



Short term seasonal effects of airborne fungal spores on lung function in a panel study of schoolchildren residing in informal settlements of the Western Cape of South Africa[☆]

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ABSTRACT

Background: The individual effects of biological constituents of particulate matter (PM) such as fungal spores, on lung function in children are not well known. This study investigated the seasonal short-term effect of daily variation in *Alternaria* and *Cladosporium* fungal spores on lung function in schoolchildren. **Methods:** This panel study evaluated 313 schoolchildren in informal settlements of the Western Cape of South Africa, exposed to spores of two commonly encountered fungi, *Alternaria* and *Cladosporium* species. The children provided forced-expiratory volume in 1-s (FEV₁) and peak-expiratory flow (PEF) measurements thrice daily for two consecutive school-weeks in summer and winter. Daily PM₁₀ levels, from a stationary ambient air quality monitor and fungal spore levels using spore traps were measured in each study area throughout the year. The effects of *Alternaria* and *Cladosporium* spores, on lung function were analysed for lag periods up to five-days, adjusting-for PM₁₀, other pollen exposures, study area, and other host and meteorological factors. Same-day exposure-response curves were computed for both fungal species.

Results: There was more variability in *Alternaria* spores level with noticeable peaks in summer. There were consistent lag-effects for *Alternaria* on PEF compared to *Cladosporium*, with the largest PEF deficit observed in winter (mean deficit: 13.78 L/min, 95%CI: 24.34 to −3.23 L/min) per 10spores/m³ increase in *Alternaria* spores on lag day-2. Although there were no observable lag-effects for *Alternaria* and *Cladosporium* on FEV₁, same-day effects of *Cladosporium* spores on FEV₁ was present across both seasons. Threshold effects of *Alternaria* on both PEF and FEV₁ deficits were apparent at levels of 100 spores/m³, but could not be explored for *Cladosporium* beyond the levels observed during the study.

Conclusion: The study provides evidence for the independent effects of daily exposure to ambient fungal spores of *Alternaria* and *Cladosporium* on lung function deficits, more especially in winter for PEF.

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1. Introduction

Several epidemiological studies that have investigated the short-term effects of ambient particulate matter on children's lung function have focused mainly on its chemical components such as sulphates, minerals, and dust particles (Anderson et al., 2012; Li et al., 2012; Ward, 2004; Weinmayr et al., 2010). However, solid particles and droplets of liquid from various natural and anthropogenic sources, comprises an important component of airborne particulate matter (Wilson et al., 2002). Those of biological origin such as fungi and pollen contribute up to 25% of airborne particulates (Jaenicke, 2005). The components of airborne particulate most responsible for reduced lung function remains unclear, as varying effects have been reported previously (Anderson et al., 2012; Li et al., 2012; Ward, 2004; Weinmayr et al., 2010). Furthermore, most studies investigating the effects of biological particulates have used time-series data. The outcome of interest in these studies were obtained mainly at the population level, using hospital records for asthma visits, over-the counter asthma medication or emergency visits for asthma attacks (Atkinson et al., 2006; DellaValle et al., 2012; Gleason et al., 2014; Harley et al., 2009; Héguy et al., 2008; Rosas et al., 1998). These outcome measures limit the exploration of the respiratory health effects at the individual level. Furthermore, data on exposures to pollen or fungal spores in these studies were obtained mainly 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 outdoor fungal spores on respiratory health also need to take into consideration concomitant exposure to particulate matter as well as other meteorological factors.

Very few studies have investigated the association between fungal spores and lung function, taking into consideration co-exposure to 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 interquartile range (IQR) increase of 1.3 log spores/ m^3 was associated with a deficit of 0.11 L (95% CI: 0.19 to -0.03 L) in forced-expiratory volume in 1-s (FEV_1), and a deficit of 0.14 L (95%CI: 0.23 to -0.05 L) in forced vital capacity (FVC), independent of $PM_{2.5}$ (Chen et al., 2011). 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 each doubling increase of *Cladosporium* spores at lag day-1, above an identified threshold of 1514 spores/ m^3 , was associated with reduced FEV_1 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-h exposure to air pollutants such as CO , O_3 , SO_2 , NO_2 , $PM_{2.5}$ and $PM_{10-2.5}$ (Chen et al., 2014). Another study of children in the United States of America ($n = 108$) reported a deficit of 1.03 L/min (95% CI: 1.86 to -0.20 L/min) in morning peak expiratory flow (PEF) associated with a 10,000 spores/ m^3 increase in *Cladosporium* spores level, adjusting for meteorological variables (Neas et al., 1996). However, none of these studies reported effects on lung function deficits across longer lag-days (Chen et al., 2011; Neas et al., 1996). Furthermore, these studies purposefully included selected schoolchildren based on their asthmatic or allergic status, to enable the detection of acute effects on vulnerable populations. Watanabe et al., found greater association of outdoor fungi with pulmonary dysfunction among children with asthma, compared to those without asthma (Watanabe et al., 2016).

The overall findings of a recent review of the literature (Olaniyan

et al., 2016) indicated that there was little evidence from epidemiological studies of the independent effects of aeroallergen from fungal spores on respiratory health outcomes using objective measures such as lung function among otherwise healthy schoolchildren in the presence of co-exposure to particulate matter. There were also no reports on the time lag of these effects, nor the confounding effect of time-varying factors such as meteorological variables on these associations. Furthermore, to our knowledge, the association between fungal spores and lung function has not been conducted in sub-Saharan Africa, where climatic conditions are different to previously reported studies. This study, therefore, sought to examine the effect of daily variations in ambient fungal spores level independent of particulate matter of aerodynamic diameter of $10 \mu g/m^3$ or less (PM_{10}) and other pollen exposures on lung function during winter and summer seasons in a panel study of otherwise healthy schoolchildren in the Western Cape Province of South Africa.

2. Methods

2.1. Study population and design

The panel study was conducted from 15th February to February 26, 2016 and from 25th July to August 5, 2016, representative of the summer and the winter sampling periods, respectively. It should be noted that these seasons are peculiar to the southern hemisphere. The participants in this study were part of a larger cohort study, which investigated 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 (Olaniyan et al., 2017). 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)] that had a comprehensive set of detailed PM_{10} measurements, to enable the construction of a multi-pollutant model with fungal spores as the main exposure of interest. The study areas have similar and comparable socio-demographic profiles, with Khayelitsha and Marconi-Beam being an urban settlement within 20 km radius of the Cape Town metropolis, and having a coastal Mediterranean climate, experiencing cold wet winters and hot dry summers.

All participating schoolchildren walked and resided within a 1-km radius of their respective school. Participant's host characteristics – including age, gender, height, weight, child birthweight, maternal smoking status, atopic status, reported doctor-diagnosed asthma, and asthma medication-use, were all recorded in February 2016. Details on each of these measures have been previously published in the methodology article on this cohort (Olaniyan et al., 2017).

The University of Cape Town's Human Health Research Ethics Committee granted approval (HREC REF: 697/2014) to conduct the study, and permission to include schoolchildren was obtained from the Western Cape Department of Education (REFERENCE: 20140917-36653). Consent was obtained from each participating child's parent or guardian prior to questionnaire administration and performance of health outcome tests. All information was kept confidential and anonymity was maintained through unique participant identifiers used throughout the study.

2.2. Data collection

2.2.1. PM₁₀ and fungal spores data

Daily average hourly concentrations of PM₁₀ air quality data were measured with T640 Teledyne API particulate instrumentation available from a stationary ambient air quality monitor positioned at the Cape Town International airport (operated by the Airports Company South Africa and managed contractually via outsourcing) located approximately 16 km and 19 km from Khayelitsha and Marconi-Beam, respectively.

Ambient fungal spores including other pollens of weeds, trees, and grass were measured in each of the study areas close to the schools. However, only fungal spores (*Alternaria* and *Cladosporium*) and pollen that were predominant during the panel study period were investigated further in this study. These measurements were conducted using the Burkard 7-day recording volumetric spore trap, located on a flat rooftop of minimum height of 1.5 m to avoid dust contamination. The spore trap in each study area was located within a 10-km radius from each child's home and school address. Trained counters conducted microscopic analysis of pollen and spores, and the resulting daily counts were used to estimate the average daily concentrations in particles per cubic meter of air for each study area. The daily concentration of each spore and pollen taxa were extracted for the monitoring periods of the panel study.

Hourly meteorological data were obtained from the South African Weather Services (SAWS) for each of the study areas for throughout the monitoring period. The daily averages were computed from the hourly observations. The meteorological variables of interest included daily average temperature, wind speed, relative humidity and atmospheric pressure.

2.2.2. Lung function assessments

Each child was required to perform serial peak expiratory flow (PEF) and forced expiratory volume in 1 s (FEV₁) measurements bihourly (just before school started, during the first break and 2 h 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 school days during which lung function indices were obtained in summer was ten, and nine in winter, since there was a national public holiday on the August 3, 2016. In total, observations (number of school days x number of participants) of 3130 and 2817 were anticipated for 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 bihourly 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.

2.3. Statistical analyses

The lag effect of fungal spores of *Alternaria* and *Cladosporium* of up to day-5, on daily average PEF and FEV₁, considering co-exposure to PM₁₀ and other pollen exposures, was evaluated using random-effects models (equation (1)). This is based on the assumption that the variation across the children is random and uncorrelated with the fungal spores and other covariates in the model. In addition, we hypothesized that differences across each children, as observed in the preliminary analysis on this cohort (Olaniyan et al., 2018), may influence the fungal spores' effects on the measured lung function indices. The random-effect models included a random intercept (the individual child), and both time-variant and time-invariant covariates, in addition to the exposure variables. *Apriori*

confounders based on previous literature entailed host characteristics such as time-invariant variables obtained from questionnaires (childbirth weight and maternal smoking status) and variables obtained during lung function tests (age, gender, height, atopy status as assessed by the Phadiatop test) conducted during the overall cohort study (Olaniyan et al., 2018). Additional *apriori* confounders such as study area were included to adjust for any clustering or unmeasured characteristics at the area level, as well as time-varying meteorological variables (temperature, humidity, pressure and wind speed) obtained from each study area. Additional pollen variables such as *Ascospores*, *Betula*, *Poaceae*, and *Cupressaceae* were tested as possible potential confounders through bivariate testing (p -value < 0.1) and step-wise model building, but only *Poaceae* pollen was positively identified. The final model therefore consisted of all the *apriori* confounders and *Poaceae* pollen in addition to the exposure variables included in the model in a saturated manner. Absolute difference in PEF and FEV₁ values were used to present the estimated effect, with 95% confidence intervals (CI) per increase of 10 spores/m³ of *Alternaria* and 1-log spores/m³ of *Cladosporium*. Fractional polynomial prediction plot was used to calculate the prediction for both the average PEF and FEV₁ from estimation of a fractional polynomial of either *Alternaria* or *Cladosporium* at lag-0 to plot the resulting exposure-response curve. The random-effects models and exposure-response relationship analyses were performed using STATA statistical software (version 15.1).

$$\text{PEF (FEV}_1\text{)}_{it} = \beta_0 + \beta(\text{Alternaria/Cladosporium})_{it} + \sum \beta_k X_{k,it} + U_{it} + \varepsilon_{it} \quad (1)$$

where β_0 = the intercept.

$i = 1 \dots N$ (number of individuals)
 t = daily time measure
 k = number of adjusted covariates
 β_k = coefficients for the adjusted covariates
 $X_{k,it}$ = each adjusted covariates
 U_{it} = between-individual error
 ε_{it} = within-individual error

Before running the random-effects models, imputations (assignments) for missing PM₁₀ data was done on less than 5% of the PM₁₀ data using the 'Multivariate Imputation by Chained Equation (MICE)' package in the R statistical package (van Buuren and Groothuis-Oudshoorn, 2007). 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.

In addition to evaluating the individual effect of fungal spores of *Alternaria* and *Cladosporium*, in the single pollutant models, a multi-pollutant model was applied to assess the independent effect of these fungal spores after adjusting for the other fungal spores, as well as PM₁₀, *Poaceae* pollen, host characteristics, meteorological variables, and study area. The difference in the estimated effects on PEF and FEV₁ were explored between schoolchildren with and without complete follow-up in summer and winter, in order to assess if observed effects were associated with school absenteeism. Furthermore, differences in effect estimates were also assessed in schoolchildren with and without current clinical/symptomatic asthma using reported current asthma medication-use as an indicator since not all sensitized individuals were symptomatic. All analyses were estimated with a two-sided p -value of 0.05.

3. Results

The measured concentrations for PM₁₀, airborne fungal spores, *Poaceae* pollen and meteorological data are presented in Table 1 and Fig. 1. Overall, spore levels of *Alternaria* and *Cladosporium* were higher during the dry summer weather phase (Table 1). In Marconi Beam, there were 6 days (5 in summer and 1 in winter) during which the allergic health effect threshold of 80 spores/m³ for *Alternaria* (Kasprzyk et al., 2015) were exceeded (Fig. 1). However, all measured levels of *Cladosporium* spores were under the allergic health effect threshold of 1500 spores/m³ (Kasprzyk et al., 2015) during summer and winter study periods. The PM₁₀ levels were relatively similar during the summer and winter phases of the panel study (Table 1). All daily average PM₁₀ levels were below the National Ambient Air Quality Standard of 75 µg/m³ over a 24-h averaging period (Department of Environmental Affairs, 2009). A correlation matrix of the day-to-day concentrations or levels of the pollutants and meteorological variables are presented in appendix Table A1. There was a moderate positive correlation between *Alternaria* and *Cladosporium* (Spearman's correlation, $r = 0.51$, $p < 0.001$), *Alternaria* and *Poaceae* ($r = 0.45$, $p < 0.001$), including a moderate positive association between *Cladosporium* and *Poaceae* ($r = 0.56$, $p < 0.001$). The host characteristics and the measured lung function indices are also presented in Table 1. The lung function parameters were, on average, similar for both summer and winter (Table 1). A total of 2542 and 2162 lung function measurements were utilised for the analysis of 276 and 258 children in summer and winter, respectively. The missing lung function measurements (61 and 79 participants in summer and winter, respectively) were the result of participants being absent from school on these specific test days.

3.1. Effect of alternaria on lung function

There were consistent lag-effects of *Alternaria* spores on PEF

deficit in summer, independent of co-exposure to *Cladosporium*, PM₁₀, and *Poaceae* pollen, as evident in the single- and multi-pollutant models (Fig. 2A). Regarding PEF, this lagged effect demonstrated an increasing deficit across lag day-2 to lag day-5 per 10 spores/m³ increase for *Alternaria* in summer (Fig. 2A and Table A2). However, the largest PEF deficit was observed in winter (mean deficit: 13.78 L/min, 95%CI: 24.34 to −3.23 L/min) per 10 spores/m³ increase in *Alternaria* on lag day-2. Furthermore, there were no consistent lag-effects observed for FEV₁ (Fig. 3A). Nonetheless, there were observable threshold effects of *Alternaria* on both PEF and FEV₁ deficits at levels of 100 spores/m³ (Fig. 4). All observed lag-effect estimates were consistent even after restricting the analyses to schoolchildren with complete follow-up in summer and winter (Figure appendix A2A and A2B).

3.2. Effect of Cladosporium on lung function

There was an increase deficit in PEF from lag day-3 to lag day-5 per 1-log spores/m³ increase in *Cladosporium* in winter. These effects were independent of co-exposure to *Alternaria*, PM₁₀, and *Poaceae* pollen (Fig. 2B). The largest deficit in PEF was observed on lag day-5 in winter (mean deficit: 8.74 L/min, 95%CI: 12.51 to −4.97 L/min) per 1 log spores/m³ increase in *Cladosporium*. In summer, the effects of *Cladosporium* on PEF deficit were consistent across all the lag-periods investigated (i.e. lag day-0 to lag day-5) per 1 log spores/m³ increase (Fig. 2B and Table A2). However, these consistent effects of *Cladosporium* were not observed for FEV₁, since the lag-effects demonstrated an inverted U-shape with significant same-day (lag day-0) and latter lag day effects, both in summer and winter (Fig. 3B and Table appendix A3). Although the effects of *Cladosporium* could not be explored beyond the known threshold levels of 1500 spores/m³ (equivalent to 7.3 log spores/m³), the exposure-response curve demonstrated an increasing deficit in both PEF and FEV₁, with increasing *Cladosporium* levels (Fig. 4B).

Table 1
Levels of 24-h average for PM₁₀, airborne fungal spores, pollen, meteorological factors, including host characteristics and lung function indices measured during the sampling phases.

Parameter	Summer (15 Feb - Feb 26, 2016)	Winter (25 July - Aug 5, 2016)	Combined
Exposures (period)	8 Feb-26 Feb	18 July-5 Aug	
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 (0.0–2.2)	4.3 (1.4–21.6)
<i>Cladosporium</i> (spores/m ³)	27.4 (7.9–66.9)	4.3 (0.7–16.6)	12.2 (2.2–50.4)
<i>Poaceae</i> pollen (grains/m ³)	0.7 (0.0–1.4)	0.2 (0.0–0.1)	0.4 (0.0–0.72)
Meteorological factors (period)	8 Feb-26 Feb	18 July-5 Aug	
Temperature (°C)	21.7 (20.9–23.4)	14.2 (12.9–15.2)	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 (n1)	313	313	313
Age (years)	10.0 (9.0–10.0)	10.0 (9.0–10.0)	10.0 (9.0–10.0)
Gender (Male) ^a	149 (50.5)	149 (50.5)	149 (50.5)
Weight (kg)	31.2 (27.9–36.3)	31.2 (27.9–36.3)	31.2 (27.9–36.3)
Height (cm)	135 (130.0–140.0)	135 (130.0–140.0)	135 (130.0–140.0)
Body mass index (kg/m ²)	17.2 (15.8–19.4)	17.2 (15.8–19.4)	17.2 (15.8–19.4)
Atopy (positive Phadiatop) ^a	131 (42.1)	131 (42.1)	131 (42.1)
Prenatal maternal smoking ^a	49 (15.8)	49 (15.8)	49 (15.8)
Birth weight (kg)	3.2 (3.0–3.8)	3.2 (3.0–3.8)	3.2 (3.0–3.8)
Lung function indices (n2)	276	258	288
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 meteorological variables are presented as 24-h daily average across the summer and winter study period. The exposures were lagged for 5-days prior to the commencement of the lung function test in the various schools. n1 is the total sample size of the schoolchildren in the two study areas. n2 is the number of schoolchildren with available lung function indices that were analysed across summer and winter. Exposures, meteorological factors and continuous host characteristics are presented as median (interquartile range).
^a Categorical variable presented as number (%).

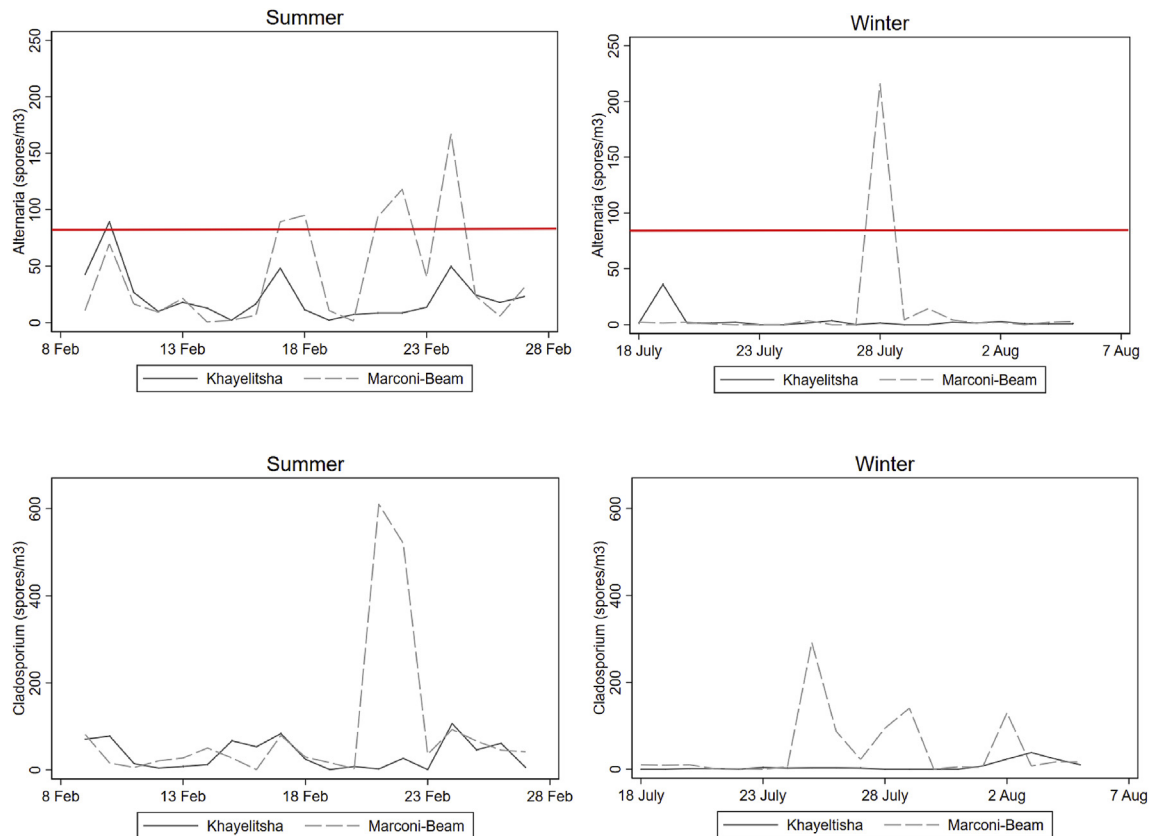


Fig. 1. Concentration of *Alternaria* – top panel, and *Cladosporium* – bottom panel, in summer and winter across the two study areas over a 2-week period in 2016 (red line represents the allergenic threshold of 80 spores/m³ - Kasprzyk et al., 2015). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

4. Discussion

This is the first study, to the best of our knowledge, to estimate longer lag period effects (of up to 5-days) on lung function due to short-term exposure to fungal spores of *Alternaria* and *Cladosporium*, independent of other concomitant particulate and pollen exposures. That the study was conducted in a middle-income country in sub-Saharan Africa, is important since low- and middle-income countries globally have the highest global burden of lung disease resulting from air pollution (Coker and Kizito, 2018; World Health Organization, 2016). Most previous epidemiological studies on the short-term respiratory health effects of fungal spores with co-exposure to air pollutants used time-series analyses and air quality/pollen data often from single or few stationary monitors. Furthermore, the outcomes were based on number of asthma hospital admissions or reported asthma symptoms (Atkinson et al., 2006; Dales et al., 2000; Endre et al., 2007; Héguay et al., 2008; Newson et al., 2000), rather than individual effects as has been reported in few epidemiological studies (Chen et al., 2014, 2011; Neas et al., 1996). However, the short-term effects of outdoor fungal spores on lung function in schoolchildren observed in this latter group were not reported beyond 1-day lag.

The most important finding in this study was the consistent independent effect of fungal spores resulting in a PEF deficit. The results indicate that the PEF deficit associated with exposure to *Alternaria* spores increased across lag day-2 to lag day-5 in summer (Fig. 2A and appendix Table A2) therefore suggesting a lagged effect. The results also show an increased deficit in PEF from lag day-3 to lag day-5 per 1-log spores/m³ increase in *Cladosporium* in winter.

The lagged associations with lung function indices suggest that these exposures may likely trigger sustained (immediate and delayed) reactions in the airways. It is possible that such lagged lung function effects could decline, remain the same or have a delayed increase over time, as observed in this study. For instance, the lag-effects of *Cladosporium* spores exposure on FEV₁ deficit were not consistently declining with increasing lag period. Rather, these effects (i.e. *Cladosporium* spores exposure on FEV₁ deficit) demonstrated an inverted U-shape with significant deficit observed on lag day-0 and lag day-5, and approaching a null to positive associations observed at lag day-1 through lag day-4, both in summer and winter (Fig. 3B and appendix Table A3). While the consistent same-day FEV₁ deficits demonstrates an immediate hypersensitivity (type-1 allergic reaction) expected following exposure to fungal allergens, the delayed effects observed which were discrepant between PEF and FEV₁, could be due to a dual reaction but this warrants further investigation. This highlights the importance of considering lag-response relationships in epidemiological studies that explore the effect of variation in environmental factors on respiratory health outcomes.

Another interesting finding in the study was the increased deficit in PEF following exposure to fungal spores of *Alternaria* and *Cladosporium* when controlling for PM₁₀ and *Poaceae* pollen in the multi-pollutant model (Fig. 2A and B) compared to the single-pollutant model, which is unexpected and indicates a complex interaction between these pollutants. The factors that play a role in these interactions include the increased duration of allergens in air carried by fungal spores or pollen, the increased allergenic effect of pollen or spores in the presence of particulate matter, which may

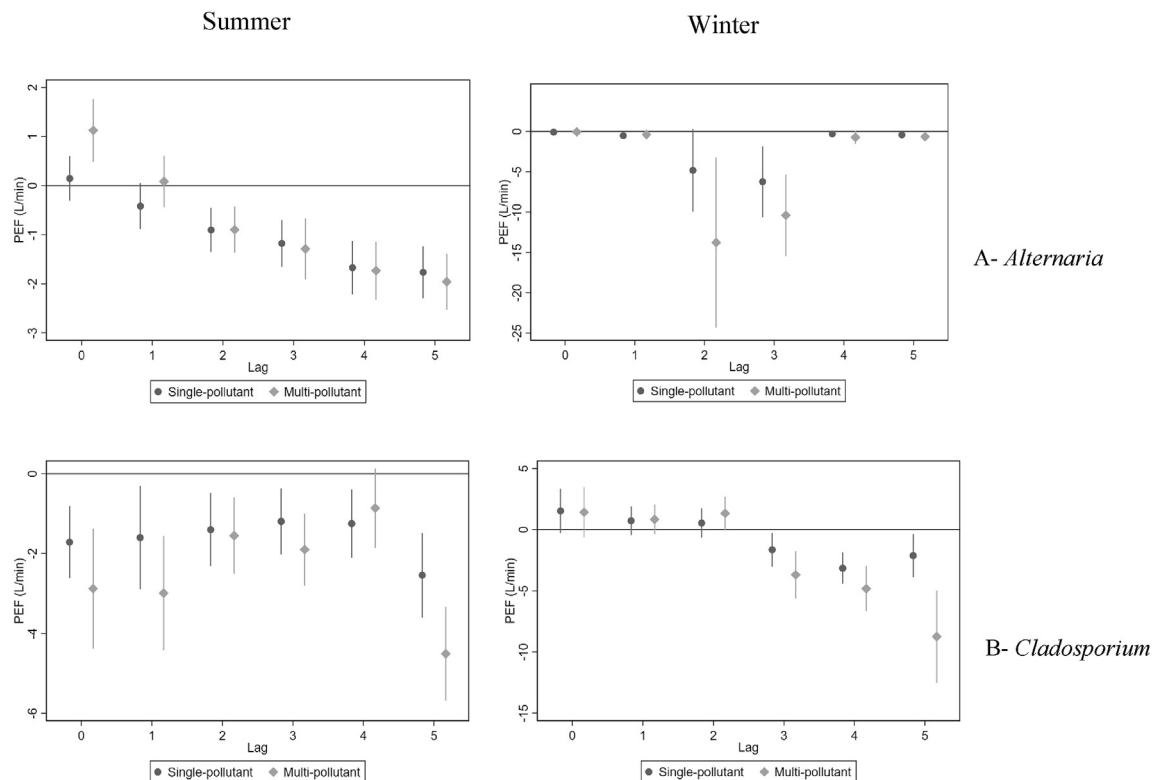


Fig. 2. A, B: Each panel represents the single- and multi-pollutant lagged effect on peak expiratory flow (PEF) from random-effects models across summer and winter for A) an increase of 10 spores/m³ for *Alternaria*; B) an increase of 1 log spores/m³ for *Cladosporium*. Single pollutant model includes only either *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, gender, height, childbirth weight, maternal smoking status, atopy, study area. Multi-pollutant model includes *Alternaria*, *Cladosporium*, PM₁₀, and pollen of *Poaceae* adjusting for time-varying confounders such as humidity, temperature, pressure and wind speed at lag 0 and other covariates such as age, gender, height, childbirth weight, maternal smoking status, atopy, study area.

promote airway sensitization (D'Amato, 2011; García-Gallardo et al., 2013), and/or the increased exposure to allergenic constituents given that ambient particulate matter also function as a carrier of allergens. Allergens released from pollen and spores adhere to particulate matter due to their porous surfaces and intrinsic electrostatic properties (D'Amato et al., 2002). Under experimental conditions, *Phleum pratense* pollen has been reported to release more allergen-containing granules on contact with air pollutants, compared to exposure to air only (Motta et al., 2006). Traffic-related pollutants have also been documented to increase the bioavailability of airborne pollen allergens (Motta et al., 2006). 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 (D'Amato et al., 2002).

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 μ m) (Reponen et al., 1996) 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 Th2 and non-Th2 cytokine types have been reported when human epithelial cells are exposed to *Cladosporium* extracts during in-vitro culture (Shin et al., 2006, 2004). Chronic respiratory effects including allergic inflammation in the lungs on administration of fungal spores have also been reported in animal studies (Denis et al., 2007). The lung function effects observed in this study

may therefore have resulted from inflammatory responses to unique biological properties (Green et al., 2003; Horner et al., 1995) and *Cladosporium* antigen activity (Kauffman et al., 2000). Although, specific sensitization to *Alternaria* and *Cladosporium* was not tested in the children who participated in this study, consistent effect estimates were observed when children with and without current clinical asthma (using asthma medication-use as proxy) were excluded from the analyses (data not shown).

This study has also demonstrated that time-varying covariates such as meteorological factors, and more broadly seasonality, have an impact on the association between biological pollutants (such as ambient fungal spores) on the respiratory health of children. There were seasonal differences in the levels of fungal spores, with a study area (Marconi-Beam) having five days with levels above the allergic threshold of 80 spores/m³ of *Alternaria* spores in summer (Kasprzyk et al., 2015). However, the largest PEF deficit was observed in winter (mean deficit: 13.78 L/min, 95%CI: 24.34 to -3.23 L/min) per 10 spores/m³ increase in *Alternaria* on lag day-2. Similarly, for *Cladosporium*, the largest deficit in PEF was observed on lag day-5 in winter (mean deficit: 8.74 L/min, 95%CI: 12.51 to -4.97 L/min) per 1 log spores/m³ increase in *Cladosporium*. Furthermore, there were observable threshold effects of *Alternaria* on both PEF and FEV₁ deficits at levels of 100 spores/m³ (Fig. 4). This threshold effect was adjusted for season, study area and all other covariates in the random-effects models. With regard to the threshold for the *Cladosporium* spores effect, a previous study by Chen et al., found that each doubling increase of *Cladosporium* spores at lag day-1, above an identified threshold of 1514 spores/m³, was associated with reduced FEV₁ of -0.23 L (95% CI: 0.35

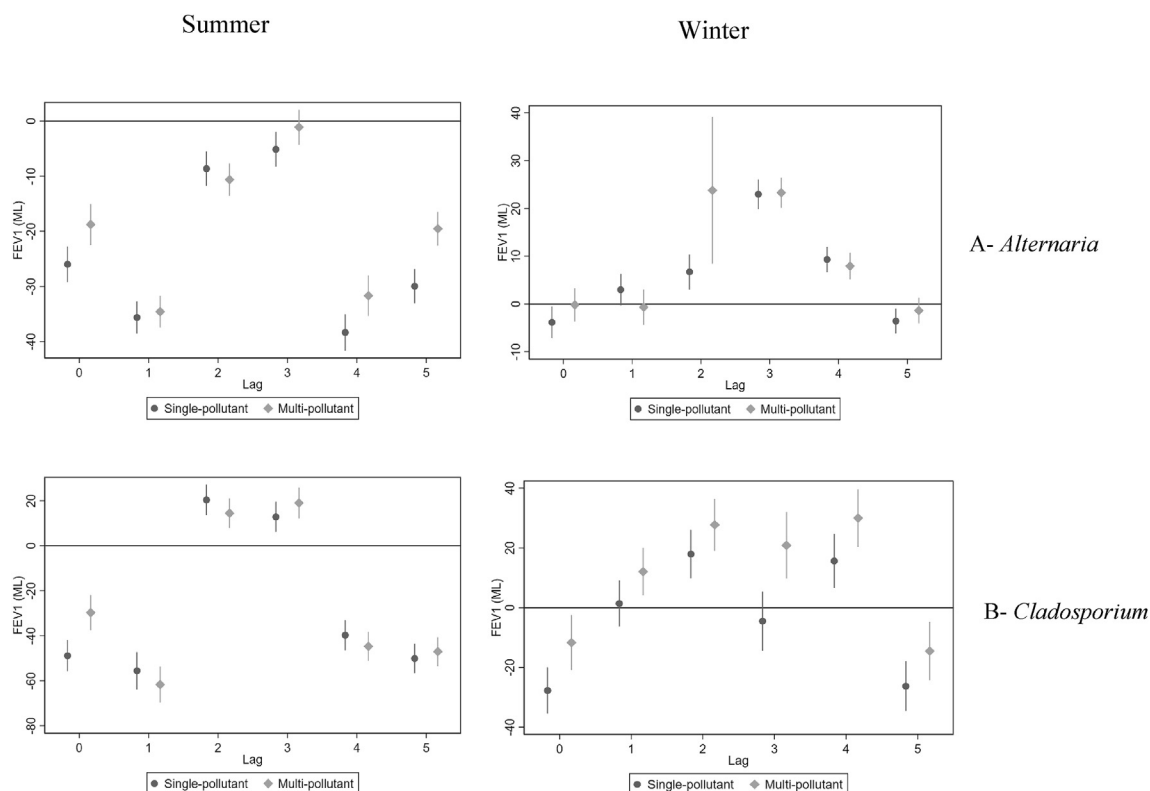


Fig. 3. A, B: Each panel represents the single- and multi-pollutant lagged effect on forced expiratory volume in 1 s (FEV_1) from random-effects models across summer and winter for A) an increase of 10 spores/ m^3 for *Alternaria*; B) an increase of 1 log spores/ m^3 for *Cladosporium*. Single pollutant model includes only either *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, gender, height, childbirth weight, maternal smoking status, atopy, study area. Multi-pollutant model includes *Alternaria*, *Cladosporium*, PM_{10} , and pollen of *Poaceae* adjusting for time-varying confounders such as humidity, temperature, pressure and wind speed at lag 0 and other covariates such as age, gender, height, childbirth weight, maternal smoking status, atopy, study area.

to -0.11 L), independent of air pollutants (Chen et al., 2014). However, in this current study, the effects of *Cladosporium* spores could not be explored beyond the known threshold levels of 1500 spores/ m^3 (equivalent to 7.3 log spores/ m^3), but the exposure-response curve showed an increasing deficit in both PEF and FEV_1 , with increasing *Cladosporium* spores level (Fig. 4B). Furthermore, the observed association found with *Cladosporium* at this low level may partly be due to underlying susceptibility previously reported in this current study population, that present with a relatively higher proportion of under-diagnosed and under-recognised asthma (50% without asthma medication), as well as a high proportion (38%) with airway inflammation as assessed using exhaled-nitric oxide (FeNO) (Olaniyan et al., 2018).

There are some limitations in this study worth nothing. Firstly, the estimation of exposures to PM_{10} levels were based on a stationary ambient air quality monitor that was not located immediately within the vicinity of the two study areas (i.e. located 16 km from Khayelitsha and 19 km 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 ambient air quality monitor located at the Cape Town International Airport provided background data that was used to estimate particulate matter exposure throughout the monitoring period in this panel study. Although health data were collected in four study areas, two study areas were excluded from the analysis, since complete ambient air quality data for those areas were not available. 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, there was sufficient level of spatial variability and exposure gradient across the study areas to assess the fungal spores effect. Nonetheless, it is difficult to rule out the possibility of random variation as an explanation for the inconsistent lag effects observed in this study (for example; that of *Cladosporium* spores exposure on FEV_1).

The use of stationary monitors in assessing exposures often introduces exposure misclassification since time-activity patterns were not available (Steinle et al., 2013). Personal monitors are more representative of personal exposures to various pollutant (Steinle et al., 2013). 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 over-estimated since differential exposure misclassification would only exist if the proportion of time spent outdoors or indoors was related to the fungal spore level. Furthermore, all participating schoolchildren walked and resided within a 1-km radius of their respective school.

5. Conclusion

The current study has provided evidence that exposure of schoolchildren to *Alternaria* and *Cladosporium* spores negatively

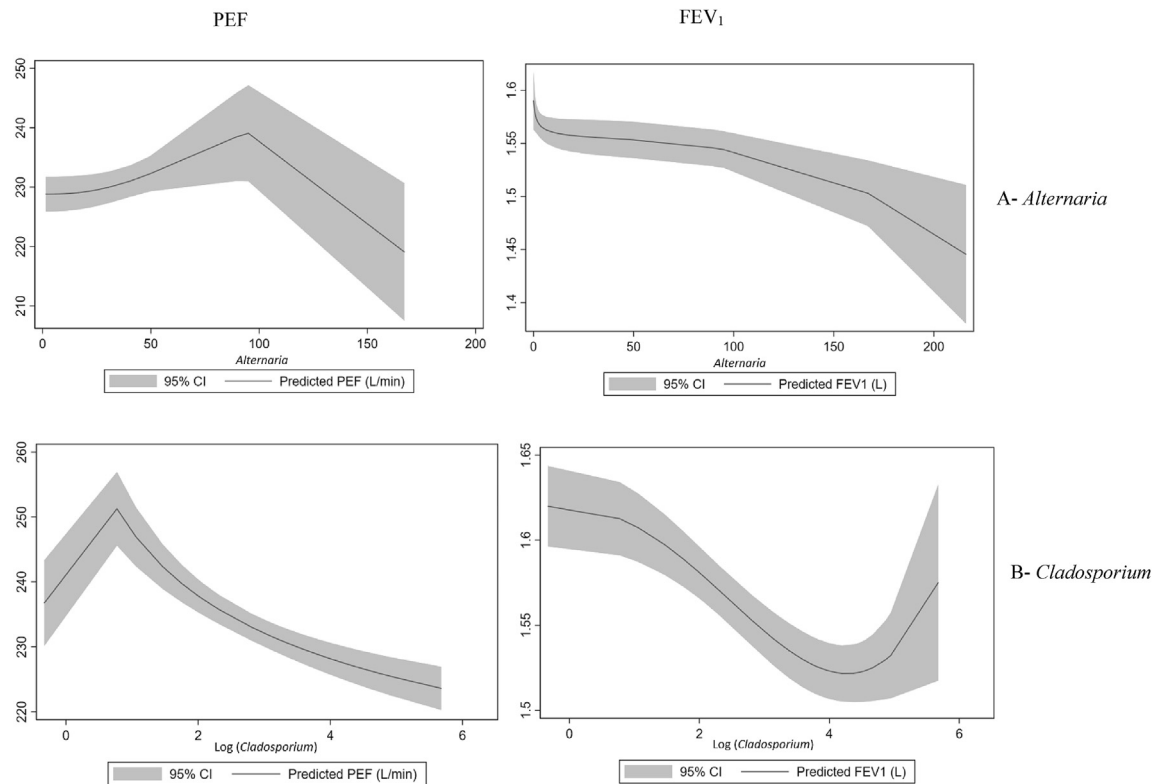


Fig. 4. Adjusted exposure-response curve of PEF and FEV₁ over *Alternaria* (spores/m³) – top panel (A), and log of *Cladosporium* (spores/m³) – bottom panel (B), using same-day exposure spores level.

impacted lung function parameters beyond the day of exposure. Although a consistent increasing PEF deficit was observed in summer with a long lag-period, the largest deficit in PEF was observed in winter. There was also an apparent threshold effect at 100 spores/m³ of *Alternaria* on PEF and FEV₁ deficit. Should current climate predictions hold, longer sporulation of dry weather spores 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. This increased risk may be due to longer exposure duration even at low levels of exposure to these spores. Moreover, it is likely that increasing levels of air pollutants associated with variability in meteorological conditions may lead to greater allergenic potency of fungal spores as suggested by the observed increased deficit in lung function indices in the presence of co-exposure to PM₁₀. It is therefore important that the public be provided with real-time information and alerts on ambient 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 should incorporate specific allergen sensitization tests with more repeated monitoring of particulate matter levels (to account for spatiotemporal variations), which would enhance further investigations of co-exposure of biological and particulate matter association on short-term adverse respiratory health effects in children.

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Appendices

Table A1

Correlation matrix of the day-to-day concentrations or levels of the pollutants and climatic variables

	<i>Alternaria</i>	<i>Cladosporium</i>	PM ₁₀	<i>Poaceae</i>	Temperature	Humidity	Pressure	Wind Speed
<i>Alternaria</i>	1.000	0.506*	0.169*	0.468*	0.737*	−0.420*	−0.477*	0.355*
<i>Cladosporium</i>		1.000	0.055*	0.556*	0.432*	−0.409*	−0.161*	0.181*
Particulate matter (PM ₁₀)			1.000	0.165*	0.243*	−0.469*	−0.122*	−0.199*
<i>Poaceae</i>				1.000	0.565*	−0.515*	−0.212*	0.235*
Temperature					1.000	−0.597*	−0.575*	0.248*
Humidity						1.000	0.074*	−0.191*
Pressure							1.000	−0.301
Wind Speed								1.000

* $p < 0.001$.

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

Total exposure measurements = 76: which is the available measurement applicable to all variables simultaneously across 2 school weeks (14 days) plus 5 days lag constituting a total of 19 measurement days per season for each of the 2 study areas.

Table A2

Estimated effects of fungal spores of *Alternaria* and *Cladosporium* on peak expiratory flow (PEF) in a single and multi-pollutant lagged random-effects models after adjusting for particulate matter $\leq 10 \mu\text{m}$ in diameter (PM₁₀) and pollen

		Effects estimated as beta coefficients (95% Confidence interval) on PEF (l/min)			
		<i>Alternaria</i>		<i>Cladosporium</i>	
		Summer	Winter	Summer	Winter
Single pollutant	Lag 0	0.14 (−0.31–0.60)	−0.09 (−0.41–0.24)	−1.72 (−2.62–−0.81)	1.53 (−2.83–3.34)
Multi-pollutants		1.13 (0.49–1.76)	−0.06 (−0.45–0.34)	−2.88 (−4.38–−1.38)	1.42 (−0.63–3.47)
Single pollutant	Lag 1	−0.42 (−0.89–0.05)	−0.51 (−0.85–−0.18)	−1.60 (−2.89–−0.31)	0.72 (−0.44–1.88)
Multi-pollutants		0.08 (−0.44–0.61)	−0.39 (0.80–0.03)	−2.99 (−4.42–−1.56)	0.83 (−0.38–2.04)
Single pollutant	Lag 2	−0.90 (−1.35–−0.46)	−4.83 (−9.94–0.29)	−1.40 (−2.32–−0.49)	0.54 (−0.66–1.74)
Multi-pollutants		−0.90 (−1.38–−0.43)	−13.78 (−24.34–−3.23)	−1.55 (−2.51–−0.60)	1.33 (−0.03–2.69)
Single pollutant	Lag 3	−1.18 (−1.65–−0.70)	−6.23 (−10.62–−1.84)	−1.20 (−2.02–−0.37)	−1.65 (−3.03–−0.26)
Multi-pollutants		−1.29 (−1.91–−0.67)	−10.40 (−15.45–−5.35)	−1.90 (−2.80–−0.99)	−3.70 (−5.62–−1.77)
Single pollutant	Lag 4	−1.67 (−2.21–−1.13)	−0.30 (−0.61–0.01)	−1.25 (−2.10–−0.40)	−3.15 (−4.43–−1.88)
Multi-pollutants		−1.73 (−2.32–−1.14)	−0.73 (−1.54–0.09)	−0.86 (−1.86–−0.13)	−4.83 (−6.68–−2.98)
Single pollutant	Lag 5	−1.77 (−2.29–−1.24)	−0.42 (−0.72–−0.11)	−2.54 (−3.60–−1.48)	−2.13 (−3.89–−0.36)
Multi-pollutants		−1.96 (−2.53–−1.39)	−0.64 (−0.99–−0.30)	−4.51 (−5.68–−3.34)	−8.74 (−12.51–−4.97)

Each estimates represents the adjusted lag-response relationship for an increase of 10 spores/m³ for *Alternaria* and 1 Log spores/m³ for *Cladosporium*.

PM₁₀: Particulate matter of aerodynamic diameter 10 μm or less.

PEF: Peak expiratory flow.

Single pollutant model includes only either *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, gender, height, childbirth weight, maternal smoking status, atopy, study area.

Multi-pollutant model includes *Alternaria*, *Cladosporium*, PM₁₀, and pollen of *Poaceae* adjusting for time-varying confounders such as humidity, temperature, pressure and wind speed at lag 0 and other covariates such as age, gender, height, childbirth weight, maternal smoking status, atopy, study area.

Bold text denotes statistical significance at $p < 0.05$.

Table A3

Estimated effects of fungal spores of *Alternaria* and *Cladosporium* on Forced expiratory volume in 1 s (FEV₁) in a single and multi-pollutant lagged random-effects models after adjusting for particulate matter $\leq 10 \mu\text{m}$ in diameter (PM₁₀) and pollen

		Effects estimated as beta coefficients (95% Confidence interval) on FEV ₁ (ml)			
		<i>Alternaria</i>		<i>Cladosporium</i>	
		Summer	Winter	Summer	Winter
Single pollutant	Lag 0	−26.01 (−29.24–−22.77)	−3.85 (−7.17–−0.54)	−48.89 (−55.79–−41.98)	−27.75 (−35.45–−20.04)
Multi-pollutants		−18.77 (−22.49–−15.06)	−0.20 (−3.67–3.27)	−29.74 (−37.57–−21.90)	−11.72 (−20.92–−2.53)
Single pollutant	Lag 1	−35.64 (−38.54–−32.74)	2.97 (−0.37–6.30)	−55.59 (−63.91–−47.26)	1.38 (−6.35–9.10)
Multi-pollutants		−34.59 (−37.41–−31.77)	−0.68 (−4.40–3.04)	−61.71 (−69.69–−53.71)	12.04 (4.12–19.96)
Single pollutant	Lag 2	−8.65 (−11.76–−5.54)	6.71 (3.03–10.40)	20.31 (13.56–27.06)	17.94 (9.83–26.05)
Multi-pollutants		−10.63 (−13.57–−7.69)	23.79 (8.46–39.13)	14.43 (7.85–21.01)	27.74 (19.03–36.45)
Single pollutant	Lag 3	−5.15 (−8.31–−2.00)	22.98 (19.85–26.10)	12.77 (6.08–19.47)	−4.51 (−14.39–5.36)
Multi-pollutants		−1.12 (−4.31–2.06)	23.30 (20.14–26.46)	18.99 (12.09–25.88)	20.86 (9.70–32.02)
Single pollutant	Lag 4	−38.36 (−41.66–−35.06)	9.31 (6.66–11.97)	−39.77 (−46.42–−33.11)	15.61 (6.54–24.68)
Multi-pollutants		−31.69 (−35.36–−28.03)	7.94 (5.17–10.71)	−44.71 (−51.12–−38.30)	29.99 (20.34–39.64)
Single pollutant	Lag 5	−29.98 (−33.08–−26.87)	−3.58 (−6.17–−0.99)	−50.12 (−56.61–−43.63)	−26.29 (−34.63–−17.96)
Multi-pollutants		−19.55 (−22.62–−16.48)	−1.38 (−4.03–1.26)	−47.05 (−53.46–−40.65)	−14.54 (−24.35–−4.73)

Each estimates represents the adjusted lag-response relationship for an increase of 10 spores/m³ for *Alternaria* and 1 Log spores/m³ for *Cladosporium*.

PM₁₀: Particulate matter of aerodynamic diameter 10 μm or less.

FEV₁: Forced expiratory volume in 1 s.

Single pollutant model includes only either *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, gender, height, childbirth weight, maternal smoking status, atopy, study area.

Multi-pollutant model includes *Alternaria*, *Cladosporium*, PM₁₀, and pollen of *Poaceae* adjusting for time-varying confounders such as humidity, temperature, pressure and wind speed at lag 0 and other covariates such as age, gender, height, childbirth weight, maternal smoking status, atopy, study area.

Bold text denotes statistical significance at $p < 0.05$.

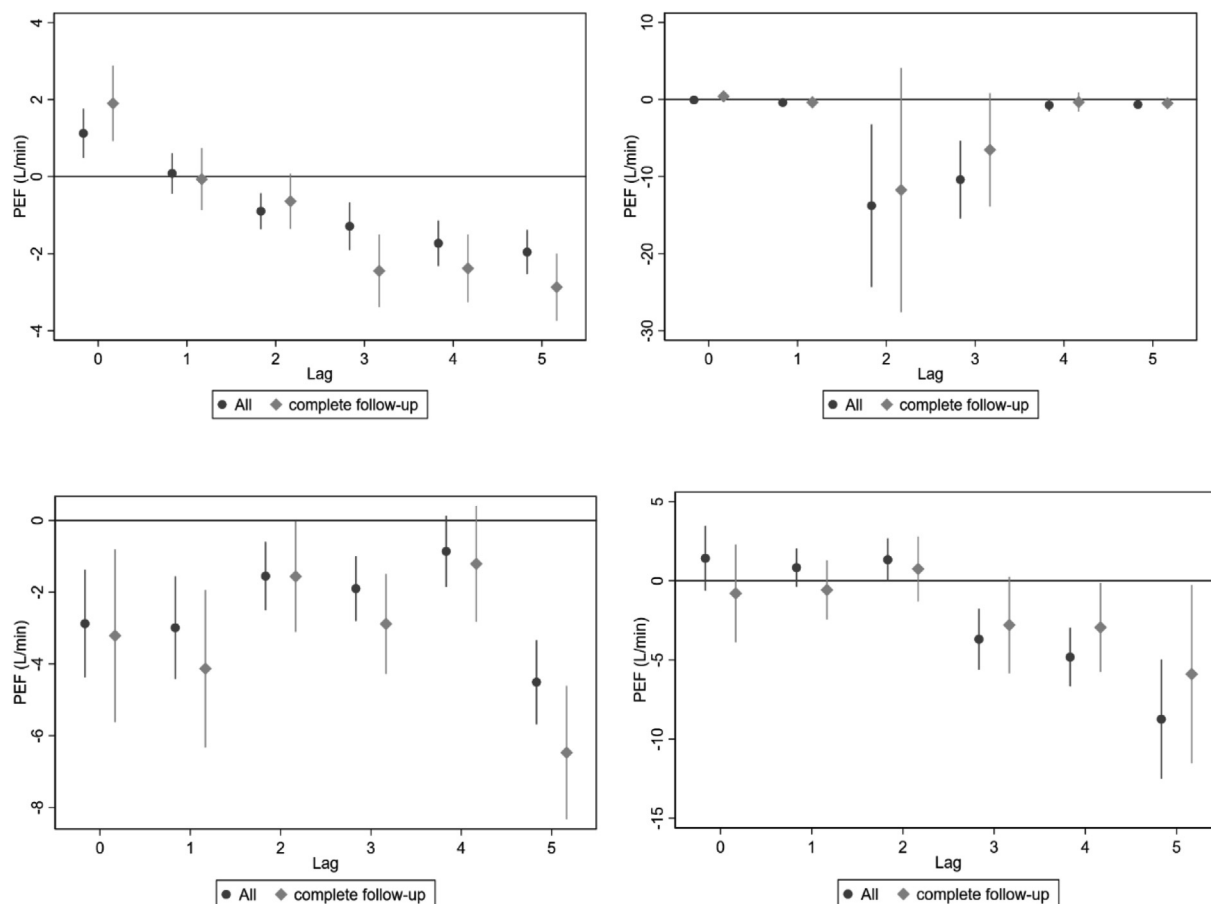


Fig. A2. A, B: Each panel represents the multi-pollutant lagged effect on peak expiratory flow (PEF) from random-effects models comparing school children with and without complete follow-up (ALL), across summer and winter for A) an increase of 10 spores/m³ for *Alternaria*; B) an increase of 1 log spores/m³ for *Cladosporium*. Multi-pollutant model includes *Alternaria*, *Cladosporium*, PM₁₀, and pollen of *Poaceae* adjusting for time-varying confounders such as humidity, temperature, pressure and wind speed at lag 0 and other covariates such as age, gender, height, childbirth weight, maternal smoking status, atopy, study area.

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