, has child changed what they eat for any medical reason or health condition?
Medical_Reason	What was the medical reason or health condition that caused the child to change what he/she eats?
Child_Weight_Status	Do you consider the child to be overweight, underweight, or about the right weight?
Chronic_illness	Does the child have any of the following illnesses? (CLICK ALL THAT APPLY)
Other_Chronic_illnesses	If the child has other illnesses, please specify.....
Child_Immunization	Do you have a vaccination record/card for the child that I can see?
Child_DPT_Shot	Has the child ever received a DPT or tetanus shot? A DPT shot is to prevent diphtheria, tetanus, and pertussis or whooping cough (verify with record/card)
Child_Cough	Does the child cough for more than 3 months a year?
Child_Winter_Cough	Does the child usually cough first thing in the morning in the winter?
Child_Daily_Cough	Does the child usually cough at all during the rest of the day?[IGNORE AN OCCASIONAL COUGH]
Child_Cough_Period	For how many years has the child had this cough?
Child_Winter_Morning_Phlegm	Does the child usually bring up any phlegm/sputum/mucus from his/her chest first thing in the morning in the winter?
Child_Winter_Day_Phlegm	Does the child usually bring up any phlegm/sputum/mucus from his/her chest during the day in the winter?
Child_Phlegm_Frequency	Does the child bring up phlegm like this for more than 3 months?
Child_Coughs_Blood	Has the child ever coughed up blood?
When_Child_Coughed_Blood	Was this in the past year?

Episodes_Of_Cough_And_Phlegm	Has the child had a problem with cough and phlegm lasting for 3 weeks in any season?
Child_Breath_Shortness_When_Hurrying	Does the child has shortness of breath when hurrying on level ground?
Shortness_Of_Breath_When_Walking	Does the child get short of breath walking with other children of his/her own age on level ground?
Child_Wheezing	Does the child chest ever sound wheezy or whistling?
Child_Cold	Does the wheezing or whistling occurs when the child has a cold?
Wheezing_Frequency	Does the child get a wheeze or whistle in the chest when he/she do not have a cold?
Wheezing_Period	For how many years has this wheezing or whistling been present?
Wheezing_Episodes	How many episodes of wheezing or Whistling has the child had in the past 12 Months?
Weather_Effect_On_Chest	Does the weather affect the child's chest? [Only record YES if adverse weather definitely and regularly causes chest symptoms]
Shortness_Of_Breath_Due_To_Weather	Does the weather make the child short of breath?
Type_of_Weather	What kind of weather?
Other_Symptoms	During the past 12 months, has the child had any of the following? (CLICK ALL THAT APPLY)
Cause_Of_Symptoms_	Are these symptoms brought on by any of the following? (CLICK ALL THAT APPLIES)
Specify	Please Specify Other things that cause the symptoms
When_Pollen_Worsens_Symptoms	During which months of the year does pollen make the child's symptoms worse? (CLICK ALL THAT APPLY)
Food_Allergy	Within an hour after eating something, has the child ever had trouble with any of the following? (CLICK ALL THAT APPLY)
Allergy_Shot_Or_Tests_Reaction	Within an hour after receiving allergy shots or allergy tests, has the child ever had trouble with any of the following? (CLICK ALL THAT APPLY)
Pet_Allergy	Has the child ever given up or had to avoid a pet because of allergies?
Days_Child_Absent	During the past 12 months, about how many full days was the child absent from school because of illness, playing truant, or for other reasons?
Repeating_Of_Grades	Has the child repeated any grades for any reason?
Grade_Child_Repeated	What grade(s) did the child repeat? (CLICK ALL THAT APPLY)
Reason_For_Grade_Repeat	Why did the child repeat the grade(s)? (CLICK ALL THAT APPLY)
Specify_Reason	Please specify if there's other reasons for repeating a grade
Child_Has_Asthma	Has a doctor or nurse ever told you that the child has asthma?
Age_When_Asthma_Diagnosed	How old was the child when a doctor or nurse told you that he/she had asthma?
Frequency_Of_Continuous_Cough	In the past 12 months, how many times has the child had a cough that won't go away?
Wheezing_With_Cold	In the past 12 months, how many times has the child had wheezing (a whistling sound from the chest) with a cold?
Wheezing_Without_Cold	In the past 12 months, how many times has the child had wheezing (a whistling sound from the chest) without a cold?
Attack_Of_Wheezing	In the past 12 months, how many times has the child had wheezing that made it hard for him or her to breathe or catch his or her breath?
Wheezing_With_Physical_Activity	In the past 12 months, how many times has the child wheezed with exercise or running or playing hard?
Coughing_With_Physical_Activity	In the past 12 months, how many times has the child coughed with exercise or running or playing hard?

Tight_Chest	In the past 12 months, how many times has the child complained that his or her chest felt tight or heavy?
Disturbance_Of_Sleep	In the past 12 months, how many times has the child's sleep been disturbed due to wheezing, coughing, chest tightness or shortness of breath?
Child_Worse_Season	Are there any particular seasons or months when the child's symptoms are worse?
Season_Child_Has_Breathing_Problems	During which season (or months) does the child have the most breathing problems? [CLICK ALL THAT APPLY]
Breathing_Problem_Symptoms	I am going to read a list of things that might bring on wheezing, tightness in the chest, cough, or shortness of breath in some children. I would like to know whether each of these things brings on these symptoms for the child.[CLICK ALL THE RESPONSES THAT ARE MENTIONED, REMEMBER TO REPEAT QUESTION FROM TIME TO TIME]
Specify_Causes	Please specify if there's other things that cause symptoms
Child_Allergies	Has a doctor ever told you that the child has Allergies?
Child_Eczema	Has a doctor ever told you that the child has eczema?
Child_Reactive_Airway_Disease	Has a doctor ever told you that the child has Reactive airway disease?
Child_Asthmatic_Bronchitis	Has a doctor ever told you that the child has Asthmatic bronchitis?
Child_Lung_Condition	Has a doctor ever told you that the child has any other lung/breathing condition?
If_YES	If YES above, please specify any other lung/breathing condition
Family_Doctor	Not including the emergency room, does the child have a regular family doctor or health care provider that you usually go to for his/her health care?
Child_Doctor_Or_Clinic	If YES please specify the child's Doctor or Clinic's name
Last_Doctor_Visit	When is the last time you visited this doctor or clinic?
Asthma_Medication	Has the child ever taken any medication for asthma?
Period_Medication_Was_Last_Used	Can you tell me when the child last used this medicine?
Medication_Names	Please list all medication name.
Medication_Costs	How much does a 1 month supply of the medication [If taken as prescribed] cost you? (RANDS)
Itchy_Rash	Has the child ever had an itchy rash which was coming and going for at least six months?
Rash_Past_12months	Has the child had this rash at any time in the past 12 months?
Places_Rash_Affected	Has this itchy rash at any time affected any one of the following places? (CLICK ALL THAT APPLY)
Rash_Clearing	Has this rash cleared up completely at any time during the past 12 months?
Sneezing_Problems	Has the child ever had a problem with sneezing, or a runny or blocked nose when he/she DID NOT have a cold or flu?
Intereference_Caused	In the past 12 months, how much did this nose problem interfere with child's daily activities?
Hayfever	Has the child ever had hayfever?
Residence	Do you rent or own your home or neither?
Standing_water	At any time during the year is there standing water or puddles in the following places (CLICK ALL THAT APPLY)
Leaks	In the past year have you had any other problem with water damage or leaking water in your home, such as from a leaking roof or leaky plumbing in any of the following (CLICK ALL THAT APPLY)
Pets_At_Home	Do you have pets in the house?
Pets_In_Child_Bedroom	Do any of these pets spend any time in the child's bedroom?

Pest_Home_Treatment	Have you or someone else (your landlord, another family member, a professional) treated your home for cockroaches or mice (rat) in the past year?
Last_Treatment	When was the last time it was treated?
Type_Of_Treatment	What was used to treat your home for cockroaches or mice (rats)?
Specify_Treatment	Please specify if other type of treatment not specified was used.
Use_Of_Heater	During the past 12 months was a room heater used to heat one or more rooms in this house?
Heating_Cooking_Fuel	What is the fuel used for cooking and heating?
Specify_Other_Fuel	Please specify if used fuel not specified
Working_Around_Chemicals	Is there anyone whose paying job is working around chemicals (such as pesticides, paints) or dust living in the home?
Work_Clothes	If yes, do they usually wear their work clothes home?
Location_Of_Job	Is there anyone in the house whose job involved working with chemicals?
Dusting_Of_Child_Room	During the last 2 weeks, how many times was the room in which the child sleeps dusted?
Dusting	What do you use when you dust the child's room?
Other_Dusting_Tools	Please specify if use other dusting tools not specified.
Frequency_Of_Dusting	During the last 2 weeks, how many times were other rooms in the house dusted?
Changing_Child_Bedding	How many times do you change the child's Bedding in a month?
Washing_Temperature	When the child's bedding is washed, is it washed in hot or cold water?
Getting_Rid_Dust_Mites	Do you or any member of your family add anything to the wash to help get rid of dust mites? [PROMPT: Such as eucalyptus oil.]
Specify_Additional_Thing	Please Specify if using additional things to get rid of dust mites
Stuffed_Animals	Does the child have stuffed animals in his or her bedroom?
Washing_Of_Stuffed_Animals	Do the child's stuffed animals get washed?
Mold_Growth	In the last 12 months, have you removed any visible mould growth from your house?
Cigarette_Smoking	Have you changed cigarette-smoking rules in the home?
Change_Of_Things	Did you do any other things around the house because of the child's asthma?
Smokers	Are there any people who live in the child's home who smoke?
Occasional_Smoking	Do you smoke cigarettes, even occasionally?
Amount_Of_Cigarettes	About how many cigarettes a day do you now smoke?
Smoking_Outside	How often do you go outside the home to smoke?
Smoking_Visitors	Do any frequent visitors smoke?
Keeping_Child_Away	Many people have difficulties keeping their children away from cigarette smoke. Do you have problems keeping the child away from people who are smoking?
Current_Residence	Has the child lived anywhere else apart from his/her current home?
Number_Town_lived	In how many towns has the child lived before?
Name_Town_Lived	What is the name of the town the child lived before?
Duration_Town_Lived	How many years did the child live in the town mentioned above?
Any_Question	You did a great job! Thank you for helping us! Do you have any questions for me?

Appendix Table S5.4: Respiratory-related allergic outcomes of school children residing in informal settlements of the Western Cape Province

	Boys (n1=286, n2=271)	Girls (n1=294, n2=270)	Overall (n1=580, n2=541)
<i>Range of Ns for the variables presented</i>			
Past medical history, n1 (%)			
Doctor-diagnosed asthma *	12 (4.2)	7 (2.4)	19 (3.3)
Doctor-diagnosed eczema	10 (3.5)	21 (7.1)	31 (5.3)
Doctor-diagnosed hay fever	20 (7.0)	16 (5.4)	36 (6.2)
Asthma symptom score, n2 (%)			
Score 0	233 (86)	229 (84.8)	462 (85.4)
Score 1	19 (7.0)	22 (8.2)	41 (7.6)
Score >1	19 (7.0)	19 (7.0)	38 (7.0)
Asthma-like symptoms, n1 (%)			
Shortness of breath walking with other children on level ground *	16 (5.6)	8 (2.7)	24 (4.1)
Symptoms history in the past 12 months, n1 (%)			
Current wheezing *	44 (15.4)	30 (10.2)	74 (12.8)
Wheezing during physical activities *	8 (2.8)	3 (1.0)	11 (1.9)
Cough during physical activities *	6 (2.1)	3 (1.0)	9 (1.6)
Chest tightness *	12 (4.2)	6 (2.1)	18 (3.1)
Sleep disturbances due to wheeze, chest tightness or shortness of breath *	11 (3.9)	6 (2.1)	17 (2.9)
Use of asthma medication *	7 (2.5)	2 (0.7)	9 (1.6)
Upper airway symptoms, n1 (%)			
Ocular-nasal symptoms	76 (26.6)	73 (24.8)	149 (25.7)
Rhinitis	60 (21.2)	58 (19.7)	118 (20.5)

Categorical variables, number (%).

* Asthma symptom score ranging from 0 -8 derived from a sum of positive answers to eight main asthma symptoms and bronchial hyperresponsiveness questions from the ISAAC questionnaire

Appendix Table S5.5: Associations between indoor household exposures and asthma-related outcomes among school children using stepwise adjusted logistic regression models

Outcomes	Dampness (leaks)	Visible mould	Pets in the home	Paraffin use	Smokers in the home
	OR (95% CI)				
Doctor-diagnosed asthma ^a	NE	1.84 (0.38 – 8.95)	1.24 (0.38 – 4.08)	1.47 (0.52 – 4.19)	0.33 (0.07 – 1.47)
Asthma symptom score ≥ 2 ^b	2.46 (0.92 – 6.59)	0.73 (0.21 – 2.54)	1.45 (0.62 – 3.38)	0.73 (0.37 – 1.47)	0.58 (0.25 – 1.37)
Current wheeze ^c	2.58 (1.22 – 5.45)	0.95 (0.40 – 2.26)	0.89 (0.45 – 1.78)	1.44 (0.85 – 2.46)	1.71 (1.01 – 2.90)
Ocular-nasal symptoms ^d	1.07 (0.51 – 2.23)	1.91 (1.01 – 3.61)	1.10 (0.67 – 1.79)	1.05 (0.70 – 1.58)	0.64 (0.40 – 1.01)
Rhinitis ^e	2.78 (1.39 – 5.58)	3.09 (1.60 – 5.97)	1.00 (0.54 – 1.82)	1.62 (1.02 – 2.57)	1.18 (0.72 – 1.93)
FEV ₁ < LLN ^f	0.35 (0.10 – 1.18)	0.78 (0.33 – 1.82)	1.32 (0.74 – 2.34)	1.00 (0.61 – 1.62)	1.10 (0.66 – 1.83)
FVC < LLN ^g	0.43 (0.10 – 1.86)	1.47 (0.61 – 3.53)	1.05 (0.52 – 2.12)	1.13 (0.63 – 2.03)	0.71 (0.36 – 1.37)
FEV ₁ /FVC < 0.80 ^g	1.01 (0.40 – 2.57)	0.67 (0.25 – 1.80)	1.54 (0.84 – 2.83)	1.45 (0.85 – 2.49)	0.75 (0.42 – 1.36)
FEV ₁ /FVC < LLN ^f	0.89 (0.33 – 2.41)	0.87 (0.35 – 2.17)	1.74 (0.95 – 3.17)	1.51 (0.87 – 2.61)	1.03 (0.59 – 1.82)
FEF ₂₅₋₇₅ < LLN ^f	1.07 (0.47 – 2.46)	0.84 (0.37 – 1.89)	1.10 (0.62 – 1.96)	1.10 (0.69 – 1.76)	0.78 (0.46 – 1.31)
FeNO > 35 ppb ^h	0.93 (0.29 – 2.93)	0.42 (0.08 – 2.09)	0.81 (0.29 – 2.24)	2.31 (1.05 – 5.06)	0.52 (0.21 – 1.29)

OR: Odds ratio; each OR is a separate adjusted regression model

^a controlling-for age, gender, body mass index, atopy and study area

^b controlling-for age, gender, body mass index and study area

^c controlling-for age, gender, birthweight and study area

^d controlling-for age, gender, atopy and study area

^e controlling-for age, gender, prenatal maternal smoking, birthweight and study area

^f controlling-for only study area, as age, gender and height were already used in computing the cut-off

^g controlling-for only atopy and study area, as age, gender and height were already used in computing the cut-off

^h controlling-for age, gender, body mass index, prenatal maternal smoking, birthweight, atopy and study area

Bold text denotes statistical significance at $p < 0.05$

FeNO: Fractional exhaled nitric oxide

FEV₁: Forced expiratory volume in 1 sec; FVC: Forced vital capacity; PEF: peak expiratory flow; FEF₂₅₋₇₅: Forced mid expiratory flow

LLN: lower limit of normal below the 5th percentile

Appendix Table S6.1: Correlation matrix of the day-to-day concentrations or levels of the pollutants and climatic variables

	PM₁₀	Alternaria	Cladosporium	Temperature	Humidity	Pressure	Wind Speed
Particulate matter (PM₁₀)	1.000	0.135	-0.151	0.413	-0.499	-0.216	-0.147
<i>Alternaria</i>		1.000	0.078	0.375	-0.315	-0.020	0.104
<i>Cladosporium</i>			1.000	-0.083	0.011	-0.173	-0.105
Temperature				1.000	-0.703	-0.285	-0.134
Humidity					1.000	0.337	0.028
Pressure						1.000	0.388
Wind Speed							1.000

Bold text represents correlations above 70% correlation

The above matrix was computed using Spearman correlation due to the skewness of the parameters

Appendix Table S6.2: Estimated effects per interquartile range increase in PM₁₀, *Alternaria* and *Cladosporium* on PEF from single and multi-pollutant distributed lags covariate-adjusted linear regression models

		Effects estimated as beta coefficients (95% Confidence interval) on PEF (l/min)					
		PM ₁₀		<i>Alternaria</i>		<i>Cladosporium</i>	
		Summer	Winter	Summer	Winter	Summer	Winter
Single pollutant	Lag 0	-8.28 (-42.69 - 26.12)	-83.41 (-614.20 - 447.38)	-2.18 (-4.47 - 0.12)	-0.20 (-2.39 - 1.99)	-5.99 (-12.07 - 0.08)	-2.54 (-39.60 - 34.52)
Multi-pollutants		-8.28 (-42.69 - 26.12)	-9.67 (-33.20 - 13.86)	1.70 (-20.06 - 23.46)	2.95 (0.15 - 5.74)	-2.11 (-18.90 - 14.68)	21.31 (9.95 - 32.66)
Single pollutant	Lag 1	2.79 (-17.39 - 22.97)	-16.41 (-113.92 - 81.10)	-0.41 (-1.67 - 0.85)	-0.26 (-2.72 - 2.21)	-4.64 (-7.88 - -1.40)	-3.77 (-100 - 92.70)
Multi-pollutants		2.79 (-17.39 - 22.97)	41.22 (-0.76 - 83.20)	-0.47 (-22.09 - 21.16)	8.66 (2.84 - 14.47)	-0.65 (-16.02 - 14.72)	16.17 (5.67 - 26.67)
Single pollutant	Lag 2	9.49 (-9.60 - 28.58)	27.89 (-175.84 - 231.61)	0.49 (-1.97 - 2.95)	-0.35 (-3.16 - 2.46)	-3.99 (-7.15 - -0.82)	-4.06 (-127.94 - 119.81)
Multi-pollutants		9.49 (-9.60 - 28.58)	63.45 (4.77 - 122.13)	-2.13 (-21.72 - 17.46)	12.73 (4.61 - 20.85)	0.65 (-19.59 - 20.89)	-0.18 (-7.11 - 6.75)
Single pollutant	Lag 3	11.81 (-6.71 - 30.33)	49.48 (-284.65 - 383.61)	0.52 (-2.17 - 3.21)	-0.49 (-3.49 - 2.52)	-4.02 (-7.92 - -0.12)	-3.41 (-121.94 - 115.12)
Multi-pollutants		11.81 (-6.71 - 30.33)	57.02 (1.20 - 112.85)	-3.27 (-17.19 - 10.64)	15.17 (5.72 - 24.63)	1.80 (-16.92 - 20.52)	-27.74 (-44.34 - -11.14)
Single pollutant	Lag 4	9.76 (-5.47 - 24.99)	48.37 (-263.39 - 360.61)	-0.32 (-2.15 - 1.51)	-0.66 (-3.74 - 2.43)	-4.74 (-8.86 - -0.63)	-1.82 (-82.92 - 79.28)
Multi-pollutants		9.76 (-5.47 - 24.99)	21.93 (-13.46 - 57.32)	-3.91 (-13.92 - 6.10)	15.98 (6.14 - 25.82)	2.80 (-9.86 - 15.46)	-66.51 (-104.84 - -28.18)
Single pollutant	Lag 5	3.34 (-19.51 - 26.18)	24.57 (-112.51 - 161.65)	-2.03 (-3.44 - -0.61)	-0.87 (-4.16 - 2.42)	-6.16 (-10.31 - -2.01)	0.72 (-25.52 - 26.95)
Multi-pollutants		3.34 (-19.51 - 26.18)	-41.82 (-77.44 - -6.20)	-4.04 (-27.24 - 19.17)	15.15 (5.74 - 24.57)	3.64 (-18.61 - 25.89)	-116.49 (-118.67 - -48.31)
Single pollutant	Overall	28.91 (-50.82 - 108.64)	50.49 (-369.19 - 470.17)	-3.93 (-10.73 - 2.87)	-2.83 (-17.91 - 12.26)	-29.54 (-48.94 - -10.14)	-14.89 (-477.61 - 447.82)
Multi-pollutants		28.91 (-50.82 - 108.64)	132.13 (-55.69 - 319.95)	-12.11 (-61.58 - 37.36)	70.63 (26.22 - 115.05)	6.13 (-41.08 - 53.34)	-173.44 (-276.83 - -70.05)

Each estimates represents the adjusted lag-response relationship for an interquartile range increase in pollutants (20 µg/m³ for PM₁₀; 10 spores/m³ for *Alternaria*; 50 spores/m³ for *Cladosporium*)

The overall cumulative effect per interquartile range increase in pollutants is defined as the total contributions up to the maximum lag

PM₁₀: Particulate matter of diameter 10 micrograms

PEF: Peak expiratory flow

Single pollutant model includes only either PM₁₀, *Alternaria* or *Cladosporium*, adjusting for time-varying confounders such as humidity, temperature, pressure and wind speed at lag 0 and other covariates such as age, age-square, gender, height, height-square, childbirth weight, maternal smoking status, atopy, study area including a smoothing function (natural cubic spline) of 'day of test' with 4 degrees of freedom

Multi-pollutant model includes PM₁₀, *Alternaria* and *Cladosporium*, adjusting for time-varying confounders such as humidity, temperature, pressure and wind speed at lag 0 and other covariates such as age, age-square, gender, height, height-square, childbirth weight, maternal smoking status, atopy, study area including a smoothing function (natural cubic spline) of 'day of test' with 4 degrees of freedom

Bold text denotes statistical significance at $p < 0.05$

Appendix Table S6.3: Estimated effects per interquartile range increase in PM₁₀, *Alternaria* and *Cladosporium* on FEV₁ from single and multi-pollutant distributed lags covariate-adjusted linear regression models

		Effects estimated as beta coefficients (95% Confidence interval) on FEV ₁ (ml)					
		PM ₁₀		<i>Alternaria</i>		<i>Cladosporium</i>	
		Summer	Winter	Summer	Winter	Summer	Winter
Single pollutant	Lag 0	-34.33 (-177.20 - 108.53)	204.93 (142.05 - 267.82)	31.08 (-57.55 - 119.70)	-13.04 (-19.86 - -6.23)	-62.12 (-120.54 - -3.71)	-53.63 (-65.78 - -41.48)
Multi-pollutants		-51.08 (-197.59 - 95.44)	168.93 (100.11 - 237.75)	-26.84 (-127.84 - 74.15)	-6.70 (-13.67 - 0.27)	-63.19 (-125.67 - -0.71)	-45.84 (-58.91 - -32.77)
Single pollutant	Lag 1	-15.41 (-91.01 - 60.19)	102.57 (68.10 - 137.03)	57.16 (7.85 - 106.48)	-9.42 (-13.61 - -5.23)	-55.99 (-108.54 - -3.44)	-26.24 (-33.89 - -18.59)
Multi-pollutants		-33.08 (-110.60 - 44.44)	85.46 (48.97 - 121.95)	9.87 (-51.35 - 71.09)	-3.08 (-7.77 - 1.61)	-58.20 (-121.72 - 5.32)	-21.90 (-30.56 - -13.24)
Single pollutant	Lag 2	5.62 (-63.47 - 74.71)	41.46 (0.42 - 82.50)	59.34 (0.33 - 118.35)	-6.59 (-11.26 - -1.92)	-54.34 (-119.88 - 11.19)	-10.01 (-18.93 - -1.10)
Multi-pollutants		-13.06 (-83.69 - 57.57)	39.36 (-1.93 - 80.65)	30.99 (-32.82 - 94.82)	-1.54 (-6.75 - 3.66)	-56.46 (-133.58 - 20.65)	-6.47 (-16.42 - 3.47)
Single pollutant	Lag 3	28.74 (-41.45 - 98.92)	21.61 (-20.91 - 64.14)	37.61 (-23.35 - 98.56)	-4.55 (-9.24 - 0.14)	-57.18 (-117.60 - 3.23)	-4.95 (-13.90 - 3.99)
Multi-pollutants		8.99 (-62.97 - 80.97)	30.63 (-11.99 - 73.26)	36.53 (-27.70 - 100.77)	-2.09 (-7.36 - 3.19)	-57.98 (-128.28 - 12.33)	0.44 (-9.68 - 10.56)
Single pollutant	Lag 4	53.96 (-12.09 - 120.00)	43.03 (-0.71 - 86.76)	-8.04 (-71.11 - 55.03)	-3.31 (-7.67 - 1.05)	-64.51 (-119.50 - -9.53)	-11.06 (-20.02 - -2.10)
Multi-pollutants		33.08 (-34.80 - 100.96)	59.28 (15.66 - 102.90)	26.48 (-47.27 - 100.24)	-4.72 (-9.77 - 0.34)	-62.74 (-129.95 - 4.47)	-1.16 (-11.54 - 9.23)
Single pollutant	Lag 5	81.27 (-28.82 - 191.36)	105.70 (26.08 - 185.32)	-77.59 (-192.69 - 37.51)	-2.86 (-10.01 - 4.29)	-76.33 (-182.02 - 29.37)	-28.33 (-44.17 - -12.48)
Multi-pollutants		59.20 (-52.80 - 171.19)	125.31 (44.89 - 205.72)	0.84 (-133.78 - 135.46)	-9.43 (-17.14 - -1.71)	-70.76 (-198.64 - 57.12)	-11.26 (-28.54 - 6.01)
Single pollutant	Overall	119.84 (-190.83 - 430.51)	519.30 (337.79 - 700.82)	99.56 (-139.26 - 338.38)	-39.77 (-58.95 - -20.59)	-370.48 (-590.09 - -150.87)	-134.23 (-171.04 - -97.42)
Multi-pollutants		4.06 (-317.43 - 325.55)	508.96 (320.77 - 697.15)	77.89 (-202.05 - 357.83)	-27.56 (-50.60 - -4.51)	-369.34 (-650.91 - -87.77)	-86.19 (-131.69 - -40.70)

Each estimates represents the adjusted lag-response relationship for an interquartile range increase in pollutants (20 µg/m³ for PM₁₀; 10 spores/m³ for *Alternaria*; 50 spores/m³ for *Cladosporium*)

The overall cumulative effect per interquartile range increase in pollutants is defined as the total contributions up to the maximum lag

PM₁₀: Particulate matter of diameter 10 micrograms

FEV₁: Forced expiratory volume in one second

Single pollutant model includes only either PM₁₀, *Alternaria* or *Cladosporium*, adjusting for time-varying confounders such as humidity, temperature, pressure and wind speed at lag 0 and other covariates such as age, age-square, gender, height, height-square, childbirth weight, maternal smoking status, atopy, study area including a smoothing function (natural cubic spline) of 'day of test' with 4 degrees of freedom

Multi-pollutant model includes PM₁₀, *Alternaria* and *Cladosporium*, adjusting for time-varying confounders such as humidity, temperature, pressure and wind speed at lag 0 and other covariates such as age, age-square, gender, height, height-square, childbirth weight, maternal smoking status, atopy, study area including a smoothing function (natural cubic spline) of 'day of test' with 4 degrees of freedom

Bold text denotes statistical significance at $p < 0.05$

Appendix Table S7.1: Basic data comparing school children who were lost to follow-up with those with complete follow-up

	Baseline (n1)	Lost to follow-up (n2)	<i>p-value</i>
Age, years (n1 = 522, n2 = 68)	9.93 ± 0.92	10.09 ± 0.81	0.172
Gender, males (n1 = 522, n2 = 68)	258 (49.4)	35 (50)	0.751
Atopy, positive Phadiatop (n1 = 561)	215 (38.3)	-	-
Weight, kg (n1 = 520, n2 = 68)	32.29 ± 7.97	31.26 ± 5.89	0.304
Height, cm (n1 = 522, n2 = 68)	134.41 ± 7.83	134.43 ± 6.86	0.984
Rhinitis (n1 = 474, n2 = 60)	84 (17.7)	10 (16.7)	0.840
Doctor-diagnosed asthma (n1 = 476, n2 = 60)	16 (3.4)	2 (3.3)	0.991
Ocular-nasal symptoms (n1 = 537, n2 = 53)	132 (24.6)	19 (35.9)	0.073
Wheezing ** (n1 = 475, n2 = 60)	53 (11.2)	5 (8.3)	0.507
Asthma-symptom score ≥ 2 *** (n1 = 491, n2 = 50)	36 (7.3)	2 (4)	0.380
FEV ₁ , litres (n1 = 463, n2 = 55)	1.57 ± 0.29	1.59 ± 0.31	0.631
FVC, litres (n1 = 463, n2 = 55)	1.83 ± 0.33	1.85 ± 0.36	0.674
FEF ₂₅₋₇₅ , litre/sec (n1 = 464, n2 = 55)	1.95 ± 0.62	1.96 ± 0.66	0.911
FEV ₁ < LLN (n1 = 463, n2 = 55)	81 (17.5)	10 (18.2)	0.899
FVC < LLN (n1 = 463, n2 = 55)	55 (11.9)	9 (16.4)	0.339
FEV ₁ /FVC < 0.8 (n1 = 463, n2 = 55)	68 (14.7)	9 (16.4)	0.741
FEF ₂₅₋₇₅ < LLN (n1 = 464, n2 = 55)	86 (18.5)	13 (23.6)	0.363
FeNO, ppb* (n1 = 520, n2 = 69)	12.5 (9 – 19)	12 (9.5 – 23)	0.249
FeNO > 15 ppb (n1 = 520, n2 = 69)	199 (38.3)	30 (43.5)	0.404
FeNO > 35 ppb (n1 = 520, n2 = 69)	49 (9.4)	9 (13)	0.343

Continuous variable expressed as mean ± SD; Categorical variable expressed as n (%)

n1, number at baseline; n2, number lost to follow-up;

*skewed data represented as median (interquartile range)

** presence of wheeze in the past 12 months

*** Asthma symptoms score of more than 2 or more computed from 8 asthma associated symptoms questions

Appendix Table S7.2: Distribution of estimated annual average air pollutants levels, pollen levels, and meteorological variables across the study areas

Variables	Study areas				Mean ± SD	Overall Median	IQR
	Khayelitsha	Oudtshoorn	Marconi-Beam	Masiphumulele			
<i>Air pollutants</i>							
PM _{2.5} (µg/m ³)	11.89 ± 3.0	8.08 ± 6.3	10.90 ± 2.4	9.56 ± 2.8	10.01 ± 4.4	9.4	4.95
NO ₂ (µg/m ³)	24.22 ± 3.0	7.58 ± 1.8	23.3 ± 2.1	12.8 ± 0.8	16.62 ± 7.6	14.0	14.22
<i>Airborne pollen and fungal spores</i>							
<i>Alternaria</i> (spores/m ³)	140.03	66.06	158.28	56.30	107.3 ± 43.9	140.1	83.1
<i>Ascospores</i> (spores/m ³)	865.30	3143.76	1086.45	2498.31	1884.6 ± 982.7	1086.5	2278.5
<i>Cladosporium</i> (spores/m ³)	263.66	2090.55	629.63	385.47	896.5 ± 763.2	629.6	1826.9
<i>Poaceae</i> (counts/m ³)	10.8	13.75	23.87	14.92	15.7 ± 4.9	13.7	8.6
<i>Betula</i> (counts/m ³)	0.28	0.65	0.12	0.04	0.3 ± 0.2	0.3	0.5
<i>Cupressaceae</i> (counts/m ³)	5.25	47.42	5.91	3.45	17.0 ± 19.2	5.9	42.2
<i>Myrtaceae</i> (counts/m ³)	2.46	1.12	2.02	0.97	1.7 ± 0.6	2.0	1.3
<i>Oleaceae</i> (counts/m ³)	2.14	5.72	2.63	1.97	3.2 ± 1.6	2.6	3.6
<i>Pinus</i> (counts/m ³)	2.99	5.02	2.49	17.73	6.1 ± 5.8	2.5	2.7
<i>Myricaceae</i> (counts/m ³)	15.77	3.23	1.66	19.19	9.4 ± 7.4	3.3	13.3
<i>Meteorological factors</i>							
Maximum temperature (°C)	22.78	23.6	26.31	19.68	23.3 ± 2.2	23.6	2.2
Temperature (°C)	17.57	18.84	18.73	16.28	17.9 ± 0.9	18.7	1.3
Humidity (%)	71.67	78.33	64.42	74.08	72.2 ± 5.2	71.7	10.3
Pressure (hPa)	1012.19	1016.34	979.55	1017.04	1006.2 ± 15.5	1012.2	20.5

Max: Maximum; Min: Minimum; IQR: interquartile range, airborne pollen and meteorological factors do not vary within area as only one monitoring site was used per area