

Characterization of Allergens and Airborne Fungi in Low and Middle-Income Homes of Primary School Children in Durban, South Africa

Nkosana Jafta, MMedSc,¹* **Stuart A. Batterman, PhD,²** **Nceba Gqaleni, PhD,¹**
Rajen N. Naidoo, PhD,¹ and **Thomas G. Robins, MD²**

Background *The South Durban Health Study (SDHS) is a population-based study that examined the relationship between exposure to ambient air pollutants and respiratory disease among school children with high prevalence of asthma who resided in two purposely selected communities in north and south Durban, KwaZulu-Natal, South Africa.*

Methods *From the SDHS participants, a subgroup of 135 families was selected for investigation of household characteristics potentially related to respiratory health. In these households, a walkthrough investigation was conducted, and settled dust and air samples were collected for allergen and fungal measurements using standardized techniques.*

Results *Asp f1 allergen was detected in all homes, and Bla g1 allergen was detected in half of the homes. House dust allergens, Der f1 and Der p1 exceeded concentrations associated with risk of sensitization and exacerbation of asthma in 3% and 13%, respectively, of the sampled homes, while Bla g1 exceeded guidance values in 13% of the homes. Although airborne fungal concentrations in sleep areas and indoors were lower than outdoor concentrations, they exceeded 1,000 CFU/m³ in 29% of the homes. Multivariate analyses identified several home characteristics that were predictors of airborne fungal concentrations, including moisture, ventilation, floor type, and bedding type. Airborne fungal concentrations were similar indoors and outdoors, which likely reduced the significance of housing and indoor factors as determinants of indoor concentrations.*

Conclusion *Allergen concentrations were highly variable in homes, and a portion of the variability can be attributed to easily recognized conditions.* Am. J. Ind. Med. 55:1110–1121, 2012. © 2012 Wiley Periodicals, Inc.

KEY WORDS: *allergens; home characteristics; indoor air pollution; moisture*

¹Discipline of Occupational and Environmental Health, School of Nursing and Public Health, College of Health Sciences, University of KwaZulu-Natal, Durban 4041, South Africa

²Department of Environmental Health Sciences, School of Public Health, University of Michigan, Ann Arbor, Michigan

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*Correspondence to: Nkosana Jafta, MMedSc, Discipline of Occupational and Environmental Health, School of Nursing and Public Health, University of KwaZulu-Natal, 321 George Campbell Building, Howard College Campus, Durban 4041, South Africa. E-mail: jaftan@ukzn.ac.za

INTRODUCTION

Studies in both developing and developed countries have found rapid increases in the prevalence of pediatric asthma [Ehrlich, 2002; Abu-Ekteish et al., 2009]. Exposure to indoor air pollutants, including allergens, has been recognized as an important factor in asthma and other respiratory diseases. In developed countries, exposure and sensitization to indoor allergens is considered a possible cause of asthma and known trigger of asthma attacks, especially in children [Pearce et al., 2000]. Asthma exacerbation in children is associated with exposure to high concentrations of cockroach allergen (Bla g1, Bla g2, and Per f1), house dust mite (HDM) allergen (Der p1 and Der f1), animal allergen (Mus m1, Rat n1, Can f1, and Fel d1), and fungal allergen (Asp f1, Alt a1, Cla h1, and S. chartarum) [Rosenstreich et al., 1997; Garret et al., 1998; Gold et al., 1999; Lau et al., 2000; Carlsten et al., 2010; Olmedo et al., 2011]. Understanding the household risk factors associated with proliferation of allergens is an initial and important step in controlling exposure and reducing morbidity [IOM, 2000; Pearce et al., 2000; Arshad, 2010].

House dust mites are common in warm and humid climates, and their allergens are found in reservoirs, such as bedding, floor coverings, and fabric covered furnishings [Peroni et al., 1994; van Strien et al., 1994; Custovic et al., 1998a]. High surface concentrations of animal and insect allergens, for example, mouse and cockroach allergens, are most likely in spaces where food is abundant, for example, kitchens, dining rooms, and living rooms [Rosenstreich et al., 1997; Olmedo et al., 2011]. Numerous fungal species are abundant outdoors, but indoor infestations require spaces and surfaces with moisture or high water activity [Arruda et al., 1992; Dales et al., 1997; Garret et al., 1998].

In developing countries, studies of residential indoor environments have focused on biomass fuels and combustion pollutants. Biological pollutants, such as allergens, have received scant attention [Manjra et al., 1995; Ezzati and Kammen, 2001; Tielsch et al., 2009], particularly in Africa. In this study, concentrations of allergens and airborne fungi are characterized in homes of primary school children in Durban, KwaZulu-Natal, South Africa. Also examined is the association of household characteristics and concentrations of these pollutants with the aim of contributing to the understanding of the relationship between allergens and childhood asthma.

Durban is an industrial port city located on the east coast of South Africa with a population of approximately three million people. Due to the prevailing subtropical climate, both the temperature and humidity are generally high, creating an environment that encourages the growth and proliferation of biological agents and pollutants. Very high prevalence rates of asthma (32%) have been reported

among children attending schools in south Durban, an area containing numerous air pollutant-emitting industries, as compared to a prevalence rate of 17% for children living outside this area [Kistnasamy et al., 2008].

METHODS

Sample Selection, and Recruitment of Participants

The homes selected for indoor environment assessment represent a subset of households participating in the South Durban Health Study (SDHS), an epidemiological study that examined the relationship between ambient air pollution and respiratory disease among school children (grades 3–6 [Naidoo et al., 2007]). The SDHS recruited children attending seven schools in two communities: four schools in the highly industrialized south Durban area, and three schools in northern Durban, an area without major industry. At each of these schools, a screening questionnaire was completed by caregivers and used to identify children with known or probable persistent asthma. Of the 423 children who participated in the SDHS, 81 (19%) were classified as persistent asthmatics, based on the screening questionnaire.

From the participating schools, households of children with known and probable asthma were given priority for recruitment into an indoor environment assessment sub-study. A total of 20 households from each school list were targeted. Child caregivers or other responsible adults living with the child were recruited by telephone or house visits. Individuals giving consent were then scheduled for indoor assessments. Their homes were visited during the day by trained technicians who could speak the local languages (isiZulu or English). Home visits for walkthrough assessment, settled dust collection, and airborne fungal sampling were done from May 2004 to September 2005.

Each household recruited for the indoor environmental assessment provided written consent. All procedures in the study were approved by the respective ethics committees of the University of KwaZulu-Natal (South Africa) and the University of Michigan (USA) participating in the SDHS.

Home Walkthrough Assessment

The walkthrough assessment utilized a standardized instrument that was previously field-tested and used in Detroit, USA to investigate household characteristics as indicators of allergen concentrations [Parker et al., 2004]. This instrument was modified to include questions about informally constructed homes common in Durban. It also included direct observations, questions directed to the child caregiver or the adult respondent regarding

household characteristics in each room of the house, and activities of the child and other occupants. Information collected on housing characteristics included the type of house (single family house or flat/apartment, single or double storey, etc.), building age, moisture problems (sources or indicators), fungal growth, ventilation, and heating practices. Information on the types of floors, furnishings, bedding used by the child, and presence of stuffed toys was also collected. The rooms investigated in each house were the child's sleep area, the child's play area, the kitchen, and the bathroom.

Settled Dust Sampling and Allergen Quantification

Following the walkthrough assessment, two settled dust samples from the child's sleep area were collected. One sample was collected from the bedding (pillows and mattress), and, if present, a second sample was collected from a carpet or rug near the bed. A vacuum cleaner (WapVs300s, Bellenberg, Germany) equipped with a specialized nozzle and a 40 mm diameter filter paper (Macherey-Nagel, Dueren, Germany) on the inlet was used. Dust samples were collected from bedding materials by vacuuming for 2 min. Carpet/rug samples were collected by vacuuming a 1.0 m² area for 2 min. After sampling, the dust-loaded filter was folded, wrapped with aluminium foil, and placed in a sealed polyethylene bag for transport. Additional settled dust samples were collected from fabric furniture and carpet or rugs in the child's play area using similar procedures. All settled dust samples were transported to the laboratory on the day of collection and stored at 4°C until analysis.

In the laboratory, settled dust samples were sieved through a No. 45 mesh screen (355 µm sieve) to remove large particles and fibers. If two settled dust samples were collected from a single room (sleep area or play area), they were pooled and analyzed as a single sample. For extraction, 100 mg of fine settled dust sub-samples were weighed in test tubes and 2.0 ml of 0.05% Tween20 in phosphate-buffered saline was added. The samples were then suspended using a vortex mixer, mixed for 2 hr using a laboratory mixer, and then centrifuged at 2,500 rpm for 20 min at 4°C. Supernatants for allergen analysis were removed and stored at -20°C [Earle et al., 2007]. Allergens were measured using ELISA monoclonal antibodies assays (Indoor Biotechnologies, Wiltshire, UK) for HDM (Der p1 and Der f1) and fungi (Asp f1) on the children's sleep area samples, and for cockroach allergen (Bla g1) on the children's play area samples. Airborne fungi and mite allergen concentrations in settled dust were expressed as µg/g dust, and Bla g1 concentrations were expressed as U/g dust [Chapman et al., 1987]. The estimated minimum detectable level (MDL) for Der p1 and Der f1 was

0.30 µg/g dust and 0.60 U/g for Bla g1, and the reproducibility was 18% for Der p1, 3% for Der f1, and 5% for Bla g1, based on previous inter-laboratory studies [Pollart et al., 1991; Ovsyannikova et al., 1994; Sawyer et al., 1998; van Strien et al., 2003; Arbes et al., 2005]. Because of the few number of censored data points for HDM allergens (10% and 6% of Der p1 and Der f1 respectively), ½ MDL value (0.15 µg/g dust) was used to replace censored data for statistical analysis [Hewett and Ganser, 2007]. The characteristics of the Bla g1 concentrations meant using a more robust (more precise and less bias) β-substitution method to substitute Bla g1 concentrations below MDL was necessary [Ganser and Hewett, 2010].

Airborne Fungal Sampling, Identification, and Quantification

A sterilized two-stage Andersen sampler (Tisch Environmental, Cleves, OH) was used to collect viable airborne fungi from the child's sleep area, the room most used by the child (play area), the kitchen, and the bathroom of each home. During sampling, any external windows and doors of the sampled rooms were closed. The sampler was placed at the center of the room at least 0.5 m above the floor and away from obstacles that could affect airflow. Samples were collected for 5 min at 15 L/min using a calibrated vacuum pump (SKC Ltd, Eighty Four, PA) onto malt extract agar (MEA) and dichloran (18%) glycerol agar (DG18) plates (Oxoid Ltd, Basingstoke, Hampshire, UK). One to four airborne indoor fungal samples, and one airborne outdoor fungal sample were on each media collected during each home visit. After sampling, the media plates were sealed, marked, transported to the laboratory, and incubated in a temperature-regulated microbial cabinet between 25 and 28°C for 3–5 days. After incubation, fungal colonies were counted and visually identified as *Cladosporium*, *Aspergillus*, *Fusarium*, *Penicillium*, and *Rhizopus* species. Unknown species were grouped and classified as "unknown". Each of the five identified airborne fungal species was further isolated for confirmation by culturing onto MEA and DG18 agar plates for further observations of cellular, hyphal, and spore morphology. Morphologies were defined by visual observation and microscopy, and organisms were identified using Samson et al. [2000] as a reference. Concentrations of viable airborne fungi were expressed as colony forming units per cubic meter of air (CFU/m³). Airborne fungal concentrations below the MDL (13 CFU/m³) were set to ½ MDL for statistical analysis. Confirmatory analyses of the fungal genera were performed by the Medical Microbiology Laboratory of Inkosi Albert Luthuli Central Hospital in Durban, South Africa.

Airborne concentrations of each fungal species and the total fungal concentration measured in different rooms

of the same house were averaged to create new variables: indoor *Cladosporium*, indoor *Aspergillus*, indoor *Penicillium*, indoor *Fusarium*, indoor *Rhizopus*, indoor unknown, and indoor total. Several culture samples from the sleep area ($n = 4$) and outdoors ($n = 9$) showed excessively high concentrations of several species that made identification and quantification of other species impossible. For these samples, the concentrations were reported as the highest concentration found for each species and for the total. Airborne fungal species and total fungal concentrations on MEA and DG18 were similar, therefore only results of MEA are presented in this study.

Data Analyses

Descriptive analyses were performed for home characteristics and other factors potentially associated with allergen and airborne fungal concentrations. Nonparametric Spearman's rank correlations were used to investigate the association between concentrations of airborne fungi and allergens in settled dust. Multivariate analyses used step-wise regression and dust allergen concentrations and airborne fungal concentrations as dependent variables to test association with home characteristics variables, such as season, neighbourhood and region, used as independent variables. Stata/IC version 11.0 for Windows (StataCorp LP, College Station, TX) was used for statistical analysis. Before a multivariate regression analysis was computed for different outcome variables, bivariate analyses between outcome variables and home characteristics, were done. Variables that were significant at the 95% confidence level in the bivariate analyses, together with other variables that were considered important potential determinants of indoor pollution concentrations as shown in other studies, were included in the final multivariate models.

RESULTS

A total of 135 homes were visited for the indoor environment study: 53% ($n = 71$) of them were situated in south Durban region, and the rest in north Durban. Using data from the screening questionnaire administered to caregivers for SDHS, 103 (77%) of the participating households had children classified as asthmatics or probable asthmatics.

Characteristics of Children's Homes

Most homes (70%) were single-family homes of formal brick construction. Relatively few (7%) were informally constructed with walls made of different materials from wood, corrugated steel, cardboard boxes, etc. Typically, homes had 4 or 5 rooms, but the range in the sample was from 1 to 10 rooms.

The frequency of selected home characteristics in children's sleep and play areas, based on the walkthrough checklist, are shown in Table I. The bedroom was the room used by the majority (87%) of children as a sleep area; the remainder of the children used a living room (6%) or other room (7%) in the house. Living rooms were frequently (80%) used as an indoor play area. Few of the sleep areas were heated during the cold season (10%) or cooled during the hot season (36%). In most sleep and

TABLE I. House Characteristics in 135 Sleep and 112 Play Areas of Visited Homes of School Children

Conditions found in the households	n (%)	
	Sleep area [N = 135]	Play area [N = 112]
Heating	13 (9.6)	
Use of fan/air conditioner	50 (44.6)	60 (53.6)
Chairs present	46 (34.1)	105 (93.7)
Cluttered spaces	15 (11.1)	4 (3.5)
Evidence of cockroaches	49 (36.3)	27 (27.3)
Evidence of rodents	26 (19.3)	16 (14.32)
Opening windows	131 (97.0)	107 (95.5)
Fungal or mildew smell	49 (36.3)	11 (9.8)
Ceiling/interior roof		
Presence of ceiling	86 (63.7)	81 (71.7)
Visible water stains/moisture	48 (35.6)	34 (30.1)
Visible fungal growth	36 (26.7)	18 (15.9)
Wall		
Visible water stains/moisture	30 (22.2)	14 (12.4)
Visible fungal growth	16 (11.9)	4 (3.5)
Floor		
Smooth nonporous	83 (61.2)	93 (93.9)
Cement	18 (13.3)	4 (4.0)
Wall-to-wall carpet	34 (25.2)	14 (12.5)
Dirt floor	1 (0.7)	
Hard floor with rug/s	32 (23.7)	34 (30.4)
Type of bedding		
Bed with mattress	129 (95.6)	
Mattress on the floor	5 (3.7)	
Sofa/couch	1 (0.7)	
Blankets on the floor	1 (0.7)	
Type of blankets		
Cotton blankets	40 (29.6)	
Wool blankets	65 (48.1)	
Comforter	67 (49.6)	
Acrylic blankets	2 (1.5)	
Type of pillows		
Feather filled	4 (3.0)	
Polyester filled	58 (43.0)	
Foam filled	68 (50.4)	
Other type	4 (3.0)	

play areas, smooth and/or impervious floors (cement, floor tiles, or linoleum) were dominant (56% and 87%, respectively). Sleep areas had more evidence of moisture and visible fungal growth than play areas, which occurred mostly on interior roofs and/or ceilings rather than on walls and floors. Most children slept on beds with mattresses (93%); a few of them used mattresses on the floor (4%). Most children used either foam (44%) or polyester-filled (40%) pillows on their beds.

Occurrence and Concentrations of Allergens and Airborne Fungi in Children's Homes

Settled dust samples could not be collected from 9 (7%) of the homes visited because of the absence of electricity. In one-room homes, only a single settled dust sample from the sleep area could be collected. In these homes, 126 and 99 valid (composite) settled dust samples for allergen analysis were collected and analyzed from sleep areas and play areas, respectively, and 129 indoor and 129 outdoor air samples were collected and analyzed.

Table II shows allergen concentrations in settled dust. Detection frequencies varied from 51% for Bla g1 to 100% for Asp f1. The average Asp f1 concentrations in south Durban were higher than concentrations in north Durban. Only one household had both HDM allergen concentrations in children's settled dust bed samples below MDL. Of the HDM allergen concentrations, 3% (4 of 126) of homes had Der f1 concentrations above 10 µg/g dust, a concentration sometimes used as a threshold for the development and exacerbation of asthma [Platts-Mills et al., 1997], compared to 13% (13 of 126) Der p1 concentrations exceeding this value. The maximum Der f1 concentration (39.7 µg/g dust) exceeded the next highest value (22.9 µg/g dust) by almost twofold. Although for cockroach allergen 49% of the homes were below MDL, 13% (13 of 99) exceeded 2 µg/g dust, a guideline value for asthma exacerbation [Platts-Mills et al., 1997]. The highest Bla g1 concentration (24.4 U/g dust) detected in a home's play area settled dust was over three times higher

than the second highest concentration (7.8 U/g) observed in another home.

Mean airborne concentrations of different fungal species in children's sleep areas, indoors, and outdoors are shown in Figure 1. Indoor and outdoor fungal compositions were similar, and *Cladosporium* was by far the most abundant of the identified species. *Cladosporium* concentrations averaged around 60% of the total airborne fungal concentration, and were typically 10 or more times higher than other identified fungal species. *Penicillium* concentrations were slightly higher than other species identified, namely *Aspergillus*, *Fusarium*, and *Rhizopus*. Airborne fungal aerosol compositions in the children's sleep areas and indoor spaces were similar to those outdoors, however, outdoor concentrations were higher (Fig. 2).

Airborne fungal concentrations in Durban homes exceeded concentrations that have been associated with health effects in several studies [WHO, 2009]. The median concentration was 667 CFU/m³ for *Cladosporium*, a dominant species in the samples collected from these homes. Thirty percent of the homes had *Cladosporium* concentrations above 1,000 CFU/m³, a concentration which has been associated with adverse respiratory health outcomes in other studies [WHO, 2009]. The highest *Cladosporium* concentrations were 3,500 CFU/m³ indoors and 4,040 CFU/m³ outdoors, but these represent underestimates due to sampler overload. The number of homes with total airborne fungal concentrations that exceeded 1,000 CFU/m³ was 66 (51% of the 129) homes. Although *Penicillium* and *Aspergillus* were frequently detected in the sleep areas, the concentrations averaged 520 and 147 CFU/m³, respectively.

Home Characteristics, Allergen Concentrations in Dust, and Airborne Fungal Concentrations

There were no significant correlations observed between Asp f1 settled dust concentrations and airborne fungal concentrations in sleep areas, indoors, and outdoors. However, analysis stratified by some home characteristics

TABLE II. Fungal (Asp f1) and House Dust Mite (Der p1, Der f1) Allergen Concentrations in Settled Dust Collected From Children's Sleep Areas and Cockroach (Bla g1) Allergen Concentrations in Settled Dust Collected From Children's Play Areas

Allergen	Sample size	Detection frequency (%)		Mean (SD)	Median	Minimum	Percentile		
							90th	95th	Maximum
Asp f1(µg/g)	126	99	0.58(0.24)	0.47	0.32	0.99	1.09	1.37	
Der p1(µg/g)	126	90	6.89(13.51)	1.98	0.15	18.26	46.57	71.42	
Der f1(µg/g)	126	94	1.96(4.40)	1.00	0.15	2.63	5.39	39.71	
Bla g1(U/g)	99	51	1.23(2.65)	0.66	0.25	2.87	3.31	24.40	

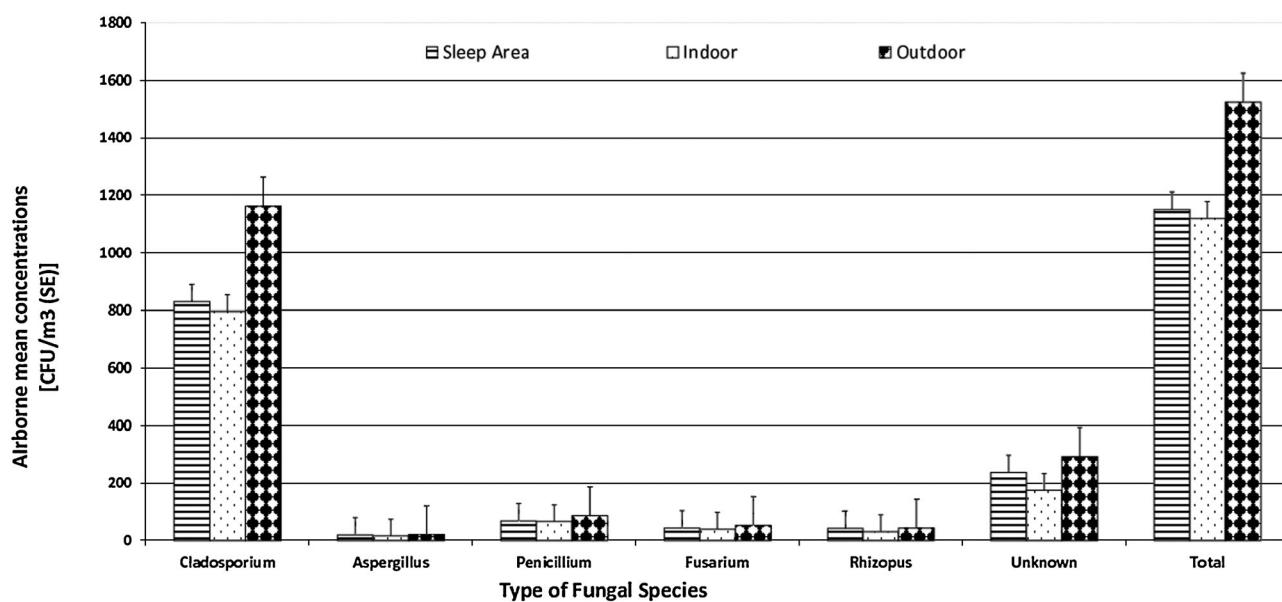


FIGURE 1. Mean airborne concentrations (standard error) of different fungal species (identified and unknown) sampled from sleep areas, indoors, and outdoors of the children's homes.

showed significant correlations. Homes with rugs, or usage of fans in the sleep areas, had significant positive correlations between these two variables, namely, Asp f1 and airborne total fungal concentrations ($r = 0.53$, $P = 0.011$, $n = 22$; and $r = 0.36$, $P = 0.032$, $n = 35$, respectively).

In informal constructed homes, Asp f1 concentrations in settled dust were negatively correlated with airborne total fungal concentrations ($r = -0.80$, $P = 0.005$, $n = 10$).

Tables III and IV show results of the multivariate models for different allergen and airborne fungal

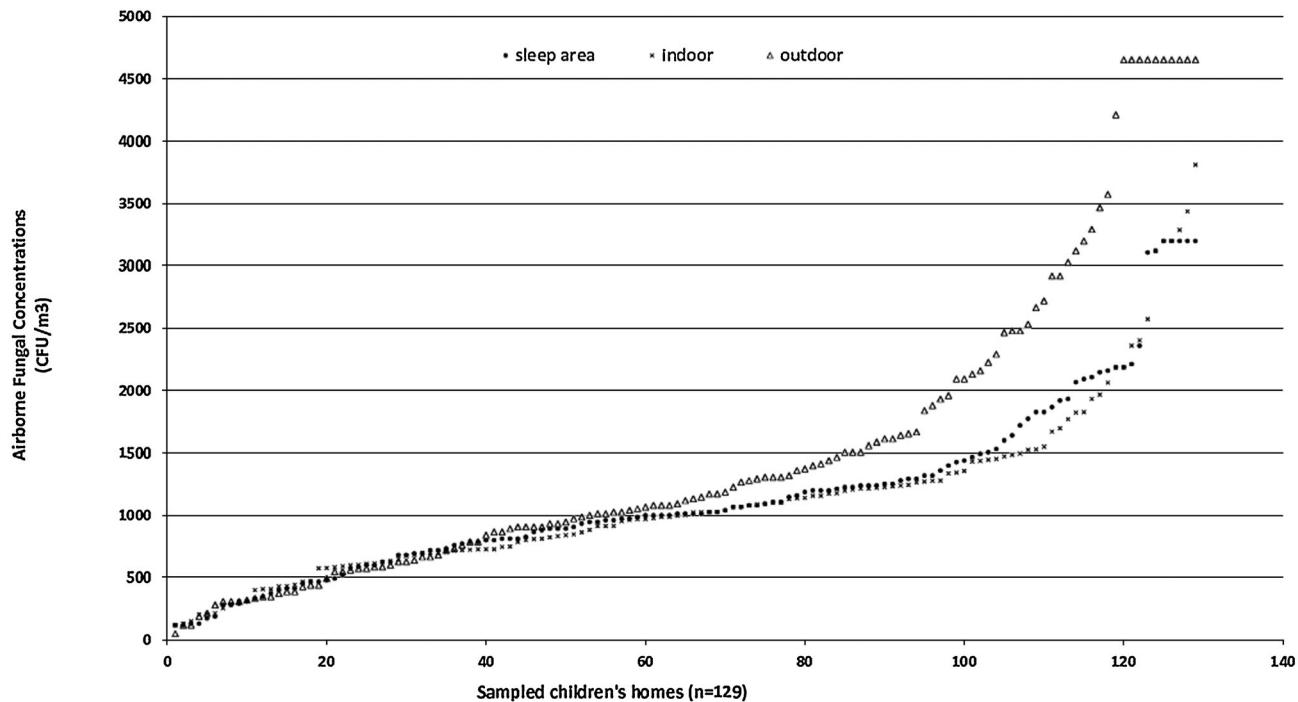


FIGURE 2. Distributions of total airborne viable fungal concentrations sampled in sleep, indoor, and outdoor areas of visited children's homes.

TABLE III. Determinants of Different Allergen Concentrations in Children's Sleep Areas (Asp f1 and HDM) and Play Areas (Bla g1) Predicted Using Multivariate Stepwise Regression Models

Dependent variable (model adjusted R ²)	Home characteristics (predictor variables)	Coefficient	95% CI	P-value
<i>Aspf1</i> (0.2808)	Flat/duplex home	0.16	0.07 to 0.26	0.001*
	Opening windows	-0.48	-0.72 to -0.24	<0.001*
	Comforter	0.07	<0.01 to 0.15	0.065
<i>Derf1</i> (0.0529)	Cement floor	2.51	0.17 to 4.86	0.036*
	Wall-to-wall carpet	1.89	0.01 to 3.77	0.048*
	Cotton blanket	1.94	-0.36 to 4.24	0.097
<i>Derp1</i> (0.1363)	Opening windows	16.80	0.43 to 33.17	0.044*
	Comforter	-36.95	-56.82 to -17.08	<0.001*
	Wool blankets	-39.18	-58.82 to -1954	<0.001*
	Cotton blanket	-30.83	-51.33 to -10.34	0.004*
<i>Blag1</i> (0.0511)	Blend of blankets	-30.88	-51.20 to -10.57	0.003*
	Smooth floors	0.67	-0.56 to 1.40	0.070
	Visible roof or ceiling mould	-0.71	-1.37 to -0.04	0.038*

Variables included in the models: type of house, house age, opening windows, moisture and dampness signs, fungal growth, bedding, heating, and presence of a ceiling.

Table showing variables with a P-value of <0.1 on the multivariate analysis.

All models adjusted for season and region (south or north Durban).

*P < 0.05.

TABLE IV. Factors Influencing Concentrations of Various Airborne Fungal Species and Total Fungal Concentrations From Children's Sleep Areas—From Multivariable Regression Models

Dependent variable (model adjusted R ²)	Home characteristics (predictor variables)	Coefficient	95% CI	P-value
<i>Cladosporium</i> (0.1008)	Opening windows	701	-129 to 1530	0.097
	Informal constructed home	499	82 to 916	0.019*
	Visible ceiling or interior roof moisture	321	77 to 565	0.010*
<i>Penicillium</i> (0.2320)	Visible ceiling or interior roof moisture	-54	-92 to -16	0.006*
	House made of bricks	156	20 to 293	0.025*
	Informal constructed home	246	148 to 344	<0.001*
	Presence of a ceiling	-55	-96 to -15	0.008*
	House made of wood	-142	-297 to 12	0.071
<i>Fusarium</i> (0.1286)	Opening windows	54	6 to 101	0.028*
	Visible wall fungal growth	-20	-42 to 1	0.065
	Home <25 years old	20	6 to 34	0.004*
<i>Rhizopus</i> (0.1034)	House made of bricks	70	1 to 140	0.045*
	Informal constructed home	62	-2 to 126	0.057
Unknown fungal species(0.0945)	Home >25 years	-116	-239 to 7	0.064
	Opening windows	550	114 to 985	0.014*
Total airborne fungi(0.1179)	House made of bricks	792	45 to 1539	0.038*
	Visible wall fungal growth	-358	-741 to 25	0.067
	Informal constructed home	1193	510 to 1875	0.001*
	Visible ceiling or roof moisture	337	86 to 589	0.009*

Variables included in the models: type of house, house age, opening windows, moisture and dampness signs, fungal growth, bedding, heating, and presence of a ceiling.

Table showing variables with a P-value of <0.1 on the multivariate analysis.

All models adjusted for season and region (south or north Durban).

*P < 0.05.

concentrations adjusted for season and region. Each of the final models had different significant predictors for each allergen, and the coefficients of determination (R^2) were less than 0.3. Surprisingly, the presence of open windows and cement floors in sleep areas were significant and positive predictors of higher Der p1 and Der f1 allergen dust concentrations, respectively. Predictors of Bla g1 dust concentrations included smooth floors and visible roof and ceiling mould. No home characteristics were identified as significant predictors of airborne *Aspergillus* concentrations. Moisture and visible fungal growth on interior roof surfaces were negative predictors of higher airborne *Penicillium* and total fungal concentrations, but positive predictors of *Cladosporium* concentrations in air.

DISCUSSION

Durban homes showed allergen concentrations and compositions in settled dust that resemble those in some studies in both developing and developed countries [e.g., Chew et al., 1998; Addo-Yobo et al., 2001; Perfetti et al., 2004], but concentrations were lower than those seen in inner cities in the USA [e.g., Eggleston et al., 1999; Breysse et al., 2005]. A moderate fraction of Durban homes had allergen and airborne fungal concentrations that exceeded suggested risk thresholds for sensitization and/or asthma exacerbation and other respiratory illnesses [Platts-Mills et al., 1997; WHO, 2009]. This is an important finding because most of the sampled homes had children classified as probable or known to be asthmatic. Total airborne fungal concentrations in sleep areas and total airborne indoor fungal concentrations were significantly associated with fungal allergen concentrations in settled dust, but only when some home characteristics were present (or absent), suggesting that dust as a reservoir of allergenic materials may be influenced by the airborne fungi only under certain conditions. From the observed correlations it could be true that airborne fungal concentrations are influenced by reservoir fungal dust contamination. Overall, the lack of correlation among dust and air measurements across different homes in Durban suggests that multiple factors unaccounted for in this study may be affecting these measurements.

Fungal Allergen (Asp f1) and Airborne Fungi in Sleep Areas

Asp f1 was detected in settled dust samples in all homes. The abundance of this fungus supports the hypothesis that fungi can rapidly proliferate when conditions are suitable, as found in Durban's subtropical climate. Fungi have been reported to grow from 6 to 196 hr after spore deposition in appropriate conditions, and allergens can be produced within 12 hr after this growth [Arruda et al.,

1992]. This observation is supported by our findings of an association between interior roof or ceiling moisture damage and total airborne fungal and *Cladosporium* concentrations found in the children's sleep areas.

Dust concentrations of fungal allergen were significantly affected by housing type. Elevated concentrations of Asp f1 and total airborne fungi were seen in attached homes (flat/apartment/duplex) and in informal constructed homes (as compared to detached and formal family homes). One factor that may influence this association is the close proximity between attached dwellings, which can promote air exchange between dwellings that transports airborne fungi. Conditions such as high water activity and indoor climate in informal constructed homes can also promote indoor fungal growth and lead to higher concentrations [Danaviah et al., 2000].

The similarity of indoor and outdoor airborne fungal concentrations at the time of sampling suggests only limited indoor sources of fungi. However, the associations observed between home characteristics and airborne fungal concentrations suggest that several characteristics are important determinants of indoor fungal concentrations (Table IV). Opening windows in sleep areas was a significant determinant of lower concentrations of Asp f1 and total airborne fungi in sleep areas. Although natural ventilation possible with opened windows allows entry of outdoor airborne fungi, ventilation can help to reduce by dilution indoor air contaminants, and it can reduce the build-up of humidity, which can be especially important in smaller and more crowded houses and bedrooms. Opening windows may provide a practical approach to provide ventilation and control airborne fungi in the study communities, especially considering that none of the Durban homes used mechanical ventilation or particle filtration.

Bedding type was a significant determinant of Asp f1 concentrations in children's beds. Unlike other microbes, fungal proliferation on surfaces needs sufficient water activity and nutrients. These fungal growth requirements are unlikely to be found in bedding, hence this finding appears coincidental. Possibly, bedding concentrations of Asp f1 may be a surrogate for household maintenance practices, which are likely to be worse in poorer households.

Other determinants of individual airborne fungal species concentrations identified in children's sleep areas were not consistent with the determinants of total airborne fungal concentrations. Concentrations of different airborne fungi were inversely associated with visible moisture, visible fungal growth, and age of the homes, which is also inconsistent with the conditions fungi need to proliferate. Again, these results appear coincidental. Because indoor and outdoor concentrations of airborne fungi were very similar, these associations may reflect seasonality or some other unmeasured or uncontrolled factor. It is clear that airborne fungal concentrations determined using short-

term indoor measurements require careful and cautious interpretation given the influence of outdoor concentrations and the great variation that can occur very rapidly [Chew et al., 2003; Cho et al., 2006].

Earlier studies examining indoor environments in Durban showed that *Cladosporium* is the dominant airborne fungal genus in the region [Danaviah et al., 2000; Berman, 2007]. In the present study, association of *Cladosporium* allergen concentrations was not associated with airborne *Cladosporium* concentrations was not investigated. Airborne *Cladosporium* concentrations were not associated with visible fungal growth or moisture on surfaces. Asp f1 allergen concentrations in dust are relevant due to their potentially profound health effects.

House Dust Mite Allergens (Der p1 and Der f1) in Sleep Areas

The occurrence of HDM allergens in the SDHS homes was high (90% and 94% for Der p1 and Der f1, respectively) in comparison to homes in the USA [80.2%; Leaderer et al., 2002] and elsewhere [Custovic et al., 1998b; Moscato et al., 2000]. This is not surprising given that year-round climatic conditions in Durban are ideal for the proliferation of these allergen-producing organisms, for example, the annual average temperature and humidity is 23°C and 75%, respectively. Potter [2010] also documented that coastal areas of South Africa have higher HDM concentrations compared to (typically drier and cooler) inland areas. Although Der p1 concentrations were not correlated to Der f1 concentrations in the children's sleep areas, both allergens seem to thrive in Durban homes. Der p1 had higher concentrations, which supports observation that only a single type of HDM allergen is dominant in a house [Moscato et al., 2000; Perfetti et al., 2004]. Proliferation of one type of species in an environment is a likely result of the competition for food and water.

Few factors were associated with HDM allergen concentrations. The observed association of blanket type with HDM concentrations is similar to findings elsewhere. All blanket types (both natural and synthetic materials) were associated with lower Der p1 concentrations, contrary to expectations. Blankets made of cotton and other natural materials have been associated with lower concentrations of Der p1, suggesting that such material is not conducive for HDM proliferation [Hallam et al., 1999; Custovic et al., 2000]. However, natural materials can provide protein as food for HDM [Sawyer et al., 1998]. In the present study, although the association between Der f1 allergen concentrations and cotton blankets was not statistically significant, the positive association observed supports this hypothesis of natural fibers as a food source for HDMs. However, the higher HDM concentrations associated with

cement floors (smooth floors) and presence of opened windows found in this study are inconsistent by Carrer et al. [2001], who shows lower concentrations with these factors. Further, that visible moisture and dampness were not significant predictors of HDM concentrations might imply that these factors did not significantly affect humidity inside Durban homes, possibly because ambient conditions are already very humid.

Cockroach Allergen (Bla g1) in Play Areas

Although Manjra et al. [1995] indicated that Per f1 allergen was the most prevalent cockroach allergen in Durban homes, 51% of the samples in the present study showed the presence of Bla g1 allergen. A much lower occurrence of cockroach allergen has been in homes in the USA in several studies, for example, 30% for Bla g1 [Chew et al., 1998; Leaderer et al., 2002] and 24% for Bla g in family room floors and/or furnishings [Gold et al., 1999]. Although the Bla g1 detection frequency was high, concentrations were much lower than those reported elsewhere [Eggleston et al., 1999]. Because settled dust samples for Bla g1 analysis were collected from children's "play areas" and most of these were living rooms, it is likely that higher detection frequency could have been observed from samples collected from the kitchens.

Although almost 50% of the samples had Bla g1 below detection limit, we modeled concentrations against home characteristics. Cockroach allergen proliferation has been associated with socioeconomic status and housekeeping practices, for example, food storage and trash maintenance, and with older homes and informally constructed homes [Leaderer et al., 2002]. In our study, observed associations, such as smooth floors in children's play areas associated with higher concentrations of Bla g1 allergen, and visible interior roof and ceiling mold growth associated with lower Bla g1 concentrations are contrary to expectations. The low R^2 (0.0511) for the Bla g1 model in Table III suggests that our independent variables do not fully explain the variability in the allergen concentration.

Study Limitations

The sample size of the sub-study limits our ability to observe relationships and account for interactions among variables. Moreover, the home assessments were conducted in different seasons, and seasonal influences on allergen concentrations, dampness, visible moisture, and ventilation practices may influence the findings.

All of the dust and air sampling data were cross-sectional in nature. Longitudinal data would have provided a more comprehensive assessment of allergen and airborne fungal concentrations. Additionally, repeated measurements in each household would help to ensure that

observations were representative of everyday practise and conditions. The fungal aerosol sampling used a single growth media and incubation condition, and it represents a very short-term (5 min) sample that may not be representative. The general approach of collecting and incubating airborne fungi may underestimate some species due to the different media and incubation temperatures needed for optimal determination of these species [Samson et al., 2000]. This applies to incubation temperatures needed for optimal growth of *Aspergillus* and *Penicillium* species which are between 35 and 37°C [Samson and Hoekstra, 1994]. Also, only five fungal genera were identified in air samples, and some species may be misclassified as "unknown" although they may belong to one of the target genera. We may have missed the association between airborne fungal concentrations and allergen concentrations in settle dust allergen because we investigated *Aspergillus* allergen instead of the *Cladosporium* allergen which is the most prevalent.

Analysis, such as modeling Bla g1 concentrations, using data with approximately 50% of the dust samples having concentrations not detectable is problematic. In our dataset with characteristics that include single MDL, small sample size ($n = 99$), high left censored points (49%) and large standard deviation (2.65, β -substitution was a method of choice compared to maximum likelihood estimation (MLE), log-probit regression (LPR), and common substitution methods (MDL/2 and MDL/ $\sqrt{2}$). Our dataset was not lognormal which is another important characteristic for using β -substitution as a substitution method [Hewett and Ganser, 2007; Ganser and Hewett, 2010]. Because of the few censored data points seen for the airborne fungal concentrations and HDM and fungal dust allergens concentrations, our substitution method of choice is justified for these variables.

CONCLUSIONS

This study characterized concentrations of allergens in homes in a developing country, and included many homes in which a vulnerable population lived: children with asthma. Settled dust allergen and airborne fungal concentrations frequently exceeded concentrations and guidelines linked to respiratory illness, including asthma exacerbation and asthma development. While few of the "classical" determinants of high pollutant concentrations were identified, and results are somewhat limited due to the imprecision of environmental sampling of airborne fungi and ELISA measurements of Asp f1 allergen in settled dust, these two biological contaminants require further investigation in indoor environments in developing countries. Because allergen concentrations varied with certain home characteristics, such as house age and presence of opening windows, some low cost interventions to

reduce allergen concentrations may be feasible. The study results suggest that developing countries should view allergen exposure as an important public health issue.

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